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Physiological Relevance and Functional Potential of Central Nervous System-Derived Cell Lines

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Abstract

Central nervous system (CNS)-derived neural cell lines have proven to be extremely useful for delineating mechanisms controlling such diverse phenomena as cell lineage choice and differentiation, synaptic maturation, neurotransmitter synthesis and release, and growth factor signalling. In addition, there has been hope that such lines might play pivotal roles in CNS gene therapy and repair. The ability of some neural cell lines to integrate normally into the CNS following transplantation and to express foreign, often corrective gene products *in situ* might offer potential therapeutic approaches to certain neurodegenerative diseases. Five general strategies have evolved to develop neural cell lines: isolation and cloning of spontaneous or mutagenically induced malignancies, targeted oncogenesis in transgenic mice, somatic cell fusion, growth factor mediated expansion of CNS progenitor or stem cells, and retroviral transduction of neuroepithelial precursors. In this article, we detail recent progress in these areas, focusing on those cell lines that have enabled novel insight into the mechanisms controlling neuronal cell lineage choice and differentiation, both in vitro and in vivo.

Index Entries: Cell lines; retroviral transduction; somatic cell fusion; stem cells; progenitor/precursor cells; transgenic mice; targeted oncogenesis; differentiation; cell lineage; transplantation; gene therapy; neurodegeneration.

Introduction

The marked cellular diversity that occurs both within a specific central nervous system (CNS) region and between discrete structures arises from a limited number of precursor cells in the developing neural ectoderm. Delineating the complex mechanisms underlying that process of maturation is a daunting prospect, especially in intact animals. Although the terminally differentiated fate of these pluripotent precursors has been exquisitely detailed (for reviews, *see* refs. *1–6*), the specific effectors that regulate that differentiation have not been

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clearly defined. It has also proven very difficult to identify in vivo the molecular events that underlie neural cell development and maturation. Primary cell culture has facilitated significant progress in both areas, but is often limited by the heterogeneity of the cultures and the difficulties in obtaining sufficient tissue. One approach to model neural cell differentiation in vitro that obviates many of the concerns of primary cell culture is the development of CNS-derived neural cell lines. The underlying assumption in such studies is that the closer a cell line mimics the behavior of primary neural cells, the more valid are the conclusions one can draw from those studies regarding the behavior of cells within the normal, intact CNS. The degree to which this has been successful is one focus of this article. One central conclusion we reach is that many of these neural cell lines show markedly enhanced differentiation in vivo, indicating the complex epigenetic signals needed for mature neuronal development. These cell lines should provide excellent model systems with which to begin to delineate these signals.

Five general approaches to the development of neural cell lines have evolved. Various aspects of neural cell line isolation have been reviewed previously *(3,7–10)*, but are not inclusive and the field has been expanding rapidly. Initially, neural cell lines were derived from spontaneous or mutagenically induced tumors. Over the last decade, the development of sophisticated molecular biological methodologies has enabled neural cell line isolation by retroviral transduction of neuroepithelial precursors and from targeted oncogenesis of specific neural populations in transgenic mice. The recent recognition that the CNS harbors stem cells that can be induced to proliferate in vitro by appropriate cytokine and/or growth factor treatment has allowed the isolation of large numbers of pluripotent neural cell populations, some of which may be clonal. Finally, somatic cell hybrid techniques, initially developed in the 1960s, have recently been applied to CNS tissues to develop neural cell lines.

Numerous investigators have utilized these approaches to generate nonneuronal cell lines displaying properties of astrocytes *(11–22)*, oligodendrocytes *(23–26)*, astrocyte–oligodendrocyte progenitor cells *(27–32)*, microglia *(33)*, and olfactory ensheathing cells *(20,34)*. Although these cell lines have provided important new information regarding the development and physiology of nonneuronal CNS cells, it is our intention here to discuss only those CNS-derived cell lines that have the potential for neuronal differentiation. Specifically, our primary focus is on those cell lines that have enabled insights into the mechanisms controlling CNS neuronal cell lineage choice and differentiation, both in vitro and in vivo.*

Generation of CNS-Derived Neural Cell Lines

Each of the strategies to neural cell line development outlined above have both advantages and limitations with respect to the stage of precursor that can be isolated, the potential lineage choice(s) of the cell line, and the degree to which the terminal differentiated phenotype(s) resembles that of mature CNS neurons and glia. In this article, we have summarized much of the data that highlight the potential applications of neural cell lines derived by these various approaches. We have attempted to be comprehensive in discussing neural stem cells and cell lines propagated by cytokine and/or growth factor treatment, retroviral transduction, somatic cell fusion, and targeted oncogenesis in transgenic mice. However, a large number of neuronal and nonneuronal cell lines have been isolated from spontaneously arising or exogenously induced tumors. We

*Because of the focus of this article, we will not discuss an important autonomic nervous system-derived neural cell line (MAH) generated by retroviral transduction of embryonic sympathoadrenal progenitors. MAH cells mimic many of the properties of primary progenitors that generate sympathetic neurons and adrenal chromaffin cells and have provided novel insight into their developmental biology *(35–39)*.

unfortunately cannot be as inclusive in those discussions. Rather, we have described a few examples of those cell lines that have pluripotent differentiative capacities and/or provide specific insight into neuronal function.

Neural Cell Lines Derived from Spontaneous and Exogenously Induced Tumors

The majority of these cell lines are represented by various neuroblastoma and glioblastoma cell lines. Many of these tumor-derived cell lines are of ill-defined origin, do not constitute a developmental spectrum of cells from defined locations and stages, and have lost many of the properties and developmental programs of the region from which they originated. Typically they are also tumorigenic, which precludes their use for transplantation experiments. We will highlight but a few pertinent examples of these cell lines.

Although not strictly defined as CNS-derived, embryonic stem (ES) cells and embryonal carcinoma (EC) cells have properties that mimic CNS neural cells and may provide models for CNS development. ES and EC cells are multipotent cells that can be derived from early mammalian embryos. ES cells are derived directly from early embryos cultured in vitro *(40)*. EC cells arise from germ cell tumors or the induction of teratocarcinoma tumors from ectopically transplanted primitive ectoderm. One mouse-derived EC cell line, P19, yields not only nonneural cells, but also differentiates into multiple CNS cell types, neurons, astrocytes, oligodendrocytes, and microglia, in response to retinoic acid *(41)*. The regulatory mechanisms that control P19 cell fate once treated with retinoic acid are at present unknown, and these cells may be very useful to delineate those early signals for CNS lineage determination. Differentiated P19 neurons synthesize multiple neurotransmitters, form synapses, and contain both small clear and large dense core vesicles *(42)*. Following transplantation of retinoic acid-differentiated P19 cells into the ibotenic acid-lesioned striatum of young adult rats, both astrocytes and neurons were observed *(43)*. The transplanted neurons displayed the same neurotransmitter heterogeneity observed in vitro and developed appropriate electrophysiological properties. However, no evidence of functional interaction between the grafted cells and host neurons was observed. The relationship of P19 cells to normal development is being actively investigated.

Another murine EC cell line, PCC7-5-aza-R-1009, can be induced to differentiate in vitro into both neurons and glia by treatment with retinoic acid and dibutyryl cAMP (dbcAMP), and some of the neurons are tyrosine hydroxylase (TH)-positive *(44)*. Following transplantation of undifferentiated PCC7-5-aza-R-1009 cells into the kainic acid lesioned thalamus of the adult rat, only neurons were detected, some of which were TH-positive. Although these grafts were rejected in the absence of immunosuppression, the results underscore a recurrent theme in this article: Some neural cell lines often show markedly divergent lineage restriction in vitro than that observed in vivo, most likely reflective of the lack of essential cues for differentiation in vitro.

The NTera-2 cell line was derived from a human teratocarcinoma emerging from an EC of the testis *(45)*. Treatment of NTera-2 cells with retinoic acid combined with differential plating and treatment with antimitotic agents resulted in a highly enriched population of differentiated neurons *(46)*. These cells exhibited parceling of axonal and dendritic proteins similar to those of primary CNS neurons and should allow detailed biochemical and molecular studies of the control of neuronal polarity. Moreover, because NTera-2 cells are of human origin, they represent one reproducible model system for human neurons that can be utilized for a wide range of pharmacological and toxicological studies. Following transplantation of differentiated NTera-2 cells into the adult and neonatal rat brain, the cells survive up to 4 wk without immunosuppression and acquire the molecular phenotype and axonal-dendritic polarity seen in vitro *(47)*. Within these short time periods, the transplanted NTera-2 cells

fail to synthesize and express proteins of mature CNS neurons, such as the heavily phosphorylated 210-kDa neurofilament protein (NF-H) and adult CNS tau. However, NTera-2 cells did express these mature CNS neuronal proteins after chronic survival (6–12 mo) in the adult nude mouse brain *(48)*. Despite the lack of synaptic integration of these grafts with host neurons, the location of engraftment markedly influenced the degree of neuritic outgrowth, indicating that these neurons may have the capacity to respond to extrinsic developmental cues. Interestingly, if undifferentiated NTera-2 cells were transplanted into adult CNS, superficial transplants (e.g., into subarachnoid space, lateral ventricles, or upper layers of the cerebral cortex) formed lethal tumors. In contrast, deep parenchymal transplants into caudate-putamen did not form tumors and differentiated into postmitotic neurons *(49)*, suggesting either the presence of mitotic-inhibitory or differentiation-induction factors in the caudate-putamen. Proliferating NTera-2 cells *(46)* as well as P19 cells *(43)* can be stably transfected, raising the possibility that they could be used to supply lost endogenous neurotransmitters or neurotrophic factors and maintain the potential for regulated release. The degree to which their oncogenic potential has genuinely been abrogated and the extent to which these cell lines faithfully model primary CNS neurons in vivo remain intriguing and active areas of investigation.

Two human cell lines, HCN1 and HCN2, were recently isolated from the cerebral cortex of a patient with unilateral megalencephaly *(50)*. These cells are likely neuroepithelial precursors, expressing both neuronal and non-neuronal properties *(51)*. Treatment with nerve growth factor (NGF), isobutylmethylxantine (IBMX), and dbcAMP resulted in morphological differentiation to a multipolar or bipolar phenotype as well as the expression of neurofilament, GABA, somatostatin, vasoactive intestinal polypeptide, cholecystokinin, and glutamate immunoreactivities *(50)*. These cells have been utilized to examine the control of HIV-1 infection and replication in developing human neurons *(52,53)*. HCN-1 cells survived poorly following transplantation into the adult rat CNS, but that may reflect the lack of immunosuppression in that study *(51)*. Since differentiated HCN-1 cells downregulate class I major histocompatibility antigens *(51)*, similar to rodent CNS neuronal cell lines *(54)*, it may be possible to achieve enhanced survival by transplanting predifferentiated cells *(10)*.

The various cell lines described in this section represent distinct points in the CNS lineage. Defining the effectors and genes that control the progression from a totipotent EC or ES cell to a neurally restricted cell fate may allow novel insight into CNS development and plasticity. Moreover, because they are of human origin, HCN-1 and NTera-2 cells provide a potentially more clinically relevant model of immature and differentiated human CNS neurons, respectively. Whereas the class of neural cell lines discussed in this section will be extremely useful for in vitro studies that dissect mechanisms of differentiation at the cellular and molecular level, their utility for examining brain development and/or repair in vivo remains an open issue. Clearly they survive under certain conditions following transplantation. However, the degree to which they can integrate into host parenchyma and develop reciprocal synaptic connections must be better established.

Neural Cell Lines Derived from Somatic Cell Hybrids of Primary CNS Neurons and Immortal Fusion Partners

It was demonstrated over 30 yr ago that fusion of somatic cells could be mediated by inactivated Sendai virus *(55)* or polyethylene glycol *(56)*. Over the last decade, this approach has been utilized to generate cell lines from rodent CNS. In all of the cell lines published to date, the fusion partner has been the N18TG2 neuroblastoma cell line, which is deficient in the enzyme hypoxanthine phosphoribosyltransferase (HPRT). HPRT converts hypox-

anthine and guanine to their corresponding nucleosides and functions as an alternative pathway for purine synthesis. N18TG2 cells are fused to primary CNS cells, which synthesize HPRT, and viable hybrids selected in HAT (Hypoxanthine, Aminopterin, Thymidine) media. The aminopterin blocks *de novo* purine synthesis and forces successful cell fusions to utilize their HPRT pathway. Neuronal cell lines have been developed from a number of embryonic and postnatal regions of the rodent CNS (Table 1).

In general, these neuronal hybrid cell lines are more mature than those established by the techniques described later. This results from the use of differentiated neurons as the fusion partner. For example, neurotransmitter synthesis appropriate for those neurons has been detected: The spinal cord and septal cell lines are cholinergic and the mesencephalic cell lines are dopaminergic. These cell lines also generate action potentials *(57–59)*, whereas most alternatively generated neuronal cell lines do not. Thus, hybrid neuronal cell lines are potentially excellent models of primary CNS neurons and have been used to examine motoneuron killing in amyotrophic lateral sclerosis (ALS) *(60)*, the regulation of dopamine synthesis *(61,62)* and *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity *(59)* in mesencephalic neurons, and the control of acetylcholine (ACh) synthesis in septal neurons *(63)*. The limitations of these neuronal cell lines result from the use of the N18TG2 cells as the fusion partner. These cell lines show marked chromosomal abnormalities *(59,62, 64,65)*. Moreover, they continuously divide and thus do not attain a stable, differentiated neuronal phenotype. A preliminary report described a somatic cell hybrid that utilized a hippocampal neuroepithelial precursor cell line, conditionally immortalized with the temperature-sensitive mutant of SV40 large T-antigen (ts-T-ag) as the fusion partner *(66)*. This approach may ultimately allow development of cell lines that differentiate with mature neuronal phenotype and whose proliferation can be controlled.

Neural Cell Lines Derived from Transgenic Mice with Neural-Specific Promoters Driving Oncogenes

Although limited to murine studies, the significant advantage of this approach to neural cell-line development is that discrete cellular populations can be specifically targeted for oncogenic transformation. A number of different promoters have been utilized in attempts to drive SV40 large T-antigen (T-ag) expression in specific populations of proliferating CNS neuroepithelial precursors. Although this approach is relatively straightforward in principle, targeted oncogenesis of CNS neurons has not been entirely successful.

Transgenic mice, in which the promoter region for the hypothalamic peptide growth hormone-releasing factor was fused to T-ag, had normal hypothalamic function, but instead suffered from massive thymic hyperplasia *(67)*. Using the regulatory region of the preproglucagon gene to drive T-ag, Efrat et al. *(68)* observed T-ag expression in both pancreatic α cells and neurons of the nucleus tractus solitarius (NTS), but neither phenotypic changes nor tumors were observed and the mice appeared normal. When the phenylethanolamine-N-methyl transferase (PNMT, the enzyme that catalyzes the formation of epinephrine from norepinephrine) promoter was utilized to drive T-ag expression, animals developed both retinal and adrenal tumors *(69)*. A cell line derived from one retinal tumor differentiated with a neuronal phenotype, expressed neuronal-specific proteins, but did not have catecholaminergic properties; neither PNMT mRNA or protein nor TH immunoreactivity were observed *(70)*. These results underscore the fact that complex regulatory mechanisms control gene expression in CNS neurons. Multiple elements, both enhancers and silencers, are essential for appropriate neuronal gene expression *(71)* and the promoter constructs utilized may not have included all the transcriptional control elements essential for specific neuronal expression.

However, a number of neural cell lines have been generated utilizing this approach. The

Table 1
CNS Cell Lines Derived by Somatic Cell Fusion

Tissue source (cell line)	Differentiating conditions	Neuronal properties	Ref.
E14 mouse striatum (SN5, SN6)	10% Serum	NF-M,[a] NSE,[a] ChAT[a] activity	64
P21 mouse striatum (SN41–SN59)	Serum-free	NF-H,[a] ChAT activity	65
E18 mouse hippocampus (HN9.10e)	1% Serum, retinoic acid	NF-M, ChAT activity, action potentials	57
E14 mouse mesencephalon (MN9D)	10% Serum	NF-H, dopamine and norepinephrine synthesis, action potentials	59
E12–14 mouse spinal cord (NSC-34, NSC-15)	10% Serum, 1 m*M* dbcAMP	NF-L, NF-M, NF-H; ChAT activity, action potentials, high affinity choline uptake, synapse with myotubes	58
E16 rat mesencephalon (MES23.5)	2% Serum, N2,[b] 1 m*M* dbcAMP	TH[a] activity, dopamine synthesis, voltage-gated Ca^{2+} channels	62
E15 rat spinal cord (VSC4.1)	2% Serum, N2, 1 m*M* dbcAMP	NF-H, NSE; ChAT activity	60
E18 mouse striatum (X52–X62)	10% Serum	ChAT activity, D_1 or D_2 receptors coupled to adenylate cyclase	163

[a]Abbreviations: NF-L, NF-M, NF-H: low-, medium-, and high-mol-wt neurofilament proteins; NSE: neuron-specific enolase; ChAT: choline acelyltransferase; TH: tyrosine hydroxylase.

[b]N2 serum-free components of Bottenstein and Sato *(164)*.

GT1 cell line was isolated from a hypothalamic tumor of a mouse expressing T-ag under the control of the gonadotropin-releasing hormone (GnRH) promoter. These cells express neuron-specific enolase, the 68-kDa neurofilament protein (NF-L), and synaptic vesicle proteins; they also extend neurites, and release GnRH *(72)*. These cells have proven extremely useful in delineating the regulatory mechanisms that control GnRH release *(73–77)*. In addition, the enhancer that confers tissue-specific expression of GnRH, as well as specific transcription factors involved, have been identified with GT1 cells *(78)*. Moreover, GT1 cells have been utilized in transplantation experiments to induce LH release in the hypogonadal mouse that lacks a functional GnRH gene *(79)*.

Suri et al. *(80)* isolated neural cell lines from the brain and adrenal gland of transgenic mice carrying a construct in which T-ag was fused to the promoter region of the TH gene. These cell lines differentiate neuronally and synthesize both dopamine and norepinephrine. Largent et al. *(81)* utilized the regulatory elements of the olfactory marker protein gene to direct T-ag expression in the olfactory bulb. Neuronally differentiating cell lines were obtained with properties similar to primary olfactory neurons. Both of these cell lines should prove useful to define molecular and biochemical mechanisms that control the differentiated phenotype of the respective CNS neurons.

One other approach has enabled the isolation of neural cell lines from the CNS of transgenic mice. Jat et al. *(82)* developed a transgenic mouse in which ts-T-ag expression is directed by the mouse major histocompatibility complex H-2K^b promoter, which can be induced by interferons. The advantage of this approach is that constitutive expression of the oncogene is controlled both by the need for interferon induction and rodent body temperature, which is nonpermissive for ts-T-ag expression. In principle, cell lines can be generated from any proliferating tissue by incubation at permissive temperature (33°C) in the presence of interferon. Kershaw et al. *(83)* utilized these mice to generate populations of neural cells from embryonic hippocampus that have both neuronal and astrocytic properties. Eves et al. *(84)* isolated cell lines from the hippocampus, septum, and cerebral cortex of an 8-wk-old H-2K^b-tsT mouse. Although these cell lines likely are derived from neural progenitor cells present in the postnatal CNS (*see* the following two sections), they document the usefulness of this transgenic mouse as a means to generate neural cell lines.

Growth Factor-Mediated Expansion of Neural Progenitor Cells

The rationale for "perpetuating" neural progenitors and/or stem cells in culture derived from lineage analyses in vivo that utilized retroviral vectors and tracer dyes to track *in situ* the progeny of individual progenitors in certain structures of the vertebrate CNS (e.g., retina, tectum, spinal cord, cortex, cerebellum). These studies indicated that some progenitors give rise to multiple neural cell types, both neuronal and glial, quite late into development, whereas others become committed much earlier to their cell type fate (reviewed in refs. *1–6*). Important information would be gained from being able to isolate these progenitors in vitro to study mechanisms of differentiation. However, attempts to isolate and propagate pure populations of these various progenitors in cell culture have, until recently, been problematic. Progenitors removed from the brain do not normally remain in a constant proliferative state in vitro. After a limited number of mitoses, if any, they cease dividing and differentiate. To maintain them in a proliferative state requires chronic exposure of progenitors to mitogenic growth factors and/or cytokines or transduction of immortalizing genes into neural progenitors. As this and the following section will discuss, the extent to which "freeze frames" of development have actually been created through various perpetuation techniques has been both fascinating and perplexing.

Chronic cytokine or growth factor exposure, e.g., to epidermal growth factor (EGF), fibro-

blast growth factor 2 (FGF2), and/or platelet-derived growth factor (PDGF), represents an important approach to the perpetuation of at least a subset of neural progenitors (i.e., those with appropriate receptors). These progenitors appear to maintain or reacquire proliferative potential in the presence of these factors and withdraw from the cell cycle on their removal. Multipotent precursors derived with FGF2 have been characterized for the E13 rat *(57)* and E17 mouse *(85)* cerebral cortex, E14 rat striatum *(86)*, E14–16 rat spinal cord *(87,88)*, E10 mouse mesencephalon and telencephalon *(89)*, E16 rat hippocampus *(90,91)*, and E15 olfactory epithelium *(92)*. Reynolds et al. *(93)* found similar precursors from E14 striatum but these were induced to proliferate by epidermal growth factor (EGF). Another population of stem cells from E14 rat cerebral cortex was induced to proliferate by media conditioned by astrocytes and meningeal cells *(94)*. All of these precursors underwent at least one round of cell division and subsequently exited the cell cycle, either constitutively or after withdrawal of the mitogenic signal, and had the potential to differentiate into neurons or glia.

Importantly, these mitotic embryonic neural precursor cells can be serially passaged *(88, 90,93)*. E14 striatal precursors can be expanded by culturing at very low cell density in serum-free medium containing EGF *(93)*. These proliferating neural precursors grew as clusters of cells floating in the media or loosely attached to the substratum and have been called "neurospheres." In dissociated cell cultures from E18 rat hippocampus *(90)* and E15 rat spinal cord *(88)* grown at low density on polyornithine-laminin coated dishes, FGF2 was the most potent mitogen, and the cells were passaged by selective mild trypsinization. In contrast to EGF-generated neurospheres, the number of neuronal differentiating cells was >50%. Immunostaining was observed with NF-H, which recognizes more mature neurons, as well as for choline acetyltransferase (ChAT) in the spinal cord cells. Thus, it appears that EGF-driven stem cells represent different precursor populations than the FGF2-derived cell populations. Kilpatrick and Bartlett *(85)* found that neural precursors propagated from the E17 mouse cerebral cortex with FGF2 generated both a neuronal-astrocytic precursor and a glial-restricted precursor, whereas those similarly derived with EGF generated only a glial-restricted precursor. The differences between these latter results and those cited previously may also reflect differences in culture conditions and perhaps the region of derivation.

Mitotically active precursor populations can be generated from the adult CNS using similar cytokines. Reynolds and Weiss *(95)* cultured dissociated adult mouse striatum at low density on uncoated tissue culture plates in serum-free medium containing EGF. Spheres of proliferating precursor cells, immunopositive for the neural stem cell intermediate filament nestin *(96)*, grew under these conditions. These neurospheres could also be passaged multiple times following dissociation into single cells and, when plated on adherent substrates, differentiated into neurons and astrocytes. However, the percentage of cells that differentiated with neuronal or astrocytic phenotype was very low, consistent with results seen with EGF-driven neurospheres derived from embryonic striatum *(93)*. Richards et al. *(97)* generated pluripotent precursors from adult mouse brain by initially plating cells for 1 wk at low density in medium containing 10% fetal calf serum (FCS) and FGF2. The cultures were then passaged with trypsin and grown in serum-free medium in coculture with an immortalized astrocyte cell line Ast-1.

One emerging theme is that multiple trophic factors or cell surface signals in a precise temporal cascade are necessary for optimal neural progenitor cell proliferation and differentiation. E10 mesencephalic neural precursors were grown initially in 10% FCS followed by subsequent expansion in FGF2 and Ast-1-conditioned medium *(98)*. E10 cortical progenitors required either media conditioned by astrocytes and meningeal cells or membrane fragments from these cells for continued proliferation *(94,99)*. Neuronal proliferation of E10

mesencephalic precursors appears to involve an interaction between exogenous FGF2 and intrinsic insulin-like growth factor I (IGF-I), perhaps released in an autocrine loop *(100)*, as well as laminin synthesized by concurrently differentiating astrocytes *(101)*. Cattaneo and McKay *(86)* also demonstrated multiple growth factor interactions on CNS neuroepithelial precursors, since NGF only induced proliferation in striatal precursors that had initially been treated with FGF2. FGF2 treatment of EGF-generated neurospheres from E14 mouse striatum induced the proliferation of a bipotential neuron/astrocyte precursor as well as a neuronally restricted progenitor *(102)*. Brain derived neurotrophic factor (BDNF) treatment of these EGF-generated neurospheres resulted in enhanced neuritic outgrowth and neuronal differentiation *(103)*. FGF2 also has a differential effect on two populations of E16 rat hippocampal neural precursors, inducing one population of nestin-positive cells to proliferate and a second to differentiate into calbindin-positive granule neurons *(91)*. Neural precursor cells derived from E13–14 mouse cerebral cortex proliferate in response to FGF2, whereas the addition of NT-3, induces neuronal differentiation *(104)*.

With respect to the degree of differentiation of these various cytokine- and growth factor-responsive neuroepithelial precursors, both ChAT-positive *(87)* and GABA-positive *(88)* spinal neurons were identified following chronic FGF2 exposure, and the EGF-driven striatal neurons demonstrated GABA, substance P, and met-enkephalin immunoreactivity, but not other striatal neurotransmitters *(93)*. The lack of certain classes of neurons likely reflects the absence of the appropriate differentiative signal(s).

Although both EGF and FGF2 have the potential to generate large clonal populations of CNS neural progenitor or stem cells, it has proven very difficult to establish culture conditions that enable isolation of pure populations of specific cell phenotypes, although neuroblast proliferation may be preferential with FGF2 *(88,90)*. Without exception, expanded cell populations from both neonate and adult CNS generated neural precursors with multiple phenotypic potentials. As the temporal combinations of extracellular effectors that control specific lineage choices are better delineated, it may be possible to generate pure populations of defined classes of neuronal and nonneuronal cells. Genes have recently been cloned that control neural fate, such as *Mash-1 (105)* and NeuroD *(106)*, which are involved in neurogenesis and terminal neuronal differentiation, respectively. It is likely that more will be identified in the future. Stable transfection and expanded populations of expression of such genes in neural progenitors *(88)* may provide an alternative approach to developing defined neural cell populations.

Data are beginning to emerge on how these cytokine and/or growth factor expanded neural progenitors behave in vivo following transplantation. They can be engrafted back into structures of origin *(107)* as well as into a few heterotopic regions in the adult rat CNS *(108)*. Interestingly, the potential of at least some cell populations appears to be broader than initially suspected from in vitro studies alone. For example, FGF2-expanded precursors from adult hippocampus express immature glial and neuronal antigens in culture, but following transplantation, both neurons and glia develop, expressing the mature markers neuN and GFAP, respectively *(108)*. The ability to transplant progenitors such as these raises the question of whether such cells might have therapeutic potential. It is still uncertain whether cell populations that have been successfully expanded and passaged have been enriched for a subset of cells with upregulated EGF and/or FGF receptors. Neural cells with oncogenic potential have been defined by aberrations in the EGF receptor *(109,110)* and FGF2 can transform cells under certain conditions *(111)*, raising the specter of possible tumorigenicity in vivo. Nonetheless, recent data are encouraging and it is expected that rapid progress will be made in this area in the immediate future.

Neural Cell Lines Derived by Retroviral Transduction of Neuroepithelial Precursors

Retroviral transduction has been widely utilized as a means to develop neural cell lines from the CNS (Table 2). Excellent reviews detailing the methodologies to generate and characterize these cell lines have recently appeared *(9,10,112,113)*. What is initially apparent from Table 2 is that multiple, single oncogenes, both constitutively expressed (T-ag, neu, myc) and conditionally expressed (the temperature-sensitive mutant of Rous sarcoma virus [ts-RSV], ts-T-ag), have been utilized successfully to generate these neural cell lines. It is important to distinguish among "immortalization" of a cell, defined here as the ability to proliferate beyond crisis and avoid senescence (roughly 50 passages in a human fibroblast), "transformation," which refers to morphological alterations in growth patterns and the loss of anchorage-dependent growth, and "tumorigenic," defined as the capacity of the cells to form invasive tumors in nude mice. Cell lines can be immortalized and not transformed or tumorigenic, as well as transformed but not immortalized or tumorigenic. However, once tumorigenic, cells are usually both immortal and transformed, although metastatic human tumors that are not immortal in vitro have been reported.

Neural Cell Line Differentiation In Vitro

Stable integration of a retrovirus into the genome of neuroepithelial precursors requires that mitotically active cells be infected *(114)*. The physiological consequences of this are that, when utilizing fetal or neonatal dissociated CNS as a tissue source, neuroepithelial precursors from multiple stages of differentiation will be infected. Consistent with this assertion, many investigators isolated numerous clones that variably expressed distinct cell type-specific markers and antigens reflecting various maturation stages of neural cells *(115–125)*. Most importantly, the majority of the neural cell lines detailed in Table 2 are pluripotent, retaining the ability to differentiate along either a neuronal or nonneuronal lineage. Moreover, multiple pluripotent phenotypes have been obtained. Although the most frequent bipotential lineage observed was a neuronal-astrocytic fate, neuronal-oligodendrocytic, neuronal-Schwann cell, and even tripotential neuronal-astrocytic-oligodendrocytic precursors have been described. These diverse phenotypes are not artifacts of immortalization or cell culture, as similar pluripotent precursors have been described in vivo *(1–6,126–129)*. Whereas some of these neural cell lines constitutively differentiated in serum-containing media, the majority required reduced or serum-free conditions and/or the presence of additional effectors to initiate differentiation. Mehler et al. *(125)* described a temporal hierarchy of growth factor and cytokine regulation of immortalized neuroepithelial precursor cell lines from the E17 mouse hippocampus. Maximal neuronal differentiation was seen with initial culture in FGF2 followed by cotreatment with TGFα and IL-5, -7, or -9. These data suggest that the terminal phenotype(s) of immortalized neuroepithelial precursor cell lines can reflect both intrinsic developmental programs and determination by exogenous effectors. That some of the neural cell lines are restricted to neuronal differentiation in vitro likely reflects their immortalization at a later stage in their development when they had already committed to a neuronal lineage. However, optimal differentiation of these neuronal cell lines also required a combination of genetic determination and exogenous factors. Although the relative contribution of each of these mechanisms to differentiation must be empirically determined for each neural cell line, these cell lines should prove to be useful models with which to delineate epigenetic signals that modulate lineage decisions.

One example of the interaction of intrinsic and extrinsic differentiation signals can be seen with RN46A cells, derived with ts-T-ag from E13 rat medullary raphe nucleus. RN46A cells constitutively differentiate along the neuronal lineage, expressing neuron-specific enolase

Table 2
CNS Cell Lines Derived by Retroviral Transduction

Tissue source (cell line)	Immortalizing protein	Cell phenotype in vitro	Differentiation conditions	Ref.
E14 mouse hypothalamus (Ht9-C7)	T-ag[a]	Primitive neurosecretory cells (neurophysin)[b]	17.5% Serum	*122*
E7 quail neuroretina (QNR/D)	ts-RSV[c]	Amacrine/ganglion neurons (GABA)[b]	5% Serum	*121*
P2 rat cerebellum (ST15E, M15B)	ts-T-ag[d]	Bipotential: neuron/astrocyte	Serum-free	*123*
	neu	Bipotential: neuron/astrocyte	Serum-free + dbcAMP/retinoic acid	
E10 mouse mesencephalon (2.3D, Nzen5–NZen37)	c-myc/N-myc	Undifferentiated neuroepithelial precursors	10% Serum	*115*
		Bipotential: neuron/astrocyte	10% Serum/FGF2	*117*
E9 mouse neural crest (NC4.10.1C–NC15.8.S1)	c-myc/N-myc	Neurons, Schwann cells	10% Serum	*116*
		Bipotential: neuron/Schwann cell		
P1 mouse olfactory bulb (OLB13–OLB24)	Avian myc	Bipotential: neuron/ oligodendrocyte	10% Serum	*118*
		Bipotential: neuron/astrocyte		
P4 mouse cerebellum (C17-2, C27-3)	Avian myc	Biopotential: neuron/ oligodendrocyte	10% Serum	*118*
		Tripotential:neuron/ oligodendrocyte/astrocyte		
P1 mouse striatum (Str. SVLT.3.8)	T-ag	Astrocytes	10% Serum	*124*
		Bipotential: neuron/astrocyte	Serum-free	
E16 rat hippocarnpus[e] (HiB5)	ts-T-ag	Undifferentiated neuroepithelial precursors	10% Serum/FGF2	*142*
E17 rat hippocampus[e] (H19-7, WH19-4)	ts-T-ag	Neurons	1% Serum/phorbol ester	*119*
		Bipotential: neuron/astrocyte	1% Serum/phorbol ester	
E13 rat medullary raphe nucleus (RN33B)	ts-T-ag	Neurons (glutamate)[b]	Serum-free	*137*
E14 rat striatum (M213-20, M26-1F)	ts-T-ag	Neurons (GABA)[b]	10% Serum/NGF/ dbcAMP/IBMX	*120*
E7 mouse hippocampus (MK31)	ts-T-ag	30% Neurons	Serum-free/FGF2 d 1, then TGFα, IL9	*125*
E18 rat hippocampus[f] (H19-7, WH19-4)	ts-T-ag	Neurons	1% Serum + glial feeder layer	*84*
E13 rat medullary raphe nucleus (RN46A)	ts-T-ag	Neurons (serotonin)[b]	Serum-free	*130*
P5 mouse cerebellar external germinal layer cells (GC-B6)	ts-T-ag	Bipotential: neuron/astrocyte	Serum-free coculture with primary EGL cells	*138*

[a]SV40 large T antigen.
[b]If known, neurotransmitter synthesized is indicated in parentheses.
[c]Temperature-sensitive mutant of Rous sarcoma virus.
[d]Temperature-sensitive mutant of SV40 large T antigen.
[e]Cells were grown in culture for 24 h before infection.
[f]Cells were infected on d 1 and grown at 37°C for 12 d in the presence of cytosine arabinoside before switching to permissive growth conditions.

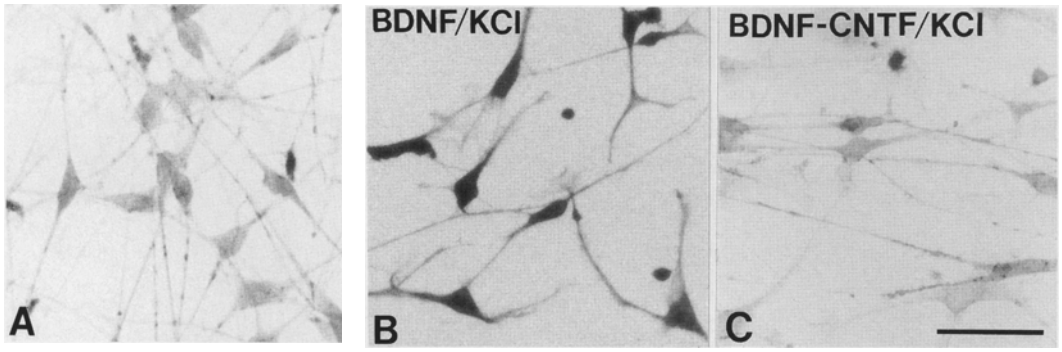


Fig. 1. Interaction of BDNF and CNTF in mediating the expression of 5-HT in RN46A cells. RN46A cells were differentiated in the presence of **(A)** no effectors, **(B)** 20 ng/mL BDNF for d 0–8 with 40 m*M* KCl added for d 5–8 (BDNF/KCl), or **(C)** 20 ng/mL BDNF and 40 ng/mL CNTF for d 0–8 with 40 m*M* KCl added for d 5–8 (BDNF-CNTF/KCl) and immunohistochemically analyzed for the expression of serotonin (5-HT). Note the marked increase in 5-HT synthesis induced by BDNF/KCl that was completely blocked by cotreatment with CNTF. Magnification bar = 50 μm.

and neurofilament proteins, but not glial-specific proteins *(130)*. These cells synthesize low levels of tryptophan hydroxylase (TPH, the rate-limiting enzyme in serotonin synthesis) but serotonin is not detected (Fig. 1A). Treatment with BDNF markedly increases TPH levels but serotonin is still not synthesized. It is only when BDNF is combined with partial membrane depolarization that serotonin synthesis is initiated (Fig. 1B). In parallel with this initial finding, it was subsequently demonstrated that BDNF and depolarization similarly regulate serotonin synthesis in primary raphe neurons *(131)*. Concurrent treatment with ciliary neurotrophic factor (CNTF) blocks the induction of serotonin synthesis in RN46A cells (Fig. 1C), an effect also seen in primary serotonergic neurons *(165)*. Thus, novel regulatory mechanisms initially defined in an immortalized raphe neuron cell line have been shown to function in primary neurons as well. TPH synthesis and 5HT uptake in RN46A cells are also increased by adrenocorticotropic hormone (ACTH) *(132)*, similar to that observed with primary raphe neurons *(133,134)*. FGF2 and EGF interact to affect the proliferation of C17-2 cells *(135)*, an immortalized multipotential cerebellar precursor cell line *(118)*, consistent with the overlapping effects of these factors on cerebral cortical neural precursors *(136)*. It is certain that other retrovirally derived neural cell lines will provide similar new insight into CNS cellular function.

Two recent observations have brought into question the issue of neuronal differentiation as a terminal phenotypic commitment. Eves et al. *(84)* infected dissociated E17 rat hippocampus with a retrovirus encoding ts-T-ag. The cultures were then grown for 12 d at nonpermissive temperature and pyramidal neurons selected in the presence of a mitotic inhibitor. Following a subsequent shift to a temperature permissive for ts-T-ag-driven cell proliferation, neuronally-restricted cell lines were detected. These authors concluded that postmitotic neurons can re-enter the cell cycle. Other investigators could not induce a ts-T-ag-derived neuronal cell line to reinitiate cell division following differentiation *(137)*, although this discrepancy may reflect differences in culture conditions. Gao and Hatten *(138)* observed that immortalization of enriched cerebellar granule neuron precursors, which were restricted to granule neuron differentiation in vitro, resulted in pluripotent neural cell lines, expressing both neuronal and astrocytic properties. These authors concluded that retroviral transduction could subvert the normal neuronal differentiation program inherent in granule neuron precursors. These data suggest that the expression of

a relatively mature neuronal phenotype, even in a mitotically quiescent cell, may not reflect a terminally differentiated cell fate.

The genetic homogeneity and ready abundance of immortalized neural progenitors, as well as the fact that they share many in vitro properties with primary stem cells and progenitors, make them attractive models for multiple neurobiologic inquiries. Are these neural cell lines truly representative of progenitors in the CNS? The extent to which such lines are representative of progenitor cells in vivo remains to be empirically determined.

Neural Cell Line Differentiation In Vivo

Why transplant neural cell lines? Both primary culture and immortalized CNS cell lines suffer from the same inherent limitation: removal of a cell from its in vivo context. The most compelling evidence for the physiological relevance of neural progenitor cell lines would be their incorporation into the normal cytoarchitecture of the appropriate brain region at the appropriate host age in a functionally meaningful manner. Transplantation of clonal progenitors may also help to trace in vivo the lineage relationships of neural cell types. Finally, as graft material for therapeutic purposes, clonal neural cell lines may have many advantages over primary fetal neural tissue as well as nonneural cells *(139–141)*. If progenitor cell lines can integrate normally within a host brain and express a foreign gene within CNS parenchyma for prolonged periods, then using such cell lines as a transduction agent for exogenous, therapeutic gene products and/or to become an integral member of the CNS cytoarchitecture to replace dysfunctional neural cell types may be feasible for clinical applications.

Some of the retrovirally derived CNS cell lines show maximal differentiation following transplantation back into the neonatal *(138, 142–144a,145)* or adult *(144a,145,146)* CNS. Moreover, progenitor clones that were multipotent in vitro often recapitulated that multipotency in vivo. The HiB5 cell line, derived from E17 rat hippocampus, has properties of undifferentiated neuroepithelial precursors in vitro. After transplantation into the neonatal hippocampus and cerebellum, HiB5 cells integrated into host parenchyma and assumed differentiated morphologies consistent with those of endogenous hippocampal dentate gyrus granule neurons and cerebellar granule neurons, respectively *(142)*. In addition, HiB5 cells in the cerebellum adopted morphologies identical to Bergman glia. Thus, these transplanted neuroepithelial precursor cells showed markedly enhanced differentiation in vivo relative to their phenotype in vitro. Snyder et al. *(143)* similarly showed that C17-2 cells differentiated into granule and basket cell neurons, astrocytes, and oligodendrocytes following transplantation into the neonatal cerebellum. In both of these studies, the neuron cell types into which the transplanted multipotent cells differentiated were those normally undergoing neurogenesis at the time and region of transplantation. However, using an identical paradigm, Gao and Hatten *(138)* described differentiation of transplanted GC-B6 cells, derived from enriched P5 cerebellar granule neuron precursors, into multiple neuronal phenotypes, both mitotically active and postmitotic, as well as multiple glial morphologies. An understanding of the mechanisms responsible for these differences in differentiative potential will be exceedingly instructive for understanding the various steps in the fate restriction of neural progenitors throughout development. Following transplantation of C17-2 cells into various CNS germinal zones at various developmental timepoints (from embryo to adult), these postnatally-derived immortalized cerebellar progenitors engrafted extensively and participated in the development at multiple stages of multiple regions along the neuraxis *(145)*. Although they gave rise to multiple cell types in these various regions, they differentiated only into the types of neurons and glia expected for the respective region at the particular developmental stage of the transplant. For instance, C17-2 cells differentiated into neocortical pyramidal neurons when engrafted into the ventricular zone of a midembryonic mouse brain during normal corticogenesis, but

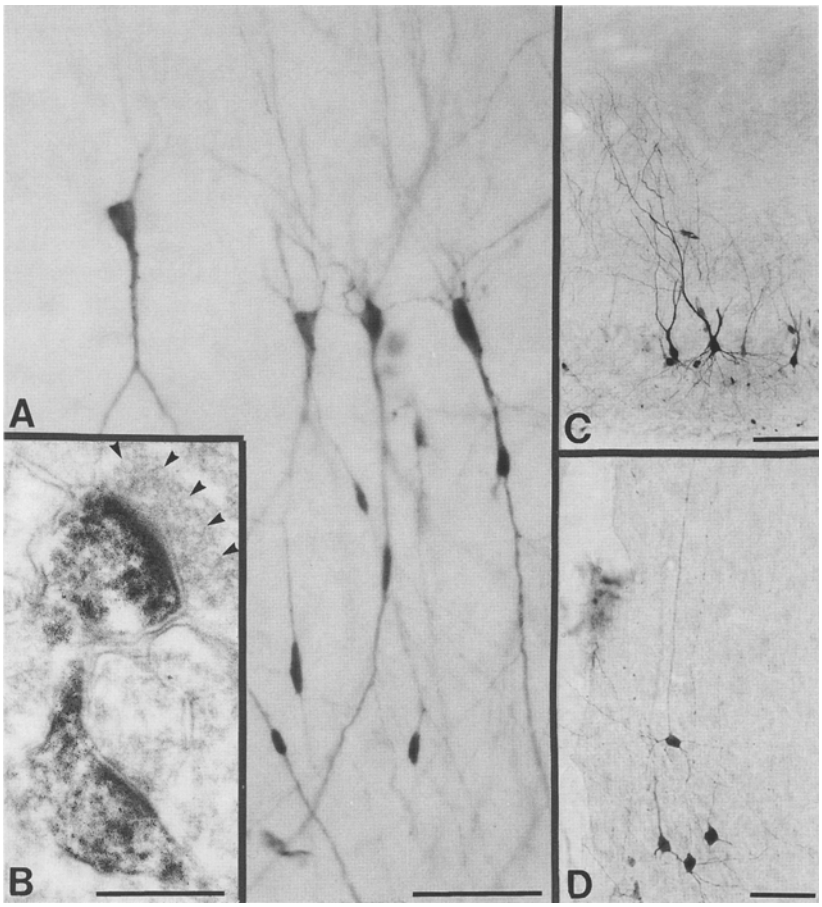


Fig. 2. Differentiation of RN33B cells in the neonate and adult CNS. Photomicrographs of β-galactosidase (β-gal)-labeled RN33B cells 8 wk after transplantation into the **(A,B)** neonatal or **(C,D)** adult CNS; cells are detected immunohistochemically with an antibody against β-gal. (A) RN33B cells located in the CA1 pyramidal cell layer of the hippocampus. The morphological differentiation of RN33B cells is identical to endogenous CA1 pyramidal neurons. (B) Electron micrograph of the apical dendrites of the cells in (A) showing that labeled dendritic spines of RN33B cells receive synaptic contacts from host axons. Note the presence of synaptic vesicles on the presynaptic side of the synapse (arrowheads). RN33B cells located in (C) the CA3 pyramidal cell layer and (D) layer V of the cerebral cortex. These cells are morphologically identical to endogenous CA3 and cortical pyramidal neurons, respectively. Magnification bars = 50 μm in (A,D), 1 μm in (B), and 100 μm in (C).

did not undergo neuronal differentiation in the neocortex at a later stage when neurogenesis has normally ceased and gliogenesis predominates. In regions where gliogenesis is the predominant developmental process, C17-2 cells differentiated with glial phenotypes.

In contrast, following transplantation of the raphe-derived and neuronally restricted RN33B cell line *(137)* into neonatal hippocampus and cerebral cortex, Shihabuddin et al. *(144a)* observed differentiated RN33B cells identical to endogenous hippocampal CA1 (Fig. 2A) and CA3 pyramidal neurons and dentate gyrus granule neurons and cortical pyramidal and stellate neurons, respectively; cell types that were both mitotically active and postmitotic. Moreover, these cells made synaptic connections with host axons (Fig. 2B) and were induced to express mature pyramidal cell proteins not expressed in vitro *(144b)*. Nonneuronal differentiation

was not observed. These differences in the lineage restriction of the various immortalized neural cell lines following transplantation into the neonatal CNS are likely reflective of the differentiation potential of the individual cell lines. Determination of the point at which a stem-like cell line and a neuronally restricted cell line have diverged will require collaborative investigations using these distinct cell lines in similar experimental paradigms.

Collectively, these results demonstrate that neural cell lines have substantial plasticity and differentiate in vivo according to local microenvironmental signals, rather than follow a predetermined developmental fate. Moreover, the extent of differentiation is maximal in vivo where substrate cues, endogenous trophic factors, cell topography, and afferent input provide an optimal environment to direct that precise differentiation. Importantly, the immortalization process did not subvert the ability of these progenitors to respond to normal cues and withdraw from the cell cycle, differentiate, and interact with host cells. The fact that transplanted neural precursor cell lines, even those derived with constitutive immortalizing proteins, never generated tumors in vivo suggests that the CNS represents a restrictive environment for proliferation of these specific cell lines.

One crucial issue is whether the adult CNS can similarly support specific differentiation of transplanted neural precursor cell lines. RN33B cells transplanted into the adult rat hippocampus, cerebral cortex, or spinal cord differentiate with neuronal morphologies identical to those of endogenous neurons in those regions *(144a,146)* (Fig. 2C,D). These transplants survived up to 6 mo postgrafting, the longest time-point examined. However, not all neural cell lines similarly engraft. In contrast to RN33B cells, transplants of the identically derived RN46A cell line *(130)* were localized predominantly in the white matter, underwent some proliferation in vivo, did not exhibit extensive morphological differentiation, and survived only up to 6 wk *(9)*. Moreover, it appears that other progenitor cell lines respond differently, even in the adult CNS, if certain novel signals are provided or the environment otherwise perturbed. Targeted photolytic cell death is a technique for experimentally eliminating a particular subpopulation of neurons through apoptotic degeneration in the adult CNS *(147,148)*. C17-2 cells were transplanted into the neocortex of either intact adult mice or adult mice rendered selectively deficient of layer II/III pyramidal neurons. In the intact adult neocortex, donor progenitors differentiated exclusively into glia. However, in adult recipients with targeted neuronal apoptosis, C17-2 cells within regions of neuronal degeneration differentiated into pyramidal neurons, extending axons and dendrites, and establishing afferent synaptic contacts, as if replacing the degenerated neuronal population *(149)*. Apoptosis had presumably reactivated developmental signals in the adult, normally expressed only during fetal corticogenesis, to which the C17-2 cells now responded.

It is clear, therefore, that the adult CNS retains the potential to direct specific integration and differentiation of certain transplanted neuroepithelial precursor cell lines, either constitutively or following certain injuries. Ultimately, appropriately derived immortalized neural cell lines may prove therapeutically useful as a means to replace endogenous CNS neurons lost as a result of trauma or neurodegenerative disease.

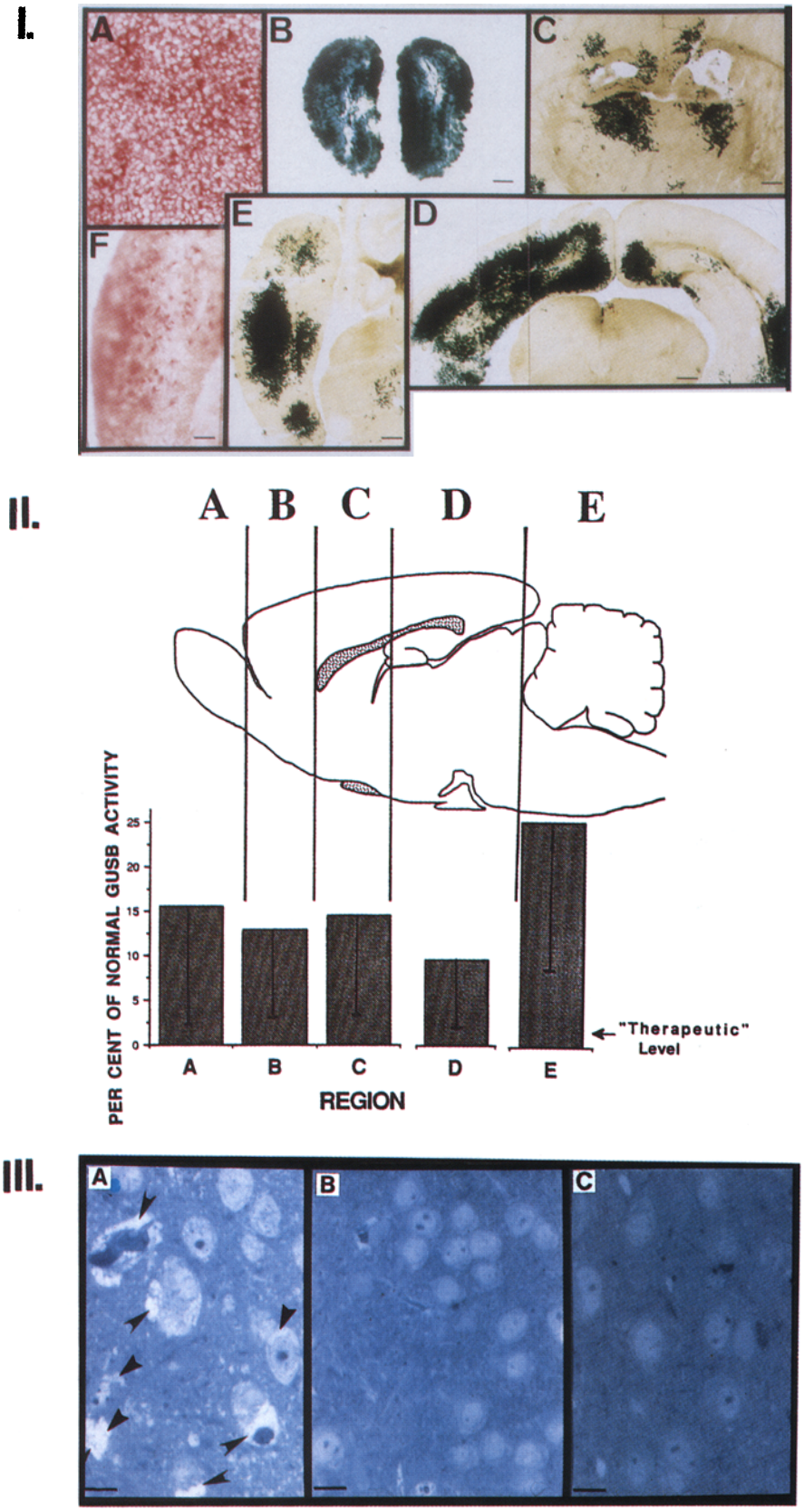
Neural Cell Lines for Gene Therapy

Neuronal replacement is not the only potential therapeutic utility of neural cell lines. Many gene transfer methods are under study for the brain, including transplantation of genetically engineered nonneuronal cells *(140,150)* and direct injection of recombinant, replication-deficient herpes simplex virus 1 *(151,152)* or adenovirus *(153)* vectors. Exploiting the properties of neural progenitors and stem-like cells to become integral members of normal structures throughout the host CNS might allow foreign gene products, including some with therapeutic potential, to be delivered in a sustained, direct, and perhaps regulated fashion. As these cell lines are dividing in vitro, they can be easily infected by retroviruses or trans-

fected by other means to stably express exogenous genes. Anton et al. *(154)* transplanted a conditionally immortalized rat nigral cell line transfected with a TH expression vector into the caudate-putamen of hemiparkisonian rats and monkeys and observed an amelioration of behavioral deficits. Snyder et al. *(155)* tested the feasibility of using neural progenitors to treat the widespread CNS neuropathology of the murine lysosomal disease mucopolysaccharidosis type VII (MPS VII), an inherited neurodegenerative disease attributable to the absence of β-glucuronidase (GUSB). C17-2 cells, stably expressing GUSB, were implanted into the cerebral ventricles of MPS VII newborn mice and the transplanted cells corrected lysosomal storage throughout the CNS (Fig. 3). A similar study is ongoing utilizing C17-2 cells overexpressing the α-subunit of β-hexosaminidase A (HexA) *(156)* in a mouse model of Tay-Sachs disease. HiB5 cells have been genetically engineered to secrete NGF, and transplantation of these cells into the basal forebrain of adult rats with fimbria-fornix lesions or cognitively impaired aged rats can prevent cholinergic neuronal atrophy *(157)* and reverse deficits in spatial learning *(158)*, respectively.

There are multiple advantages of utilizing neural cell lines as a means to deliver exogenous genes to the CNS. The cells are endogenous to the CNS, will integrate into the host parenchyma with regional specificity, and show widespread dispersion. Whereas neural transplantation has traditionally been viewed as a therapy for localized defects, the use of progenitor cell lines may now enable transplantation strategies for global and multifocal neuropathologies *(159)*. Furthermore, given neuronal differentiation, there is the potential for the formation of appropriate circuitry and gene product release regulated by afferent input. This is of importance if a deficient neurotransmitter is being expressed. Lastly, the immunological properties of neuronal CNS cell lines are such that differentiation results in down-regulation of cell surface molecules necessary for recognition by cytotoxic T lymphocytes *(51,54)*. Thus, neuronal cell lines can be utilized as allogeneic grafts with limited concern for potential immunological rejection.

Fig. 3. *(opposite page)* Widespread engraftment of neural progenitors expressing β-glucuronidase (GUSB) throughout the brain of the mucopolysaccaridosis type VII (MPS VII) GUSB-deficient mouse. **(I)** Brain of a mature MPS mouse after receiving a neonatal intracerebral ventricle transplant of C17-2 neural progenitor cells expressing human GUSB. **(A)** GUSB staining (red reaction product) of C17-2 cells in culture before transplantation (expressing ≈ 800 U of GUSB activity/mg protein in vitro). **(B–E)** Identification in vivo of donor-derived C17-2 cells by X-gal histochemical reaction (blue precipitate) for expression of the *lacZ* marker gene. The blue cells have engrafted throughout the recipient mutant brain. Representative regions are shown proceeding clockwise from rostral to caudal: (B) olfactory bulbs, (C) telencephalon at the level of the striatum, (D) telencephalon at the level of the caudal aspect of the hippocampus, (E) posterior telencephalon and midbrain. **(F)** Expression of GUSB (red cells) in a sister section to (E) showing that GUSB (red) cells colocalized precisely with the distribution of the donor X-gal-positive (blue) cells in (E). Untreated MPS VII mice show no GUSB staining. Scale bars: 400 μm in (B–E) and 320 μm in (F). **(II)** Distribution of GUSB enzymatic activity throughout brains of mature MPS VII recipients of GUSB C17-2 transplants as newborns. Serial sections were collected throughout the brains of MPS VII transplant recipients and GUSB activity assayed. Serial coronal slices were pooled to reflect the activity present within the regions demarcated in the figure. The regions were defined by anatomical landmarks in the anterior to posterior plane (*see* ref. *155*) to permit comparisons among animals. The mean levels of GUSB enzyme activity for each region ($N = 17$) are presented as the average normal levels for each region. GUSB activity is not detected in untreated MPS VII mice. Enzyme activity ≥2–5% of normal is corrective based on data from liver and spleen. **(III)** Decreased lysosomal storage in treated MPS VII mouse brain at 8 mo of age. **(A)** Extensive vacuolation representing distended lysosomes (arrowheads) in both neurons and glia in the cerebral cortex of an 8-mo-old, untransplanted MPS VII mouse. **(B,C)** Decrease in lysosomal storage in the neocortex of an MPS VII mouse treated at birth from a region analogous to the untreated control section in (A). Other regions of this animal's brain showed a similar decrease in lysosomal storage compared to untreated, age-matched controls. Scale bars: 21 μm in (A,C) and 31 μm in (B). (Figure adapted with permission from ref. *155*).


I.
A
B
C
D
E
F
II.
A B C D E
PER CENT OF NORMAL GUSB ACTIVITY
0
5
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A
B
C
D
E
REGION
"Therapeutic" Level
III.
A
B
C

Fig. 3.

Whether immortalized neural progenitors are representative of the majority of mammalian CNS progenitors, or merely an unusual subtype, is uncertain. This uncertainty, in fact, represents one of the limitations in the use of progenitor lines (immortalized by any means) for addressing questions of normal development. Gao and Hatten *(138)* voice caution in assuming that immortalized progenitors accurately reflect the behavior of endogenous progenitors in vivo. Whereas immortalized progenitors derived from the external granular layer (EGL) of the neonatal cerebellum could, on transplantation, give rise to a wide range of interneurons and glia in the neonatal cerebellum, cultures of primary EGL cells, similarly implanted, yielded only granule cells. Immortalization, therefore, although clearly not promoting abnormal behavior by progenitors, may artificially suspend their commitment and differentiation. In forestalling phenotypic restriction, immortalization may maintain some progenitors in their normal antecedent stem cell-like state; self-renewing and multipotent. Another concern is that immortalization, especially with SV40 large T antigen, leads to marked chromosomal abnormalities *(11,160–162)*, and may affect specific genes of interest. Future challenges in the transplantation of neural progenitor cell lines into the CNS include the following: to understand better the basic biology of the cells that have been perpetuated, to determine the parameters that optimize engraftment, to discern the triggers that direct the phenotypic fate of neural progenitors in the brain, and to identify the mechanisms that dictate efficiency of foreign gene expression by engrafted neural cells. These goals all will require a better understanding of fundamental neural progenitor cell biology.

Summary

It is clear from the above discussion that CNS-derived neural cell lines have proven to be extremely useful to begin to understand the plasticity of the developing CNS. As additional extracellular effectors and genes involved in cell fate decisions are identified, pluripotent stem cell populations and precursor cell lines will provide model systems to examine the cellular and molecular mechanisms underlying lineage decisions and cell differentiation. CNS cell lines derived by targeted oncogenesis and somatic cell hybridization have enabled the establishment of clonal cell lines of defined phenotype. These cell lines, and those similarly derived in the future, should continue to facilitate studies on the controls of neurotransmitter synthesis and release, synaptic formation and maturation, neurotoxicity and intracellular signaling mechanisms to name but a few applications. It can be argued that the characterization of neural cell line function in clonal cell culture does not accurately reflect similar phenomena in vivo. However, the ability of many CNS-derived neural cell lines to survive, integrate, and differentiate following transplantation into the neonate and adult CNS has enabled direct validation of these in vitro observations. These studies have revealed not only an even greater pluripotentiality of the respective cell lines than that observed in vitro but also the retention in the adult CNS of the epigenetic cues necessary to direct that differentiation. These latter results lend credence to the ultimate potential therapeutic utility of human CNS cell lines in the treatment of trauma or neurodegenerative disease. As cellular and molecular neurobiology develops a more detailed knowledge of CNS function, CNS-derived neural cell lines should prove increasingly important model systems with which to examine those phenomena, both in vitro and in vivo.

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