Adult neurogenesis and cellular brain repair with neural progenitors, precursors and stem cells

U. Shivraj Sohur[†], Jason G. Emsley[†], Bartley D. Mitchell and Jeffrey D. Macklis*

MGH-HMS Center for Nervous System Repair, Departments of Neurosurgery and Neurology, and Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital; and Harvard Stem Cell Institute, Harvard University, Edwards 410 (EDR 410), 50 Blossom Street, Boston, MA 02114, USA

Recent work in neuroscience has shown that the adult central nervous system (CNS) contains neural progenitors, precursors and stem cells that are capable of generating new neurons, astrocytes and oligodendrocytes. While challenging the previous dogma that no new neurons are born in the adult mammalian CNS, these findings bring with them the future possibilities for development of novel neural repair strategies. The purpose of this review is to present the current knowledge about constitutively occurring adult mammalian neurogenesis, highlight the critical differences between 'neurogenic' and 'non-neurogenic' regions in the adult brain, and 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 presently 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 be cautious 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 towards molecular and cellular manipulation of endogenous neural precursors *in situ*, without transplantation. We conclude this review with a discussion of what might be the function of newly generated neurons in the adult brain, and provide a summary of present thinking about the consequences of disturbed adult neurogenesis and the reaction of neurogenic regions to disease.

Keywords: dentate gyrus; subventricular zone; rostral migratory stream; olfactory bulb

1. INTRODUCTION

Over most of the past century of modern neuroscience, it was thought that the adult brain was incapable of generating new neurons or having neurons added to 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 & Das 1965; Altman 1969; Reynolds & Weiss 1992; Lois & Alvarez-Buylla 1993; Palmer *et al.* 1995; and see reviews in McKay (1997), Gage (2000), van der Kooy & Weiss (2000) and 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 towards therapeutic neuronal or glial repopulation. Elucidation of the relevant molecular controls might allow both the 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); and what is known about the location, behaviour 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: (i) adult neurogenesis and precursor cell function might play an important role for the function of the healthy and diseased brain, specifically, they may underlie particular aspects of neuronal plasticity as defined by the adaptation of circuitry in response to functional demands; (ii) 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.

* Author for correspondence (jeffrey_macklis@hms.harvard.edu).

[†] These authors contributed equally to this work.

One contribution of 13 to a Theme Issue 'The regenerating 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 behaviour of endogenous adult precursors: this includes their ability to migrate to selected brain regions, differentiate into neurons and finally integrate into normal adult neural brain circuitry. Identification of molecular activity-related controls over constitutively occurring neurogenesis is underway. The location, identity and differentiation potential of endogenous adult precursors have started to be understood. In this review, we will outline a few examples of normally occurring neurogenesis in the adult mammalian CNS; briefly describe adult neural precursors; and 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 many challenges facing the 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, multilevel and critical analyses.

2. DEFINING NEURAL STEM CELLS, PROGENITORS AND PRECURSORS

Rigorously defined, adult CNS 'stem cells' exhibit three cardinal features: (i) they are 'self-renewing', with the theoretically unlimited ability to produce progeny indistinguishable from themselves; (ii) they are proliferative, continuing to undergo mitosis (though perhaps with quite long cell cycles); and (iii) they are multipotent for the different neuroectodermal lineages of the CNS, including the multitude of different neuronal and glial subtypes. 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 and oligodendroglial). Together, CNS stem cells and all precursor/progenitor types are broadly defined 'precursors'. We favour reserving the term 'stem cell' as rigorously defined earlier 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 completely 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 these criteria might apply in many cases, it is not clear how many of the rare site- 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 not yet known, limiting some conclusions, the heterogeneity and seeming variety of neural precursor cells are also important aspects 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 may, in some cases, allow far more efficient production of desired cell types. The brain itself may 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 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 ([Brookes 1997](#)). Goldfish undergoes retinal neurogenesis throughout life ([Johns & 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 2002](#)). Lesioned postnatal avian retina undergoes some neurogenesis with the new neurons most likely arising from Müller glia ([Fischer & Reh 2001](#)). In songbirds, new neurons are constantly added to the high vocal centre (HVC; [Goldman & Nottebohm 1983](#); [Nottebohm 1985](#); [Kirn *et al.* 1991](#); [Alvarez-Buylla *et al.* 1998](#)), a portion of the brain necessary for the production of learned song ([Nottebohm & Arnold 1976](#); [Simpson & 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 zebra finch (Scharff *et al.* 2000), which does not normally seasonally replace HVC—robust nucleus of the archistriatum (RA) projection neurons in the song production network, e.g. as canaries do. Inducing cell death of HVC—RA neurons in zebrafishes 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 behaviour.

4. THE DISCOVERY OF CONSTITUTIVELY OCCURRING ADULT MAMMALIAN NEUROGENESIS

Ramon y Cajal has been widely quoted as writing that 'in the adult centres, 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 adult brain. Using tritiated thymidine as a mitotic label, he published evidence that neurogenesis constitutively occurs in the hippocampus (Altman & Das 1965) and olfactory bulb (Altman 1969) of the adult mammalian brain. These results were later replicated using tritiated thymidine labelling 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 & 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 subregion of the hippocampus have 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 the dentate gyrus, it would severely limit the potential for neuronal replacement therapies based on *in situ*

manipulation of endogenous precursors. 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 & 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; Weiss *et al.* 1996a; Shihabuddin *et al.* 1997; Palmer *et al.* 1999; 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 & Weiss 1992). While it has become standard for some groups to use this so-called 'neurosphere-forming' assay as a key criterion for 'neural stem cells' (NSCs), the ability to form a 'neurosphere' alone is not a sufficiently reliable hallmark of stem cells and does not completely differentiate between various mitotic and precursor cell populations (Seaberg & van der Kooy 2003). Clonal analysis provides important additional information. By subcloning 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 still. Not surprisingly, the importance of tissue conditions for precursor cell function, for example, factors contributed by astroglia, has become 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 & Weiss 1992; Palmer *et al.* 1995; Craig *et al.* 1996). The general effectiveness of both the factors as mitogens for precursors has also been demonstrated *in vivo* (Craig *et al.* 1996; Kuhn *et al.* 1997), and they have been used recently to enhance induced neurogenesis in the normally non-neurogenic 'cornu Ammon (CA1)' region of the hippocampus after focal ischaemia (Nakatomi *et al.* 2002). However, their effects *in vivo* are not identical to those *in vitro*, and consequently,

their physiological 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 can be induced to divide by themselves (Brewer 1999; Gu *et al.* 2000). Although it seems unlikely that a neuron could maintain the elaborate neurochemical and morphological differentiation states of a mature neuron while replicating its DNA and remodelling 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 and assessment of double labelling using immunocytochemical labels; and the importance of understanding the *in vivo* microenvironment in which neural precursors reside.

In essentially all these researches, the 'stemness' of certain cells *in vivo* has been inferred from: (i) the fact that after being labelled as dividing cells, the cells give rise to neurons and glia and (ii) *ex vivo* evidence regarding precursor cells in the same brain region. Thymidine analogues are typically used as proliferation markers, to label dividing cells permanently and to pass labelling on to the progeny. Most of the earlier studies used tritiated thymidine, which is incorporated into the DNA of dividing cells during S-phase of mitosis and can be detected autoradiographically (Altman & Das 1965; Cameron *et al.* 1993). In recent studies, tritiated thymidine has usually been replaced by the thymidine analogue bromodeoxyuridine (BrdU) because BrdU can be detected immunocytochemically and thus allows for double- and triple-labelling with cell type-specific markers and confocal microscopic analysis (Kuhn *et al.* 1996). However, the use of thymidine analogues and particularly BrdU has raised some

technical issues. One question is whether BrdU labelling might identify cells undergoing DNA repair rather than proliferation; this possibility has recently been ruled out for standard BrdU labelling, and has been discussed in detail elsewhere (Cooper-Kuhn & Kuhn 2002). There is no evidence 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 an abortive cell cycle that includes an S-phase (Yang *et al.* 2003; Kuan *et al.* 2004) and thus could potentially incorporate BrdU. However, such terminally sick neurons do not survive for long periods (Kuan *et al.* 2004), hence experiments with long survivals do not risk confusion. An additionally important approach is to study the development of labelled 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 mature neurons are labelled, this eliminates confusion by ruling out BrdU incorporation by dying cells. Pre-labelling 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 labelling can also identify mitotic cells and allow complete cellular morphological and/or electrophysiological 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, labelling 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 the olfactory bulb (Carleton *et al.* 2003; Magavi *et al.* 2005) by labelling the dividing cells with retroviruses.

A second methodological issue concerns criteria for identification of cellular phenotype. Presently, NeuN has become the standard immunocytochemical marker to identify neurons, and glial fibrillary acidic protein (GFAP) and S100 β are used as astroglial markers. In mice, nestin (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 upregulated. The development of transgenic mice expressing GFP under a neural-specific element of the nestin promoter has been very helpful in identifying precursor cells *in vivo* (Yamaguchi *et al.* 2000; Filippov *et al.* 2003), but conclusions can only be drawn regarding those cells that are labelled and 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 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-labelled 'neuron' solely by its expression of NeuN is insufficient (Magavi & Macklis 2002a,b). 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 labelling of axons (Stanfield & Trice 1988; Hastings & Gould 1999; Markakis & 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 progressive differentiation in the sense of progression through different markers of increasing neuronal (or other cell type) differentiation over time after the cell division (Magavi *et al.* 2000; Arvidsson *et al.* 2002; Magavi & 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 marker 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), polysialylated form of neural cell adhesion molecule or β -III-tubulin. In all these cases, the specificity of markers is not clear, and from neuronal development and studies of induced adult neurogenesis, it is known 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 there are no marker proteins as of yet that allow an 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 completely known. Other markers are of great interest, e.g. SOX-2 (D'Amour & Gage 2003) and brain lipid-binding protein (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 unknown 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 olfactory bulb and the dentate gyrus of the hippocampus. In the olfactory system, precursor cells reside in the anterior aspect of the 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 (rostral migratory stream, RMS) into the olfactory bulb, and differentiate into granule or periglomerular interneurons (Lois & Alvarez-Buylla 1993; Luskin 1993; Goldman 1995; Doetsch *et al.* 1999). In the dentate gyrus of 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 brain regions are thus referred to as 'neurogenic regions' of the brain. As will be discussed later in more detail, a report of extremely low-level constitutive neurogenesis in the normal neocortex (Gould *et al.* 1999c, 2001) has been disputed (Kornack & Rakic 2001; Koketsu *et al.* 2003), primarily for methodological reasons. In addition, the same group later modified their interpretation by stating that the existence of these neurons was only transient (Gould *et al.* 2001). Additionally, there has also been a single report 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 neurogenesis in regions currently described as non-neurogenic is impossible. Rather, recent isolated reports of such a phenomenon have not sufficiently overcome many of the methodological issues described earlier.

Transplantation studies support the principle of defining neurogenic and non-neurogenic regions and provide evidence about the role of microenvironment in influencing the potential of neural precursors. If the precursor cells are transplanted into neurogenic regions, they can differentiate into neurons in a region-specific way (Gage *et al.* 1995a; Suhonen *et al.* 1996; Takahashi *et al.* 1998; Shihabuddin *et al.* 2000). The SVZ precursor cells generate hippocampal neurons when transplanted into the hippocampus and the 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 behaviour 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 is surprising, to many in the field, that the 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 & 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 & 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 homogenous, with notably different growth kinetics and differentiation potential. Neither their function outside the classical neurogenic regions nor their relation to the precursor cells of the neurogenic regions is known. For example, neural precursor cells from the spinal cord behave much like their counterparts from the SGZ and SVZ *in vitro* (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 summary, the extent of neurogenesis in a region is a function of the local microenvironment on precursor cells with (varying) neurogenic potential.

Therefore, there is 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 & 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. As will be described later in this review, Magavi *et al.* (2000) 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 (Chen *et al.* 2004). 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 ischaemia 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 controversial examples of reportedly extremely low-level constitutive neurogenic regions to date might also support this possibility. This phenomenon would be '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 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 & 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-existent 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.* (1999a–c), neurogenesis of 2–3 new neurons per 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 recent reports, analysed with more rigorous methods as noted earlier, are unable to reproduce these findings, and report a complete absence of constitutive cortical neurogenesis in both rodents and primates (Magavi *et al.* 2000; Kornack & Rakic 2001; Ehninger & 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). However, examination of the potential for constitutive neurogenesis in what are considered normally to be non-neurogenic regions will be required perhaps to assess definitively the potential existence of extremely low-level neurogenesis.

The situation in the literature is not clear, but it is important to remain critical and cautious in interpretation, maintaining 'reserved optimism'. After all, the brain does regenerate poorly. Although these newer results might fundamentally change our view on pathology-induced neuroplasticity, it should not be forgotten that there is little evidence to date that neurogenesis outside the normally neurogenic regions could by itself significantly contribute to brain repair. Attempts towards endogenous repair might require exogenous support. Restorative neurobiology focusing

on this approach aims at awakening the potential that lies in precursor cells in the neurogenic and non-neurogenic regions of the adult brain.

8. NEUROGENESIS IN THE 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) and 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 & Alvarez-Buylla 1993; Kornack & Rakic 1999; Pencea *et al.* 2001a). *In vitro* (Kirschenbaum *et al.* 1994; Pincus *et al.* 1998) and *in vivo* (Sanai *et al.* 2004) evidences suggest that such neuronal precursors also exist in humans. When retroviruses (Lois & Alvarez-Buylla 1994), tritiated thymidine (Lois & Alvarez-Buylla 1994), vital dyes (Lois & Alvarez-Buylla 1994; Doetsch & Alvarez-Buylla 1996) or virally labelled SVZ cells (Luskin & Boone 1994; Doetsch & Alvarez-Buylla 1996) are microinjected into the anterior portion of the SVZ of postnatal animals, labelled cells are eventually found in the olfactory bulb. Upon reaching the olfactory bulb, these labelled neurons differentiate into interneurons specific to the olfactory bulb, olfactory granule neurons and to a much lesser extent, peri-glomerular interneurons (Luskin 1993; Lois & 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 & 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 of the cells born in the SVZ die before they mature completely into neurons (Winner *et al.* 2002). Thus, a principle of embryonic development is repeated here: new neurons (or cells with neuronal potential) are generated 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 the addition of new neurons. In contrast to the hippocampus (to be discussed later), 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 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 the rodents, and the introduction of new neurons into such a complex pre-existing system may be a far more challenging feat in humans than in rodents or non-human primates. 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 a ciliated process touching the ventricular surface (Doetsch *et al.* 1999). These multipotent neural precursors have astrocytic morphology and express 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). Since 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 presently available evidences suggest 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 serve 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 (Malatesta *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 EGF or basic 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 the fate of the precursors. EGF and FGF-2 (Gritti *et al.* 1995; Gritti *et al.* 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 towards a neuronal fate (Whittemore *et al.* 1999). Bone morphogenetic proteins promote differentiation of SVZ precursors into astroglial fates (Gross *et al.* 1996), while platelet-derived growth factor (Williams *et al.* 1997; Whittemore *et al.* 1999) and insulin-like growth factor-I (IGF-I; Arsenijevic & Weiss 1998) promote SVZ precursors to differentiate into neurons. There are ambiguous results regarding whether brain-derived neurotrophic factor (BDNF) promotes the survival (Kirschenbaum & 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 infused EGF or transforming growth factor- 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, most notably vascular endothelial growth factor (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 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 vivo* in the adult to replace neurons lost to diseases or degeneration in brain areas that do not normally undergo neuronal replacement. Some scepticism regarding the feasibility of such approaches is also justified; however, 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, several molecular and extracellular controls have been identified that can influence neural precursor behaviour. 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, several proteins, including the mRNA-binding protein, Musashi1 (Sakakibara *et al.* 2001), the glycosylated form of cystatin C (Taupin *et al.* 2000), and the orphan nuclear receptor 'tailless' (TLX) (Shi *et al.* 2004), have been implicated in regulating adult SVZ proliferation and differentiation.

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 haematopoietic 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). Labelled 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 environments 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, partially at least due 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 started to be answered, but our understanding of hippocampal neurogenesis is already quite significant (Kempermann *et al.* 2004a,b). The fact that hippocampal neurogenesis can be modulated by physiological and behavioural events, such as aging, stress, seizures, learning and exercise, is of particular interest. 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.

Hippocampal neurogenesis has been described *in vivo* in adult rodents (Altman & Das 1965), monkeys

(Gould *et al.* 1998, 1999b; Kornack & Rakic 1999) and adult humans (Eriksson *et al.* 1998). Newborn cells destined to become neurons are generated along the innermost aspect of the granule cell layer, the 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 & Trice 1988; Hastings & Gould 1999; Markakis & 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 extend their processes into the CA3 region as early as 4 days after division (Hastings & 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.* 2004b): (i) radial glia-like precursors termed 'B cells' or 'type 1 cells'; (ii) nestin-expressing 'type 2 cells', which lack glial features; and (iii) DCX-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 & van der Kooy 2002). These cells, termed B cells or 'type 1 cells' by different groups, have astrocytic properties among themselves, 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 DCX-positive 'type 2a' and DCX-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 physiological mitogenic stimuli (such as physical activity) seem to affect these early types of precursors primarily. Type 1 cells rarely divide, and their proliferation is not induced by the experimental manipulations studied so far. Behavioural stimuli such as in environmental enrichment (discussed later), 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, i.e. nestin-negative stages. A subset of type 3 cells become postmitotic and begin 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 fibre 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 identical to older granule neurons over one or two 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 are apparently recruited into a functional role, or die (Kempermann *et al.* 2004a,b).

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 may be important in assessing the possible utility of potential future neuronal replacement therapies based on manipulation of the 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 & 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 (Represa *et al.* 1995; Parent *et al.* 1997, 1999, 2002a).

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 & 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 either the hippocampal granule cells suppress neurogenesis or they or the surrounding cells produce signals that induce neurogenesis as they die. However, since neurogenesis is also increased by low 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 behaviour 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 further assess 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 two 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 surplus neurons are 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 & Gage 2002).

Some of the molecular mechanisms that mediate behavioural influences on hippocampal neurogenesis have started 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 & 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 & 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 the local ones alone, may be useful in developing potential future neuronal replacement therapies involving manipulation of endogenous precursors. As with the adult SVZ, 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, and Wnt signalling might also play a critical role in controlling the fate of hippocampal NSCs (Lie *et al.* 2004). Finally, the developmentally critical basic helix-loop-helix transcription factor, *NeuroD*, has been shown to be involved in adult hippocampal neurogenesis (Hsieh *et al.* 2004).

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 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 microenvironments 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 NSCs 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 in some way participate 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 electrophysiological integration by newborn granule neurons (van Praag *et al.* 2002) and an increasing responsiveness of new neurons to external stimuli (Jessberger & 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 & 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 & Daszuta 1999; Herrlinger *et al.* 2000). If adult neurogenesis is blocked by irradiation, the behavioural 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 the only aspect of a general failure of neural plasticity (Kempermann & Kronenberg 2003). Additionally and importantly, adult hippocampal neurogenesis is limited to the dentate gyrus subregion 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: (i) local parenchymal precursor cells might be activated to generate new neurons upon a stimulus associated with either a pathological event or a set of directed molecular and/or genetic interventions; or (ii) precursor cells from normally neurogenic regions, e.g. the SVZ, might respond to either type of stimulus and migrate into the parenchyma. Already, there are examples for both the possibilities.

The first evidence that regenerative neurogenesis is generally possible was reported by 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 earlier, these precursors have broad potential; they can differentiate into astroglia, oligodendroglia and neurons; receive afferents; and 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 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 Magavi *et al.* 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 the 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* ischaemia model by which it was found that repopulation of neurons in the CA1 region of the hippocampus is possible following the elimination of these neurons in the CA1 region (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, [Chen *et al.* \(2004\)](#) demonstrated the induction of low-level neurogenesis of corticospinal motor neurons, with progressive extension of axons into the cervical spinal cord over three to four months. 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 the 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.* 2004b](#); [Magavi *et al.* 2005](#)). Possibly, these functions differ fundamentally from functions 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. This characteristic of the mammalian CNS is a disadvantage in cases of neuronal loss, which therefore cannot be replaced, but one theory is that this extremely limited ability 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 been at odds for years with the fact that, even without considering actual cellular replacement or addition via neurogenesis, there is amazing structural and synaptic plasticity in the brain. Nonetheless, the fact remains that there is normally no (or extremely low) 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, functionally, the hippocampus and olfactory system would appear to have little in common. Whereas, the hippocampus is one of the best-studied areas of the brain, and has been named the 'gateway to memory' owing to 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 very 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 optimization of the mossy fibre connections between the dentate gyrus and CA3 in accordance with the level of complexity and novelty frequently encountered by an individual. Since 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 & 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 these changes in population response. These results indicate that adult-born granule neurons provide a novel form of synaptic plasticity via insertion of entirely new cellular units into the existing functional circuitry.

12. CONSEQUENCES OF DISTURBED ADULT NEUROGENESIS AND THE REACTION OF NEUROGENIC REGIONS TO DISEASE

Since very 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 several studies characterizing the response of the SVZ and the 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](#)). Owing to this, dysregulated adult neurogenesis might provide an explanation for the development of 'granule cell dispersion' found in many cases of temporal lobe epilepsy. There is some evidence that ectopic neurons can be formed 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 (PD) has been reported to decrease precursor cell proliferation in both the SVZ and the dentate gyrus (Hoglinger *et al.* 2004). The reproducibility and functional significance of these phenomena in neurological disorders have 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 PD. It remains to be investigated whether these neurogenic changes are in direct response to pathogenic conditions or whether they are more 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 extent 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 may 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 & Duman 2002; Kempermann & 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.* (2003) eliminated cell proliferation in the SGZ by selective irradiation and showed that this manipulation attenuated the effects of antidepressants on performance in an anxiety task. However, the interpretation of these experiments is somewhat controversial because this behavioural 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 & 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 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 towards 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 may be able to direct the processes of *in situ* neurogenesis and *ex vivo* precursor differentiation towards therapies using cellular and molecular controls of cell-type specific differentiation (Arlotta *et al.* 2005; Molyneaux *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. A better understanding of these issues may enable the prevention of disease and dysfunction, beyond the existing 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 the damaged or diseased areas of the nervous system (Wichterle *et al.* 2002; Arlotta *et al.* 2005; Molyneaux *et al.* 2005). The future prospect of directing the development and integration of precursors in the adult mammalian brain, towards the replacement of lost neurons or glia, is exciting indeed, and several recent lines of work provide remarkable progress towards 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 towards the goal of cellular repopulation and repair.

Although constitutive neurogenesis normally occurs in only two areas of the adult mammalian brain (SVZ and dentate gyrus), 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, towards 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 to be capable of differentiation 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 to 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 behavioural manipulations, and have started to provide key information towards 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 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 may be 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 very successfully by urodeles and other amphibians, i.e. de-differentiation of seemingly mature cells and subsequent repopulation of damaged areas. While there are no data as yet 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 the normally neurogenic regions of the brain, it might be feasible to induce them to proliferate and differentiate from small populations that are widely distributed throughout the neuraxis. There is accumulating evidence that endogenous neural precursors are regionally heterogeneous, and that subsets of partially fate-restricted precursors might exist local to regions in which neuronal replacement is desired. Such precursors might offer advantages in directing lineage-specific differentiation for cellular repair (e.g. [Arlotta et al. 2005](#); [Molyneaux et al. 2005](#)). 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 learnt about precursor heterogeneity and how to take advantage of what may 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 forthcoming decades promise to offer significant insight into these and other critical issues for the field.

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REFERENCES

- Aberg, M. A., Aberg, N. D., Hedbacker, H., Oscarsson, J. & Eriksson, P. S. 2000 Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J. Neurosci.* **20**, 2896–2903.
- Ahmed, S., Reynolds, B. A. & Weiss, S. 1995 BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. *J. Neurosci.* **15**, 5765–5778.
- Altman, J. 1969 Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neurol.* **137**, 433–457. (doi:10.1002/cne.901370404)
- Altman, J. & Das, G. D. 1965 Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* **124**, 319–335. (doi:10.1002/cne.901240303)
- Alvarez-Buylla, A., Garcia-Verdugo, J. M., Mateo, A. S. & Merchant-Larios, H. 1998 Primary neural precursors and intermitotic nuclear migration in the ventricular zone of adult canaries. *J. Neurosci.* **18**, 1020–1037.
- Alvarez-Buylla, A., Garcia-Verdugo, J. M. & Tramontin, A. D. 2001 A unified hypothesis on the lineage of neural stem cells. *Nat. Rev. Neurosci.* **2**, 287–293. (doi:10.1038/35067582)
- Alvarez-Dolado, M., Pardal, R., Garcia-Verdugo, J. M., Fike, J. R., Lee, H. O., Pfeffer, K., Lois, C., Morrison, S. J. & Alvarez-Buylla, A. 2003 Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* **425**, 968–973. (doi:10.1038/nature02069)
- Arlotta, P., Molyneaux, B. J., Chen, J., Inoue, J., Kominami, R. & Macklis, J. D. 2005 Neuronal subtype-specific genes that control corticospinal motor neuron development *in vivo*. *Neuron* **45**, 207–221. (doi:10.1016/j.neuron.2004.12.036)
- Arsenijevic, Y. & Weiss, S. 1998 Insulin-like growth factor-I is a differentiation factor for postmitotic CNS stem cell-derived neuronal precursors: distinct actions from those of brain-derived neurotrophic factor. *J. Neurosci.* **18**, 2118–2128.
- Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. & Lindvall, O. 2002 Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat. Med.* **8**, 963–970. (doi:10.1038/nm747)
- Bauer, S., Hay, M., Amilhon, B., Jean, A. & Moyse, E. 2005 *In vivo* neurogenesis in the dorsal vagal complex of the adult rat brainstem. *Neuroscience* **130**, 75–90. (doi:10.1016/j.neuroscience.2004.08.047)
- Belachew, S., Chittajallu, R., Aguirre, A. A., Yuan, X., Kirby, M., Anderson, S. & Gallo, V. 2003 Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. *J. Cell Biol.* **161**, 169–186. (doi:10.1083/jcb.200210110)
- Bengzon, J., Kokaia, Z., Elmer, E., Nanobashvili, A., Kokaia, M. & Lindvall, O. 1997 Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc. Natl Acad. Sci. USA* **94**, 10 432–10 437. (doi:10.1073/pnas.94.19.10432)
- Benraiss, A., Chmielnicki, E., Lerner, K., Roh, D. & Goldman, S. A. 2001 Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J. Neurosci.* **21**, 6718–6731.
- Bernier, P. J., Bedard, A., Vinet, J., Levesque, M. & Parent, A. 2002 Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proc. Natl Acad. Sci. USA* **99**, 11 464–11 469. (doi:10.1073/pnas.172403999)
- Betarbet, R., Zigova, T., Bakay, R. A. & Luskin, M. B. 1996 Dopaminergic and GABAergic interneurons of the olfactory bulb are derived from the neonatal subventricular zone. *Int. J. Dev. Neurosci.* **14**, 921–930. (doi:10.1016/S0736-5748(96)00066-4)
- Biebl, M., Cooper, C. M., Winkler, J. & Kuhn, H. G. 2000 Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci. Lett.* **291**, 17–20. (doi:10.1016/S0304-3940(00)01368-9)
- Brandt, M. D., Jessberger, S., Steiner, B., Kronenberg, G., Reuter, K., Bick-Sander, A., von der Behrens, W. & Kempermann, G. 2003 Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol. Cell. Neurosci.* **24**, 603–613. (doi:10.1016/S1044-7431(03)00207-0)
- Brewer, G. J. 1999 Regeneration and proliferation of embryonic and adult rat hippocampal neurons in culture. *Exp. Neurol.* **159**, 237–247. (doi:10.1006/exnr.1999.7123)
- Brezun, J. M. & Daszuta, A. 1999 Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* **89**, 999–1002. (doi:10.1016/S0306-4522(98)00693-9)
- Brezun, J. M. & Daszuta, A. 2000 Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. *Eur. J. Neurosci.* **12**, 391–396. (doi:10.1046/j.1460-9568.2000.00932.x)
- Brockes, J. P. 1997 Amphibian limb regeneration: rebuilding a complex structure. *Science* **276**, 81–87. (doi:10.1126/science.276.5309.81)
- Brown, J. P., Couillard-Despres, S., Cooper-Kuhn, C. M., Winkler, J., Aigner, L. & Kuhn, H. G. 2003 Transient expression of doublecortin during adult neurogenesis. *J. Comp. Neurol.* **467**, 1–10. (doi:10.1002/cne.10874)
- Cameron, H. A. & Gould, E. 1994 Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* **61**, 203–209. (doi:10.1016/0306-4522(94)90224-0)
- Cameron, H. A. & McKay, R. D. 1999 Restoring production of hippocampal neurons in old age. *Nat. Neurosci.* **2**, 894–897. (doi:10.1038/13197)
- Cameron, H. A., Woolley, C. S., McEwen, B. S. & Gould, E. 1993 Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience* **56**, 337–344. (doi:10.1016/0306-4522(93)90335-D)
- Carleton, A., Petreanu, L. T., Lansford, R., Alvarez-Buylla, A. & Lledo, P. M. 2003 Becoming a new neuron in the adult olfactory bulb. *Nat. Neurosci.* **6**, 507–518.
- Carro, E., Nunez, A., Busiguina, S. & Torres-Aleman, I. 2000 Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J. Neurosci.* **20**, 2926–2933.
- Catapano, L. A., Magavi, S. S. & Macklis, J. D. 2002 Neuroanatomical tracing of neuronal projections with Fluoro-Gold. *Methods Mol. Biol.* **198**, 299–304.
- Chen, J., Magavi, S. S. & Macklis, J. D. 2004 Neurogenesis of corticospinal motor neurons extending spinal projections in adult mice. *Proc. Natl Acad. Sci. USA* **101**, 16 357–16 362. (doi:10.1073/pnas.0406795101)
- Chiasson, B. J., Tropepe, V., Morshead, C. M. & van der Kooy, D. 1999 Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. *J. Neurosci.* **19**, 4462–4471.
- Clarke, D. L., Johansson, C. B., Wilbertz, J., Veress, B., Nilsson, E., Karlstrom, H., Lendahl, U. & Frisen, J. 2000 Generalized potential of adult neural stem cells. *Science* **288**, 1660–1663. (doi:10.1126/science.288.5471.1660)

- Cooper-Kuhn, C. M. & Kuhn, H. G. 2002 Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. *Brain Res. Dev. Brain Res.* **134**, 13–21. (doi:10.1016/S0165-3806(01)00243-7)
- Cooper-Kuhn, C. M., Vroemen, M., Brown, J., Ye, H., Thompson, M. A., Winkler, J. & Kuhn, H. G. 2002 Impaired adult neurogenesis in mice lacking the transcription factor E2F1. *Mol. Cell. Neurosci.* **21**, 312–323. (doi:10.1006/mcne.2002.1176)
- Craig, C. G., Tropepe, V., Morshead, C. M., Reynolds, B. A., Weiss, S. & 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.
- Curtis, M. A., Penney, E. B., Pearson, A. G., Van Roon-Mom, W. M., Butterworth, N. J., Dragunow, M., Connor, B. & Faull, R. L. 2003 Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proc. Natl Acad. Sci. USA* **100**, 9023–9027. (doi:10.1073/pnas.1532244100)
- D'Amour, K. A. & Gage, F. H. 2003 Genetic and functional differences between multipotent neural and pluripotent embryonic stem cells. *Proc. Natl Acad. Sci. USA* **100** (Suppl. 1), 11 866–11 872. (doi:10.1073/pnas.1834200100)
- D'Sa, C. & Duman, R. S. 2002 Antidepressants and neuroplasticity. *Bipolar Disord.* **4**, 183–194. (doi:10.1034/j.1399-5618.2002.01203.x)
- Dawson, M. R., Polito, A., Levine, J. M. & Reynolds, R. 2003 NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol. Cell. Neurosci.* **24**, 476–488. (doi:10.1016/S1044-7431(03)00210-0)
- Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T. D. & Malenka, R. C. 2004 Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron* **42**, 535–552. (doi:10.1016/S0896-6273(04)00266-1)
- Derrick, B. E., York, A. D. & Martinez Jr, J. L. 2000 Increased granule cell neurogenesis in the adult dentate gyrus following mossy fiber stimulation sufficient to induce long-term potentiation. *Brain Res.* **857**, 300–307. (doi:10.1016/S0006-8993(99)02464-6)
- Doetsch, F. & Alvarez-Buylla, A. 1996 Network of tangential pathways for neuronal migration in adult mammalian brain. *Proc. Natl Acad. Sci. USA* **93**, 14 895–14 900. (doi:10.1073/pnas.93.25.14895)
- Doetsch, F., Caille, I., Lim, D. A., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. 1999 Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* **97**, 703–716. (doi:10.1016/S0092-8674(00)80783-7)
- Ehninger, D. & Kempermann, G. 2003 Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. *Cereb. Cortex* **13**, 845–851. (doi:10.1093/cercor/13.8.845)
- Emsley, J. G., Mitchell, B. D., Kempermann, G. & Macklis, J. D. 2005 Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells. *Prog. Neurobiol.* **75**, 321–341. (doi:10.1016/j.pneurobio.2005.04.002)
- Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A. & Gage, F. H. 1998 Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313–1317. (doi:10.1038/3305)
- Filippov, V., Kronenberg, G., Pivneva, T., Reuter, K., Steiner, B., Wang, L. P., Yamaguchi, M., Kettenmann, H. & Kempermann, G. 2003 Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol. Cell. Neurosci.* **23**, 373–382. (doi:10.1016/S1044-7431(03)00060-5)
- Fischer, A. J. & Reh, T. A. 2001 Muller glia are a potential source of neural regeneration in the postnatal chicken retina. *Nat. Neurosci.* **4**, 247–252. (doi:10.1038/85090)
- Fricker-Gates, R. A., Shin, J. J., Tai, C. C., Catapano, L. A. & Macklis, J. D. 2002 Late-stage immature neocortical neurons reconstruct interhemispheric connections and form synaptic contacts with increased efficiency in adult mouse cortex undergoing targeted neurodegeneration. *J. Neurosci.* **22**, 4045–4056.
- Frielingsdorf, H., Schwarz, K., Brundin, P. & Mohapel, P. 2004 No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **101**, 10 177–10 182. (doi:10.1073/pnas.0401229101)
- Fukuda, S., Kato, F., Tozuka, Y., Yamaguchi, M., Miyamoto, Y. & Hisatsune, T. 2003 Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *J. Neurosci.* **23**, 9357–9366.
- Gage, F. H. 1998 Stem cells of the central nervous system. *Curr. Opin. Neurobiol.* **8**, 671–676. (doi:10.1016/S0959-4388(98)80098-6)
- Gage, F. H. 2000 Mammalian neural stem cells. *Science* **287**, 1433–1438. (doi:10.1126/science.287.5457.1433)
- Gage, F. H., Coates, P. W., Palmer, T. D., Kuhn, H. G., Fisher, L. J., Suhonen, J. O., Peterson, D. A., Suhr, S. T. & Ray, J. 1995a Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proc. Natl Acad. Sci. USA* **92**, 11 879–11 883. (doi:10.1073/pnas.92.25.11879)
- Gage, F. H., Ray, J. & Fisher, L. J. 1995b Isolation, characterization, and use of stem cells from the CNS. *Annu. Rev. Neurosci.* **18**, 159–192. (doi:10.1146/annurev.ne.18.030195.001111)
- Garcia-Verdugo, J. M., Doetsch, F., Wichterle, H., Lim, D. A. & Alvarez-Buylla, A. 1998 Architecture and cell types of the adult subventricular zone: in search of the stem cells. *J. Neurobiol.* **36**, 234–248. (doi:10.1002/(SICI)1097-4695(199808)36:2<234::AID-NEU10>3.0.CO;2-E)
- Goldman, J. E. 1995 Lineage, migration, and fate determination of postnatal subventricular zone cells in the mammalian CNS. *J. Neurooncol.* **24**, 61–64. (doi:10.1007/BF01052660)
- Goldman, S. A. 1998 Adult neurogenesis: from canaries to the clinic. *J. Neurobiol.* **36**, 267–286. (doi:10.1002/(SICI)1097-4695(199808)36:2<267::AID-NEU12>3.0.CO;2-B)
- Goldman, S. A. & Nottebohm, F. 1983 Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc. Natl Acad. Sci. USA* **80**, 2390–2394. (doi:10.1073/pnas.80.8.2390)
- Gotz, M., Hartfuss, E. & Malatesta, P. 2002 Radial glial cells as neuronal precursors: a new perspective on the correlation of morphology and lineage restriction in the developing cerebral cortex of mice. *Brain Res. Bull.* **57**, 777–788.
- Gould, E., Cameron, H. A., Daniels, D. C., Woolley, C. S. & McEwen, B. S. 1992 Adrenal hormones suppress cell division in the adult rat dentate gyrus. *J. Neurosci.* **12**, 3642–3650.
- Gould, E., Tanapat, P., McEwen, B. S., Flugge, G. & Fuchs, E. 1998 Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl Acad. Sci. USA* **95**, 3168–3171. (doi:10.1073/pnas.95.6.3168)

- Gould, E., Beylin, A., Tanapat, P., Reeves, A. & Shors, T. J. 1999a Learning enhances adult neurogenesis in the hippocampal formation. *Nat. Neurosci.* **2**, 260–265. (doi:10.1038/6365)
- Gould, E., Reeves, A. J., Fallah, M., Tanapat, P., Gross, C. G. & Fuchs, E. 1999b Hippocampal neurogenesis in adult old world primates. *Proc. Natl Acad. Sci. USA* **96**, 5263–5267. (doi:10.1073/pnas.96.9.5263)
- Gould, E., Reeves, A. J., Graziano, M. S. & Gross, C. G. 1999c Neurogenesis in the neocortex of adult primates. *Science* **286**, 548–552. (doi:10.1126/science.286.5439.548)
- Gould, E., Vail, N., Wagers, M. & Gross, C. G. 2001 Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. *Proc. Natl Acad. Sci. USA* **98**, 10 910–10 917. (doi:10.1073/pnas.1813 54698)
- Gritti, A., Cova, L., Parati, E. A., Galli, R. & Vescovi, A. L. 1995 Basic fibroblast growth factor supports the proliferation of epidermal growth factor-generated neuronal precursor cells of the adult mouse CNS. *Neurosci. Lett.* **185**, 151–154. (doi:10.1016/0304-3940(95)11247-T)
- Gritti, A. *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.
- Gritti, A., Frolichsthal-Schoeller, P., Galli, R., Parati, E. A., Cova, L., Pagano, S. F., Bjornson, C. R. & Vescovi, A. L. 1999 Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. *J. Neurosci.* **19**, 3287–3297.
- Gross, R. E., Mehler, M. F., Mabie, P. C., Zang, Z., Santschi, L. & Kessler, J. A. 1996 Bone morphogenetic proteins promote astroglial lineage commitment by mammalian subventricular zone progenitor cells. *Neuron* **17**, 595–606. (doi:10.1016/S0896-6273(00)80193-2)
- Gu, W., Brannstrom, T. & Wester, P. 2000 Cortical neurogenesis in adult rats after reversible photothrombotic stroke. *J. Cereb. Blood Flow Metab.* **20**, 1166–1173. (doi:10.1097/00004647-200008000-00002)
- Guazzi, S., Strangio, A., Franzini, A. T. & Bianchi, M. E. 2003 HMGB1, an architectural chromatin protein and extracellular signalling factor, has a spatially and temporally restricted expression pattern in mouse brain. *Gene Expr. Patterns* **3**, 29–33. (doi:10.1016/S1567-133X(02)00093-5)
- Handler, M., Yang, X. & Shen, J. 2000 Presenilin-1 regulates neuronal differentiation during neurogenesis. *Development* **127**, 2593–2606.
- Hastings, N. B. & Gould, E. 1999 Rapid extension of axons into the CA3 region by adult-generated granule cells. *J. Comp. Neurol.* **413**, 146–154. (doi:10.1002/(SICI)1096-9861(19991011)413:1<146::AID-CNE10>3.0.CO;2-B) [published erratum appears in *J. Comp. Neurol.* 1999 Dec 6; 415 (1):144].
- Heins, N., Malatesta, P., Cecconi, F., Nakafuku, M., Tucker, K. L., Hack, M. A., Chapouton, P., Barde, Y. A. & Gotz, M. 2002 Glial cells generate neurons: the role of the transcription factor Pax6. *Nat. Neurosci.* **5**, 308–315. (doi:10.1038/nn828)
- Herrera, D. G., Garcia-Verdugo, J. M. & Alvarez-Buylla, A. 1999 Adult-derived neural precursors transplanted into multiple regions in the adult brain. *Ann. Neurol.* **46**, 867–877. (doi:10.1002/1531-8249(199912)46:6<867::AID-ANA9>3.0.CO;2-Z)
- Herrlinger, U., Wojciechowski, C., Sena-Esteves, M., Aboody, K. S., Jacobs, A. H., Rainov, N. G., Snyder, E. Y. & Breakefield, X. O. 2000 Neural precursor cells for delivery of replication-conditional HSV-1 vectors to intracerebral gliomas. *Mol. Ther.* **1**, 347–357. (doi:10.1006/mthe.2000.0046)
- Herzog, C. & Otto, T. 1999 Regeneration of olfactory receptor neurons following chemical lesion: time course and enhancement with growth factor administration. *Brain Res.* **849**, 155–161. (doi:10.1016/S0006-8993(99)02075-2)
- Hitchcock, P. F., Lindsey Myhr, K. J., Easter, S. S., Mangione-Smith, R. & Jones, D. D. 1992 Local regeneration in the retina of the goldfish. *J. Neurobiol.* **23**, 187–203. (doi:10.1002/neu.480230209)
- Hoglinger, G. U., Rizk, P., Muriel, M. P., Duyckaerts, C., Oertel, W. H., Caille, I. & Hirsch, E. C. 2004 Dopamine depletion impairs precursor cell proliferation in Parkinson disease. *Nat. Neurosci.* **7**, 726–735. (doi:10.1038/nn1265)
- Horner, P. J., Power, A. E., Kempermann, G., Kuhn, H. G., Palmer, T. D., Winkler, J., Thal, L. J. & Gage, F. H. 2000 Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J. Neurosci.* **20**, 2218–2228.
- Hsieh, J., Nakashima, K., Kuwabara, T., Mejia, E. & Gage, F. H. 2004 Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc. Natl Acad. Sci. USA* **101**, 16 659–16 664. (doi:10.1073/pnas.0407643101)
- Huang, L., Cilio, M. R., Silveira, D. C., McCabe, B. K., Sogawa, Y., Stafstrom, C. E. & Holmes, G. L. 1999 Long-term effects of neonatal seizures: a behavioral, electrophysiological, and histological study. *Brain Res. Dev. Brain Res.* **118**, 99–107. (doi:10.1016/S0165-3806(99)00135-2)
- Jacobs, B. L., Praag, H. & Gage, F. H. 2000 Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol. Psychiatry* **5**, 262–269. (doi:10.1038/sj.mp.4000712)
- Jessberger, S. & Kempermann, G. 2003 Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur. J. Neurosci.* **18**, 2707–2712. (doi:10.1111/j.1460-9568.2003.02986.x)
- Jin, K., Zhu, Y., Sun, Y., Mao, X. O., Xie, L. & Greenberg, D. A. 2002 Vascular endothelial growth factor (VEGF) stimulates neurogenesis *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **99**, 11 946–11 950. (doi:10.1073/pnas.182296499)
- Jin, K., Galvan, V., Xie, L., Mao, X. O., Gorostiza, O. F., Bredesen, D. E. & Greenberg, D. A. 2004a Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APP^{Sw,Ind}) mice. *Proc. Natl Acad. Sci. USA* **101**, 13 363–13 367. (doi:10.1073/pnas.0403678101)
- Jin, K., Peel, A. L., Mao, X. O., Xie, L., Cottrell, B. A., Henshall, D. C. & Greenberg, D. A. 2004b Increased hippocampal neurogenesis in Alzheimer's disease. *Proc. Natl Acad. Sci. USA* **101**, 343–347. (doi:10.1073/pnas.2634794100)
- Johansson, C. B., Momma, S., Clarke, D. L., Risling, M., Lendahl, U. & Frisen, J. 1999 Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* **96**, 25–34. (doi:10.1016/S0092-8674(00)80956-3)
- Johns, P. R. & Easter, S. S. 1977 Growth of the adult goldfish eye. II. Increase in retinal cell number. *J. Comp. Neurol.* **176**, 331–341. (doi:10.1002/cne.901760303)
- Kaplan, M. S. 1981 Neurogenesis in the 3-month-old rat visual cortex. *J. Comp. Neurol.* **195**, 323–338. (doi:10.1002/cne.901950211)
- Kempermann, G. 2002 Why new neurons? Possible functions for adult hippocampal neurogenesis. *J. Neurosci.* **22**, 635–638.
- Kempermann, G. & Gage, F. H. 2002 Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. *Brain Res. Dev. Brain Res.* **134**, 1–12. (doi:10.1016/S0165-3806(01)00224-3)

- Kempermann, G. & Kronenberg, G. 2003 Depressed new neurons—adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol. Psychiatry* **54**, 499–503. (doi:10.1016/S0006-3223(03)00319-6)
- Kempermann, G., Kuhn, H. G. & Gage, F. H. 1997 More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**, 493–495. (doi:10.1038/386493a0)
- Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M. & Gage, F. H. 2003 Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* **130**, 391–399. (doi:10.1242/dev.00203)
- Kempermann, G., Jessberger, S., Steiner, B. & Kronenberg, G. 2004a Milestones of neuronal development in the adult hippocampus. *Trends Neurosci.* **27**, 447–452. (doi:10.1016/j.tins.2004.05.013)
- Kempermann, G., Wiskott, L. & Gage, F. H. 2004b Functional significance of adult neurogenesis. *Curr. Opin. Neurobiol.* **14**, 186–191. (doi:10.1016/j.conb.2004.03.001)
- Kirn, J. R., Alvarez-Buylla, A. & Nottebohm, F. 1991 Production and survival of projection neurons in a forebrain vocal center of adult male canaries. *J. Neurosci.* **11**, 1756–1762.
- Kirschenbaum, B. & Goldman, S. A. 1995 Brain-derived neurotrophic factor promotes the survival of neurons arising from the adult rat forebrain subependymal zone. *Proc. Natl Acad. Sci. USA* **92**, 210–214. (doi:10.1073/pnas.92.1.210)
- Kirschenbaum, B., Nedergaard, M., Preuss, A., Barami, K., Fraser, R. A. & Goldman, S. A. 1994 *In vitro* neuronal production and differentiation by precursor cells derived from the adult human forebrain. *Cereb. Cortex* **4**, 576–589.
- Koketsu, D., Mikami, A., Miyamoto, Y. & Hisatsune, T. 2003 Nonrenewal of neurons in the cerebral neocortex of adult macaque monkeys. *J. Neurosci.* **23**, 937–942.
- Kondo, T. & Raff, M. 2000 Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* **289**, 1754–1757. (doi:10.1126/science.289.5485.1754)
- Kornack, D. R. & Rakic, P. 1999 Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proc. Natl Acad. Sci. USA* **96**, 5768–5773. (doi:10.1073/pnas.96.10.5768)
- Kornack, D. R. & Rakic, P. 2001 Cell proliferation without neurogenesis in adult primate neocortex. *Science* **294**, 2127–2130. (doi:10.1126/science.1065467)
- Kronenberg, G., Reuter, K., Steiner, B., Brandt, M. D., Jessberger, S., Yamaguchi, M. & Kempermann, G. 2003 Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J. Comp. Neurol.* **467**, 455–463. (doi:10.1002/cne.10945)
- Kuan, C. Y. *et al.* 2004 Hypoxia-ischemia induces DNA synthesis without cell proliferation in dying neurons in adult rodent brain. *J. Neurosci.* **24**, 10 763–10 772. (doi:10.1523/JNEUROSCI.3883-04.2004)
- Kuhn, H. G., Dickinson-Anson, H. & Gage, F. H. 1996 Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* **16**, 2027–2033.
- Kuhn, H. G., Winkler, J., Kempermann, G., Thal, L. J. & 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.
- Kukekov, V. G., Laywell, E. D., Suslov, O., Davies, K., Scheffler, B., Thomas, L. B., O'Brien, T. F., Kusakabe, M. & Steindler, D. A. 1999 Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. *Exp. Neurol.* **156**, 333–344. (doi:10.1006/exnr.1999.7028)
- Laywell, E. D., Rakic, P., Kukekov, V. G., Holland, E. C. & Steindler, D. A. 2000 Identification of a multipotent astrocytic stem cell in the immature and adult mouse brain. *Proc. Natl Acad. Sci. USA* **97**, 13 883–13 888. (doi:10.1073/pnas.250471697)
- Lendahl, U., Zimmerman, L. B. & McKay, R. D. 1990 CNS stem cells express a new class of intermediate filament protein. *Cell* **60**, 585–595. (doi:10.1016/0092-8674(90)90662-X)
- Lie, D. C., Dzieczapolski, G., Willhoite, A. R., Kaspar, B. K., Shults, C. W. & Gage, F. H. 2002 The adult substantia nigra contains progenitor cells with neurogenic potential. *J. Neurosci.* **22**, 6639–6649.
- Lie, D. C., Song, H., Colamarino, S. A., Ming, G. L. & Gage, F. H. 2004 Neurogenesis in the adult brain: new strategies for central nervous system diseases. *Annu. Rev. Pharmacol. Toxicol.* **44**, 399–421. (doi:10.1146/annurev.pharmtox.44.101802.121631)
- Lim, D. A., Fishell, G. J. & 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**, 14 832–14 836. (doi:10.1073/pnas.94.26.14832)
- Lois, C. & Alvarez-Buylla, A. 1993 Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc. Natl Acad. Sci. USA* **90**, 2074–2077. (doi:10.1073/pnas.90.5.2074)
- Lois, C. & Alvarez-Buylla, A. 1994 Long-distance neuronal migration in the adult mammalian brain. *Science* **264**, 1145–1148.
- Lopez-Garcia, C., Molowny, A., Martinez-Guijarro, F. J., Blasco-Ibanez, J. M., Luis de la Iglesia, J. A., Bernabeu, A. & Garcia-Verdugo, J. M. 1992 Lesion and regeneration in the medial cerebral cortex of lizards. *Histol. Histopathol.* **7**, 725–746.
- Luskin, M. B. 1993 Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11**, 173–189. (doi:10.1016/0896-6273(93)90281-U)
- Luskin, M. B. 1998 Neuroblasts of the postnatal mammalian forebrain: their phenotype and fate. *J. Neurobiol.* **36**, 221–233. (doi:10.1002/(SICI)1097-4695(199808)36:2<221::AID-NEU9>3.0.CO;2-3)
- Luskin, M. B. & Boone, M. S. 1994 Rate and pattern of migration of lineally-related olfactory bulb interneurons generated postnatally in the subventricular zone of the rat. *Chem. Senses* **19**, 695–714.
- Magavi, S. S. & Macklis, J. D. 2002a Identification of newborn cells by BrdU labeling and immunocytochemistry *in vivo*. *Methods Mol. Biol.* **198**, 283–290.
- Magavi, S. S. & Macklis, J. D. 2002b Immunocytochemical analysis of neuronal differentiation. *Methods Mol. Biol.* **198**, 291–297.
- Magavi, S. S., Leavitt, B. R. & Macklis, J. D. 2000 Induction of neurogenesis in the neocortex of adult mice. *Nature* **405**, 951–955. (doi:10.1038/35016083)
- Magavi, S. S. P., Mitchell, B. D., Szentirmai, O., Carter, B. S. & Macklis, J. D. 2005 Adult-born and pre-existing olfactory granule neurons undergo distinct experience-dependent modifications of their olfactory responses *in vivo*. *J. Neurosci.* **25**, 10 729–10 739.
- Magloczky, Z. & Freund, T. F. 1993 Selective neuronal death in the contralateral hippocampus following unilateral kainate injections into the CA3 subfield. *Neuroscience* **56**, 317–335. (doi:10.1016/0306-4522(93)90334-C)

- Malatesta, P., Hartfuss, E. & Gotz, M. 2000 Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* **127**, 5253–5263.
- Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. 2000 Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J. Neurosci.* **20**, 9104–9110.
- Markakis, E. A. & Gage, F. H. 1999 Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *J. Comp. Neurol.* **406**, 449–460. (doi:10.1002/(SICI)1096-9861(19990419)406:4<449::AID-CNE3>3.0.CO;2-I)
- McKay, R. 1997 Stem cells in the central nervous system. *Science* **276**, 66–71. (doi:10.1126/science.276.5309.66)
- Molyneaux, B. J., Arlotta, P., Hirata, T., Hibi, M. & Macklis, J. D. 2005 Fezl is required for the birth and specification of corticospinal motor neurons. *Neuron* **47**, 817–831. (doi:10.1016/j.neuron.2005.08.030)
- Monje, M. L., Mizumatsu, S., Fike, J. R. & Palmer, T. D. 2002 Irradiation induces neural precursor-cell dysfunction. *Nat. Med.* **8**, 955–962. (doi:10.1038/nm749)
- Monje, M. L., Toda, H. & Palmer, T. D. 2003 Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760–1765. (doi:10.1126/science.1088417)
- Montaron, M. F., Petry, K. G., Rodriguez, J. J., Marinelli, M., Aurousseau, C., Rougon, G., Le Moal, M. & Abrous, D. N. 1999 Adrenalectomy increases neurogenesis but not PSA-NCAM expression in aged dentate gyrus. *Eur. J. Neurosci.* **11**, 1479–1485. (doi:10.1046/j.1460-9568.1999.00579.x)
- Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. & Nakafuku, M. 2002 Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell* **110**, 429–441. (doi:10.1016/S0092-8674(02)00862-0)
- Noctor, S. C., Flint, A. C., Weissman, T. A., Wong, W. S., Clinton, B. K. & Kriegstein, A. R. 2002 Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J. Neurosci.* **22**, 3161–3173.
- Nottebohm, F. 1985 Neuronal replacement in adulthood. *Ann. NY Acad. Sci.* **457**, 143–161.
- Nottebohm, F. 2002 Why are some neurons replaced in adult brain? *J. Neurosci.* **22**, 624–628.
- Nottebohm, F. & Arnold, A. P. 1976 Sexual dimorphism in vocal control areas of the songbird brain. *Science* **194**, 211–213.
- Nottebohm, F., Alvarez-Buylla, A., Cynx, J., Kirn, J., Ling, C. Y., Nottebohm, M., Suter, R., Tolles, A. & Williams, H. 1990 Song learning in birds: the relation between perception and production. *Phil. Trans. R. Soc. B* **329**, 115–124.
- Oppenheim, R. W. 1991 Cell death during development of the nervous system. *Annu. Rev. Neurosci.* **14**, 453–501. (doi:10.1146/annurev.ne.14.030191.002321)
- Palmer, T. D., Ray, J. & 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. (doi:10.1006/mcne.1995.1035)
- Palmer, T. D., Markakis, E. A., Willhoite, A. R., Safar, F. & Gage, F. H. 1999 Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *J. Neurosci.* **19**, 8487–8497.
- Palmer, T. D., Willhoite, A. R. & Gage, F. H. 2000 Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol.* **425**, 479–494. (doi:10.1002/1096-9861(20001002)425:4<479::AID-CNE2>3.0.CO;2-3)
- Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S. & Lowenstein, D. H. 1997 Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* **17**, 3727–3738.
- Parent, J. M., Tada, E., Fike, J. R. & Lowenstein, D. H. 1999 Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. *J. Neurosci.* **19**, 4508–4519.
- Parent, J. M., Valentin, V. V. & Lowenstein, D. H. 2002a Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. *J. Neurosci.* **22**, 3174–3188.
- Parent, J. M., Vexler, Z. S., Gong, C., Derugin, N. & Ferriero, D. M. 2002b Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann. Neurol.* **52**, 802–813. (doi:10.1002/ana.10393)
- Pencea, V., Bingaman, K. D., Freedman, L. J. & Luskin, M. B. 2001a Neurogenesis in the subventricular zone and rostral migratory stream of the neonatal and adult primate forebrain. *Exp. Neurol.* **172**, 1–16. (doi:10.1006/exnr.2001.7768)
- Pencea, V., Bingaman, K. D., Wiegand, S. J. & Luskin, M. B. 2001b Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. *J. Neurosci.* **21**, 6706–6717.
- Petreanu, L. & Alvarez-Buylla, A. 2002 Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. *J. Neurosci.* **22**, 6106–6113.
- Pincus, D. W. et al. 1998 Fibroblast growth factor-2/brain-derived neurotrophic factor-associated maturation of new neurons generated from adult human subependymal cells. *Ann. Neurol.* **43**, 576–585. (doi:10.1002/ana.410430505)
- Pollard, H., Charriaud-Marlangue, C., Cantagrel, S., Represa, A., Robain, O., Moreau, J. & Ben-Ari, Y. 1994 Kainate-induced apoptotic cell death in hippocampal neurons. *Neuroscience* **63**, 7–18. (doi:10.1016/0306-4522(94)90003-5)
- Rakic, P. 1985 Limits of neurogenesis in primates. *Science* **227**, 1054–1056.
- Rakic, P. 2002 Neurogenesis in adult primate neocortex: an evaluation of the evidence. *Nat. Rev. Neurosci.* **3**, 65–71. (doi:10.1038/nrn700)
- Represa, A., Niquet, J., Pollard, H. & Ben-Ari, Y. 1995 Cell death, gliosis, and synaptic remodeling in the hippocampus of epileptic rats. *J. Neurobiol.* **26**, 413–425. (doi:10.1002/neu.480260313)
- Reynolds, B. A. & Weiss, S. 1992 Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707–1710.
- Reynolds, R. & Hardy, R. 1997 Oligodendroglial progenitors labeled with the O4 antibody persist in the adult rat cerebral cortex *in vivo*. *J. Neurosci. Res.* **47**, 455–470. (doi:10.1002/(SICI)1097-4547(19970301)47:5<455::AID-JNR1>3.0.CO;2-G)
- Reynolds, B. A., Tetzlaff, W. & Weiss, S. 1992 A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. *J. Neurosci.* **12**, 4565–4574.
- Richards, L. J., Kilpatrick, T. J. & Bartlett, P. F. 1992 *De novo* generation of neuronal cells from the adult mouse brain. *Proc. Natl Acad. Sci. USA* **89**, 8591–8595. (doi:10.1073/pnas.89.18.8591)
- Rietze, R., Poulin, P. & Weiss, S. 2000 Mitotically active cells that generate neurons and astrocytes are present in multiple regions of the adult mouse hippocampus. *J. Comp. Neurol.* **424**, 397–408. (doi:10.1002/1096-9861(20000828)424:3<397::AID-CNE2>3.0.CO;2-A)

- Rousselot, P., Lois, C. & Alvarez-Buylla, A. 1995 Embryonic (PSA) N-CAM reveals chains of migrating neuroblasts between the lateral ventricle and the olfactory bulb of adult mice. *J. Comp. Neurol.* **351**, 51–61. (doi:10.1002/cne.903510106)
- Rousselot, P., Heintz, N. & Nottebohm, F. 1997 Expression of brain lipid binding protein in the brain of the adult canary and its implications for adult neurogenesis. *J. Comp. Neurol.* **385**, 415–426. (doi:10.1002/(SICI)1096-9861(19970901)385:3<415::AID-CNE6>3.0.CO;2-6)
- Roy, N. S. *et al.* 2000 *In vitro* neurogenesis by progenitor cells isolated from the adult human hippocampus. *Nat. Med.* **6**, 271–277. (doi:10.1038/73119)
- Sakakibara, S., Nakamura, Y., Satoh, H. & Okano, H. 2001 RNA-binding protein Musashi2: developmentally regulated expression in neural precursor cells and subpopulations of neurons in mammalian CNS. *J. Neurosci.* **21**, 8091–8107.
- Sanai, N. *et al.* 2004 Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. *Nature* **427**, 740–744. (doi:10.1038/nature02301)
- Santarelli, L. *et al.* 2003 Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809. (doi:10.1126/science.1083328)
- Scharff, C., Kirn, J. R., Grossman, M., Macklis, J. D. & Nottebohm, F. 2000 Targeted neuronal death affects neuronal replacement and vocal behavior in adult songbirds. *Neuron* **25**, 481–492. (doi:10.1016/S0896-6273(00)80910-1)
- Scharfman, H. E., Goodman, J. H. & Sollas, A. L. 2000 Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *J. Neurosci.* **20**, 6144–6158.
- Seaberg, R. M. & van der Kooy, D. 2002 Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. *J. Neurosci.* **22**, 1784–1793.
- Seaberg, R. M. & van der Kooy, D. 2003 Stem and progenitor cells: the premature desertion of rigorous definitions. *Trends Neurosci.* **26**, 125–131. (doi:10.1016/S0166-2236(03)00031-6)
- Seri, B., Garcia-Verdugo, J. M., McEwen, B. S. & Alvarez-Buylla, A. 2001 Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* **21**, 7153–7160.
- Sheen, V. L., Arnold, M. W., Wang, Y. & Macklis, J. D. 1999 Neural precursor differentiation following transplantation into neocortex is dependent on intrinsic developmental state and receptor competence. *Exp. Neurol.* **158**, 47–62. (doi:10.1006/exnr.1999.7104)
- Sheline, Y. I., Wang, P. W., Gado, M. H., Csernansky, J. G. & Vannier, M. W. 1996 Hippocampal atrophy in recurrent major depression. *Proc. Natl Acad. Sci. USA* **93**, 3908–3913. (doi:10.1073/pnas.93.9.3908)
- Shi, Y., Chichung Lie, D., Taupin, P., Nakashima, K., Ray, J., Yu, R. T., Gage, F. H. & Evans, R. M. 2004 Expression and function of orphan nuclear receptor TLX in adult neural stem cells. *Nature* **427**, 78–83. (doi:10.1038/nature02211)
- Shihabuddin, L. S., Ray, J. & Gage, F. H. 1997 FGF-2 is sufficient to isolate progenitors found in the adult mammalian spinal cord. *Exp. Neurol.* **148**, 577–586. (doi:10.1006/exnr.1997.6697)
- Shihabuddin, L. S., Horner, P. J., Ray, J. & Gage, F. H. 2000 Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. *J. Neurosci.* **20**, 8727–8735.
- Shin, J. J., Fricker-Gates, R. A., Perez, F. A., Leavitt, B. R., Zurakowski, D. & Macklis, J. D. 2000 Transplanted neuroblasts differentiate appropriately into projection neurons with correct neurotransmitter and receptor phenotype in neocortex undergoing targeted projection neuron degeneration. *J. Neurosci.* **20**, 7404–7416.
- Shors, T. J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T. & Gould, E. 2001 Neurogenesis in the adult is involved in the formation of trace memories. *Nature* **410**, 372–376. (doi:10.1038/35066584)
- Shors, T. J., Townsend, D. A., Zhao, M., Kozorovitskiy, Y. & Gould, E. 2002 Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* **12**, 578–584. (doi:10.1002/hipo.10103)
- Simpson, H. B. & Vicario, D. S. 1990 Brain pathways for learned and unlearned vocalizations differ in zebra finches. *J. Neurosci.* **10**, 1541–1556.
- Snyder, E. Y., Yoon, C., Flax, J. D. & Macklis, J. D. 1997 Multipotent neural precursors can differentiate toward replacement of neurons undergoing targeted apoptotic degeneration in adult mouse neocortex. *Proc. Natl Acad. Sci. USA* **94**, 11 663–11 668. (doi:10.1073/pnas.94.21.11663)
- Song, H., Stevens, C. F. & Gage, F. H. 2002a Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417**, 39–44. (doi:10.1038/417039a)
- Song, H. J., Stevens, C. F. & Gage, F. H. 2002b Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat. Neurosci.* **5**, 438–445.
- Soria, J. M., Tagliatela, P., Gil-Perotin, S., Galli, R., Gritti, A., Verdugo, J. M. & Bertuzzi, S. 2004 Defective postnatal neurogenesis and disorganization of the rostral migratory stream in absence of the Vax1 homeobox gene. *J. Neurosci.* **24**, 11 171–11 181. (doi:10.1523/JNEUROSCI.3248-04.2004)
- Stanfield, B. B. & Trice, J. E. 1988 Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Exp. Brain Res.* **72**, 399–406.
- Suhonen, J. O., Peterson, D. A., Ray, J. & Gage, F. H. 1996 Differentiation of adult hippocampus-derived progenitors into olfactory neurons *in vivo*. *Nature* **383**, 624–627. (doi:10.1038/383624a0)
- Takahashi, M., Palmer, T. D., Takahashi, J. & Gage, F. H. 1998 Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina. *Mol. Cell. Neurosci.* **12**, 340–348. (doi:10.1006/mcne.1998.0721)
- Tanapat, P., Galea, L. A. & Gould, E. 1998 Stress inhibits the proliferation of granule cell precursors in the developing dentate gyrus. *Int. J. Dev. Neurosci.* **16**, 235–239. (doi:10.1016/S0736-5748(98)00029-X)
- Tao, Y., Black, I. B. & DiCicco-Bloom, E. 1996 Neurogenesis in neonatal rat brain is regulated by peripheral injection of basic fibroblast growth factor (bFGF). *J. Comp. Neurol.* **376**, 653–663. (doi:10.1002/(SICI)1096-9861(19961223)376:4<653::AID-CNE11>3.0.CO;2-N)
- Taupin, P., Ray, J., Fischer, W. H., Suhr, S. T., Hakansson, K., Grubb, A. & Gage, F. H. 2000 FGF-2-responsive neural stem cell proliferation requires CCg, a novel autocrine/paracrine cofactor. *Neuron* **28**, 385–397. (doi:10.1016/S0896-6273(00)00119-7)
- Trejo, J. L., Carro, E. & Torres-Aleman, I. 2001 Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. *J. Neurosci.* **21**, 1628–1634.
- Tropepe, V., Coles, B. L., Chiasson, B. J., Horsford, D. J., Elia, A. J., McInnes, R. R. & van der Kooy, D. 2000 Retinal stem cells in the adult mammalian eye. *Science* **287**, 2032–2036. (doi:10.1126/science.287.5460.2032)

- van der Kooy, D. & Weiss, S. 2000 Why stem cells? *Science* **287**, 1439–1441. (doi:10.1126/science.287.5457.1439)
- van Praag, H., Kempermann, G. & Gage, F. H. 1999 Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2**, 266–270. (doi:10.1038/6368)
- van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D. & Gage, F. H. 2002 Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030–1034. (doi:10.1038/4151030a)
- Wang, Y., Sheen, V. L. & Macklis, J. D. 1998 Cortical interneurons upregulate neurotrophins *in vivo* in response to targeted apoptotic degeneration of neighboring pyramidal neurons. *Exp. Neurol.* **154**, 389–402. (doi:10.1006/exnr.1998.6965)
- Weiss, S., Dunne, C., Hewson, J., Wohl, C., Wheatley, M., Peterson, A. C. & Reynolds, B. A. 1996a Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J. Neurosci.* **16**, 7599–7609.
- Weiss, S., Reynolds, B. A., Vescovi, A. L., Morshead, C., Craig, C. G. & van der Kooy, D. 1996b Is there a neural stem cell in the mammalian forebrain? *Trends Neurosci.* **19**, 387–393. (doi:10.1016/S0166-2236(96)10035-7)
- Whittemore, S. R., Morassutti, D. J., Walters, W. M., Liu, R. H. & Magnuson, D. S. 1999 Mitogen and substrate differentially affect the lineage restriction of adult rat subventricular zone neural precursor cell populations. *Exp. Cell Res.* **252**, 75–95. (doi:10.1006/excr.1999.4621)
- Wichterle, H., Lieberam, I., Porter, J. A. & Jessell, T. M. 2002 Directed differentiation of embryonic stem cells into motor neurons. *Cell* **110**, 385–397. (doi:10.1016/S0092-8674(02)00835-8)
- Williams, B. P., Park, J. K., Alberta, J. A., Muhlebach, S. G., Hwang, G. Y., Roberts, T. M. & Stiles, C. D. 1997 A PDGF-regulated immediate early gene response initiates neuronal differentiation in ventricular zone progenitor cells. *Neuron* **18**, 553–562. (doi:10.1016/S0896-6273(00)80297-4)
- Winner, B., Cooper-Kuhn, C. M., Aigner, R., Winkler, J. & Kuhn, H. G. 2002 Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. *Eur. J. Neurosci.* **16**, 1681–1689. (doi:10.1046/j.1460-9568.2002.02238.x)
- Yamaguchi, M., Saito, H., Suzuki, M. & Mori, K. 2000 Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice. *Neuroreport* **11**, 1991–1996.
- Yamamoto, S., Yamamoto, N., Kitamura, T., Nakamura, K. & Nakafuku, M. 2001 Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. *Exp. Neurol.* **172**, 115–127. (doi:10.1006/exnr.2001.7798)
- Yang, J., Cheng, L., Yan, Y., Bian, W., Tomooka, Y., Shiurba, R. & Jing, N. 2001 Mouse nestin cDNA cloning and protein expression in the cytoskeleton of transfected cells. *Biochim. Biophys. Acta* **1520**, 251–254.
- Yang, Y., Mufson, E. J. & Herrup, K. 2003 Neuronal cell death is preceded by cell cycle events at all stages of Alzheimer's disease. *J. Neurosci.* **23**, 2557–2563.
- Young, M. J., Ray, J., Whiteley, S. J., Klassen, H. & Gage, F. H. 2000 Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats. *Mol. Cell. Neurosci.* **16**, 197–205. (doi:10.1006/mcne.2000.0869)
- Zhao, M. *et al.* 2003a Evidence for neurogenesis in the adult mammalian substantia nigra. *Proc. Natl Acad. Sci. USA* **100**, 7925–7930. (doi:10.1073/pnas.1131955100)
- Zhao, X. *et al.* 2003b Mice lacking methyl-CpG binding protein 1 have deficits in adult neurogenesis and hippocampal function. *Proc. Natl Acad. Sci. USA* **100**, 6777–6782.
- Zigova, T., Pencea, V., Wiegand, S. J. & Luskin, M. B. 1998 Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb. *Mol. Cell. Neurosci.* **11**, 234–245. (doi:10.1006/mcne.1998.0684)