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NEUROGENESIS IN THE ADULT BRAIN: New Strategies for Central Nervous System Diseases

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■ **Abstract** New cells are continuously generated from immature proliferating cells throughout adulthood in many organs, thereby contributing to the integrity of the tissue under physiological conditions and to repair following injury. In contrast, repair mechanisms in the adult central nervous system (CNS) have long been thought to be very limited. However, recent findings have clearly demonstrated that in restricted areas of the mammalian brain, new functional neurons are constantly generated from neural stem cells throughout life. Moreover, stem cells with the potential to give rise to new neurons reside in many different regions of the adult CNS. These findings raise the possibility that endogenous neural stem cells can be mobilized to replace dying neurons in neurodegenerative diseases. Indeed, recent reports have provided evidence that, in some injury models, limited neuronal replacement occurs in the CNS. Here, we summarize our current understanding of the mechanisms controlling adult neurogenesis and discuss their implications for the development of new strategies for the treatment of neurodegenerative diseases.

INTRODUCTION

> "Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, and immutable: everything may die, nothing may be regenerated." Santiago Ramon y Cajal (1).

Since the early 1900s, it has been generally believed that the adult central nervous system (CNS) of mammals has very limited regenerative capacity (1). The

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predominant repair mechanisms in the CNS were thought to be postmitotic, such as sprouting of axon terminals, changes in neurotransmitter-receptor expression, and synaptic reorganization; no replacement of dying/degenerating neurons was believed to occur. However, almost four decades ago, pioneering work by Altman & Das (2) suggested continuing neurogenesis throughout adulthood (2), and since the early 1990s, a large body of work has demonstrated that new neurons are indeed born in restricted regions of the adult mammalian CNS (3–5). The addition of new neurons throughout life not only provides a unique model system to understand basic mechanisms of neural development in the mature CNS but also raises the exciting possibility that stimulation of this process can be applied as a new strategy for therapy for CNS diseases that had hitherto been thought intractable.

In this review, we first summarize our current understanding of the cellular and molecular mechanisms that control sequential steps in adult neurogenesis. We then focus our attention on the challenges and strategies for treating CNS diseases by mobilizing endogenous adult neural stem cells to undergo these steps in a regulated fashion. For information on the treatment of neurodegenerative diseases utilizing transplantation of stem cell–derived neural cells, we refer the reader to several recent comprehensive reviews (6–12).

BIOLOGY OF NEUROGENESIS IN THE ADULT CNS

Neural Stem Cells May Exist Along the Entire Adult Neuraxis

Neural stem cells are defined as cells that have the ability to self-renew and to give rise to the three major cell types of the mammalian CNS: neurons, astrocytes, and oligodendrocytes (4). During development of the mammalian CNS, neurons and glia arise from multipotent neural stem cells in a stereotyped sequence in which neurons are generated first, primarily during the embryonic period, followed by glia, the majority of which differentiate after most neurons are born (13, 14).

In the adult CNS, the continued generation of glia has been observed in many different regions (15–17). The new glial cells are thought to derive from neural stem cells. This concept is based primarily on in vitro analysis of proliferating cells isolated from different adult CNS regions. Cells with the ability to differentiate into all three major lineages and/or to self-renew can be isolated from the adult rodent and—more importantly with regard to therapy for CNS diseases—from the adult human CNS (17–26). Taken as a whole, these studies have suggested the presence of adult neural stem cells throughout the entire neuraxis. One caveat to this interpretation, however, is the fact that proliferating cells derived from the adult brain have been primarily analyzed following long-term exposure to high concentrations of growth factors/mitogens that can potentially lead to changes of their epigenetic program (23, 27, 28). Unlike the hematopoietic and the neural crest fields, where stem cells can be isolated based on surface antigens, the field of adult neural stem cells is hampered by our current inability to identify and acutely isolate them (29). Thus, the relationship between the in vivo proliferating cells and

the in vitro cultured cells cannot be firmly established. However, new protocols have been developed that allow the enrichment of neural stem/progenitor cells in culture, thereby allowing in vitro characterization soon after isolation (23, 30). In vitro studies using these methods have confirmed that in vivo proliferating cells from gliogenic regions have the ability to give rise to neurons in culture without exposure to growth factors/mitogens (17), thereby providing additional support for the idea of a broad presence of neural stem cells in the adult mammalian CNS.

Active Neurogenesis is Limited to Specific Neurogenic Regions of the Intact Adult CNS

Although neural stem cells with the potential to give rise to neurons in vitro appear to be ubiquitously present within the adult mammalian CNS, adult neurogenesis has consistently been found only in the subventricular zone (SVZ) of the lateral ventricle (3) and in the hippocampal subgranular zone (5). Importantly, the generation of new neurons throughout adulthood has not only been demonstrated in rodents but also in humans (dentate gyrus of the hippocampus) (31).

Some authors have suggested that neurogenesis occurs also in other regions of the intact adult mammalian CNS such as the cortex (31a) and the substantia nigra (31b). However, these results are contradictory to other published reports (17, 31c, 127) and currently lack confirmation.

Recent studies in rodents have shown that the newly generated neurons derived from neural stem cells in the SVZ and the hippocampal subgranular zone, become electrically active, are capable of firing action potentials, and receive synaptic inputs (32–35), showing that they can become physiologically mature. Future studies will need to determine whether they also make functional synapses with their downstream target neurons and release appropriate neurotransmitters in order to unequivocally demonstrate their integration into adult circuitries.

Integration of functional neurons in the neural networks is believed to be achieved through sequential steps in a highly regulated fashion: proliferation of the neural stem cell, generation of a rapidly amplifying progenitor cell, differentiation into an immature neuron, migration to the final location, growth of axon and dendrites and formation of synapses with other neurons in the circuits, and ultimately maturation into a fully functional neuron. Although these steps are equivalent to the ones that newborn neurons have to undergo during development, the fundamental difference between developmental and adult neurogenesis is that new adult neurons undergo these processes in an already mature environment and therefore have to integrate into preexisting circuits.

In adult hippocampal neurogenesis (Figure 1), neural progenitors located in the subgranular zone proliferate and give rise to immature neurons. Many of these newly generated cells die between the first and second week after they are born. The surviving neurons then migrate into the molecular layer (36). Within four weeks, they send axons to the CA3 region to form mossy fibers and project dendrites to the outer molecular layer (34, 35, 37, 38). During this period, the newly generated

neurons become electrically active and capable of firing action potentials. Electrophysiological studies have shown that these newly generated granule neurons start to receive synaptic inputs from the cortex within four to six weeks after birth, appearing to become functionally integrated in the circuit (35). The complexity and density of their dendritic spines, however, continue to grow for at least several months. Thus, the course of neuronal development for granule neurons born in the adult brain appears much more protracted than for those generated during embryonic stages (39).

New neurons in the adult olfactory bulb go through similar stages in their development (Figure 1). Neural stem cells located in the SVZ of the lateral ventricle proliferate and give rise to neuroblasts, which then migrate in the so-called rostral migratory pathway (RMP) through mature neural tissue. In contrast to the developing CNS, the newborn neurons are not guided by radial glia but migrate tangentially in chains through tubular structures formed by specialized astrocytes (40, 41). As early as 14 days after birth, some of the new neurons have reached the olfactory bulb and migrate radially in the olfactory bulb to their final positions. At this stage, they already display dendritic spines, which suggests that they are receiving synaptic inputs. Indeed, spontaneous synaptic activity emerges soon after migration is completed. Surprisingly, however, their spiking activity does not occur until late into the maturation process (32). This delayed maturation of excitability may serve to prevent the newborn cells from disrupting the function of the adult preexisting circuitry. These findings further illustrate differences in neuronal development during embryonic stages and adulthood.

The function of adult neurogenesis is currently not known. It has been hypothesized that, in the adult hippocampus, neurogenesis is involved in learning and memory (42). Moreover, some correlative evidence has been accumulated that suggests that neurogenesis in these areas compensates for dying neurons (43) and is necessary for the functional integrity of these structures (44). Adult neurogenesis can therefore serve as a model system for neuronal replacement in the adult brain, and its characterization can provide strategies for the eventual stimulation of endogenous neural stem cells to replace dying neurons in other brain areas.

Environmental Control of Adult Neurogenesis

How is neurogenesis restricted to the hippocampus and the SVZ, given that neural stem/progenitor cells have been isolated from many CNS regions? Transplantation studies have provided evidence for the role of environmental factors in neural stem/progenitor cell fate choice. For example, although adult hippocampus-derived neural stem/progenitor cells generate only granule cell neurons when transplanted back into hippocampus, when introduced into the RMP they generate site-specific tyrosine-hydroxylase-positive interneurons in the olfactory bulb, a phenotype never seen in the hippocampal granular cell layer (45). Strikingly, although neural stem/progenitor cells derived from gliogenic (i.e., nonneurogenic) regions will differentiate into glial cells when transplanted back into the region of their origin, they will give rise to neurons when transplanted into the neurogenic hippocampus (17, 46). These results have two important implications.

First, they indicate that the neuronal differentiation observed in cultured neural stem/progenitor cells from gliogenic regions is not a culture artifact but reflects the potential of these cells to give rise to neurons in vivo. Second, these findings demonstrate that adult neural stem/progenitor cells from different regions are not fate-restricted by intrinsic programs but that extrinsic cues derived from the local environment control adult neural stem cell fate.

CELLULAR CONTROL OF ADULT NEUROGENESIS What are the cellular elements that create a neurogenic niche in the regions of the adult CNS that are permissive for neurogenesis? Anatomical analysis has identified the vasculature as one potential candidate that may constitute the neurogenic niche. In the adult hippocampus, but not in nonneurogenic areas of the adult CNS, proliferating cell clusters are found in close proximity to blood vessels, indicating the possibility that vasculature- or blood-derived factors are regulators of neurogenesis (47). The finding that factors that promote endothelial cell proliferation also increase neurogenesis in the mammalian forebrain has suggested an important relationship between these two processes (48, 49). Indeed, recent studies in the adult male songbird brain have shown a causal interaction between angiogenesis, an increase in endothelial cell-derived growth factors, and the generation of new neurons in the adult forebrain (50). Within the rodent hippocampus, approximately one third of the newly generated cells in the proliferating clusters express markers of endothelial cells, suggesting that angiogenesis and neurogenesis are closely interlinked in the hippocampus and, even more provocatively, that a lineage relationship may exist between the two proliferating populations.

Regional differences in the astrocyte population have recently also been demonstrated to be important for the neurogenic microenvironment in the adult hippocampus (51). Adult hippocampal astrocytes actively regulate neurogenesis by promoting the proliferation of neural stem/progenitor cells and by instructing them to adopt a neuronal fate. In contrast, astrocytes from nonneurogenic regions, such as the adult spinal cord, do not promote neurogenesis, indicating that the characteristics of the local astrocyte population may play a major role in the creation of a neurogenic environment. In addition to the regulation of proliferation and fate specification of adult neural stem cells, hippocampal astrocytes are also likely to contribute to later steps in hippocampal neurogenesis, such as the maturation and synapse formation of the newly generated neurons (52, 53).

With respect to the neurogenic niche that generates the adult-born olfactory neurons, it has been found that astrocytes in the SVZ have similar effects upon the proliferation and neuronal differentiation of neural stem/progenitor cells as their hippocampal counterparts (54). Additionally, ependymal cells in the lateral ventricle regulate neurogenesis by secreting factors that bias/instruct the cell fate of the SVZ stem/progenitor cells (55) (see below).

MOLECULAR CONTROL OF ADULT NEUROGENESIS The recent advances in the identification of cell types that contribute to the neurogenic microenvironment (see above) will provide an experimental framework to test the involvement and

interaction of the many different signals implied in the regulation of adult neurogenesis (Figure 2). Based on their ability to influence different aspects of neurogenesis, such as the size of the proliferative pool, fate choice of adult neural stem cells, and survival of newly generated neurons in vitro and in vivo, large numbers of growth factors (17, 22, 48, 49, 56–72), hormones (73–81), and neurotransmitters (66, 76, 80, 82–87) have been implicated in the control of neurogenesis. The relationship between these different factors is not understood and it is not clear whether all of them play a physiological role in the regulation of neurogenesis or whether these factors are acting directly on stem cells or through secondary signals. In the following, we summarize some of the most important molecular players in the regulation of neurogenesis known to date.

Regulation of proliferation of adult neural stem cells FGF-2 and EGF-receptor ligands are the primary mitogens used to propagate adult neural stem cells in vitro and are hypothesized to be very important for the control of in vivo proliferation of neural stem/progenitor cells (22, 57–62). Indeed, at least a subpopulation of proliferating cells in the SVZ express the EGF-receptor (88, 89), and a null-mutation for the EGF-receptor ligand TGFα (56) leads to significantly decreased stem/progenitor cell proliferation within the SVZ. Moreover, delivery of either EGF or FGF-2 to the adult rodent CNS by different routes has been demonstrated to increase the proliferation of progenitor cells in the SVZ (15, 90, 91).

The analysis of the neurogenic niche in adult songbirds has provided some interesting insights into the interaction of hormones and growth factors (50). In this system, testosterone induces the expression of vascular endothelial growth factor (VEGF), thereby increasing angiogenesis. Newly generated endothelial cells then stimulate neurogenesis by increasing the levels of brain-derived neurotrophic factor (BDNF) in the neurogenic areas, which enhances the proliferation of progenitors. Given the stimulatory effects of intraventricularly infused or virally overexpressed BDNF and VEGF on mammalian neurogenesis (48, 49, 65, 69), it is possible that this molecular interaction between endothelial cells and neural stem cells also exists in the adult mammalian CNS.

Neural stem cell–derived factors are also essential for the regulation of proliferation in an autocrine fashion. The glycosylated form of Cystatin C (CCg) is expressed in hippocampal neural stem cells and is necessary as a cofactor for FGF-2-dependent proliferation (60).

Fate specification of adult neural stem cells The molecular mechanisms underlying fate specification of adult neural stem cells have just begun to be revealed. Adult neural stem cells express members of the bone morphogenetic protein (BMP) family that instruct them to adopt a glial cell fate (55, 92–95). However, in the neurogenic SVZ, the BMP inhibitor noggin is secreted by ependymal cells in the lateral ventricle and presumably serves to block the gliogenic effects of BMPs (55).

Although noggin blocks gliogenic signals, it is by itself not sufficient to induce the neuronal differentiation of adult neural stem cells (55). Recently, we

have identified Wnt-signaling as one candidate pathway that instructs neural stem cells in the adult hippocampus to adopt a neuronal fate (L. Desire, H. Song, D.C. Lie, S.A. Colamarino & F.H. Gage, unpublished observations). Wnts are expressed in adult hippocampal astrocytes, and blocking of astrocyte-derived Wnt-signaling leads to a significant decrease in neuronal differentiation of adult neural stem cells. Interestingly, neural stem cells themselves secrete the Wnt-antagonist sFRP3, which blocks Wnt-induced neuronal differentiation. The balance of competing autocrine/paracrine signaling pathways like BMPs/noggin and Wnts/sFRP3 might represent a general principle by which the environment interacts with neural stem cells to control their proliferation and differentiation in order to ensure that new neural cells are generated in a spatially and temporally coordinated fashion.

Neuronal migration, nerve guidance, synapse formation, and survival Factors controlling later steps in neurogenesis, such as functional maturation, synapse formation and integration into the neuronal circuit, and survival, are currently unknown. However, some mechanisms and molecules that are essential for the remarkable long-distance migration of newly generated neurons through the adult CNS have been described. The interaction of the migrating neurons with their environment through expression of the polysialated glycoprotein neural cell adhesion molecule (PSA-NCAM) is necessary for proper migration, as null mutation for NCAM or the deletion of the polysialic acid moiety results in migratory defects (96–98). Members of the ephrin-B family (99), Slit (100) integrin family members (101), and astrocyte-derived factors of unknown identity (102) have also been demonstrated to direct the migration through the RMP.

NEURAL STEM CELLS IN THE ADULT CNS: AN ENDOGENOUS SOURCE FOR REPAIR?

During the past decade, the progress in the field of stem cells has fueled our hopes to be able to cure currently intractable diseases by cell replacement. In this regard, adult neural stem cells have been proposed as an endogenous cellular source for the treatment of CNS diseases. The use of endogenous sources for cell replacement offers several potential advantages. Many ethical concerns and political restrictions that have been raised regarding the use and manipulation of fetal tissue and embryonic stem cells do not apply for endogenous stem cells. In addition, the use of endogenous neural stem cells for cell replacement offers a unique advantage over other cell sources: Immunological reactions are avoided. The stimulation of endogenous neural stem cells for cell replacement, however, does pose multiple specific challenges and problems to be overcome. Next, we critically review the current literature on neuronal cell replacement from endogenous stem cells and provide a framework for the design of new treatment strategies for CNS diseases based on mobilization of endogenous neural stem cells.

Repair from Adult Stem Cells in Neurogenic CNS Regions

There is reason to believe that stem cells in neurogenic areas respond to injury. Precursor cell proliferation in the SVZ and the number of migrating neurons in the RMP are temporarily augmented after ischemia and seizure (103, 104). Similarly, transient increase of cell proliferation in the SGZ is observed shortly after seizure, ischemia, and excitotoxic and mechanical lesions of the hippocampal dentate gyrus (103, 105–112) and leads to a transitory, up to eightfold, increase in the rate of hippocampal neurogenesis.

Although these findings are suggestive of endogenous stem cells contributing to repair and integrity of the lesioned circuits by cell replacement, it is premature to conclude that increased neurogenesis equals the regeneration of the compromised circuit. First, there is no direct evidence that the new neurons are replacing neurons that degenerate owing to the injury. Second, the generation of new neurons in neurogenic areas has not been proven to be causally linked to functional recovery. Third, lesion-induced neurogenesis might also contribute to pathological alterations in the hippocampal formation. Aberrant migration and network reorganization, as well as altered physiological properties of the newly generated ectopic neurons, were demonstrated following seizure activity (104, 110, 113). This observation in particular highlights the argument that the pure addition of new neurons into a compromised neuronal circuit may not be beneficial per se. For injury-induced neurogenesis to be advantageous, the new neurons have to integrate appropriately into the injured neuronal circuit, display functional properties that are similar to the characteristics of the neurons that were lost owing to the disease, and be generated in numbers that are comparable to the number of neurons that were lost.

Repair from Adult Stem Cells in Nonneurogenic Regions

Are the neurogenic regions unique in their ability to generate new neurons following injury? Several studies have suggested that factors and mechanisms regulating proliferation, neuronal migration, differentiation, survival, and connectivity during development are reactivated in the injured adult environment. Following hypoxia, factors that stimulate the in vitro and in vivo proliferation of adult neural stem cells and their respective receptors are upregulated (114, 115). Interneurons surrounding dying neurons in the adult neocortex can upregulate the expression of neurotrophins (116), which can direct the fate of adult neuronal precursors in vitro (70). Glial cells in diseased areas can acquire properties of radial glia (117), which act as a substrate for neuronal migration during development. In some specialized models, these environment-derived factors are upregulated by injury and appear to be sufficient to direct transplanted immature neurons or immortalized, neonate-derived neural precursors to migrate toward the lesioned area, to differentiate into neurons, and to establish synaptic contacts (118–121).

Can these lesion-induced environmental signals also direct the fate of endogenous neural stem cells? Magavi and colleagues (122) investigated the effects of neuronal cell death on cell genesis in the adult neocortex of mice, a region where

neurogenesis normally does not occur. Their study applied a lesion model that results in the selective and synchronous cell death of neocortical projection neurons without affecting surrounding nonneuronal cells. The lesion did not have a significant impact on the proliferation of endogenous stem/progenitor cells; surprisingly, though, approximately 2% of the newly generated cells in the lesioned neocortex expressed neuronal markers. Comparable numbers of newborn neurons were found at two weeks and six months following lesion, indicating significant survival of these cells. Moreover, retrograde labeling studies suggested that some of the newly generated neurons extended appropriate long-distance connections.

Two other recent studies reported increased cell proliferation and generation of immature neurons in normally nonneurogenic regions following focal stroke by occlusion of the middle cerebral artery (123, 124). In this clinically more relevant lesion model, newly generated cells that expressed markers for immature neurons were observed in the lesioned striatum of adult rats at two weeks after ischemia. Interestingly, at this early time point, the majority of these new neurons expressed markers of developing striatal neurons, indicating that they were differentiating into the neuronal phenotype that was destroyed by the striatal lesion and might potentially be able to contribute to cell replacement and repair following ischemic lesion.

Restrictions on Neuronal Cell Replacement from Endogenous Stem Cells

The studies showing neuronal cell replacement following injury even outside the neurogenic CNS areas are encouraging for the prospect of repair from the endogenous stem cell population. However, they also illustrate that restrictions in all key steps of neurogenesis have to be overcome in order to achieve functionally relevant neuronal cell replacement (Figure 3).

Magavi and colleagues (122) found neuronal differentiation of endogenous stem/progenitor cells, survival, and potential integration into circuits of newborn neurons in a lesion model that is unparalleled in human disease. It is not clear how the number of newborn neurons compared to the number of dying neurons in this lesion model; however, the density of new neurons was very low ($\sim$100 cells/mm^3), reflecting in particular the very low neuronal differentiation rate of endogenous stem/progenitor cells in the lesioned neocortex, the limited proliferation in this area, and the limited migration of immature neurons derived from other germinal zones toward the lesion.

In the study of Arvidsson and colleagues (124), only 20% of the new striatal neurons that were initially generated survived longer than two weeks, and only half of these cells expressed markers that were consistent with a mature, striatal neuronal phenotype. Compared to the number of striatal neurons that were lost, this amounted to an estimated replacement of only 0.2% of the population, a number probably too low to have a significant impact on functional recovery. Even in the case of maximum survival and maturation of the entire newly generated population, only a very small fraction of the lost neuronal population would have been replaced.

Finally, the important question about whether the new neurons were functional and integrated into the circuit remains unanswered.

Perhaps more troubling, neuronal cell replacement from endogenous stem cells does not appear to be a consistent feature of the adult mammalian CNS. Despite the potential of low-level neurogenesis in the neocortex following a specific lesion (122), in models of stroke (123–125), all but one study (126) found no evidence for cortical neurogenesis. Degeneration of dopaminergic neurons in the substantia nigra has also produced inconsistent results (17, 31c, 127), with only one study describing limited dopaminergic neurogenesis in the substantia nigra (31c). Injuries to the spinal cord (20, 26) led to increased stem/progenitor cell proliferation but without de novo neurogenesis.

Cell Replacement from Endogenous Progenitor Cells: The Importance of Understanding the Disease Process

What explains the inconsistency in the occurrence of lesion-induced neurogenesis and the discrepancies in differentiation and survival of new neurons? At present, we cannot answer this question with certainty; however, analysis of the present literature provides some clues as to what aspects could be causal for the observed differences.

Neurogenesis following injury has been reported consistently in areas close to the SVZ (123, 124). In these studies, the appearance of immature neurons expressing the migratory neuron-marker doublecortin between the lesion and the SVZ has been observed; moreover, Arvidsson and colleagues described a gradient of newborn neurons in the striatum, with the highest density closest to the SVZ. These findings suggest the possibility that new neurons from the SVZ are attracted by lesion-derived migratory cues. Moreover, they indicate that new neurons in the lesion may be primarily derived from stem cells in the SVZ and less so from resident stem/progenitor cells, which might account for the lack of neurogenesis in lesions distant from the SVZ (17, 20, 26, 123–125, 127).

The conflicting reports on lesion-induced neurogenesis in the neocortex illustrate another important point. Neocortical neurogenesis and long-time survival of new neurons were observed in a lesion model that specifically targeted pyramidal cortical neurons but not their surrounding environment (122). In contrast, nonselective lesions, such as stroke, failed to induce cortical neurogenesis (123–125). This finding suggests that de novo neurogenesis in the neocortex and potentially in other CNS regions is highly dependent on the lesion model or, more specifically, on its effect on the environment, which presumably generates environmental signals that can direct migration, differentiation, maturation, and survival of newborn neurons.

Implications for the Treatment of Human Diseases

Lesion-induced neurogenesis and its apparent dependence on CNS region and lesion model highlight a central problem for the treatment of human CNS diseases

using endogenous stem/progenitor cells: There are many diseases with different pathophysiologies affecting diverse regions of the human CNS. Obviously, not every disease will be suitable for this treatment strategy. It is also evident that each disease poses specific problems and will require specifically tailored strategies for the mobilization of endogenous stem cells. Given that we are far from understanding the processes regulating physiological neurogenesis, and even less so lesion-induced neurogenesis, it is too early to comment on the suitability of cell replacement from endogenous stem cells in the context of specific diseases or to outline specific treatment strategies. However, we can outline specific aspects of CNS diseases that are likely to have a major impact on both the potential for cell replacement therapy and the design of strategies.

INJURY/DISEASE TIMING Enhanced or de novo neuronal cell replacement has been observed following lesions that induce the synchronous cell death of a regionally confined neuronal population (122–124), thereby resembling the timing of acute CNS diseases and injuries. Whether synchronicity of neuronal death is necessary to induce neurogenesis is not known; however, it is possible that massive synchronous cell death leads to significant regional increases in environmental signals that support the neuronal cell replacement process.

There is currently no evidence that de novo neurogenesis is occurring following the death of small neuronal populations over an extended period of time, a timeframe that would resemble the timing of neuronal death in neurodegenerative diseases. Are there signals present in these diseases that can potentially support the replacement process? And how can we achieve gradual cell replacement in light of continued neuronal cell loss?

DISEASE LOCATION Neuronal cell replacement has been primarily observed in regions close to the lateral ventricle and has been ascribed to neurons that have migrated in from the SVZ. However, many diseases affect CNS regions that are so distant from the SVZ that it seems unlikely that significant migration from the SVZ can be achieved. As we have discussed, numerous studies have suggested that neural stem/progenitor cells are present in CNS areas other than just the SVZ (17–26). There is currently no evidence that these cells contribute to neuronal cell replacement following lesion, and there is no definite proof that they can give rise to functional neurons in vitro or in vivo. Can we recruit these populations for cell replacement?

EXTENT OF CELL LOSS The degree of neurogenesis in nonneurogenic regions following lesion is limited and is not sufficient to have a significant impact on functional recovery. At this point, it is unclear whether the low rate of lesion-induced neurogenesis is due to the limited presence or proliferative capacity of stem cells and/or restrictions in differentiation, migration, and survival. Whatever the mechanism, it is apparent that the extent of cell loss will greatly influence whether functional regeneration from endogenous stem cells can be achieved.

GENETIC BASIS OF DISEASE In many diseases, a genetic basis for the neuronal cell death has either been proposed or has been uncovered. For example, single gene defects have been described in amyotrophic lateral sclerosis and Parkinson's disease that are causal to a small percentage of familial cases (128–130), and it is likely that in the future, other genetic defects associated with neurodegenerative diseases will be discovered. In these cases, genetic defects will also be present in the new neurons derived from the endogenous stem cell population and/or in their environment. Will this lead to the degeneration of the newly generated neuronal population?

EFFECTS OF THE DISEASE ON THE ENVIRONMENT Injury and disease may create a milieu that is hostile to the generation of new neurons. Acute injuries, such as stroke, increase the presence of signals (131, 132) that induce scar formation by reactive gliosis (133, 134). These gliogenic factors (55, 92–95) potentially inhibit or compete with the neuronal differentiation of endogenous stem cells.

Gliotic scar (135–137); extracellular matrix molecules (138–142); and myelin-derived growth inhibitors, such as Nogo, MAG, and OMgp (143–146), are known to inhibit axonal growth in the adult CNS and potentially also inhibit the migration of immature neurons (147, 148). It has been speculated that new neurons in the adult CNS might be capable of escaping the inhibition of myelin-derived growth inhibitors (149). Although this point remains unproven, it is apparent that barriers, such as scar tissue and extracellular matrix, will interfere with the formation of axonal and dendritic connections and possibly prevent the survival of the new neurons by depriving them of target-derived trophic support (150).

We also have to abandon our neurocentric view of the CNS. It is important to recognize that not only neurons but also glia are affected in many diseases, such as stroke. Glial cells create the necessary milieu for neuronal function (151–155). In addition, adult neurogenesis occurs in microenvironments that consist of multiple, regionally specialized cell types, some of which may be glia (51, 53, 54, 156). It is therefore very important to focus not only on the generation of new neurons but also on the restitution of a complete cellular environment that supports the maturation, survival, and function of new neurons.

A Glimpse of Hope: Modulating the Repair from Endogenous Stem Cells

With these complex challenges in mind, it is not clear what promises the strategy of neural cell replacement from endogenous stem cells hold for the repair of the adult CNS in the immediate future. However, two recent studies have provided some grounds for a more positive outlook. Both studies reported beneficial effects of the delivery of growth factors on functional outcome in different lesion models and suggested a causal link between recovery and neuronal cell replacement from endogenous stem cells.

In the first study, Fallon and coworkers investigated the effects of exogenous TGFα in a rodent model for Parkinson's disease (157). Dopaminergic neurons in the substantia nigra, which are the main afferents of the striatum, were killed

by administration of 6-hydroxydopamine. TGFα, an EGF-receptor ligand and mitogen for stem/progenitor cells in the SVZ, was subsequently infused into the striatum. Following this treatment, animals showed improvement of behavioral deficits, which was ascribed to the appearance in the striatum of new neurons of a dopaminergic phenotype. The generation of these neurons in the striatum is highly surprising and warrants further confirmation, given that neuronal cell death occurred in a different CNS region (i.e., substantia nigra pars compacta) and that under physiological circumstances dopaminergic neurons are never observed in the adult striatum (15).

The role of TGFα in the emergence of these new dopaminergic neurons is not known. It was reported that proliferation and migration from the SVZ toward the striatum of EGF-receptor-positive progenitor cells were observed following TGFα infusion. Similar results had been previously reported following infusion of EGF into the lateral ventricle, although the effect on neuronal differentiation of progenitor cells in the striatum remained controversial (15, 90).

As a cautionary note, it needs to be mentioned that the authors did not evaluate other potential effects of TGFα, such as survival of the lesioned dopaminergic neurons in the substantia nigra or sprouting of remaining dopaminergic axon terminals in the striatum, for their contribution to functional recovery.

In the second study, Nakatomi and colleagues examined the effects of a combined infusion of FGF-2 and EGF into the lateral ventricle of adult rats following selective degeneration of hippocampal CA1 pyramidal neurons by global ischemia (158). In growth factor–treated animals, the authors observed an increased proliferation of endogenous progenitor cells and a significant number of new neurons, which was estimated to regenerate approximately 40% of the pyramidal neurons in the CA1 region, and they also reported some evidence for the generation of new neurons in the temporal cortex. The new neurons appeared to have originated primarily in the caudal extension of the SVZ adjacent to the CA1 region. Importantly, the authors found that the new pyramidal neurons survived at least up to six months after ischemia, and behavioral studies showed an improved behavioral recovery of growth factor–treated animals.

The study by Nakatomi and colleagues represents the most complete study on the potential of endogenous neural stem cells in CNS repair and provides evidence that modulation of the endogenous stem/progenitor cells' response to injury (e.g., via growth factor infusion) can be beneficial for repair and behavioral recovery. However, some open questions remained in this study. The authors found evidence for synpase formation on the new neurons and for the formation of appropriate axonal projections, suggesting that these neurons were integrated into the hippocampal circuitry. The functional properties of the new neurons, however, are unclear. Even after three months—a time point when behavioral recovery was documented—the new neurons formed synapses that were morphologically immature and displayed altered electrophysiological properties. In addition, other mechanisms, such as trophic support of injured neurons, axonal sprouting, and synaptic modulation by the growth factors, were not completely excluded in this experiment.

Therapeutic Recruitment of Endogenous Neural Stem Cells: The Most Pressing Questions

One of the central questions that we need to answer is whether and how new neurons after lesion contribute to functional recovery. At first glance, the solution to this question appears trivial: More new neurons are better and will lead to more complete recovery. However, the caveats for this simplistic view have been outlined and we have to unequivocally demonstrate the benefits of neurogenesis following any type of lesion.

At this stage, we envision that our findings about the regulation of neural stem cell behavior and normal neurogenesis will eventually result in approaches that allow us to control the replacement of cells in the adult CNS. It is therefore absolutely mandatory to continue our investigation into the basic biology of adult neural stem cells and adult neurogenesis. In the context of repair, we propose that it is most important to answer the following questions in the near future.

Are neural stem cells truly present throughout the entire neuraxis? Although the current literature is highly suggestive of the ubiquitous presence of stem-like cells that can give rise to neurons, we have not shown that this differentiation potential is present in vivo and that the neurons derived from these cells are functional. What degree of plasticity do adult neural stem cells have, and do adult neural stem cells in different regions display the same degree of plasticity? At the heart of this question is the uncertainty about whether endogenous stem cells have the potential to differentiate into all of the different neuronal and glial populations that are affected in CNS diseases. Thus far, it has been demonstrated that adult neural stem cells can give rise to the functional neuronal phenotypes of the olfactory bulb and the hippocampal dentate gyrus (32, 35). But can endogenous stem cells also generate other neuronal phenotypes, such as functional dopaminergic neurons of the substantia nigra pars compacta or motoneurons in the ventral horn?

Other important information has to be acquired from fields complementary to the stem cell field. The question is not only what the characteristics of the neurogenic and gliogenic environment are but also what the differences and similarities between the normal and diseased environment are. Without taking these parameters into account, cell replacement will persist as an in vitro phenomenon.

Finally, we cannot assume that our findings in animal models are readily translatable into the human system. The human adult neurogenesis field and neural stem cell field are even less advanced. Our optimism is based on findings that adult neurogenesis occurs in the human dentate gyrus (31) and that a few groups have been successful in isolating and culturing cells with stem-like characteristics (21, 24, 159). But even a seemingly trivial question such as the occurrence of neurogenesis in the human SVZ is not answered, and we will have to characterize human neural stem cell biology in much greater detail before we can realistically propose cell replacement using endogenous stem cells as a potential therapy.

CONCLUDING REMARKS

The adult mammalian CNS poses unique challenges for repair, given the diversity of neural cell types, the complexity of networks, and the limited spontaneous regenerative capacity. However, the demonstration of constant neurogenesis in some areas of the CNS and of the presence of proliferating cells with the ability to give rise to neurons in multiple CNS regions has reinvigorated our hopes of regenerating the diseased CNS by neuronal cell replacement. The first exciting step on this long road has been taken by demonstrating that, in principle, new neurons can be generated following injury in regions that, under physiological conditions, do not show neurogenesis. The tasks ahead are challenging: The amount of neuronal cell replacement from endogenous stem cells without additional manipulation is minimal, and strategies need to be defined to support this process in order to make it functionally relevant. These strategies should be based on our insights into mechanisms of both adult neurogenesis and development, given that neuronal cell replacement has to recapitulate elements of developmental processes in an adult CNS environment. Understanding the specific disease mechanism is another prerequisite for the development of successful strategies because each disease poses unique problems and obstacles for cell replacement. Finally, the conceptual development of strategies has to go hand in hand with the development of new tools that allow the easy and safe manipulation of the injured CNS.

It will take a broad, multidisciplinary approach to meet these challenges, and with continuing progress, we will meet more obstacles and questions. However overwhelming the task appears, the prospect of functional restoration of the diseased brain at the end of the road makes our efforts worthwhile.

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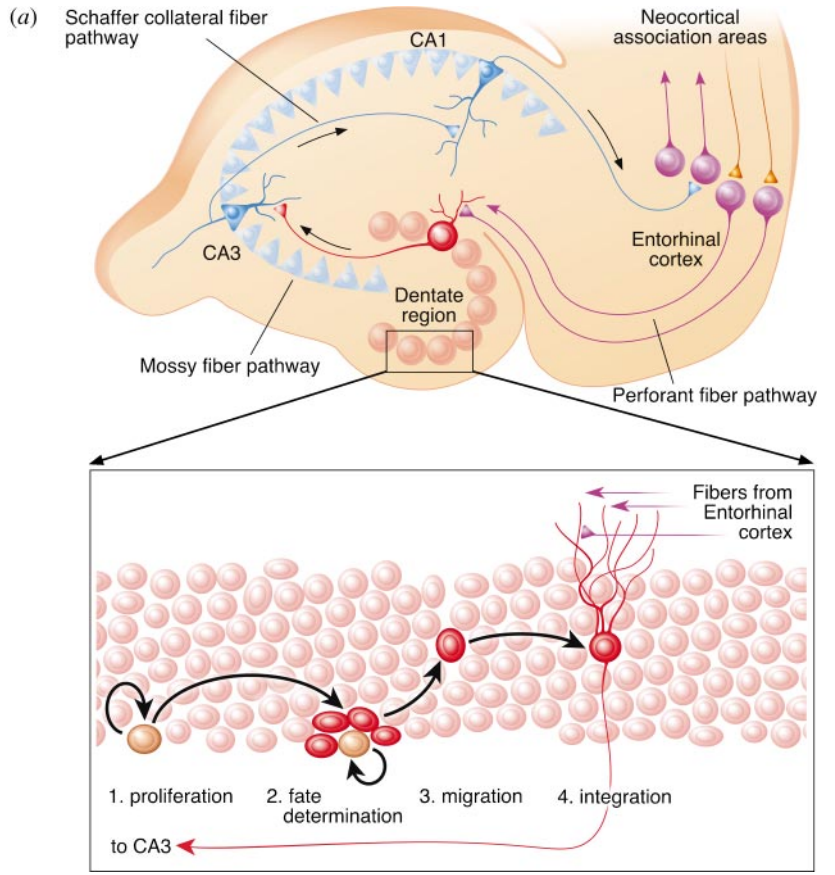
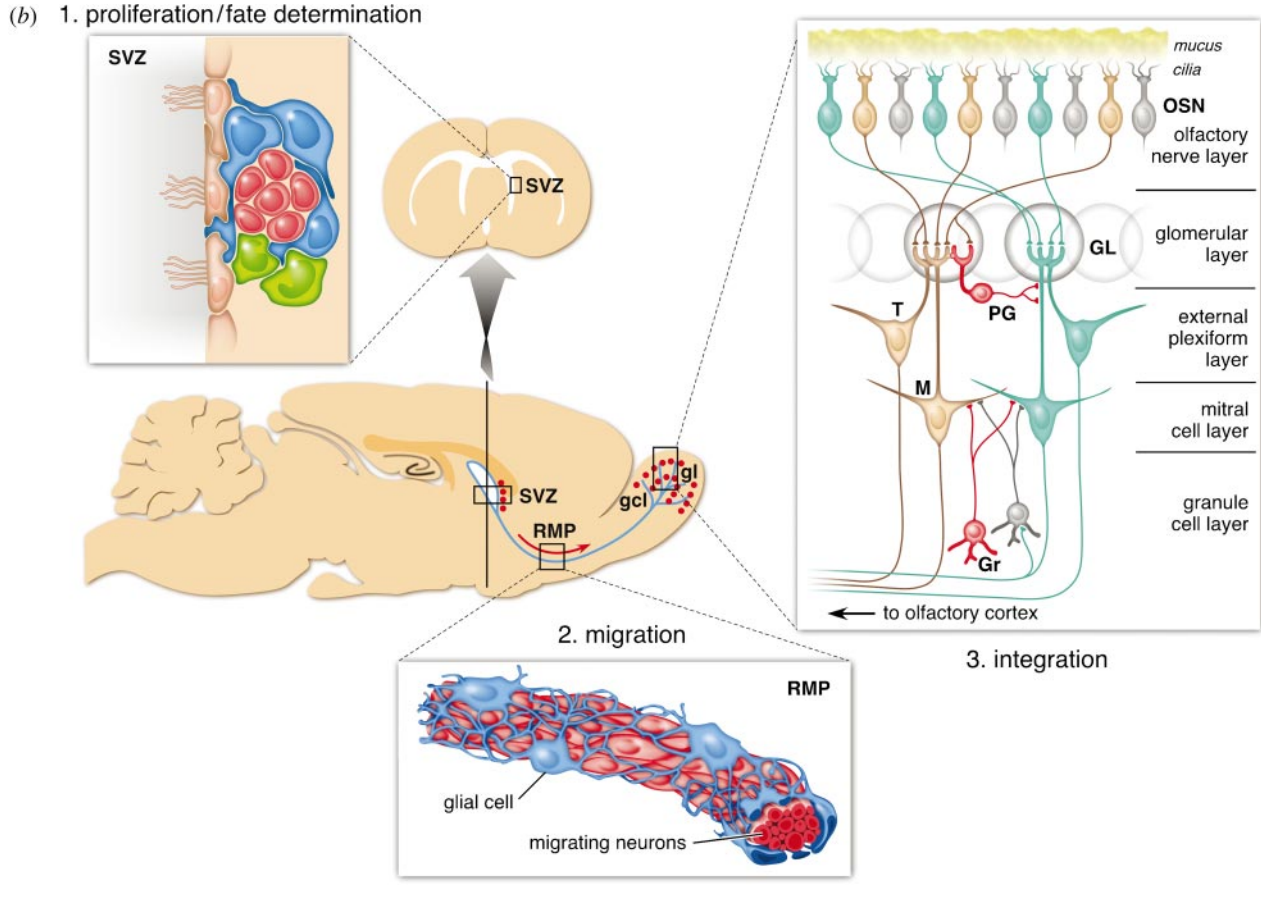


Figure 1 Neurogenic zones of the adult mammalian CNS. (*a*) Hippocampal dentate gyrus. 1. Proliferation and fate determination: Stem cells (*beige*) in the subgranular zone of the dentate gyrus give rise to transit amplifying cells that differentiate into immature neurons. 2. Migration: Immature neurons migrate into the granule cell layer of the dentate gyrus. 3. Integration: Immature neurons mature into new granule neurons, receive inputs from the entorhinal cortex, and extend projections into CA3. (*b*) Subventricular zone (SVZ)/olfactory bulb system. 1. Proliferation and fate determination: Stem cells in the SVZ of the lateral ventricle (*blue*) give rise to transit amplifying cells (*green*) that differentiate into immature neurons (*red*). Adjacent ependymal cells (*light brown*) of the lateral ventricle are essential for the neuronal fate determination by providing inhibitors of glial differentiation. 2. Migration: Immature neurons (*red)* migrate along each other in chains through the rostral migratory pathway (RMP). The migrating neurons are ensheathed by astrocytes (*blue*). 3. Integration: Immature neurons differentiate local interneurons (*red*) in the granule cell layer and the periglomerular layer. Olfactory sensory neurons (OSN); tufted neurons (T); mitral neurons (M); granule neurons (Gr); periglomerular neurons (PG).



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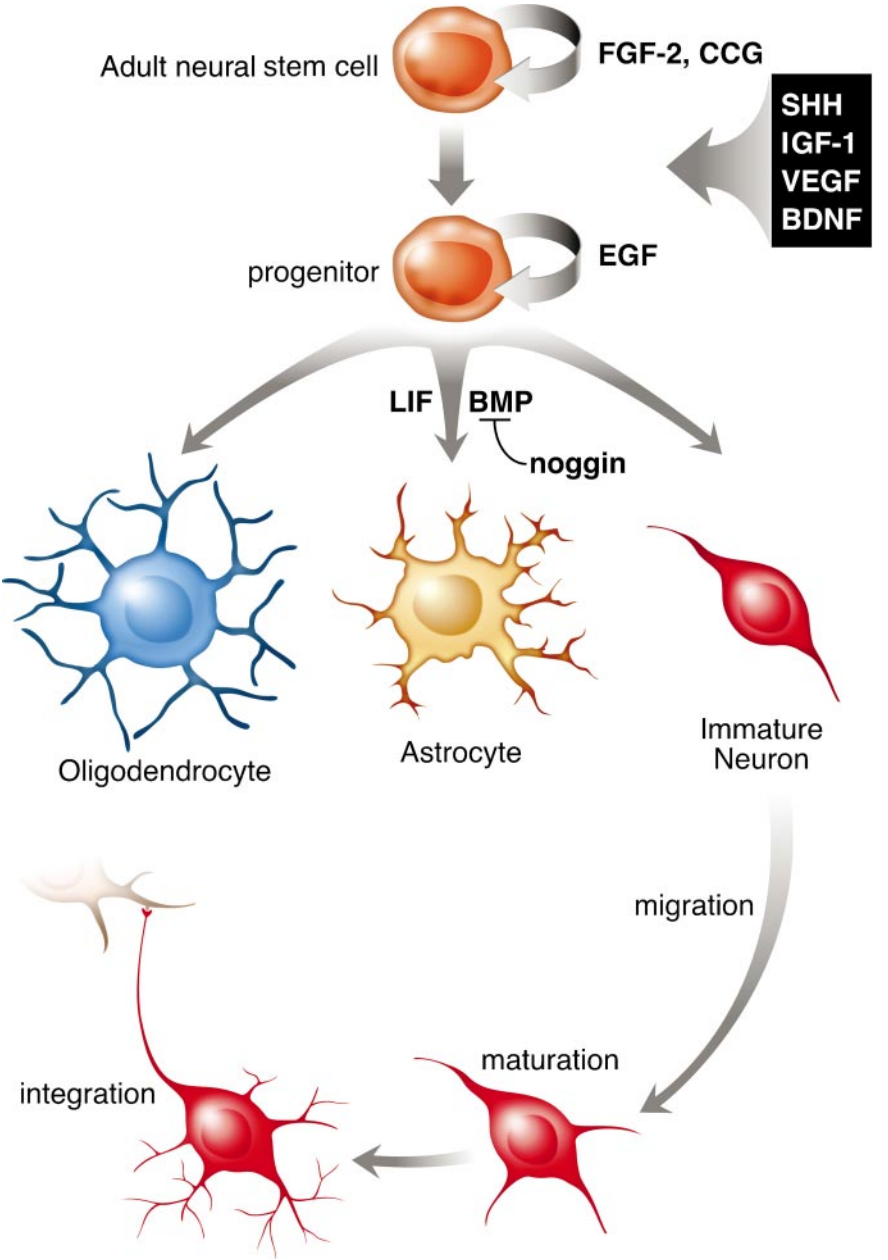

Figure 2 Molecular regulation of adult neurogenesis.

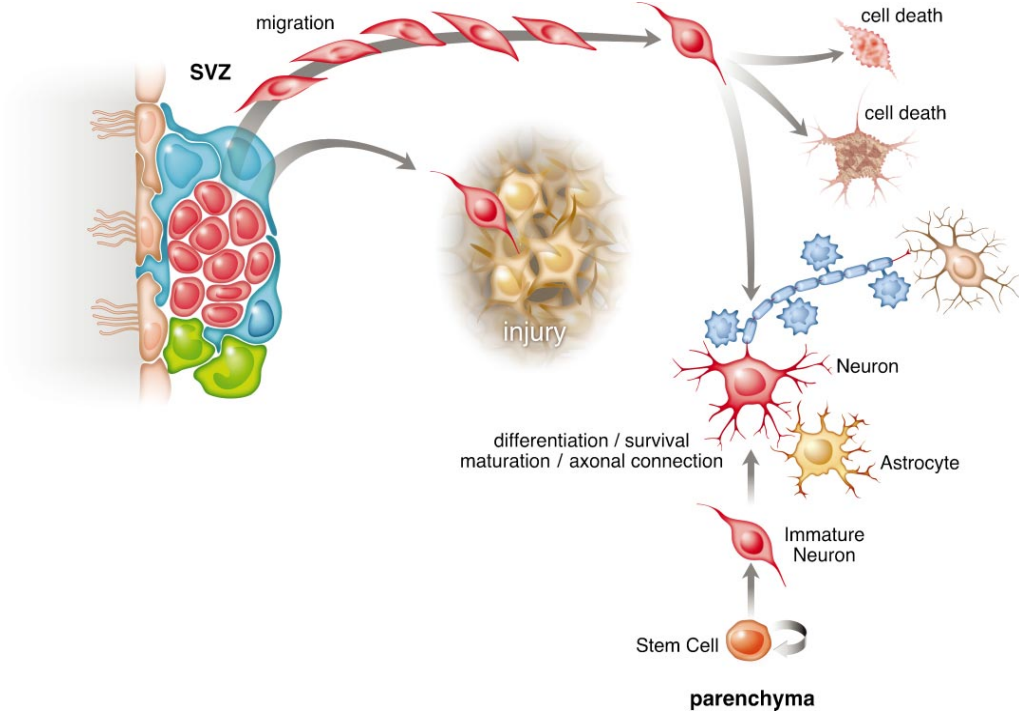

Figure 3 Endogenous neural stem cells in repair. New neurons for replacement of dying neurons in injury and disease can be potentially derived from stem cells in the neurogenic zones, such as the SVZ. These new neurons have to undergo directed migration toward the lesioned CNS region. Alternatively, these new neurons may be derived from resident parenchymal neural stem cells. New neurons have to survive, differentiate/mature into site-specific functional neurons, and form appropriate axonal and dendritic connections in order to contribute to functional repair of the lesioned CNS region. Moreover, it is important to reconstitute other cell types of the lesioned CNS region, such as astrocytes and oligodendrocytes, which provide the necessary environment for neuronal function.

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CONTENTS