Molecular and Cellular Neuroscience 6, 2–15 (1995)

MCN

REVIEW

The Regulation of Neural Precursor Cells within the Mammalian Brain

T. J. Kilpatrick,[1] L. J. Richards,[2] and P. F. Bartlett

The Walter and Eliza Hall Institute, Grattan Street, Parkville, Melbourne 3052, Australia

INTRODUCTION

A central aim in developmental neurobiology is to determine how the processes of neurogenesis and gliogenesis within the central nervous system (CNS) are regulated. At the cellular level, the initial phase of CNS development is characterized by the proliferation of neural precursor cells (Cowan, 1979), followed by the generation of neurons and glia. An essential question with regard to these early developmental events is whether the precursors, cells which lack the phenotypic characteristics of terminally differentiated cells, but from which the differentiated cells are derived, have multipotential capacity. In addition, this question has bearing on another fundamental issue, that is whether environmental influences have the capacity to direct lineage commitment or whether their affects are confined to the potentiation of the differentiation of precursors already committed to either the neuronal or glial lineages. This review will address these issues and also will attempt to elucidate how an understanding of these processes has given us new insight into cellular kinetics within the adult CNS and the potential for novel therapeutic strategies to repopulate the damaged brain.

LINEAGE COMMITMENT IN THE DEVELOPING BRAIN

In recent years, several investigators have assessed cell lineage within the developing CNS and the operational framework they have used to study the issue has relied heavily upon the nomenclature developed to define lineage within the hematopoietic system. Study of the latter system has established the existence of discrete subsets of precursor cells, each with differing potentiality with respect to their ability to selfrenew, to divide, and to differentiate. The most primitive hematopoietic cell is the stem cell, defined as a precursor with both the capacity to selfrenew and with multipotentiality, such that it can repopulate the entire hematopoietic system. The undifferentiated progeny of the stem cell are termed progenitors. These cells are also capable of selfreplication but only for a limited number of cell divisions. There is also a hierarchy of progenitor cells with respect to lineage potentiality: the immediate progeny of stem cells may themselves have multipotentiality but progenitors generated later in ontogeny show lineage restriction and, ultimately, commitment to a single lineage. The term "blast" also has been widely used and refers to the capacity of a precursor cell to undergo selfreplication, independent of its lineage potentiality. Although this nomenclature has been applied to early CNS development, it often has been used inconsistently and somewhat imprecisely. One particular difficulty is the complexity of the mammalian CNS, such that large numbers of differentiated phenotypes, including a vast diversity of neuronal subtypes are generated during ontogeny. As a result, most assessments of lineage within the CNS have been restricted to the basic issue of how the neuronal lineage and the two major macroglial cell types (astrocytes and oligodendrocytes) are generated, rather than addressing the question of how specification of the various phenotypes (e.g., neuronal subtypes) within a particular lineage is achieved.

It has been widely accepted that commitments to the neuronal and glial lineages are temporally dispersed, such that neurogenesis precedes gliogenesis. In the mouse, cells committed to the neuronal lineage can be

[1] To whom correspondence should be addressed at The Salk Institute for Biological Sciences, 10010 North Torrey Pines Road, La Jolla, CA 92037.

[2] Current address: The Salk Institute for Biological Sciences, 10010 North Torrey Pines Road, La Jolla, CA 92037.

1044-7431/95 $6.00

first detected at about Embryonic Day 11 (E11) (Nornes and Das, 1974; Bailey *et al.*, 1994) and neurogenesis continues until around E20 in the rat (Angevine and Sidman, 1961; Frederiksen and McKay, 1988). In comparison, the generation of astrocytes is delayed, and has been reported to commence at E16 in the rat (Abney *et al.*, 1981), continuing well into postnatal development (Miller *et al.*, 1985; Frederiksen and McKay, 1988). Birth date studies, however, fail to address the issue of the lineage potentiality of the precursor cells from which the neurons and astrocytes are derived. For example, the lineage commitment of the differentiated progeny of a multipotential precursor undergoing repetitive cycles of asymmetrical division could vary according to the developmental stage and, in order to assess this and related issues, formal lineage studies are required.

An assessment of cell lineage within the developing CNS was first undertaken over a century ago, when His (1989) suggested that there was a heterogeneous population of precursor cells which exhibited a variety of lineage commitments. This conclusion was based on the observation that there were two morphologically distinct cell types present in early CNS development, that is round cells thought to be neuronal precursors and columnar cells, hypothesized to be glial progenitors. It was, however, subsequently shown that these morphologies reflected different phases of the cell cycle, rather than specifying lineage restriction (Schaper 1897). Levitt *et al.* (1981) provided more convincing evidence of preexisting lineage determination, by showing that at midgestation in the fetal rhesus monkey, a subset of the morphologically undifferentiated ventricular cells expressed glial fibrillary acidic protein (GFAP), an astrocyte specific lineage marker. Current opinion, however, favors that at least some precursor cells retain a multipotential capacity, with the ability to differentiate into both neurons and glial. Evidence for this view has accumulated from immunohistochemical staining (De Vitry *et al.*, 1980), immortalization (Bartlett *et al.*, 1988; Frederiksen *et al.*, 1988), clonal analysis (Temple, 1989; Kilpatrick and Bartlett, 1993; Davis and Temple, 1994), and *LacZ* tagging (Price *et al.*, 1987; Walsh and Cepko, 1992) of precursors. De Vitry *et al.* (1980) presented circumstantial evidence to support multipotentiality, by demonstrating that murine hypothalamic precursor cells expressed both neuron-specific enolase and S100, reported to be neuronal and glial restricted markers, respectively. More recently, fluorescent tracers have been injected into precursor cells in the developing frog retina and provide strong evidence to support the presence of multipotential precursors in this CNS tissue (Holt *et al.*, 1988; Wetts and Fraser, 1988). However, the technique is limited to short-term studies and it is not easily adapted to use within less accessible regions of the CNS.

The lineage potential of precursor cells also has been studied by analyzing immortalized cell lines produced by infection of precursors with retroviral constructs containing protooncogenes. Bartlett *et al.* (1988) generated cell lines from embryonic mouse neuroepithelium, by infection with a retrovirus containing the *c-myc* oncogene. The immortalized cells had the morphological characteristics of primary neuroepithelial cells and did not spontaneously differentiate, but both GFAP positive and neurofilament positive cells were generated when clonal derivatives of the parental cell line were cultured with fibroblast growth factor (FGF) (see below). Frederiksen *et al.* (1988) utilized the temperature sensitive SV40 large T antigen and found that the resultant immortalized cells retained the morphological characteristics of precursor cells at the permissive temperature but, at restrictive temperatures, both GFAP positive cells and neuronal-like cells were generated. The phenotypic potential of retrovirally infected neural precursor cell lines has been explored more recently by transplantation experiments (Renfranz *et al.*, 1991; Snyder *et al.*, 1992). These studies confirm that the transplanted cells have a multipotential capacity and that either they, or their differentiated progeny (including both neuronal and glial-like cells), have the ability to migrate to specific zones of the postnatal rodent brain, including the dentate gyrus of the hippocampus and the internal granular layer of the cerebellum. Retroviral-induced alteration of the genome could, however, alter the developmental potential of the immortalized cells and, thus, it is unclear if these results reflect the lineage potential of primary cells.

The infection of very low numbers of cells with replication-incompetent retroviruses containing the *LacZ* gene, also has been employed to assess lineage, both *in vivo* and *in vitro* (Sanes *et al.*, 1986; Price *et al.*, 1987; Price and Thurlow, 1988; Luskin *et al.*, 1988; Williams *et al.*, 1991). When this technique was applied to the developing chick spinal cord (Leber *et al.*, 1990), tectum (Gray *et al.*, 1988; Galileo *et al.*, 1990), and forebrain (Gray *et al.*, 1990) and to rodent retinal development (Turner and Cepko, 1987; Turner *et al.*, 1990), the presence of multipotential precursor cells was established. However, when applied to the study of precursor cells in the mammalian cerebral cortex, the retroviral labeling technique produced conflicting results. This may be attributed, in part, to the variable expression of β-galactosidase by infected cells; in addition, until recently, the full migration potential of clonally related cells may not have been appreciated (Walsh and Cepko, 1992), making interpretation of the reported data difficult. Luskin *et al.* (1988) reported that the vast majority of clones derived by the *in vivo* infection of E12–

14 murine cortical cells with constructs containing the *LacZ* gene were homogeneous with respect to cell type; 81% of the derived clones were composed of neurons, 13% contained oligodendrocytes, and 4% contained astrocytes. Price and Thurlow (1988) studied cell lineage in the embryonic (E16) rat cerebral cortex and reported similar findings. Williams *et al.* (1991) used retroviral infection to study the *in vitro* development of cells isolated from embryonic rat cerebral cortex and found that most proliferating clones were once again composed of cells restricted to a single lineage; after 7–8 days *in vitro*, 39% of clones contained oligodendrocytes, 18% contained neurons, and 3% contained GFAP positive astrocytes. In addition, 36% of the clones were composed of undifferentiated cells and 5% contained both neurons and oligodendrocytes but no astrocytes. These studies suggested that neuronal precursors are more numerous than astrocytic precursors within the developing cerebral cortex, although another view would be that astrocytic precursors incorporate the retrovirus at a lower efficiency than neuronal precursors. Alternatively, Davis and Temple (1994) have suggested that some glial progeny of ventricular zone cells could migrate and establish subclones elsewhere within the developing cerebral cortex which may not be identified as related to the parental clone; this could result in underestimation of the diversity of progeny generated from a given precursor. Another important observation was that although astrocytic clones were less frequent in the immortalization studies, they usually contained more cells than neuronally restricted clones, suggesting that neurons and astrocytes might accumulate from precursor cells at either different rates or by different mechanisms (Luskin *et al.*, 1988; Price and Thurlow, 1988).

Although these results suggested significant lineage restriction among precursors within the developing rodent cerebral cortex, both the studies of Luskin *et al.* (1988) and of Williams *et al.* (1991) identified that, in a subset of clones, some of the progeny were of undifferentiated morphology; consequently, the full lineage potential of the precursor cells may not have been apparent, possibly because the appropriate environmental conditions for the differentiation of astrocytes were not present (see Fig. 1). This issue has been addressed by assessing the phenotype of clonally related cells, using ultrastructural and immunohistochemical criteria; the results reaffirmed the view that most neurons, astrocytes, and oligodendrocytes arise from separate progenitor cells within E15 and E16 rat cerebral cortex (Grove *et al.*, 1993; Luskin *et al.*, 1993). However, recent data indicate that proviral integration is into postreplication DNA; thus, only half of the progeny of an infected cell will carry a copy of the provirus. If the infection is into asymmetrically dividing stem cells which generate another stem cell and a committed progeny, half of the infected clones will contain only committed progeny, potentially biasing the assessment, by underestimating the diversity of progeny generated (Hajihosseini *et al.*, 1993). Clonal boundaries and thus, lineage, now also have been more rigorously analyzed, by infecting the developing brain with a library of genetically distinct viruses and amplifying single viral genomes, using the polymerase chain reaction (Walsh and Cepko, 1992). This study has suggested that at least 5% of clones labeled from E15 rat neocortex contained both neurons and astrocytes and, thus, that they were derived from multipotential precursors. However, even this study may have methodological difficulties, given that the true incidence of multiple infections by a given retroviral tag cannot be accurately calculated (Kirkwood *et al.*, 1992).

It also should be noted that the results of *in vivo* clonal analysis between E14 and E16 do not necessarily reflect the lineage potential of the earliest precursor cells, present within the neuroepithelium. The concept of progressive restriction to a single lineage is supported by *in vitro* studies, which have identified neuronal precursors on the basis of loss of an inducible marker (MHC class 1 molecules), as the precursors become committed to the neuronal lineage (Bailey *et al.*, 1994). In fact, from E11 onwards in the mouse, there is an increasing number of precursors committed to the neuronal lineage, up to 40% by E12. Furthermore, it is possible that the restricted range of phenotypes observed within clones *in vivo* does not reflect the full lineage potential of the founder cell, due to environmental constraints (see below). Finally, it is possible that the proliferative capacity of multipotential precursors could diminish *in vivo* as development proceeds, as occurs in the hematopoietic system (Metcalf and Moore, 1971) and *in vivo* clonal analysis, utilizing retroviral constructs, would be biased against the detection of such a quiescent subset of precursors.

In vitro clonal analysis of embryonic CNS cells has confirmed the presence of multipotential cells. Temple (1989) analyzed E13.5–14.5 rat septal precursor cells and found that there was not only heterogeneity within the isolated population but that 22% of the plated cells exhibited multipotentiality. In addition, Kilpatrick and Bartlett (1993) undertook clonal analysis of E10 murine neuroepithelial cells, and confirmed heterogeneity, in that two predominant and morphologically distinct types of clone were identified. The first type consisted of large amorphous cells (37% of clones) of undetermined lineage potential and the second type contained cuboidal, epithelial-like cells (54% of clones). Clones of the latter type contained very large numbers ($>5 \times 10^4$) of precursor cells, suggesting that their differentiation did not occur

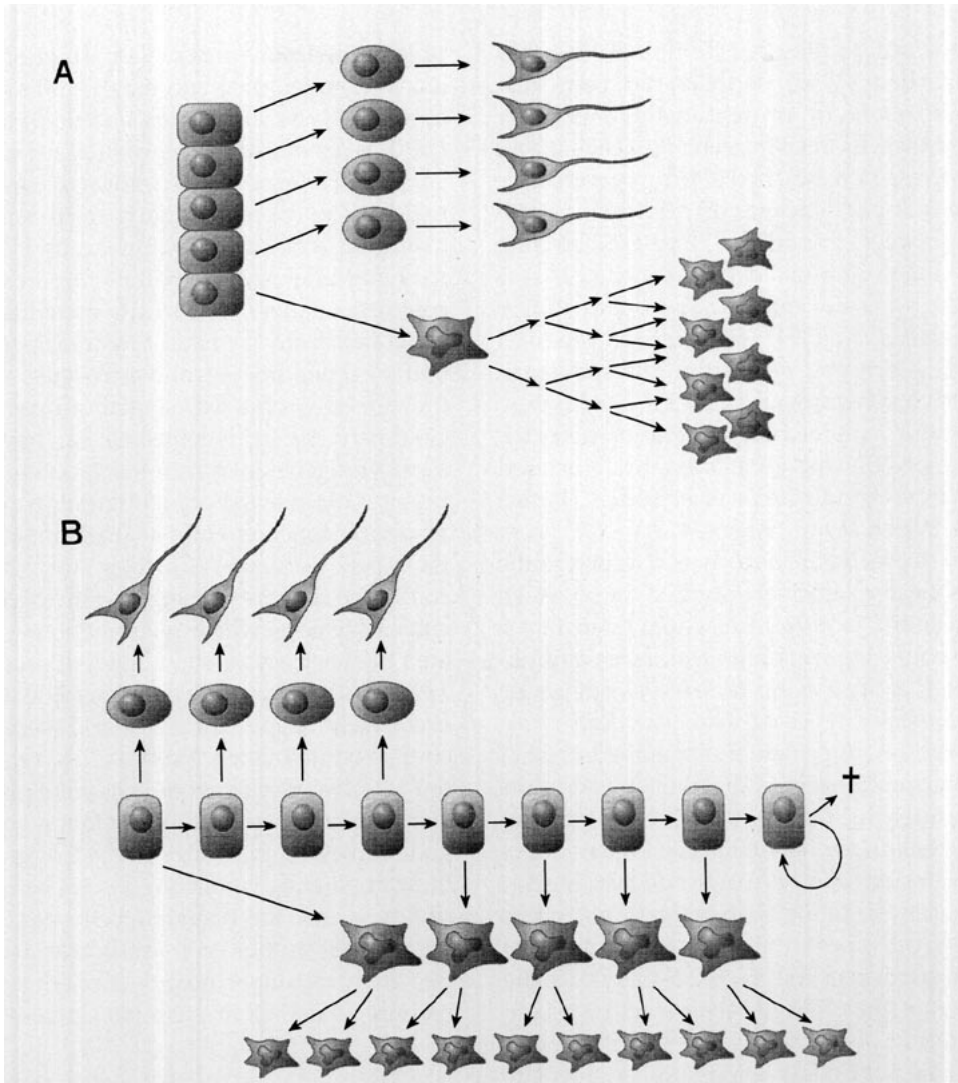

FIG. 1. Alternative models for the generation of neurons and astrocytes within the mammalian brain. In model A, neurons and astrocytes are generated from precursor cells already committed to the neuronal and astrocytic lineages. Neurons are generated first and astrocytic progenitors subsequently proliferate before differentiating into mature astrocytes. In model B, there is a steady-state complement of multipotential precursor cells present throughout ontogeny. Initially, under the influence of temporally restricted environmental signals, the precursors generate neuroblasts which, in turn, differentiate into mature neurons. A small number of glioblasts is also generated at this early developmental stage but they are also generated from the multipotential precursor subsequent to the completion of neurogenesis. Further expansion of the astrocytic progenitor population occurs by proliferation, prior to astrocytic differentiation. In this model, the multipotential precursor survives into adult life and continues to generate committed progeny as a result of asymmetrical division but, in the absence of the appropriate environmental signals, the newly generated progenitor cells die.

by default after a predetermined number of cell divisions. Although the vast majority of these clones contained undifferentiated cells, 24% contained a small number of neurons (<1% of the total cell number). In addition, some 59% of the clones that contained neurons also contained GFAP positive astrocytes, indicating that many of these clones were derived from bipotential precursor cells.

Very recently, Davis and Temple (1994), by following the fate of single ventricular zone cells in culture, have shown that selfrenewing, multipotential precursor cells are present in the E12 and E14 rat cerebral cortex. These precursor cells produced about 7% of the cortical clones generated, whereas over 80% of the clones consisted of small numbers of either neurons or glia. The authors defined the multipotential precursors as stem cells because of their ability to self renew, as determined by the fact that some of their subcloned progeny were themselves able to generate multiple cell types, including neurons, astrocytes, and oligodendrocytes. Overall, these re-

sults and the previous findings of Temple (1989) and Kilpatrick and Bartlett (1993) support the view that multipotential precursors are present in the embryonic CNS and suggest that as development proceeds, these cells become progressively restricted to progenitors of either the neuronal or glial lineages. In addition, the apparent paradox between the *in vivo* retroviral labeling and the *in vitro* clonal data could be reconciled if it is interpreted that the retroviral studies have identified two types of cells (Walsh *et al.*, 1994). The first cell type is a multipotential cell which migrates within the subventricular zone and which generates cells of the second type, a committed progenitor with restricted migration potential and which is responsible for generating the clustered clones identified in many of the *in vivo* studies (Walsh and Cepko, 1993; Walsh *et al.*, 1994).

The potentiality of precursors already committed to the glial lineages has been extensively studied in postnatal development and initial *in vitro* results suggested that a single progenitor could generate both astrocytes and oligodendrocytes (Raff *et al.*, 1983a). It also was suggested that there are two major types of astrocyte (Raff *et al.*, 1983b), type-1 astrocytes, important in the maintenance of the blood–brain barrier (Janzer and Raff, 1987) and in the production of neurotrophic factors (Raff, 1989), and type-2 astrocytes, important in the maintenance of the nodes of Ranvier (ffrench-Constant and Raff, 1986). *In vitro* lineage studies of cells isolated from the postnatal rat optic nerve suggested these two cell types were derived from different precursors and, in particular, that type-2 astrocytes share a common progenitor (the O-2A progenitor cell) with oligodendrocytes (Raff *et al.*, 1983a). However, it remains uncertain if these *in vitro* observations are pertinent to development, principally because it has been difficult to definitively identify type-2 astrocytes *in vivo* (Lillien and Raff, 1990). Further, the expression of GFAP by type-2 astrocytes *in vitro* is sometimes transient and, thus, does not provide definitive evidence of lineage commitment (Hughes *et al.*, 1988). Two studies have further addressed this issue by determining the expression of transcripts for putative oligodendrocyte lineage specific markers such as the platelet-derived growth factor α receptor and 2′, 3′-cyclic-nucleotide 3′-phosphodiesterase in the embryonic rat CNS (Pringle and Richardson, 1993; Yu *et al.*, 1994). As early as E14, discrete clusters of cells expressing these markers were found within the ventricular zone, suggesting that specialized oligodendrocyte progenitors may already be present at this developmental stage. Studies utilizing the *lacZ* reporter gene to assess the glial lineage have, however, yielded conflicting results. Williams *et al.* (1991) labeled cultures derived from E12–E18 cortex and failed to identify astrocytes and oligodendrocytes together in a single clone. Instead, this study revealed a previously unidentified clonal association, suggesting the presence of a common neuronal and oligodendrocyte progenitor (see above). Vaysse and Goldman (1990) assessed lineage in cultures generated from neonatal rat striatum and the results were interpreted to confirm that astrocytes and oligodendrocytes were generated from separate progenitors, a finding confirmed by Luskin and McDermott (1994), studying glial lineage within the neonatal rat brain. In contrast, another *in vivo* study found that 15% of the clones generated from neonatal forebrain precursors contained both oligodendrocytes and astrocytes, strongly suggesting the presence of a bipotential progenitor (Levison and Goldman, 1993); it remains unclear, however, whether the astrocytes generated from this bipotential progenitor represent a discrete subpopulation of astrocytes, subserving a specific function akin to that previously attributed to the type-2 astrocyte. A further study by the same group confirmed that the labeled progeny of neonatal subventricular zone cells, which migrated into gray matter, generated both astrocytes and oligodendrocytes (Levison *et al.*, 1993). In contrast, the progeny of P14 precursors were exclusively oligodendrocytes and were restricted to subcortical white matter. Nevertheless, when either neonatal or P14 subventricular zone cells were placed in dissociated culture, some heterogeneous clones, containing both oligodendrocytes and astrocytes, were generated. How can these apparently conflicting results be resolved? One possibility is that the disparate cell fates observed between the various studies and also between the *in vitro* and *in vivo* analyses reflect the influences of temporally and spatially regulated environmental signals.

THE ROLE OF EPIGENETIC FACTORS IN REGULATING THE DEVELOPMENTAL FATE OF NEURAL PRECURSOR CELLS

Once it was established that a proportion of neural precursor cells lack predetermined lineage capacity, it was clearly important to address the issue of how cell fate could be modulated by environmental influences. Epigenetic factors have been shown to act upon neuroepithelial cells either to potentiate their survival, to induce their proliferation, or to facilitate their commitment and differentiation into mature cell phenotypes (Table 1). Insulin-like growth factor-1 (IGF-1) acts *in vitro* to potentiate neuroepithelial cell survival (Drago *et al.*, 1991). The proliferation of neuroepithelial cells *in vitro* also has been shown to be stimulated by growth factors (Gensburger *et al.*, 1987; Murphy *et al.*, 1990; Cattaneo and McKay, 1990; Anchan *et al.*, 1991); in particular, fibroblast growth factors 1 and 2 (FGF-1 and FGF-2) stimu-

TABLE 1
Summary of the Effect of Growth Factors upon Cultured Neural Precursor Cells

Type of precursor cell	Response		
	Survival	Proliferation	Differentiation
Neuroepithelial cells	IGF-1	FGF-1, FGF-2, EGF (NGF)	+ve. FGF-1
Neuronal progenitors	?	FGF	+ve. FGF
Oligodendrocytic progenitors	PDGF, IGF-1, CNTF, LIF, NT-3	FGF, PDGF, NT-3	+ve. (CNTF, LIF) −ve. PDGF, FGF, IGF-1
Astrocytic progenitors	?	FGF, EGF	+ve. (LIF)

Note. Where growth factors are in parentheses the observed effect is known to be dependent upon the provision of additional extracellular signals (see text for details). Abbreviations used: IGF-1, insulin-like growth factor-1, FGF, fibroblast growth factor, EGF, epidermal growth factor, NGF, nerve growth factor, PDGF, platelet derived growth factor, CNTF, ciliary neurotrophic factor, LIF, leukemia inhibitory factor, NT-3, neurotrophin-3.

late the proliferation of neuroepithelial cells isolated from E10 murine telencephalon and mesencephalon (Murphy *et al.*, 1990). In addition, Cattaneo and McKay (1990) reported that neuronal precursor cells isolated from E13.5 to E14.5 rat striatum not only responded to FGF but that proliferation was further potentiated by nerve growth factor (NGF), which correlated with the finding that embryonic striatal cells express the NGF receptor (Gage *et al.*, 1989). Murphy *et al.* (1990) also showed that FGF could induce the differentiation of primary neuroepithelial cells into neurons and astrocytes, when the precursors were plated at high cell density. These latter observations raised the issue of whether FGF was acting primarily as a mitogen or whether it also was integrally involved in the differentiative processes occurring within these cultures; for example, either neuronal or astrocytic differentiation could have conceivably resulted from the endogenous production of secondary factors within the mixed cultures (Mehler *et al.*, 1993). Clonal studies provide a more precise means to distinguish between direct and indirect influences and using this approach, Kilpatrick and Bartlett (1993) established that the primary response of FGF was to induce the proliferation of the neuroepithelial population, given that the vast majority of cells in the derived clones were of an undifferentiated phenotype. Further, in these studies, the removal of the proliferative signal was inadequate, by itself, to induce differentiation, a process which required separate and discrete epigenetic signals (see below). However, when the precursor cells were exposed, in the absence of FGF, to secondary soluble activities produced by an N-*myc* immortalized astrocyte precursor cell line (Kilpatrick *et al.*, 1993), significant numbers of differentiated neurons were generated. This is consistent with the finding that astroglial cells induce the terminal differentiation of CNS neuronal progenitors (Gao *et al.*, 1991), although this latter effect was dependent upon a membrane-associated activity. The above findings differed from those of Cattaneo and McKay (1990), who found that in the rat, the withdrawal of FGF and NGF was sufficient to invoke the differentiation of colonies of precursor cells into neurons. The different findings could reflect either ontogenic or phylogenetic differences or alternatively, accessory cells in heterogeneous cultures could have produced secondary factors which potentiated differentiation and whose effects were inhibited by FGF and/or NGF.

There also has been debate in the literature concerning whether the affects of FGF are predominantly upon multipotential precursors or, alternatively, progenitors restricted to the neuronal lineage. Gensburger *et al.* (1987) had initially reported that precursor cells isolated from the cerebral hemispheres of E13 rat proliferated in response to FGF-2. Although high density, mixed cultures were studied, the majority of differentiated cells generated were neurofilament positive neurons. This suggested that the predominant affect of FGF-2 was to induce the proliferation of neuronal, rather than glial progenitors. A recent study has readdressed this issue and also suggests that FGF-2 induces the differentiation of neuronally restricted progenitors isolated from the hippocampus (Ray *et al.*, 1993; Table 1). However, although the vast majority of the progeny was clearly neuronal, without clonal analysis it is impossible to know whether the majority of the plated precursors was already committed to the neuronal lineage, whether FGF-2 exerted an affect upon multipotential precursors or whether environmental conditions failed to support either the survival or the proliferation of nonneuronal cells. Indeed, it is possible that secondary factors, produced endogenously within the hippocampal derived cultures, could be involved in selectively promoting neurogenesis rather than gliogenesis.

It also has been shown that FGF-1 and FGF-2 are pro-

duced by neuroepithelial cells (Drago *et al.*, 1991; Nurcombe *et al.*, 1993), with the onset of FGF-1 expression corresponding, temporally, to the commencement of neurogenesis. This has led to the hypothesis that FGF-1 could play a pivotal role in initiating neurogenesis, even though exogenous FGF-1 did not invoke the neuronal differentiation of E10 neuroepithelial clones (Kilpatrick, unpublished observations). It has been suggested that the presence of extracellular matrix molecules, known as heparan sulfate proteoglycans (HSPGs), is also necessary for either FGF-1 or FGF-2 to activate the appropriate high affinity receptor (Nurcombe *et al.*, 1993). Indeed, at the onset of FGF-1 production within the CNS, the synthesis of the appropriate HSPG (that which binds FGF-1 with the highest affinity) is also upregulated. These findings suggest that the inability of FGF-1 to stimulate neurogenesis within neuroepithelial clones could reflect an *in vitro* interaction between FGF-1 and an "inappropriate" heparan sulfate side-chain, resulting in suboptimal presentation of the ligand to its high affinity receptor. Indeed, preliminary evidence suggests that when E10 neuroepithelial clones are stimulated with FGF-1, as well as the appropriate HSPG, a significant percentage of the precursors differentiate into neurons, implicating a role for FGF-1 in neurogenesis (Bartlett, unpublished observations, Table 1).

The nature of the regulatory molecules that invoke the commitment of multipotential precursors to the glial lineage is largely unknown. Epigenetic regulations and in particular, cell–cell interactions could, however, play a role, as the generation of GFAP positive astrocytes among clonal populations derived from multipotential precursors occurs predominantly in regions of the cultures composed of multiple layers of cells (Kilpatrick and Bartlett, 1993). Preliminary evidence also suggests that leukemia inhibitory factor (LIF), in combination with serum-associated factors, could be implicated in promoting the generation of astrocytes from primary neuroepithelial precursors (Richards *et al.*, 1995), while it also has been suggested that both LIF and ciliary neurotrophic factor (CNTF) can induce the differentiation of the astrocytic progenitor cell line, AP-16 (Yoshida *et al.*, 1993). Furthermore, both FGF and EGF can stimulate the proliferation of committed astrocytic progenitors isolated from the E17 mouse cerebrum (Kilpatrick and Bartlett, 1995; Table 1 and see below).

In contrast to the limited information available pertaining to the regulation of gliogenesis in embryogenesis, there has been extensive investigation of the influence that environmental factors exert upon committed glial progenitors in postnatal development (Table 1). The survival of progenitors isolated from postnatal optic nerve is potentiated by neurotrophin-3 (NT-3) (Barres *et al.*, 1993), IGF-1 and platelet derived growth factor (PDGF) (Barres *et al.*, 1992). Proliferation of O-2A progenitors is stimulated by multiple growth factors, including PDGF (Noble *et al.*, 1988; Raff *et al.*, 1988; Richardson *et al.*, 1988), FGF-2 (McKinnon *et al.*, 1990; Bögler *et al.*, 1990; Mayer *et al.*, 1993), and NT-3, when administered in combination with PDGF (Barres *et al.*, 1994a), although only NT-3 has been shown to play a role in stimulating O2-A progenitor proliferation *in vivo* (Barres *et al.*, 1994a). In addition, CNTF and LIF promote oligodendrocyte maturation *in vitro* (Mayer *et al.*, 1994); they also promote oligodendrocyte survival (Barres *et al.*, 1993; Louis *et al.*, 1993; Mayer *et al.*, 1994) as do insulin, the IGFs (Barres *et al.*, 1992), and NT-3 (Barres *et al.*, 1993). On the other hand, O-2A progenitors have been reported to be stimulated to differentiate into type-2 astrocytes by either CNTF (Hughes *et al.*, 1988) or LIF (Mayer *et al.*, 1994), when the cells are cocultured with extracellular matrix (Lillien *et al.*, 1990). These findings suggest that CNTF and LIF enhance the differentiation of bipotential O-2A progenitors per se and that the choice of lineage is dependent upon other regulatory influences within the extracellular environment. Although the above data document the molecular mechanisms that potentially regulate glial development, they give little insight into how epigenetic influences interact to regulate the timing of progenitor differentiation. It has been hypothesized that oligodendrocyte differentiation depends on an intrinsic clock in O2-A progenitor cells which counts cell divisions or, more particularly, elapsed time after exposure to mitogens and which limits precursor proliferation (Temple and Raff, 1986; Bögler and Noble, 1994). It has been further suggested that hydrophobic signals such as thyroid hormone, glucocorticoids, and retinoic acid, although by themselves not required for differentiation, act as effector molecules, to coordinate the timing of differentiation, by inhibiting cellular proliferation (Barres *et al.*, 1994b). Thyroid hormone is a candidate *in vivo* signal, as the thyroid gland becomes active around the time when oligodendrocytes first appear in the rat CNS (Dussault and Ruel, 1987; Abney *et al.*, 1981).

It is also possible that a cell intrinsic developmental program is important in regulating cell lineage. In particular, subsets of committed but morphologically homogeneous and undifferentiated progenitor cells could be produced by the asymmetrical division of multipotential precursors both *in vivo* and among clonal populations generated *in vitro* (Kilpatrick and Bartlett, 1993; Davis and Temple, 1994). If this were so, epigenetic factors could provide the necessary requirements to invoke differentiation but may not, by themselves, instruct cell type. Evidence for such a combinatorial influence is derived from the study of rat retinal neuroepithelial cell

development (Watanabe and Raff, 1990), in which cell–cell interactions and a cell autonomous developmental program combine to instruct both the timing and the nature of precursor cell differentiation.

LINEAGE POTENTIAL VERSUS CELL FATE WITHIN THE DEVELOPING BRAIN

Although *in vivo* lineage studies suggest that, after E14, the cell fate of most proliferating precursors within the rodent forebrain is restricted, it is still possible that they maintain a multipotential capacity and that it is local environmental cues which regulate and ultimately restrict the lineage of their progeny (see Fig. 1B). Two recent *in vitro* studies have addressed this issue. Reynolds *et al.* (1992) isolated cells from E14 mouse striatal primordia, grew the cells at a density of 1000 cells per 35-mm culture dish and found that epidermal growth factor (EGF) stimulated the proliferation of cells within clusters called, "neurospheres." The majority of the cells within the neurospheres expressed the intermediate filament, nestin, previously reported to be expressed in neuroepithelial stem cells (Lendahl *et al.*, 1990; Cattaneo and McKay, 1990) and, in the absence of EGF, differentiation into neurons and astrocytes was observed. It also was possible to subculture the EGF-responsive cells, and after multiple passages, a small but undefined proportion of these cells maintained the capacity to differentiate. These findings suggested that an EGF-responsive multipotential cell, with the capacity to selfrenew and, thus, with some of the cardinal features of a stem cell, had been identified.

In a second study, clonal analysis of murine cerebral cells from E17 was performed and it was established that either FGF-2 or EGF induced the proliferation of a subpopulation of the cells (Kilpatrick and Bartlett, 1995). On closer analysis, it was shown that glial-restricted progenitors were stimulated by either FGF-2 or EGF, whereas clones containing both neuronal cells and astrocytes (i.e., derived from a multipotential precursor) could be generated only with FGF-2. These observations argue strongly for the presence of at least two distinct populations of precursor cells within the E17 mouse cerebrum; precursor cells which vary not only in their factor responsiveness but also in their lineage potential.

Unlike E10 precursors, E17 multipotential precursors cultured with FGF-2 exhibited the intrinsic capacity to generate large numbers of neurofilament positive cells. This finding indicates that multipotential precursors have an age dependent variability in their capacity to generate progeny which, by phenotypic criteria, are committed to the neuronal lineage (Fig. 2). Furthermore, the proliferative potential of cells within clones derived from multipotential precursors isolated at E17 (Kilpatrick and Bartlett, 1995) was much lower than from those isolated at E10 (Kilpatrick

FIG. 2. Multipotential precursors have an age dependent variability in their capacity to generate differentiated progeny, as shown by the disparate phenotypes of clones generated from precursor cells isolated from the mouse brain at E10 and E17. Shown in (a) is a clone derived from an E10 neuroepithelial cell which is composed predominantly of cuboidal precursors which fail to express either neurofilament (NF) or glial fibrillary acidic protein (GFAP), lineage markers for neurons and astrocytes, respectively. Shown in (b) and (c) are photomicrographs of a single clone generated from a multipotential precursor cell, isolated at E17. Unlike the clone generated from the E10 precursor, the E17 derived clone contains large numbers of NF + ve (b) and GFAP + ve (c) cells. Bar, 50 μm.

and Bartlett, 1993), suggesting, first, that the E17 precursors have limited selfrenewal capacity and second, that their progeny are more likely to be further down the differentiation pathway, with more limited proliferative capacity. In support of these contentions, Lillien and Cepko (1992) have found that the proliferative potential of precursors isolated from the retina reduces with age. Thus, there may be an ontogenic hierarchy of multipotential precursors within the CNS, analogous to the well defined hierarchy of precursors within the hematopoietic system (Metcalf and Moore, 1971).

How does one account for the apparent differences in factor responsiveness of multipotential precursors isolated by the two groups of investigators? The differences could be explained if EGF stimulated glial progenitors in the low density cultures of Reynolds *et al.* (1992) to produce endogenous factors, including FGF, which, in turn, directly induced the proliferation of multipotential precursors. An alternative explanation for the differences would be that there are EGF-responsive precursors that were not detected in the E17 clonal analysis because the frequency of this population is very low; consistent with this view is the observation that the frequency of the EGF-responsive cells is less than 1 in 1000 (Reynolds *et al.*, 1992).

Vescovi *et al.* (1993) have postulated that FGF-2 induces the proliferation of both a bipotential neuronal/astroglial progenitor and a committed neuronal progenitor. The authors have suggested that these data support the view that EGF and FGF act sequentially, with EGF stimulating *bona fide* stem cells; if this is so, it remains unclear why others have failed to observe EGF responsiveness among neuroepithelial cells isolated from the earliest phase of CNS development, while it has been observed that neuroepithelial cells will respond to FGF (Murphy *et al.*, 1990; Kilpatrick and Bartlett, 1993). On the other hand, experiments using FGF have not definitively isolated a stem cell and it remains formally possible that an FGF responsive and bipotential neuronal/astrocytic progenitor has been identified, especially since oligodendrocytes are not generated within these cultures (Kilpatrick and Bartlett, 1993, 1995). One view of the available data would be that EGF induces the proliferation of a very restricted population of multipotential precursors which exhibit many of the phenotypic characteristics of a stem cell, FGF induces the proliferation of a more abundant bipotential neuronal/astrocytic progenitor and both factors act to induce the proliferation of glial restricted progenitors.

THE IDENTIFICATION OF PRECURSORS IN THE ADULT MAMMALIAN CNS

The ability to isolate multipotential precursors in late embryogenesis (Kilpatrick and Bartlett, 1995) raises the question as to whether either neuronal or multipotential precursor cells might persist within the cerebrum of the postnatal mammalian brain (Fig. 1). In the rodent CNS, the majority of neurons are clearly formed prenatally, although there are well identified exceptions, including granule neurons in the hippocampus (Altman, 1963; Schlessinger *et al.*, 1975; Altman and Bayer, 1990) and cerebellum (Altman, 1972) and the olfactory bulb (Bayer, 1985). In contrast, it is well established that cells within the subventricular zones of the brains of adult fish, amphibians, and some birds retain the capacity for proliferation (Alvarez-Buylla *et al.*, 1988). In canaries, these proliferating cells maintain the capacity to differentiate into neurons and to populate the higher vocal centers in the forebrain, in response to hormonal stimuli (Nordeen and Nordeen, 1989). Further, it has been shown that proliferating cells can be identified in the subventricular zone of adult mammals, as assessed by tritiated thymidine incorporation studies (Morshead and van der Kooy, 1992). When cells in the subventricular zone were labeled with the *LacZ* reporter gene, it was shown that the resultant clones, identified adjacent to the lateral ventricle, were almost never more than two cells in size, whereas some 33% of the subventricular cellular population was shown to be undergoing cell division. This finding suggested that the proliferating cells were dividing asymmetrically, with one progeny maintaining an undifferentiated phenotype, thereby facilitating the self-renewal of the precursor population, whereas the other daughter cell invariably died (see Fig. 1B). Two recent studies, however, suggest that many of their progeny migrate to the olfactory bulb, where they undergo differentiation into interneurons (Luskin, 1993; Lois and Alvarez-Buylla, 1994), raising the possibility that either the appropriate migratory pathway or the necessary stimulus for migration is only present within this region of the brain. If the latter is correct, it will be important to define the environmental conditions which facilitate the selective migration of precursors to the olfactory bulb. It is relevant that in mutant mice exhibiting homozygous deficiencies of the neural–cell adhesion molecule (N-CAM) gene, the predominant phenotypes observed are atrophy of the olfactory bulbs (Tomasiewicz *et al.*, 1993; Cremer *et al.*, 1994) and hypertrophy of the adjacent subventricular zone (Tomasiewicz *et al.*, 1993), suggesting that N-CAM is involved in precursor migration. It remains to be established whether there are chemotactic gradients that facilitate precursor migration to the olfactory bulb, and whether there are environmental signals within the olfactory bulb which promote neuronal commitment and/or differentiation. The discovery of epigenetic conditions which induce the neuronal differentiation of precursors

during development has provided the means to address the latter issue.

Three recent reports provide further evidence that neuronal precursor cells do, indeed, exist in the adult mammalian brain (Reynolds and Weiss, 1992; Richards *et al.*, 1992; Lois and Alvarez-Bullya, 1993). In the last study, slice cultures containing rostral subventricular tissue were grown in the absence of exogenous growth factors. Precursor cells within these cultures had the constitutive capacity to differentiate upon migration out of the explant, which could suggest either a role for inhibitory factors *in vivo* or that these cells normally migrate to the olfactory bulbs and then differentiate. The other studies suggest that exogenous factors can influence precursor cell fate. Reynolds and Weiss (1992) isolated cells from adult mouse striatum, induced the proliferation of precursors within the cultures by administering EGF, and found that the precursors differentiated into neurons and glia, when EGF was subsequently withdrawn. Richards *et al.* (1992) reported that precursors could be induced to differentiate into neuronal cells when initially stimulated *in vitro* with FGF-2 and then with medium conditioned by an astrocytic precursor cell line (Kilpatrick *et al.*, 1993, see above). In this latter study, neuronal induction was not potentiated by initial culture with EGF and the reason for the disparity in the results, with regard to the efficacy of EGF, remains unclear. However, as for the multipotential precursors identified in late embryogenesis, it may be that EGF stimulates the production of secondary factors, including FGF, which in turn act directly upon the precursor population (see above). Alternatively, it is possible that the differences relate to a disparity in the expression of the EGF and FGF receptors by different subpopulations of precursor cells.

An important question which arises is whether the findings of Reynolds and Weiss (1992) and of Richards *et al.* (1992) were obtained due to the selective stimulation of a subpopulation of the cultured precursors, specifically those isolated from the rostral tip of the lateral ventricle. If this were so, it might indicate a restricted distribution of neuronal precursors within the subventricular zone and raises the possibility of a restricted lineage potentiality (i.e., to olfactory bulb interneurons). Morshead *et al.* (1994) suggest that there is, indeed, a rostrocaudal gradient, in that cells isolated from the subventricular zone of the rostral forebrain generate more neurospheres in response to EGF stimulation than cells isolated from caudal regions of the brain. On the other hand, a preliminary report by Kirschenbaum *et al.* (1993) indicates that neuron-like cells can be generated *in vitro* from human temporal lobe periventricular tissue, which is adjacent to the inferior limb, and not the rostral tip, of the lateral ventricle. In addition, recent *in vitro* work has shown that precursors isolated from both the rostro-dorsal and rostro-ventral regions of the lateral ventricles, when stimulated with FGF-1, generate clones containing neurofilament positive cells. This observation further supports the view that some of the precursors which do not migrate to the olfactory bulbs also have the potential to differentiate into neurons (Bartlett, unpublished observations).

Bipotential O-2A progenitor cells have been identified within the adult rodent CNS (Wolswijk and Noble, 1989) and it has been suggested that these cells are derived directly from a subpopulation of perinatal O-2A progenitors (Wren *et al.*, 1992). The adult progenitors are capable of prolonged selfrenewal *in vitro* under the mitogenic influence of PDGF (Wolswijk *et al.*, 1991; Wren *et al.*, 1992). These cells generate oligodendrocytes, primarily by asymmetrical division and differentiation, in contrast to the selfextinguishing pattern of symmetrical division and programmed differentiation displayed by perinatal progenitors *in vitro* (Wren *et al.*, 1992). The properties of the adult rodent progenitor would thus appear well suited to a functional role designed to replenish oligodendrocytes at a slow, basal, rate throughout life. A recent report also has documented plasticity in the adult human oligodendrocyte lineage (Gogate *et al.*, 1994). However, unlike the rodent progenitor, the human counterpart exhibited phenotypic plasticity, rather than mitogenic activity, in response to the growth factors, FGF-2 and PDGF. This either indicates fundamental interspecies differences in the proliferative potential of adult oligodendrocytic progenitors or, alternatively, disparate, and incompletely defined, spectra of growth factor responsiveness.

FUTURE DIRECTIONS

The results of adult neural cell culture, together with those of clonal analysis of embryonic neuroepithelial cells, suggest that the lineage determination and subsequent differentiation of multipotential precursor cells is invoked by specific environmental conditions. These observations raise the possibility of utilizing precursor cell populations, present *in situ*, to replace degenerating neurons by the *in vivo* administration of growth factors. In order to effectively study this possibility, a number of issues will need to be clarified. First, it will be necessary to establish whether precursors within the adult brain include dormant cells or whether they are exclusively the proliferating, subventricular cells identified by Morshead and van der Kooy (1992); in other words, will it be necessary to induce the proliferation of precursor cells *in situ*

in order to facilitate repopulation? Morshead *et al.* (1994) have very recently suggested that there are, indeed, at least two populations of precursor cells within the adult mammalian brain, first, the constitutively proliferating population previously described and second, a relatively quiescent population which has been hypothesized to be the *in vivo* source of neural stem cells. The lineage relationships between these two populations will, however, have to be more formally assessed before it will be possible to answer the above question. Second, it will be necessary to determine whether precursors can be induced to migrate within the adult cerebral cortex, in the absence of the radial glia which facilitate neuronal migration in the developing brain. Third, it will be important to determine whether precursors can autonomously assume the specific phenotype of the degenerating neuronal population or, alternatively, if they can be induced to assume this phenotype when provided with appropriate environmental signals.

Multiple reports document the successful transplantation of neural progenitors into the postnatal CNS (reviewed in Rosenfeld *et al.*, 1991; Fisher and Gage, 1993; Groves *et al.*, 1993). While these reports have speculated that transplantation of progenitors could be a practical way to repopulate the damaged brain, the recent acquisition of the methodology to generate large clonal populations from multipotential precursors *in vitro* suggests a more effective way to realize this aim. Renfranz *et al.* (1991) and Snyder *et al.* (1992) have transplanted retrovirally immortalized multipotential cells into postnatal rat brain and have provided convincing evidence of *in vivo* differentiation of these cells into neuronal and glial-like cells (see above). The methodology is now available to repeat the above transplantation experiments with primary cells and such experiments would have obvious advantages over those using cell lines. First, lineage could be studied using cells which have not been affected by genetic manipulation and second, if precursor cell transplantation is to be applied to human degenerative disease, primary cells will be the preferable source. Transplantation experiments also could be performed on primary cells after exposure to either specific growth factors or to antisense oligonucleotides designed to inhibit the effects of either specific growth factors or their receptors. Such experiments also would provide further insight into how exogenous and endogenous mechanisms interact to regulate the differentiation of neural cells.

Recent experiments have shown that primary neural populations, which are able to undergo cell division and to generate both neurons and glia for extended periods *in vitro* (see above), can successfully engraft into the postnatal mammalian brain (Kilpatrick *et al.*, 1994; Ray, 1994; Gage *et al.*, 1994). In the former study, murine neuroepithelial cells expanded *in vitro* with FGF-2 were transplanted into the hippocampus and were found to have selectively migrated into regions of continuing neurogenesis in the host brain, specifically the granular layer of the dentate gyrus. Within this region, some of the transplanted cells elaborated processes which extended toward the CA2 region of the pyramidal layer, indicating that the donor cells can adopt similar morphologies to those of host granule neurons. Ray (1994) has reported similar results after transplantation of fetal rat hippocampal cells precultured and expanded *in vitro* with FGF-2. These cells were labeled with tritiated thymidine and transplanted into the hippocampi of adult rats; many of the transplanted cells were found distributed within the host dentate gyrus and some expressed the granule cell marker, calbindin. Similar findings also can be obtained using cultured precursor cells isolated from the hippocampus of adult rats (Gage *et al.*, 1994). Precursor cells isolated directly from the subventricular zone also could represent a useful source of neuronal cells, given that Lois and Alvarez-Buylla (1994) have shown that cells from this region can be transplanted back into the lateral ventricles of adult mice and that the progeny of these grafted cells can migrate to the olfactory bulbs, where they differentiate into interneurons (see above). Collectively, these results suggest the possibility of novel and more refined approaches to repopulating the damaged brain; the data are, however, preliminary and many challenges remain. In particular, it will be important to determine whether the cell fate of primary precursors can change according to the region of the host brain into which they are transplanted. Furthermore, if these techniques are ever to be applied to human neurological disease, it will be necessary to establish that precursor cells can engraft, differentiate, and repopulate either injured or degenerating regions of the adult host brain.

ACKNOWLEDGMENTS

T.J.K. is a Howard Hughes Medical Institute Physician Research Fellow and is a recipient of the Bushell Fellowship, awarded by the Royal Australasian College of Physicians. L.J.R. is a Lucille P. Markey Visiting Fellow and is supported by a grant from the Lucille P. Markey Charitable Trust. We also thank Dr. Peter Maycox and Dr. David Weinstein for critically reviewing this manuscript.

REFERENCES

Abney, E. R., Bartlett, P. F., and Raff, M. C. (1981). Astrocytes, ependymal cells and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain. *Dev. Biol.* **83**: 301–310.

Altman, J. (1963). Autoradiographic investigation of cell proliferation in the brains of rats and cats. *Anat. Rec.* **145:** 573–591.

Altman, J. (1972). Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. *J. Comp. Neurol.* **145:** 353–398.

Altman, J., and Bayer, S. A. (1990). Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J. Comp. Neurol.* **301:** 365–381.

Alvarez-Buylla, A., Theelen, M., and Nottebohm, F. (1988). Birth of projection neurons in the higher vocal center of the canary forebrain, before, during and after song learning. *Proc. Natl. Acad. Sci. USA* **85:** 8722–8726.

Anchan, R. M., Reh, T. A., Angello, J., Balliet, A., and Walker, M. (1991). EGF and TGF-α stimulate retinal neuroepithelial cell proliferation *in vitro*. *Neuron* **6:** 923–936.

Angevine, J. B., Jr., and Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* **192:** 766–788.

Bailey, K. A., Drago, J., and Bartlett, P. F. (1994). Neuronal progenitors identified by their inability to express class I histocompatibility antigens in response to interferon-γ. *J. Neurosci. Res.* **39:** 166–177.

Barres, B. A., Hart, I. K., Coles, H. S., Burne, J. F., Voyvodic, J. T., Richardson, W. D., and Raff, M. C. (1992). Cell death and control of cell survival in the oligodendrocyte lineage. *Cell* **70:** 31–46.

Barres, B. A., Schmid, R., Sendtner, M., and Raff, M. C. (1993). Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* **118:** 283–295.

Barres, B. A., Raff, M. C., Gaese, F., Bartke, I., Dechant, G., and Barde, Y. A. (1994a). A crucial role for neurotrophin-3 in oligodendrocyte development. *Nature* **367:** 371–375.

Barres, B. A., Lazar, M. A., and Raff, M. C. (1994b). A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. *Development* **120:** 1097–1108.

Bartlett, P. F., Reid, H. H., Bailey, K. A., and Bernard, O. (1988). Immortalization of mouse neural precursor cells by the c-myc oncogene. *Proc. Natl. Acad. Sci. USA* **85:** 3255–3259.

Bayer, S. A. (1985). Neuron production in the hippocampus and olfactory bulb of the adult rat brain: Addition or replacement? *Ann. N. Y. Acad. Sci.* **457:** 163–72.

Bögler, O., Wren, D., Barnett, S. C., Land, H., and Noble, M. (1990). Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (0-2A) progenitor cells. *Proc. Natl. Acad. Sci. USA* **87:** 6368–6372.

Bögler, O., and Noble, M. (1994). Measurement of time in oligodendrocyte-type 2 astrocyte (0-2A) progenitors is a cellular process distinct from differentiation or division. *Dev. Biol.* **162:** 525–538.

Cattaneo, E., and McKay, R. (1990). Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. *Nature* **347:** 762–765.

Cowan, W. M. (1979). The development of the brain. *Sci. Am.* **241:** 112–133.

Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., Brown, R., Baldwin, S., Kraemer, P., Scheff, S., Barthels, D., Rajewsky, K., and Wille, W. (1994). Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature* **367:** 455–459.

Davis, A. A., and Temple, S. (1994). A self-renewing multipotential stem cell in embryonic rat cerebral cortex. *Nature* **372:** 263–266.

De Vitry, F., Picart, R., Jacque, C., Legault, L., Dupouey, P., and Tixier-Vidal, A. (1980). Presumptive common precursor for neuronal and glial cell lineages in mouse hypothalamus. *Proc. Natl. Acad. Sci. USA* **77:** 4165–4169.

Drago, J., Murphy, M., Caroll, S. M., Harvey, R. P., and Bartlett, P. F. (1991). Fibroblast growth factor-mediated proliferation of central nervous system precursors depends on endogenous production of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **88:** 2199–2203.

Dussault, J. H., and Ruel, J. (1987). Thyroid hormones and brain development. *Annu. Rev. Physiol.* **49:** 321–334.

ffrench-Constant, C., and Raff, M. C. (1986). The oligodendrocyte-type-2 astrocyte cell lineage is specialized for myelination. *Nature* **323:** 335–338.

Fisher, L. J., and Gage, F. H. (1993). Grafting in the mammalian central nervous system. *Physiol. Rev.* **73:** 583–616.

Frederiksen, K., and McKay, R. D. G. (1988). Proliferation and differentiation of rat neuroepithelial precursor cells in vivo. *J. Neurosci.* **8:** 1144–1151.

Frederiksen, K., Jat, P. S., Valtz, N., Levy, D., and McKay, R. (1988). Immortalization of Precursor Cells from the Mammalian CNS. *Neuron* **1:** 439–448.

Gage, F. H., Batchelor, P., Chen, K. S., Chin, D., Higgins, G. A., Koh, S., Deputy, S., Rosenberg, M. B., Fischer, W., and Bjorklund, A. (1989). NGF receptor reexpression and NGF-mediated cholinergic neuronal hypertrophy in the damaged adult neostriatum. *Neuron* **2:** 1177–1184.

Gage, F. H., Coates, P. W., Ray, J., Peterson, D. A., Suhr, S. T. *et al.* (1994). Adult hippocampal neural cells survive in vitro and following grafting to the adult hippocampus. *5th Int. Symp. Neural Transplant.*, in press.

Galileo, D. S., Gray, G. E., Owens, G. C., Majors, J., and Sanes, J. R. (1990). Neurons and glia arise from a common progenitor in chicken optic tectum: Demonstration with two retroviruses and cell type-specific antibodies. *Proc. Natl. Acad. Sci. USA* **87:** 458–462.

Gao, W.-Q., Heintz, N., and Hatten, M. E. (1991). Cerebellar granule cell neurogenesis is regulated by cell-cell interactions in vitro. *Neuron* **6:** 705–715.

Gensburger, C., Labourdette, G., and Sensenbrenner, M. (1987). Brain basic fibroblast growth factor stimulates the proliferation of rat neuronal precursor cells in vitro. *FEBS Lett.* **217:** 1–5.

Gogate, N., Verma, L., Zhou, J. M., Milward, E., Rusten, R., O'Connor, M., Kufta, C., Kim, J., Hudson, L., and Dubois-Dalcq, M. (1994). Plasticity in the adult human oligodendrocytic lineage. *J. Neurosci.* **14:** 4571–4587.

Gray, G. E., Glover, J. C., Majors, J., and Sanes, J. R. (1988). Radial arrangement of clonally related cells in the chicken optic tectum: Lineage analysis with a recombinant retrovirus. *Proc. Natl. Acad. Sci. USA* **85:** 7356–7360.

Gray, G. E., Leber, S. M., and Sanes, J. R. (1990). Migratory patterns of clonally derived cells in the developing central nervous system. *Experientia* **46:** 929–940.

Grove, E. A., Williams, B. P., Li, D-Q., Hajihosseini, M., Friedrich, A., and Price, J. (1993). Multiple restricted lineages in the embryonic rat cerebral cortex. *Development* **117:** 553–561.

Groves, A. K., Barnett, S. C., Franklin, R. J., Crang, A. J., Mayer, M., Blakemore, W. F., and Noble, M. (1993). Repair of demyelinated lesions by transplantation of purified O-2A progenitor cells. *Nature* **362:** 453–455.

Hajihosseini, M., Iavachev, L., and Price, J. (1993). Evidence that retroviruses integrate into post-replication host DNA. *EMBO J.* **12:** 4969–4974.

His, W. (1989). *Abh. Math. Phys. Cl. Kgl. Sach. Ges. Wiss.* **15:** 313–372.

Holt, C. E., Bertsch, T. W., Ellis, H. M., and Harris, W. A. (1988). Cellular determination in the Xenopus retina is independent of lineage and birth date. *Neuron* **1:** 15–26.

Hughes, S. M., Lillien, L. E., Raff, M. C., Rohrer, H., and Sendtner, M. (1988). Ciliary neurotrophic factor induces type-2 astrocyte differentiation in culture. *Nature* **335:** 70–72.

Janzer, R. C., and Raff, M. C. (1987). Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* **325:** 253–257.

Kilpatrick, T. J., and Bartlett, P. F. (1993). Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. *Neuron* **10:** 255–265.

Kilpatrick, T. J., Talman, P. S., and Bartlett, P. F. (1993). The differentiation and survival of murine neurons *in vitro* is promoted by soluble factors produced by an astrocytic cell line. *J. Neurosci. Res.* **35:** 147–161.

Kilpatrick, T. J., Cheema, S. S., Koblar, S., Tan, S-S., and Bartlett, P. F. (1994). The engraftment of transplanted primary neuroepithelial cells within the postnatal mouse brain. *Neurosci. Lett.* **181:** 129–133.

Kilpatrick, T. J., and Bartlett, P. F. (1995). Cloned multipotential precursors from the mouse cerebrum require FGF-2, whereas glial restricted precursors are stimulated with either FGF-2 or EGF. *J. Neurosci.*, in press.

Kirkwood, T. B., Price, J., and Grove, E. A. (1992). The dispersion of neuronal clones across the cerebral cortex. *Science* **258:** 317–320.

Kirschenbaum, B., Nedergaard, M., Preuss, A., Bahramian, K., and Goldman, S. A. (1993). *In vitro* neuronal and glial production and differentiation by precursor cells derived from the adult human brain. *Soc. Neurosci. Abstr.* 704.3.

Leber, S. M., Breedlove, M., and Sanes, J. R. (1990). Lineage, arrangement and death of motoneurons in chick spinal cord. *J. Neurosci.* **10:** 2451–2462.

Lendahl, U., Zimmerman, L. B., and McKay, R. D. G. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell* **60:** 585–595.

Levison, S. W., and Goldman, J. E. (1993). Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* **10:** 202–212.

Levison, S. W., Chuang, C., Abramson, B. J., and Goldman, J. E. (1993). The migrational patterns and developmental fates of glial precursors in the rat subventricular zone are temporally regulated. *Development* **119:** 611–22.

Levitt, P., Cooper, M. L., and Rakic, P. (1981). Coexistence of neuronal and glial precursor cells in the cerebral ventricular zone of the fetal monkey: an ultrastructural immunoperoxidase analysis. *J. Neurosci.* **1:** 27–39.

Lillien, L. E., and Raff, M. C. (1990). Differentiation signals in the CNS: type-2 astrocyte development in vitro as a model system. *Neuron* **5:** 111–119.

Lillien, L. E., Sendtner, M., and Raff, M. C. (1990). Extracellular matrix-associated molecules collaborate with ciliary neurotrophic factor to induce type-2 astrocyte development. *J. Cell Biol.* **111:** 635–644.

Lillien, L., and Cepko, C. (1992). Control of proliferation in the retina: temporal changes in responsiveness to FGF and TGFα. *Development* **115:** 253–266.

Lois, C., and Alvarez-Buylla, A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc. Natl. Acad. Sci. USA* **90:** 2074–2077.

Lois, C., and Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science* **264:** 1145–1148.

Louis, J.-C., Magal, E., Takayama, S., and Varon, S. (1993). CNTF protection of oligodendrocytes against natural and tumor necrosis factor-induced death. *Science* **259:** 689–692.

Luskin, M. B., Pearlman, A. L., and Sanes, J. R. (1988). Cell lineage in the cerebral cortex of the mouse studied in vivo and in vitro with a recombinant retrovirus. *Neuron* **1:** 635–647.

Luskin, M. B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron* **11:** 173–189.

Luskin, M. B., Parnavelas, J. G., and Barfield, J. A. (1993). Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: An ultrastructural analysis of clonally related cells. *J. Neurosci.* **13:** 1730–1750.

Luskin, M. B., and McDermott, K. (1994). Divergent lineages for oligodendrocytes and astrocytes originating in the neonatal forebrain subventricular zone. *Glia* **11:** 211–226.

Mayer, M., Bögler, O., and Noble, M. (1993). The inhibition of oligodendrocytic differentiation of O-2A progenitors caused by basic fibroblast growth factor is overriden by astrocytes. *Glia* **8:** 12–19.

Mayer, M., Bhakoo, K., and Noble, M. (1994). Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro. *Development* **120:** 143–153.

McKinnon, R. D., Matsui, T., Dubois-Dalcq, M., and Aaronson, S. A. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5:** 603–614.

Mehler, M. F., Rozental, R., Dougherty, M., Spray, D. C., and Kessler, J. A. (1993). Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. *Nature* **362:** 62–65.

Metcalf, D., and Moore, M. A. S. (1971). Embryonic aspects of haemopoiesis. In *Haemopoietic Cells* (A. Neuberger and E. L. Tatum, Eds.), pp. 70–271. North-Holland, Amsterdam.

Miller, R. H., David, S., Patel, R., Abney, E. R., and Raff, M. C. (1985). A quantitative immunohistochemical study of macroglial cell development in the rat optic nerve: In vivo evidence for two distinct astrocyte lineages. *Dev. Biol.* **111:** 35–41.

Morshead, C. M., and van der Kooy, D. (1992). Postmitotic death is the fate of constitutively proliferating cells in the subependymal layer of the adult mouse brain. *J. Neurosci.* **12:** 249–256.

Morshead, C. M., Reynolds, B. A., Craig, C. G., McBurney, M. W., Staines, W. A., Morassutti, D., Weiss, S., and van der Kooy, D. (1994). Neural stem cells in the adult mammalian forebrain: A relatively quiescent subpopulation of subependymal cells. *Neuron* **13:** 1071–1082.

Murphy, M., Drago, J., and Bartlett, P. F. (1990). Fibroblast growth factor stimulates the proliferation and differentiation of neural precursor cells *in vitro*. *J. Neurosci. Res.* **25:** 463–475.

Noble, M., Murray, K., Stroobant, P., Waterfield, M. D., and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333:** 560–562.

Nordeen, E. J., and Nordeen, K. W. (1989). Estrogen stimulates the incorporation of new neurons into avian song nuclei during adolescence. *Dev. Brain. Res.* **49:** 27–32.

Nornes, H. O., and Das, G. D. (1974). Temporal pattern of neurogenesis in spinal cord of rat. I. An autoradiographic study-time and sites of origin and migration and settling patterns of neuroblasts. *Brain Res.* **73:** 121–138.

Nurcombe, V., Ford, M. D., Wildschut, J. A., and Bartlett, P. F. (1993). Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. *Science* **260:** 103–106.

Price, J., Turner, D. L., and Cepko, C. L. (1987). Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* **84:** 156–160.

Price, J., and Thurlow, L. (1988). Cell lineage in the rat cerebral cortex: A study using retroviral-mediated gene transfer. *Development* **104:** 473–482.

Pringle, N. P., and Richardson, W. D. (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development* **117:** 525–533.

Raff, M. C., Miller, R. H., and Noble, M. (1983a). A glial progenitor

cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* **303:** 390–396.

Raff, M. C., Abney, E. R., Cohen, J., Lindsay, R., and Noble, M. (1983b). Two types of astrocytes in cultures of developing rat white matter: differences in morphology, surface gangliosides, and growth characteristics. *J. Neurosci.* **3:** 1289–1300.

Raff, M. C., Lillien, L. E., Richardson, W. D., Burne, J. F., and Noble, M. D. (1988). Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* **333:** 562–565.

Raff, M. C. (1989). Glial cell diversification in the rat optic nerve. *Science* **243:** 1450–1455.

Ray, J., Peterson, D. A., Schinstine, M., and Gage, F. H. (1993). Proliferation, differentiation and long-term culture of primary hippocampal neurons. *Proc. Natl. Acad. Sci. USA* **90:** 3602–3606.

Ray, J. (1994). Basic fibroblast growth factor responsive primary fetal CNS cells express neural markers in vitro and after grafting into adult animals. *Neuropsychopharmacology* **10:** 9085.

Renfranz, P. J., Cunningham, M. G., and McKay, R. D. (1991). Region-specific differentiation of the hippocampal stem cell line HiB5 upon implantation into the developing mammalian brain. *Cell* **66:** 713–729.

Reynolds, B. A., Tetzlaff, W., and Weiss, S. (1992). A multipotent EGF-responsive striatal embryonic progenitor cell produces neurons and astrocytes. *J. Neurosci.* **12:** 4565–4574.

Reynolds, B. A., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255:** 1707–1710.

Richards, L. J., Kilpatrick, T. J., and Bartlett, P. F. (1992). De novo generation of neuronal cells from the adult mouse brain. *Proc. Natl. Acad. Sci. USA* **89:** 8591–8595.

Richards, L. J., Kilpatrick, T. J., Tan, S.-S., Gearing, D. P., Bartlett, P. F., and Murphy, M. (1995). Leukemia inhibitory factor (LIF) promotes the differentiation of neuronal and astrocytic precursors within the developing spinal cord. *Eur. J. Neurosci.*, submitted for publication.

Richardson, W. D., Pringle, N., Mosley, M. J., Westermark, B., and Dubois-Dalcq, M. (1988). A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell* **53:** 309–319.

Rosenfeld, J. V., Kilpatrick, T. J., and Bartlett, P. F. (1991). Neural transplantation for Parkinson's disease: a critical appraisal. *ANZ J. Med.* **21:** 477–84.

Sanes, J. R., Rubenstein, J. L. R., and Nicolas, J.-F. (1986). Use of a recombinant retrovirus to study post-implantation cell lineage in mouse embryos. *EMBO J.* **5:** 3133–3142.

Schaper, A. (1897). *Science* **5:** 430–431.

Schlessinger, A. R., Cowan, W. M., and Gottlieb, D. I. (1975). An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *J. Comp. Neurol.* **159:** 149–175.

Snyder, E. Y., Deitcher, D. L., Walsh, C., Arnold-Aldea, S., Hartwieg, E. A., and Cepko, C. L. (1992). Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. *Cell* **68:** 33–51.

Temple, S., and Raff, M. C. (1986). Clonal analysis of oligodendrocyte development in culture: evidence for a developmental clock that counts cell divisions. *Cell* **44:** 773–779.

Temple, S. (1989). Division and differentiation of isolated CNS blast cells in microculture. *Nature* **340:** 471–473.

Tomasiewicz, H., Ono, K., Yee, D., Thompson, C., Goridis, C., Rutishauser, U., and Magnuson, T. (1993). Genetic deletion of a neural cell adhesion molecule variant (N-CAM-180) produces distinct defects in the central nervous system. *Neuron* **11:** 1163–1174.

Turner, D. L., and Cepko, C. L. (1987). A common progenitor for neurons and glia persists in rat retina late in development. *Nature* **328:** 131–136.

Turner, D. L., Snyder, E. Y., and Cepko, C. L. (1990). Lineage-independent determination of cell type in the embryonic mouse retina. *Neuron* **4:** 833–845.

Vaysse, P. J.-J., and Goldman, J. E. (1990). A clonal analysis of glial lineages in neonatal forebrain development in vitro. *Neuron* **5:** 227–235.

Vescovi, A. L., Reynolds, B. A., Fraser, D. D., and Weiss, S. (1993). bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. *Neuron* **11:** 951–966.

Walsh, C., and Cepko, C. L. (1992). Widespread dispersion of neuronal clones across functional regions of the cerebral cortex. *Science* **255:** 434–440.

Walsh, C., and Cepko, C. L. (1993). Clonal dispersion in proliferative layers of developing cerebral cortex. *Nature* **362:** 632–635.

Walsh, C., Cepko, C. L., Reid, C., and Liang, I. (1994). Cell lineage and cell phenotype in rat cerebral cortex studied with an alkaline phosphatase (AP) retroviral library. *Soc. Neurosci. Abstr.* 613.8.

Watanabe, T., and Raff, M. C. (1990). Rod photoreceptor development in vitro: Intrinsic properties of proliferating neuroepithelial cells change as development proceeds in the rat retina. *Neuron* **4:** 461–467.

Wetts, R., and Fraser, S. E. (1988). Multipotent Precursors Can Give Rise To All Major Cell Types of the Frog Retina. *Science* **239:** 1142–1145.

Williams, B. P., Read, J., and Price, J. (1991). The generation of neurons and oligodendrocytes from a common precursor cell. *Neuron* **7:** 685–693.

Wolswijk, G., and Noble, M. (1989). Identification of an adult-specific glial progenitor cell. *Development* **105:** 387–400.

Wolswijk, G., Riddle, P. N., and Noble, M. (1991). Platelet-derived growth factor is mitogenic for O-2A^{adult} progenitor cells. *Glia* **4:** 495–503.

Wren, D., Wolswijk, G., and Noble, M. (1992). In vitro analysis of the origin and maintenance of O-2A^{adult} progenitor cells. *J. Cell Biol.* **116:** 167–176.

Yoshida, T., Satoh, M., Nakagaito, Y., Kuno, H., and Takeuchi, M. (1993). Cytokines affecting survival and differentiation of an astrocyte progenitor cell line. *Dev. Brain Res.* **76:** 147–150.

Yu, W. P., Collarini, E. J., Pringle, N. P., and Richardson, W. D. (1994). Embryonic expression of myelin genes: evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube. *Neuron* **12:** 1353–1362.

Received for publication November 7, 1994