Molecular Endocrinology and Physiology of the Aging Central Nervous System

Roy G. Smith, Lorena Betancourt, and Yuxiang Sun

Huffington Center on Aging and Department of Molecular and Cellular Biology and Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

Aging is associated with a progressive decline in physical and cognitive functions. The impact of age-dependent endocrine changes regulated by the central nervous system on the dynamics of neuronal behavior, neurodegeneration, cognition, biological rhythms, sexual behavior, and metabolism are reviewed. We also briefly review how functional deficits associated with increases in glucocorticoids and cytokines and declining production of sex steroids, GH, and IGF are likely exacerbated by age-dependent molecular misreading and alterations in components of signal transduction pathways and transcription factors. (Endocrine Reviews 26: 203–250, 2005)

I. Introduction

II. Complex Behavior of Neurons in Aging
   A. Aging and the dynamics of neuronal behavior
   B. Hormone pulsatility and aging
   C. Dopaminergic system as an example of age-related change in neuronal dynamics

III. Age-Dependent Changes in Biological Rhythms
   A. Aging and disruption of circadian rhythms
   B. Altered circadian rhythms modify sleep patterns
   C. Restoration of normal rhythms in aged animals
   D. Age-associated changes in circadian rhythms influence metabolism

IV. Aging, Memory, and Cognitive Decline
   A. Age-related neuronal structural and functional changes
   B. Hippocampus and neurogenesis
   C. Aging and neurogenesis
   D. Steroids and neurogenesis
   E. IGF-I and neurogenesis
   F. Neurosteroids and memory
   G. Gene expression in memory and learning

V. GH Axis
   A. Age-associated decline in GH pulse amplitude
   B. Increase in longevity in GH-deficient rats and mice
   C. GH in the CNS
   D. Relationship of GH and IGF-I to age-related cognitive impairment
   E. Potential mechanisms of GH/IGF-I-mediated neuroprotection
   F. GHRH and cognition
   G. GH, GHRH, and sleep
   H. Somatostatin in the CNS

VI. GHS-R, Ghrelin, and Ghrelin Mimetics
   A. Identification of the GHS-R and synthetic agonists
   B. GHS-R endogenous ligands, ghrelin, and adenosine
   C. Aging is associated with ghrelin insensitivity
   D. Ghrelin and inflammatory cytokines
   E. Ghrelin and the aging brain

VII. Aging and Metabolism
   A. Aging, ghrelin, and energy balance
   B. Ghrelin production in CNS orexigenic centers
   C. Metabolism and changes in ghrelin activity during aging
   D. Leptin, metabolism, and aging
   E. Leptin resistance and aging

VIII. Hypothalamic-Pituitary Gonadal Axis and Aging
   A. NPY and GnRH
   B. Evidence that CNS changes likely precede ovarian changes in the onset of menopause
   C. Estradiol receptors in the CNS
   D. Estradiol and POMC
   E. Estradiol and synaptic communication
   F. Estrogen and neurodegeneration
   G. Estrogen in learning and memory
   H. Estradiol, galanin, and cognition
   I. Estradiol and AD
   J. Estradiol and inflammatory responses in the CNS
   K. Andropause and CNS

IX. Sexual Behavior and Aging
   A. Sex steroids and age-related deficits
   B. Dopamine and age-related deficits
   C. GH and ED

X. HPA Axis and Aging
   A. Decreased sensitivity to negative feedback regulation
   B. Corticosteroid receptors
   C. Stress response differs according to gender
   D. CRH
   E. AVP
   F. 11β-Hydroxysteroid dehydrogenase (HSD)
   G. Counterregulatory effects of GH and IGF-I on glucocorticoid action
   H. Serotoninergic system and glucocorticoids

XI. Transcriptional Regulation and Aging
   A. Overview and relevance to neuroendocrinology of aging
   B. Molecular misreading and aging
   C. Coactivators and corepressors of gene transcription
   D. Heat shock proteins
   E. Protein kinase C (PKC) isozymes
   F. Helix-loop-helix (HLH) proteins
   G. NOS and aging

XII. Summary and Conclusions
I. Introduction

This review focuses on recent developments in our molecular understanding of the effects of aging on relationships between the endocrine system and central nervous system (CNS). The declining blood levels of GH and sex steroids during aging are commonly referred to as the somatopause, menopause, and andropause (1–4). Because these hormonal changes are associated with declines in cognitive and physical abilities, attempts are often made to rescue the aging phenotype by hormone replacement; however, the relative risk/benefit ratio of hormone replacement continues to be debated.

It has been argued that the age-dependent decline in sex steroid, GH, and IGF-I production is nature’s way of protecting us from cancer and heart disease, but a more likely scenario is that once we reach our reproductive capacity, nature begins programming us for death. This is clearly illustrated by the marked decline in immune function (5, 6) and by the increased production of glucocorticoids and cytokines that negatively impact metabolism, bone density, strength, exercise tolerance, cognitive function, and mood (3, 7–11); similarly, the production of sex steroids, dehydroepiandrosterone (DHEA), GH, and IGF-I that have positive impact on these functions declines (1–4, 12). Hence, if we wish to maintain quality of life during aging we must oppose nature. However, simply replacing hormones pharmacologically does not recapture the endocrine profiles of young adults; therefore, an ideal method of intervention awaits a fundamental understanding of the underlying mechanisms causing age-dependent hormonal changes.

Altered CNS function appears to precede the metabolic, reproductive, and cognitive deficiencies associated with aging. We speculate that the underlying basis is a progression of neuroendocrine changes characterized by altered biological rhythms, reduced amplitude, altered frequency, and decreased orderliness of hormone, neuropeptide, and neurotransmitter release. Indeed, attenuation of overall functional activity in the CNS accompanies aging (13–22) (Fig. 1). For example, monoamine oxidase activity increases, causing a decrease in the concentrations of serotonin (5-HT) and dopamine (13), and this is paralleled by alterations in concentrations of receptors for hormones, neuropeptides, and neurotransmitters in the CNS. Reduced secretion of hypothalamic GnRH results in altered LH pulse amplitude, thus attenuating pulsatile gonadal steroid secretion (23). Similarly, a decrease in hypothalamic GHRH secretion causes reduced GH pulse amplitude and reduced IGF-I levels in GH target tissues (24, 25). Increases in amplitude of hormone release have also been noted and include ACTH and PTH (26–28).

Preventing or slowing the age-dependent changes in CNS and function of the pituitary gland has the potential to maintain the quality of our lives as we age. Precedents for reversing age-dependent endocrine and behavioral changes have been described. For example, transplantation of hypothalamic fetal tissue into the hypothalamus of old rodents restores aspects of neuronal activity typical of young adult rats (29–35). Rejuvenation of the GH/IGF-I axis has been achieved by administering specific small molecules. For example, treatment of old rats with l-dopa stimulates GHRH release to produce a pulsatile GH profile typical to that observed in young rats (36, 37). Furthermore, rejuvenation of the GH/IGF-I axis can also be accomplished in elderly humans by chronic treatment with a long acting synthetic agonist for the GH secretagogue receptor (GHS-R) (38). Therefore, it appears that important endocrine or paracrine factors essential for maintaining a youthful phenotype are not optimally produced during aging. Indeed, although function deteriorates during aging, because tissues retain inherent plasticity, function can be restored if the appropriate signal is provided.

We favor a hypothesis of aging based on alterations in the dynamics of neuronal behavior. Such dynamic changes are consistent with a destabilizing effect on CNS function, which potentially increases the vulnerability of the aging brain to trauma. In this review, we address the significance of age-related changes in biological rhythms and the benefits of restoring normal rhythms. Age-associated changes in cognitive decline, which appear to be associated with disruption of endocrine pathways, are described. We also discuss the underlying age-dependent alterations in components of feedback pathways governing the release of hormones, neuropeptides, and neurotransmitters. Finally, because hormones signal by modulating gene transcription, we review age-related changes in factors involved in regulating the transcription of genes intimately involved in endocrine and CNS function. Although much excellent science has been done, the reductionist approach makes it impossible to
clearly determine causality. Effects can be readily defined, but causes are likely multifactorial. Having made the reader cognizant of this caveat, we present an overview of selected topics of relevance to the molecular endocrinology of the aging CNS with the objective of providing the stimulus for continued investigation using whole systems approaches.

II. Complex Behavior of Neurons in Aging

A. Aging and the dynamics of neuronal behavior

What underlying principle might explain the progressive physiological changes that lead to an “old” phenotype? In general, physiology is governed by complex interactions arising from feedback loops of nonlinear systems; it has been proposed that a reduction in the complexity of physiological or behavioral control systems occurs with age and disease (39–44). A hypothetical advantage for biological systems to exist at the “edge of chaos” is that it allows synchronized neuronal networks to be more resistant to disruption than systems with either periodic or stochastic behavior. Hence, the nonlinear dynamics of ordered chaotic systems facilitates neural systems to adapt according to environment (40, 41). The aging phenotype reflects reduced ability of an organism to adapt to stress and trauma, which is consistent with a transition toward reduced complexity of the underlying regulatory systems.

Reduced complexity could occur through loss or defect in a component and/or altered nonlinear coupling (feedback) between components of the system (45). A loss of neuronal components and coupling between components of neuronal networks is characteristic of aging. For example, a relative increase in the concentration of glucocorticoids compared with sex steroids, GH, and IGF-I is associated with shrinkage of the hippocampus, loss of neurons, and declining neurogenesis; loss of estradiol production is associated with fewer neuronal connections. The number of dopaminergic neurons also gradually declines during aging, producing deficits in the nigrostriatal dopamine system of rodents, monkeys, and humans (19, 46, 47). Such changes would predictably result in reduced complexity and efficiency of signaling within neural networks and reduced adaptive capability. An example of a decline in adaptive capacity of neurons during aging is the increased vulnerability of the brain to anoxia and ischemia, which in rats is associated with reduced glycolytic capacity of neurons (48). On this basis, we speculate that the onset of functional deficits associated with aging is a consequence of altered behavior of underlying regulatory pathways in the CNS.

B. Hormone pulsatility and aging

One of the most significant age-related events is an alteration in amplitude and pulsatile pattern of hormone release. The frequency of release of a hormone is as important, or more important in some cases, than the amount of hormone released. Target cells respond most effectively to exogenous hormonal stimulation when the frequency of stimulation approaches the endogenous frequency (49). Age-related changes in the endocrine system can appear superficially as apparent increases in complexity (45, 50–54). Veldhuis and colleagues (27, 55–58) made extensive evaluations of age-related changes in the dynamics of pulsatile hormone release. They applied mathematical approaches to investigate the synchrony and pulsatility of GH, LH, testosterone, ACTH, cortisol, and insulin release during aging. By calculating the approximate entropy (ApEn) statistic as a measure of orderliness of synchronicity of hormone release, they showed that individual orderliness declined progressively during healthy aging. However, ApEn calculations do not directly distinguish between contributions of stochastic and deterministic behavior toward the observed regularity (45, 53). Therefore,
the less ordered rhythmic patterns of hormone release observed during aging could result from a transition of the regulatory neuronal network controlling the ordered frequency of hormone release from adaptive complex behavior to stochastic behavior.

The ApEn calculations in concert with clinical data support the concept that aging is tightly associated with disruption of the time-delayed positive and negative feedback pathways controlling synchrony of hormone release. Therefore, application of nonlinear dynamics and mathematical analyses for analyzing the behavior of neurons that regulate the endocrine system and how this behavior changes as a function of age is important and reinforces our awareness of the limitations of reductionist methods.

C. Dopaminergic system as an example of age-related change in neuronal dynamics

In aging rats, dopamine production decreases as reflected by reductions in tyrosine hydroxylase mRNA (TH) in the pars compacta of the substantia nigra, and ventral tegmental area (59). The number of cells expressing TH mRNA is the same in young and old rats, but aging is associated with reduced TH gene expression per cell. Reduced production of dopamine during aging increases the susceptibility of neurons toward glutamate neurotoxicity, resulting in seizures and neuronal cell death (60). Dopaminergic neurons in the caudate-putamen, substantia nigra, and nigrostriatal pathway also show increased susceptibility to degeneration induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment (61). This gradual loss of neurons from the neuronal network will be accompanied by progression toward reduced complexity in neuronal behavior.

Studies of the behavioral dynamics of the dopamine neurons are consistent with age-related progressive changes toward reduced complexity. When the electrophysiological behavioral characteristics of dopaminergic neurons were compared in the brains of young and old animals, two different firing modes (single-spike and bursting) that interweave to produce irregular interspike patterns were identified (62–65). Mathematical analysis to discriminate nonlinear deterministic from either stochastic or linear oscillations showed that interspike intervals recorded from dopaminergic neurons exhibited a transition toward stochastic behavior during aging (65, 66). Although irregular stochastic behavior could also organize the irregular behavior of neurons, the rapid synchronization and processing of irregular input signals is less readily accommodated (41).

In summary, during aging there appears to be an increased susceptibility of physiological systems to trauma and stress. It has been speculated that this is a consequence of a transition of physiological systems from ordered adaptive complex behavior toward more stochastic behavior (40–42). The application of nonlinear dynamics to physiology is relatively new; therefore, until more work is done, the conclusions must be considered preliminary. Despite this caveat, the characterization of age-related changes in the electrophysiological dynamics of neuronal behavior, as observed with dopaminergic neurons, paves the way to test strategies designed to reverse or prevent age-related changes; furthermore, it allows us to determine whether experimental manipulation to improve adaptive capacity by restoring the behavioral complexity of the system will prevent increased vulnerability to trauma and stress. Hence, the application of dynamic measures of complexity offers the potential to quantify physiological aging, to predict the outcome of molecular endocrine changes, and to provide a method for evaluating intervention strategies.

III. Age-Dependent Changes in Biological Rhythms

A. Aging and disruption of circadian rhythms

Aging of the neuroendocrine system is manifested by changes in pulse amplitude and increased irregularity in the periodicity of hormone and neurotransmitter release. Indeed, the onset of menopause is accompanied by changes in biological rhythms (29, 67). In addition to effects on proopiomelanocortin (POMC) and reproductive hormones, 24-h profiles of GH, cortisol, and rhythms of body temperature change during aging. These effects likely result from age-related changes in the circadian pacemaker of the suprachiasmatic nucleus (SCN) (68). By the time humans reach middle age, regulation of their biological rhythms is compromised.

Circadian rhythms exhibiting erratic firing and reduced amplitude are observed in aged rats and appear to be primarily controlled at the level of gene transcription in the SCN (69, 70). The effect of light pulses on modifying circadian rhythm differs in young vs. old animals. In young hamsters maintained under conditions of constant light, or after 6 h exposure to darkness, induction of phase advance and phase delay in circadian rhythm of locomotor activity is induced by treatment with the short-acting benzodiazepine, triazolam, whereas old hamsters are refractory (71).

In patients with Alzheimer’s disease (AD), day and nighttime levels of arginine vasopressin (AVP) mRNA in the SCN are identical, but in normal subjects, daytime levels are more than three times higher than at night (72). Liu et al. (72) speculated that the neuronal basis of the circadian rhythm disturbances in AD patients is located in the SCN, which perhaps explains the beneficial effect of light therapy on relieving restlessness at night. Clearly, these data do not establish a direct relationship between AVP circadian rhythms and AD; however, the results are intriguing and invite further clinical studies.

The fundamental mechanism underlying age-related alterations in biological rhythms of hormone release continue to be investigated. In the case of LH, the noradrenergic system is an important regulator of episodic release and provides an example of age-dependent changes in neurotransmitter action (73). Middle-aged rats show decreased levels of α1-adrenergic receptors in the SCN. The diurnal rhythm of α1-adrenergic receptors expression, characteristic of young rats, disappears by middle age (73). Similarly, aging alters the rhythmic expression of vasoactive intestinal peptide (VIP) in the SCN (74). In young female rats, but not in middle-aged rats, VIP mRNA exhibits a 24-h rhythm. By contrast, the 24-h rhythm of AVP mRNA expression persists during aging.
Thus, regulatory components of the SCN are differentially modified by aging.

B. Altered circadian rhythms modify sleep patterns

One of the most common features of aging is impairment in the quality of sleep with increased wakefulness and reduced slow-wave sleep (SWS) (75, 76). Biological clocks in the SCN of mammals are important regulators of sleep-wake cycles. SCN neurons produce VIP, AVP, and somatostatin. GHS-R is also expressed in the SCN, and the synthetic GHS-R ligand MK-0677 improves quality of sleep in healthy elderly subjects (77, 78).

Inputs to the SCN from retinal ganglion neurons and neurons of the lateral geniculate and raphe nuclei play an important role in entrainment and shift of circadian rhythms (79). Lesioning of the SCN causes a loss of circadian rhythms of hormone release and sleep-wakefulness (79–81). However, the SCN is not the only regulatory influence, because increases in SWS and slow-wave activity that follow sleep deprivation are not reduced in SCN-lesioned animals (82). There is strong evidence that regulation of sleep homeostasis involves adenosine activity in the basal forebrain (83). Indeed, adenosine may play a broader role in regulating sleep patterns, because it is an agonist for the GHS-R, which is also expressed in CNS and is involved in sleep regulation (78, 84–86).

To investigate a potential link among aging, circadian rhythms of hormone release, and sleep patterns, 24-h pulsatile profiles of cortisol, TSH, melatonin, prolactin, GH, and sleep patterns in healthy elderly men and young men were monitored (87). Mean cortisol levels were unaffected by age; however, the amplitude of circadian rhythm was reduced in elderly men. Daytime and nighttime levels of TSH and GH were markedly diminished according to age, whereas prolactin and melatonin concentrations were decreased only at nighttime. These age-dependent decreases were a result of reduced amplitude rather than a change in pulse frequency. The circadian increase of cortisol, TSH, and melatonin occurred 1–1.5 h earlier in the elderly men and was accompanied by a similar advance in rapid eye movement (REM) stage sleep. Healthy elderly subjects experience earlier clock time for melatonin circadian rhythms, body temperature, and cortisol peaks. Wake time is advanced relative to both clock time and internal circadian rhythms. The basis of such differences between young and old subjects remains to be elucidated, but it likely involves age-related changes in factors known to regulate sleep patterns. These include GHRH, adenosine, CRH, galanin, neuropeptide Y (NPY), vasopressin, and hypocretin or perhaps the endogenous ligand of the GHS-R, ghrelin (88–92).

C. Restoration of normal rhythms in aged animals

Age-dependent changes in biological rhythms can be reversed by implanting the SCN from rat or hamster fetus into the brain of the appropriately aged host (30, 31, 93). In young hamsters, repeated injection of benzodiazepines entrains the circadian clock to the exact injection period, whereas old hamsters are resistant to efficient entrainment (71). However, transplantation of fetal SCN into the hypothalamus of old hamsters partially rescues the aging phenotype by restoring phase shifts that are responsive to triazolam and restoring rhythmic c-fos expression in response to light (71). Similarly, fetal SCN transplantation modifies circadian rhythms of the CRH/ACTH axis in middle-aged rats to mimic those of young animals.

The demonstration that the young phenotype is restored in an old animal by transplanting fetal SCN tissue is fundamentally important because it shows that the aging SCN retains latent functional capacity. Furthermore, these results suggest that important factors regulating the temporal pattern of expression in the SCN are lost by the time rats reach middle age. Intriguingly, the fetal SCN either provides these factors or induces their expression in the host. Restoration of the host SCN can also be demonstrated when the transplanted fetal SCN cells are encapsulated, showing that an SCN rejuvenating factor(s) is secreted by the fetal cells (31, 94).

In addition to sex steroids that modulate dopamine signaling, catecholamine levels in the brain decline during aging (95–103). The aging hypothalamus has a reduced capacity to secrete dopamine and norepinephrine (104). Indeed, certain aspects of aging are induced by treating rats with drugs that reduce catecholamine levels in the hypothalamus, whereas drugs that elevate hypothalamic catecholamine levels reverse certain physiological aspects of aging (104). For example, when young hamsters are treated with reserpine to lower concentrations of 5-HT, norepinephrine, and dopamine in the hypothalamus, striatum, and pons/medulla, their circadian rhythms are altered and their responses to phase shifting stimuli are modified to produce a phenotype identical to that occurring spontaneously in old hamsters (71). Hence, reductions in monoaminergic activity in the brain probably contribute to the age-associated changes in the circadian clock system. Because this aging model can be manipulated by altering catecholamine levels, it allows experimental testing of the hypothesis that aging is coupled to decreased complexity of neuronal behavior.

D. Age-associated changes in circadian rhythms influence metabolism

The development of age-related reduced glucose tolerance, obesity, and peripheral insulin resistance accompanies alterations in the circadian rhythms of glucose regulation (68). Remarkably, when the daily rhythms of endogenous corticosterone and prolactin in old rats are modified by administering these hormones at times of the day corresponding to peak levels observed in young rats, the age-associated increases in insulin resistance and body fat are reversed (105). Resetting of the rhythms by appropriately timed hormone replacement restores the young phenotype. Most importantly, these experimental results emphasize the physiological importance of circadian rhythms on metabolism and are consistent with changes in behavioral complexity of regulatory neurons that produce altered rhythmicity of factors controlling glucose regulation.

Changes in circadian endocrine rhythms during aging are associated with altered carbohydrate and lipid metabolism,
which causes increased deposition of fat at the expense of muscle. Neuroendocrine perturbations involving the hypothalamic-pituitary-adrenal (HPA) axis produce insulin resistance and development of syndrome X (107, 108). Visceral fat (VF) accumulates and is an important contributing factor for age-associated insulin resistance and α2-adrenergic receptors in the thalamic-pituitary-adrenal (HPA) axis produce insulin resistance and development of syndrome X (107, 108). VF is a rich source of 11β-hydroxysteroid dehydrogenase type 1 (HSD1), which reduces 11-keto steroids to produce active glucocorticoids (109, 110). Hence, increased VF provides a rich source of the counterregulatory hormones for glucose homeostasis.

IV. Aging, Memory, and Cognitive Decline

The effect of aging on neuronal structure, memory, cognition, and neurotransmitter activity can be linked to changes in hormone action. Specific relationships are addressed, but causality in most cases has not been established. For clearer interpretation, a whole-systems approach to provide links between hormonal changes and CNS function is needed.

A. Age-related neuronal structural and functional changes

The endocrine system affects neuronal signaling and neuronal integrity; therefore, age-dependent endocrine changes influence structure and function of the CNS. Morphological studies of the hippocampus in young and old rats reveal that pyramidal neurons in old rats are smaller and contain fewer dendritic branches and spines (111). The density of presynaptic terminals per unit length of postsynaptic membrane is also lower (Fig. 2). Such changes are reminiscent of age-associated shrinkage of pyramidal neurons in the human brain (112, 113).

The electrophysiological properties of layer V pyramidal hippocampal neurons in young and old rats were evaluated by recording spontaneously occurring postsynaptic currents (PSCs) (111). There was no preferential decline in the frequency of excitatory compared with inhibitory PSCs. The reduction in inhibitory PSCs is consistent with a change in surface area of the cell bodies. These are speculated to be targets of y-aminobutyric acid (GABA) inhibitory synapses and correlate with age-related reductions in GABA<sub>A</sub> receptor mRNA expression and GABA<sub>A</sub> receptor density (114–116). The reduced amplitude of excitatory serum PSCs in old rats is probably a result of desynchronization of neurotransmitter release from presynaptic terminals, which is likely exacerbated by the greater separation of presynaptic terminals in the aged brain (111). These observations support the hypothesis that aging results in a transition toward increased stochastic behavior of neurons that leads to less robust synchrony of neurotransmitter release. Surprisingly, aside from amplitude changes, it appears that compensatory mechanisms maintain comparable input to the pyramidal neurons despite significant synaptic loss during aging. Activation of the compensatory pathways may explain why cognitive impairment in normal aging is relatively modest compared with that observed in pathological conditions such as AD.

As they age, rats and mice show changes consistent with age-dependent cognitive deficits in spatial memory and working memory (117–121). Spatial learning is dependent on the integrity of the hippocampal structures and is evaluated according to performances in the Morris water maze and Barnes maze. The early stage of memory is associated with early-phase long-term potentiation (LTP), which does not involve protein synthesis, whereas later stages that consolidate short-term to long-term memory are associated with late-term LTP requiring new mRNA and protein synthesis (120). Comparative studies in 3, 6, 12, and 18-month-old C57BL/B6 male mice showed that spatial memory was impaired in the majority of aged mice (12 and 18 months old) and was correlated with late-term LTP deficits in CA1 neurons of the hippocampus. Comparing performance in spatial tasks with neurobiological evaluation allows discrimination between a detrimental neurobiological change from compensatory adaptation (122).

B. Hippocampus and neurogenesis

Neurogenesis in the dentate gyrus (DG) of rats was first reported in 1965 (123), and recent results in primates extend these findings across species (124–126). The significance of the production of new neurons during adulthood is unknown, although studies in rodents suggest that neurogenesis plays an important role in learning (125, 127). By placing rats and mice in a stimulating environment, neurogenesis is
induced (128, 129). Furthermore, training in a task that requires hippocampal function stimulates granule cell proliferation in the DG (127, 130). However, a decline in neurogenesis occurs during aging (131–133).

Production of neurons in the mature CNS is affected by trauma. New neurons are generated in the hippocampus after seizures (of variable amplitudes), stroke, and local lesions, suggesting that they may be involved in recovery from injury (134–136). Ischemia increased the production of neuronal cells in the subgranular zone of the DG that coexpress both markers of DNA replication and mature neurons. These results support a role for neurogenesis in what may be a process that leads to recovery after stroke (135). Excitotoxic and mechanical lesions of the granule cell layer performed in the adult rats showed an increase in proliferating cells on the lesioned side compared with the unlesioned side 24 h after surgery. There was also a significant positive correlation between the extent of damage and the number of proliferating cells. Three weeks after the lesion, the majority of cells produced as a result of this insult had morphological and immunohistochemical characteristics of mature granule neurons and were located in the granule cell layer (136).

C. Aging and neurogenesis

It is not surprising that attenuated neurogenesis is observed during aging because positive regulators such as the sex steroids, DHEA, GH, and IGF-I decline, and glucocorticoids, which inhibit neurogenesis, increase (137–146). The important issue is whether these hormonal changes and reduced neurogenesis contribute to increased susceptibility of the CNS to irreversible damage and increased incidence of CNS-linked disorders. If cause and effect are linked, timely hormone replacement would be most beneficial.

D. Steroids and neurogenesis

Specific neural mechanisms alter the production of granule cells in the DG. The perforant path is the main excitatory afferent to the granule neurons and provides glutamatergic input, which appears to suppress the proliferation of granule cell precursors. Lesion of the entorhinal cortex, source of the perforant path, increases neurogenesis in the DG (147). In contrast to young rats, in old rats even acute stress produces an exaggerated release of glutamate in the hippocampus (148). Both corticosterone and glutamate N-methyl-D-aspartate (NMDA) receptor agonists inhibit neurogenesis. Treating adult rats with the NMDA-receptor antagonist dizocilpine maleate (MK-801) stimulates neurogenesis and increases the density of neurons in the granule cell layer. MK-801 also counters the corticosterone-induced decrease in cell proliferation. Hence, corticosterone and NMDA receptor activation appear to inhibit granule cell production in the rat DG through a common pathway, and NMDA-receptor activation is downstream of adrenal steroid effects (149). Overall, although the evidence remains associative, the collective findings in rodents and nonhuman primates argue that decreased neurogenesis is caused by elevations in plasma and locally produced corticosteroids. The effects of elevated glucocorticoids during aging are exacerbated by decreases in estradiol and IGF-I and likely contribute to age-related memory deficits observed in humans.

The stimulatory effects of estradiol and inhibitory effects of corticosterone on neurogenesis has been clearly demonstrated in rats. Regulation of neurogenesis by estradiol was tested by measuring the incorporation of bromodeoxyuridine (BrdU) into cell nuclei of the dentate granule cell layer at different estrus stages, and after ovariectomy with and without estradiol replacement (141). Figure 3 illustrates the beneficial effects of estradiol on stimulation of cell proliferation and cell survival in the DG of ovariectomized rats. Old age is accompanied by a marked decrease in production of hippocampal granule neurons (132, 150). Lowering corticosterone levels in old rats restores neurogenesis. Most importantly, this result shows that the neuronal precursor population is unaffected by old age, indicating that neurogenesis is inhibited by age-associated increases in basal corticosteroid levels, but the deficit can be rescued (132).

Treatment of adult male rats with sc pellets of DHEA stimulated neurogenesis in the DG and antagonized suppressive caused by administration of corticosterone (145). The precursor of DHEA, pregnenolone, and DHEA’s major metabolite, androstenediol, were both unable to replicate this property. These results show that DHEA, a steroid prominent in the human brain that decreases markedly with age, regulates neurogenesis in the hippocampus and antagonizes...
the inhibitory effect of glucocorticoids on survival and formation of new neurons.

Testosterone also plays a role in neurogenesis, and one of the best examples is from the avian system. Stimulation of neurogenesis in the adult canary incorporates neurons into the high vocal center, which is a nucleus in the adult canary brain that is important in the acquisition and presentation of learned song (151). This process occurs seasonally and is regulated by testosterone. Testosterone acts by stimulating production of brain-derived neurotrophic factor (BDNF) in the female high vocal center. BDNF mimics the effect of testosterone, and infusion of a neutralizing antibody to BDNF blocks the testosterone-induced increase in new neurons. Hence, testosterone regulation of neuronal replacement in the adult canary brain is dependent on BDNF.

E. IGF-I and neurogenesis

Growth and development of neurons in the DG are modulated by IGF-I. Marked reductions in IGF-I and neurogenesis in the DG accompany aging (133). Stereological analysis of brain sections from transgenic mice that overexpress IGF-I postnatally in the brain shows increases in neuronal and synaptic density in the DG (146). To model somatic IGF-I deficiency, rats were hypophysectomized and maintained on glucocorticoid and T4 replacement (142). These rats were subjected to short-term (6 d) and long-term (20 d) infusion of IGF-I, and cell proliferation was monitored in the DG by incorporation of BrdU (142). Both short- and long-term IGF-I infusion maintained neurogenesis in the hypophysectomized rats. These results support the notion that IGF-I regulates neurogenesis in the dentate granule cell layer and suggest that age-associated decreases in IGF-I might be involved in age-related neurodegeneration.

Recent studies show that the rate of neurogenesis in the DG of the hippocampus declines as a function of age perhaps contributing to age-related cognitive changes. Intracerebroventricular (icv) IGF-I infusion ameliorates this age-associated decline. Lichtenwalner et al. (152) used BrdU labeling and multilabel immunofluorescence to evaluate age-dependent changes in neuronal production in the DG of adult Brown Norway (BN)/Fischer 344 rats. They found an age-dependent reduction in the generation of new cells in the adult dentate subgranular proliferative zone and a 60% reduction in the differentiation of newborn cells into neurons. Intracerebroventricular infusion of IGF-I to restore IGF-I levels in senescent rats restored neurogenesis to provide a 3-fold increase in neuronal production. This study highlighted the possibility that IGF-I is an important mediator of neurogenesis in the adult and suggested that the age-dependent decline in IGF-I-regulated neurogenesis could contribute to cognitive deficits.

Exercising is also beneficial for maintaining neurogenesis. In the adult mouse, running promotes neurogenesis in the DG, which is believed to be mediated by IGF-I (153). BDNF also increases in mice after running, which counteracts the negative impact of stress on BDNF production in the hippocampus (154–156). Indeed, exercise-induced increases in BDNF enhanced the rate of learning in the Morris water maze test (157, 158). Furthermore, BDNF mediates testosterone-induced survival of new neurons in the adult brain (151). However, neuroprotection requires IGF-I, because the protective effect of exercise is antagonized by the central infusion of IGF-I antibodies (159).

F. Neurosteroids and memory

Steroidogenic acute regulatory protein (StAR) controls adrenal and gonadal steroidogenesis. It was recently shown unequivocally that STAR mRNA and protein are expressed within glia and neurons in discrete regions of the mouse brain (160). Consistent with its role in de novo neurosteroidogenesis, STAR colocalizes with the cholesterol side chain cleavage enzyme P450(scc) in both mouse and human brains (160). These data support a role for StAR in the production of neurosteroids and identify potential sites of active de novo steroid synthesis in the brain (160). Neurosteroids synthesized in the CNS appear to attenuate age-related memory and learning impairments (161–165). For example, impairments induced by aging or by an NMDA receptor antagonist were inhibited by neurosteroids (164). When young (3 months old) and old mice (16 months old) were tested in two different behavioral models of long-term memory, the performance of aged mice in step-down passive avoidance and elevated plus-maze paradigms was markedly impaired compared with the performance of young mice; however, treatment with pregnenolone sulfate (PS) and DHEA sulfate attenuated the decline in performance. To determine whether the mechanism of attenuation was mediated through the nitric oxide (NO) synthase (NOS) signal transduction pathway, mice were pretreated with the NOS inhibitor, NG-nitro-l-arginine methyl ester (l-NAME) at doses that were predetermined to have no disruptive effect on cognition. l-NAME inhibited the beneficial and antiamnesic effects of PS and DHEA sulfate, and the effect of l-NAME was blocked by the competitive substrate for NOS, l-arginine. Hence, the beneficial effects of PS and DHEA sulfate on age-related learning and memory deficits appear to be mediated by inhibition of a NO-dependent pathway.

PS synthesis in the rat hippocampus declines during aging, and performance in two different spatial memory tasks (the Morris water maze and Y-maze) was found to correlate with levels of PS in the hippocampus (163). Old rats with the greatest memory deficits had the lowest hippocampal PS levels. A possible cause-and-effect relationship was suggested by showing that impaired memory was transiently improved by ip or bilateral intrahippocampal injection of PS (163, 166).

PS is a GABA_A receptor antagonist and an allosteric activator of the NMDA receptor. Administration of PS into the CNS enhances acetylcholine (ACh) release in basolateral amygdala, cortex, and hippocampus and stimulates neurogenesis (166). ACh neurotransmission is involved in regulation of memory processes and modulation of the sleep-wake cycle and neurodegenerative diseases (166). These findings suggest that PS is at least partly involved in maintaining cognitive abilities, sleep patterns, and neurogenesis. It remains to be determined whether local neurosteroid synthesis declines during aging, or whether lower levels of neurosteroids in the CNS is a result of reduced peripheral...
steroid production. Studies are underway to address these questions through selective neurosteroid synthesis inhibition in the brain.

G. Gene expression in memory and learning

One caveat of drawing conclusions from behavioral tests on laboratory rodents is that the animals are housed in an artificial, sterile environment. If rats are exposed to an enriched environment during their youth, they perform better in memory tasks when they get older (167). Indeed, providing mice with toys, wooden blocks, spin wheels, and small houses produces biochemical and structural changes in the cortex, DG, and CA1 hippocampal structures (128).

Candidate genes that specifically contribute to memory and learning were identified by using high-density oligonucleotide microarray analysis to compare gene expression in mice maintained in standard housing with mice exposed to a stimulating environment (168). Many of the genes identified were known to play a role in neurotransmission, neuronal structure, and neuroplasticity. Certain of these genes are related to hormonally regulated genes (168). For example, the expression of estrogen-responsive finger protein was increased by 2.2-fold, and DNA methyltransferase increased 26-fold; by contrast, retinoid X receptor-α expression decreased by 26-fold. The expression of genes involved in apoptosis, such as Bcl-2 associated protein, Bax, caspase-6, and caspase-4 genes decreased by 3- to 4-fold. Expression of transcription factor X-box binding-protein, which interacts with cAMP response elements of genes to increase gene expression, increased 2.4-fold. Furthermore, expression of the cAMP-dependent protein kinase regulatory subunit was reduced 2.5-fold, which is relevant to aging because overexpression of this regulatory subunit compromises both hippocampal LTP activity and long-term memory (169). Interestingly, levels of apolipoprotein E (apoE) increased 2.3-fold (168). ApoE signaling is involved in the dephosphorylation of β protein and hyperphosphorylated τ is a component of the plaques and tangles characteristic of AD; hence, apoE may have neuroprotective properties. Although it must be remembered that these observations are merely associative, they provide important links with hormones and aging of the CNS. For example, IGF-I production declines during aging; however, increasing IGF-I levels in the brain mimics enrichment by protecting against apoptosis and neurodegeneration.

V. GH Axis

A. Age-associated decline in GH pulse amplitude

Neuroregulation of pulsatile GH release from the anterior pituitary gland secretion has been reviewed (170). The amplitude of the GH pulses is attenuated as we age because of suboptimal signaling from the hypothalamus. This is partially explained by an age-associated reduction in receptor density for the positive regulator of GH release, GHRH (171–174). Reduced receptor density would require higher levels of GHRH to normalize the pituitary response. Rudman et al. (175) addressed the question of whether GH replacement in the elderly might have functional benefits. They administered GH chronically to elderly men for 6 months and found improvements in body composition and skin thickness that were consistent with reversal of the aging process (175). Unfortunately, adverse side effects such as carpal tunnel syndrome and gynecomastia were relatively common (175). However, the incidence of adverse events is reduced if lower doses of GH are used (176–178).

It is important to note that GH replacement by bolus injection overrides the episodic physiological profile (179). Biochemical and biological data support the importance of the episodic profile. In the liver, different signal transduction pathways are activated when pulsatile vs. sustained administration of GH are compared (180). Male rats exhibit pronounced high amplitude pulses, whereas in females the profile is flatter and is reflected by distinctly different patterns of GH-regulated gene expression. In males, whole body puerperal growth rate is dependent on GH activation of signal transducer and activator of transcription 5b (STAT5b). In STAT5b knockout mice, males exhibit a female GH phenotype (181).

In humans, GH pulse amplitude and plasma IGF-I levels decline during aging (24, 38). Young adults exhibit a gender difference, in which women have the same pulse frequency as men but with a 2.4-fold increase in burst mass (182). However, these gender differences disappear during aging, and elderly men and women have equally low amplitude GH pulses and reduced IGF-I levels (24, 38). Based on the known properties of GH and IGF-I in vivo, this reduced amplitude, in combination with reduced sex steroid production, likely explains the observed age-dependent change in metabolism, increased fat/lean ratio, decreased muscle strength, reduced exercise tolerance, and increased bone loss. Hence, the functional deficits that result from aging are probably caused by suboptimal signaling from the hypothalamus. An ideal approach for modifying the aging phenotype would be to restore activity of the hypothalamic neurons that control GH pulse amplitude.

A recent study (183) describes the use of DNA microarray chip technology to relate the physiological decline in GH with molecular mechanisms underlying the aging process. Gene expression was compared in the liver of old rats, with or without GH replacement. Of 1000 genes detected in male rat liver, 47 transcripts were affected by aging and about 40% of the differentially expressed genes were normalized by GH treatment. This study is notable and refreshing because the authors evaluated gene expression in the animals after compensating for changes in GH. However, because of age-dependent changes in other hormones, such as sex steroids, and the difficulty of replacing hormones in a way that recaptures the physiology of a young animal, it is impossible to precisely differentiate hormone-dependent from hormone-independent age-related changes. Despite this caveat, studies using DNA microarray analysis of brain tissue from rats that show improvements in memory following GH replacement should be particularly informative.

B. Increase in longevity in GH-deficient rats and mice

It seems reasonable to speculate that restoration of GH release in a way that mimics the physiology of a young adult
will provide functional benefits, not necessarily by increasing longevity, but by improving the quality of life. This is based on observations in GH-deficient humans showing that GH increases bone density and improves body composition, cognitive function, cardiac function, and exercise tolerance. However, despite this evidence, the likelihood of achieving beneficial effects by rejuvenating the GH/IGF-I axis physiologically is not universally accepted. One of the reasons is based on laboratory animal studies, where the data suggest that reducing GH, IGF-I, or insulin signaling increases longevity (184–194). However, these studies do not address quality of life in elderly subjects, which is more important than longevity. Indeed, although caloric restriction has also been shown to improve longevity in a variety of species, a recent, careful study and review of the literature shows that prolonged caloric restriction impairs cognitive function in rats (195).

The evidence for a negative impact of GH and IGF-I on longevity is based largely on studies in mutant mice that are either GH deficient or lack receptors for GH. All of these models exhibit dwarfism, reduced body temperature, and reduced fertility. Certain of these mouse models, such as the Ames dwarf (dv/dv) mouse are GH, IGF-I, prolactin, and thyroid hormone deficient. Although recent studies (188) indicate that the life span of hormone-deficient dwarf mice housed under stress-free laboratory housing conditions is 50% longer than their normal littersmates, these observations are unlikely to predict survival in the natural environment. For example, earlier studies indicated that dv/dv mice had markedly reduced life span (45-60 d) and are immunocompromised (196, 197). The reduced longevity was suggested to be a result of more stressful animal housing conditions, typical of 30 yr ago, and poor adaptation to stress (198, 199). Indeed, it has been speculated that the negative effects of prolonged stress, which causes suppression of the immune system by glucocorticoids, are balanced in wild-type animals by the positive effects of GH, IGF-I, prolactin, and thyroid hormone (198, 200).

It is important to recognize the beneficial roles of the GH/IGF-I axis in human physiology. In addition to antagonizing the adverse effects of chronic stress on the immune system, GH and IGF-I may play a similarly protective role in the CNS, thereby potentially improving quality of life. Indeed, Koo et al. (201) reported that restoring GH levels produced beneficial effects on the immune system of old normal mice. They showed that when old mice are treated with an oral GH secretagogue, restoration of GH and IGF-I reversed the age-dependent shrinkage of the thymus and improved T-cell production. The advantage of rejuvenating the GH/IGF-I axis was illustrated by implanting aggressively growing tumors into the mice. Treatment with a GHS-R agonist reduced the rate of tumor growth, inhibited metastasis, and increased longevity (201).

C. GH in the CNS

The characteristics and significance of GH binding in the human brain have been reviewed by Nyberg and Burman (202). In addition to reduced GH release during aging, the concentration of GH receptors in the brain also declines. The highest density of GH binding is in the choroid plexus, with significant binding in the hippocampus, hypothalamus, amygdala, putamen, and thalamus (202). Although GH receptors are widely expressed in the CNS, and anecdotal reports claim that GH improves mood in the elderly, relatively few studies have investigated GH’s functional role in the brain (203). Indirect evidence suggests that GH plays an important role in CNS function. GH-deficient children have an increased incidence of anxiety, depression, and attention deficits, which may contribute to their observed learning disabilities in arithmetic, spelling, and reading compared with age-matched controls (202, 204). GH deficiency in adults is reported to be associated with reduced energy, unfulfilled personal life, low self-esteem, problems controlling emotional reactions, social isolation, impaired social function, mental fatigue, impaired general and mental health, and deficits in cognitive function (205–211). Markedly reduced GH levels, particularly the integrated nocturnal levels, have also been associated with major depressive illness (212). This may explain the increased incidence of depression and poor sleep quality in the elderly population.

The neuroprotective property of GH was documented in rats. Hypoxic-ischemic damage causes increased GH transport into the brain as manifested by an increase in the number of GH-immunopositive neurons (213). To demonstrate GH binding by immunostaining, the authors went to extraordinary lengths to document specificity. The immunohistochemical evidence showing that GH migrates to the sites of injury following hypoxic-ischemic injury is most persuasive. The authors also demonstrated that icv administration of GH was neuroprotective in the cortex and hippocampus (Table 1). Thus, the decline in the amplitude of GH secretion during aging likely attenuates GH-mediated neuroprotection.

D. Relationship of GH and IGF-I to age-related cognitive impairment

A comparison was made of the expression of IGF-I mRNA and protein and IGF-I receptor mRNA in the brain of Fisher 344 × BN rats during aging (214). Age-related changes in IGF-I mRNA were not evident in cortical tissue. However, between the ages of 11 and 32 months, IGF-I protein levels were reduced by 36.5% in the cortex. Although IGF-I receptor mRNA concentrations were unchanged, IGF-I receptor binding was reduced by 27% in the cortex and 31% in the hippocampus. When different age groups of rats were compared (6 months vs. 23 months), a modest decrease in IGF-I mRNA was reported in the hippocampus (215). A decrease in IGF-I

Table 1. Protective effect of icv injection of GH on cortical and hippocampal neurons following hypoxic-ischemia

| Score group   | Cortex neuronal loss | Hippocampus neuronal loss |
|--------------|---------------------|-------------------------|
| Vehicle-treated | 1.02 ± 0.12       | 1.82 ± 0.26         |
| GH-treated   | 0.46 ± 0.07        | 0.97 ± 0.18         |

N = 12 per group. [Reproduced with permission from A. Scheepens et al.: Neuroscience 104:677–687, 2001 (213). © Elsevier Science.]

a P < 0.05.

b P < 0.001.
concentrations and IGF-I binding in the hippocampus is consistent with age-related neurodegeneration.

GH administration increases IGF-I gene transcription in the CNS. Whether a causal relationship between age-associated deficiencies in cognitive function and declining brain IGF-I levels exists is the subject of continuing debate. However, an association is supported by the observation that when IGF-I is administered icv to rats for 28 d, the age-dependent decline in spatial reference and working memory is reversed (216). GH studies in adults with childhood onset GH deficiency showed that GH replacement benefited CNS function (217, 218). Doses of GH that produced supraphysiological levels of IGF-I normalized memory function after 6 months of treatment. Lower doses selected to provide physiological IGF-I concentrations in the blood improved memory function more slowly, but normal function was restored after 12 months of treatment.

Reduced IGF-I levels are characteristic of aging (2, 215, 219–221). Endogenous IGF-I plays a significant role in recovery from insults such as hypoxia-ischemia (222). Neurons die within hours or days following initial injury because of activation of cell death pathways. However, IGF-I with its binding proteins and receptors is induced within damaged areas following brain injury, which suggests that IGF-I plays a neuroprotective role. Administration of IGF-I within a few hours after brain injury confers protection on gray and white matter; by contrast, IGF-I pretreatment is ineffective, probably because of limited intracerebral penetration into the uninjured brain. This important neuroprotective property of IGF-I argues for the maintenance of young adult IGF-I levels during aging.

It has been suggested that IGF-I deficiency could be involved in cognitive deficits seen with aging. In elderly humans (aged 65–86 yr), a correlation between a subject’s performance in the Mini Mental State Examination and plasma IGF-I was reported (223). To investigate this observation in more detail, cognitive functions known to decline during aging were compared with those insensitive to aging. The outcome was consistent with a protective effect of IGF-I on the onset of age-dependent cognitive deficiencies, particularly in speed of information processing (208, 224). Similarly, Dik et al. (225) investigated whether IGF-I was associated with cognitive performance and cognitive decline over a 3-yr period in 1318 subjects, aged 65–88 yr. Although cross-sectionally IGF-I was directly related to information processing speed, memory, fluid intelligence, and Mini Mental State Examination score, these statistics were not significant after adjusting for age and other factors. However, analysis in quintiles of IGF-I illustrated a threshold effect of low IGF-I on information processing speed, with lower speed in those subjects in the lowest quintile of IGF-I ($\leq 9.4\ \text{nmol/liter}$) vs. those in the other four quintiles. A low IGF-I threshold was also observed during a 3-yr decline in information processing speed. In conclusion, this study suggested that IGF-I levels below 9.4 nmol/liter are associated with the level and decline of information processing speed.

Serum IGF-I appears to regulate brain amyloid-$\beta$ ($\text{A}\beta$) levels (226). During aging, IGF-I levels fall and A$\beta$, which is involved in the pathogenesis of AD, accumulates in the brain. Elevations in brain A$\beta$ levels are found at an early age in mutant mice having low circulating IGF-I. A$\beta$ burden can be reduced in aging rats by increasing serum IGF-I, and it reflects the ability of IGF-I to induce the clearance of brain A$\beta$. This is probably mediated by enhancing the transport of A$\beta$ carrier proteins such as albumin and transthyretin into the brain. The enhanced uptake is antagonized by TNF-$\alpha$. IGF-I treatment of mice overexpressing mutant amyloid markedly reduces their brain A$\beta$ burden; therefore, IGF-I appears to play an important role in modulating brain amyloid levels.

Studies on centenarians showed increased prevalence of dementia in those with lowest serum IGF-I levels (227). Collectively, these results are consistent with a causal link between the age-related decline of GH and IGF-I levels and cognitive deficits, which reinforces the need for continued investigation of IGF-I and CNS function. Ghrelin mimetics have been shown to normalize IGF-I levels in the elderly and to increase IGF binding protein 3; therefore, these compounds may prove beneficial as neuroprotective agents during aging (25, 38). The fact that IGF binding protein 3 levels are also increased suggests that the risk/benefit ratio regarding cancer risk may not be increased by such treatment.

### E. Potential mechanisms of GH/IGF-I-mediated neuroprotection

The age-associated decline in GH and IGF-I is likely to cause deficits in functioning of the CNS because both hormones play an important role in vascular maintenance and remodeling. The cerebrovasculature is a source of IGF-I and nerve growth factor (NGF), which are known to play an important role in memory (216, 228–230). During aging, cerebral blood flow decreases and, together with reduced production of sex steroids, correlates with the age-related decline in plasma GH and IGF-I levels. In BN rats, arteriolar density and anastomoses decline markedly between the ages of 7 and 29 months. However, GH treatment produces increases in IGF-I, reverses the age-dependent changes, and increases the number of cortical arterioles (231). These data suggest that preventing the decline in GH and IGF-I during aging would help prevent age-related reductions in vascular density.

The continued viability of adult neurones requires neurotrophic factors to support plasticity and provide neuroprotection. A decline in production of such factors probably contributes to the age-related functional deficits that occur in the aging brain. Through its property as a potent stimulator of myelination, IGF-I should protect against the demyelinating effects of increased levels of TNF-$\alpha$ (232). In mouse glial cultures, TNF-$\alpha$ increases apoptosis of oligodendrocytes, whereas IGF-I acts as a neuroprotectant by stimulating the differentiation and proliferation of oligodendrocyte precursors and inducing myelin-specific protein gene expression.

Production of specific NMDA receptor subtypes in the hippocampus of rats and mice falls during aging and appears to be regulated by IGF-I (233, 234); NMDA receptors have been implicated in memory and learning (235–237). Although NMDA1 receptor expression in the hippocampus is unaffected by aging, expression of receptor subtypes NMDAR2a (NMDA receptor 2a) and NMDAR2b decrease (233). In contrast to the hippocampus, in the cortex, an age-
related decline of NMDAR2a and NMDAR2b is not evident, and IGF-I treatment does not influence the concentration of either receptor subtype. The reduced expression of specific NMDA receptor subtypes in the hippocampus, which is reversible by IGF-I treatment, probably affects cognitive function. By contrast, in a study of aging rhesus monkeys (6-26 yr), the levels of NMDAR2b were unchanged in the hippocampus but reduced in the prefrontal cortex and caudate nucleus (238). Hence, we must remain cognizant of the need for caution when extrapolating data from rodent models to humans.

F. GHRH and cognition

GHRH secreted from arcuate neurons activates somatotrophs in the anterior pituitary gland to elicit GH release, and GH stimulates increased production of IGF-I. Hence, administering exogenous GHRH to old animals restores GH and IGF-I levels. Indeed, chronic administration of a GHRH analog (D-Ala²-GHRH) prevents age-dependent decline in memory in rats (239). D-Ala²-GHRH or saline was injected daily into 9-month-old rats until the rats were 30 months old. At this stage, spatial learning and reference memory were compared in the treated and control groups using the Morris water maze. The performances of the aged rats were also compared with 6-month-old rats. The results confirmed that spatial memory declined during aging and that chronic D-Ala²-GHRH treatment prevented this decline. The authors hypothesized that GH and/or IGF-I mediated the beneficial effects on memory, because the age-related decline in GH and IGF-I was preventable by chronic D-Ala²-GHRH treatment. GH treatment also improved mental activity, psychomotor function, behavior, and humor in elderly human subjects (240). These results suggest that orally active GHS-R ligands would also prove beneficial because they act upstream of GHRH (25).

G. GH, GHRH, and sleep

The CNS effects of GH and GHRH are believed to regulate sleep. SWS and secretion of GH decrease proportionality during aging (241). The major peak of GH release associated with sleep is markedly reduced in elderly subjects, and the amplitude of the nighttime cortisol peak increases (68, 87, 241–243). The effect of fasting on the amplitude of GH release and on sleep patterns was investigated in a small group of elderly subjects (244). GH levels were increased to levels about 50% of that in young adults, SWS was unaffected, and REM sleep was decreased (244). Therefore, although age-associated hyposomatotropism was partially restored, fasting did not induce changes in SWS.

In addition to having stimulatory effects on GH release, GHRH promotes SWS (245–248). However, the beneficial effect of exogenous GHRH on sleep has been questioned because of poor reproducibility. A possible reason for the disparities might relate to the modes of GHRH administration used in different studies. The route of administration is particularly relevant if the sleep-promoting property of GHRH is by direct action on the CNS. Bolus iv injections and intranasal administration are more efficient at delivering molecules rapidly to the CNS than slow iv infusion. Indeed, bolus and intranasal delivery of GHRH increased REM and SWS in old and young human subjects, whereas slow, continuous infusion was ineffective (245, 247, 249). Because GH secretagogues like ghrelin and its synthetic mimetics stimulate the release of GHRH from hypothalamic neurons (250–254), improvements in sleep quality elicited by the GH secretagogue MK-0677 are likely mediated by direct stimulation of hypothalamic GHRH neurons (78).

H. Somatostatin in the CNS

Increased somatostatin tone might cause the reduced amplitude of GH release observed in aging hypothalamus. However, although expression of somatostatin mRNA is reduced in frontal cortex, parietal cortex, striatum, and hippocampus, it is unchanged in the hypothalamus (255, 256). The age-related decline in somatostatin gene expression in the frontal and parietal cortex of rats paralleled impaired memory performance in a modified Morris water maze test (257).

To further investigate the consequences of reducing somatostatin in the CNS, somatostatin was depleted by treating rats with cysteamine (258). Cysteamine-treated rats exhibited significantly impaired performance in the Morris water maze, suggesting that somatostatinergic neurotransmission is important in brain functions that include learning and memory processes (258). Somatostatin-null mice have impairments in motor learning; however, because somatostatin and its receptors are present in the developing cerebellum, such impairments might be a consequence of developmental changes (259). Like rodents, an age-related decrease in somatostatin gene expression occurs in the CNS of the macaque monkey (Macaca fuscata) (255, 256). In macaques aged from 2 to over 30 yr, somatostatin mRNA levels decreased by 60-70% in the hippocampus, frontal cortex, temporal cortex, motor cortex, somatosensory cortex, and visual cortex. Although an association between declining somatostatin and impaired memory exists, causality remains to be established.

In the rat, administration of BDNF increases somatostatin expression in the CNS (260, 261). To determine whether the age-related decrease in somatostatin mRNA correlates with changes in BDNF in aging primates, BDNF mRNA was measured in macaque monkeys of different ages (256). Two BDNF transcripts (1.6 and 4.0 kb) are produced and expression of the 1.6-kb transcript was 60% lower in the hippocampus of old macaques (>30 yr old) compared with young macaques (2 yr old); the 4.0-kb transcript was unchanged. These results suggest a potential relationship between reduced BDNF and somatostatin expression during aging of primates; however, before entertaining the possibility of causal relationships, more detailed studies are needed.

If somatostatin plays an important role in the aging process, it is possible that somatostatin receptor (sst) expression also changes as a function of age. Ssts exist as six different subtypes encoded by five genes (262). Of these, subtype-2 (sst2) and subtype-5 (sst5) are primarily involved in the regulation of GH release, and both sst2 and sst5 mRNA expression in the pituitary gland decline during aging (262–268). Sst2 is also abundantly expressed in the CNS (269, 270). In
stress situations, compared with wild-type mice, sst2−/− mice release more ACTH, show increased anxiety, and exhibit reduced locomotor and exploratory behavior (264, 270). Hence, sst2 is apparently involved in regulation of locomotor, exploratory, and emotional reactivity (270). sst2 is also expressed in the retina, and treating a mouse model of diabetic retinopathy with the sst2 selective agonist MK678 inhibited neovascularization (271).

As discussed above, somatostatin tone is decreased during aging. Although reductions in sst2 and somatostatin expression do not explain attenuated GH pulsatility, they may contribute toward exaggerated anxiety-related behavior and CNS pathology associated with aging (272–274). Similarly, reduced somatostatin tone during aging may be involved in the etiology of diabetic retinopathy (271). Again, extensive studies are needed before concluding that somatostatin explains certain age-dependent pathological changes.

VI. GHS-R, Ghrelin, and Ghrelin Mimetics

The major issue facing traditional therapeutic agents is that they fail to treat the underlying altered physiology. Ideally, intervention in the aging process should maintain or restore the physiological function of young adults. The GH/IGF-I axis plays an important role in regulating metabolism, thymic function, bone density, muscle strength, cardiac function, reproductive function, and CNS function (see Section V). Although rejuvenation of the GH/IGF-I during aging may not have a profound impact on a single function, subtle improvement in all of these important physiological parameters is likely to have a significant impact on quality of life (see Section V). Reduced amplitude of GH pulsatility during aging causes decreases in serum IGF-I levels and is a result of attenuated GHRH signaling (171–174). Therefore, restoration of GH pulse amplitude should be possible by increasing endogenous GHRH release from arcuate neurons, by amplifying the stimulatory effect of GHRH on GH release, and/or by antagonizing the negative regulator somatostatin.

A. Identification of the GHS-R and synthetic agonists

By focusing on the hypothalamic-pituitary axis that regulates GH pulsatility, an orphan receptor, GHS-R, was identified that regulates GH pulse amplitude. Synthetic agonists of the GHS-R stimulate GH release from the hypothalamus, amplify the action of GHRH on the pituitary gland, and functionally antagonize somatostatin (25, 275, 276). Chronic activation of the GHS-R by the small molecule agonist MK-0677 sustained rejuvenation of the GH/IGF-I axis in elderly subjects (25, 38, 277–280). This is accompanied by increased lean body mass and increased bone mass (38, 281–283). In addition to beneficial effects on peripheral tissues, restoring young adult levels of GH and IGF-I is anticipated to be neuroprotective (213, 284).

In addition to being expressed in GHRH neurons of the hypothalamic arcuate nucleus, the GHS-R is expressed in brain centers that control biological rhythms, memory, learning, cognition, and mood (Fig. 4) (25, 86). Because GHS-R agonists restore young adult profiles of GH pulsatility by stimulating arcuate neurons, activating the GHS-R in other brain centers may restore sleep patterns, memory, cognition, and amplitude of neuropeptide and neurotransmitter release in the elderly (Fig. 1). In particular, GHS-R agonists may prevent age-related deficits in memory and learning by stimulating GHS-Rs in hippocampal structures.

B. GHS-R endogenous ligands, ghrelin, and adenosine

After cloning of the GHS-R, cell lines that stably expressed the receptor were developed and used to screen fractionated tissue extracts for endogenous ligands. The first natural ligand disclosed was ghrelin, an acylated 28-amino acid peptide isolated from extracts of stomach tissue (92).
prising feature of this peptide ligand is that octanoylation on a serine residue is essential for biological activity. Ghrelin was found to mimic the well-characterized synthetic ligands for the GHS-R by causing the release of GH from pituitary cells in vitro, stimulating GH release in vivo, and activating c-fos expression in hypothalamic neurons (92, 285).

Two groups independently identified adenosine as an agonist for the GHS-R (84, 286). In HEK293 cells engineered to stably express the GHS-R, adenosine behaves as a partial agonist and activates a signal transduction pathway distinct from that of ghrelin (286, 287). In contrast to ghrelin, adenosine fails to induce secretion of GH from cultured pituitary cells, but like ghrelin, adenosine increases food intake (84). The concentration of adenosine required for activation of the GHS-R (EC\textsubscript{50}, 2 μM) is similar to that required for activation of adenosine receptors in the brain (288, 289). By contrast, based on in vitro studies with the cloned GHS-R, it is not clear that the concentration of free ghrelin in the blood is high enough to activate the GHS-R in vivo. However, in addition to signaling the CNS via the vagus nerve (290, 291), it is possible given the widespread expression of ghrelin (292) that it functions as a paracrine or autocrine hormone.

Based on the low circulating levels of ghrelin, it has been suggested that the physiological target for ghrelin is a putative GHS-R subtype rather than the receptor cloned by the Merck group (277). We generated ghrelin- and Ghsr-knockout mice to investigate the consequences of deleting the ghrelin/ Ghsr signaling pathway and to determine directly whether the Ghsr was the ghrelin receptor that mediated ghrelin’s orexigenic and GH-releasing properties (293–295). Both genotypes are viable and visibly indistinguishable. We showed directly that the Ghsr is the ghrelin receptor that: 1) regulates the activity of GHRH neurons and GH release; 2) maintains normal IGF levels during aging of young adults; and 3) mediates ghrelin’s orexigenic property through activation of agouti-related protein (AGRP)/NPY neurons.

Although adenosine fails to stimulate GH release from pituitary cells, investigation must continue before ruling out a physiologically important role for adenosine on GHS-R expressed in the CNS (286). Adenosine levels in the CNS do not appear to decrease during aging (296, 297); however, a reduction in GHS-R expression would attenuate adenosine signaling through the GHS-R. Adenosine as a GHS-R ligand should be considered according to the important integrative role of adenosine on pathways regulated by dopamine and GABA (288, 289, 298). Adenosine, produced by the pituitary gland, increases the production of tyrosine hydroxylase in hypothalamic cells and stimulates the secretion of catecholamines by dopaminergic neurons (299–301).

Aging is accompanied by a decline in the capacity for neurons to secrete dopamine (15, 302–305). In old rats, L-dopa administration restores the amplitude of GH release to that typical of young rats (36), which is reminiscent of the effects of the GHS-R ligand MK-0677 in elderly humans (38). Furthermore, both L-dopa and GHS-R ligands have been shown to increase GHRH levels (37, 250, 251). MK-0677 lacks dopaminergic activity, but the GHS-R is expressed in areas of the brain enriched in dopaminergic neurons (86, 306). It is therefore tempting to speculate that activation of the GHS-R, either by endogenous adenosine or MK-0677, causes dopamine release from hypothalamic neurons, which increases GHRH release, resulting in an increase in GH pulse amplitude. Hence, age-related declines in dopamine resulting in attenuation of pulsatile GH release could be rescued by treatment with MK-0677.

C. Aging is associated with ghrelin insensitivity

The GH response to ghrelin shows a clear age-related decrease in both genders (307); this response agrees with previous findings showing that the GH response to either peptidyl or nonpeptidyl synthetic ghrelin mimetics in the elderly is lower than in young adult subjects (38, 308, 309). Age-related attenuation of both spontaneous and stimulated GH secretion reflects age-dependent changes in the neural control of somatotroph function as reflected by reduced GHRH activity. This potentially explains the reduced response to ghrelin and its mimetics during aging (170). Indeed, the GH-releasing activity of ghrelin and its mimetics is dependent on the functional integrity of the hypothalamic-pituitary axis involving GHRH-secreting neurons (25). Therefore, somatotroph insufficiency in aging would also reflect some impairment in the ghrelin-signaling pathway. Indeed, expression of GHS-R mRNA is reduced in the aged human hypothalamus of both genders, which is consistent with their reduced GH response to ghrelin (308). The concentration of ghrelin in plasma is reported to decline in adult rats and in humans as they age (310, 311); therefore, lower ghrelin production in addition to reduced GHS-R levels may explain the decline in GH pulse amplitude during aging.

One explanation for ghrelin resistance is through reduced synthesis of ghrelin receptors caused by hormonal changes during aging. Kaji et al. (312) investigated hormonal regulation of the human GHS-R (also known as ghrelin receptor) expression in GH3 cells transfected with the GHS-R 5′-flanking region inserted into a luciferase reporter vector. Glucocorticoids caused a weak but significant inhibition of the luciferase activity through a site in the GHS-R gene upstream between 2530 and 2475 bp. This inhibition appears to be regulated by glucocorticoid-dependent synthesis of a protein(s) that attenuates human GHS-R/Luc activity.

Because aging is associated with increased glucocorticoid levels (313), a link between the age-dependent reduced response to exogenous GHS-R agonists and glucocorticoid attenuated expression of the GHS-R can be made (38, 307–309). In humans undergoing prednisone treatment, injection of a nonpeptide mimic of ghrelin, L-692,429, produced dose-dependent GH responses; however, higher doses of L-692,429 were required compared with non-prednisone-treated subjects (314). Although alternative mechanisms can be proposed, this result in humans is consistent with increased glucocorticoid tone causing ghrelin resistance by reducing the concentrations of GHS-R on target cells.

D. Ghrelin and inflammatory cytokines

The discovery of ghrelin precipitated a major interest in determining the physiological role of this new hormone. Perhaps the most exciting recent observation is that ghrelin activation of the GHS-R on T cells antagonizes production of
IL-6 (315). This has extraordinary significance to aging because IL-6 levels increase during aging and in diseases common in the elderly, whereas production of the normal counterregulatory hormones, the sex steroids, GH, and IGF-I, decline (3, 316–319). Exogenous administration of ghrelin appears to prove effective in models of endotoxic shock, congestive heart failure, and cancer cachexia, presumably by antagonizing the effects of inflammatory cytokines (320–328). In addition to having negative effects on CNS function, increases in the IL-6/IGF-I ratio is predictive of mortality in frail, elderly women (3, 316, 329, 330). Hence, treating frail elderly subjects chronically with ghrelin mimetics should improve their quality of life and reduce mortality by lowering IL-6 and increasing IGF-I production.

E. Ghrelin and the aging brain

By using ghrelin knockout mice as negative controls it was shown unambiguously that ghrelin is expressed in the brain (294, 331). Ghrelin was shown to improve memory retention when injected at different doses into the hippocampus, amygdala, and dorsal raphe nucleus (332, 333). Anxiogenesis was induced at the highest dose tested irrespective of the injection site, but at lower doses, the incidence of anxiogenesis was dependent on the dose and the site of injection. The different sensitivities of each brain structure suggest specific roles according to the particular behaviors studied and provide intriguing results regarding the functional role of extrahypothalamic ghrelin receptors in the brain.

An indirect neuroprotective effect of a ghrelin mimetic has also been reported (334). When adult male rats were treated with the ghrelin mimetic GHRP-6 or GH for 1 wk, IGF-I mRNA levels increased in the hypothalamus, cerebellum, and hippocampus (334). In these same brain centers, phosphorylation of Akt and Bax was stimulated without a change in MAPK or glycogen synthase kinase-3β; the antiapoptotic protein Bcl-2 was also augmented in these same areas, with no change in the proapoptotic protein Bax. This suggests that GH and the ghrelin mimetic activate phosphatidylinositol kinase intracellular pathways that are involved in cell survival. Indeed, this is reminiscent of intracellular signaling pathways used by IGF-I to mediate cell survival and neuroprotection.

VII. Aging and Metabolism

The earliest manifestations of aging are metabolic changes that result in increased fat deposition and reduced muscle mass, which lead to increased likelihood of developing “metabolic disease” (type II diabetes, hyperlipidemia, arteriosclerosis, and hypertension) (107, 108, 335). Increased fat deposition in young (5 months old), middle-aged (14 months old), and old (26 months old) male BN rats illustrating increased fat deposition during aging. [Reproduced with permission from (14), copyright 2000 from Elsevier Science.]

Normal aging is associated with a decrease in appetite and energy intake, which has been termed the anorexia of aging (339, 340). Generally, after age 70-75 yr, the reduction in energy intake exceeds energy expenditure, resulting in weight loss where loss of muscle (sarcopenia) predominates and predisposes older subjects to protein energy malnutrition (340, 341). The observed malnutrition and sarcopenia correlate with increased morbidity, mortality, and a number of hospitalizations with extended stays (342). The causes of the physiological anorexia typified during aging are unknown; they are probably multifactorial and include a reduction in feeding drive with increased activity of satiety signals.

Healthy elderly subjects apparently retain their sensitivity to the satiating effects of cholecystokinin (CCK) and have higher fasting and postprandial CCK concentrations than young adults (343, 344). Indeed, it has been reported that CCK concentrations are higher in undernourished elderly subjects compared with the healthy elderly (345). Although circulating ghrelin concentrations increase between early adulthood and middle age in humans, there is evidence that old age is associated with decreased ghrelin concentrations in rodents and in humans (311, 346). Therefore, enhanced effects of CCK and/or reduced effects of ghrelin may contribute to the development of anorexia and, in some cases, protein malnutrition during aging.

A. Aging, ghrelin, and energy balance

Ghrelin, which is mainly produced and secreted by the gastric mucosa, stimulates food intake as well as GH secretion (92, 347). It is possible that circulating ghrelin levels decline during aging because of impaired function of the gastric mucosa. Indeed, the thickness of the membrane, the length of the glands, and the number of the endocrine cells in the gastric mucosa decrease in animals between puberty and old age (348, 349). If indeed this mechanism is operative in old subjects, we must elucidate how peripheral and central components of ghrelin action are functionally interrelated.
The age-related decline of plasma ghrelin concentrations might be related to the anorexia often observed in aged subjects. However, before we can make definitive conclusions, much larger cohorts of subjects must be evaluated to support the finding that ghrelin decreases during aging.

We discussed previously that chronic treatment of elderly subjects with ghrelin mimetics restores the age-related decline in amplitude of GH pulsatility and circulating IGF-I to levels typical of young adults (25, 38, 279). These results suggest that during aging either ghrelin production declines or ghrelin resistance occurs. The orexigenic property of ghrelin coupled with its anabolic effects via the GH/IGF-I axis or ghrelin resistance occurs. The orexigenic property of ghrelin coupled with its anabolic effects via the GH/IGF-I axis and its inhibition of the production of inflammatory cytokines (315) indicate that rescue of reduced GHS-R activity by treatment with exogenous ghrelin or ghrelin mimetics may prove beneficial in the anorexia of aging.

B. Ghrelin production in CNS orexigenic centers

Ghrelin produced by A cells in the stomach appears to be an important peripheral orexigenic signal to the brain (350). By using a selective antibody for ghrelin and using ghrelin knockout mice as controls, the question of whether ghrelin was expressed in areas of the hypothalamus involved in regulating energy balance was addressed (294, 351). Ghrelin-immunoreactive cells were identified that fill the interstitial clear space between the lateral arcuate hypothalamus (LAH), ventral medial hypothalamus (VMH), dorsomedial hypothalamus, paraventricular nucleus (PVN), and the ependymal layer of the third ventricle. This unique distribution does not overlap with known hypothalamic cell populations, such as those that produce NPY, AGRP, POMC, melanin-concentrating hormone, orexin, dopamine, and somatostatin 8–14. These observations suggest specific roles for locally produced ghrelin in the CNS.

Immunoelectron microscopy showed that ghrelin is located in axons where it is associated with dense-cored vesicles in presynaptic terminals (294). These axon terminals innervate the arcuate nucleus, dorsomedial hypothalamus, LAH, PVN, and ghrelin boutons and appear to make synaptic contact with cell bodies, dendrites of NPY/AGRP, POMC neurons in the arcuate nucleus, and NPY and GABA axon terminals in the arcuate nucleus and PVN. Such interactions suggest a presynaptic mode of action for ghrelin in the hypothalamus. Some ghrelin axons in the PVN innervate CRH cells, which is consistent with the increase in ACTH and glucocorticoid secretion observed following treatment with ghrelin and its mimetics. These observations delineate an anatomical basis for pre- and postsynaptic interactions between ghrelin and NPY/AGRP, POMC, and CRH circuits.

Hypothalamic localization of the GHS-R was investigated in coronal slices of rat brain using biotin-labeled ghrelin (294). Binding of biotinylated ghrelin was observed in the arcuate nucleus, LAH, and PVN was mainly associated with presynaptic boutons. Axon terminals that bound ghrelin were frequently found to contain NPY. Together, the binding data and the localization of expression of ghrelin in axons adjacent to presynaptic nerve terminals support the notion that ghrelin modulates neurotransmission.

In summary, ghrelin is produced in the hypothalamus where it is localized to a previously uncharacterized group of neurons adjacent to the third ventricle between the dorsal, ventral, paraventricular, and arcuate hypothalamic nuclei (Fig. 6) (294). These neurons send efferents onto key hypothalamic circuits, which include those producing NPY, AGRP, POMC products, and CRH. In the hypothalamus, ghrelin binds mainly to presynaptic terminals of NPY neurons. Electrophysiological recordings showed that ghrelin stimulated the activity of arcuate NPY neurons and mimicked the effect of NPY in the PVN. We propose that at these sites release of ghrelin stimulates the release of orexigenic peptides and neurotransmitters, thus representing a novel regulatory circuit controlling energy homeostasis (Fig. 6).

The involvement of NPY/AGRP neurons was confirmed by Chen and colleagues (293, 295), who showed that like Ghsr knockout mice, AGRP/NPY double knockout mice were insensitive to the orexigenic effects of ghrelin.

C. Metabolism and changes in ghrelin activity during aging

One possible explanation for altered metabolism during aging is reduced ghrelin/GHS-R signaling caused by lower production of ghrelin. Rigamonti et al. (311) found that plasma ghrelin values in old subjects (67-91 yr, n = 7) of normal weight were similar to those of young (16-36 yr, n = 7) morbidly obese, but were markedly lower than in young adults (27-39 yr, n = 12) of normal weight. Therefore, because body mass index was within normal limits, an altered nutritional state was not implicated in the old subjects. The lower ghrelin levels in the old subjects were accompanied by increased insulin levels and low serum IGF-I. The former was a predicted compensatory mechanism for age-related insulin resistance to the orexigenic effects of ghrelin.
resistance, and the latter is consistent with age-dependent hyposomatotropism rather than malnutrition. Had the elderly subjects been malnourished, the low IGF-I level would have been coupled to high rather than low circulating levels of ghrelin as observed in anorexia nervosa.

Sturm et al. (352) evaluated healthy young and older women and undernourished older women. Plasma ghrelin concentrations (total active ghrelin and inactive des-octanoyl-ghrelin) were higher in undernourished older than in the well-nourished older and young subjects. Despite the fact that ghrelin stimulates appetite and food intake (92, 347), the highest circulating ghrelin concentrations were found in underweight, undernourished, older women (352). However, this does not preclude the possibility that ghrelin activity is reduced in the undernourished older subjects because of ghrelin resistance and/or increased ratio of des-octanoylated ghrelin/ghrelin. When ghrelin concentrations were compared in well-nourished young and old women, they were found to be 20% lower in older women (352). Although this difference was not statistically significant, another study evaluated a similar number of well-nourished young and old men and women and found that plasma ghrelin concentrations were significantly (~35%) lower in older subjects (311). A caveat is that although these studies suggest ghrelin production declines during adult aging, the assays used did not discriminate active ghrelin from desacyl-ghrelin.

D. Leptin, metabolism, and aging

Leptin, through its action on the hypothalamus, regulates food intake and metabolism (353–355). Mutations identified in the leptin gene of rodents and humans are associated with altered metabolism and obesity (356). Secretion of leptin is subject to ultradian pulsatile rhythmicity, although the episodic profile is not as distinct as that illustrated by pituitary hormones. However, the pulsatile pattern becomes more organized at night, where fluctuations become synchronous with those of LH and estradiol (355).

In contrast to the reproductive hormones, variations in circadian and ultradian rhythms of leptin are inversely related to ACTH and cortisol rhythms (357, 358). In vitro studies have shown that leptin regulates biosynthesis of TSH-releasing hormone, and recent studies on the synchrony of circadian/ultradian rhythms of TSH suggest that leptin also regulates TSH oscillations (359). Clearly, the compelling data in support of such a relationship do not preclude the possibility that a common pulse generator in the hypothalamus controls both leptin and TSH rhythms. The collective findings imply a permissive role for leptin in linking nutritional status and pulsatile activity of the hypothalamic-pituitary peripheral axis, but they do not prove causality.

Leptin decreases food intake and increases energy expenditure in rodents by inhibiting neuropeptides in the hypothalamic arcuate nucleus (360). Ghrelin stimulates appetite, and its receptor (GHS-R), like the leptin receptor (Ob-Rb), is expressed in the arcuate nucleus. Ghrelin induces activation of c-fos expression in the arcuate nucleus, and 57% percent of these cells stain positive for Ob-Rb. Electrophysiology studies on hypothalamic slices show that ghrelin dose-dependently stimulates the electrical activity of these cells. Leptin is inhibitory, and ghrelin increases the electrical activity in 76% of all cells that are inhibited by leptin (360). These results show that ghrelin interacts with the leptin hypothalamic network in the arcuate nucleus and illustrate that ghrelin and leptin serve as mutual functional antagonists. Hence, ghrelin resistance can potentially be induced by increased activity of leptin and leptin-receptor in hypothalamic neurons.

E. Leptin resistance and aging

Animal models of aging have been used to investigate changes in leptin sensitivity. In rats, leptin administration selectively decreases VF by approximately 60% and inhibits hepatic glucose production by approximately 80%. Surgical removal of VF improves hepatic insulin action and decreases leptin and TNF-α gene expression in sc adipose tissue (107). Therefore, the relationship between the age-related increase in VF and increased insulin resistance may involve the failure of centrally acting leptin to regulate fat distribution.

Manipulation of serum leptin levels by fasting causes hypothalamic NPY mRNA to increase in young but not in old rats (361). Leptin infusion (7 d) reduces food consumption and hypothalamic NPY concentrations by 50% in young rats; however, in old rats, food consumption is reduced by only 20% and NPY is unaffected (362). A comparison of pair-fed rats with infused with saline or leptin showed that leptin caused a 24% increase in oxygen consumption in young rats but produced no change in oxygen consumption in old rats. These results support the conclusion that aged rats are less responsive to leptin because of impaired suppression of hypothalamic NPY synthesis.

The age-related altered response to leptin has also been investigated in Zucker diabetic fatty rats, where leptin was delivered by adenovirus-mediated leptin gene transfer (361). Leptin caused markedly different responses in old (18 months old) compared with young rats (2 months old). For example, free fatty acid and triacylglycerol levels fell precipitously in the young rats but were unaffected in the old animals. Although leptin reduced food intake, body weight, and fat deposition in old rats, the effects were less pronounced than in young animals. Similarly, important metabolic markers, such as acyl coenzyme A oxidase, carnitine palmitoyl transferase-1, and peroxisome proliferator receptor α markedly increased in response to leptin in young rats but not in old rats, confirming that the beneficial metabolic effect of leptin is attenuated during aging. The mechanism of age-dependent leptin resistance is unknown. However, one possibility is that leptin receptor signaling is attenuated because of an age-dependent increase in the expression of suppressor of cytokine signaling-3 (SOCS-3) (361).

In addition to aging, leptin resistance accompanies obesity and in most cases insulin resistance. In nonobese animals, both insulin and leptin act on the hypothalamic to inhibit feeding behavior. If the anorexic action of leptin is dependent on normal insulin signaling, insulin resistance would also present as leptin resistance. To test this hypothesis, Matsu-moto et al. (14) chronically administered the insulin sensitizer troglitazone (a peroxisome proliferator-activated receptor γ agonist) to old BN male rats. Troglitazone reduced their high insulin, high leptin, and high body fat; furthermore, their
body weight gain in response to fasting was corrected (14). Interestingly, restoration of this metabolic phenotype did not alter NPY gene expression in the arcuate nucleus. These results provide an important link between insulin and leptin resistance that apparently contributes to impairments in energy and weight regulation. Important questions must now be addressed: 1) is the mechanism independent of improved insulin sensitivity; 2) is normalization of leptin action mediated by cross talk between the insulin and leptin receptor signal transduction pathways; or 3) by improving insulin sensitivity, do asynchronous interdependent pathways essential for optimizing the biological responses to leptin become resynchronized? Clearly, additional studies are necessary to establish the mechanism of the apparent link among insulin, leptin resistance, and aging.

VIII. Hypothalamic-Pituitary-Gonadal Axis and Aging

A. NPY and GnRH

The neuroendocrine axis plays a major role in the reproductive aging of female rats. In particular, hypothalamic NPY neurosecretion appears crucial for the preovulatory LH surge in young rats (363-367). To investigate the possibility of age-related changes, NPY mRNA was quantitated by ribonuclease protection assays in microdissected hypothalamic nuclei from young (2.5-3 months old) and middle aged (7-9 months old) regularly cycling rats (368). In contrast to young rats, where prepro-NPY mRNA levels increased before and during a robust LH surge, mRNA levels in the middle-aged rats remained unchanged. Because hypothalamic NPY participates in GnRH release, the attenuated GnRH and LH surges in middle-aged rats might be a result of reduced NPY secretion.

To address the relevance of a relationship among NPY, GnRH and reproductive function, the reproductive endocrinology of NPY knockout and wild-type mice were compared (369, 370). Under basal conditions and after ovariectomy, reproductive hormone levels were identical in both genotypes. However, during proestrus, LH levels were 66% lower in NPY knockout mice; similarly, when estradiol was administered to stimulate an LH surge in ovariectomized mice, the magnitude of LH release was 50% lower in NPY knockout mice compared with wild-type mice; the NPY-null mice were also less responsive to exogenous GnRH. Although these results show that NPY is not essential for regulation of basal gonadotropin secretion, they suggest that preovulatory release of NPY is important for amplifying the LH surge. However, despite an attenuated LH response, timing of vaginal opening, pregnancy rate, litter size, and gender ratio of pups are no different in NPY-null mice and wild-type mice. This apparent paradox might be related to environmental factors, because in contrast to a native environment, under laboratory housing conditions where the metabolic energy for reproduction is not limiting, perhaps the LH threshold for triggering ovulation is lower (370).

In male rats, the age-related decline in pituitary-testicular function is consistent with a gradual decrease in NPY release that provides an excitatory signal to GnRH-expressing neurons (371). Evidently, NPY amplifies the stimulatory effects of GnRH on gonadotrophs (372). Indeed, a feed-forward role for testosterone is implied by studies showing that the decline is a result of an age-related refractoriness of NPY producing neurons to testosterone (371).

Ghrelin may also regulate GnRH function during aging through its effect on the NPY pathway. Ghrelin signaling declines as a function of age in rats and humans, and ghrelin mimetics activate a subset of NPY neurons in the arcuate nucleus that project outside the blood-brain barrier (373); NPY amplifies the stimulatory effects of GnRH on gonadotrophs in the anterior pituitary gland (374). Hence, a link among ghrelin, NPY, and GnRH is tantalizing because it suggests that, in addition to their rejuvenating effects on the GH/IGF-I axis, ghrelin mimetics may play a regulatory role on the reproductive axis by increasing secretion of NPY into the hypothalamic portal vessels.

B. Evidence that CNS changes likely precede ovarian changes in the onset of menopause

The onset of menopause correlates with changes in biological rhythms and alterations in CNS function associated with reduced exposure to estradiol (29, 67, 375, 376). Wise et al. (29) proposed that changes in the CNS likely precede changes in ovarian function during perimenopause. Prior (377) also reviewed the complex endocrinology associated with perimenopause and hypothesized that a reduction in inhibin production causes increases in FSH secretion and excessive follicular stimulation. Aging of the reproductive system correlates with changes in the pattern of GnRH secretion and changes in neurotransmitters and neuropeptides in the hypothalamic regions involved in regulating GnRH neuronal activity; such changes are perhaps a consequence of age-dependent altered behavioral complexity of the regulatory neurons as discussed in Section II.

The evidence to support the notion that aging is associated with dynamic changes in the hypothalamic and pituitary components of the reproductive axis, which are independent of changes in gonadal hormone secretion, is accumulating. Estradiol induction of a gonadotropin surge is less effective in perimenopausal women, which suggests that alterations in the hypothalamic-pituitary axis precede the loss of regular cyclicity. A change in the pulsatile pattern of LH secretion is apparent during the pre- and perimenopausal periods, implying that the GnRH pulse generator and/or the pattern of the release of neurotransmitters is altered early during the menopausal transition. Such a change is associated with acceleration of follicular depletion in the decade before menopause, which can be explained either by increased atresia of resting follicles or by increased numbers of resting follicles entering the growth phase (378, 379). After the age of 30 years, the latter prevails and is consistent with an age-related change in the central control of FSH secretion and the signals that modify local control of follicular development during the perimenopausal state (380). As suggested by Wise and others, if the increase in the rate of follicular depletion was prevented, follicular reserve would not be exhausted until later in life. Therefore, appropriate intervention would delay the onset of menopause, avoid the need for pharmacological...
hormone replacement, and have obvious benefits for women regarding osteoporosis, cognition, memory, mood, and cardiovascular function.

Aging of the hypothalamic-pituitary-gonadal axis has been investigated by comparing the effects of estrogen replacement on the hypothalamic-pituitary axis in young women (25-40 yr old) having well-defined idiopathic premature ovarian failure with postmenopausal women (51-70 yr old) (381). Estradiol was replaced transdermally and dosed to produce a serum concentration of approximately 100 pg/ml. Blood was sampled 10 min before and after estradiol replacement. Before estrogen replacement, the young women with premature ovarian failure had higher 24-hour mean LH concentrations than women with age-appropriate menopause. Despite lower serum LH in the older group, LH pulse frequency was virtually identical. Estrogen replacement caused LH levels, LH pulse amplitude, and LH pulse frequency to decrease in both groups of women, but a greater reduction in pulse frequency was evident in the postmenopausal group. Estradiol-dependent changes in FSH levels were also evident. The more pronounced LH and FSH responses to estradiol in women over the age of 50 yr support the notion that age-associated decreases in gonadotropin secretion are related to alterations in the hypothalamic-pituitary axis rather than being explained solely by changes in the ovary (381).

The effect of age on GnRH pulse frequency, in the absence of gonadal feedback, was evaluated in women (17). Gonadotropin free a-subunit (FAS) and LH were used as neuroendocrine markers of endogenous GnRH secretion in healthy, euthyroid postmenopausal women. To assess interval and duration of pulse frequency, blood samples were collected at 5-min intervals for 12 h. To determine whether frequency and amplitude of pulsatile hormone secretion were altered during aging, the results from younger (45-55 yr old) and older (70-80 yr old) postmenopausal women were compared. Young postmenopausal women had higher gonadotropin levels and higher FAS pulse frequency and amplitude than older postmenopausal women, but estrone and estradiol levels were the same in both groups. Therefore, it appeared that the marked age-dependent decrease in FAS pulse frequency was independent of gonadal function and was likely caused by age-related changes in the hypothalamic component of the reproductive axis.

Although disagreement arose as to whether a decrease in LH pulse frequency occurs during aging (17), the issue was resolved by assaying blood samples collected at 5-min rather than at 10-min intervals. This more frequent sampling provided improved resolution and was particularly important in the elderly population. Remarkably, LH clearance rate is 2.5-fold higher in postmenopausal than in premenopausal women (382). Furthermore, although LH frequency has been used as a marker of GnRH secretion, monitoring of FAS levels at 5-min intervals provides a more accurate estimate because the half-life of FAS does not change and the pulses are more clearly defined (17).

The expression of subtype-specific NMDARs as a function of age and reproductive status was compared with investigate the relationship between GnRH neurons and NMDARs in rats (383). The GnRH perikarya and neuroterminals are located in the preoptic area (POA)-anterior hypothalamus and medial basal hypothalamus (MBH), respectively. In the POA-anterior hypothalamus, NMDAR1 mRNA was generally unaffected by age or reproductive cycling, whereas NMDAR2a and NMDAR2b were lower in acyclic animals. During aging, expression of NMDAR subtype genes increased in the MBH. Administration of N-methyl-D,L-aspartate increased GnRH mRNA in young rats but decreased GnRH mRNA in middle-aged animals. Thus, the subunit composition of the NMDAR changes during reproductive aging and appears to be associated with a switch from a stimulatory to an inhibitory effect on GnRH gene expression.

During aging, progressive disintegration of several regulatory components controlling GnRH secretion contributes to diminished function of the hypothalamic-pituitary-gonadal axes. For example, the influence of the inhibitory opioids linked to a neuronal clock that is important for the preovulatory LH surge in young rats appears to be lost during aging (371). The hypothalami of young and old rats release about the same amounts of GnRH in vitro in response to high K+ concentrations; therefore, the ultrashort feedback loop regulated by GnRH appears to function similarly. However, in the anterior pituitary gland the concentration of GnRH receptors is inversely correlated with age and must be considered a factor that contributes to reduced levels of serum gonadotropins.

Catecholamines are important regulators of reproductive function. Norepinephrine is essential for the hypothalamic release of GnRH and for maintaining normal reproductive cycles in young female rats (384). To investigate the possible regulatory role of estradiol, norepinephrine was measured by microdialysis in the brain of rhesus macaques following estradiol treatment (385). Infusion of estradiol to mimic the preovulatory surge of estradiol caused an increase in epinephrine in hypothalamic dialysates (385). When norepinephrine and dopamine activities were monitored in rats from adulthood through middle age to senescence, the reductions in catecholamines associated with old age were preceded by a transitory increase of norepinephrine in middle age. The cyclic increase in norepinephrine activity associated with the LH surge begins to diminish during middle age and disappears completely in old age to coincide with cessation of estrous cycles. Reinduction of cycling is possible by treating old rats with the drug deprenyl. Deprenyl is speculated to restore estrous cycles by increasing dopamine and epinephrine production and by reducing serum prolactin levels (386).

C. Estradiol receptors in the CNS

The significance of ovarian steroids and their receptors in areas of the CNS that are not directly associated with reproductive function, such as hippocampus, basal forebrain, midbrain raphe, caudate putamen, and brainstem locus coeruleus, has been reviewed (140, 376). Localization of estrogen receptors (ERs) in these areas is particularly relevant to the relationships of hormonal changes during aging that affect memory processes and neurodegeneration. In the CNS estradiol is involved in neuroprotection, neuroendocrine regulation, and reproductive behavior. Autoradiographic stud-
ies show an abundance of neurons that bind $^3$H-estradiol in rat neocortex, hippocampus, POA, and spinal cord. Immunohistochemistry using selective antibodies against ERα and ERβ reveal colocalization of ERα and ERβ in approximately half of the neurons in cultures from neocortex and hippocampus (387). However, in the POA and spinal cord, relatively little double-staining is observed.

Selective estrogen receptor modulators (SERMs) bind to the nuclear ER, but according to cell type, a SERM may function as agonist or antagonist (388). If estradiol replacement provides benefits toward CNS function in postmenopausal women, as it appears to do in rodents, it is important to establish whether a SERM behaves as an ER agonist or antagonist in the brain. A recent study compared the effects of two SERMs, raloxifene and tamoxifen, with estradiol benzoate on choline acetyltransferase (ChAT) activity in the brain of ovariectomized Sprague-Dawley rats (389). Raloxifene, tamoxifen, and estradiol benzoate reversed the decrease in ChAT activity in the hippocampus that resulted from ovariectomy but had no effect on ChAT activity in the hypothalamus. Despite their similar properties in the hippocampus, in the uterus raloxifene behaved as an antagonist and tamoxifen as an agonist. Hence, in ovariectomized rats, both SERMS provide benefits in cholinergic neurotransmission in the hippocampus, whereas they exhibit differential effects according to structure on the uterus.

The activity of SERMs on 5-HT pathways must also be considered. Serotonergic mechanisms play an important role in depressive illness, and declining estradiol levels during menopause have been associated with increased incidence of depression. Estrogen treatment appears to be effective in treating mood disorders in postmenopausal women by modifying expression of genes involved in serotonergic neurotransmission. However, the effectiveness of estradiol compared with different SERMs was largely unknown. Zhou et al. (390) compared the effects of six SERMS (tamoxifen, raloxifene, levormeloxifene, NNC 45-0781, NNC 45-0320, NNC 45-1506) and estradiol on the regulation of mRNA encoding ERα, ERβ, 5-HT1A, and 5-HT reuptake transporter (SERT) in midbrain, amygdala, and hypothalamus of ovariectomized rats. The study showed that none of the SERMS precisely mimicked estradiol action in the rat brain and each SERM produced unique transcriptional activity in the different brain areas. For example, tamoxifen increased ERβ mRNA in the hypothalamus, whereas raloxifene increased ERα mRNA in the amygdala. Estradiol decreased SERT mRNA in the midbrain but had no effect on 5-HT1A mRNA expression in midbrain, hypothalamus, or amygdala. None of the SERMS had significant effects on 5-HT1A or SERT mRNA expression in the brain areas investigated. These results suggest that SERMS could be tailored to target specific estrogen actions in the brain, which would allow development of selective compounds to fine-tune estrogen replacement in the postmenopausal woman.

Sex steroids also mediate their potent effects on mood and mental state by 5-HT action on the 5-HT2A receptor. Following castration of male Wistar rats, 5-HT2A expression declines (391). Treatment with either testosterone or estradiol restores 5-HT2A mRNA levels in dorsal raphe nucleus and increases 5-HT2A binding sites in frontal, cingulate, primary olfactory cortex, and the nucleus accumbens. In the caudate putamen, by contrast to estradiol, neither 5α-dihydrotestosterone nor testosterone increases 5-HT2A binding sites. Aromatase activity is scarce in the caudate putamen; therefore, it is concluded that expression of 5-HT2A is regulated by estradiol. Hence, declining production of estradiol and testosterone during aging has differential effects on the regulatory role of 5-HT and 5-HT2A in different areas of the CNS.

D. Estradiol and POMC

The POMC-derived neuropeptide, β-endorphin, is believed to be important for maintaining normal patterns of LH secretion. To investigate whether the expression of POMC changes during aging and whether it is related to reproductive function, POMC mRNA levels were compared in the periaqueductal region of young (3-4 months), middle-aged (10-12 months), and old (17-19 months) ovariectomized rats (392). POMC mRNA in middle-aged rats was 20-30% lower than in young rats. No further decline was evident in the older animals. Interestingly, the decline in POMC gene expression and a 30-40% decline in the number of cells expressing POMC mRNA in middle-aged and old animals occurred irrespective of their reproductive status before ovariectomy, which indicates that the age-dependent decline in POMC gene expression is independent of reproductive status (392). Similar age-related decreases in POMC mRNA levels are observed in male rats (393).

Aging affects the biological rhythms of POMC expression (394). Estradiol treatment of young rats produces a diurnal rhythm and suppresses POMC expression. By contrast, in middle-aged and old rats, the rhythm and the ability to suppress POMC expression are abolished. Importantly, age-related changes in serum levels of LH, prolactin, and corticosterone do not correlate with changes in POMC expression, illustrating that age-associated changes in pituitary hormone secretion are not determined by alterations in hypothalamic POMC expression. Rather, the data implicate age-dependent changes in biological rhythms or complexity of neuronal behavior as differential regulators of POMC expression, and of LH, prolactin, and corticosterone secretion.

Older women exhibit marked changes in neuronal morphology and neuropeptide gene expression in the MBH (395). There is hypertrophy of substance P and neuropeptide B-containing neurons but a reduced number of neurons expressing POMC mRNA. Indeed, the number of POMC mRNA-containing neurons/hypothalamic sagittal section detected in the infundibular nucleus by in situ hybridization is 65% lower in postmenopausal than in premenopausal women (396). In a subpopulation of neurons in the MBH, GnRH expression is increased. Stereological methods showed that neuronal hypertrophy in postmenopausal women is not accompanied by degeneration of the arcuate nucleus; therefore, the loss of rhythmicity of the reproductive cycle is not explained by neuronal loss within the hypothalamus.

E. Estradiol and synaptic communication

Estradiol has marked effects on synaptic communication in the hippocampal neurons involved in cognitive processing.
Therefore, estradiol probably reduces complexity of neuronal behavior similar to that discussed in Section II. Hence, reduced estradiol production during menopause is likely to reduce cognitive function. Indeed, in postmenopausal women, estrogen replacement improves verbal memory and the ability to make new associations (398). Most studies of the effects of estrogen withdrawal and replacement in the CNS of rats (67, 399, 400) were conducted in young animals. Therefore, it is important to recognize that young and old rats respond differently to estrogen withdrawal. Dendritic spines on granule cells in the DG show a decline in aged rats with long-term, high- or low-dose estrogen replacement (401). However, with short-term estrogen replacement, spine density increased to levels typical of young adults. Hence, the effect of estrogen on spine density in dentate granule cells is dependent on the temporal pattern of replacement (401).

F. Estrogen and neurodegeneration

The decline in estradiol levels during menopause is associated with increased neurodegeneration. A recent review describes the role of estrogen as a neuroprotective hormone (402). The various mechanisms by which estradiol might inhibit neurodegeneration of mesencephalic dopaminergic neurons have also been reviewed (98). These pathways include the antioxidant properties of estradiol, which appears to be dependent on its phenolic A ring (403), attenuation of glutamate-induced Ca\(^{2+}\) entry through Ca\(^{2+}\) channels, and cross talk between estrogen and neurotropic factors such as NGF, BDNF, and glial-derived neurotropic factor.

Interestingly, a nonclassical ER associated with the plasma membrane apparently mediates estradiol’s neuroprotective effect on glutamate toxicity, because the beneficial effect was not antagonized by tamoxifen (404). In ovariectomized mice, estradiol protects dopamine neurons from methamphetamine-induced toxicity, but this effect was antagonized by tamoxifen, which is consistent with the involvement of a classical ER (405). If translatable to humans, the antagonism of the neuroprotective effect of estradiol on the nigrostriatal dopaminergic system by tamoxifen has important implications relating to gender differences observed in Parkinson’s disease. Furthermore, this CNS effect is an important issue for consideration before proposing use of tamoxifen in premenopausal women who might be at risk for breast cancer.

More recently, tamoxifen was evaluated an antagonist of estradiol’s neuroprotective properties in intact mice. In contrast to the results in ovariectomized mice, tamoxifen was protective against methamphetamine-induced nigrostriatal neurotoxicity (406). Despite the different experimental protocols, it is difficult to explain the opposing results as they relate to tamoxifen. Because of the clinical significance of these discrepancies concerning the mechanism of estradiol-mediated neuroprotection, particularly as it relates to the classical nuclear ER vs. non-classical plasma membrane associated ERs, further investigation is needed.

In addition to controlling axonal sprouting and enhancing synaptic transmission, estradiol’s neuroprotective role appears to be mediated by increasing cell survival and regeneration. Retrospective studies suggest a correlation between dose and duration of estrogen replacement therapy with beneficial effects on memory. The protective effects of estradiol replacement on stroke-related injury was evaluated in young ovariectomized rats (3-4 month old) and middle aged (9-12 months) ovariectomized rats treated with estradiol for 1 wk before middle cerebral artery occlusion (407). Infarct volume was measured 24 h after middle cerebral artery occlusion and showed that estradiol replacement reduced ischemic injury by 50%. Most importantly, estradiol was protective at both low and high physiological doses irrespective of the age of the rats; therefore, the neuroprotective pathway is retained in old animals (407).

Neuroprotection in the cerebral cortex is apparently mediated by ERs, because estradiol is not protective in the ERα knockout mouse (407). A putative estrogen response element is present in the bcl-x gene, and in cultured hippocampal neurons estradiol increases expression of the antiapoptotic protein Bcl-xL (408). Co-localization of ER and Bcl-xL immunoreactivities is most prominent in hippocampal subfield CA3. These data suggest that estrogen may act as a neuroprotective agent by inhibiting apoptosis of hippocampal neurons. Indeed, long-term treatment with estrogen at physiological doses prevents apoptosis and ischemia-induced injury to CA1 hippocampal neurons (409). The potential clinical significance of these results argues that extensive early intervention studies for the prevention of age-related neurodegeneration with estradiol and its precursor testosterone should be conducted with high priority in both men and women.

G. Estrogen in learning and memory

To evaluate the benefits of estrogen replacement on reducing the age-related increased incidence of learning and memory deficits, a multiple-trial passive avoidance paradigm was used that allowed effects on acquisition to be distinguished from effects on retention (410). To introduce deficits in learning and memory, rats were treated with the muscarinic antagonist scopolamine and with lorazepam. Estradiol replacement attenuated the scopolamine-induced deficit on passive avoidance acquisition; surprisingly, this beneficial effect was only observed at serum estradiol levels less than 200 pg/ml. Estradiol was also protective against lorazepam-induced impairments in passive avoidance retention (410).

A consequence of reduced estradiol production during aging is that neuronal function becomes suboptimal. In ovariectomy models, the reduced production of estradiol produces a deficit in passive avoidance behavior (411). When performance in the Morris water maze was used as a measure of spatial memory, ovariectomized rats showed normal spatial learning but were deficient in spatial memory; this deficit was prevented by treating the ovariectomized rats with estradiol. Estradiol replacement increased the synthesis of BDNF and NGF and prevented the decrease in high-affinity choline uptake and ChAT activity in the hippocampus and frontal cortex. Both BDNF and NGF are neuroprotective of cholinergic neurons, which provides a potential mechanism for the neuroprotective role of estradiol and supports a role for estradiol replacement during menopause.
The effects of aging and gonadectomy on genes regulating the function of basal forebrain cholinergic neurons projecting to the hippocampus and cortex were evaluated (412). A decrease in high affinity NGF receptor mRNA, which encodes the high-affinity NGF receptor, was detected in the medial septum (MS) of intact females but not in males aged 13 to 25 months. Aging had no effect on ChAT mRNA expression. In ovariectomized rats, decreases in both ChAT and TrkA mRNA in the MS and nucleus basalis magnocellularis (NBM) was evident at 6 months, but not at 3 months after ovariectomy. Six months after ovariectomy, treatment with estrogen for 3 d partially restored ChAT mRNA levels in the MS and TrkA mRNA levels in the NBM. The reduction in ChAT mRNA 6 months after ovariectomy is consistent with long-term loss of ovarian function, causing deficiencies in basal forebrain cholinergic neurons. Furthermore, the age-related decrease in trkA mRNA in the MS of intact females and the decrease in the MS and NBM after ovariectomy predict decreased responsiveness of the cholinergic neurons to endogenous NGF. Therefore, long-term loss of ovarian function in rats exacerbates the effects of aging and has negative impact on the function of basal forebrain cholinergic neurons.

H. Estradiol, galanin, and cognition

A coexistence of ERα, ERβ, and cholinergic, muscarinic, or nicotinic sites occurs in many neurons of the neocortex and hippocampus (387). Autoradiography and immunohistochemistry also illustrate colocalization of ER with receptors for the neuropeptide galanin (387). The coexpression of estrogen, cholinergic, and galanin receptors on the neurons has important relevance to age-related neurodegeneration, particularly in postmenopausal women.

Estradiol regulates galanin gene expression in GnRH neurons (413). Consistent with this, a comparison of galanin expression in a subset of GnRH neurons in the medial POA and the diagonal band of Broca of male and female rats showed that expression was four to five times higher in female rats. When galanin expression was compared in 2-, 10-, 18-, and 24-month-old Fischer rats, the number of galanin/GnRH-coexpressing cells markedly declined as a function of age and was undetectable in GnRH neurons of 24-month-old female rats. Interestingly, although galanin expression was absent in the GnRH neurons of these old female rats, their GnRH content was unaltered, and estradiol treatment was ineffective in modifying the low incidence of colocalization with galanin. By contrast, estradiol treatment of 24-month-old ovariectomized rats increased the incidence of coexpression to that observed in young cycling animals. Remarkably, these results show that although galanin expression in GnRH neurons declines during aging and that estradiol stimulates galanin expression in GnRH neurons of 24-month-old ovariectomized rats, the positive response to estradiol is somehow prevented in 24-month-old intact rats. Hence, estradiol is capable of inducing galanin gene expression in GnRH neurons, but in aged rats the ovary appears to produce an inhibitory factor.

The distribution of galanin-specific binding sites was measured in brain sections from young (3-4 months old), middle-aged (14-15 months old), and aged (26-27 months old) male Sprague-Dawley rats in an attempt to correlate distribution with performance in the Morris water maze task (414). Increased binding observed in the piriform and entorhinal cortex, ventral subiculum, and dorsal DG of the aged animals correlated with impaired performance in the Morris water maze. Although increased binding of exogenous galanin could be interpreted as an increase in receptor density, it more likely reflects reduced galanin receptor occupancy as a consequence of reduced production/secretion of endogenous galanin.

Galanin colocalizes with acetyltransferase in a subset of basal forebrain neurons that mainly project to the hippocampus and with ACh in the majority of neurons of the rat medial septal nucleus and the nucleus of the diagonal band of Broca. During aging the galanin-positive cells progressively decrease (415). Galanin cell loss in the medial septal area is associated with parallel but smaller cholinergic cell loss. The significance of galanin expression in these particular neurons is exemplified by the fact that galanin-null mice have one third fewer cholinergic neurons in the MS and vertical limb diagonal band of the basal forebrain; this is associated with increased apoptosis on postnatal d 7 and an age-related deficit in ACh release. At 4 months of age, galanin-null and wild-type mice exhibit identical performances in the Morris water maze; however, performance deficits become evident in the former at 10 months of age. Interestingly, the phenotype of galanin-null mice, as illustrated by a deficiency of cholinergic neurons in the basal forebrain, is similar to that seen in NGF receptor TrkA-null mice (416) and is consistent with the demonstration that icv infusion of NGF increases galanin expression in forebrain neurons that coexpress galanin and ChAT. Hence, NGF and galanin apparently function together to control both cholinergic survival and function.

The results described above suggest that declining galanin production partially explains the age-related decline in spatial memory. However, based on pharmacological evidence, this conclusion appears invalid. For example, centrally administered galanin produces performance deficits in tests of memory and learning (417–419). Galanin infused in the ventral, but not the dorsal, hippocampus impairs spatial learning and reduces basal ACh release. One explanation for the paradoxical findings is that different galanin receptor subtypes are involved. Indeed, galanin receptor subtype-1 (GAL-R1) is expressed in the ventral hippocampus, whereas GAL-R2 expression is more evident in the basal hippocampus, especially in the granular cell layer of the DG (420). Galanin’s inhibitory effects on ACh release, cognition, and LTP can only be blocked by galanin antagonists when galanin is administered exogenously (421–423), which suggests that endogenous galanin does not modulate ACh release under steady-state conditions. However, interpretation is confounded because the galanin antagonists M40 and M15 possess weak agonist activity (424–426). These experiments emphasize the need for caution when interpreting data derived from pharmacology studies alone.

Another interpretation of the above results is that endogenous galanin is not released under basal conditions. Electrical excitation of the basal forebrain stimulates galanin release from the hippocampus (427); therefore, galanin may
play a protective role when neuronal firing rates are highest because of injury or anoxic damage (428). Under these conditions, galanin might act as a trophic factor and as an inhibitor of the release of excitatory amino acids (428, 429). Collectively, the experimental findings support the notion that decreased expression of galanin in cholinergic neurons projecting to the hippocampus potentially contributes to the onset of age-related deficiencies in spatial memory. It remains to be determined whether the age-related changes result from changes in production of sex steroids, and whether estradiol, in addition to regulating galanin expression in GnRH neurons, stimulates galanin expression in cholinergic neurons of the basal forebrain.

I. Estradiol and AD

Epidemiological studies indicate that estrogen replacement therapy might be protective against AD in postmenopausal women (430). A central factor implicated in the pathophysiology of AD is processing of amyloid precursor protein (APP) to produce the plaque-forming Aβ peptides. Full-length human APP undergoes proteolytic cleavage, either within the Aβ domain to produce secreted APPα peptide or at the N- and C-terminal domain(s) to generate the Aβ peptides. Estradiol appears to modulate the metabolism of APP in vitro, resulting in a decrease in brain-derived Aβ peptides (431–434). Recent studies (435) in guinea pigs showed that ovariectomy caused a 1.5-fold increase in Aβ peptides, and estradiol treatment reversed the effect of ovariectomy on Aβ levels. These results are consistent with the idea that low estradiol production, typical of postmenopausal women, facilitates production of Aβ peptides at the expense of alpha cleavage in the brain.

The perceived protective effect of estrogen in delaying the onset of AD in postmenopausal women might involve multiple in vivo activities of estrogen. For example, it has been discussed above that estradiol plays an important role in the plasticity of maintaining neuronal connections. As discussed in Section II, reduced interactions between neurons can reduce complexity of neuronal behavior, which, according to complexity theory, would produce a system that is more susceptible to disruption. Indeed, electroencephalograms recorded from AD patients who were required to rest with eyes open and closed and while performing mental arithmetic, showed reduced complexity as a function of disease severity (436–438).

Estradiol appears to be neuroprotective in familial AD and in AD associated with Down’s syndrome. In primary cortical neurons carrying the APP Swedish mutation, estradiol increases the ratio of secreted APPα/APPβ (439). However, coculturing neurons with astrocytic cells or addition of astrocyte-conditioned medium prevents the estrogen-induced increase in APPα. Hence, secreted factors from astrocytes apparently antagonize the benefits of estradiol on producing APPα. Physiological doses of estradiol attenuate endogenous Aβ production in primary cortical neurons; furthermore, in N2a cells and rat primary cerebrocortical neurons, testosterone increases the secretion of APPα, and decreases secretion of Aβ peptides (433). These results suggest that replacing testosterone and estradiol during aging might be beneficial in delaying or preventing onset of AD.

The manifestation of accelerated aging in Down’s syndrome provides a link between onset and progression of AD and estrogen deficiency. In females with Down’s syndrome, the average age of menopause (44.7 yr; n = 42) is younger than in the general population, and onset of dementia correlates with the age of menopause (440). The results of a multicenter clinical trial in women with established AD showed that estrogen treatment for 12 months did not halt the decline in cognitive function (441), but the average age of the women studied was 75 yr; and it remains to be determined whether younger women would benefit. Indeed, the failure of estrogen to inhibit progression of the disease suggests that estrogen is neuroprotective rather than restorative. However, by inhibiting Aβ peptide production, Aβ-mediated irreversible neurotoxicity should be reduced.

J. Estradiol and inflammatory responses in the CNS

Gliaal cell function and inflammatory responses are affected by changes in steroid hormone production and are therefore important factors involved in aging of the CNS (442). To evaluate the role of estrogen, primary cultures of rat microglia and N9 microglial cell lines were treated with increasing doses of estradiol, either before or during stimulation by phorbol ester, lipopolysaccharide, or interferon-γ (443). Estradiol attenuated microglial superoxide release, phagocytic activity, and inducible NOS production. Estradiol also induced phosphorylation of p42/p44 MAPK, and the MAPK inhibitor, PD98059, blocked the anti-inflammatory properties of estradiol. Consistent with a mechanism involving the nuclear ER, the antiestrogen, ICI 182,780, inhibited the anti-inflammatory properties of 17β-estradiol. Hence, in microglial cells, the age-related reduction in estradiol production results in reduced activation of a MAPK pathway that is normally neuroprotective.

A recent review (3) discusses the association of increased IL-6 expression to age-associated disorders. This tightly regulated proinflammatory cytokine is expressed at low levels except during infection, trauma, or stress (3, 329). An age-associated rise in IL-6 is linked to lymphoproliferative disorders, multiple myeloma, osteoporosis, and AD (3). Furthermore, overexpression of IL-6 in the brain establishes a state permissive for the onset of neurodegenerative disease (316, 444, 445). Although IL-6 levels do not appear to change in the hypothalamus, concentrations of IL-6 in the cerebellum, cerebral cortex, and hippocampus of mice increase with age (316).

A comparison of IL-6 production in glial cells cultured from brains of neonate, adult, and aged mice showed age-dependent increases in IL-6 (316). More microglia and the proportion of microglia positive for IL-6 expression function (446–448). These changes in IL-6 expression provide a further link among the andropause, menopause, and increased
production of factors associated with age-related frailty and neurodegeneration (3, 316, 317, 319, 330, 449).

K. Andropause and CNS

In men, aging is associated with a progressive decline in testosterone production, GH secretion, and DHEA. These endocrine changes are accompanied by fatigue, depression, decreased libido, erectile dysfunction (ED), and decreased intellectual and physical ability. The attenuation of these symptoms by androgen replacement therapy implicates reductions in testosterone as causative (4). Indeed, transdermal testosterone gel applied to hypogonadal men improved their sexual function, mood, strength, and body composition (450). Testosterone replacement also has the potential to inhibit neurodegeneration by maintaining expression of BDNF in the aging brain (151). This is particularly important because of the importance of BDNF in maintaining noradrenergic innervations during aging as well as its proposed significance in Parkinson’s disease and AD (451, 452).

Aging male BN rats exhibit both primary and secondary testicular failure, similar to that described during the andropause in humans. To determine whether these might be a consequence of hypothalamic changes, the concentrations of hypothalamic preproGnRH (ppGnRH) mRNA were compared in young, middle-aged, and old rats by in situ hybridization; GnRH peptide content in microdissected brain areas was also compared (16). During aging, GnRH levels declined and castration decreased ppGnRH mRNA content as a function of age, but the number of neurons expressing ppGnRH mRNA remained constant. Aging had no effect on pituitary responsiveness to GnRH with respect to LH secretion, but the FSH response appeared to increase. Despite this similar LH response, the stimulatory effect of LH on testosterone production declined, LH circadian rhythmicity was blunted, and testosterone levels over 24 h declined progressively with age. Hence, deficits in testicular function in aging male BN rats is attributable, at least in part, to decreased GnRH rather than decreased responsiveness of the anterior pituitary gland to GnRH; this property and the attenuated circadian rhythmicity of LH and testosterone secretion are reminiscent of age-dependent changes in the GH axis.

The fact that the responsiveness of gonadotrophs to exogenous GnRH treatment is preserved, whereas the amplitude of endogenous LH pulses is attenuated during aging, suggests that GnRH feed-forward signaling is impaired. To assess whether this could be caused by reduced androgen production, LH pulsatility was compared in old and young men after blocking androgen biosynthesis by ketoconazole administration (453); inhibition of glucocorticoid synthesis by ketoconazole was compensated for by administration of low-dose dexamethasone. In contrast to young men made hypergonadotropic, in older men the reduced testosterone levels did not enhance LH pulsatility. Because pituitary LH stores and responsiveness to GnRH are preserved, the deficit in older men is consistent with impaired feed-forward drive (453). When the orderliness of LH release patterns was monitored by ApEn, older men were found to have more irregular pulse patterns. Hence, the hypothalamic-pituitary response to reduced androgen production is muted during aging in males (453).

The application of pulsatile GnRH infusion to elderly men unmasks hypothalamic and Leydig cell defects. Elderly men were evaluated to determine whether reductions in serum testosterone, in the absence of increased LH, were a reflection of hypothalamic GnRH deficiency (454). Five young (ages 20-34 yr) and five older (ages 60-78 yr) men were given randomized infusions of saline or pulsatile GnRH iv at 90-min intervals for 24 h. Older men infused with saline produced more LH pulses with lower pulse amplitude than did younger men, and pulsatility was more disordered as judged by ApEn calculations. Remarkably, during pulsatile GnRH infusions, serum LH increased equivalently in both young and older men; furthermore, LH pulse frequency, amplitude, and ApEn were similar. This indicates that hypogonadotropism associated with aging is a consequence of altered endogenous hypothalamic GnRH release. However, in contrast to the LH profile, 24-h testosterone concentrations failed to increase equivalently in the older men, implicating Leydig cell deficiency. Although the authors concluded that a dual defect in the CNS-pituitary-Leydig cell axis marks aging in men, the significance of reduced GH in this population cannot be ignored; for example, the characteristics of transgenic mice that lack functional GH receptor signal transduction highlight the subtle role that GH plays in enhancing the response of Leydig cells to LH (455).

Dietary restriction has an adverse effect on fertility in many species from Caenorhabditis elegans to humans (456). Short-term fasting was used to unmask age-related neuroendocrine changes in the GnRH/LH axis and the dynamics of LH release in young (28 ± 3 yr) and older men (67 ± 2 yr) (457). In fed older men, basal LH peak frequency and free testosterone concentrations were lower than those of young men. Fasting for 3.5 d suppressed pulsatile LH secretion and enhanced orderliness of LH release as measured by ApEn in young, but not in older, subjects. As discussed in Section II, increased disorderliness of pulsatile LH secretion in elderly subjects is consistent with the notion that alterations in the nonlinear dynamic behavior of the regulatory neurons produce a less robust phenotype (39, 42, 43, 65). Clearly, the metabolic stressor of short-term fasting unmasks age-related neuroendocrine differences in the regulation of both the pulsatile and nyctohemeral control of the male hypothalamic-pituitary-gonadal-axis. How these changes affect function in the young animals is an important question. For example, does sustained enhanced orderliness during continued fasting produce a benefit or deficit on the reproductive system? Clearly, prolonged food restriction will negatively affect reproductive function.

Aging also has impact on the opioid control of gonadotropin secretion. GnRH secretion was compared in hypothalamic tissue fragments from young (75-90 d) and old rats (18-20 months) in the absence or presence of the opiate antagonist naloxone (458). Serum concentrations of testosterone and LH were lower in the old animals, but basal GnRH secretion was similar for both age groups. Naloxone produced a significant dose-dependent increase in the release of GnRH from the hypothalamic tissue fragments that was age dependent. These results suggest that age-related changes in
endogenous opioid systems likely contribute to differences in secretion of GnRH, which in turn affects the dynamics of LH secretion and testosterone production.

Excitatory amino acids also regulate pulsatile secretion of hypothalamic GnRH and LH. To explore the significance of aging on this pathway, the effects of GnRH and the glutamate receptor agonist NMDA on gonadotropin and prolactin release were investigated in prepubertal (35 d), young (3-4 months), middle-aged (12-13 months), and old (21-23 months) BN rats (459). The release of gonadotropins in response to GnRH was not age dependent, and NMDA increased LH and prolactin secretion in all age groups. An FSH response was observed in young and middle-aged, but not in old, rats. However, the NMDA enhancement of LH, FSH, and prolactin release was lowest in old rats. To investigate whether the reduced LH response to NMDA in old rats occurred at the hypothalamic level, the direct effects of NMDA on GnRH release were evaluated in tissue fragments from the preoptic medial basal hypothalamus. The magnitude of GnRH release from these fragments was inversely related to age, and determination of amino acid content showed that aged animals had the lowest concentrations of glutamate, taurine, and GABA. Hence, the attenuated responsiveness of GnRH neurons to NMDA and reductions in excitatory amino acids likely contribute to the diminished pulsatile LH secretion typically observed in old rats.

IX. Sexual Behavior and Aging

A. Sex steroids and age-related deficits

The sex steroids dopamine, oxytocin, and 5-HT regulate sexual behavior through actions in the CNS. Because the production of these hormones and neurotransmitters declines during aging, it is hardly surprising that aging is commonly associated with a decline in libido and sexual performance. Based on studies with aromatase knockout mice, ERα knockout mice, and ERβ knockout (βERKO) mice, estradiol plays an important role in male sexual behavior. Aromatase knockout mice are fertile, but sexual behavior of the males is strongly modified (460, 461). Male sexual behavior is partially disrupted in ERα knockout mice and ERβ knockout mice, and the ERα or β knockout male mice fail to exhibit any component of normal sexual behavior (462). However, conclusions about the link between the phenotype and estradiol in the sexually mature knockout mice must be tempered because of the possibility of altered embryonic development. For example, sexual differentiation of dopaminergic neurons in the periventricular nucleus of the hypothalamus during development appears to be ER specific (463). A less ambiguous picture of the role of specific ERs and the decline in testosterone and estradiol production during aging requires the generation of mice where ERs are conditionally inactivated after the mice reach sexual maturity.

Sexual behavior in male mice following castration varies according to genotype; therefore, sexual behavior is not solely dependent on testosterone production. For example, in contrast to C57BL/6j and DBA/2j mouse strains, 30% of B6D2F1 mice retain the ability to ejaculate for at least 25 wk following castration. As expected, the levels of testosterone and nuclear ERs are lower in castrated males, but they copulate without the stimulatory effects of gonadal hormones (464).

The decline in sexual arousal, copulatory activity, and fertility observed in old male rats can be rescued by grafting tissue isolated from the POA of fetal rats into the third ventricle of aged males (33–35). Typically, copulatory behavior is sustained for at least 2-4.5 months, and serum testosterone and LH levels are similar to those of young males (35). In contrast, no improvement in sexual performance occurs when fetal cerebral cortex neurons are grafted into the POA, or when POA neurons are grafted into the VMH. Hence, these results show that a decrease in copulatory activity, sexual motivation, and neuroendocrine function in aged male rats are at least partially because of dysfunction of the POA; restoration probably involves the combined effects of sex steroids and dopamine. Importantly, these studies again illustrate the latent plasticity of the aging phenotype.

Lesioning studies show that in addition to the POA, the central tegmental field (CTF) is an important area controlling sexual behavior (465, 466). The CTF appears to be involved in translating sexual motivation into action (467). Giordano et al. (468) evaluated the effects of combined fetal homotopic tissue transplants into the POA and CTF of rats with electrolytic lesions of the POA and CTF. They observed recovery of sexual behavior in the lesioned animals following bilateral, but not unilateral, transplants. These results support the notion that both POA and CTF play an important role in regulation of sexual behavior. However, the fact that sexual activity of old rats can be restored efficiently by transplanting the POA alone argues that changes in function of the POA rather than CTF occur during aging.

B. Dopamine and age-related deficits

Through their actions in the medial POA, testosterone and dopamine are important mediators of male sexual behavior. Two approaches for restoration of sexual function have been compared. In the first, rats were castrated at 22 months and then administered testosterone for 2 months; for the second, rats were castrated at 12 months and administered testosterone for 12 months. The latter was the more effective for sustaining mount rate. Effectiveness correlated positively with NMDA-responsive dopamine secretion from the medial POA (MPOA) (469), which is consistent with experiments showing that the dopamine agonist apomorphine facilitates copulatory activity in male rats, whereas administration of the dopamine antagonist cis-flupenthixol into the MPOA of male rats inhibits copulation and ejaculation (470). Flupenthixol also attenuates the facilitative property of apomorphine. These results support the notion that long-term testosterone replacement inhibits the decline in sexual activity during aging by restoring dopamine activity in the medial POA.

Interestingly, administration of the monamine oxidase-B inhibitor, 1-deprenyl, increased the sexual activity of old rats, retarded the age-related decline in learning and memory function, and increased life span (471–473). Deprenyl’s beneficial effects on sexual activity included reducing the latency to mounting and intromission, increasing the frequency of
intromissions, and delaying the age-related loss in the ability to ejaculate (474, 475); these benefits are probably associated with stimulation of dopamine and norepinephrine release from the medial basal hypothalamus (476). The sustained stimulation of release of these two neurotransmitters is likely mediated through inhibition of monoamine oxidase by deprenyl.

The mechanism of deprenyl action on reproductive function in rats was investigated during lifetime treatment. At a crucial developmental phase between weaning and the second month of age, the release of neurotransmitters was markedly increased until sexual development was complete. These results show that deprenyl enhances catecholaminergic activity in the brain, which stimulates sexual activity (473). In female rats, chronic treatment of old acyclic animals (aged 15-16 months) with deprenyl temporarily reestablished estrous cycles and reduced the incidence of pituitary and mammary tumors (386). Increased longevity is perhaps mediated by enhancing superoxide dismutase and catalase activity in the striatum and by preventing characteristic age-dependent morphological changes in neurocytes in the substantia nigra (475); alternatively, improved longevity might be a consequence of increased sexual activity.

In female rats and mice, sexual receptivity as measured by a lordosis response is dependent on progesterone, dopamine, and the presence of a functional progesterone receptor (PR) in the hypothalamus (96, 97). For example, dopamine and progesterone fail to induce a lordosis response in PR knockout mice. Similarly, the dopamine and cAMP-regulated phosphoprotein-32 (DARPP-32) knockout mouse is insensitive to the lordosis effects of dopamine and progesterone. Hence, both PR and DARPP-32 are essential components of progesterone- and dopamine-mediated lordosis (477). Estradiol treatment of female rats also stimulates phosphorylation of DARPP-32 in the hypothalamus (95). Because estradiol, progesterone, the PR, and dopamine concentrations in the hypothalamus all decline during aging, age-related deficits in sexual receptivity of female rats and mice are not surprising. For a detailed account of the broad significance of phosphorylation/dephosphorylation of DARPP-32 and its link with neurotransmitter activity in the CNS, the reader is referred to Fig. 7 and Greengard’s review (478).

C. GH and ED

The reduced levels of GH caused by attenuation of hypothalamic-pituitary signaling during aging may also contribute toward ED, in addition to affecting testosterone production and spermatogenesis (479). Serum GH levels measured in normal subjects increased during penile tumescence. During the transition from rigidity to detumescence, a transient decline in GH occurs. In patients with ED, GH levels during penile flaccidity were 7-fold lower than in normal men. When GH was measured in blood isolated from the corpus cavernosum and cubital vein during penile tumescence, the average GH increase in psychogenic ED patients was similar to that seen in normal men; however, in organogenic ED this increase was negligible. The effect of GH on erectile function is probably mediated locally and through stimulation of NO production because GH enhances the regeneration of NOS-containing penile nerves and neurons following cavernous nerve neurotomy (480). These results suggest that the central control of GH release plays a contributory role in normal erectile function.

X. HPA Axis and Aging

The HPA axis provides a defense against stress. However, chronic stimulation of this axis leading to hypersecretion of glucocorticoids, particularly in the elderly, is implicated in the pathology of systemic, neurodegenerative, and affective disorders.

A. Decreased sensitivity to negative feedback regulation

In addition to changes in sex steroid production, the most significant age-associated endocrine change during aging resides in the corticosteroid pathway (26, 313). Corticosteroids secreted episodically by the adrenal gland increase in response to stress (481). A tightly controlled feedback loop involving CRH from the CNS and ACTH from the anterior pituitary gland regulates the amplitude of glucocorticoid secretion (Fig. 8). The hippocampus plays the major inhibitory role on HPA activity, and the sensitivity of the HPA axis to glucocorticoid feedback suppression becomes attenuated as humans age (482, 483). Decreased sensitivity toward corticosteroid negative feedback is detrimental, because prolonged elevation of glucocorticoids impairs cognitive function, inhibits LTP, and reduces dendritic density (484–489).

The stress-induced increase in glucocorticoid levels persists longer in old animals (139, 148, 485). This persistence is also reflected in the stress-induced increase in amplitude and duration of glutamate release from the hippocampus in old compared with young rats (Fig. 9) (148). It is unclear whether this altered response to stress, as a function of age, is explained by vascular changes that result in slower uptake of glucocorticoids into the brain, by changes in glucocorticoid receptor (GR) concentrations, or by altered signal transduction pathways (110, 490).

The role of glucocorticoids in the CNS is complicated because of the concentration dependence of neuroprotective and neurodegenerative effects of glucocorticoids (491). Mice that have a GR that fails to bind DNA because of a point mutation in the receptor exhibit impaired spatial memory (492). Normal basal levels enhance synaptic plasticity with beneficial effects on memory, whereas continued exposure to elevated levels produced during chronic stress has the opposite effect (493). Deficits in performance in the water maze test, similar to that seen during normal aging, are attenuated if corticosterone levels are maintained at low basal levels throughout life by treating adrenalectomizing rats with low-dose corticosterone replacement (494, 495). However, when corticosterone levels are increased by chronic stress or by administering elevated levels of corticosterone, deficits in hippocampal function are induced (481).

To investigate age-dependent alterations in negative feedback in humans, ACTH and cortisol were measured in blood collected at frequent intervals after cortisol injections (496). In healthy older men (aged 65-88 yr), although plasma cortisol levels were maximal after 2 min, ACTH levels did not change significantly for the first 15 min, after which a pro-
nounced and significant decline occurred. By direct contrast, in healthy young men (aged 18-26 yr), in synchrony with the increase in plasma cortisol, ACTH decreased markedly within the first 15 min followed by a less pronounced decline. Hence, cortisol mediated ACTH inhibition is biphasic where the first phase (0-60 min) is clearly different between old and young men; the second phase (from 60 to 180 min) is the same for both age groups. The slower response to glucocorticoid feedback inhibition of ACTH in old men is consistent with altered central regulation during aging.

**B. Corticosteroid receptors**

During aging, the levels of GR in the CNS decline (497–500). Two corticosteroid receptors, the GR and mineralocorticoid receptor (MR) are involved in feedback regulation of the HPA axis. Corticosterone has a 10-fold higher affinity for the MR. The hippocampus, in contrast to the hypothalamus and anterior pituitary gland, has high concentrations of MR and GR, which facilitate feedback regulation over a wide range of steroid concentrations (483). Basal activity is partially regulated by hippocampal inhibition of AVP. Levels of AVP in portal blood correlate with low levels of corticosteroid receptor occupancy in the hippocampus; AVP expression in CRH neurons is sensitive to very low corticosteroid levels (482). Basal HPA activity is apparently mediated via the MR, whereas at high levels of glucocorticoids typically seen as a result of stress, negative feedback correlates with occupancy of the GR.

Estrogen replacement attenuates the stress-induced elevation of cortisol in postmenopausal women (501). Estrogen treatment also increases GR mRNA in the hippocampus and amygdala (137, 138); therefore, the altered response to stress with age might be related to alterations in GR concentrations in the CNS. The response to acute stress was monitored in young and old male rats by measuring corticosterone levels at 15, 60, and 120 min following ether stress (1 min). In young rats, corticosterone reached a maximum after 15 min and...
returned to almost prestress levels by 120 min, but in old rats corticosterone increased slowly, reached a maximum after 60 min, where it was sustained for at least 120 min (139). How-

ever, when old male rats were treated chronically with es-

tradiol, their stress response was indistinguishable from that of young rats (139). Quantitative immunohistochemistry on brains of estradiol-treated old rats indicated that normalization of the stress response by estradiol treatment was accompanied by restoration of GR concentrations in CA1 and CA2 hippocampal structures, subiculum, and PVN (139).

Presumably, by increasing GR concentrations, the sensi-
tivity to corticosterone is greater because more receptors become occupied. Hence, estrogen treatment enhances the glucocorticoid feedback signal by increasing GR in hippocampus, correcting the age-related alterations in regulation of the HPA axis. This result supports the concept that reduced concentrations of GR explain the altered feedback response to stress in old animals. However, a caveat is that pharmacological concentrations of estradiol were attained in this study; therefore, the physiological relevance remains unclear. Indeed, sites other than those in the hippocampus must be involved in feedback regulation because removal of hippocampal input by lesioning reduces, but does not elim-

inate, glucocorticoid negative feedback (482).

The role of corticosteroid receptors in the brain has also been addressed using GR- and MR-null and transgenic mice (502). Experiments with these genetically manipulated mice confirm that antagonism of the GR activates the HPA axis and that increases in GR levels inhibit the axis. A comparison of GR and MR knockouts suggests that MR, but not GR, is required for maintenance of granule cell populations in the DG. However, the cellular properties of CA1 neurons and hippocampal-dependent explicit memory are different in GR mutant mice. Hence, age-related reductions in either or both GR and MR would likely contribute toward deficits in brain function during aging.

C. Stress response differs according to gender

The stress response is influenced by cross talk between the gonadal and adrenal axes according to gender, which might explain why certain stress-related diseases are sex depen-
dent. Females produce a bigger cortisol response than males (503). Indeed, testosterone inhibits and estradiol enhances function of the HPA axis. In rats, basal ACTH is regulated by testosterone-induced AVP synthesis. The influence of go-

nadal steroids on basal and stress-induced activity appears to be mediated primarily by AVP secretory neurons (504, 505). Under basal conditions, AVP synthesis is low, but syn-
thesis increases in response to chronic stress. Testosterone does not appear to modify GR or MR binding in the brain other than in the MPOA. Implanting testosterone or corti-

costerone pellets into the MPOA reduces AVP, but not CRH levels in the median eminence, and decreases the ACTH release in response to stress (505).

In postmenopausal women, AVP neurons in the PVN are larger than in young women, and the secretion of AVP appears to be influenced by sex steroids (506). In both men and women, AVP cell size correlates positively with age (507). Curiously, these size changes are more pronounced on the right side of the brain (507). Estrogen inhibits activity of AVP neurons in the supraoptic nucleus (SON) and PVN (506). It has been presumed that inhibition is mediated by genomic effects of estradiol on either ERα or ERβ. ERβ is localized in the PVN of male mice. Recent studies in βERKO mice

![Fig. 8. Aging and feedback pathways that regulate activity of the HPA axis. Hypothalamic CRH and AVP stimulate ACTH release from the anterior pituitary gland, which increases production of cortisol from the adrenal gland. Basal cortisol production action is regulated centrally by cortisol interacting through feedback pathways mediated by the high-affinity type 1 GR/MR. Under conditions of stress, the feedback pathways controlling elevated cortisol production are reg-

ulated via the lower-affinity type 2 GR. During aging, the concen-

tration of GRs decreases, which is associated with reduced sensitivity of feedback inhibition, and the activity of hippocampal HSD increases, which exposes hippocampal neurons to higher levels of cortisol. Age-
dependent decreases in brain levels of GH and IGF-I allow increased HSD1 activity and increased apoptosis to occur.](https://thinkingpub.com/fig8.png)

![Fig. 9. Stress-induced glutamate release is sustained in old (22-24 months) but not in young (3- to 4- month old) rats. [Reproduced with permission from (148).](https://thinkingpub.com/fig9.png)
showed that estrogen treatment reduced AVP mRNA in the PVN of wild-type mice but was without effect in βERKO mice (508). Hence, the inhibitory effect of estradiol on AVP in the PVN appears to be mediated by ERβ.

D. CRH

The elderly phenotype exhibits lower resistance to traumatic insult. In rats, following acute stress, the response of catecholaminergic systems and the HPA axis is attenuated as a function of age (102, 509). One possible explanation for the attenuation is reduced CRH expression. Indeed, in rats, aging decreases CRH mRNA in the PVN, in the amygdala and in the bed nucleus of the stria terminalis (BNST) (510). These alterations in CRH gene expression are consistent with an age-dependent decrease in neuroendocrine reserve toward stress. Neurons in the amygdala and BNST project onto CRH neurons (511), and because reduced expression of CRH mRNA in the amygdala and BNST precede changes in the PVN, it is speculated that age-related changes in the PVN are a consequence of age-dependent changes in the amygdala and BNST.

To evaluate how the amygdala stress system changes with age, Fischer 344 rats of different ages (4, 12, or 24 months) were tested for anxiety-like behaviors using the elevated plus maze after 14 d of hourly restraint (512). The levels of CRH and CRH-binding protein mRNA in the amygdala of old rats were significantly lower relative to controls. In young rats no significant differences were observed. Decreased expression of CRH in the amygdala accompanies decreased anxiety-like behaviors following restraint and is consistent with the known behavioral effects of exogenous CRH applied to the amygdala.

Basal levels of glucocorticoids are essential for normal function, but chronically high levels have adverse effects in the CNS; excessive CRH production is also detrimental. A transgenic mouse line overexpressing CRH in neural tissues was developed as a model of stress-related hypersecretion of CRH (513). These mice exhibit adrenal hypertrophy with elevated plasma corticosterone levels but normal levels of plasma ACTH. Stress induces a normal corticosterone response, but the mice are unresponsive in a dexamethasone challenge in old rats relative to young rats (518). In old rats were accompanied by higher ACTH and corticosterone levels, which supports the notion that aging is associated with a hyperactive HPA axis (517). Further support for the role of AVP in the age-related changes in the HPA axis was provided by experiments with a selective AVP antagonist. Treatment with an AVP type 1 receptor antagonist reduces the magnitude of the ACTH response to a dexamethasone/CRH challenge in old rats relative to young rats (518). In old rats, dexamethasone increases the number of neurons expressing AVP mRNA and CRH mRNA in the paraventricular area of the PVN. The expression of CRH mRNA/neuron also increases, whereas the expression of AVP mRNA/neuron is unchanged. These age-related changes are likely secondary to age-dependent impaired functioning of corticosteroid receptor negative feedback signaling (519, 520).

E. AVP

It has been speculated that synergism between CRH and AVP contributes toward age-associated changes in the HPA axis. AVP is predominantly expressed in magnocellular neurons of the PVN and SON that project to the neurohypophysis. AVP also colocalizes with CRH in parvocellular neurons of the PVN that project to the median eminence. To evaluate the effects of stress as a function of age, intracerebral release of AVP was measured in young adult (3 months old) and middle-aged rats (22-24 months old) before and after a forced swim test. An increase in basal release of AVP in the PVN and a blunted intranuclear response to the swim stress were observed according to age (517). Interestingly, these responses are site specific because neither basal nor stress-induced AVP age-dependent differences were identified in the SON. When aged subjects are treated with a combination of dexamethasone and CRH, the release of ACTH is higher than that in young subjects, suggesting that endogenous AVP is elevated in the elderly population (518).

Plasma levels of both AVP and oxytocin are elevated in aged rats, and similar observations have been observed in humans, which is consistent with disinhibition of magnocellular neurons (517). Increased basal levels of AVP in older rats were accompanied by higher ACTH and corticosterone levels, which supports the notion that aging is associated with a hyperactive HPA axis (517). Further support for the role of AVP in the age-related changes in the HPA axis was provided by experiments with a selective AVP antagonist. Treatment with an AVP type 1 receptor antagonist reduces the magnitude of the ACTH response to a dexamethasone/CRH challenge in old rats relative to young rats (518). In old rats, dexamethasone increases the number of neurons expressing AVP mRNA and CRH mRNA in the paraventricular area of the PVN. The expression of CRH mRNA/neuron also increases, whereas the expression of AVP mRNA/neuron is unchanged. These age-related changes are likely secondary to age-dependent impaired functioning of corticosteroid receptor negative feedback signaling (519, 520).

F. 11β-Hydroxysteroid dehydrogenase (HSD)

HSD exists as two isoforms (HSD1 and HSD2), which are important metabolic determinants of glucocorticoid action. HSD1 produces an active glucocorticoid by reducing the 11-keto steroid moiety to the 11β-hydroxy-steroid (cortisol in humans and corticosterone in rodents), and HSD2 acts exclusively as a dehydrogenase to inactivate glucocorticoids. In the hippocampus, only the HSD1 isoform appears to be produced (521, 522). GH decreases HSD1 activity (523); therefore, the age-related reduction in the amplitude of GH pulsatility indirectly increases HSD1 activity to increase local production of glucocorticoids in the hippocampus. This increased activity of HSD1 in hippocampal neurons during
aging likely contributes toward age-related neurodegeneration (524).

Increased local production of glucocorticoids is apparently involved in age-related learning and memory deficits. The learning impairment exhibited by mice as they age, illustrated by performance in the water maze, is not evident in HSD1 knockout mice (525). Surprisingly, plasma corticosterone levels are higher in the young knockout mice (13.4 ± 2 μg/dl) compared with wild-type mice (2.5 ± 0.5 μg/dl). However, in contrast to wild-type mice, corticosterone does not increase in HSD1 knockout mice during aging. Both wild-type and knockout mice had identical plasma corticosterone levels at 18-20 months of age, but hippocampal corticosterone levels are significantly lower in aged HSD1 knockout mice (525). Although quantitation of corticosterone in the hippocampus was indirect and accuracy of the assay might be questioned, it is clear that elimination of HSD1 activity protects against age-related decline in hippocampal function. The results also reinforce the notion that local rather than peripheral levels of glucocorticoids are associated with adverse effects on the hippocampus.

**G. Counterregulatory effects of GH and IGF-I on glucocorticoid action**

The age-related increase in glucocorticoid production is accompanied by a progressive decline in production of anabolic hormones such as GH, IGF-I, and sex steroids. These hormones appear to counteract the negative effects of chronically elevated cortisol on muscle, bone mass, and hippocampal neurons. Hence, during aging the increased production of glucocorticoids caused by age-related deficits in the glucocorticoid negative feedback pathway and increased HSD1 activity in VF and the hippocampus cause an imbalance, as indicated in Fig. 10. This age-dependent imbalance, in addition to affecting peripheral metabolism and hypothalamic function, also affects the integrity of hippocampal structures (8, 11, 485, 486, 526–530).

Aging is associated with a decline in immune responsiveness, the consequences of which are increased susceptibility to infectious diseases, the emergence of cancer, and increased incidence of autoimmune disease (531, 532). Prolactin, GH, IGF-I, and/or thyroid hormone play an important modulatory role on the immune system. It has been hypothesized that the immunosuppressive effects of glucocorticoids are normally kept in balance by counterregulatory actions of these hormones (198, 200). A counterregulatory pathway for glucocorticoid action is necessary under conditions of chronic environmental stressors. In this case, the concentrations of catecholamines and glucocorticoids are likely higher than those encountered during a normal immune response. In the absence of modulating hormones, chronic immunosuppression is debilitating. For example, Snell dwarf mice become immunocompromised under conditions of severe stress.

Reactivation of counterregulatory pathways for glucocorticoid excess during aging is likely to prove beneficial. Age-related reductions in GH and IGF-I are associated with involution of the thymus and declining T-cell production (5). Koo et al. (201) showed that restoration of GH and IGF-I levels in old mice by treatment with a GHS-R ligand increased both the cellularity of the thymus and T-cell production. Functional benefit was illustrated by the demonstration that growth and metastases of tumors implanted into the old mice were inhibited by treatment with the GHS-R ligand. Similarly, HIV patients experienced improvements on thymic function after 6-month treatment with GH (533), which supports the relevance of the mouse studies described by Koo et al. (201) and stresses the importance of intervening centrally to restore physiological profiles of GH in the elderly.

**H. Serotonergic system and glucocorticoids**

The raphe-hippocampal 5-HT neuronal system is sensitive to glucocorticoids and plays an important regulatory role in mood, memory, and neuroendocrine responses. Treating old rats with the monoamine uptake inhibitor amitriptyline
caused a modest decrease in the concentration of 5-HT$_{1A}$ receptor mRNA in the dorsal raphe nucleus, whereas in young rats HT$_{1A}$ receptor mRNA levels were unchanged (534). Irrespective of age, administration of amitriptyline did not affect expression of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{2C}$, or 5-HT$_{7}$ receptor subtypes in any hippocampal subregion. Therefore, the differential responsiveness to amitriptyline according to age originates at the level of raphe 5-HT$_{1A}$ autoreceptor gene expression.

Young (3 months old) and old (18 months old) adrenalectomized rats respond differently to corticosterone replacement. In the former, expression of 5-HT$_{1A}$ receptors is inhibited, whereas in the latter corticosterone fails to suppress 5-HT$_{1A}$ receptor expression. This alteration in 5-HT$_{1A}$ receptor expression in response to corticosterone is another example of age-associated differences associated with the HPA axis and altered adaptation to stress (535). Interpreting the significance of modestly reducing expression of 5-HT$_{1A}$ receptors is complicated, because mice lacking 5-HT$_{1A}$ receptors have impaired hippocampal learning (536). However, the complete lack of these receptors throughout life may compromise hippocampal development and function; therefore, the phenotype of the 5-HT$_{1A}$ receptors knockout mice may not be relevant to the aging phenotype of wild-type mice.

XI. Transcriptional Regulation and Aging

A. Overview and relevance to neuroendocrinology of aging

It would be oversimplistic to expect that the changing concentration of hormones and their receptors during aging occurs in a vacuum. Because some of the changes are subtle, their contribution toward the aging phenotype might be underestimated. However, regulation of the neuroendocrine system is dependent on neuropeptides and neurotransmitters, and in target tissues the response to hormones is dependent on activation of specific signal transduction pathways. The interplay among regulators of hormone release, the hormones, and signal transduction pathways establishes fine control over transcription, translation, and posttranslational mechanisms. Consequently, age-related changes in the overall physiology of an animal that are endocrine related are complex and not simply correlated with alterations in hormone concentrations. For example, age-dependent decreases in the efficiency of the signal transduction pathways activated by GH and leptin contribute to the decline in expression of IGF-I and phosphorylation of signal transducer and activator of transcription 3 (362, 537). Moreover, in addition to the decline in production of sex steroids during aging, increased methylation of ER$\alpha$, resulting in inactivation of ER$\alpha$ production, can occur (538). Hence, a number of factors (other than the hormones themselves) that are associated indirectly with hormone action contribute to the aging phenotype; these factors cannot be ignored if we are to understand the consequences of neuroendocrine changes on CNS function during aging.

B. Molecular misreading and aging

The impact of mutagenesis and molecular misreading of endocrine pathways in the brain during aging deserves consideration. Perhaps molecular misreading is a consequence of age-related changes in hormone concentrations. Studies with lacZ transgenic mice indicate that spontaneous mutation frequency increases during aging in all tissues, including brain (539). The complexity of mutations identified in old tissues implicates a unique mechanism compared with young tissues. However, even more intriguing than mutations in DNA is the concept of molecular misreading in neurons, which causes an age-associated increase in mutations because of inaccurate transcription of a normal gene; as a consequence, nonsense transcripts and translation mutant proteins are produced (540). For example, dinucleotide deletions within and adjacent to GAGAG motifs in mRNA cause a reading frameshift to the +1 frame, hence +1 proteins are synthesized. These proteins have a wild-type N terminus, but, according to the site of dinucleotide deletion, they have an altered nonfunctional COOH terminus. Molecular misreading occurs in the rat vasopressin gene, and in the APP and ubiquitin-B genes associated with AD. Besides the brain, +1 proteins have been found in liver, epididymis, parotid gland, and neuroblastoma cell lines. These +1 proteins have been found in elderly but not in younger subjects (< 72 yr old), suggesting that molecular misreading is a factor in aging.

A creative approach to identify hot spots of molecular misreading using a bacterial expression system containing green fluorescent protein was developed (541). Total RNA was isolated from cortical regions of human brain and subjected to RT-PCR, and the cDNA products were subcloned into green fluorescent protein. Insert size was determined and then sequenced to identify frameshift mutations. Most of the mutations identified are in close proximity to short repeats: for example, GAGAG, GGUGGU, GAAGAAGAA, and UCAUCAUCA. New frameshift mutations occur at a number of locations in the transcripts of ubiquitin-B and APP genes. Interestingly, some of the new APP fragments have the potential to produce neurotoxic A$\beta$ peptides. Hence, molecular misreading is a source of transcript errors involved in age-related pathologies. Whether molecular reading errors occur because of age-related endocrine changes depends on the results of experiments designed to rescue the phenotype by hormonal replacement.

C. Coactivators and corepressors of gene transcription

As discussed above, the decline in production of sex steroids during aging produces impairment in neuronal properties. The steroid hormones have profound effects on gene transcription and play a significant role during development and in adulthood. Coactivators, such as SRC-1, and corepressors play an important regulatory role in defining activity of nuclear receptor complexes in a cell-specific manner, thereby controlling development, behavior, and neuroendocrine function (388, 542–544). Any age-related change in the concentrations of nuclear receptors and their coactivators or corepressors will alter the magnitude of signaling in re-
response to ligands. For example, mice deficient in SRC-1 exhibit attenuated responses to estradiol and thyroid hormone (542, 545). Increasing the concentrations of circulating hormones through exogenous hormone administration will compensate for an age-related decline in both the production of steroid hormones and reduced expression of their receptors. However, compensating for age-related changes in the expression of cell specific coactivators or corepressors is more problematic because it will depend on the relative magnitude of the changes.

Phosphorylated cAMP regulatory element-binding protein (CREB) and its binding protein play a very important and broad role in neuroendocrine regulation of gene transcription (544); therefore, changes in the concentration or localization of these proteins during aging of the CNS would compromise neuronal function. The subcellular immunohistochemical localization of CREB in motoneurons of the spinal nucleus of the bulbocavernosus in young and old male rats shows that CREB is exclusively localized in the nucleus (546). In old animals, both the number of CREB immunoreactive nuclei and the intensity of the immunoreactivity are significantly reduced compared with young animals. This marked decline in nuclear localization of CREB, which is such an important signal for cAMP-mediated regulation of gene expression, will exacerbate the functional deficiencies caused by altered production of steroid hormones during aging. For example, disruption of the gene encoding type 1 adenylate cyclase, which is involved in cAMP production and is expressed predominantly in the brain, results in decreased LTP in CA1 of the hippocampus and a deficit in spatial memory (547).

D. Heat shock proteins

Binding sites for heat shock proteins are present on steroid receptors. These proteins behave as chaperones and appear to be essential for optimal steroid receptor function in vivo. Age-related changes in expression of heat shock cognate proteins are associated with impaired retinal function during aging. When cDNA microarray analysis was used to compare patterns of gene expression in the human retina of a 4-yr-old subject and an 80-yr-old subject, one of the age-related, differentially expressed genes was identified as heat shock cognate 70 (HSC70) (548). Northern analysis of total retinal RNA from human donors suggested a 2- to 3-fold decrease in HSC70 mRNA levels in the human retina by the eighth decade of life. Western blot analyses show that reduced expression of HSC70 in the retina during aging is also seen in nonhuman primates (548). Both HSC70 and the related chaperone heat shock protein-90 are necessary for steroid activation of GRs (549). In addition to having relevance to declining glucocorticoid responsiveness during aging, the reduced production of heat shock-related proteins in the retina may contribute to the age-related increased susceptibility of the retina to disease. Indeed, according to studies conducted in a cell culture model of the blood-retinal barrier and results from a pilot clinical trial, the glucocorticoid triamcinolone acetonide shows potential for treatment of age-related macular degeneration (550). These studies provoke speculation that attenuation of specific endogenous glucocorticoid functions during aging contributes to the marked increased incidence of macular degeneration in the elderly.

E. Protein kinase C (PKC) isoforms

PKC is a common transducer of hormone messages. Investigation of the effect of age on expression of PKC isoforms in the brain is important because of the potential role of PKC in signal transduction mechanisms that involve memory function. The distribution of PKC-α, -β, and -γ was examined in the brains of young and old rats by in situ hybridization histochemistry (551). Although the concentrations of the mRNA encoding the three PKC isoforms were different in cortical compared with hippocampal regions, aging caused no detectable changes in expression. Therefore, if altered phosphorylation associated with age-related neurodegeneration is PKC mediated, enzyme activity would have to be regulated posttranscriptionally.

PKC protein was compared in the senescence-accelerated P8 mouse (SAMP8) model of aging. Calcium-dependent PKC and calcium-calmodulin-dependent protein kinase were measured in the hippocampus of SAMP8 mice at different ages (4, 8, and 12 months). Western blot analysis showed that total hippocampal PKC-γ declined linearly with age (552). The cellular distribution of PKC-γ also changed with age. Indeed, a decrease in the amount of PKC in the particulate fraction relative to the soluble PKC fraction correlated with previous observations of the age-related decline in retention but not with acquisition. Therefore, perhaps changes in the distribution of these kinases exacerbate the age-related decline in hippocampal function that is caused by decreasing levels of sex steroids, GH, and IGF-I and increasing levels of glucocorticoids.

F. Helix-loop-helix (HLH) proteins

The expression pattern of HLH transcriptional regulatory proteins factors are altered in the aging brain. Expression of NeuroD and ME2 change differentially according to brain region (553). During aging, the expression of HLH E-protein ME2 decreases in both the cerebellum and hippocampus, whereas NeuroD expression is sustained at high levels in the cerebellum but markedly declines in the hippocampus (553). NeuroD has importance in endocrinology because it associates with coactivators of the steroid receptors, CBP and p300, and has been shown to be an important positive regulatory factor of POMC expression (554). Consistent with this role of NeuroD, the levels of both NeuroD and POMC decline as a function of age (392).

The expression of NeuroD also declines during aging in the DG, which may have relevance to age-related cognitive decline. Figure 11 illustrates the distribution of NeuroD transcripts in cerebellum and hippocampus of rats aged 12 and 24 months and illustrates a marked decline in expression in the hippocampus (553). An age-dependent decrease in cell proliferation in the DG decreases during aging (see Section IV) and is associated, but not necessarily causal, with cognitive decline and depression. Mice homozygous for a deletion at the NeuroD locus provide a correlation between NeuroD expression and proliferation of the granule cell layer.
of the DG; the granule layer fails to develop in these mice (555). A decrease in hippocampal *NeuroD* expression is likely to exacerbate the effects of declining neurogenesis caused by age-related decline in GH, IGF-I, and sex steroid production. Hence, the reduced production of HLH proteins provides a link between age-dependent changes in hormonal control of gene expression with progressive functional decline of the brain.

**G. NOS and aging**

The NMDA receptors are the main neurotransmitter receptors involved in fast synaptic excitation in the CNS. Ligand activation of NMDA receptors stimulates neuronal NOS (nNOS) to enhance NO production. NO is an important signal transducer that appears to be involved in regulating the synaptic events required for pulsatile GnRH release (556). Decreased LH pulse amplitude and reduced GnRH and LH responsiveness to NMDA typify aging of the hypothalamic-pituitary-ovarian axis. In young rats, nNOS mRNA levels increase 4 h before the LH surge; however, in middle-aged rats an increase in nNOS mRNA does not precede the attenuated LH surge (368).

When the effects of aging on the NMDA and NO pathways were evaluated in BN male rats aged 1, 3, and 24 months, NMDA receptor binding and NMDAR content were 66% lower in the hypothalamus from old rats compared with adult animals. However, NOS activity in the hypothalamus was 67% higher in old rats. Paradoxically, it was speculated that excessive production of NO and its cytotoxic metabolites cause apoptosis resulting in neuronal loss. Hypothalamic nNOS content was unchanged, and the increase in NOS activity is explained by a 3.8-fold higher concentration of inducible NOS (iNOS) in 24-month-old rats compared with 3-month-old animals. The increase in hypothalamic iNOS is accompanied by higher iNOS in the frontal cortex, parietal cortex, and cerebellum. Hence, aging is associated with
higher NO production in the brain independent of the NMDA receptor and nNOS activation pathways. The marked age-dependent increase of iNOS in the CNS potentially explains age-associated impairment of GnRH secretion and neuronal loss leading to an age-related decline in cognitive function (556).

XII. Summary and Conclusions

In 1900, the population of the United States over the age of 65 was approximately 3 million, growing to approximately 35 million in the year 2000. There is a perceived need to improve the quality of life for this elderly population. The age of onset and rate of functional decline vary widely among the aging population, consistent with a regulatory role by genetic factors. We reviewed age-related changes that occur in hormones, neuropeptides, neurotransmitters, and their signaling pathways. Figure 12 provides a summary of the more significant age-dependent changes that have been measured in the CNS. At first glance, correcting or preventing these complex changes might seem insurmountable; however, by identifying the underlying pivotal regulatory factors involved and by understanding their function, appropriate intervention is feasible.

The hormones having the most significant pivotal roles in the CNS are estradiol, testosterone, cortisol, GH, and IGF-I. In addition to their interplay with neurotransmitters and the complexity of their interactions, they act upstream of the most important regulators of neuronal function. Not surprisingly, hormone replacement has been exploited to improve and maintain quality of life in aging subjects by attenuating the decline in sexual function, memory, learning, mood, quality of sleep, and physical abilities. However, today’s hormone replacement therapies, with the exception of GHS-R agonists for the GH/IGF-I axis, are not ideal because they fail to restore the physiological hormone profile of young adults. Realization of the complex interdependence of the regulatory pathways involved emphasizes the limitations of reductionism. The application of chaos and complexity theories, as introduced briefly in Section II, to biological aging should provide new tools for hypothesis testing and accelerate our understanding of the molecular endocrinology of aging of the CNS.

In the search for a CNS receptor that would restore hormone pulsatility in the elderly to the physiological profile observed in young adults, a group at Merck developed synthetic agonists and then characterized and cloned the orphan GHS-R that regulates the pulse amplitude of GH release (25, 276–278, 280). Following cloning of the GHS-R, an endogenous hormone, ghrelin, was discovered (92). The discovery of the GHS-R established a precedent for the discovery of CNS receptors: they are pivotal regulators of physiological centers affected by aging. Agonists of the GHS-R reverse physiological aging of the GH/IGF-I axis partially restore thymic function, increase bone density, are neuro- and cardioprotective, and attenuate production of inflammatory cytokines (25, 201, 280, 315, 557–559). Based on this precedent and renewed emphasis on aging research, we are optimistic that methods of endocrine intervention to delay or prevent age-related endocrine and CNS changes that precede detrimental effects on function in the elderly will be forthcoming.

To understand the biological basis of functional aging, it is critically important to combine a systems approach with reductionist methods. Most of the published work we discussed describes correlations rather than causal relationships. Our challenge is to design experimental paradigms with a biological systems approach in mind for incorporation into a neuroendocrinology model of aging. Such an approach will allow identification of pivotal points in aging pathways that lend themselves to intervention.

Acknowledgments

Address all correspondence and requests for reprints to: Roy G. Smith, Ph.D., Huffington Center on Aging, Baylor College of Medicine, One Baylor Plaza, M320, Houston, Texas 77030. E-mail: rsmith@bcm.tmc.edu

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248 Endocrine Reviews, April 2005, 26(2):203–250

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