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Developmental Mechanisms in Aging and Age-Related Diseases of the Nervous System

Mark P. Mattson and Tobi L. Limke

INTRODUCTION

This chapter provides developmental neurobiologists with an overview of cellular and molecular changes that occur in the nervous system during aging, describes the current state of understanding of how aging impacts developmental processes operative in the adult nervous system, and considers how developmental mechanisms may contribute to the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Although studies of invertebrates, particularly *Caenorhabditis elegans* and *Drosophila*, have provided vital information on the molecular regulation of development, they have not yet been tapped to study mechanisms of nervous system aging. This chapter, therefore, focuses almost exclusively on the aging of mammalian nervous systems. While many age-associated changes in the nervous system also occur in other tissues, we will focus on those that have the highest impact (such as oxidative stress and protein accumulation) and those that are relatively unique to the nervous system (such as the age-associated alterations in the Notch–Delta signaling pathway). We will then explore some of the mechanisms that not only regulate development of the nervous system, but also play a role in aging in both the normal and diseased brain.

We now know that a spectrum of developmental processes operates in the adult mammalian nervous system. The adult nervous system is not "hard-wired"; instead, neuronal circuits undergo structural remodeling in response to environmental demands. Like other tissues, there are cells in the nervous system capable of undergoing proliferation, differentiation, and programmed cell death (apoptosis), as well as a number of more subtle changes that alter neural structure and function. For example, hippocampal synapses may form, disassemble, or change their shape in response to learning, stress, and fluctuations in levels of sex steroids (McEwen, 2001). In neurogenic regions of the adult brain, there are dynamic populations of stem cells capable of dividing and differentiating into neurons or glial cells (Gage, 2000). Programmed cell death (apoptosis) also occurs in the adult nervous system, at a low level under normal conditions, and at an accelerated pace following injury or in certain neurological disorders (Mattson, 2000). As far as is known, developmental processes in the mature nervous system are regulated by similar, if not identical, signaling mechanisms to those employed during embryonic development. Thus, members of each of the major types of signaling systems employed in embryonic development are operative in the adult. The impact of aging on these signaling pathways, and the consequences for age-related alterations in the cytoarchitecture and function of the nervous system, will therefore be given considerable attention in this chapter. In order to understand how developmental mechanisms may contribute to normal aging and age-related dysfunction and diseases in the nervous system, it is first necessary to understand the cellular and molecular changes that occur during aging.

CELLULAR AND MOLECULAR CHANGES DURING NORMAL AGING

Aging in all tissues, including the nervous system, involves a progressive loss of normal function as a result of intrinsic and extrinsic forces (Fig. 1). These processes occur during normal aging, in the absence of disease; however, as will be discussed later, many of these processes are exacerbated during age-related neurodegenerative disorders and often accelerate the damage and/or inhibit effective repair. Changes that occur in the nervous system during normal aging include increased oxidative damage to proteins and DNA, accumulation of protein and lipid byproducts (e.g., lipofuscin and advanced glycation end products), reduced metabolic activity, mitochondrial dysfunction, and cytoskeletal alterations. These processes affect terminally differentiated cells as well as proliferating and maturing stem/progenitor populations. However, there are also age-related changes that are unique to the nervous system that are likely the result of the molecular complexity of neurons and glial cells, which express approximately 50–100 times more genes than cells in other

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Developmental Neurobiology, 4th ed., edited by Mahendra S. Rao and Marcus Jacobson. Kluwer Academic / Plenum Publishers, New York, 2005.

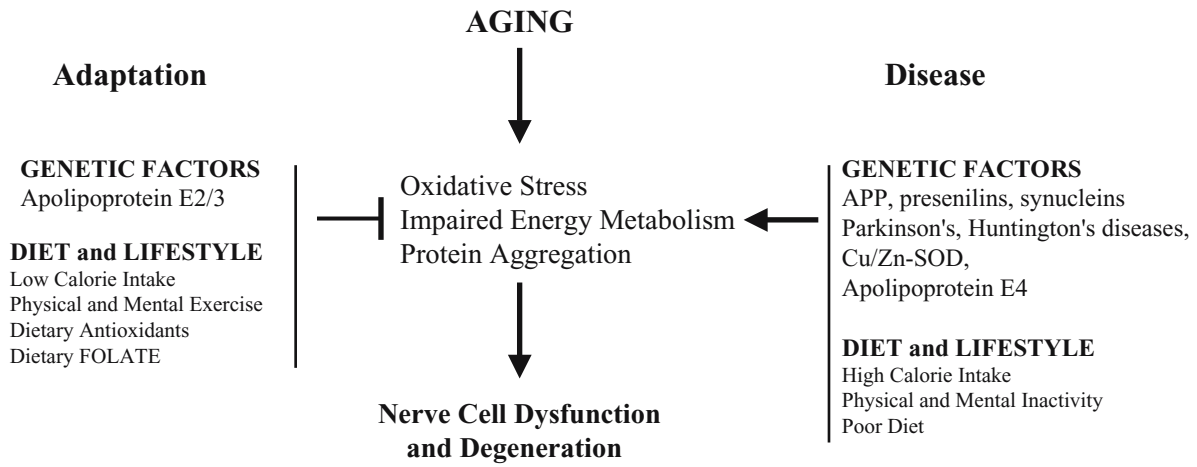

FIGURE 1. The nervous system may age successfully, or may suffer disease, depending upon its ability to adapt to adversity. Both intrinsic (genetic) and extrinsic (environmental) factors influence the outcome of aging. Successful aging of the nervous system is achieved when cells are able to adapt by enhancing their ability to resist degeneration and restore damaged neuronal circuits.

TABLE 1. Mechanisms that Regulate Successful and Unsuccessful Development and Aging in the Nervous System

Trophic factors (bFGF, BDNF)	Oxidative stress
Adhesion molecules (integrins)	Metabolic stress
Neurotransmitters (glutamate)	Diet (caloric intake)
Gases (nitric oxide)	Behavior (exercise)

tissues. The many different signal transduction pathways for neurotransmitters, trophic factors, and cytokines are examples of such complex regulatory systems that may be particularly prone to modification by aging. Many different genetic and environmental factors undoubtedly play roles in determining whether the nervous systems ages successfully by adapting to the aging process, or unsuccessfully resulting in disease. Interestingly, many of these determinant factors also play a critical role in developmental processes (Table 1).

Age-Related Cytoarchitectural Changes in the Nervous System

While the most dynamic structural changes in the cellular composition of the nervous system occur during embryonic and early postnatal development, there are similar but more subtle changes that occur throughout adult life. The changes include neurogenesis and gliogenesis, cell death, dendritic and axonal growth or retraction, synapse loss and remodeling, and glial cell reactivity. Alterations in cellular signaling pathways that control cell growth and motility may contribute to both adaptive and pathological structural changes in the aging brain. A prime example is glutamate, the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate plays important roles in regulating dendritic growth cone motility and synaptogenesis during brain development (Mattson *et al*., 1988a, b, 1989) and in regulating synaptic plasticity in the adult (Izquierdo, 1994), but may also contribute to synaptic degeneration and cell death in aging and age-related disorders such as Alzheimer's disease and stroke (Hugon *et al*., 1996; Mattson and Furukawa, 1998).

Because cellular structure is controlled by the cytoskeleton, many architectural changes in the brain with aging result from alterations in cytoskeletal proteins. The primary cytoskeletal components of cells are actin microfilaments (6 nm diameter); intermediate filaments (10–15 nm diameter), made of one or more cell type-specific intermediate filament proteins (e.g., neurofilament proteins in neurons and glial fibrillary acidic protein in astrocytes); and microtubules (25 nm in diameter), which are made of tubulin. In order to control the polymerization dynamics of cytoskeletal filaments and their interactions with other cytoskeletal components and membranes, cells express an array of cytoskeleton-associated proteins that are particularly complex in neurons. For example, several different microtubule-associated proteins (MAPs) are expressed in neurons where they are differentially distributed within the complex neuritic architecture of the cell. A well-known example is the presence of MAP-2 in dendrites and its absence in the axon, whereas an MAP called tau is present in axons but not in dendrites (Mandell and Banker, 1995). Alterations in the subcellular localization and phosphorylation state of MAPs are widely documented in aging and neurodegenerative disorders (Mandelkow and Mandelkow, 1995).

Studies of rodents and primates have revealed several changes in the cytoskeleton of neurons and glial cells during aging (Fig. 2). Overall levels of cytoskeletal proteins (tubulin, actin, and neurofilament proteins) do not change appreciably with normal aging, with a few exceptions. One cytoskeletal protein that does increase consistently during normal brain aging in humans and laboratory animals is the astrocytic intermediate filament protein glial fibrillary acidic protein (Morgan *et al*., 1999); this increase is characteristic of activated astrocytes and may therefore result from a reaction to subtle neurodegenerative changes. Several changes in the cytoskeletal organization and in posttranslational modifications of cytoskeletal proteins occur in the aging nervous system. Neurites may become distorted or dystrophic,

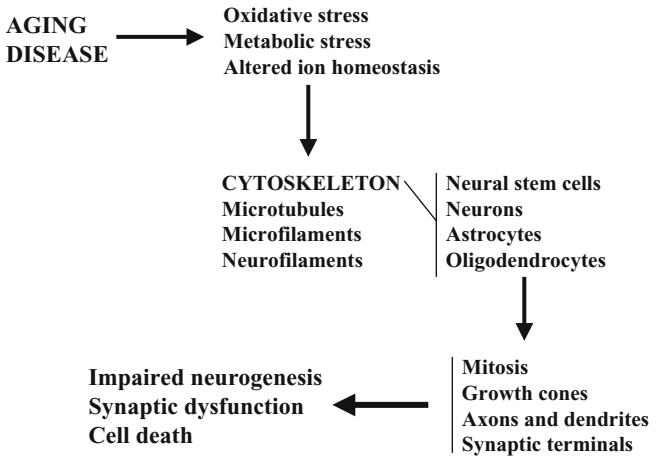

FIGURE 2. Roles of the cytoskeleton in aging and disorders of the nervous system. Increases in oxidative stress, impaired energy metabolism, and perturbed cellular ion homeostasis result in modifications of the cytoskeleton of neurons, glia, and neural stem cells. The modifications may include increased or decreased protein phosphorylation, oxidative modifications, and changes in polymerization state and interactions with cytoskeleton-associated proteins. The alterations in the cytoskeleton may adversely affect neurogenesis, neurite outgrowth, and synaptic plasticity, and may ultimately result in the death of neurons, glia, and neural stem cells.

while astrocytes may assume a more ramified structure. One prominent type of posttranslational alteration that occurs during aging is an increase in phosphorylation of several cytoskeletal proteins. For example, increased phosphorylation of the MAP tau occurs in neurons in some brain regions, particularly those involved in learning and memory, such as the hippocampus and basal forebrain. Increased or decreased proteolysis of cytoskeletal proteins may result in localized loss or accumulation of the proteins. Calcium-mediated proteolysis of cytoskeletal proteins, such as MAP-2 and spectrin, increases in some neuronal populations during aging (Nixon *et al*., 1994). On the other hand, aggregates of several proteins occur during aging in humans including tau, amyloid beta-peptide, alpha-synuclein, and ubiquitin (Johnson, 2000). As the result of increased levels of oxidative stress during aging, there is increased oxidative modification of cytoskeletal proteins which can manifest as carbonyls, glycation, and covalent binding of lipid peroxidation products such as 4-hydroxynonenal (Keller and Mattson, 1998). Cytoskeletal alterations are also a prominent feature of Parkinson's disease, with abnormal accumulations of neurofilaments, associated MAPs (particularly MAP-1b), alpha-synuclein, and actin-related proteins such as gelsolin, forming in neurons (Braak and Braak, 2000). Lower motor neurons are also vulnerable to age-related disease; in amyotrophic lateral sclerosis, motor neurons become filled with massive accumulations of neurofilaments that are concentrated in proximal regions of the axon (Julien and Beaulieu, 2000).

Synaptic remodeling occurs in the adult nervous system with the extent of remodeling depending on the particular neuronal circuits involved and the environmental demands that are placed upon those circuits. For example, synaptic connections in the hippocampus are modified by learning and memory (Muller *et al*., 2000), physical activity (Cotman and Berchtold, 2002), psychosocial stress (Fuchs *et al*., 2001), and even changes in diet (Prolla and Mattson, 2001). Studies of synapses during the aging of rodents and humans suggest that in some brain regions there may be decreases in synaptic numbers, but that such decreases may be offset by increases in synaptic size, whereas in other brain regions, no changes in synapse numbers or size can be discerned (Bertoni-Freddari *et al*., 1996). There may be a preferential loss of synapses and neurons with particular neurotransmitter phenotypes during aging. For example, cholinergic synapses on dendrites of cortical layer V pyramidal neurons are reduced in numbers during aging to an extent greater than other types of synapses (Casu *et al*., 2002). Studies of cerebellar circuitry indicate that the numbers of synapses on Purkinje cell dendrites decrease during aging, but the size of each synapse increases (Chen and Hillman, 1999). Thus, there is considerable evidence that synaptic remodeling occurs in the CNS during aging (DeKosky *et al*., 1996).

Age-Related Molecular Changes in the Nervous System

Many of the molecular alterations that occur in the nervous system also occur in other tissues and can therefore be considered typical of aging. However, some age-related molecular changes may be confined to specific regions of the nervous system, or to specific neuronal circuits. For example, a progressive loss of D2 dopamine receptors occurs during aging and may contribute to age-related deficits in motor function (Roth, 1995). In humans, the protein content of the brain typically decreases with aging, which likely plays a major role in the progressive decrease in overall brain weight that occurs with aging. Insoluble aggregates of proteins accumulate in the brain during aging, with the cytoskeletal protein tau and Aβ being the two most closely linked to age-related neurodegeneration. Changes in membrane lipids during aging have been documented in numerous studies, with one prominent change being an increase in the levels of sphingomyelin (Giusto *et al*., 1992). A conspicuous lipid alteration during aging is the intracellular accumulation of damaged membrane lipids which form autofluorescent lipofuscin granules. Although there is little or no change in overall DNA content in the brain during aging, brain region-specific changes in RNA levels have been documented. Thus, levels of RNA decrease in the basal nucleus of Meynert, in several regions of cerebral cortex, and in some cranial nerve nuclei with advancing age, whereas RNA levels increase in the subiculum (Naber and Dahnke, 1979). While global changes in the molecular composition of the nervous system do not change dramatically during aging, numerous alterations in specific molecules have been identified.

Oxidative Damage during Aging

The most widely documented changes during aging are those resulting from increased oxidative stress. Free radicals are molecules with an unpaired electron in their outer orbital, which

makes them highly reactive and capable of damaging other molecules by abstracting hydrogen ions. A prominent free radical produced in cells is the superoxide anion radical ($O_2^-\cdot$), which is generated in mitochondria during the electron transport process, as well as by the activities of various oxygenases (e.g., cyclooxygenases). Superoxide is normally eliminated from cells via the activity of manganese- and copper/zinc superoxide dismutases (MnSOD and Cu/ZnSOD), which convert $O_2^-\cdot$ to hydrogen peroxide (H_2O_2). However, hydrogen peroxide is a source of a damaging free radical called hydroxyl radical ($\cdot OH$), formed in a reaction catalyzed by Fe^{2+} and Cu^+. Because of its potential to be toxic, cells possess enzymes called glutathione peroxidases and catalases that eliminate hydrogen peroxide. Another free radical in cells of the nervous system is nitric oxide which is formed as the result of calcium-mediated activation of enzymes called nitric oxide synthases. A related reactive oxygen molecule called peroxynitrite is formed as the result of the interaction of superoxide with nitric oxide. The importance of oxyradicals in aging is emphasized by compelling evidence that there is an increase in production and accumulation of oxyradicals in essentially all tissues in the body during the aging process, including the brain (Sastre *et al*., 2000). As a result, there is progressive oxidative damage to membrane lipids, proteins, and nucleic acids that apparently contributes to neural impairments during aging.

During aging, free radicals can attack the double bonds of membrane lipids in a process called lipid peroxidation. This process impairs the function of various types of membrane proteins in neurons and glial cells including receptors, ion-motive ATPases, glucose and glutamate transporters, and GTP-binding proteins (Mattson, 1998). This may occur as the result of covalent modification of the membrane proteins by an aldehydic product of lipid peroxidation called 4-hydroxynonenal. Lipid peroxidation-related changes may also contribute to a variety of age-related changes throughout neurons and other cells. For example, covalent modification of cytoskeletal proteins by 4-hydroxynonenal can alter protein phosphorylation resulting in abnormalities in cytoskeletal dynamics (Mattson *et al*., 1997). In addition, functions of mitochondria and the endoplasmic reticulum can be adversely affected by lipid peroxidation. By altering the function of ion channels and ion-motive ATPases, lipid peroxidation can have a particularly damaging effect on cellular ion homeostasis (Mattson, 1998; Lu *et al*., 2002).

Oxidative damage to nuclear and mitochondrial DNA occurs in cells of the nervous system during development and throughout adult life. In the nucleus, damaged DNA is normally repaired by highly efficient DNA repair enzyme systems, whereas in mitochondria, damaged DNA is less readily repaired. During aging, and particularly in age-related neurodegenerative disorders, DNA damage may become excessive and may trigger cell death (Rao, 1993; Mattson, 2000). DNA damage can also cause cell cycle arrest and/or death of mitotic cells including glia and neural progenitor cells (LeDoux *et al*., 1996; Cheng *et al*., 2001). Many age-related oxidative processes are greatly enhanced in neurodegenerative disorders. Studies of brain tissues of patients with Alzheimer's and Parkinson's diseases have revealed increased levels of protein oxidation in vulnerable brain regions and, in particular, in degenerating neurons. Two proteins shown to be heavily glycated in AD are Aβ and tau, the major components of plaques and neurofibrillary tangles, respectively.

Mitochondrial DNA damage can be extensive during normal aging, largely because mitochondria are sites where the vast majority of free radicals are generated and because cells do not possess effective systems for repair of damaged mitochondrial DNA. Damage to mitochondrial DNA can lead to failure of mitochondrial electron transport and reduced ATP production, and can impair calcium-regulating functions of mitochondria. These changes can render neurons vulnerable to excitotoxic and metabolic insults. The importance of mitochondrial oxyradical production in aging in general is underscored by recent studies of the mechanism whereby caloric restriction extends lifespan in rodents and nonhuman primates. Levels of cellular oxidative stress (as indicated by oxidation of proteins, lipids, and DNA) are decreased in many different nonneural tissues of rats and mice maintained on a calorie-restricted diet (30–40% reduction in calories). Recent studies suggest that levels of oxidative stress are also reduced in the brains of calorie-restricted rodents (Dubey *et al*., 1996). The current dogma for the underlying mechanism is that reduced mitochondrial metabolism due to reduced energy availability results in a net decrease in mitochondrial ROS production over time, and hence less radical-mediated cellular damage. Thus, one factor contributing to brain aging is simply the constant production of oxyradicals and resultant progressive damage to cells.

Alterations in Signaling Pathways during Aging

Additional alterations of aging that may be more specific to the nervous system are impaired calcium signaling and neurotrophic factor signaling, which may promote perturbed synaptic function and neuronal degeneration. Alterations in neuronal calcium regulation and expression of certain Ca^{2+}-binding proteins are observed in aged rodents (Disterhoft *et al*., 1994); such changes in the hippocampus are associated with age-related deficits in learning and memory. Changes in the levels of voltage-dependent calcium channels and glutamate receptors may also occur during aging (Clayton *et al.*, 2002). An age-related decrease in nerve growth factor (NGF) levels and levels of NGF receptors in the aging rodent brain apparently contributes to age-related cognitive impairment (Koh *et al.*, 1989; Nabeshima *et al.*, 1994). Brain-derived neurotrophic factor (BDNF) signaling also decreases during aging, with an associated decline in learning and memory (Lapchak *et al.*, 1993; Croll *et al.*, 1998). Similarly, neurotrophin-3 and neurotrophin-4 levels decrease in the targets of sensory neurons during aging (Bergman *et al.*, 2000), which may play a role in age-related sensory deficits. The ability of the nervous system to modulate neurotrophic factor signaling in response to stress may be compromised during aging (Smith and Cizza, 1996). Analysis of gene expression in individual neurons in the basal forebrain of young and old rats revealed significant decreases in the percentage of neurons expressing choline acetyltransferase and of neurons expressing glutamate decarboxylase (Han *et al.*, 2002), suggesting that neurons cease producing acetylcholine and GABA during aging, and/or that neurons

expressing these neurotransmitters are preferentially lost during aging.

Aging and Programmed Cell Death

The programmed cell death of neurons that occurs during development is easily documented in many regions of the nervous system as relatively large numbers of cells die during a brief time window (Johnson and Oppenheim, 1994). Apoptosis is the predominant form of programmed developmental cell death; it is characterized by cell shrinkage, membrane blebbing, and nuclear chromatin condensation and fragmentation. A biochemical cascade involving pro-apoptotic Bcl-2 family members such as Bax and Bad, mitochondrial alterations resulting in the release of cytochrome *c*, and activation of death effector enzymes called caspases mediates apoptosis (Fig. 3). It is believed that one important trigger of developmental neuronal death is insufficient access to target-derived neurotrophic factors that occurs at the time synapses are being formed. Neural precursor cells may also undergo apoptosis (de la Rosa and de Pablo, 2000), but the factors that control their survival remain to be determined.

Considerable evidence suggests that many neurons die during adult life, and that such cell deaths are increased during aging and even more so in neurodegenerative disorders (Mattson, 2000). Age-related decreases in number of neurons have been documented in some brain regions, but not in others (West *et al.*, 1994). Age-related neuronal death presumably results from apoptosis or a related form of programmed cell death, but this has not been conclusively established. It is unlikely that neurons undergo necrosis because this form of cell death, which is characterized by cell swelling and rupture, usually involves large numbers of cells dying in clusters; this phenomenon has not been observed in the nervous system during normal aging. In an immunohistochemical study of the cerebellum and hippocampus of young adult and old rats, it was shown that levels of the apoptotic protein p53 are increased in Purkinje cells and hippocampal CA1 neurons of old rats (Chung *et al.*, 2000). Many neurons and glial cells may undergo adaptive responses during aging that allow them to survive. Levels of the anti-apoptotic protein Bcl-2 are increased in hippocampal and cerebellar neurons during aging, and this increase appears to be a cytoprotective response to age-related increases in levels of oxidative stress (Kaufmann

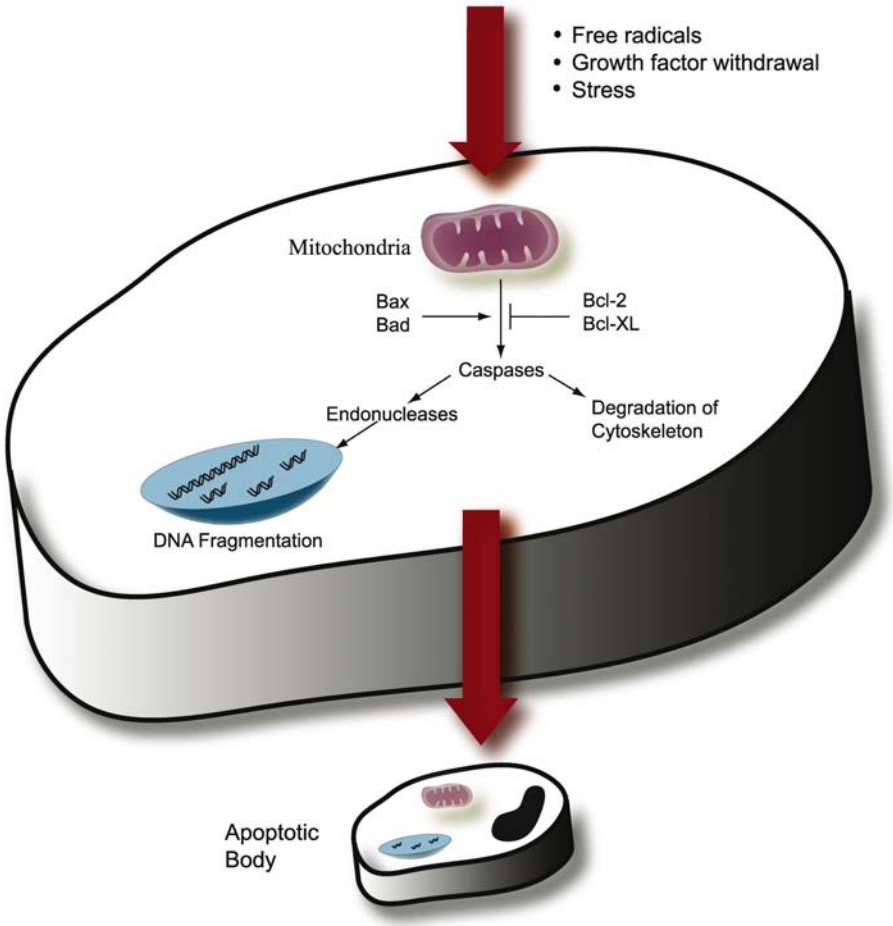

FIGURE 3. Simplified outline of apoptosis pathway. When cells are exposed to severe stress (in the form of free radicals, growth factor withdrawal, etc.), a signaling cascade is activated in which pro-apoptotic factors (such as Bax and Bad) activate caspases, which then activate other proteins which degrade the cytoskeleton and gauge fragmentation of nuclear DNA. Apoptosis is a common feature in both normal development and in several neurodegenerative disorders.

et al., 2001). The strongest evidence for neuronal apoptosis during aging comes from studies of neurodegenerative disorders in which numbers of cell deaths are greatly increased. Numerous studies of postmortem brain tissues from patients with Alzheimer's disease, Parkinson's disease, and stroke have provided evidence that neurons die by apoptosis. Hallmarks of apoptosis, including increased levels of p53, Par-4, Bax, activated caspases, are present in neurons affected in these disorders (Mattson, 2000). In addition, interventions known to prevent apoptosis, such as inhibitors of p53 and caspases, and agents that stabilize mitochondria, can prevent neuronal death in animal and cell culture models (Robertson *et al.*, 2000; Culmsee *et al.*, 2001; Liu *et al.*, 2002). The factors that trigger neuronal apoptosis during normal aging are not known, but may involve oxidative and metabolic stress, and reduced trophic support.

Neural Control of Aging

As described above, the brain undergoes profound changes during the aging process. Interestingly, there is increasing evidence suggesting that the brain also plays a role in regulating lifespan as well as health status during the aging process. The nervous system contains several signaling pathways that influence and possibly regulate lifespan in individuals. One such pathway is the insulin-like signaling pathway in mammals, in which activated plasma membrane receptor kinases phosphorylate tyrosine residues on an intracellular adapter protein termed insulin receptor substrate-1 (IRS-1). IRS-1 then activates phosphatidylinositol-3-kinase (PI3K), which activates Akt (protein kinase B), a regulator of several targets including forkhead transcription factors (van Weeren *et al.*, 1998; Tang *et al.*, 1999). In the mammalian brain, this pathway influences several aspects of neural development, including neuronal growth and differentiation, retinal axon pathfinding (Song *et al.*, 2003) and growth factor-mediated neuronal survival (Vaillant *et al.*, 1999; Gary and Mattson, 2001). Insulin-like signaling decreases in the rat brain during aging (Sonntag *et al.*, 1999), while infusion of insulin-like growth factor-1 (IGF-1) into the lateral ventricle of aged rats can ameliorate age-related deficits in brain energy metabolism (Lichtenwalner *et al.*, 2001) and memory (Markowska *et al.*, 1998). Thus, insulin-like signaling apparently plays a critical role in both neural development and age-related neural decline; additionally, it may play a role in determining the lifespan of an individual, as demonstrated by studies in nonmammalian species. Mutations in the insulin receptor (Tatar *et al.*, 2001) and the IRS homolog CHICO (Clancy *et al.*, 2001) result in an increased lifespan in *Drosophila*. In *C. elegans*, there are several homologs of members of the insulin-like signaling pathway including the insulin receptor (daf-2), PI3K (age-1), and the forkhead transcription factor (daf-16). Mutations in *daf-2* and *age-1* increase lifespan in *C. elegans*. When cell-type specific promoters are used to overexpress wild-type daf-2 or age-1 in *daf-2* or *age-1* mutants, the increased longevity of the mutants is reversed but only when overexpression occurs in the nervous system, but not when overexpression is targeted to muscle or intestinal cells (Wolkow *et al.*, 2000). Similar increases in lifespan are reported for mutations in the *C. elegans* tryptophan hydroxylase homolog *tph-1* (Sze *et al.*, 2000), suggesting that more than one pathway regulates lifespan.

In mammals, there is indirect evidence that neural signaling pathways can influence lifespan. Dietary restriction extends lifespan in animals and causes a corresponding decrease in circulating insulin levels and increased insulin sensitivity and glucose tolerance (Kalant *et al.*, 1988; Weindruch and Sohal, 1997; Wanagat *et al.*, 1999). Dietary restriction also increases levels of BDNF in several brain regions in rodents (Duan *et al.*, 2001; Prolla and Mattson, 2001; Lee *et al.*, 2002b). BDNF interacts with the trkB receptor, whose signaling pathway is remarkably similar to the insulin pathway (Foulstone *et al.*, 1999) and is generally considered to be a neuroprotective trophic factor. Significantly, dietary restriction delays age-related deficits in learning and memory in rodents (Ingram *et al.*, 1987) and can protect neurons against dysfunction and death in rodent models of Alzheimer's disease, Parkinson's disease, and stroke (Bruce-Keller *et al.*, 1999; Duan and Mattson, 1999; Yu and Mattson, 1999; Zhu *et al.*, 1999). Dietary restriction also increases neural levels of antioxidant enzymes, stress proteins (such as HSP-70 and GRP-78), and anti-apoptotic proteins (such as Bcl-2), suggesting that the lifespan-increasing effect of dietary restriction may result from decreased oxyradical production and enhanced cellular stress resistance (Bruce-Keller *et al.*, 1999; Duan and Mattson, 1999; Yu and Mattson, 1999). The dual effect on oxidative stress and trophic factors emphasizes the point that aging is a complex process with many overlapping and converging pathways that play a role in the aging process. Further, the ability of alterations in specific signaling pathways to alter aspects of aging indicates that the nervous system is not only affected during the aging process, but may also play an active role in determining an individual's lifespan.

DEVELOPMENTAL MECHANISMS UNDERLYING AGE-RELATED ALTERATIONS IN NEUROGENESIS

As described in the previous sections, many of the mechanisms that regulate neural development are believed to play a role in the aging of the nervous system. This is especially true for neural stem cells, which continue dividing in the adult brain long after most neural cells have undergone terminal differentiation, albeit at a lower rate than in the developing brain. Neural stem cells are defined as cells that can self-renew through cell division and are multipotent (i.e., they can produce differentiated progeny of all three mature neural cells: neurons, astrocytes, and oligodendrocytes). Neural stem cells in the adult brain have several potential fates (Fig. 4). The first is to remain quiescent and not re-enter the cell cycle, thus preventing self-renewal and differentiation into mature neurons and glia. This process has the additional consequence of reducing the stem cell pool as it is not renewed by new cell divisions. Stem cells may also enter the cell cycle but undergo apoptosis and die, or they may re-enter the cell cycle and successfully produce differentiated progeny.

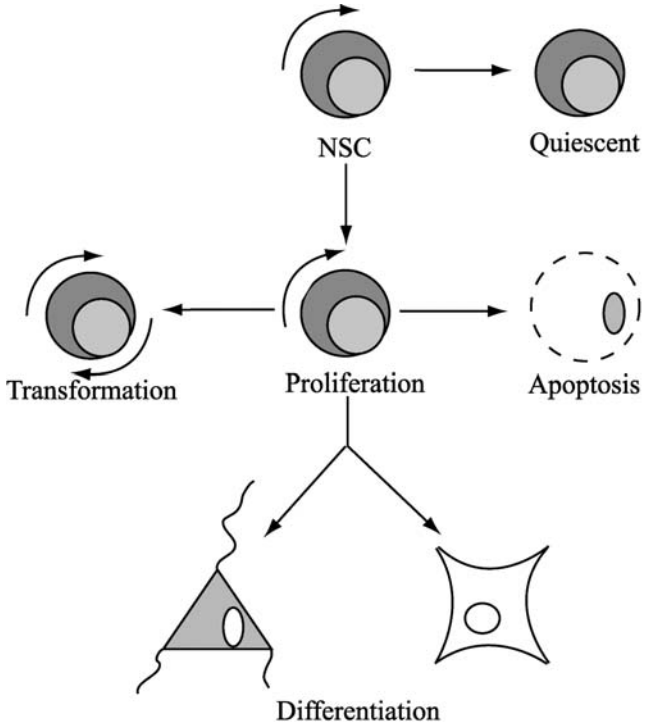

FIGURE 4. A neural stem cell has several fates. It can remain quiescent and not undergo any further cell divisions. Alternatively, it can re-enter the cell cycle and divide symmetrically (to produce more neural stem cells) or asymmetrically (to produce differentiated neurons and/or glia). If the stem cell becomes transformed, it will divide uncontrollably and may contribute to tumor formation. Finally, a stem cell and/or its progeny can undergo apoptosis and be removed from the tissue.

Finally, stem cells may successfully undergo division to produce healthy, functional daughter cells. Such division may be symmetric, to produce two identical daughter cells, or asymmetric, to produce one new stem cell and one daughter cell that will become a differentiated cell. The final outcome depends on the convergence of many intrinsic and extrinsic signals received by the cell and is influenced by factors such as cell density, receptor expression, and cross-talk between various signaling pathways (Sommer and Rao, 2002). Many of the mechanisms driving neural stem cell proliferation, differentiation, and survival in the adult and aging brain are similar to those regulating neural development and are often implicated in the pathogenesis of age-related neurodegenerative disorders.

Neurogenesis in the Developing and Adult Nervous System

The two primary populations of neural stem cells in the adult brain are located in the subventricular zone adjacent to the lateral ventricles and in the dentate gyrus of the hippocampus. Stem cells in the subventricular zone give rise to interneurons of the olfactory bulb, a population of neurons that die and are replaced throughout life. Stem cells in the dentate gyrus can form either granule cell layer neurons or astrocytes. Cells with a more restricted developmental potential than neural stem cells also exist in the CNS and can give rise to differentiated progeny. Such cells are generally restricted to a neuronal fate (neuronal restricted progenitors) or a glial fate (glial restricted progenitors) (Fig. 5). In the developing spinal cord, it has been demonstrated that multipotent neural stem cells give rise to lineage-restricted progenitor cells, as assessed by differential expression of -lineage specific markers (Mayer-Proschel *et al.*, 1997; Kalyani and Rao, 1998; Quinn *et al.*, 1999). The ultimate fate of these stem and progenitor cells depends on a number of factors, including but not limited to the presence or absence of trophic factors; system stress from oxidative or metabolic stress; and diet.

Many of the characteristics of adult neural stem cells are similar to those of fetal neural stem cells, including the two defining characteristics of a stem cell: the capacity for self-renewal through cell division, and the ability to produce differentiated progeny of all three types of mature neural cells. Both fetal and adult stem cells respond to a variety of growth factors and cytokines, including epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Additionally, adult neural stem cells give rise to neurons that are integrated into existing neuronal circuitry and appear to be fully functional, as determined by electrophysiological recordings from newly formed neurons in the adult mouse hippocampus (van Praag *et al.*, 2002). However, there is some evidence to suggest that the mechanisms that regulate stem cell processes change as the organism matures. For example, the early embryonic spinal cord is derived from multipotent neuroepithelial cells. At early developmental stages (E10.5 in the rat), neuroepithelial cells in the neural tube express neural stem cell-specific markers, such as fibroblast growth

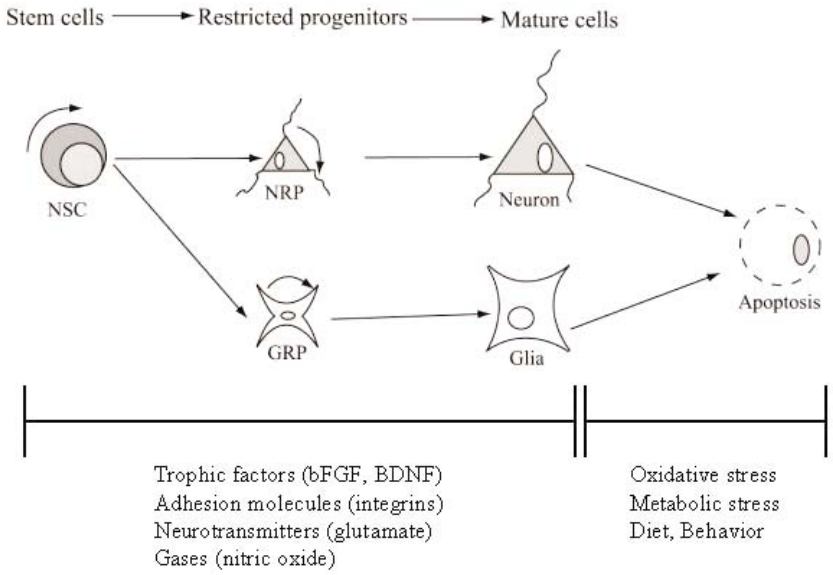

FIGURE 5. Mechanisms that regulate neurogenesis and gliogenesis in the adult nervous system. Multipotent neural stem cells can give rise to neuron-restricted progenitor cells (NRP) and glia-restricted progenitor cells (GRP). Differentiated neurons and glia may become functional and endure, or may undergo apoptosis; GRP and NRP may also undergo apoptosis. Proliferation, differentiation, and survival are regulated by a myriad of factors, including trophic support and environmental conditions (oxidative stress, metabolic stress, etc.).

factor receptor-4 (FGFR4), Frizzled 9 (Fz-9), and Sox-2 (Cai *et al.*, 2002). Expression of Fz-9 and FGFR4 is downregulated as neuroepithelial cells become committed to a specific cell linage (neuronal or glial) and are virtually undetectable by E14 (Kalyani *et al.*, 1999; Cai *et al.*, 2002), suggesting they are uniquely expressed by early but not late embryonic neural stem cells. This is supported by the lack of FGFR4 expression in neural stem cells of the late embryonic and adult rat hippocampus (Limke *et al.*, 2003). In contrast, the transcription factor Sox-1, a HMG-box protein related to SRY, is expressed in ectodermal cells fated to become neural cells (Pevny *et al.*, 1998) and is also found in late embryonic and young adult hippocampus in proliferative cells (Limke *et al.*, 2003), suggesting that some factors may regulate both developmental and adult stem cell populations.

Neurogenesis in the Aging Nervous System

Neural stem and progenitor cells are subject to many of the same environmental stressors other neural cells experience during aging, which can alter their capacity for self-renewal as well as their survival (Fig. 6). Stem cells appear to be affected by at least some of these factors, as there is decreased incorporation of bromodeoxyuridine in the aged rat hippocampus, suggesting a decline in the neurogenic capacity of the adult nervous system with age (Kuhn *et al.*, 1996). Neurogenesis might be impaired as the result of reduced proliferation or differentiation of neural stem cells, increased quiescence of cells as they mature, or increased death of newly generated neurons. Even in the young adult brain, studies in which neural stem cells were labeled with bromodeoxyuridine provide evidence that most newly generated cells in the hippocampus and subventricular zone eventually die, with some of them dying before they differentiate into functional

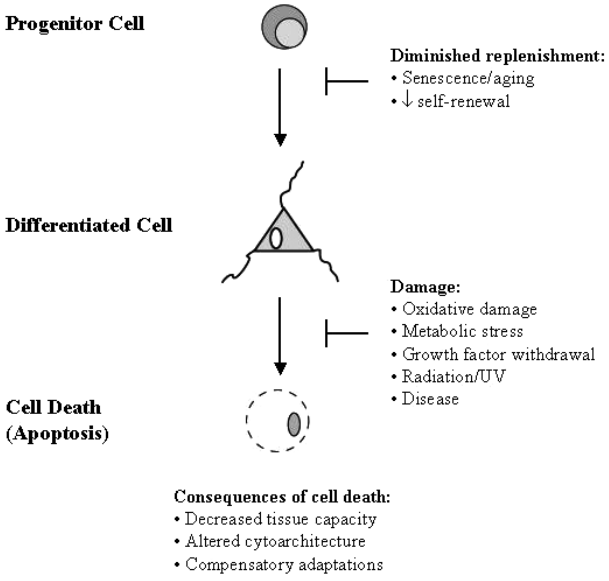

FIGURE 6. Neural stem cells and their progeny are exposed to stressors which may affect their ability to function and can ultimately lead to cellular senescence or, in severe situations, apoptosis. These stressors are present during normal aging and are often heightened during neurodegenerative disorders. As stem and mature cells are removed from the brain, there is a decreased capacity for proliferation/cell replacement, as well as alterations in the brain's structure and plasticity.

neurons or glial cells (Levison *et al.*, 2000; Lee *et al.*, 2002a). The decline in hippocampal neurogenesis does not appear to be caused by metabolic impairment, but may result from decreased proliferation or a decrease in the numbers of neural stem cells (Kuhn *et al.*, 1996). Presumably, age-related increases in cellular

oxidative stress or decrements in neurotrophic factor levels contribute to the decline in neurogenesis during aging (Haughey *et al.*, 2002), although this remains to be established.

Various growth factors and cytokines drive the formation, maturation, and survival of the neural cells during development; modification of these factors may influence neurogenesis in the aged brain. Adult neural stem cells respond to several growth factors, particularly EGF and bFGF, which promote proliferation of stem cells and progenitor cells derived from the adult subventricular zone (Kuhn *et al.*, 1997). Factor bFGF also induces the proliferation of hippocampal neural progenitor cells (Ray *et al.*, 1993); the responsiveness of these cells to bFGF may decrease during aging (Cheng *et al.*, 2002). EGF has a similar mitogenic effect in proliferating cells in the subventricular zone, although its effects appear to promote gliogenesis rather than neurogenesis (Kuhn *et al.*, 1997; Gritti *et al.*, 1999). Injection of EGF and NGF into the lateral ventricle of aged mice promotes proliferation of subventricular zone cells (Tirassa *et al.*, 2003). Interestingly, this protocol also causes an upregulation of mRNA for BDNF, a trophic factor which promotes survival of newly born neurons. BDNF itself promotes the differentiation and survival of newly generated neurons in the hippocampus (Lee *et al.*, 2002a, b). Age-related declines in BDNF and the BDNF receptor, TrkB, have been described in the rat and primate brain (Hayashi *et al.*, 1997; Katoh-Semba *et al.*, 1998; Romanczyk *et al.*, 2002). Interestingly, when mice are maintained on dietary restriction, hippocampal neurogenesis is increased (Lee *et al.*, 2002b), possibly as a result of a BDNF-mediated increase in survival of newly generated neurons (Lee *et al.*, 2002a). Another growth factor that declines in the aging brain is IGF-1, which is reduced in the hippocampus of aged rats (Lai *et al.*, 2000). Age-associated diminishment of hippocampal neurogenesis in the aged rat can be reversed by administration of IGF-1 (Lichtenwalner *et al.*, 2001), suggesting that its receptor plays a role in the aging process.

Other molecules that drive development, including cytokines, neurotransmitters, and hormones, are also critical regulators of neurogenesis and gliogenesis during development and aging. Leukemia inhibitory factor (LIF) and ciliary neuro-trophic factor (CNTF) act through gp130 heterodimer receptors to promote maintenance of an undifferentiated state in mouse embryonic stem cells, but promote gliogenesis in the adult mouse brain (Williams *et al.*, 1988; Conover *et al.*, 1993; Yoshida *et al.*, 1993). Neurotransmitter signaling may also play important roles in regulating adult neurogenesis. For example, antidepressants that enhance serotonergic signaling stimulate hippocampal neurogenesis by a mechanism that may involve upregulation of BDNF (Duman *et al.*, 2001). Neurogenesis can also be stimulated by adrenalectomy, suggesting that endocrine signals can also modulate neurogenesis in the aged brain (Cameron and Gould, 1994; Cameron and McKay, 1999). Interestingly, sex hormones (estrogen and testosterone) may directly and/or indirectly affect neurogenesis in the aging brain. For example, estrogen levels decline abruptly in post-menopausal women not receiving hormone replacement therapy. Estrogen deprivation significantly reduces hippocampal BDNF levels in the female rat hippocampus; interestingly, exercise and/or hormone replacement therapy restore BDNF mRNA and protein content to normal levels (Berchtold *et al.*, 2001). Estrogen alone promotes proliferation of both embryonic and adult neural stem cells (Brannvall *et al.*, 2002). Similarly, men experience an age-related decline in testosterone levels. Testosterone promotes neurogenesis in the adult songbird neostriatum (Louissaint *et al.*, 2002) and, like estrogen, causes an upregulation of survival-promoting BDNF (Rasika *et al.*, 1999). Thus, age-related declines in neurogenesis may be linked to loss of hormone levels associated with normal aging.

Other manipulations which increase neurogenesis in the aged rodent hippocampus include physical exercise (van Praag *et al.*, 1999) and enriched environments (Kempermann *et al.*, 1998; Nilsson *et al.*, 1999), consistent with beneficial effects of exercise and intellectual activities in preserving brain function during aging in humans. Increased hippocampal neurogenesis creates new neurons with apparently functional circuitry (Snyder *et al.*, 2001; van Praag *et al.*, 2002) and is associated with cognitive improvement in aged rodents (Kempermann *et al.*, 2002). Reduced hippocampal neurogenesis is associated with loss of ability to form trace memories, which is regained when neurogenesis is restored (Shors *et al.*, 2001). Additionally, exercise-induced neurogenesis significantly improves learning, exploratory behavior, and locomotion in aged mice (Kempermann *et al.*, 2002). What cannot be determined from these studies is the contribution of increased neurogenesis to the observed changes, as compared to other beneficial effects of exercise (increased trophic support, etc.). Interestingly, age-related reductions in neurogenesis do not correlate with spatial memory impairment (Merrill *et al.*, 2003), suggesting that increased neurogenesis is not a "cure-all" for all age-related hippocampal impairments.

While the level of neurogenesis can be modulated by factors such as diet, environmental stimulus, and trophic factor levels, there is little information to date regarding the intrinsic mechanisms underlying the age-related decline in neural stem function. Proliferating, non-transformed cells will undergo a certain number of cell divisions before exiting the cell cycle to become senescent. This number of divisions, termed the "Hayflick limit," is controlled by telomerase, an enzyme that adds a six-base DNA repeat onto the ends of chromosomes (telomeres) and thereby prevents their shortening during successive rounds of cell division. Telomerase levels are high in developing neural progenitor cells, but then decrease as cells differentiate into neurons and glia (Klapper *et al.*, 2001). Telomerase has been suggested to play a role in aging because its absence in somatic cells results in telomere shortening and cell senescence. Telomeres are generally shorter in older people than in younger people, suggesting that telomere length may provide a molecular clock for measuring lifespan. Alterations in telomere length can dramatically affect the onset and maintenance of aging. For example, accelerated shortening of telomeres in disease such as Werner's syndrome and Down's syndrome is associated with early onset of aging. Recent studies have shown that telomerase promotes the survival of neurons and neuronal precursor cells (Fu *et al.*, 2000; Lu *et al.*, 2001) and its reduction during aging may therefore play a role in age-related neuronal loss and impaired neurogenesis.

DEVELOPMENTAL MECHANISMS IN AGE-RELATED NEURODEGENERATIVE DISORDERS

As described in the previous sections, aging involves a series of changes within the brain that are a normal part of the aging process. These include elevation of reactive oxygen species, increased oxidative damage to proteins and DNA, accumulation of protein and lipid byproducts, reduced metabolic activity, and cytoskeletal changes. Such changes are distinct from the effects of age-related neurological disorders which often exacerbate the factors contributing to the general decline observed during aging. A number of neurodegenerative disorders exist which are positively correlated with aging (Table 2). Some diseases, such as Parkinson's disease, target a distinct population of neurons (in this case, the dopaminergic neurons of the substantia nigra), while others, such as Alzheimer's disease, affect a more diffuse set of cells (in this case, primarily the cortex and hippocampus). What is of interest is that, like the normal alterations in brain physiology that accompany aging, many of the age-related neurological disorders also have a foundation in developmental processes.

Inherited Disorders with Abnormal Aging Phenotype

Inherited disorders that are characterized by premature aging are providing insight into the overlap of mechanisms of aging and development in the nervous system. Werner's syndrome is an autosomal recessive disorder caused by mutations in a DNA helicase that manifests accelerated aging of tissues throughout the body (van Brabant *et al.*, 2000). Age-related alterations in the brains of Werner's patients have been documented and include amyloid deposition and neurofibrillary tangles in frontal and temporal lobes (Leverenz *et al.*, 1998). Cockayne syndrome is characterized by a defect in DNA repair (van Gool *et al.*, 1997) and manifests widespread aging-like changes in the nervous system including retinal and cochlear degeneration, peripheral neuropathies, and neurodegenerative changes in the brain (Rapin *et al.*, 2000). Patients with progeria exhibit a dramatic acceleration of age-related pathologies including cerebrovascular disease and neuronal degeneration (Rosman *et al.*, 2001). A more common inherited disorder that manifests premature age- and Alzheimer-like pathologies in the brain is Down's syndrome (trisomy of chromosome 21). Patients with Down's syndrome exhibit extensive amyloid deposition in the brain with associated neurofibrillary pathology and cognitive dysfunction, as well as degeneration of cholinergic and noradrenergic systems (Coyle *et al.*, 1986; Sawa, 1999). Although the gene(s) responsible for the phenotypes of Down's syndrome has not been clearly established, those encoding amyloid precursor protein (APP) and proteins involved in oxyradical metabolism are located on chromosome 21. In particular, a role for APP is suggested by studies showing that APP plays important roles in regulating neuronal plasticity (dendrite outgrowth and synaptic plasticity) and cell survival (Mattson, 1997). Thus, disruption of mechanisms that regulate development can result in symptoms which mimic changes observed during aging, supporting the idea that the mechanisms driving development and aging are often the same.

TABLE 2. Age-Related Diseases of the Nervous System

Disease	Primary symptoms
Alzheimer's disease	β-amyloid plaques and neurofibrillary tangles, primarily in hippocampus and cortex; results in memory deficits
Parkinson's disease	Loss of dopaminergic neurons in the substantia nigra and striatum; results in motor control problems
Huntington's disease	Cell death in neostriatum and cortex, with accompanying movement and cognitive dysfunction; results in severely reduced lifespan
Werner's syndrome	Amyloid deposition and neurofibrillary tangles in frontal and temporal lobes; results in accelerated aging
Cockayne syndrome	Defect in DNA repair causing retinal and cochlear degeneration, peripheral neuropathies, and neurodegenerative changes in the brain; symptoms resemble nervous system changes observed in aging
Down's syndrome	Amyloid deposition, neurofibrillary tangles, and cognitive dysfunction, degeneration of cholinergic and noradrenergic systems

Developmental Mechanisms Underlying Age-Related Neurodegenerative Disorders

How might developmental mechanisms contribute to the pathogenesis of neurodegenerative disorders? Each neurodegenerative disorder is characterized by selective vulnerability of particular populations of neurons (Fig. 7). The mechanisms that regulate the survival and plasticity of neurons and glia during aging are not well understood, but studies of age-related neurodegenerative disorders have revealed novel genes and environmental factors that influence both the development of the nervous system and its susceptibility to dysfunction and degeneration during aging.

Studies of the brains of Alzheimer's disease patients have revealed several development-related processes occurring in association with amyloid plaques and neurofibrillary tangles, the major pathological lesions in this disease. For example, fetal forms of MAPs are present in dystrophic neurites (Joachim *et al.*, 1987) and aberrant axonal sprouting occurs in some brain regions (Larner, 1995). In addition, growth factors such as bFGF and transforming growth factor-beta are present at high levels in amyloid plaques (Cummings *et al.*, 1993; Finch *et al.*, 1993). Damage to nuclear DNA in striatum of Huntington's disease patients, and in hippocampus and vulnerable cortical regions of Alzheimer's patients, has been documented. For example, levels

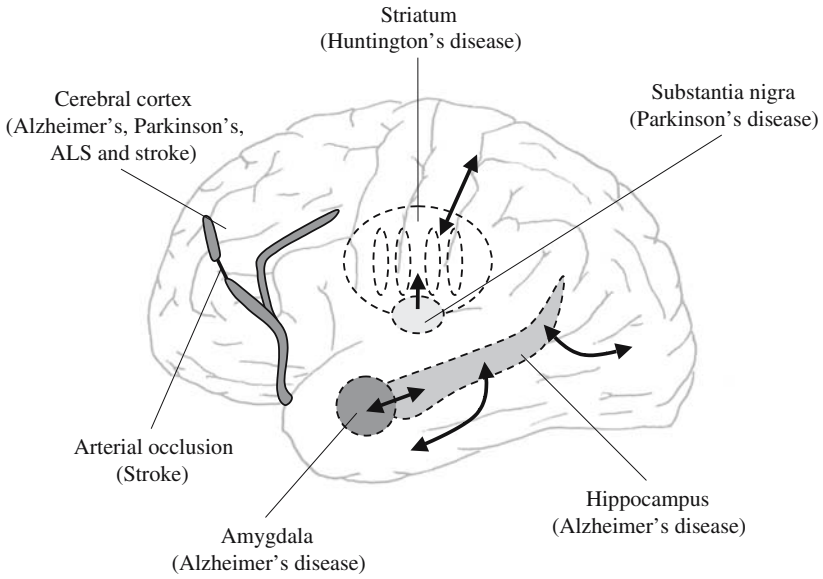

FIGURE 7. Brain regions affected in age-related neurodegenerative disorders. Synaptic dysfunction and degeneration and neuron death occur in the affected brain regions in the indicated disorders. Accordingly, the symptoms of each disorder are directly related to the functions of the affected brain regions. For example, brain regions involved in cognitive processes (hippocampus and cerebral cortex) and emotional behaviors (amygdala) are affected in Alzheimer's disease, while brain regions involved in controlling body movements (substantia nigra and striatum) are affected in Parkinson's disease.

of 8-hydroxyguanosine are increased suggesting DNA damage caused by reactive oxygen molecules such as hydroxyl radical and peroxynitrite. Interestingly, a dietary deficiency of folate can have striking adverse effects on the developing nervous system and may also increase the risk of Alzheimer's disease and Parkinson's disease by promoting DNA damage in neurons (Duan *et al.*, 2002; Kruman *et al.*, 2002).

The most striking links between development and neurodegenerative disorders comes from studies of Alzheimer's disease. Although the cause of most cases of Alzheimer's disease is unknown, some cases result from genetic mutations. Three disease-causing genes have been identified; they encode the APP, presenilin-1 (PS1), and presenilin-2 (PS2). APP is a transmembrane protein that is the source of the amyloid beta-peptide that forms insoluble plaques in the brains of Alzheimer's patients (Mattson, 1997). Cleavage of APP within the amyloid beta-peptide sequence by an enzyme activity called alpha-secretase releases a soluble form of APP (sAPP) from the cell surface; this cleavage occurs normally and is stimulated by various growth factors and by electrical activity in neurons. In Alzheimer's disease, there is a decrease, in the production of sAPP; instead, APP is cleaved by enzymes that cut it at the N- (beta-secretase) and C- (gamma-secretase) termini of amyloid beta-peptide to generate the full-length amyloidogenic peptide. APP, PS1, and PS2 mutations increase the production of amyloid beta-peptide. Presenilin and APP mutations may alter neuronal plasticity and promote neuronal degeneration by perturbing cellular calcium homeostasis (Mattson, 1997).

Recent studies have revealed important roles for APP and presenilins in the development of the nervous system and in adult neuroplasticity. The secreted form of APP has been shown to regulate neurite outgrowth and cell survival in embryonic rat hippocampal neurons (Mattson *et al.*, 1993; Mattson, 1994) and can protect neurons against death in experimental models of Alzheimer's disease and stroke (Goodman and Mattson, 1994; Smith-Swintosky *et al.*, 1994). Studies of synaptic transmission in hippocampal slices showed that sAPP can enhance long-term potentiation (Ishida *et al.*, 1997), suggesting that sAPP facilitates learning and memory, a possibility consistent with *in vivo* studies (Roch *et al.*, 1994). In addition to its neurotrophic effects and roles in synaptic plasticity, sAPP may play a role in neurogenesis. When cultured embryonic cortical stem cells were exposed to sAPP, their proliferation rate increased (Hayashi *et al.*, 1994; Ohsawa *et al.*, 1999). The signal transduction pathway that mediates the biological activities of sAPP may involve cyclic GMP and the transcription factor NF-κB (Furukawa *et al.*, 1996; Guo *et al.*, 1998).

Notch is a type 1 membrane protein that, when activated by cell-associated ligands, is proteolytically processed in a manner that releases an intracellular C-terminal fragment of Notch which then translocates to the nucleus where it may regulate gene expression (Fig. 8). The developmental roles of presenilins are thought to result from a function in the Notch signaling pathway because the phenotype of PS1 null mice is essentially identical to that of Notch knockout mice (Conlon *et al.*, 1995; Shen *et al.*, 1997). In addition, the cellular expression of PS1 and Notch in the developing rodent nervous system is very similar, being high during neurogenesis and decreasing as the embryo develops. Levels of Hes5, a gene induced by activation of the Notch signaling pathway, are decreased in the ventricular zone of PS1 null mice, whereas levels of a Notch ligand are elevated. The Drosophila PS1 homolog is highly expressed in neurons during development; mutations of PS1 alter the

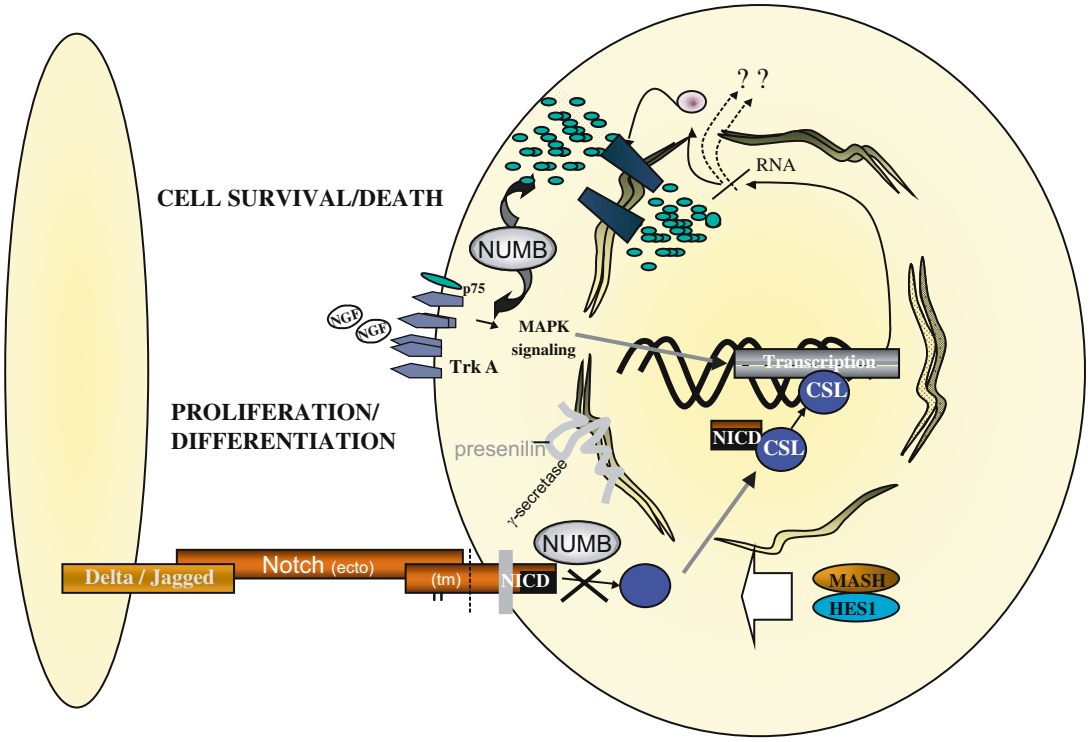

FIGURE 8. Model for the mechanisms whereby Notch and Numb regulate neuronal differentiation and survival. Activation of Notch by cell–cell interactions results in a proteolytic cleavage of an intracellular domain (NICD), which interacts with a protein called CSL and thereby regulates gene transcription. Numb can antagonize Notch signaling. Numb may also enhance NGF signaling by facilitating activation of the high-affinity receptor trkA resulting in activation of mitogen-activated protein kinases (MAPK). In neural progenitor cells, Notch signaling promotes cell proliferation, whereas Numb promotes cell differentiation. In differentiated neurons, Notch may promote cell survival, while Numb can facilitate apoptosis. Notch and Numb may play important roles in aging and neurodegenerative disorders.

subcellular localization of Notch and result in defects in eye development and neuronal differentiation. PS1 and PS2 have considerable homology to two genes in the nematode *C. elegans* called *spe*-4 and *sel*-12; *spe*-4 functions in spermatogenesis and *sel*-12 plays a role in the process of egg-laying by a mechanism involving the Notch signaling pathway. The *sel*-12 mutants can be rescued by PS1 demonstrating a conserved function for these two genes. Moreover, human PS1 can rescue defective egg-laying resulting from mutations in *sel*-12, strongly suggesting similar functions of PS1 and *sel*-12 (Levitan and Greenwald, 1995). PS1 is necessary for ligand-induced transmembrane cleavage of Notch (Hartmann *et al.*, 2001), and may thereby regulate cell fate decisions.

Numb is an evolutionarily conserved protein identified by its ability to control cell fate in the nervous system of Drosophila, wherein Numb may act by antagonizing Notch signaling (Artavanis-Tsakonas *et al.*, 1999) (Fig. 8). Mammals express four isoforms of Numb that differ in the composition of a phosphotyrosine-binding domain (PTB) and a proline-rich region (PRR). Numb regulates the sensitivity of cells to neurotrophin-induced differentiation and cell survival dependency in an isoform-specific manner (Pedersen *et al.*, 2002). Numb isoforms containing a short PTB enhance the differentiation response to NGF, and enhance apoptosis in response to NGF withdrawal by a mechanism dependent upon release of calcium from endoplasmic reticulum stores. These findings suggest that isoform-specific modulation of neurotrophin responses by Numb may play important roles in the development and plasticity of the nervous system. Additional studies have examined the possible roles of Numb in the pathogenesis of Alzheimer's disease. Numb isoforms containing a short PTB domain increase the vulnerability of neural cells to death induced by amyloid beta-peptide (Chan *et al.*, 2002). Dysregulation of cellular calcium homeostasis occurs in cells expressing Numb isoforms with a short PTB domain, and the death-promoting effect of Numb is abolished by pharmacological inhibition of calcium release. The levels of Numb are increased in cultured primary hippocampal neurons exposed to Aβ, suggesting a role for endogenous Numb in the neuronal death process. Furthermore, higher levels of Numb were detected in the cortex of mice expressing mutant APP relative to age-matched wild-type mice. These findings suggest that the effects of Numb on cell fate decisions, both during development of the nervous system and in neurodegenertive disorders, are mediated by changes in cellular calcium homeostasis.

Deficits in neurotrophic factors may contribute to neurodegenerative processes in aging and disorders of aging. Analyses of neurotrophic factor expression in brain tissues from young and old rodents, and from patients with age-related neurodegenerative disorders, suggest that neurotrophic support of neurons declines

with advancing age and more so in neurodegenerative disorders. It was reported that transgenic mice that express an antibody against NGF exhibit neuronal degeneration with features of AD including amyloid deposits and neurofibrillary tangle-like pathology in the hippocampus and cerebral cortex (Capsoni *et al.*, 2000). Although depletion of a neurotrophic factor or impaired neurotrophic signal transduction has not yet been shown to cause a neurodegenerative disorder, recent findings suggest major contributions of diminished neurotrophic support in Alzheimer's, Parkinson's, and Huntington's diseases. It was reported that the normal huntingtin protein induces the expression of BDNF, and that disease-causing mutations in huntingtin result in a marked decrease in BDNF expression (Zuccato *et al.*, 2001).

The evidence that developmental mechanisms are involved in Alzheimer's disease is now quite strong, and investigations of other age-related neurodegenerative disorders are revealing similar processes. Sprouting of nitric oxide synthase-positive neurites occurs in Parkinson's disease (Sohn *et al.*, 1999), suggesting a role for aberrant nitric oxide signaling in the pathogenesis of this disorder. Glial cell-line-derived neurotrophic factor (GDNF) can promote the survival, production of dopamine, and neurite sprouting in dopaminergic neurons in experimental models of Parkinson's disease (Gash *et al.*, 1998) and is currently being tested in clinical trials in human patients. Parkinson's disease can be caused by mutations in alpha-synuclein, and studies of songbirds and mammals have provided evidence that alpha-synuclein functions in the regulation of synaptic plasticity (Clayton *et al.*, 2002). Ischemic stroke involves a complex set of neurodegenerative and neurorestorative cellular responses. Apoptosis appears to be a prominent form of neuronal death in stroke, while neurogenesis and neurite outgrowth are compensatory responses that likely influence the extent of recovery from a stroke (Stroemer *et al.*, 1995; Jin *et al.*, 2001). Thus, there is significant evidence that developmental mechanisms play a role in many neurodegenerative disorders of the aging brain.

SUMMARY

The mechanisms driving development of the nervous system are complex and involve the integration of many intrinsic and extrinsic signals. Many of the mechanisms which regulate development, including trophic factors, cytokines, and hormones, are the same mechanisms that dysfunction during aging and contribute to the pathogenesis of neurodegenerative disorders. A better understanding of how abnormalities in developmental signaling mechanisms may contribute to the pathogenesis of neurodegenerative disorders, and how developmental mechanisms might be tapped to restore damaged neuronal circuits are important areas for future investigations.

ACKNOWLEDGMENTS

The authors would like to thank the members of their respective laboratories for insightful discussion and suggestions during the preparation of this chapter. T.L. was supported by an NIA IRTA fellowship. The authors thank Lance A. Edwards and Sic L. Chan for the contribution of several figures presented in this publication.

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