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NEUROGENESIS IN ADULT BRAIN

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HISTORIC PERSPECTIVE

The idea that the adult brain retains the capacity to generate new neurons has been proposed several times over the last 40 years, and in each case both conceptual and technical constraints have led to resistance. Joseph Altman first reported that some dividing cells in the adult brain survived and differentiated into cells with morphology similar to neurons using tritiated thymidine autoradiography (1). Over the subsequent years he and his colleagues confirmed these initial observations and focused on the few areas where neurogenesis was apparent in the adult, at the light microscopic level, while systematically documenting the birth dates of neurons throughout the brain during development (2). When contrasting adult neurogenesis with the extensive neurogenesis in development, adult neurogenesis seemed almost like an epiphenomenon. The continued skepticism surrounding adult neurogenesis and the absence of definitive phenotypic markers limited the development of the field. In the mid-1970s and early 1980s Michael Kaplan reexamined the initial observations using the electron microscope and added substantial confidence that not only could neurogenesis occur in the adult brain, but also that the cells appear ultrastructurally, similar to sister cells in the dentate gyrus of the hippocampus, one of the structures shown to be neurogenic (3). In the mid-1980s, Fernando Nottebohm and his student Steve Goldman further stimulated this fledgling field by showing that songbirds experience a seasonal cell death and neurogenesis in a region of the brain important for song production (4). They have continued to reveal more about the environment and molecular regulators of this process in the adult avian brain (5). Despite these observations of neurogenesis in the adult brain, confusion over the mechanism origin of cell genesis in the adult brain persisted. In the early 1990s a series of papers in the adult mouse and rat revealed that cells with stem cell properties could be isolated and expanded in culture. Under a variety of culture conditions with different factors, these isolated cells can be induced to differentiate into glia and neurons (6–9). This later observation provided a mechanism for the neurogenesis in the adult. Mature committed neurons were not dividing, but rather a population of immature stem-like cells exists in the brain and it is likely that it is the proliferation and differentiation of this population that is resulting in neurogenesis. With this conceptual framework the original statement by Cajal that, "Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In adult center, the nerve paths are something fixed and immutable: everything may die, nothing may be regenerated," still holds true for most areas of the adult brain. The new lesson that we have learned is that development never ended in some areas of the brain.

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CHARACTERIZATION OF CELL GENESIS *IN VIVO*

Areas of Neurogenesis

Neurogenesis is a process that includes cell division, migration, and differentiation. There appear, at present, to be only two areas of the brain where stems cells initially reside and proliferate prior to migration and differentiation. Those areas are the lateral subventricular and subgranular zones of the dentate gyrus. The exact cell that corresponds to the initiating stem cell in the lateral ventricle is a point of contention. One view is that this cell is the ependymal cell facing the ventricle (10), whereas an alternative view is that a glial population one cell layer in from the ependyma are the stem cells (11). In any case, after cell division one of the stem cells begins to differentiate and migrate in what Alvarez-Buylla has described as "chain migration" along the rostral migratory stream toward the olfactory bulb where they differentiate into interneurons in the bulb (12). This process continues throughout life; the functional importance and consequences of this process are not understood. The stem cell in the adult mammalian subgranular zone of the dentate gyrus is likely an ectopically displaced cell

originating from the developing ventricular zone. It remains formally possible that a more primitive cell exists elsewhere in the adult brain in a quiescent state, and migrates to the dentate gyrus where the cells begin to divide. However, from the subgranular zone, one of the progeny migrates into the granule cell layer once the cells divide; there the majority become neurons with axons extending to the CA3 pyramidal neurons and receiving synaptic connections. The function of these newly born cells is being investigated.

Although these are the two principal areas where neurogenesis occurs in the mammalian brain, cell genesis occurs throughout the adult brain including cortex, optic nerve, spinal cord, and many brainstem and forebrain structures. To date the function of this cell genesis in the normal intact brain and spinal cord is not known, but some of these new cells can become glial cells (13). A clear challenge for the future is to document all the areas of the adult brain where cell genesis continues, and to understand the normal function as well as the factors that regulate this process.

Mammalian Species in Which Adult Neurogenesis Is Documented

The first studies demonstrating adult neurogenesis were in the rat. Subsequently, rabbit and cat were shown to exhibit similar characteristics, although little additional work has been done in those animals since the original publications (14,15). It was not until 1997 that Kempermann and associates showed the mice retain neurogenesis and that significant genetic variability exists among mouse strains (16). These findings, together with the important role for environmental stimulation as a regulator of neurogenesis, have placed adult neurogenesis as paradigm for examining the interactions of nature and nurture (17,18). Although there was debate in the mid-1980s as to whether nonhuman primates retained adult neurogenesis, a series of papers by Fuchs and associates beginning with tree shrew followed by marmosets and finally with Rhesus monkeys demonstrated and confirmed that neurogenesis in occurs in adult nonhuman primates (19,20). Recently, Gould presented data suggesting that neurogenesis occurs in the adult primate frontal cortex and concluded that the cells are derived from the subventricular zone where they migrate to specific cortical regions of the adult primate brain. This observation awaits confirmation (21). One of the markers for determining cell division is bromo-deoxyuridine (BrdU); a traceable analogy of uridine, which in incorporated into the genome of, cells undergoing cell division. Administering BrdU and then examining cell proliferation in tumor biopsies is occasionally used to monitor tumor progression in patients with cancer. Because BrdU is a small soluble molecule, it is distributed throughout the body including the brain, thus can be a marker for cell and neurogenesis in humans. In 1998 Eriksson and colleagues (22) reported that in five of the cancer patients they examined who received BrdU at between 15 days and over 2 years early, all of them showed neurogenesis as revealed by colabeling of BrdU with markers of mature neurons in the dentate gyrus. Together these studies clearly demonstrate that neurogenesis, at least in the dentate gyrus, is a process that persists throughout life and in all mammalian species (Fig. 8.1). The extent to which or whether neurogenesis can occur in other brain areas remains an area of intense investigation.

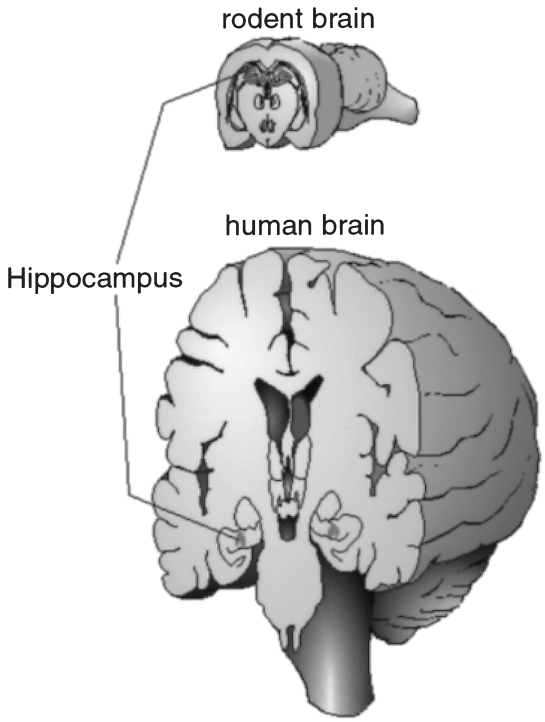

FIGURE 8.1. Birth of new neurons in the adult hippocampus has been documented in a variety of species, including rodents and humans. See color version of figure.

PROPERTIES OF STEM CELLS *IN VITRO*

CNS Areas from Which Stem Cells Can Be Isolated

Neural stem cells can be derived from the adult brain and propagated *in vitro* (6–8,10,11,23–26). Ongoing adult neurogenesis, however, has only been documented convincingly in two brain areas, the subventricular zone/olfactory system and the dentate gyrus of the hippocampus. Interestingly, stem cells are found not only in these regions, but also have been isolated from areas that are non-neurogenic such as the septum, striatum (27), spinal cord (28), cerebral cortex, corpus callosum, and optic nerve (29) and eye (30). In culture, these cells are multipotent and can give rise to neurons and glia. However, cells isolated from areas outside of the hippocampus and subventricular zone require high levels of FGF-2 in order to give rise to neurons, rather than only glial cells (29). These findings suggest that either differ-

ent populations of stem cells exist in the nervous system or that they require unique culture conditions to become multipotent. Alternatively, the cells isolated from different CNS regions may already be committed toward a specific lineage. Indeed, there are no antigenic markers that allow unambiguous identification of stem cells in the nervous system. In the subventricular zone, stem cells are suggested to divide slowly, whereas and their offspring, progenitor cells, may divide more frequently (31). Stem cells in this area have been suggested to ependymal cells (10) or a subclass of glial cells in the subependymal zone (11). The location and identity of the hippocampal stem cell remains to be determined.

Factors That Affect Proliferation and Differentiation of Stem Cells *In Vitro*

A variety of cytokines, neurotrophins, and conditioned media are used to culture neural progenitor cells (32–34). The two major factors are EGF and FGF. Progenitor cells responsive to EGF have been isolated and cultured from adult mouse subventricular zone (6,7,31). FGF-2 has been found to be mitogenic for adult neural progenitors from brain and spinal cord (9,27,28). FGF-2, however, is member of a family of 10 related, but genetically and functionally distinct polypeptides. Among those, only FGF-2 and FGF-4 are mitogens for neural progenitor cells. Moreover, a comparison of amino acid sequences between the FGFs revealed a striking similarity between a 10-amino acid sequence of FGF-2 and FGF-4. This 10-amino acid sequence was been shown to elicit the mitogenic effects of FGF-2 and FGF-4 on neural progenitor cells, whereas similar regions in FGF-1 and -5 were found to be inactive (35).

Several factors have been found to be important for neuronal differentiation in cultured progenitor cells. In particular, retinoic acid and cAMP increase neuronal differentiation (27,36,37). In addition, neurotrophins such as NGF, BDNF, and NT-3 have been found to influence neuronal differentiation and transmitter phenotype (34,37,38), whereas CNTF can regulate glial differentiation of precursor cells (39).

Transplanted Cells and Responses to Local Cues

Progenitor cells may play an important role in brain or spinal cord repair. In particular, grafting of progenitor cells into degenerated or injured areas may be used to replace cells that are no longer functional. The phenotype and function that these cells acquire appears to be very much dependent on the specific environment into which they are transplanted. Thus, cultured hippocampal progenitors become granule cell neurons when grafted into the dentate gyrus, tyrosine hydroxylase, and calbindin positive neurons in the olfactory bulb after grafting into the rostral migratory stream (40). In addition, after implantation into the developing retina these cells showed properties of several types of retinal neurons (37). Moreover, progenitors isolated from a non-neurogenic area such as the spinal cord, acquired the morphologic characteristics of granule cell neurons when grafted into the dentate gyrus, and had a glial phenotype when grafted back in to the spinal cord (41). These studies suggest that neural stem cells derived from the adult mammalian brain retain multipotentiality. Recent research suggests that neuronal stem cells are multipotent outside the CNS as well. It was reported that neural progenitor cells repopulate experimentally depleted bone marrow and reconstitute the hematopoietic system (42). It remains to be determined what the local cues are, that are driving the neuronal precursor cells to acquire such specific fates when transplanted *in vivo.*

REGULATION OF PROLIFERATION AND DIFFERENTIATION *IN VIVO*

The mechanisms that generate new granule cells in the dentate gyrus are poorly understood. A variety of environmental, behavioral, genetic, neuroendocrine, and neurochemical factors can regulate adult neurogenesis. Two critical processes that lead to neurogenesis, cell proliferation and the subsequent differentiation and survival of newborn neurons, can undergo differential regulation by these factors (Table 8.1).

Genetics

In 1997 Kempermann and colleagues found that strains of mice differ with respect to rate of cell division and amount of cell survival and neurogenesis. Comparisons were made among C57BL/6, BalB/c, CD1, and 129/SVJ strains. Proliferation was found to be highest in C57BL/6 mice; however, net neurogenesis was highest in the CD1 strain. 129/SVJ produced relatively more astrocytes and fewer neurons than other strains (16). The degree to which environmental, behavioral, and biochemical factors can affect cell proliferation and neurogenesis may also differ depending on the species or strain of animal involved. Indeed, exposure to an enriched environment (18) had different effects on two of these strains of mice, C57BL/6 and 129/SVJ, respectively. In C57BL/6 mice enrichment promoted the survival of progenitor cells but did not affect proliferation, whereas the net increase in neurogenesis in 129/SVJ mice was accompanied by a twofold increase in proliferation (18). Thus, strain differences not only influence the baseline rate of adult hippocampal neurogenesis, but also influence how adult hippocampal neurogenesis is regulated in response to environmental stimulation. Indeed, proliferation, survival and differentiation of progenitor cells and their progeny are each separately influenced by inheritable traits and are not uni-

TABLE 8.1. REGULATION OF CELL PROLIFERATION AND NEUROGENESIS IN THE DENTATE GYRUS *IN VIVO*

Factor	Proliferation	Glia	Neurons	References
FGF	→	→	→	44,46
EGF	→	↑	↓	44
IGF	↑	→	↑	47
Estrogen	↑	→	→	67
Serotonin	↑	n.d.	n.d.	73,74,75
Glutamate	↓	n.d.	n.d.	65,70
MK801	↑	n.d.	↑	
Enriched environment	→	→	↑	17,18,80
Wheel running	↑	→	↑	55,56
Learning	→	n.d.	↑	83
	→	→	→	56
Stress	↓	n.d.	n.d.	60,61
Glucocorticoids	↓	n.d.	n.d.	57,58,59,64
Adrenalectomy	↑	n.d.	↑	
Stroke	↑	n.d.	↑	90,91
Epilepsy/seizures	↑	n.d.	↑	85,86,87,88,89,100
Vitamin E deficiency	n.d.	n.d.	↑	99
Aging	↓	n.d.	↓	63,64,81

formly up-regulated in response to environmental stimulation.

Growth Factors

During development, growth factors provide important extracellular signals for regulating the proliferation and fate determination of stem and progenitor cells in the CNS (43). Several studies have been carried out to investigate progenitors in the adult brain respond to such growth factors. Intracerebroventricular infusion of EGF and FGF-2 in adult rats increased proliferation in the subventricular zone (44). Neither EGF nor FGF enhanced proliferation in the subgranular zone of the dentate gyrus. With regard to differentiation, EGF promoted glial differentiation, whereas FGF-2 did not influence phenotype distribution (44). In another series of experiments, FGF was administered systemically during the first postnatal weeks and in the adult rat. Cell proliferation was increased the dentate gyrus of infant rats but not in the adult hippocampus (45,46). Recent research has shown that intracerebral infusion of IGF increases both cell proliferation and neurogenesis in hypophysectomized rats (47). In songbirds, seasonal regulation of adult neurogenesis depends on testosterone levels that mediate their effect through BDNF (48). In addition, IGF-1, FGF mRNA, and BDNF mRNA are elevated in rodents by exercise (49–52). Expression of BDNF (53) and GDNF is increased by exposure to an enriched environment (54). Both running and enrichment increase net neurogenesis (17,55,56). The effects of intracerebral administration of trophins such as BDNF, NT-3, and GDNF remain to be determined; however, it appears that growth factors do play a role in *in vivo* regulation of proliferation and neurogenesis in the adult hippocampus. Better understanding of their mechanisms of action may lead to therapeutic application of these factors after brain injury or disease.

Neuroendocrine Factors and Stress

McEwen and Gould at Rockefeller University first investigated the effects of glucocorticoids or stress on adult hippocampal neurogenesis. The initial study reported that adrenalectomy, which leads to a reduction in serum glucocorticoid levels, elicits cell division in the dentate gyrus. This effect could be reversed by corticosterone replacement (57). Conversely, stress or increased levels of glucocorticoid hormones inhibit proliferative activity in the dentate gyrus (58,59). For example, administration of high levels of corticosterone diminishes cell division in the adult rat hippocampus (59). In addition, exposure of a rat to the odor of a natural predator (fox), causing stress and elevated corticosterone levels, transiently suppressed cell proliferation in the

adult rat dentate gyrus (60). Furthermore, exposure of marmoset monkeys to a resident intruder causes stress and results in a decrease in cell proliferation (61). In a recent study, rats that are highly reactive to novelty and exhibit a prolonged corticosterone secretion in response to novelty and stress were found have reduced dentate gyrus cell proliferation (62). Aging is accompanied by a reduction in neurogenesis (63), which may be caused in part by elevated glucocorticoid levels. Adrenalectomy in aged rodents has been shown to increase cell proliferation and neurogenesis (64). The effects of glucocorticoids on cell genesis appear to be mediated via a downstream effect on NMDA glutamate receptors (65). Thus, glucocorticoids and stress associated with increased corticosterone secretion inhibit cell genesis in the hippocampus. Enhanced stress or glucocorticoid levels therefore may impair hippocampal function, and lead to deficits in learning and memory. In contrast to the glucocorticoids, other steroid hormones, such as testosterone, enhance neurogenesis in birds (66), whereas estrogen results in a transient increase in proliferation in rats (67). Thyroid hormone can affect neuronal differentiation of hippocampal progenitor cells *in vitro* (27). *In vivo,* hypothyroidism interferes with cell migration (68), but does not affect postnatal cell proliferation (69).

Neurotransmitters

Neurotransmitters have also been suggested to play a role in adult dentate gyrus neurogenesis. Systemic injection of glutamate analogs inhibits birth of new cells, whereas an antagonist, such as MK801, enhances cell division (65,70). Recently, another class of neurotransmitters, the monoamines, has been suggested to be important as well. Prolonged administration of fluoxetine, as well as therapeutic agents acting on norepinephrine and dopamine receptors, and electroconvulsive shock enhance the number of BrdU-positive cells in rats (71–73). Acute administration of fluoxetine did not affect cell genesis (73). Grafting of fetal raphe neurons also stimulated granule proliferation in the hippocampus, whereas embryonic spinal tissue had no effect (74). Furthermore, depletion of serotonin reduces stem cell proliferation in the dentate gyrus (75). It is possible that these effects are mediated by the 1A receptor, because administration over 4 days of a specific 1A receptor antagonist (WAY) reduced basal rate of cell proliferation (Jacobs et al., unpublished observations). Taken together, these findings suggest that induction of cell proliferation is dependent on chronic administration of monoamines, consistent with the therapeutic time course for antidepressant treatments. Indeed, these studies have led to the hypothesis that therapeutic interventions that increase serotonergic transmission may act in part by augmenting dentate neurogenesis, promoting recovery from depression (76,77). It is of interest to note in this context that voluntary exercise increases cell proliferation (55), enhances monoamine levels and has an antidepressant effect (78). Thus, monoamines can affect cell genesis in the dentate gyrus. The receptors and mechanisms by which they exert their effects as well as possible interactions with other classes of neurotransmitters and/or growth factors remain to be determined.

Experience

As mentioned, stress (19) and depression may reduce the birth of new neurons. In addition, the aging process is accompanied by a decrease in neurogenesis (63); however, there are several environmental and behavioral interventions that can enhance neurogenesis (Fig. 8.2). In 1997 Kempermann and colleagues carried out the first of these studies comparing mice living under standard conditions with those housed in an enriched environment (17). Exposure to an enriched environment, consisting of larger housing; toys; and more opportunity for social stimulation, physical activity, and learning than standard laboratory conditions (79), resulted in a significant increase in neurogenesis, without affecting cell proliferation in mice and rats (17,80). Subsequent studies showed that the age-related decline in neurogenesis could be attenuated by enrichment (81). In addition, it was shown that enrichment inhibits cell death by apoptosis and prevents seizures (54). Moreover, it was determined that the most important components of enrichment are increased physical activity and possibly learning. Similar to enrichment, voluntary exercise in a running wheel increases net neurogenesis (55). In addition, running increases

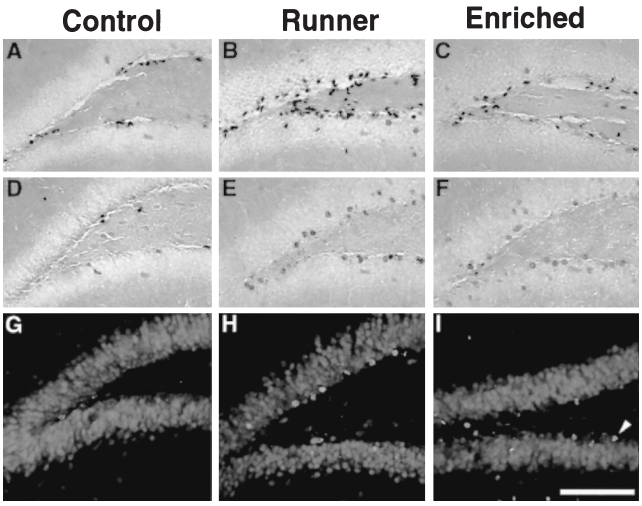

FIGURE 8.2. Proliferation and neurogenesis in the dentate gyrus. Photomicrographs of BrdU-positive cells 1 day *(a–c)* and 4 weeks *(d–f)* after the last injection in control *(a,d),* running *(b,e),* and enriched *(c,f)* mice. Confocal images of BrdU positive cells in control *(g),* running *(h),* and enriched *(i)* mice, 4 weeks after the last injection. Sections were immunofluorescent triple labeled for BrdU *(red),* NeuN indicating neuronal phenotype *(green),* and s100B selective for glial phenotype *(blue).* Orange (*arrow,* newborn neuron) is red + green. Scale bar is 100 μm. See color version of figure.

cell proliferation in the dentate gyrus (55). It is interesting to note that enrichment and running had the same net effect on neurogenesis, but that running increased proliferation, whereas enrichment did not. Thus, not only the genetic factors mentioned, but also different environmental and behavioral factors can have differential effects on cell proliferation and neurogenesis. Others reported that hippocampus-dependent tasks, such as spatial learning in the Morris water maze (82), increases the number of surviving BrdU-positive cells (83,84); however, in our laboratory there was no effect of learning on proliferation or survival of newborn hippocampal cells (55).

Apart from these rather innocuous manipulations, there are several pathologic events that can affect granule cell number. Damage to the hippocampus by kindling (85,86), seizures (87–89), ischemia (90,91), or mechanical lesions (92) enhances proliferation. Thus, both normal and pathologic circumstances can affect cell genesis. Whether increased proliferation is beneficial for function or may represent compensation for lost cells and/or function remains to be determined.

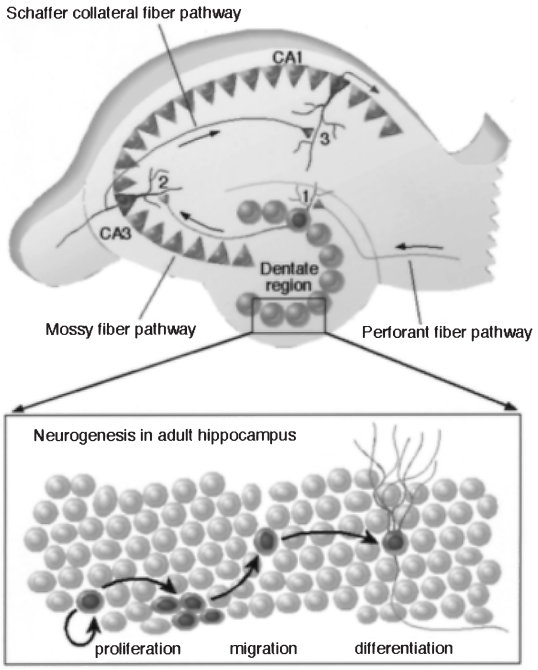

FIGURE 8.3. The three major areas of the hippocampus are the dentate gyrus, CA3, and CA1. The perforant pathway *(1)* from the subiculum forms excitatory connections with granule cells of the dentate gyrus. Both newborn and existing granule cells give rise to axons that form the mossy fiber pathway *(2)*. This pathway projects to area CA3 pyramidal cells. The CA3 cells project to CA1 pyramidal cells by Schaffer collaterals. Within the hippocampal system, only the dentate gyrus gives rise to new neurons in the adult brain. These cells proliferate, migrate, and differentiate into mature neurons in the dentate gyrus (see box). See color version of figure.

FUNCTIONAL SIGNIFICANCE OF NEUROGENESIS

Adult neurogenesis has been reported to exist over more than three decades, and to occur in a variety of species, including humans; however, the functional role of these new cells has yet to be determined. Given that the hippocampus is important for some forms of learning and memory and related mechanisms of neural plasticity such as long-term potentiation (LTP), much of the research has focused on finding a relationship between neurogenesis and memory function.

Behavior

It was found in several studies that animals living in an enriched environment not only had more new neurons, but also performed better on a spatial learning task (17,18,80). In addition, mice that were housed with a running wheel had more new neurons than their sedentary counterparts (55) and performed better on the water maze task (56). As mentioned, neurogenesis declines with aging (63); however, exposure to an enriched environment can restore some of the neurogenesis and improve performance on the water maze task in aging mice (81). Whether voluntary exercise in a running wheel would yield similar results in aged animals remains to be determined.

Electrophysiology

Although we can identify new granule cells using histologic and morphologic techniques, the question remains whether these cells are physiologic functional and if so, how similar or different are they from existing granule cells (Fig. 8.3). Moreover, what could be the functional significance of their (possible) neuronal activity? It has been shown that new hippocampal granule cells send axons along the mossy fiber tract to CA3 as do all other granule cells (93–95) and that they receive synaptic contacts (93). A recent study compared LTP, a physiologic model of certain forms of learning and memory (96) in the dentate gyrus, and CA1 in hippocampal slices from running and control mice. LTP amplitude was selectively enhanced in the dentate gyrus of running mice (56). It is possible that the newborn granule cell neurons play a role in increased dentate gyrus LTP because running increases learning and neurogenesis. Although the new cells are a small percentage of the granule cell layer, the possibility exists that they have greater plasticity than do mature cells. Indeed, dentate gyrus LTP last longer in immature rats than adults (97); however, in order to test this hypothesis it is necessary to record activity from individual new granule cells. In a recent study granule cells from the inner and outer layer of the dentate gyrus were compared. The inner layer cells were considered to be "young" cells and the outer layer "old" cells. The researchers found that the putative

young cells had a lower threshold for LTP and were unaffected by GABA-A inhibition, suggesting enhanced plasticity in the "young" cells (98); however, the problem with this study is that the definition of "young" cells is ambiguous. In our studies we have found newly generated cells throughout the granule cell layer. Moreover, the only way to be certain that a cell is a newborn neuron is by labeling the cell when it divides with a mitogenic marker. Ideally, recordings would be made from such a labeled cell and compared with preexisting neurons.

In summary, adult hippocampal neurogenesis appears to be associated with memory function. Indeed, performance on a learning task is better in animals in which neurogenesis is stimulated, such as by running or enrichment, than in controls. This link may between neurogenesis and behavior may be causal rather than correlational, given that electrophysiologically measurable changes occur in the brain region where adult neurogenesis occurs.

POTENTIAL THERAPEUTIC IMPLICATIONS

Several areas of interest emerge when considering the therapeutic potential for neurogenesis. First, a strategy that takes advantage of the ability of stem cells from the adult brain to be isolated and induced to divide in culture opens the opportunity for cellular transplantation to replace cells that have died because of injury or disease. Although evidence supports that ability of adult stem cells to survive grafting to the adult brain, the fate of the grafted cells appears to be dictated by the local environment. Thus, in order to accurately replace cells in damaged areas of the brain significant new information about the cellular and molecular mechanisms that control fate decisions is required in order to "train" the immature cells in culture to respond to the unique features of each of the environment to which they are grafted.

A second strategy emerges as a direct result of the fact that the adult brain retains stem cells *in situ* throughout life. By understanding the internal factors, molecules and mechanisms as well as the external stimuli and influences that control and regulate each of the steps in neurogenesis *in vivo,* eventually the potential of the endogenous cells could be harnessed. To this end, cell number could be amplified, direction of migration could be targeted, and finally terminal fate could be specified to the extent that a form of "self-repair" could be induced or orchestrated in the adult damaged brain. In all cases a more complete understanding the cellular and molecular events directing neurogenesis is a prerequisite for the use of this process in rational strategies for therapy.

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