Modification of Brain Aging and Neurodegenerative Disorders by Genes, Diet, and Behavior

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Mattson, Mark P., Sic L. Chan, and Wenzhen Duan. Modification of Brain Aging and Neurodegenerative Disorders by Genes, Diet, and Behavior. Physiol Rev 82: 637–672, 2002; 10.1152/physrev.00004.2002.—Multiple molecular, cellular, structural, and functional changes occur in the brain during aging. Neural cells may respond to these changes adaptively, or they may succumb to neurodegenerative cascades that result in disorders such as Alzheimer’s and Parkinson’s diseases. Multiple mechanisms are employed to maintain the integrity of nerve cell circuits and to facilitate responses to environmental demands and promote recovery of function after injury. The mechanisms include production of neurotrophic factors and cytokines, expression of various cell survival-promoting proteins (e.g., protein chaperones, antioxidant enzymes, Bcl-2 and inhibitor of apoptosis proteins), preservation of genomic integrity by telomerase and DNA repair proteins, and mobilization of neural stem cells to replace damaged neurons and glia. The aging process challenges such neuroprotective and neurorestorative mechanisms. Genetic and environmental factors superimposed upon the aging process can determine whether brain aging is successful or unsuccessful. Mutations in genes that cause inherited forms of Alzheimer’s disease (amyloid precursor protein and presenilins), Parkinson’s disease (α-synuclein and Parkin), and trinucleotide repeat disorders (huntingtin, androgen receptor, ataxin, and others) overwhelm endogenous neuroprotective mechanisms; other genes, such as those encoding apolipoprotein E, have more subtle effects on brain aging. On the other hand, neuroprotective mechanisms can be bolstered by dietary (caloric restriction and folate and antioxidant supplementation) and behavioral (intellectual and physical activities) modifications. At the cellular and molecular levels, successful brain aging can be facilitated by activating a hormesis response in which neurons increase production of neurotrophic factors and stress proteins. Neural stem cells that reside in the adult brain are also responsive to environmental demands and appear capable of replacing lost or dysfunctional neurons and glial cells, perhaps even in the aging brain. The recent application of modern methods of molecular and cellular biology to the problem of brain aging is revealing a remarkable capacity within brain cells for adaptation to aging and resistance to disease.

I. INTRODUCTION

Many persons live for nine or more decades and enjoy a well-functioning brain until the very end of life. We therefore know what the brain is capable of and, accordingly, a major goal of research in the area of the neurobiology of aging is to identify ways to facilitate successful brain aging in everyone. Studies of brains of the oldest old have provided evidence for stability as well as plasticity in successful brain aging (Fig. 1). In many
Adaptation
- protein chaperones
- growth factors
- anti-apoptotic proteins
- antioxidant enzymes
Neurogenesis
Apoptosis
Growth of axons and dendrites
Synaptogenesis

Molecular Damage
- DNA oxidation/mutations
- Protein modifications
- Protein aggregation
- Lipid peroxidation

Energy Dysregulation
- Calorie intake
- Insulin resistance
- Oxidative production
- Glycation

Glial/Immune alterations
- Cytokine cascades
- Microglial activation
- Impaired detoxification
- Demyelination

Neurodegeneration
- Synaptic dysfunction
- Synapse loss
- Dendrite regression
- Apoptosis
- Impaired neurogenesis

Studies of embryonic and early postnatal development, and of synaptic plasticity in the young adult, have made a major contribution to our current understanding of the molecular and cellular mechanisms that determine whether brain aging occurs successfully or manifests dysfunction or disease. This is because the same intercellular signals and intracellular transduction pathways that regulate neurite outgrowth, synaptogenesis, and cell survival during development are also operative throughout life (213). Although new signaling systems continue to be discovered, the major classes of signaling molecules important in brain aging include neurotrophic factors, neurotransmitters, cytokines, and steroids. Neurotrophic factors such as neurotrophins [brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotensin (NT-3, and NT-4), fibroblast growth factors, glial-derived neurotrophic factor (GDNF), and ciliary neurotrophic factor (CNTF) have been shown to promote the survival of specific populations of brain neurons under experimental conditions relevant to brain aging and neurodegenerative disorders (232). In addition to their roles as mediators of synaptic transmission, neurotransmitters such as glutamate, acetylcholine, and dopamine also play important roles in regulating the formation of neuronal circuits during development and in influencing the neurodegenerative process in brain disorders of aging (215). Sex steroids (estrogen and testosterone) and stress steroids (glucocorticoids) have been shown to have quite striking effects on brain function and structure, and alterations in regulation of their production and signaling mechanisms have been reported to occur during aging (239). The status of such neurotransmitter, trophic factor, cytokine, and steroid hormone signaling systems is likely to have a major influence on the outcome of brain aging.

While the brain can age successfully, its cells may face considerable adversity during the journey (Fig. 1). Increased oxidative stress (oxyradical production) and accumulation of oxidatively damaged molecules (proteins, nucleic acids, and lipids) promote dysfunction of various metabolic and signaling pathways (178). Neurons may also face energy deficits as the result of alterations in the cerebral vasculature and in mitochondrial function (131). As in other organ systems, cells in the brain encounter a cumulative burden of oxidative and metabolic stress that may be a universal feature of the aging process. Each of the major classes of cellular molecules, including proteins, nucleic acids, and lipids, is oxidatively modified during brain aging. Protein modifications include carbonyl formation (34, 35, 74); covalent modification of cysteine, lysine, and histidine residues by the lipid peroxidation product 4-hydroxynonenal (261, 268, 266); nitration on tyrosine residues (326); and glycation (249). DNA and RNA bases are subject to oxidative modification, with a prominent example being the formation of 8-hydroxydeoxyguanosine (331). Double bonds in mem-

![Image](http://physrev.physiology.org/DownloadedFrom)
brane lipids are oxidized resulting in the production of a variety of lipid peroxides and aldehydes (218). These modifications of proteins, nucleic acids, and lipids are greatly exacerbated in neurodegenerative disorders such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) consistent with a major role for oxidative stress of aging in the pathogenesis of those disorders (207).

Analyses of experimental animal and cell culture models of age-related neurodegenerative disorders have provided insight into the mechanisms that result in increased oxidative stress and damage to proteins, nucleic acids, and membrane lipids (Fig. 3). The pathogenesis of AD involves altered proteolytic processing of the β-amyloid precursor protein (APP) resulting in increased production of a long (42 amino acid) form of amyloid β-peptide which self-aggregates and forms insoluble plaques in the brain (216). As amyloid β-peptide aggregates, it generates reactive oxygen species that can induce membrane lipid peroxidation in neurons resulting in the impairment of the function of membrane ion-motive ATPases and glucose transporter proteins, which, in turn, disrupts cellular ion homeostasis and energy metabolism (216). These oxidative actions of amyloid β-peptide can cause dysfunction of synapses and may render neurons vulnerable to excitotoxicity and apoptosis (80, 219). In PD, degeneration of dopaminergic neurons in the substantia nigra may be triggered by mitochondrial impairment and increased oxidative stress resulting from aging and exacerbated by environmental factors (147). In the common late-onset forms of AD and PD, the neurodegenerative cascade is most likely promoted by environmental factors (see sects. IV and V) that result in increased oxidative and metabolic

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**Fig. 2.** Mechanisms involved in regulating protein turnover and their modification by cellular stress. Proteins damaged by oxidative stress or other modifications can be degraded by the proteasomal or lysosomal systems. Protein chaperones such as heat shock proteins (HSP40, HSP70, and HSP90), glucose-regulated proteins (GRP78 and GRP94), and ubiquitin play important roles in controlling protein folding and targeting proteins for proteolytic degradation. ER, endoplasmic reticulum.
stress. On the other hand, more rare inherited forms of these disorders in which disease onset occurs at an early age (30–60 years of age) are caused by specific mutations; for example, mutations in APP and presenilins that cause AD (122) and mutations in H9251-synuclein and parkin that cause PD (165, 281). Some neurodegenerative disorders are purely genetic including Huntington’s disease (HD) and related trinucleotide repeat disorders (389); such disorders may not, therefore, be considered as diseases of aging, although aging processes may affect the age of disease onset and clinical course.

In the United States and other industrialized countries, life expectancy continues to increase, and therefore, more people will suffer from age-related neurodegenerative conditions. The negative impact of age-related neurodegenerative disorders on our societies is emphasized by the fact that more dollars are required to care for patients with AD, PD, and stroke than are spent on the combined care for patients with cardiovascular disease or cancer. Each neurodegenerative disorder is characterized by dysfunction and degeneration of specific populations of neurons in the brain (246). Neurons in brain regions involved in learning and memory processes, such as the hippocampus and cerebral cortex, are afflicted in AD. In PD, dopaminergic neurons in the substantia nigra degenerate resulting in motor dysfunction (147). A stroke occurs when a cerebral blood vessel becomes occluded or ruptures resulting in the degeneration of neurons in the brain tissue supplied by that vessel (65). Several genetic and environmental factors that may initiate the neurodegenerative process in AD, PD, and stroke have been identified, and this information has led to the development of valuable animal models of these disorders. Animal models of AD include transgenic mice overexpressing mutant forms of human APP (93, 133), transgenic and knockin mice expressing mutant forms of human presenilin-1 (PS1) (75, 108), and infusion of amyloid H9252-peptide and excitotoxins into the brains of rats and mice (31, 97). Animal models of PD include administration of the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to monkeys and mice resulting in selective degeneration of substantia nigra dopamine-producing neurons and associated motor dysfunction (71), and transgenic mice expressing mutant human α-synuclein which exhibit degeneration of dopaminergic neurons and a behavioral phenotype with features similar to PD (210). Stroke models involve transient or permanent occlusion of the middle cerebral artery in rats and mice (65, 378). The mecha-
nisms that result in neuronal dysfunction and/or death in these models are beginning to be understood, with increased oxidative stress, perturbed energy metabolism, altered calcium homeostasis, and activation of apoptotic cascades playing important roles in most cases (219). Data obtained using these various models have provided valuable insight into the cellular molecular mechanisms of neurodegenerative disorders and will therefore be cited throughout the remainder of this article.

II. ADAPTIVE CELLULAR AND MOLECULAR RESPONSES IN BRAIN AGING

A goal of basic and clinical neuroscientists is to identify approaches for promoting the maintenance of cognitive, emotional, motor, and sensory functions throughout the life span. This could be accomplished by avoiding genetic (through genetic counseling or germline gene therapy, for example) and environmental (dietary and behavioral factors, for example) factors that facilitate neuronal dysfunction and death, or by enhancing the ability of neurons to adapt to the aging process. Basic research is identifying cellular signaling mechanisms that promote cell survival, neurite growth, and/or synapse formation/plasticity; understanding these signaling pathways may reveal ways of promoting successful brain aging. Clinicians, geneticists, and epidemiologists should therefore work together to identify genetic and environmental factors that cause or affect risk of age-related neurological disorders.

A. Neuroprotective Mechanisms

Intercellular signaling mechanisms mediate the second-to-second functions of neuronal circuits as well as long-term changes in the biochemistry and structure of those circuits. Three major classes of intercellular signaling proteins that regulate neuronal survival and synaptic plasticity are neurotransmitters, neurotrophic factors, and hormones. Glutamate and GABA, the major excitatory and inhibitory neurotransmitters in the brain, play pivotal roles in regulating neuronal survival (231) and synaptic plasticity (18). By inducing the expression of neurotrophic factors such as BDNF, glutamate can promote neuronal survival (203). On the other hand, overactivation of glutamate receptors can cause neuronal death, particularly under conditions of increased levels of oxidative and metabolic stress, as occurs during aging and in age-related neurodegenerative disorders (224). By reducing neuronal excitability, GABA can protect neurons in experimental models of neurodegenerative disorders (231). Other neurotransmitters that can modify neuronal vulnerability in cell culture and animal models of neurodegenerative disorders include acetylcholine, dopamine, norepinephrine, and serotonin (89, 214, 288). During brain aging, these neurotransmitters may contribute to either degeneration or adaptive responses of neurons.

Neurotrophic factors promote the survival, outgrowth, and/or synaptogenesis of neurons. Examples of neurotrophic factors that have been shown to counteract untoward aspects of aging (oxidative stress and disturbed ion homeostasis, for example) include basic fibroblast growth factor (bFGF), NGF, BDNF, NT-4, transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), and GDNF. These neurotrophic factors can protect one or more populations of brain neurons against excitotoxic, oxidative, and metabolic insults in models of stroke, AD, PD, and HD (232, 233). Signaling by cell adhesion proteins such as integrins may also play important roles in modulating neuronal survival (94). Growth factors, cytokines, and integrin ligands promote neuronal survival by inducing the expression of genes that encode proteins that suppress oxidative stress, stabilize cellular calcium homeostasis, and antagonize neurodegenerative biochemical cascades. Examples of three neuroprotective signaling cascades, activated by BDNF, bFGF, and the secreted form of APP are shown in Figure 4. bFGF, BDNF, TNF, and NGF can increase the production of one or more antioxidant enzymes [Cu/Zn-superoxide dismutase (SOD), Mn-SOD, glutathione peroxidase, and catalase] in hippocampal neurons (233, 229). NGF, BDNF, and TNF can induce expression of anti-apoptotic Bcl-2 family members (32) and inhibitor of apoptosis proteins (IAP) (364). Neurotrophic factors can also modulate the expression and/or activity of subunits of glutamate receptors and voltage-dependent calcium channels in ways that promote neuronal survival and synaptic plasticity (217, 225, 360). Kinases such as mitogen-activated protein (MAP) kinase and protein kinase C, and transcription factors such as NF-κB and CREB, transduce the cell survival signals of neurotrophic factors and cytokines.

Another type of adaptive response that may protect neurons against the adversities of aging and disease is a stress response that involves protein chaperones that exhibit neuroprotective properties. Examples of such stress proteins include heat shock proteins (e.g., HSP-70, HSP-90, and HSP-60) and glucose-regulated proteins (e.g., GRP-78 and GRP-94). These chaperone proteins interact with many different proteins in cells and function to ensure their proper folding, on the one hand, and degradation of damaged proteins, on the other hand (86, 96). They may also interact with, and modify the function of, apoptotic proteins including caspases (14, 293). Levels of some of these chaperone proteins may be increased during aging as a protective response (180, 182). Cell culture and in vivo studies have shown that HSP-70 and GRP-78 can protect neurons against injury and death in experimental models of neurodegenerative disorders (197, 380). Interestingly, caloric restriction, a dietary manipulation

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that increases life span and brain “health span” (the time window of life during which the brain maintains a level of function that permits a productive life-style), can increase the expression of chaperone proteins in the brains of rats and mice (see sect. IV).

Synapses are sites where the actions of neurotrophic factors, stress proteins, and anti-apoptotic Bcl-2 and IAP family members may play particularly important roles in preserving the integrity and function of neuronal circuits (115, 228). The impact of aging is likely to be most severe in synapses, because these compartments are sites of repetitive calcium influx and oxyradical production; it is therefore of great importance to understand how genes and environment affect synaptic homeostasis (see sect. VI).

B. Neurorestorative Mechanisms

Research performed in many different laboratories during the past 10 years has revealed that regeneration/compensation can occur in the adult brain and that populations of stem cells or neural progenitor cells (NPC) may play a role in this process by dividing and then differentiating into neurons or glia (91). Various neurotrophic factors and cytokines may promote neurogenesis, neurite outgrowth, and synaptic recovery after brain injury (146, 232). Damage to axons and dendrites can result in regrowth of those processes; however, in contrast to the peripheral nervous system, the brain contains a number of inhibitory signals that may prevent successful reinnervation of target cells (312). If synaptogenesis does occur, it may or may not replace lost function depending on the degree of specificity of neuronal connections in the circuits involved. For example, reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement (333). On the other hand, many brain functions rely on “memories” based on the past history of synaptic activity, and such memories are unlikely to be restored by new synapse formation.

Stem cell biology is a rapidly growing area in the fields of neuroscience research and aging. Embryonic stem cells have received considerable attention because of their ability to form any type of cell in the body including neurons (99). They are therefore a potential cell source for replacement of neurons lost in neurodegenerative disorders. Two major populations of pluripotent NPC are present in the adult brain, one in the subventricular zone and the other in the subgranular layer of the dentate gyrus of the hippocampus (91). These NPC cells can give rise to either neurons or astrocytes, and there is increasing evidence that some of the progeny of the NPC survive and become functional, although many may undergo programmed cell death (Fig. 5). Newly generated cells in the brain can be identified by giving animals the thymidine analog bromodeoxyuridine (BrdU); the phenotype of their differentiated progeny can then be determined by double-labeling using antibodies against neuronal (e.g., neural cell adhesion molecule or \( \beta_3 \)-tubulin) or astrocyte (glial fibrillary acidic protein [GFAP]) markers. Several signals that control the proliferation, differentiation, and survival of NPC have been identified (91, 291). bFGF and epidermal growth factor (EGF) can maintain NPC in a proliferative state, whereas BDNF and NT-3 can promote their

![FIG. 4. Examples of cellular signaling pathways that modulate neuronal plasticity and survival during aging. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) activate membrane receptor tyrosine kinases that initiate kinase signaling cascades that ultimately regulate the expression of genes that encode proteins which enhance neuronal survival and plasticity. Such gene targets include those encoding proteins that suppress apoptotic cascades, reduce oxidative stress, and stabilize cellular calcium homeostasis. AKT/PKB, Akt kinase; CaMKIV, calcium/calmodulin-dependent protein kinase IV; CREB, cAMP response element binding protein; IRS1, insulin receptor substrate-1; MAPK, mitogen-activated protein kinase; MEK, MAP kinase kinase; PI3K, phosphatidylinositol 3-kinase; PKG, protein kinase G; R, receptor; sAPP, secreted form of amyloid precursor protein; SHC, src homology domain-containing adaptor protein; trkB, high-affinity BDNF receptor.](http://physrev.physiology.org/doi/fig/10.1152/physrev.00196.2001)
differentiation and/or survival, and bone morphogenetic protein can induce NPC to become astrocytes (91, 241). Additional signals that control NPC cell fate include Notch (344), Numb (40), neurogenin (339), and the secreted form of APP (255). Brain injury is a potent stimulus for neurogenesis (194), and this effect is likely mediated by the trophic factors and cytokines induced by cell injury (232).

Considerable interest in fundamental mechanisms of cellular aging has arisen from studies of telomerase, a reverse transcriptase that adds a six-base DNA repeat onto the ends of chromosomes (telomeres) and thereby maintains their integrity during successive rounds of cell division (221). Expression of the catalytic subunit of telomerase (TERT) and telomerase activity are associated with cell immortalization and cancer and are absent from most somatic cells in the adult, suggesting an important role in the aging process. Indeed, expression of TERT in normal fibroblasts makes them immortal (without transforming them) (24). Telomerase is present in brain cells during development, where it is thought to play a role in the maintenance of NPC in a proliferative state, and in promoting survival of NPC and their neuronal and glial progeny (87, 166). Telomerase is also present in NPC in the adult brain where its expression may be influenced by brain injury and other environmental factors. Recent studies suggest that TERT expression can be induced by BDNF and sAPPα (W. Fu and M. P. Mattson, unpublished data). It has been reported that TERT can prevent apoptosis of cultured neurons in experimental models relevant to AD and stroke (87, 387), suggesting that if TERT expression could be induced in neurons or NPC in the adult brain, it may increase the resistance of neurons to age-related neurodegenerative disorders (221). DNA damage may trigger neuronal death in age-related neuregenerative processes, and telomerase may suppress such DNA damage responses (198); nuclear localization and reverse transcriptase activity appear to be critical for the antiapoptotic function of TERT (P. Zhang and M. P. Mattson, unpublished data).

The possibility that the aging process impairs neurogenesis is suggested by studies in which BrdU-labeled cells were quantified in the brains of middle-aged and old rats (174). This adverse effect of aging on neurogenesis may be counteracted by many of the same environmental conditions that promote successful brain aging. Indeed, dietary restriction can increase neurogenesis (183). Interestingly, neurogenesis can also be increased by environmental enrichment (164, 254) and physical exercise (351). Because no specific molecular markers of NPC have been established, and because NPC cannot be labeled by the usual BrdU method in clinical studies of humans, it is not known whether abnormalities in neurogenesis contribute to the pathogenesis of age-related neurodegenerative disorders. However, recent studies of experimental models of AD have shown that amyloid β-peptide can impair neurogenesis (124). Both the proliferation and survival of NPC in the dentate gyrus of the hippocampus are reduced in APP mutant mice. Infusion of amyloid β-peptide into the lateral ventricle of adult mice impairs neurogenesis of NPC in the subventricular region. Moreover, exposure of cultured human NPC to amyloid β-peptide impairs their proliferation and differentiation and can induce apoptosis (124). These experimental findings suggest that adverse effects of amyloid β-peptide on NPC may contribute to depletion of neurons and cognitive impairment in AD. Although it is not known whether a failure of neurogenesis contributes to the pathogenesis of PD, several studies in rodents, nonhuman primates, and humans suggest that functional recovery can occur after transplantation of
NPC or mobilization of endogenous NPC (21, 323). The development of methods for identifying NPC and their recent progeny in postmortem brain tissue sections from human patients would greatly facilitate our understanding of the relative contributions of neuronal degeneration and impaired neurogenesis to neurodegenerative disorders.

The implications of neurogenesis for facilitating successful brain aging and treating age-related neurodegenerative disorders are quite profound. It may be possible to mobilize endogenous NPC in the brain or to introduce exogenous NPC to replace dysfunctional or dead neurons and glia. As described above, three different behavioral modifications have been shown to enhance neurogenesis (dietary restriction and increased intellectual and physical activities). In addition, pharmacological approaches for mobilizing NPC are being developed. For example, it has been shown that antidepressant drugs such as seroton transport inhibitors can increase production of BDNF and stimulate neurogenesis (5, 201). There have already been reports of improved functional outcome following NPC transplantation in models of traumatic central nervous system injury (238), demyelinating disorders (372), and PD (21). Particularly intriguing are reports suggesting that stem cells in other organs of the body, including bone marrow, are capable of forming neurons and glial cells (242). A major clinical hurdle in interindividual transplantsations is immune attack on the transplanted cells; this could be avoided by using a person's own stem cells for transplantation. Embryonic stem cells may also prove valuable in treating various neurodegenerative disorders because of their multipotent properties and their reduced reactivity toward immune cells.

III. GENETIC FACTORS IN BRAIN AGING AND NEURODEGENERATIVE DISORDERS

The probability of living a long life with preservation of a high level of brain function is strongly influenced by the genes one inherits. Accordingly, genetic factors also play important roles in determining one's risk of age-related neurodegenerative disorders. In this section we review the evidence for the involvement of specific genes in the determination of life span and risk of neurological disorders of aging.

A. Genes That Influence Life Span and Brain Health Span

There is ample evidence that life span (52, 193, 274), intelligence (57, 240), and risk of various neurological disorders (51, 337, 355) are determined, in part, by heritable factors. However, the specific genes involved and their mechanisms of action are largely unknown. One gene that appears to have an influence on aging in general, and on risk of age-related neurodegenerative disorders, is apolipoprotein E (327). Three alleles of apolipoprotein E encode proteins that differ in two amino acids; E2 contains a cysteine in each position, E3 contains a cysteine in one of the positions, and E4 does not contain a cysteine in either position. Individuals with an E4 allele have a reduced life span (126) and are at increased risk of AD (157). The mechanism whereby E4 may accelerate brain aging has been suggested to involve a decreased antioxidant and neuroprotective properties of this isoform (Fig. 6). The cysteine residues in E2 and E3 may bind to and thereby detoxify 4-hydroxynonenal, a cytotoxic product of lipid peroxidation (266).

Other genes linked to life span that may influence brain aging are those encoding growth hormone (10) and major histocompatibility complex (MHC) proteins (36). Growth hormone levels decrease with aging, and this change is ameliorated by caloric restriction. The density of microvessels in the brains of rodents decreases during aging, and this can be reversed by treating the animals with growth hormone, which may increase production of insulin-like growth factor I (IGF-I) in the brain (332). The latter studies further showed that IGF-I can reverse age-related impairment in learning and memory. IGF-I can also protect neurons against injury and death in experimental models of AD and related neurodegenerative disorders (46, 386). MHC genes are expressed in neurons (58). Mice that are genetically deficient for class I MHC proteins exhibit incomplete synaptogenesis in the developing visual system and enhanced long-term potentiation of synaptic transmission in the hippocampus (135), suggesting important roles for MHC proteins in learning and memory.

Inherited variability of mitochondrial genes encoding proteins involved in oxidative phosphorylation and other aspects of mitochondrial function may also contribute to aging (60) and the pathogenesis of neurodegenerative disorders (258). Mitochondrial DNA damage has been shown to increase in brain cells during aging (121), and it has been proposed that accumulation of mitochondrial DNA mutations is a major factor in the aging process itself (260).

B. Genes That Cause or Increase Risk of Neurodegenerative Disorders

The past decade has been filled with major advances in our understanding of the pathogenesis of age-related neurodegenerative disorders, as the result of the combined efforts of molecular geneticists and cell and molecular biologists. More than 20 different genes have been identified in which mutations cause an inherited form of a neurodegenerative disorder. Once such a gene is discovered, the pathogenic mechanism of the mutated form of...
the gene can be elucidated in studies of cultured cells and transgenic mice expressing the mutant gene. In this section we describe how the discovery of such disease-causing genes has revealed why neurons become dysfunctional and die in several of the most prominent neurodegenerative disorders including AD, PD, HD, and amyotrophic lateral sclerosis (ALS). What emerges from the studies described below is a view of neurodegenerative diseases in which genetic compromise renders the brain vulnerable to the aging process, with specific populations of neurons being disproportionately affected. In general, disease-causing mutations appear to act mainly by accelerating the same neurodegenerative cascade that occurs in more common sporadic forms of the disease.

Although the vast majority of cases of AD are sporadic with no clear pattern of inheritance and a late age of onset (70s and 80s), some cases are inherited in an autosomal dominant manner with complete penetrance and an early age of onset (40s and 50s). The first gene linked to familial AD is located on chromosome 21 and encodes the APP, the source of the 40- to 42-amino acid amyloid β-peptide (Aβ) that forms insoluble amyloid plaques in the brains of all AD patients (122). Several different disease-causing mutations in APP have been reported, all of which are located within or adjacent to the Aβ sequence, and all of which increase production of Aβ-(1-42). The most intensely studied APP mutations are the "Swedish" mutation (a 2-amino acid substitution adjacent to the NH₂ terminus of Aβ; Ref. 177) and the "London" mutation (a missense mutation adjacent to the COOH terminus of Aβ; Ref. 42). In addition, several AD kindreds have been identified in which mutations within the Aβ sequence are
pathogenic (253). Two other genes linked to early-onset familial AD are those encoding PS1 (chromosome 14) and PS2 (chromosome 1); more than 70 different PS1 mutations (all except one are missense mutations), and 2 PS6 mutations have been reported (122). PS1 and PS2 are structurally similar integral membrane proteins with eight transmembrane domains and are localized mainly in the endoplasmic reticulum (ER). The presenilin-1 mutations tend to cluster in the cytoplasmic loop region and in or near transmembrane domain 2. The identification of the mutations in the APP and presenilin genes has led directly to experiments that have revealed, at least in part, how they cause AD (Fig. 7; Ref. 226).

A well-documented abnormality that results from APP mutations, as well as presenilin mutations, is increased production of Aβ [particularly Aβ(1–42)] and decreased production of sAPPα (6, 122, 216). Aβ can impair synaptic function and can render neurons vulnerable to excitotoxicity and apoptosis by the following mechanism. During the process of self-aggregation, Aβ generates reactive oxygen species (hydrogen peroxide and hydroxyl radical) by a mechanism that may involve metal-catalyzed oxidation of methionine (128, 134, 353). When this process occurs in the immediate vicinity of cell membranes, lipid peroxidation is initiated (204, 205). In neurons, Aβ-induced lipid peroxidation impairs the function of ion-motive ATPases (sodium and calcium pump proteins), glucose transporter proteins (204–206), and GTP-binding proteins (163). Aβ can also induce oxidative stress in astrocytes resulting in impaired glutamate trans-

FIG. 7. Mechanisms underlying the pathogenic actions of mutations in amyloid precursor protein (APP) and presenilins. Mutations in APP, as well as presenilin mutations, shift the proteolytic processing of APP such that more Aβ is produced and less sAPPα is produced. Presenilins play an essential role in γ-secretase cleavage of APP and may also facilitate Notch cleavage and release of the transcription-regulating Notch COOH-terminal domain (NICD). Presenilin mutations have a major impact on endoplasmic reticulum (ER) calcium signaling, effectively increasing the pools of ryanodine- and inositol triphosphate-sensitive stores. The normal functions of APP and presenilins, and the consequences of Alzheimer’s disease-linked mutations in these proteins, may be particularly important in synapses.
port (23). By this mechanism, Aβ disrupts neurotransmitter signaling, destabilizes cellular calcium homeostasis, and renders neurons vulnerable to excitotoxicity and apoptosis (204, 227). Oxidative stress induced by Aβ may be particularly detrimental for neuronal function and survival when it occurs in synapses (162).

Exposure of cultured neurons to Aβ can trigger programmed cell death which manifests characteristic mitochondrial membrane alterations and release of cytochrome c and caspase activation. Aβ stimulates the production of apoptotic proteins including Par-4, Bax, and the tumor suppressor protein p53 (55, 80, 109, 111, 262). Analyses of post mortem brain tissue from AD patients reveals evidence for neuronal apoptosis including upregulation of Par-4 (109) and caspase activation (38). Agents that stabilize mitochondrial function and caspase inhibitors protect neurons against Aβ-induced death (109, 111, 234).

In addition to increasing production of Aβ, APP and presenilin mutations decrease the production of sAPPα (6). A decrease in sAPPα levels may contribute to the pathogenesis of AD, because sAPPα normally functions in modulating synaptic plasticity (learning and memory) and in promoting survival of neurons (90, 142, 230). The mechanism whereby sAPPα promotes neuronal survival and synaptic plasticity involves activation of potassium channels and of the transcription factor NF-κB; these actions of sAPPα stabilize cellular Ca2+ homeostasis and suppress oxyradical production (225).

AD patients typically exhibit emotional disturbances and abnormal stress responses involving increased glucocorticoid production that likely result from pathological changes in brain regions that control such behaviors and stress responses including limbic structures such as the amygdala and hippocampus and the frontal cortex (300). Studies of APP mutant transgenic mice suggest that such abnormal stress responses are the result of increased levels of Aβ in these brain regions. APP mutant mice exhibit an age-dependent increase in sensitivity to physiological stressors, which is associated with abnormalities in hypothalamic-pituitary-adrenal function and dysregulation of blood glucose levels (267). Two related neuropeptides that may play a role in the alterations in affective behaviors in AD patients and APP mutant mice are corticotropin-releasing hormone (CRH) and urocortin. CRH is expressed in brain regions prone to degeneration in AD, and CRH can protect cultured hippocampal and cortical neurons against cell death caused by Aβ (271). Urocortin and urocortin II are CRH-related neuropeptides that act on CRH receptors expressed by neurons in the hippocampus and functionally related brain regions. Urocortin can protect hippocampal neurons against excitotoxic and oxidative injury by activating the type I CRH receptor and a signaling pathway involving cAMP-dependent protein kinase, protein kinase C, and MAP kinase (272). These findings suggest roles for CRH and urocortin in antagonizing the neurodegenerative process in AD. It remains to be determined whether pharmacological manipulations of CRH/urocortin signaling will benefit AD patients.

Two major consequences of presenilin mutations are perturbed cellular calcium homeostasis (226) and altered APP processing (122) (Fig. 6). At this point in time, it remains unclear which defect is a primary consequence of the mutations and which is secondary. PS1 and PS2 mutations increase the vulnerability of cultured cells to apoptosis and excitotoxicity (108, 110, 112, 367). Hippocampal neurons in PS1 mutant knockin mice are more vulnerable to excitotoxicity and apoptosis (108). Perturbed calcium regulation in the ER is central to the cell death-promoting effects of PS1 mutations (Fig. 7). The abnormality involves an increased pool of ER calcium resulting in increased calcium responses when cells are challenged with glutamate or agonists that stimulate calcium release from the ER (39). Abnormal ER calcium signaling caused by presenilin mutations may promote altered capacitative calcium influx through voltage-dependent channels in the plasma membrane (186, 375). Presenilin mutations result in altered APP processing, and there is evidence that presenilins are critical for γ-secretase activity (369) and Notch cleavage (63). Altered APP processing may not account for the spectrum of effects of PS mutations. Instead, altered APP and Notch processing caused by PS mutations may result from altered calcium-mediated regulation of the enzyme activities that mediate cleavage of the two proteins (191, 276, 284, 316, 361). Indeed, one or more secretaire activities are sensitive to calcium (44). Notch signaling may promote neuronal survival by enhancing cellular calcium homeostasis, an action antagonized by a protein called Numb; alterations in Notch and Numb functions may play roles in the pathogenesis of AD (40).

Mutations in genes encoding the proteins α-synuclein and Parkin can cause early-onset familial PD; α-synuclein mutations are inherited in an autosomal dominant manner, while mutations in parkin are inherited in an autosomal recessive manner (280). The α-synuclein gene is located on chromosome 4, and the Parkin gene is located on chromosome 6. α-Synuclein is a vesicle-associated protein, and Parkin is a cytoplasmic protein. α-Synuclein is axonally transported and associates with vesicles in presynaptic terminals, suggesting a role in regulation of vesicle trafficking (148). Parkin is expressed primarily in neurons where it is localized at particularly high levels in neurites (137). Studies of the pathogenic actions of three missense mutations in α-synuclein (A53T, A30P, and G209A) have provided new insight into the events that lead to the dysfunction and degeneration of dopaminergic neurons in PD (Fig. 8). Expression of α-synuclein mutations in cultured cells increases their vulnerability to ox-
Overexpression of wild-type or mutant α-synuclein induces apoptosis in cultured neurons (305); PC12 cells overexpressing mutant α-synuclein exhibit decreased proteasome activity and increased vulnerability to mitochondrial dysfunction and apoptosis (343). Masliah et al. (210) reported evidence for loss of dopaminergic neurons and Lewy body-like cytoplasmic inclusions in α-synuclein mutant mice. However, another line of mice expressing mutant α-synuclein driven by a tyrosine hydroxylase promoter did not exhibit pathology in the substantia nigra (211). α-Synuclein forms aggregates that may exhibit toxic properties similar to those of Aβ including production of reactive oxygen species (347) and increased membrane ion permeability (357). Collectively, these findings suggest that α-synuclein mutations may promote neuronal degeneration by causing abnormalities in protein degradation and oxidative stress. Some data point to a loss of function, as opposed to a gain of function in the pathogenic action of α-synuclein mutations. Thus α-synuclein knockout mice exhibit a defect in dopamine release (1), and overexpression of wild-type (but not mutant) α-synuclein protects cultured neural cells against apoptosis (56).

Parkin is a ubiquitin-protein ligase that presumably functions in protein degradation; Parkin mutations result in loss of the ubiquitin-protein ligase activity (320). It was recently reported that parkin can ubiquitinate α-synuclein (321), suggesting a link between impaired proteasomal degradation of α-synuclein and the neurodegenerative process. These observations strongly suggest that a defect in protein degradation is central to the pathogenesis of PD and further suggest strong mechanistic interactions between oxidative stress and protein degradation in neurodegenerative disorders in general, since oxidative damage
to proteins often makes them targets for ubiquitination and proteasomal degradation. In addition to disease-causing mutations, risk of PD may be influenced by genetic polymorphisms, a possibility that is currently being investigated by several laboratories (43, 356).

The genetics of HD are seemingly straightforward; all cases of this disease are believed to be caused by the expansion of trinucleotide (CAG) repeats in the huntingtin gene (located on chromosome 4) resulting in long stretches of polyglutamine repeats in the huntingtin protein (76, 199). However, the consequences of the huntingtin mutation may be subject to modification by aging, as suggested by evidence for variability in age of disease onset and progression of the clinical phenotype. HD patients manifest progressive motor dysfunction characterized by involuntary body movements due to degeneration of neurons in the basal ganglia, principally the caudate and putamen (3). As the disease progresses, the neurodegenerative process may spread to regions of the cerebral cortex, thalamus, and cerebellum resulting in cognitive dysfunction and emotional disturbances. Recently, a closely related familial HD-like disorder was described that appears to result from a trinucleotide expansion in a yet-to-be identified gene (202).

The alterations caused by polyglutamine expansions in huntingtin that result in neuronal death are beginning to be revealed (Fig. 9). Overexpression of mutant human huntingtin in cultured cells and transgenic mice can induce spontaneous cell death (apoptosis) and can increase the vulnerability of neurons to excitotoxicity (130, 192, 294, 346). Several different behavioral abnormalities have been described in mice expressing mutant huntingtin including motor deficits and cognitive dysfunction (250, 311). The reason that trinucleotide expansions in huntingtin promote degeneration of striatal neurons in HD is unclear. Mutant huntingtin self-aggregates resulting in the
formation of inclusions in the nucleus and cytoplasm (120, 311). Neurons in mice expressing mutant huntingtin exhibit increased caspase activation, and administration of caspase inhibitors to the mice can suppress the neurodegenerative process (257), suggesting that mutant huntingtin triggers programmed cell death. Cells expressing mutant huntingtin exhibit altered proteasomal function that may trigger apoptosis (145). Mutant huntingtin may trigger apoptosis by weakening the interaction of huntingtin with huntingtin interacting protein-1 (Hip-1), thereby allowing Hip-1 to interact with a protein called Hippi that then recruits caspase-8 and thereby initiates the cell death cascade (223).

Interestingly, adverse effects of mutant huntingtin on cell function are not limited to the nervous system because abnormalities in adipocytes have been described that may be related to the well-known alterations in energy metabolism in HD patients (82). The ability of dietary supplementation with creatine to delay motor symptoms and increase survival in huntingtin mutant mice (7) is consistent with impaired cellular energy metabolism in the neurodegenerative process. Finally, it was recently reported that levels of BDNF are decreased in HD (390), suggesting that decreased neurotrophic support may be a consequence of huntingtin mutations that promotes the degeneration of striatal neurons.

In each of the three neurodegenerative disorders just described (AD, PD, and HD), there is considerable evidence suggesting that abnormalities in mitochondrial function contribute to the disease process. In each disorder, alterations in activities of enzymes involved in oxidative phosphorylation have been demonstrated including a decrease in activity of the α-ketoglutarate dehydrogenase complex in AD (97a) and a defect in complex I in PD (306b). Such abnormalities in mitochondrial energy metabolism may precede and contribute to the increased oxidative stress and perturbations in neuronal calcium homeostasis that occurs in each disorder. Interestingly, the metabolic deficits, and oxidative stress and calcium dysregulation, may not be limited to the brain cells affected in the disorders, as they have been documented in peripheral cells including fibroblasts and lymphocytes (95a, 306b). Based on these and additional data, Blass (23a) has introduced the concept of a “mitochondrial spiral,” in which metabolic deficits result in oxyradical production and calcium dysregulation, to explain the pivotal role of mitochondrial alterations in neurodegenerative disorders. Both genetic and environmental factors may promote such neurodegenerative mitochondrial spirals (97a).

ALS involves degeneration of spinal cord motor neurons resulting in progressive paralysis and death (196, 301). Most cases of ALS are sporadic, but some result from mutations in the gene encoding the antioxidant enzyme Cu/Zn-SOD, which is located on chromosome 21 (62, 302). Transgenic mice expressing mutant Cu/Zn-SOD exhibit progressive motor neuron degeneration and a clinical phenotype remarkably similar to ALS patients (118, 368). Lipid peroxidation is increased in spinal cord motor neurons of ALS patients and transgenic mice (268, 269), and administration of vitamin E to Cu/Zn-SOD mutant mice delays disease onset (117), suggesting an important role for lipid peroxidation in the neurodegenerative process. In addition, creatine delayed the neurodegenerative process in a mouse model of ALS (168). ALS Cu/Zn-SOD mutations cause impairments in synaptic glucose and glutamate transport (114) and increase the vulnerability of motor neurons to excitotoxic injury by increasing oxidative stress and perturbing cellular calcium homeostasis (172, 185).

The remarkably large size of axons of motorneurons and their correspondingly high density of neurofilaments has led to the suggestion that impaired axonal transport plays a role in the pathogenesis of ALS. Studies of axonal transport in Cu/Zn-SOD mutant mice and of mice lacking or overexpressing neurofilament proteins support a role for impaired axonal transport in ALS (365, 383). Apoptosis of motor neurons in ALS is suggested by studies showing that levels of the proapoptotic protein Par-4 are increased in spinal cord motor neurons of ALS patients and Cu/Zn-SOD mutant mice (269), and levels of caspase activation are also increased in spinal cord tissue from Cu/Zn-SOD mutant mice (264). In addition, caspase inhibitors (190) and the antiapoptotic protein Bcl-2 (358) can protect motor neurons in Cu/Zn-SOD mutant mice. Moreover, neurotrophic factors that can prevent apoptosis of motor neurons in culture can also prevent motor neuron loss and disease progression in Cu/Zn-SOD mutant mice (243).

Genetic risk factors for stroke, an age-related neurological disorder, overlap with risk factors for coronary artery disease due to shared mechanisms of atherosclerosis and blood clot formation in each disorder. Familial hypercholesterolemia caused by mutations in the low-density lipoprotein receptor results in early-onset atherosclerotic vascular disease (334). Apolipoprotein E genotype affects risk of atherosclerosis (286). Polymorphisms in the apolipoprotein(s) gene and the gene for methylene tetrahydrofolate reductase have been shown to be prominent risk factors for atherosclerotic vascular disease (252). Polymorphisms in the fibrinogen and factor XIII genes have been linked to stroke (37), suggesting a contribution of genetic differences in regulation of clot formation to disease risk. An inherited stroke syndrome called Cadasil (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) was recently linked to mutations in the Notch-3 gene on chromosome 19 (59), and the cellular and molecular alterations caused by these mutations are currently being investigated.

Collectively, the data that have accumulated in stud-
ies of the age-related neurodegenerative disorders described above suggest that each disorder shares abnormalities that contribute to neuronal dysfunction and death. The alterations include increased oxidative stress, dysregulation of protein trafficking and processing, metabolic impairment, and disruption of cellular calcium homeostasis. Genetic factors that cause, or increase risk of, a disorder do so by impacting directly or indirectly one or more of the cellular systems involved in oxycradical metabolism, protein processing, energy metabolism, and calcium homeostasis. Section IV describes a rapidly growing body of evidence demonstrating that age-related neurodegenerative cascades can be influenced by environmental factors.

IV. DIETARY FACTORS IN BRAIN AGING AND NEURODEGENERATIVE DISORDERS

There are numerous dietary factors that have been reported to affect brain physiology in ways that could, in theory, modify brain aging and the pathogenesis of neurodegenerative disorders (221a). These range from amino acids such as tryptophan (16) to caffeine and related stimulants (85) to omega-3 fatty acids (348). Many such dietary factors have been shown to affect mood or cognition. However, this review focuses on a more limited number of dietary factors for which considerable supportive experimental, clinical, and epidemiological data have accrued to justify the development of recommendations to the general public vis-à-vis risk reduction for neurodegenerative disorders. These factors include caloric intake, folic acid, and antioxidants.

A. Effects of Dietary Restriction on Brain Aging: Neuroplasticity and Neuroprotection

The mean and maximum life spans of many different organisms including yeast, roundworms, rodents, and monkeys can be increased by up to 50% simply by reducing their food intake (9, 176, 362). The incidence of age-related cancers, cardiovascular disease, and deficits in immune function is decreased in rodents maintained on such dietary restriction (DR) feeding regimens (362). Data from clinical and epidemiological studies in humans support the antiaging and disease prevention effects of DR. Thus a low-calorie diet decreases the risk of the most prominent age-related diseases in humans including cardiovascular disease, diabetes, and cancers (29, 179, 187). Recent findings reviewed in this section strongly suggest that DR can delay age-related functional deficits in the brain and may reduce the risk of major neurodegenerative disorders including AD, PD, and HD. DR may also increase resistance of neurons to acute insults such as stroke and severe epileptic seizures.

Biochemical and molecular analyses of the brains of old rats and mice that had been maintained on calorie-restricted diets reveal a retardation of changes that occur during aging of animals fed ad libitum including increases in levels of GFAP and oxidative damage to proteins and DNA (74, 245). Gene array analysis of the expression levels of thousands of genes in the brains of young rats and old rats that had been maintained on control or restricted diets revealed changes in gene expression in brain cells during aging and showed that DR can suppress many of those changes (180). Age-related changes in the expression of genes that encode proteins involved in innate immune responses, oxidative stress, and energy metabolism are counteracted by DR. This retardation of brain aging at the molecular level may underlie the preservation of brain function during aging in animals maintained on DR. For example, DR attenuates age-related deficits in learning and memory ability and motor function in rodents (140, 336). Studies of human populations suggest that DR can promote successful brain aging in humans. For example, epidemiological data suggest that the risk of developing AD, PD, and stroke is lower in individuals with a low calorie intake (30, 195, 235).

Beneficial effects of DR have been demonstrated in several of the animal models just described. The two DR protocols most commonly used in such studies are every-other-day feeding (the animals must go a whole day without food and then eat ad libitum on the next day) and paired feeding (the restricted animals are given food pellets that contain 30–40% fewer calories than the pellets given to the control animals). Both of these feeding regimens increase the life spans of rats and mice by 30–40% (103, 362). Rats maintained on DR for 2–4 mo exhibit increased resistance of hippocampal neurons to kainate-induced degeneration in a model relevant to the pathogenesis of AD and epilepsy; kainate selectively damages hippocampal pyramidal neurons and there is a profound deficit in learning and memory (31). When rats were maintained for several months on DR, damage to hippocampal neurons was decreased, and learning and memory were preserved compared with rats fed ad libitum (31). Studies of PS1 mutant knockin mice showed that the PS1 mutations increase the vulnerability of hippocampal and cortical neurons to excitotoxicity and apoptosis by a mechanism involving enhanced calcium release from the ER (108, 111). When PS1 mutant knockin mice were maintained on DR, they exhibited increased resistance of hippocampal CA1 and CA3 neurons to excitotoxic injury compared with mice fed ad libitum (388). Lipid peroxidation in the hippocampus after kainate administration was decreased in DR PS1 mutant mice, suggesting that suppression of oxidative stress is one mechanism whereby DR protects neurons (388).

DR has beneficial effects in animal models of PD and HD. The vulnerability of midbrain dopaminergic neurons
to MPTP toxicity was decreased in mice maintained on dietary restriction with more dopaminergic neurons surviving exposure to MPTP; the motor function of the mice was also improved in the restricted mice (69). Administration of the succinate dehydrogenase inhibitor (mitochondrial toxin) 3-nitropropionic acid (3-NP) to rats and mice results in selective degeneration of striatal neurons and motor impairment, a model of HD. When rats were maintained on DR for several months before administration of 3-NP, more striatal neurons survived exposure to 3-NP, and their motor function was improved (31). The ability of DR to improve outcome after a stroke was demonstrated in a rat model in which the middle cerebral artery was transiently occluded resulting in damage to the cerebral cortex and striatum supplied by that artery and unilateral motor dysfunction. When rats were maintained on DR for several months and then subjected to a stroke, they exhibited reduced brain damage and improved behavioral outcome (380). The neuroprotective effects of DR in animal models of several different neurodegenerative disorders suggest that low-calorie diets may prove beneficial in reducing the incidence and/or severity of the corresponding human neurodegenerative disorders.

Although the findings described in the preceding paragraphs document quite striking neuroprotective effects of DR, this dietary manipulation has not proven beneficial in all animal models of neurodegenerative disorders. For example, when one line of APP mutant transgenic mice was maintained on an every-other-day feeding regimen or was fed ad libitum, they died within 2–3 wk (267). Additional analyses in the latter study showed that the APP mutant mice were hypersensitive to the stress associated with fasting for an entire day and became severely hypoglycemic during the days they were without food. The APP mutant mice exhibited abnormalities in the regulation of the stress-responsive hypothalamic-pituitary-adrenal axis including altered glucocorticoid and blood glucose regulation in response to restraint stress. However, when every-other-day feeding was begun in APP mutant mice that were less than 3 mo of age, they survived, suggesting a role for age-dependent amyloid deposition in the aberrant stress response. Transgenic ALS mice expressing the G93A Cu/Zn-SOD mutation did not benefit from DR. When they were maintained on an every-other-day feeding regimen, the age of disease onset was unchanged (270). Moreover, once the ALS mice on DR became symptomatic, the disease progressed more rapidly and they died sooner than did ALS mice that were fed ad libitum. These findings are interesting in that they suggest that the pathogenic mechanism of action of the Cu/Zn-SOD mutation is not subject to modification by DR and/or that DR does not exert the same kind of neuroprotective action on spinal cord motor neurons that it exerts on neurons in the brain.

There is emerging evidence from studies of human populations that is consistent with the possibility that DR can reduce the risk of human neurodegenerative disorders. The following epidemiological data suggest that individuals with a low calorie intake may have reduced risk for AD and PD. There is a strong correlation between per capita food consumption and risk for AD (105). For example, the reported incidence of AD in China and Japan is approximately one-half that in the United States and Western Europe, and this is correlated with a lower calorie intake in China and Japan (1,600–2,000 calories/day) compared with the United States and Western Europe (2,500–3,000 calories/day). Overeating is also a major risk factor for stroke (30). Although there are caveats with the latter observations (for example, per capita food consumption is a very poor measure of energy intake, and disease diagnosis may differ among the countries), they are consistent with a protective effect of low-calorie diets against age-related neurodegenerative disorders. More convincing evidence that DR can protect against neurodegenerative disorders comes from population-based case-control studies by Mayeux and colleagues who found that individuals with the lowest daily calorie intakes had the lowest risk of AD (235) and PD (195). Interestingly, the risk of PD and AD was more strongly correlated with calorie intake than with weight or body mass index. More recently, Hendrie et al. (127) reported findings from a population-based longitudinal prospective study which indicate that the incidence of AD increases among individuals living in industrialized countries compared with genetically similar individuals that live in nonindustrialized countries. Although the environmental factors that increase risk of AD in industrialized countries are not known, one clear difference between the two environments is calorie intake, which is much higher in industrialized countries. Together, the epidemiological and experimental data provide strong evidence that DR can reduce risk of AD, PD, and stroke, three of the most devastating neurodegenerative conditions in the elderly.

B. Cellular and Molecular Mechanisms Underlying the Neural Effects of Dietary Restriction

Because dietary restriction increases life span and reduces risk of many different age-related diseases including cardiovascular disease, diabetes, and cancers, it might be expected that it modifies shared biochemical cascades that lead to cell dysfunction and disease. In the case of neurodegenerative disorders, it is clear that while different genetic and environmental factors may initiate the neurodegenerative process in different disorders, a shared biochemical cascade ensues. Increased oxidative stress, perturbed cellular calcium homeostasis, and impaired energy metabolism occur in every neurodegenerative disorder studied to date (216, 219). These alterations...
render neurons vulnerable to apoptosis, a biochemical cascade of molecular interactions involving proteins such as Par-4, Bcl-2 family members, and caspases (219).

It has been shown that DR can stabilize mitochondrial function and reduce oxidative stress in brain cells of rodents (113), and this may increase the resistance of neurons to many different types of genetic and environmental factors. DR can induce the expression of genes that encode proteins that promote neuronal survival and plasticity (Fig. 10). For example, levels of heat shock protein-70 (HSP-70) and glucose-regulated protein-78 (GRP-78) are increased in cortical, striatal, and hippocampal neurons of rats and mice maintained for several months on a dietary restriction feeding regimen (69, 380). HSP-70 and GRP-78 can protect neurons against excitotoxic and oxidative injury (197, 379), suggesting that their increased levels contribute to the neuroprotective effect of dietary restriction.

DR can induce the expression of several different neurotrophic factors in brain cells. Levels of BDNF are increased in neurons in the cerebral cortex, hippocampus, and striatum of rats and mice maintained on dietary restriction (67, 183). It is known that BDNF can protect neurons in culture and in vivo against excitotoxic, metabolic, and apoptotic insults (217). Levels of NGF (67) andCNTF (W. Duan and M. P. Mattson, unpublished data) are also increased by DR in one or more brain regions. Neurotrophic factors may protect neurons by stimulating the production of proteins that suppress oxidative stress (antioxidant enzymes and Bcl-2) and stabilize cellular calcium homeostasis (calcium-binding proteins and glutamate receptor subunits) (4, 98, 141, 233). BDNF and other neurotrophic factors might also counteract the adverse effects of aging on synaptic function because they can modify synaptic plasticity in ways that facilitate learning and memory (115, 146). Evidence for an important role for neurotrophic factors in the beneficial effects of DR in the brain is suggested by studies showing that infusion of a BDNF blocking antibody into the lateral ventricles of DR mice significantly attenuates the protective effect of DR (67).

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Protein chaperones and neurotrophic factors are known to be induced by cellular stress, and it is therefore very likely that DR elicits a cellular stress response; this might result from decreased energy (glucose) availability to the cells, or from increased activity in neuronal circuits as the result of increased arousal due to hunger. Evidence supporting a role for a cellular stress response in the neuroprotective effects of DR was obtained in studies showing that the neuroprotective effects of DR can be mimicked by giving 2-deoxy-D-glucose (a nonmetabolizable analog of glucose) to rats and mice fed ad libitum. Animals given 2-deoxy-D-glucose exhibit increased levels of protein chaperones in their brain cells and increased resistance of neurons to excitotoxic, oxidative, and ischemic injury. Seizure-induced damage to hippocampal neurons is decreased, and learning and memory are preserved in rats given 2-deoxy-D-glucose (182). In the MPTP model of PD, mice given 2-deoxy-D-glucose exhibit decreased damage to dopaminergic neurons in the substantia nigra and a marked reduction in motor deficits (69). Rats administered 2-deoxy-D-glucose also exhibit reduced damage to cortical and striatal neurons and improved behavioral outcome after transient occlusion of the middle cerebral artery (380).

The adult brain contains populations of cells that are capable of dividing and then differentiating into neurons (neurogenesis) or glial cells (gliogenesis). In rodents and humans, neural stem cells are most abundant in the subventricular zone and in the subgranular layer of the dentate gyrus of the hippocampus (91). It is thought that neural stem cells in the adult brain may provide a cellular reserve to replace neurons and glia that die as the result of various injuries and diseases. In support of the latter function of neural stem cells, it has been shown that neurogenesis can be stimulated by ischemic and excitotoxic brain injuries (194, 263). We discovered that caloric restriction can increase neurogenesis in the brains of rats and mice (183, 184). Animals that had been maintained on

![Diagram](http://physrev.physiology.org/)
a restricted diet or a control ad libitum diet for 3 mo were given five daily injections of the DNA precursor BrdU and were killed either 1 day or 3–4 wk after the last BrdU injection. Numbers of BrdU-positive (newly generated) cells in the dentate gyrus were quantified by unbiased stereological methods. At the 1-day time point there was no difference in BrdU-labeled cells between calorie-restricted and control animals, indicating that calorie restriction does not affect the proliferation rate of the neural stem cells. Instead, DR resulted in a significant increase in the number of BrdU-positive cells remaining at the 3- or 4-wk time points, suggesting that DR promotes the survival of newly generated neural cells (183, 184). Many of the newly generated cells become dentate granule neurons. Although not yet established, it is conceivable that BDNF plays a role in the enhanced survival of newly generated neural stem cells in the dentate gyrus of rats maintained on DR because BDNF is known to have a similar effect on neural stem cells. When taken together with the fact that learning and memory is preserved in aging rodents maintained on dietary restriction (140), and that suppression of NPC proliferation can impair learning and memory (322), it is possible that DR promotes maintenance of cognitive function during aging by enhancing neurogenesis.

Interestingly, increasing data suggest that the brain may control life span by regulating energy metabolism of the entire organism (222). Because systems that regulate energy metabolism, such as the insulin signaling pathway, are believed to play major roles in the aging process (366), they are likely to have an important influence on brain aging. Key signaling pathways involved in the regulation of energy metabolism by the brain include BDNF, insulin-like growth factors, and neuropeptides that control feeding behavior in the brain, and insulin in peripheral tissues (Fig. 11).

Although DR clearly has beneficial effects in the nervous system, there are several aspects of its mechanism of action that remain to be explained. For example, animals maintained on DR exhibit increased levels of glucocorticoids consistent with an increased level of stress (250a). In animals fed ad libitum, increased glucocorticoids associated with chronic stress have been shown to promote neuronal degeneration (306a) and impair neurogenesis (35a). Why then does DR prevent neuronal degeneration and enhance neurogenesis? One possibility is that the specific responses of neural cells to DR stress are different from responses to the kinds of chronic psychosocial stress that have been shown to be deleterious to the brain. Indeed, we have documented a different profile of changes in the expression of glucocorticoid and mineralocorticoid receptors in the hippocampus in response to DR than are seen in animals subjected to psychosocial stress (183a). The ability of DR to increase neurotrophic factor production and the expression of cytoprotective stress proteins may override any potentially deleterious effects of elevated glucocorticoids. Indeed, stress and glucocorticoids decrease the expression of BDNF in the hippocampus (327a), and BDNF can protect neurons against the adverse effects of glucocorticoids (254a). Therefore, DR elicits cellular and molecular responses that allow enhanced activation of the hypothalamic-pituitary-adrenal axis to occur, but without the deleterious effects that might otherwise result from this heightened state of neuroendocrine function.

![FIG. 11. Regulation of energy metabolism by interactions between the brain and peripheral tissues. The brain controls feeding behaviors through complex neural circuits involving sensory inputs, cortical and hippocampal connections, and hypothalamic interactions with higher brain regions via neurotransmitters and modulators such as serotonin and neuropeptide Y as well as circulating hormones such as leptins. See text for further information.](http://physrev.physiology.org/)
C. Folic Acid

Humans cannot synthesize folic acid and therefore must obtain it in the diet; major sources of folate are green vegetables, citrus fruits, liver, and whole grains. The predominant dietary folates are 5-methyltetrahydrofolate and formyltetrahydrofolate, which are readily transported across the intestinal epithelium. The reason that many processed foods contain folic acid is that it was recognized decades ago that babies born to women that have a diet deficient in folic acid are at increased risk of birth defects. The fact that such birth defects most commonly involve the nervous system (spina bifida, meningocele, encephalocoele, and anencephaly) indicates that neural cells may be particularly sensitive to folic acid. Folic acid deficiency causes abnormalities in cell proliferation, differentiation, and survival. The important role for folic acid in development of the nervous system is further demonstrated by genetic alterations in methyltetrahydrofolate reductase (MTHFR), which decreases enzyme activity, resulting in decreased folic acid levels and increased risk of neural tube defects (319, 328). In addition, targeted gene deletion of the folic acid transporter results in embryonic lethality in mice (277). Recent studies have demonstrated direct consequences of folic acid deficiency on neurons by showing that simply depriving cultured embryonic brain cells of folate can induce apoptosis (programmed cell death) in developing neurons (171).

Folic acid acts as a cofactor in many different biochemical reactions by donating and accepting one-carbon units (314). Methionine synthase, a vitamin B₁₂-dependent enzyme, plays an important role in facilitating the conversion of extracellular 5-methyltetrahydrofolate to monoglutamyl tetrahydrofolate, a form of folic acid that can be readily used in nucleotide biosynthesis. Membrane transporters for folic acid are expressed in cells throughout the body including brain cells, and it is therefore likely that folic acid plays a critical role in one-carbon metabolism in neural cells (325). An array of biochemical reactions require methyl groups, and the normal dietary supply of methyl groups is insufficient to meet these demands and must be synthesized from the one-carbon folic acid pool. Folic acid (5'-methyltetrahydrofolate) is required for conversion of methionine to S-adenosylmethionine (SAM), the latter being the major methyl donor in most biochemical reactions (Fig. 12). Folic acid deficiency results in depletion of SAM and a reduction in the methylation of cytosine in DNA. The decreased DNA methylation that can result from folic acid deficiency may enhance gene transcription and DNA strand breakage which can trigger malignant transformation (279, 359).

During the past decade it has become clear that folic acid deficiency can increase risk for coronary artery disease and stroke (28, 77) and that this adverse effect of folic acid deficiency is associated with an elevation in plasma homocysteine levels (104). Homocysteine is produced from methionine by demethylation (Fig. 12), and homocysteine levels are kept low by its remethylation to methionine by a reaction requiring folic acid and vitamin B₁₂ or by conversion of homocysteine to cystathionine by the activity of the enzyme cystathionine-β-synthase (CBS). Alterations in the expression or enzyme activities

![Fig. 12. The involvement of folic acid and homocysteine in one-carbon metabolism. Homocysteine is a metabolite of methionine, an amino acid that plays a key role in the generation of methyl groups required for numerous biochemical reactions; homocysteine can either be remethylated to methionine by enzymes that require folic acid or catabolized by cystathionine-β-synthase (CBS), a vitamin B₁₂-dependent enzyme, to form cysteine. SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine; MTase, methyltransferase.](http://physrev.physiology.org.org)
of homocysteine and CBS can affect levels of homocysteine. Indeed, levels of homocysteine are increased in the cerebrospinal fluid of children with mutations in CBS (340) and may contribute to the abnormalities in brain function documented in such patients. Homocysteine can damage cells by inducing oxidative stress and DNA damage and impairing DNA repair (170, 171).

The importance of folic acid in the developing nervous system suggests the possibility that folic acid deficiency and elevated homocysteine levels might also have adverse effects in the adult nervous system. Possible links between folic acid, homocysteine, and neurological disorders have therefore been looked for and found. Data have accumulated that suggest links between dietary folic acid, homocysteine levels, and the pathogenesis of AD and PD. AD patients have significantly lower levels of folic acid and higher levels of homocysteine in their blood compared with neurologically normal age-matched control patients (49, 188). Elevated levels of homocysteine have also been reported in PD patients (175). A caveat with the studies just described is that all analyses were performed on blood samples from symptomatic patients, and it is therefore unclear whether decreased folic acid levels and elevated homocysteine levels precede and contribute to the neurodegenerative process. Thus the nutritional abnormalities might result from altered diet in these sick patients. Additional evidence from studies of patients and animal models described below do, however, support roles for homocysteine in the early pathogenesis of neurodegenerative disorders.

The major risk factor for AD, PD, and stroke is age, and studies have shown that homocysteine levels progressively increase with age (27). Deficiencies in folic acid and vitamin B12 may also contribute to the declines in cognitive and other neurological functions that occur during normal aging (315) including psychiatric disorders (25). A study of geriatric patients admitted to a psychiatric hospital revealed that individuals with below median values of folic acid and vitamin B12 performed worse on tests of cognitive function than did individuals with above-median levels of folic acid and vitamin B12 (17). On the other hand, elevated plasma homocysteine levels were not associated with cognitive impairment in centenarians (292). Genetic variations in the MTHFR gene may be associated with risk of PD with individuals with the C677T genotype (which increases homocysteine levels) being at increased risk (374). Mice deficient in MTHFR exhibit hyperhomocysteinemia and neuropathological alterations (45), consistent with a loss of function of the enzyme in humans with disease-promoting forms of the enzyme.

Animal and cell culture models of neurodegenerative disorders have provided evidence that folic acid deficiency and elevated homocysteine levels can render neurons vulnerable to dysfunction and death. When APP mutant mice, which develop progressive amyloid deposition in their brains, were maintained on a low-folate/high-homocysteine diet, neurons in the hippocampus degenerated (171). This contrasted with nontransgenic mice in which the experimental diet did not cause degeneration of neurons. Folic acid deficiency may render neurons vulnerable to being damaged and killed by amyloid because exposure of cultured rat hippocampal neurons to folic acid-deficient medium or to homocysteine increases the vulnerability of the neurons to being killed by Aβ (170, 171). It has also been reported that dietary folic acid deficiency sensitizes mice to MPTP-induced PD-like pathology and motor dysfunction (68). Direct application of homocysteine into either the substantia nigra or striatum exacerbated dopamine depletion, neuronal degeneration, and motor dysfunction. Moreover, homocysteine increased the vulnerability of cultured human dopamine-producing cells to rotenone and iron. The ability of folic acid deficiency and elevated homocysteine levels to sensitize neurons to amyloid and MPTP toxicities suggests a mechanism whereby dietary folic acid may modify risk of AD and PD.

The mechanism whereby homocysteine damages and kills neurons has been elucidated in recent studies. Homocysteine induced DNA damage in cultured hippocampal neurons, which resulted from a combination of impaired DNA repair and increased oxidative stress, as indicated by increased uracil misincorporation and increased oxidative modification of DNA bases (171). Homocysteine-induced DNA damage can trigger a programmed cell death pathway involving poly(ADP-ribose) polymerase and the tumor suppressor protein p53, leading to mitochondrial dysfunction and activation of death proteases (170). Impaired DNA repair may play a role in the pathogenesis of AD because fibroblasts from AD patients exhibit a defect in repair of DNA lesions (189, 297). In cultured dopaminergic cells, homocysteine can exacerbate oxidative stress, mitochondrial dysfunction, and apoptosis in cells exposed to the pesticide rotenone or the pro-oxidant Fe2+. The dopaminergic cells can be protected against the adverse effects of homocysteine by administration of the antioxidant uric acid and by an inhibitor of poly(ADP-ribose) polymerase (68).

Alterations in folate and one-carbon metabolism may also contribute to several other neurodegenerative conditions. For example, the huntingtin protein interacts with CBS (26), and alterations in folate metabolism have been documented in patients with ALS (376). ALS is a disorder in which spinal cord motor neurons degenerate resulting in progressive paralysis and death. It has been proposed that an abnormality in folate metabolism accounts for the reported reduction of RNA and the elevation of taurine in the nervous system of ALS patients (376). Homocysteine can also induce seizures in rodents (173), and alterations in homocysteine levels may occur in human
epilepsy patients (313), suggesting a possible contribution to epilepsy.

D. Antioxidants

There are tens of thousands of natural and synthetic compounds that possess antioxidant activity, and a rapidly growing number of these agents have been reported to have beneficial effects in one or more experimental models of age-related disorders. It is beyond the scope of this article to review the field of antioxidants and brain aging; instead, examples of results obtained with several of the most widely studied antioxidants are presented. These include vitamin E, coenzyme Q10, lipoic acid, creatine, and Ginkgo biloba extract.

Vitamin E (α/γ-tocopherol) is a lipid-soluble antioxidant that is very effective in suppressing membrane lipid peroxidation. Data have accumulated that suggest that dietary supplementation with vitamin E can reduce risk of cardiovascular disease and many cancers (64). Chronic treatment of rodents with vitamin E can preserve learning and memory function, which otherwise declines during aging (330). Treatment of rat hippocampal slices with α-tocopherol enhanced long-term potentiation of synaptic transmission, a cellular correlate of learning and memory (370). Clinical trials of vitamin E in AD patients have yielded positive results with patients receiving this antioxidant exhibiting a slowing of disease progression compared with those receiving placebo (106). Vitamin E may counteract the effects of aging and neurodegenerative disorders by suppressing membrane lipid peroxidation and thereby preserving membrane transporter function and stabilizing cellular ion homeostasis. As evidence, vitamin E can protect cultured neurons and synaptosomes against dysfunction and death induced by Aβ (102, 204) and can protect against amyloid-induced learning and memory deficits in adult rats (371). Vitamin E has also been reported to be effective in animal and cell culture models of ALS (117, 172, 265) and PD (299), although clinical benefit in human patients has not yet been established.

Coenzyme Q10 (ubiquinone) is associated with the mitochondrial oxidative phosphorylation enzyme complexes where it serves an antioxidant function. Administration of ubiquinone to rodents can enhance learning and memory (156). Ubiquinone protected cultured neural cells against insults relevant to the pathogenesis of AD (273). Dietary supplementation with ubiquinone resulted in increased resistance of midbrain dopaminergic neurons to MPTP-induced damage in a mouse model of PD (13) and increased resistance of striatal neurons to the succinate dehydrogenase inhibitor malonate in a rodent model of HD (12). Clinical trials of ubiquinone have proven effective in treating patients with myocardial infarction (324) and cancers (129), although it is not established that ubiquinone supplementation can reduce risk of these disorders. The potential of dietary supplementation with ubiquinone to prevent or treat age-related neurodegenerative disorders remains to be determined.

α-Lipoic acid is an endogenous disulfide compound that is present in small amounts in most animal cells where it functions as a coenzyme in the α-ketoglutarate dehydrogenase and pyruvate dehydrogenase enzyme complexes (208). The antioxidant and cytoprotective actions of lipoic acid have been documented in a variety of cell culture and animal models of age-related disease (237). A number of clinical trials of lipoic acid have been completed or are in progress in patients with cardiovascular disease, cancer, and diabetes. Dietary supplementation with lipoic acid has proven beneficial in normalizing glucose metabolism in patients with type II diabetes (143). Lipoic acid and the related compound dihydrolipoate were effective in protecting cultured neurons against death induced by hypoxia, glutamate, and iron and were effective in reducing focal ischemic brain injury in vivo (248, 283). Lipoic acid was also effective in protecting cultured rat cortical neurons against death induced by Aβ (384). Dietary supplementation with lipoic acid increased the survival of Cu/Zn-SOD mutant transgenic mice (8), suggesting that this antioxidant may prove beneficial in ALS patients.

Creatine increases phosphocreatine levels in muscle and brain cells and may thereby improve cellular energetics and reduce oxyradical production (220). Creatine is now widely employed as a dietary supplement by athletes to improve their performance (79). Creatine significantly reduced brain damage in a rat model of traumatic brain injury, suggesting that it may not only enhance the performance of athletes, but may also improve the outcome of head injuries (338). Head injury is an important cause of morbidity and mortality in the elderly (296). The possibility that creatine may protect against neurodegenerative conditions has been tested in several animal models. Dietary supplementation with creatine delayed motor dysfunction and increased survival in a transgenic mouse model of HD (7), protected dopaminergic neurons against MPTP toxicity in mice (212), and increased the survival of Purkinje cells in a transgenic mouse model of spinocerebellar ataxia type 1 (155). Preliminary results of a recent clinical trial of creatine supplementation in ALS patients suggest that it can increase muscle strength (236).

The public has recently been barraged with advertisements touting the health benefits of Ginkgo biloba with a particular emphasis on its benefits for the brain (251). Ginkgo biloba extract enhanced performance of aged mice in a learning and memory task (50). In a rat model of severe diabetes, Ginkgo biloba was effective in improving learning and memory performance (132). Ginkgo biloba supplementation in a double-blind, place-
bo-controlled, 14-wk, parallel group, repeated assessment, multi-center trial proved effective in improving cognitive function in healthy middle-aged subjects (363). However, other trials of Ginkgo extracts on memory performance in normal adults (247) and elderly patients with mild cognitive impairment (350) have not revealed a significant effect of this dietary supplement (247). Ginkgo extracts can protect cultured cortical neurons against damage induced by iron (107) and protect cultured PC12 cells against Aβ toxicity (11, 373). Ginkgo extract reduced damage to dopaminergic neurons caused by MPTP in a mouse model of PD (287). Blindness due to degeneration of photoreceptors occurs in many aged individuals, and it was shown that Ginkgo biloba extract can protect photoreceptors against light-induced damage (289). Overall, the available data suggest that Ginkgo biloba has beneficial effects in the nervous system, although further studies will be required before this dietary supplement can be recommended for primary prevention of neurodegenerative disorders.

Other antioxidants that have been associated with reduced risk or disease and/or have exhibited neuroprotective effects in cell culture and animal models relevant to age-related neurodegenerative disorders include estrogen, carotenoids, uric acid, glutathione, and N-acetylcysteine. Epidemiological data and clinical studies suggest that estrogens can improve memory and reduce risk of dementia during aging (318). Estrogen can protect cultured neurons against death induced by Aβ (101, 278) and can protect cortical neurons against ischemic injury in vivo (73). However, clinical trials of estrogen in AD patients have not resulted in clear beneficial effects and epidemiological studies aimed at determining whether estrogens can prevent AD have generated mixed results. Individuals over the age of 65 that have higher levels of β-carotene perform better in learning and memory tests compared with individuals with low β-carotene levels (275). Lycopene is a carotenoid that has been suggested to protect against heart disease, stroke, and certain cancers (290, 295). Lycopene can protect cultured hippocampal neurons against Aβ and glutamate toxicity (Fig. 13). Levels of uric acid have been reported to be decreased in patients with AD (200, 345). Uric acid administration to adult rats reduced focal ischemic brain injury and improved functional outcome in a stroke model (378). Uric acid is remarkably effective in protecting cultured neurons against insults relevant to AD and PD, including exposure to Aβ and iron (111, 160, 161, 378). Glutathione is a major antioxidant in the brain and can protect neurons against a variety of oxidative insults in experimental models relevant to the pathogenesis of AD, PD, ALS, and stroke (161, 205, 265). N-acetylcysteine has proven effective in reducing age-related deficits in learning and memory (209). Encouraging beneficial effects of N-acetylcysteine supplementation were recently reported in a clinical trial in patients with probable AD (2), suggesting that this antioxidant may soon be used as a dietary supplement for patients in the early stages of neurodegenerative disorders.

V. BEHAVIORAL MODIFICATION OF BRAIN AGING

Studies performed during the past decade strongly suggest that our daily behaviors can affect the molecular composition and cellular structure of our brain. When adult rodents are housed in complex environments or exercised on a regular basis, there are increases in the complexity of dendrites in cortical neurons and increased numbers of synapses (153). These structural changes are associated with increased production of certain neurotrophic factors and with enhanced performance on learning and memory and motor function tasks. Other behavioral factors may have a negative impact on the brain, with the best-documented example being psychosocial stress (239). The molecular and cellular mechanisms responsible for beneficial or detrimental effects of different behaviors on the brain are beginning to be understood and may involve changes in neurotrophic factor, neurotransmitter, and hormonal signaling pathways. With respect to aging and age-related neurodegenerative disorders, the available data suggest that those behaviors that enhance
dendritic complexity and synaptic plasticity also promote successful aging and decrease risk of neurodegenerative disorders. Collectively, the emerging data suggest that behavioral factors can have a major impact on the outcome of brain aging.

A. Intellectual Activity

Epidemiological studies have documented an inverse relationship between educational attainment and risk for AD such that more educated persons are at reduced risk (81). Particularly interesting are data suggesting that intellectual function in early life can affect risk of AD. Thus, in a study of a population of nuns in Minnesota, Snowdon et al. (329) showed that nuns with the best linguistic abilities as young adults were at reduced risk for AD. One interpretation of these data is that intellectual activity is neuroprotective. Animal studies are consistent with this interpretation. Greenough and co-workers (22, 167) showed that rats raised in complex environments exhibit increased complexity of dendritic arbors and synapses in the hippocampus and cerebellum, suggesting an increased functional reserve. During aging, synaptic density decreases in the hippocampus in rats, and maintenance of the rats in a complex environment can prevent such age-related synaptic loss (306). In addition, when rodents are raised in complex environments in which they have many objects to play with, neurogenesis is enhanced and learning and memory ability is improved (164, 254, 377). Further studies have shown that regular exercise can also promote maintenance of cognitive function during aging (78, 349). Other studies have shown that regular exercise in elderly men is particularly effective in improving cognitive performance on tasks that require visuospatial processing (317). Fourteen-month-old rats that exercised regularly (swimming 1 h/day, 5 days/wk for 9 wk) exhibited improved performance in a learning and memory task and reduced levels of membrane lipid peroxidation and oxidative damage to DNA (285). For example, mice allowed access to a running wheel exhibit increased neurogenesis and improved learning and memory compared with “couch potato” mice (351). Exercise results in an increase in the level of BDNF in the hippocampus in rats (304), suggesting a role for BDNF in the beneficial effects of exercise on brain function and plasticity. Another neurotrophic factor upregulated in the brain in response to learning and exercise is bFGF (100). The latter study showed that the increase in bFGF levels was associated with an increase in astrocyte density, suggesting a potential role for astrocytes in the beneficial effects of exercise in the brain. Further data supporting a beneficial effect of exercise on the brain come from epidemiological studies in humans which show that regular vigorous physical activity can reduce risk for ischemic stroke (181); although it has not been established that prior physical activity can improve outcome after a stroke, this would seem plausible. Physical activity can also benefit the brain after injury. For example, exercise after brain injury improved functional outcome in rats, and the improved outcome was associated with enhanced structural plasticity in the motor cortex (152).

B. Exercise

Exercise benefits not only the musculoskeletal and cardiovascular systems, but it also benefits the brain. Regular vigorous exercise improves mood and cognition; these effects of exercise have been clearly established in controlled studies (84). Studies of elderly populations suggest that regular exercise can also promote maintenance of cognitive function during aging (78, 349). Other studies have shown that regular exercise in elderly men is particularly effective in improving cognitive performance on tasks that require visuospatial processing (317).
VI. SYNAPSES AND AGING: EMERGING CONCEPTS

The numbers and size of synapses change during aging and response to environmental stimuli. During successful brain aging, numbers of synapses may or may not decrease depending on the brain region; in brain regions in which synapse numbers do decrease, the size of the remaining synapses may increase, perhaps as a compensatory mechanism. For example, in a study of superior-middle frontal cortex (area 9) in cognitively normal individuals ranging in age from 20 to 89 yr, there was no change in synapse density in lamina III and V (308). In the other hand, a decrease in synaptic density occurs in the lateral septal nucleus in a subset of aged rats (309). In striking contrast to normal aging, clear and profound decreases in synapse numbers occur in brain regions involved in learning and memory in AD (307). Interestingly, synapse loss also occurs in the cerebral cortex of many patients with HD and PD (382). Studies of animal models of age-related neurodegenerative disorders (AD, HD, and PD) have documented synapse loss and have provided evidence that the synapse degeneration occurs early in the disease process, well before cell death (256, 259, 298).

Abnormalities in synaptic signal transduction pathways and associated functional deficits may occur during aging and may be early and pivotal events in the pathogenesis of neurodegenerative disorders. Several findings point to synaptic alterations occurring very early in AD and being central to the cognitive deficits and neuronal death. Studies of APP (41) and PS1 (263) mutant transgenic mice have documented alterations in synaptic plasticity that occur without evidence of synapse loss or cell death. Aβ is deposited at high levels in synaptic regions, presumably because its protein precursor (APP) is abnormally transported. Exposure of isolated synaptic terminals to Aβ results in impairment of ion-motive ATPases and glucose and glutamate transporters (162). Aβ and oxidative stress can also induce apoptotic biochemical cascades in synapses and dendrites, including increased production of Par-4, mitochondrial alterations, and caspase activation (70, 234). Neurotrophic factors (115) and estrogen (159) that may protect against AD can preserve synaptic transporter functions during exposure to Aβ. PS1 mutations result in perturbed synaptic calcium homeostasis and thereby promote synapse dysfunction and degeneration (15). Although fewer studies have addressed the issue of synaptic dysfunction in PD and HD, studies of animal models are consistent with perturbations in synaptic function and initiation of apoptotic biochemical cascades occurring early in the disease process (66, 71).

The vast majority of signal transduction pathways that regulate structural and functional plasticity, and survival of neurons are localized in synapses. Some of these synaptic signaling systems may be particularly sensitive to age-related increases in levels of oxidative stress and decreases in energy availability (Fig. 14). For example, oxidative stress leading to membrane lipid peroxidation can impair the coupling of muscarinic cholinergic receptors to the GTP-binding protein Gq11, apparently as the result of covalent modification of Gq11 by the lipid peroxidation product 4-hydroxynonenal (23, 163). Age-related decreases in levels of neurotrophic factors and/or their receptors have been reported (125, 381), and on the basis of the localization of neurotrophic factor receptors in synapses, compromised neurotrophic signaling would be expected to promote synapse degeneration and cell death. On the other hand, cellular adaptations to aging may include an enhancement of neurotrophic signaling that supports synaptic plasticity (Figs. 1 and 14).

Age- and disease-related synaptic dysfunction and degeneration may be subject to modification by dietary and behavioral factors. Studies of cortical synaptosomes prepared from rats maintained on calorie-restricted or control diets have shown that calorie restriction can increase the resistance of synapses to oxidative and metabolic insults, as indicated by relative preservation of glucose and glutamate transport and mitochondrial function (113). 2-Deoxy-d-glucose administration exerted similar beneficial effects on synapses (116). The amounts of HSP-70 and GRP-78 were increased in synaptosomes from calorie-restricted rats and rats given 2-deoxy-d-glucose, demonstrating that energy restriction bolsters the ability of synapses to cope with the oxidative and metabolic stress associated with aging. As described in section v, increased activity in neuronal circuits due to intellectual or physical activities may prevent age-related dysfunction and loss of synapses. Presumably, many of the same adaptive changes that occur in neurons subjected to dietary restriction, including increased neurotrophic factor signaling and protein chaperone production, also occur in response to intellectual and physical activity. These changes benefit synapses directly by enhancing their ability to tolerate oxidative and metabolic stress.

VII. IMPLICATIONS FOR PREVENTION AND TREATMENT OF NEURODEGENERATIVE DISORDERS

Because of advances in the prevention and treatment of cardiovascular disease and cancers, many more people are living beyond the age of 70, and neurological disorders of aging have therefore become more common causes of disability and death. As described above, research efforts on neurodegenerative disorders have rapidly expanded in the past decade and have greatly advanced our understanding of the molecular and cellular mechanisms that
result in neuronal dysfunction and death, and the genetic and environmental risk factors for the disorders. Basic and clinical research in this field have reached a point where recommendations can be made to the general population concerning how they can reduce their risk of age-related neurodegenerative disorders. Moreover, effective treatments for symptomatic patients are beginning to emerge.

As with other major age-related diseases, including cardiovascular disease, type 2 diabetes, and some cancers, the most effective means of reducing risk of neurodegenerative disorder may be to modify one’s diet and behaviors. As described in section ivA, caloric restriction during adult life may be particularly effective in reducing risk of age-related neurodegenerative disorders. A link between high cholesterol levels and risk of stroke has been established, and recent findings suggest that high cholesterol levels may also increase risk of AD (54), suggesting that a reduced fat diet may help ensure a healthy brain as one ages. Links between pesticides and PD (20) and dietary toxins and ALS (335) have been reported. The latter findings suggest that reducing exposure to such environmental toxins may reduce the incidence of these disorders. New dietary and behavioral approaches for promoting healthy brain aging will undoubtedly emerge from ongoing research. By analogy with physical exercise
benefiting the musculoskeletal and cardiovascular systems, mental exercise may improve the ability of brain cells to cope with the aging process.

Can pharmacological interventions retard brain aging? Antioxidants such as vitamin E, coenzyme Q10, lipoic acid, and ginko extract, and supplements such as creatine that enhance cellular energy maintenance, may provide some degree of protection (7, 11–13, 50, 107, 119, 155, 168, 212, 354, 384). Recent studies have evaluated dietary supplements that mimic the physiological effects of caloric restriction for their ability to enhance resistance of neurons to age-related disease (69, 116, 380). Suppressing apoptosis using agents that target key proteins in the cell death process, such as p53 and caspases, is another potentially effective strategy (55, 72). It has also been suggested that cholesterol-lowering statins may protect the brain against age-related neurodegenerative disease via actions on the vasculature or directly on neurons (83, 149). In addition to preventative strategies that affect pathways of neuronal degeneration or neuroprotection that are shared among age-related neurodegenerative disorders, novel disease-specific approaches are also being developed. In the case of AD, for example, drugs that inhibit β- and γ-secretases and thereby reduce Aβ production are being developed (48), as are amyloid vaccine-based approaches (139).

Finding cures for age-related brain dysfunction and disease is a worthy, but elusive and often frustrating, goal. By the time that patients with AD, PD, HD, and stroke become symptomatic, extensive damage to neuronal circuits has already occurred. Treatment strategies must therefore focus on halting further neuronal degeneration and promoting reestablishment of neuronal circuits. Recent advances, many of which are described in this review, suggest that the goal of curing patients with age-related neurodegenerative disorders is worth pursuing. One reason for optimism is that the extent of neuronal death in PD and AD patients during the early period of the diseases may not be as great as initially thought, because many dysfunctional neurons may be able to recover (95). In addition, neuronal stem cells may be capable of replacing lost neurons (91). Moreover, it may be possible to manipulate cell adhesion and signaling pathways in ways that enhance neurite outgrowth and synaptogenesis (312). Neurotrophic factors and agents that stimulate their production as well as drugs that enhance synaptic transmission are currently in clinical trials, as are several antioxidants. In addition, trials are in progress to determine the safety and efficacy of transplantation of neural stem cells in PD patients. “Cocktail” treatments (e.g., multiple antioxidants) have proven most efficacious in preclinical studies and are therefore likely to also achieve the greatest effects in human patients. In this regard, it is of considerable interest that dietary supplementation with fruits and vegetables rich in multiple antioxidants is proving effective in counteracting age-related brain dysfunction in studies in rodents (154).

Preclinical studies in animal models of neurodegenerative disorders have identified several approaches that are efficacious in preventing neuronal degeneration and/or restoring lost function. Administration of Aβ to APP mutant transgenic mice results in reduced amyloid deposition and increased clearance of amyloid deposits in the brain and improved learning and memory performance (244, 310). The latter studies have led to a clinical trial of an amyloid “vaccine” in AD patients. Unfortunately, the clinical trial was recently halted because several patients developed encephalitis. Another promising approach for removing amyloid deposits from the brains of AD patients is based on a role for metals, copper, iron, and zinc in particular, to promote aggregation of Aβ. An older study suggested a benefit of the iron chelator desferoxamine in AD patients (53), and recent preclinical studies in APP mutant mice (47) have led to a clinical trial of a copper chelator in AD patients. Epidemiological and animal studies suggest that antioxidants (282), estrogenic steroids (239), and anti-inflammatory drugs (136) can reduce risk of AD, presumably by reducing damage to neurons and microglial activation. Amyloid deposition and oxidative stress in AD may facilitate excitotoxic neuronal death (224). Glutamate receptor antagonists are therefore being evaluated for the treatment of AD patients (144).

Several types of drugs are in development for the treatment of PD patients. Because excitotoxicity is thought to be involved in the degeneration of dopaminergic neurons, N-methyl-D-aspartate receptor antagonists are being tested in animal models of, and patients with, PD and related movement disorders (61). Similar antiexcitotoxic approaches are being applied to HD and stroke (33). Synthetic inhibitors of the proapoptotic protein p53 protected dopaminergic neurons against death and improved motor function, in a mouse model of PD (Duan). As described above, dietary supplementation with creatine has proven beneficial in mouse models of ALS and PD (168, 212).

The kinds of data obtained in the studies of rodent models of brain aging and neurodegenerative disorders described above suggest that DR can increase health span of the brain and effectively protect neurons against the dysfunction and death that occurs in neurodegenerative disorders. However, it remains to be conclusively demonstrated that the results of the animal studies are directly applicable to humans. Clinical trials of DR in humans will be difficult to perform because they will require long time periods (many years to decades) of compliance and extensive resources. Nevertheless, data emerging from a study of life-long DR in rhesus monkeys begun here at the National Institute on Aging 14 years ago strongly suggest that DR increases life span in primates (176a). In our view, there is no doubt that DR is beneficial for the nervous system of humans, just as it benefits every other organ system studied. The major hurdle
How might genetic factors that cause or increase risk of age-related neurodegenerative disorders be dealt with directly? Autosomal dominant mutations, such as those in the genes encoding APP, presenilins, α-synuclein, huntingtin, and Cu/Zn-SOD, could be eliminated in a single generation. This would be accomplished by identifying those that harbor the mutations at an early age and ensuring, by counseling abstinence or other means, that they do not pass on the defective gene. Genetic screening to identify those at increased risk of a disorder (e.g., individuals with an E4 allele of apolipoprotein E) would allow aggressive preventative dietary and pharmacological interventions. In the more distant future it may be possible to eliminate “bad” genes using gene therapy approaches, although many technical hurdles need to be crossed. Until such breakthroughs occur, we must continue to pursue dietary, behavioral, and drug-based approaches for increasing the health span of the brain.

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