NEUROGENESIS IN DISEASES OF AGING

Injury of several types can stimulate neurogenesis, or the birth of new neurons, in the adult brain. A major outstanding issue regarding the potential clinical importance of this phenomenon is whether injury-induced neurogenesis can generate functional neurons and contribute to enhanced recovery.

Acute and chronic neurodegenerative diseases are common, disabling, and poorly responsive to current treatment. Stroke, the most frequent cause of acute neurodegeneration, has a prevalence of ~4.8 million and an incidence of ~700,000 individuals per year in the United States, where it is the third leading cause of death {1}. Even among those who survive stroke, disability due to hemiparesis, gait disorders, aphasia and other deficits is common, and ~20% of these patients require institutional care at 6 months post-stroke. This long-term disability contributes to the average lifetime cost for stroke care of ~$140,000 and an annual national cost of ~$54 billion. The most recent major advance in treatment, the use of thrombolytic agents to dissolve clots in the acute aftermath of stroke, has had limited impact because it appears to be effective only within about the first 3 hours after onset of symptoms {2}.

Chronic neurodegenerations include Alzheimer's disease (AD), Parkinson's disease (PD), and hereditary polyglutamine disorders like Huntington's disease (HD). These diseases affect different, but overlapping, regions of the central nervous system and vary in prevalence, from ~4.5 million cases in AD, to ~1.5 million cases in PD, and ~30,000 cases in HD, in the United States alone. However, all typically culminate in an extended period of functional disability preceding death. Except for PD, in which drugs and surgery are available to reduce symptoms at least temporarily, even symptomatic treatment for chronic neurodegenerations is extremely limited at present. In AD, acetylcholinesterase inhibitors and the NMDA-type glutamate receptor antagonist memantine exert modest behavioral effects in some patients {3}. Perhaps most notably, no treatment exists for any of these diseases that can restore lost function.

Clinical manifestations of acute and chronic neurodegenerative diseases result primarily from irreversible cellular (especially neuronal) dysfunction and, eventually, cell death. One reason for the limited responsiveness of neurodegenerative diseases to treatment may be that it is more difficult to overcome loss of cells than impairment of selected cellular functions. As an example, among neurological disorders, the greatest therapeutic successes have come in conditions where cell loss is not a major feature, such as epilepsy and migraine. Even in PD, where cell loss is relatively circumscribed, pharmacological restoration of a key cellular function like dopaminergic neurotransmission, without the temporal, spatial and stimulus-coupled regulation that a cellular context provides, has been an imperfect stratagem.

Based on this experience, it is reasonable to conclude that cell-replacement therapy, technically challenging though it may be, is worth pursuing {4-6}. In addition to the prospect of more completely restoring brain function, cell-replacement therapy has the further advantage that it might be effective at later stages of a disease. This is an important consideration not only in disorders like stroke, which often evolve too quickly for acute treatment to be instituted, but also in chronic neurodegenerations, where cell loss is already extensive before the onset of symptoms.

Evidence for the feasibility of cell-replacement in the brain, and principles to guide cell-replacement research, come from several sources, including evolution and development. The challenge of cell replacement for neurodegenerative diseases is, in simple terms, to (re)build the brain. This is a task that is faced in one form or another (a) in evolution, as brain size increases, and (b) in ontogeny, as the brain develops from the neural tube.

As larger brains evolved, they appear to have done so primarily through an increase in neuron number, rather than, for example, neuron size or proportional connectivity {7}. This suggests that supplying new cells might also be the principal requirement for brain rebuilding. The evolutionary principle of epigenetic population matching suggests that trophic influences of surviving brain cells may help direct new neurons to reestablish appropriate connections. A related concept, the parcellation hypothesis, predicts a mechanism for pruning of exuberant axonal connections to help restore normal patterns of circuitry. Finally, the phenomenon of connectional invasion presages a capacity for restoring connections over an altered neuronal landscape and, perhaps, forming alternative, compensatory circuitry.

Development is the most extensively employed archetype for studying adult neurogenesis, providing voluminous information about mechanisms and patterns of neuronal proliferation, migration, differentiation and settling {8}. For example, molecular mechanisms of trophic factor stimulation, cell cycle regulation,

programmed cell death and neurodifferentiation, as well as pathways for the migration of newborn neurons, appear to be highly conserved between ontogeny and adult neurogenesis. These observations do not imply that principles guiding evolution or development are necessarily transferable to regeneration, only that they offer biological precedents that may be useful starting points for investigation.

Both endogenous and exogenous precursor cells are potential sources for neuronal replacement. At least two sources of cells for neuronal replacement after neurodegeneration can be envisioned: (a) cells mobilized from within the affected individual, and (b) cells obtained from an exogenous source, or donor, and transplanted into a recipient. In either case, the stage of differentiation of the cells employed could vary as well, from pluripotent, self-renewing stem cells to more developmentally restricted progenitor cells or precursors. The possibility that endogenous cells might be available for therapeutic cell replacement is based on the occurrence of physiological cell replacement in a wide range of organs.

Endogenous and exogenous sources for replacement of brain cells each have theoretical advantages and disadvantages. Endogenous cell replacement is inherently less invasive, circumvents immunologic compatibility problems, and makes maximal use of endogenous mechanisms that direct cell proliferation, survival, differentiation, migration, settling and functional integration. With exogenous replacement strategies, on the other hand, larger numbers of cells can be obtained, many sources of cells can be used (*e.g.*, embryonic *versus* adult stem cells), and the state of precursor cell differentiation can be optimized *ex vivo* prior to transplantation into the recipient.

Constitutively occurring adult neurogenesis provides a physiological substrate for endogenous cell-replacement therapy. The need to produce new cells continues beyond the primary period of development, as cells succumb to use or injury, and must be replaced. This is accomplished by adult stem cells, which preferentially reconstitute the tissues in which they reside. The best known examples are found in organs, such as bone marrow, skin and intestine, where cell turnover is rapid and continues throughout life. However, new cells are also produced throughout life in tissues like the brain, where cell turnover is more limited.

Proliferating neuronal precursors can be identified by labeling with [^{3}H]thymidine or bromodeoxyuridine (BrdU), transfection with viral vectors, or immunoreactivity for proliferation markers such as PCNA. Because these approaches may give false-positive results, as in injured cells undergoing DNA repair, convincing demonstration of neuroproliferation typically requires the use of multiple techniques. As newborn neurons mature, they express successive waves of developmentally regulated proteins, including polysialylated (embryonic) nerve cell adhesion molecule (ENCAM), the neuronal differentiation antigen NeuroD, βIII tubulin and Hu. As they migrate to their ultimate destinations, they can be identified by antibodies for doublecortin (DCX), and eventually NeuN and MAP2.

In rodents, adult neurogenesis occurs primarily in two brain regions **(FIGURE 1)** — the subventricular zone (SVZ), especially its most rostral extent in the walls of the anterior horns of the lateral ventricles {9-11}, and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) {12}. Neurons arising in the SVZ migrate primarily along the rostral migratory stream (RMS) to the olfactory bulb (OB), where they replace granule and periglomerular cells, although cells that arise in the human SVZ may not follow the same pathway {13}. Alternative routes for migration from the adult SVZ, such as the lateral cortical stream {14} and ventral migratory mass {15}, have also been described. Neurons arising in the SGZ migrate into the adjacent DG granule cell layer (GCL). Although its physiological role is incompletely understood, adult neurogenesis has the capacity to generate functional neurons {16,17}, which may help to replace cells lost to physiological cell death. Some reports suggest that additional brain regions may also generate new neurons in the adult brain {18-20}, but the extent to which this is occurs under physiological conditions, especially in primates, is unclear {21}.
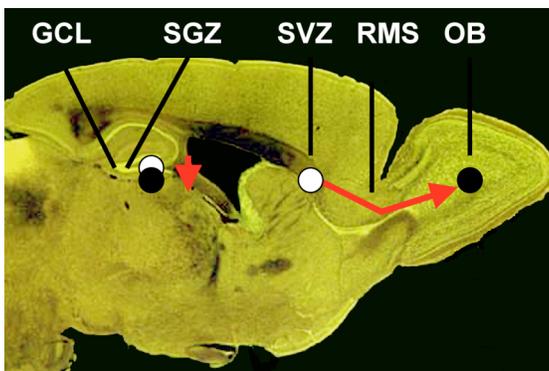
FIG 1. Sites of origin and migratory pathways of brain neurons arising through adult neurogenesis in the rodent. Neurons arising in the subgranular zone (SGZ) of the hippocampal dentate gyrus (left) travel a short distance away from the dentate hilus and into the adjacent granule cell layer (GCL), where they mature into granule neurons. Neurons that originate in the subventricular zone (SVZ) adjacent to the wall of the lateral ventricle (right) migrate *via* the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they become granule or periglomerular cells.

Adult neurogenesis can be regulated by growth factors, drugs and behavior, suggesting potential approaches for therapeutic enhancement. Neurogenesis is subject to physiological regulation by glucocorticoids, sex hormones, growth factors, neurotransmission, learning and stress {4,22,23}, and can be stimulated by drugs, including lithium, antidepressants, NMDA antagonists, phosphodiesterase inhibitors, anti-inflammatories and statins. Neuronal precursor cells can be cultured *in vitro* and several growth factors stimulate neurogenesis in such systems, including epidermal growth factor (EGF) {24}, basic fibroblast growth factor (FGF2) {25}, brain-derived neurotrophic factor (BDNF) {9} and erythropoietin (EPO) {26}. In addition, cultured progenitor cells {27-29} or tissue explants containing axons that project to neuroproliferative zones {30} release factors into conditioned medium that can regulate neurogenesis. Administration or overexpression of growth factors has also been shown to enhance neurogenesis in neuroproliferative zones of the adult brain *in vivo* {31-36}. We have used these combined *in vitro* and *in vivo* approaches to identify roles for three additional growth factors — stem-cell factor (SCF) {37}, heparin-binding EGF-like growth factor (HB-EGF) {38} and vascular endothelial growth factor (VEGF) {39,40} — in neurogenesis.

Acute injury stimulates neurogenesis, suggesting an endogenous regenerative capacity. Pathological processes can also stimulate neurogenesis in the brain {41,42}, and in some cases redirect the migration of nascent neurons from normal routes like the RMS and toward the site of pathology. For example, apoptotic degeneration of corticothalamic neurons in mice is followed by restoration of corticothalamic connections, and appears to involve neurogenesis, because the cells involved can be labeled with the cell-proliferation marker bromodeoxyuridine (BrdU) and express immature neuronal markers such as doublecortin (DCX) and Hu {43}. Similarly, injury resulting from status epilepticus in the rat both stimulates neurogenesis and diverts neuronal precursors from the RMS and into the affected forebrain {44}. Injury-induced neurogenesis, which has been observed in excitotoxic damage {41,45}, seizures {46}, and oxidative stress-induced apoptosis {43}, and which we and others have described in global {47,48} or focal {49,50} cerebral ischemia, may contribute to CNS recovery and repair. However, how brain injury stimulates neurogenesis is poorly understood.

To identify signaling factors that might be involved in the stimulation of neurogenesis by one source of cerebral injury (ischemia) we prepared cerebral cortical cultures from embryonic mouse brain and deprived these cultures of oxygen, to model ischemia *in vitro* {37}. Hypoxia increased bromodeoxyuridine (BrdU) incorporation into cells that expressed cell-proliferation markers and immature neuronal markers. Hypoxia-conditioned medium and stem cell factor (SCF), which was present in hypoxia-conditioned medium at increased levels, also stimulated BrdU incorporation into normoxic cultures. The SCF receptor, c-kit, was expressed in neuronal cultures and in neuroproliferative zones of the adult rat brain, and *in vivo* administration of SCF increased BrdU labeling of immature neurons in these regions. These results suggest that hypoxia and ischemia may stimulate neurogenesis through the release of trophic factors, including SCF {37}. Other trophic factors, such as FGF2 {45}, HB-EGF {38} and VEGF {39,40}, seem likely to be involved in ischemia-induced neurogenesis as well, and FGF2 has been implicated in neurogenesis after traumatic brain injury {51}.

One of the most striking features of injury-induced neurogenesis is its ability to redirect migrating neurons away from their normal paths of transit and into the region of injury. This is observed in epilepsy {44}, as well as in cerebral ischemia {52-55}. How migration is redirected by injury is unknown, but the altered migration of SVZ precursors into the ischemic cerebral cortex *via* the lateral cortical stream recapitulates an ontogenetic neuromigratory route, and is also reminiscent of the partial redirection that occurs in *Slit1*-knockout mice, which lack normal Slit/Robo chemorepulsive signaling {14}.

Whether different forms of cerebral injury trigger neurogenesis through the same or different mediators is unknown. Even the role of cell death in stimulating neurogenesis is uncertain — on one hand, if neurons proliferate to replace cells that are lost though injury or disease, cell loss might be a prerequisite for neurogenesis. On the other hand, neurogenesis is increased by seizures (which do not necessarily kill cells), sublethal forebrain ischemia, and physiological stimuli such as exercise, so cell death may not be required for injury-induced neurogenesis. Injury-induced neurogenesis in the absence of cell death cannot *replace* cells, but it might have other functions. For example, the new neurons could provide surviving but damaged neurons with trophic factors, or set up parallel neuronal connections to bypass or supplement malfunctioning circuits.

Chronic neurodegeneration is also accompanied by increased neurogenesis. Less is known about the effects of chronic than of acute neurodegeneration on neurogenesis. However, an increase in the number of cells that express the cell-proliferation marker PCNA and the immature neuronal marker βIII-tubulin was observed in the SVZ adjacent to the caudate nucleus in brains of patients who died with Huntington's disease (HD) {56}. In rats given intrastriatal injections of quinolinic acid, an excitotoxic model of HD, increased SVZ neurogenesis was also demonstrated by BrdU labeling and DCX expression {57}. We have found that FGF2 administration stimulates neurogenesis, leading to striatal migration of newborn cells that have phenotypic

features of medium spiny neurons and which project to globus pallidus, and also prolongs survival, in a transgenic (R6/2) model of HD {57a}.

In animal models of Parkinson's disease, 6-OHDA administration failed to stimulate the proliferation of dopaminergic neurons {19}. Lesioning with 6-OHDA combined with infusion of TGFα {58}, or MPTP-induced parkinsonism {59}, both increased the number of BrdU-labeled cells expressing tyrosine hydroxylase or dopamine transporters, although these results have been questioned {60}.

Neurogenesis in AD has also been studied using animal models. In one study, 11-14 month-old transgenic mice that express amyloid precursor protein (APP) with the Swedish (APP695[K595N/M596L]) mutation showed reduced numbers of BrdU-, ENCAM-, and BrdU/ENCAM-labeled cells in DG or SVZ, consistent with impaired neurogenesis {61}.   In another study, 24-month APP23 (APP751[K670N/M671L]) transgenic mice {62} showed a large increase in BrdU labeling in cerebral neocortex, but BrdU-immunopositive cells were NeuN-immunonegative {63}, and neither DG nor SVZ was studied. Presenilin 1 (PS1), which is mutated in some cases of familial AD, has also been implicated in neurogenesis, in that increased expression of wild-type, but not familial AD mutant, PS1 increases hippocampal neurogenesis {64}, and environmental enrichment-induced (but not basal) neurogenesis is impaired in DG-SGZ of PS1-knockout mice {65}.

In contrast, we found evidence for increased neurogenesis in hippocampus from patients with AD {66}, as well as in a transgenic mouse model {67}. PDGF-APP$_{Sw,Ind}$ mice, which express the Swedish and Indiana APP mutations, show increased incorporation of BrdU and expression of immature neuronal markers in SGZ and SVZ.   These changes, consisting of approximately twofold increases in the number of BrdU-labeled cells, were observed in SGZ at age 3 months, when neuronal loss and amyloid deposition are not detected. Because enhanced neurogenesis occurs in both AD and an animal model of AD, it appears to be due to the disease itself, and not confounding clinical factors. Since neurogenesis is increased in PDGF-APP$_{Sw,Ind}$ mice in the absence of neuronal loss, it must be triggered by more subtle disease manifestations, such as impaired neurotransmission. In support of this view, an APP mutation (D664A) that removes a caspase cleavage site involved in generating the C31 terminal peptide abolishes both neurotoxicity and enhanced neurogenesis in PDGF-APP$_{Sw,Ind}$ mice {67a}.

Increased DG neurogenesis has also been reported in anorexic (*anx/anx*) mutant mice {68}. In contrast, neurogenesis is decreased by chronic alcohol consumption {69} or vitamin E deficiency {70}.

Fundamental questions remain regarding the general phenomenon of injury-induced neurogenesis. Our previous work and that of others in this field points to many basic questions about injury-induced neurogenesis that are unanswered, and which are likely to be important in the eventual design of therapeutic strategies. Some targets for investigation suggested by these questions are illustrated in **FIGURE 2** below.
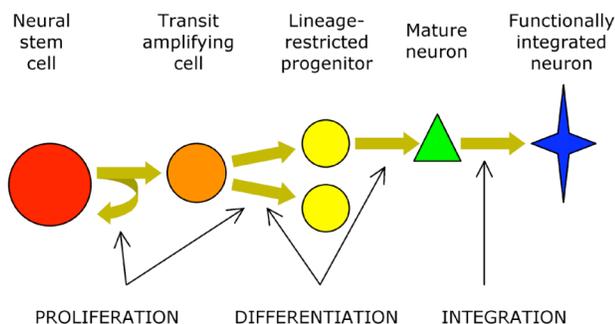
FIGURE 2. Hypothetical steps in neurogenesis, from neural stem cells to functionally integrated neurons, that might be modified by injury.

What stimulates injury-induced neurogenesis? What types of injuries can trigger neurogenesis? Are negative (ablative) and positive (irritative) lesions equally effective? How large must a lesion be to stimulate neurogenesis? Must lesions be in particular locations to do so? Is there a quantitative relationship between severity of injury and magnitude of neurogenesis, or is it an all-or-none phenomenon? What is the range of injury-induced factors that can stimulate neurogenesis? Can the injury-induced loss of neurogenesis-inhibiting influences (pathways or factors) stimulate neurogenesis?

How is injury-induced proliferation triggered in target neuronal precursor cells? How do neuronal precursors sense injury? What determines which neuroproliferative site or sites (SVZ or SGZ) respond to an injury? What determines which subpopulation of neuronal precursor cells within a proliferative site responds to injury? What changes in gene and protein expression occur following injury in neuronal precursors? Which

intracellular signaling pathways are involved in triggering neuroproliferation? Does injury modify programmed death of the progeny of neurogenesis?

How is the spatial and phenotypic fate of injury-induced new neurons determined? What directs the differentiation of newborn neurons toward a specific phenotypic fate? What directs newborn neurons to a specific spatial destination? To what extent do intrinsic versus extrinsic cues specify neuronal fate in injury-induced neurogenesis? What is the role of extrinsic (ECM or scaffolding) cues in directing the migration of new neurons? Do additional, latent neuroproliferative sites exist that can respond to local injury?

To what extent does injury-induced neurogenesis yield functional neurons? Are neuronal precursors themselves affected by neurogenetic disorders, so as to impair their ability to become functional or survive? Do immature cells of neuronal lineage, as opposed to mature neurons, exert any effects on brain function? Do mature neurons produced in response to injury exert any effects other than cell replacement (secretion, elaboration of ECM or scaffolding, ectopic compensatory influences)? What electrophysiological neuronal properties can neurons arising through adult neurogenesis assume? What neurotransmitter phenotypes do new neurons exhibit and how is this determined? Do new neurons integrate into the synaptic circuitry of surviving brain?

Does injury-induced neurogenesis contribute to improved functional outcome? Can neurogenesis be selectively inhibited prior to injury? If so, how does this modify outcome in various disease models? What proportion of lost neurons must be replaced to achieve functional benefit? In a disease known to present clinically only after, for example, 90% of vulnerable neurons are lost, is it sufficient to restore the number of neurons to >10% of the original population to reverse symptoms?

How can endogenous neurogenesis be modified to improve outcome after injury? Do exogenous factors (growth factors, drugs, behavior) produce the same types of new neurons as injury? Can inhibiting programmed cell death enhance functional neurogenesis? How long after an acute injury or how late in the course of a chronic disorder can increased neurogenesis modify outcome?

In addition to general attributes shared by injury-induced neurogenesis from diverse causes, unique features of neurogenesis are also likely to arise in different diseases. This makes finding a universally applicable approach to therapeutic neurogenesis unlikely, but may facilitate tailoring therapy to specific disease contexts. The approach we have chosen has the advantage that it will allow us to:

How can the unique features of different diseases help elucidate fundamental principles of injury-induced neurogenesis? As illustrated in **TABLE 1** below, neurodegenerative diseases exhibit differences in time course, tissue distribution, cellular vulnerability and etiology. Understanding how injury stimulates neurogenesis in each of these models will provide information about the temporal, spatial, cytopathological and pathophysiological requirements for eliciting neurogenesis in response to brain injury. The intrinsic differences among the diseases in question dictates that the approach to cell-replacement therapy is likely to require modification depending on the disease being targeted. For example, the new cells produced will need to be directed toward different phenotypic fates and regional destinations if they are to have a functional impact. An important question that has received little attention is whether endogenous neural precursor cells that might be mobilized for brain repair are themselves affected by the diseases against which they are to be targeted. For example, such cells might be functionally impaired *ab initio*, or destined for an early death. If this were the case, it might suggest that in these diseases, approaches employing exogenous sources of replacement cells will be preferable.

TABLE 1. Distinguishing features of selected acute and chronic neurodegenerations

Disease	Time course	Distribution	Cells affected	Etiology
Stroke	Acute	Focal, unilateral; vascular territory	Neurons, glia, endothelium	Ischemic
Alzheimer's	Chronic	Diffuse; cortical	Neurons	Sporadic > Genetic
Parkinson's	Chronic	Focal, bilateral; nigrostriatal tract	Dopaminergic nigrostriatal neurons	Sporadic > Genetic
Huntington's	Chronic	Focal, bilateral; striatum and cortex	Medium spiny neurons	Genetic

One example from our work to date relates to neurogenesis in experimental stroke. In this case, we found that a unilateral lesion produces a bilateral (albeit asymmetrical) increase in neurogenesis {50}.   This has

implications for the manner in which the injury signal is likely to be transmitted to neuroproliferative regions of the brain, and might be most consistent with a humoral effect transmitted through the cerebrospinal fluid.

Other examples come from our study of neurogenesis in AD. The finding that neurogenesis was increased in an animal model of stroke, which produces abrupt, massive cell death, led us to ask whether such a catastrophic lesion was required to trigger neurogenesis. More specifically, does only acute brain pathology, or only pathology associated with large-scale cell death, increase the production of new brain neurons? We found evidence for increased neurogenesis in both AD patients {66} and transgenic mice expressing mutant APP {67}, implying that a chronic pathological process can also enhance neurogenesis. In the mutant mice, we also found that increased neurogenesis preceded both extracellular amyloid deposition and cell loss, suggesting that earlier and more subtle manifestations of disease, such as synaptic dysfunction, must provide the trigger. Thus, investigation of specific diseases helped us to answer questions that are likely to be relevant to injury-induced neurogenesis in general.

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