ESTROGEN EFFECTS ON COGNITIVE AND SYNAPTIC HEALTH OVER THE LIFECOURSE

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Hara Y, Waters EM, McEwen BS, Morrison JH. Estrogen Effects on Cognitive and Synaptic Health Over the Lifecourse. Physiol Rev 95: 785–807, 2015; Published June 24, 2015; doi:10.1152/physrev.00036.2014.—Estrogen facilitates higher cognitive functions by exerting effects on brain regions such as the prefrontal cortex and hippocampus. Estrogen induces spinogenesis and synaptogenesis in these two brain regions and also initiates a complex set of signal transduction pathways via estrogen receptors (ERs). Along with the classical genomic effects mediated by activation of ERα and ERβ, there are membrane-bound ERα, ERβ, and G protein-coupled estrogen receptor 1 (GPER1) that can mediate rapid nongenomic effects. All key ERs present throughout the body are also present in synapses of the hippocampus and prefrontal cortex. This review summarizes estrogen actions in the brain from the standpoint of their effects on synapse structure and function, noting also the synergistic role of progesterone. We first begin with a review of ER subtypes in the brain and how their abundance and distributions are altered with aging and estrogen loss (e.g., ovariectomy or menopause) in the rodent, monkey, and human brain. As there is much evidence that estrogen loss induced by menopause can exacerbate the effects of aging on cognitive functions, we then review the clinical trials of hormone replacement therapies and their effectiveness on cognitive symptoms experienced by women. Finally, we summarize studies carried out in nonhuman primate models of age- and menopause-related cognitive decline that are highly relevant for developing effective interventions for menopausal women. Together, we highlight a new understanding of how estrogen affects higher cognitive functions and synaptic health that go well beyond its effects on reproduction.

I. INTRODUCTION

Estrogen receptors were first identified by radioautography in the rodent hypothalamus after the introduction of tritium-labeled steroid hormones and the discovery of the nuclear receptor mechanism of steroid hormone action (reviewed in Refs. 90, 169, 252). These receptors were tied to the regulation of reproductive behavior and neuroendocrine function. Nuclear estrogen receptors were only sparsely present outside of the hypothalamus, yet there were many actions of estrogens on brain systems and behaviors unrelated to reproduction and neuroendocrine regulation (reviewed in Ref. 133). Resolution of this inconsistency came via the identification of rapid nonnuclear actions of estrogens and identification of membrane-associated estrogen receptors, first in nonneural cells and subsequently in the brain (reviewed in Ref. 101). The hippocampal formation was the first brain region in which these membrane-associated, nonnuclear forms of the classical estrogen receptor were identified along with signaling pathways (reviewed in Ref. 134). These findings were prompted by the discovery that ovarian hormones modulate and mediate the density of spine synapses in the hippocampus of rodents and later of rhesus monkeys. Subsequent investigations revealed the presence of estrogen receptors in many, if not all, brain regions with subcellular localizations in synaptic terminals and dendritic spines, dendritic shafts, axons, mitochondria, and glial cell processes. Cell nuclear labeling in cortical areas is often in inhibitory interneurons. Further investigations of androgen and progestin receptors have revealed both nuclear and nonnuclear forms and locations of classical receptors (reviewed in Ref. 134).

The discovery that ovarian hormones regulate the turnover of a subset of synapses in the hippocampus, along with regulating signaling pathways and neurotransmitter release and actions, has triggered much further investigation showing, among other findings, that the hormonal regulation of synapse turnover is not limited to the hippocampus (re-
viewed in Ref. 133). Furthermore, the presence of a multiplicity of signaling mechanisms of estrogens at the cellular level has important implications for basic and applied neuroscience (reviewed in Ref. 134). This includes a new understanding of how peripheral hormones affect higher cognitive functions and other neural processes beyond reproduction, including mood, motor coordination, pain sensitivity, cognition, and neuroprotection from stroke as well as Parkinson’s and Alzheimer’s diseases (reviewed in Refs. 33, 133). This review considers estrogen actions in the brain from the standpoint of their effects on synapse structure and function across age and sex. We begin with a review of estrogen receptor subtypes in the brain and what is known about their changes with aging in rodent, primate, and human brain. These sections are followed by discussion on the menopausal transition and changes in structure and function of the aging brain.

II. NUCLEAR AND EXTRANUCLEAR STEROID RECEPTORS IN THE BRAIN

A. Discovery of Estrogen Receptors Outside of the Hypothalamus in Nuclear and Extranuclear Sites

Here, we review briefly the discovery and current knowledge that classical estrogen and other steroid hormone receptors exist in both nuclear and nonnuclear sites in the brain as well as in other cell types throughout the body. Early understanding of the important roles of estrogen receptors in brain regions outside the hypothalamus was stimulated by the discovery of estrogen-induced spine synapse formation in the hippocampus (42, 58, 233) (FIGURE 1). Prior to that time, a number of studies had identified nuclear estrogen receptors by light microscopy in the hippocampus and other brain regions but at much lower density than in the hypothalamus (32, 121). The demonstration of estradiol effects on hippocampal spine density was followed by light and electron microscopy studies showing the presence of steroid hormone receptors in the synapses throughout the brain.

Since the discovery of estrogen receptors (ERs) (91), and the subsequent cloning of ER α (59), the discovery of new steroid hormone receptors has continued at a rapid pace. This timeline suggests that new steroid receptors remain to be discovered along with new steroid analogs, both endogenous steroids and exogenous steroids including environmental and botanical derived agents. During this same time, the understanding of the roles of steroid receptors has expanded from its nuclear or genomic actions to a number of nonnuclear, nongenomic functions. Estrogen receptors are members of the nuclear receptor superfamily and DNA binding transcription factors. Despite extensive homology, ER α and ER β diverge in their expression and actions (see review in Ref. 29). ER α and β are differentially expressed throughout the brain (192) and are also differentially regulated by estradiol (114, 163). ER α and β are able to heterodimerize (158), suggesting that different responses are generated in the presence of both receptors (63, 64). Shifts in the ratio of ER α to ER β regulate estrogen-mediated gene transcription and memory during aging (41, 66). That many of these interactions occur outside the nucleus and connect ERs to other cellular systems, particularly second messenger systems (201), ultimately contributes to the multifaceted actions of estrogen and other steroid receptors.

B. ER α

ER α was the first steroid receptor investigated for nonnuclear actions. In regions that mediate learning and memory, mainly the prefrontal cortex (PFC) and hippocampus, nuclear estrogen receptors are sparse; however, extranuclear ER α is found throughout the cell, in both cytoplasmic and membrane locations including within dendritic spines and at the synapse (FIGURE 2).

1. Primates young versus aged

ER α mRNA and protein are widespread in the young adult human and monkey telencephalon.

In human adults, ER α mRNA is detected at multiple sites in the human telencephalon including the PFC and the hippocampal formation, especially the dentate gyrus, by microarray punch studies and in situ hybridization (161). It is yet to be determined whether ER α mRNA levels or distributions are altered with aging or are different between males and females in humans. An immunocytochemical study performed on human fetal (n = 38) and adult (n = 8) brains has revealed that protein expression of ER α is first detected in proliferating zones and the cortical plate at 9 gestational weeks, much before ER β is expressed (57). In the human hippocampus, ER α is first detected at 15 gestational weeks in the nuclei of pyramidal cells of the Ammon’s horn. During prenatal life, ER α protein is found exclusively in nuclei of cortical and hippocampal cells, while in adulthood, ER α expression is maintained but appears in both the cytoplasm and nuclei of pyramidal neurons (57). An equal number of brains from male and female adults (n = 4 for each sex; age range, 17-52 yr old) were included in this study, and no significant sex differences were observed in the cell number, immunolabeling intensity, or distributions of ER α in the cortex and hippocampus (57). As the samples were obtained from young to middle-aged adults, it is unknown whether ER α protein expression is altered in elderly people.

The distribution patterns of ER α mRNA in macaque monkeys is similar to what is observed in humans (162). ER α mRNA in monkeys is detected in many areas that lack
prominent nuclear labeling (164, 177), and this difference is reconciled by the understanding that not only does ER\textsubscript{α}/H9251 shuttle between the cytoplasm and nucleus, it also resides and acts at extranuclear locations (16). Nuclear ER\textsubscript{α}/H9251 immunoreactivity is sparse in the hippocampus and cortex, although it is located both pre- and postsynaptically in the PFC of young and aged monkeys (143) (FIGURE 2). A recent study demonstrated that the hypothalamus in aged rhesus monkeys retained youthful expression levels of ER\textsubscript{α}, GPER1, and progestin receptor (PR) protein and neuronal levels of these receptors were responsive to estradiol treatment in both young and aged monkeys (151). In ovariectomized female monkeys, estradiol treatment along with the presence of postsynaptic ER\textsubscript{α} improved cognitive performance (222), supporting a role of ER\textsubscript{α} in tasks mediated by the PFC. Nonneuronal expression of ER\textsubscript{α} may also contribute to estrogen actions, including injury-sensitive expression of ER\textsubscript{α} in glial cells (15).

2. Rodents young versus aged

Expression of ER\textsubscript{α} mRNA is widespread throughout the rodent telencephalon (192) (Allen Brain Atlas). Within the hippocampus, adult rats and mice show similar patterns of...
ER α expression in the hippocampus varies across the estrous cycle and is mostly conserved between young adult female rats and mice. In young adult mice, extranuclear ER α levels in the hippocampus peak when circulating estrogen levels are lowest either during diestrus or after ovariectomy (140, 142). However, ER α expression in rat hippocampal dendritic spines is significantly higher in proestrus females with high estrogen levels compared with males and diestrus females (182). Estradiol has no effect on the frequency of ER α-containing synapses in young or aged females, but this frequency decreases with age (2) [FIGURE 3]. In addition, young ovariectomized rats receiving estrogen replacement have less ER α in nonsynaptic compartments within presynaptic terminals and postsynaptic spines compared with vehicle-treated rats [FIGURE 3]. In male rats, the density of ER α-expressing hypothalamic cells is retained in middle-aged compared with young rats, but the density decreases in response to circulating testosterone in both young and middle-aged rats (240).

In mice, ER α protein is also expressed in the cortex of young adults but decreases with age (22). This is consistent with the high mRNA levels seen early in development that decline with age (see review in Ref. 232).

C. ER β

ER β mRNA is transcribed from a different gene than ER α (217). First cloned in 1996, ER β has ligand binding properties similar to ER α (109, 110); however, ER β may have both ligand-dependent and -independent actions (216). Splice variants that alter the DNA-binding domain (27, 144) and NH2 terminus (12) are also detected.

1. Primates young versus aged

ER β mRNA is expressed in human hippocampus and neocortex, but distribution patterns are distinct from those of ER α (76, 161). For example, ER α mRNA levels are highest in the amygdala and hypothalamus, while ER β mRNA is most abundant in the hippocampal formation, claustrum, thalamus, and cerebral cortex (161, 162). In deeper cortical lamina, ER β mRNA is found in layers V–VI, while ERα is localized specifically to layer V (161). In humans, ER β protein is first detected at 15 gestational weeks in proliferative zones, 6 wk later than ER α expression begins (57). At 17 gestational weeks, ER β is expressed in the nuclei of cortical plate cells, pyramidal cells of the Ammon’s horn, and dentate gyrus granule cells (57). At 25 gestational weeks, ER β immunostaining is widely detected in cortical neurons, with intense labeling present in the nuclei and weaker labeling in the cytoplasm. At birth and into adulthood, much of the nuclear labeling is lost and ER β is found predominantly in the cytoplasm of cortical and hippocampal pyramidal neurons (57). As with ER α, ER β levels and distributions are comparable across males and females. It is

ER α protein expression, although the levels of nuclear and extranuclear receptors vary (140, 142). Nuclear ER α is concentrated in the hilus of the dentate gyrus and CA1 stratum radiatum in both males and females (229). In dorsal hippocampus, ER α-labeled nuclei are found predominantly in interneurons (150), although in ventral hippocampus, nuclear ER α is also found in pyramidal cells (75).

Extranuclear ER α is found in most cellular compartments in young adult female and male rat hippocampus and neocortex (140, 147). ER α is found in both neurons and glial cells in the hippocampus (140, 142, 195). Pyramidal neurons in the hippocampal CA1 region have spine synapses that contain ER α (2, 147); these neurons also receive inputs from cholinergic terminals that contain ER α (213).
currently unknown whether ER β protein expression is altered in elderly people.

In monkeys, mRNA expression of ER β is present in the hippocampus, PFC, and the hypothalamus, among other brain regions (164, 177). Due to the small sample sizes in non-human primate studies, there was limited power to detect age-related effects or sex differences in ER β transcripts in the monkey. In addition, age- and sex-related differences in ER β protein expression and subcellular distribution have not yet been defined in the monkey model.

2. Rodents young versus aged

ER β distribution in rodents parallels but does not completely overlap with ER α (192). No nuclear ER β is detected in hippocampal primary cells or interneurons in young adult rats (139), although it does occur in newly born neurons in the hippocampal dentate gyrus (DG) of neonatal rats (82). In mice, sparse nuclear ER β immunoreactivity is found in the hippocampus (142).

ER β is found in pre- and postsynaptic compartments in both male and female rat (139) and mouse hippocampus (142). In the hippocampal CA1 region, postsynaptic ER β predominates, although it is also found presynaptically. There is a reduction in synaptic ER β during middle age; however, age does not affect the ability of estradiol to increase synaptic ER β labeling, particularly near the synaptic cleft (227) (FIGURE 3).

Estrogen regulates ER β in young adult rats and mice, in that levels are highest during estrus and diestrus (142). ER β expression decreases with age in the hippocampus but persists in the cortex (243). Estrogen regulation of ER β mRNA declines in middle age (231); however, postsynaptic protein expression does not decline in aged female rats and retains the ability to be upregulated by estradiol replacement (227). In mice, ER β protein expression in the cortex also decreases with age (22, 188).

The degree to which ER α and ER β are colocalized remains unclear. The similar actions of ER α and ER β agonists on hippocampal targets suggest that they may be present in the same cells if not the same cellular compartments (224), but direct protein colocalization has not been established. In the diencephalon, the Bed nucleus of the stria terminalis, medial amygdala, and preoptic area
contain neurons that coexpress ER α and ER β mRNA. In contrast, in the arcuate nucleus, cortical amygdala, and ventromedial nucleus, both receptors are expressed but rarely colocalized (193).

D. Membrane ERs

Rapid nongenomic estrogen actions can occur through extranuclear ER α and β as well as new classes of membrane ERs. G protein-coupled estrogen receptor 1 (GPER1) is a membrane ER, identified in cancer studies but is also shown to have widespread expression throughout the body and central nervous system (160, 172). It is linked to rapid membrane-initiated estrogen actions (39). GPER1 expression is regulated by insulin in cancer cells (31); however, it is currently unknown whether similar effects are seen in the brain.

In monkeys, GPER1 mRNA is present in the forebrain as well as in luteinizing hormone-releasing hormone (LHRH) neurons where it mediates fast release (156). Human GPER1 mRNA expression is also detected in the hippocampus and cortex (available from http://human.brain-map.org) (76). In the monkey hypothalamus, GPER1 is localized to cell processes and perikarya, and the number of GPER1-containing cells in the periventricular area increases with aging but is unaltered with estradiol treatment (151). A study examining the effects of aging and estradiol replacement on GPER1 distribution in monkey PFC synapses is currently underway.

In the rodent, GPER1 is described in rat and mouse hippocampus and cortex (65, 77, 87, 225). It is present on the plasma membrane of hippocampal pyramidal cells (44, 128) and medial PFC neurons (6). Electron microscopic studies have revealed that GPER1 is localized to neuronal perikarya, endoplasmic reticulum, dendritic shafts, synaptic and extrasynaptic sites on dendritic spines, and axon terminals in the mouse hippocampus (225). The overall abundance of GPER1 in hippocampal subregions (CA1, CA3, and dentate gyrus) is comparable between male and female mice. However, estrous cycle phase influences GPER1 distribution such that proestrous females with elevated estrogen levels have more GPER1-containing axonal profiles than estrous females (225). In rat hippocampal dendritic spines, GPER1 binds to the synaptic scaffolding protein postsynaptic density-95 (PSD-95) (5). GPER1 interactions with another synapse-associated protein, SAP97, have also been observed in mouse hippocampal synapses (225).

E. PRs

Estrogen-sensitive genomic and nongenomic PRs are present in the hippocampus, largely in nonnuclear locations. In mouse and rat, genomic PR expression changes across development such that in adults it is found largely in extranuclear sites (142, 226). Nuclear labeling detected early in postnatal development in rats (173) does persist in a small number of interneurons in adult mice (142). Extranuclear PR expression found in pre- and postsynaptic compartments of excitatory neurons and glial cells is inducible by estrogen (142, 226). Expression of PR mRNA is found in monkeys and humans (76), but protein localization of extranuclear sites remains to be identified.

Nongenomic membrane PRs discovered in the last two decades are classified into two families. The first is the class II progestin and adipoQ receptor (PAQR) family called membrane progestin receptors (mPRs) and includes the following: mPRα (PAQR7), mPRβ (PAQR8), mPRγ (PAQR5), mPR6 (PAQR6), and mPRε (PAQR9) (see review in Ref. 167). The second is the b5-like heme/steroid-binding protein family that includes progesterone receptor membrane component 1 (PGRMC1), PGRMC2, neudesin, and neuferricin. Receptors from the PGRMC1/S2R family and mPRs are present in forebrain structures important for neuroendocrine regulation and other nongenomic effects of progesterone in rodents (136, 167). Estrogen regulates membrane PR expression in the hypothalamus (118, 254) and hippocampus (9). PGRMC expression is also detected in human and monkey cortex and hippocampus (76).

F. Steroid Receptors Beyond ER and PR

ER and PR belong to a superfamily of nuclear receptors that include other steroid receptors, including androgen, glucocorticoid, mineralocorticoid, vitamin D, retinoic acid, thyroid hormone, and orphan receptors/transcription factors (see reviews in Refs. 24, 218). Evidence supports actions of these receptors in hippocampal function. Androgens regulate hippocampal synaptic plasticity through dihydrotosterone (DHT)-sensitive mechanisms in male rodent hippocampus (117, 199) and androgen receptors are present in rodent (135, 137), non-human primate (1, 26, 183), and human (185) forebrain. Corticosterone acts via glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) to exert genomic and nongenomic actions (see review in Ref. 148). While translocation of cytosolic MRs and GRs to the nucleus mediates gene transcription (179), nongenomic, rapid actions can be mediated by synaptic GR and MR in the hippocampus and amygdala (95, 171, 247) that modulate synaptic plasticity (93, 247). Glucocorticoids also regulate vitamin D receptor transcription in mice (83). Vitamin D receptors present in the rat hippocampus (112) have been attributed to regulation of plasticity (92) and steroid-mediated outcomes (see reviews in Refs. 38, 47).
III. ESTROGEN AND PROGESTIN ACTIONS ON RAPID SIGNALING THAT AFFECT SYNAPTIC FUNCTION

A. Rapid Nongenomic Actions of Estrogens and Other Steroid Hormones

There is now substantial evidence for estrogen activation of signaling pathways at extranuclear sites that may also have indirect effects on gene expression in addition to the rapid effects on many aspects of cell function. Beginning with work on rapid effects induced by iontophoretic application of 17β-estradiol (102), and continuing with work on neural and nonneural systems (101), it is now evident estradiol has rapid actions via receptors that in many cases are posttranslationally modified forms of the classical ER that reside in parts of the cell outside of the nucleus, including mitochondria (155), presynaptic terminals (115, 214), postsynaptic spines (2, 140), and glial cell processes (142). Caveoli associated with, but not part of the cell membrane, are involved as a reservoir of classical ERs that are activated by estradiol near the cell surface (18). An exception to the classical ERs is the GPER1 (111, 208), which associates with PSD-95 in the dendritic spine and appears to couple estrogen action to membrane progestin receptors, 5HT1A receptors, and corticotrophin releasing hormone (CRH1) receptors (35). Finally, it should be noted that rapid, nongenomic actions are recognized along with classical nuclear actions for all classes of steroid hormones: progestins (142, 230), androgens (209), glucocorticoids (210, 211), mineralocorticoids (98), and vitamin D (86).

B. Estrogen Signaling and Synapse Formation

Rapid estrogen signaling leads to axospinous synapse formation in the hippocampus through at least two pathways

(FIGURE 4). The first concerns estrogen stimulation of filopodia formation in neurons via a pathway involving estrogen-stimulated LIMK-1 phosphorylation and cofilin phosphorylation, which releases actin from cofilin inhibition so that it can polymerize and facilitate filopodia formation that may develop into mature spines (250). The second involves rapid stimulation of translation of the synaptic scaffolding protein, PSD-95, before any transcription of PSD-95 mRNA occurs; this is mediated by phosphatidylinositol 3-kinase activation of Akt, leading to phosphorylation and inhibition of a protein translation regulator, 4E-BP1 (4, 253).

Before these pathways are engaged, estrogen treatment increases glutamate NMDA receptor binding and expression (40, 228) and increases NMDA receptor-mediated synaptic input (239). In addition, blocking NMDA receptors during estrogen treatment in ovariecтомized rats prevents the estrogen-induced spine synapse formation in the CA1 region of the hippocampus (237). The upregulation of NMDA receptors in the CA1 region of female rat hippocampus by estradiol is indirect, in that it is blocked by M2 muscarinic receptor antagonists and mimicked, bypassing estradiol, by elevating cholinergic activity (30), and this appears to involve inhibition of the inhibitory GABAergic input to CA1 pyramidal neurons (184). In this connection, it is important to note that estrogens upregulate the activity of choline acetyltransferase (123), the enzyme responsible for acetylcholine synthesis, as well as potassium-stimulated acetylcholine release (54), and the latter may be mediated by nongenomic ER α in cholinergic terminals (214).

C. Rapid Estrogen Actions on Excitability

Estrogen induces spinogenesis and synaptogenesis upon the enhancement of fast glutamatergic transmission and long-term potentiation (LTP). Actin dynamics within the synaptic terminal also play important roles in the structural plas-
ticity of the postsynaptic spine. In hippocampal slices, estrogen increases spine concentrations of filamentous actin and strongly enhances actin polymerization in association with LTP, and it does so by activating the small GTPase RhoA and phosphorylates and thus inactivates coflin, a downstream target of RhoA (108). In this study, an antagonist of RhoA kinase (ROCK) blocked the effects of estrogen on synapses. According to these authors (108), estrogen does not, however, strongly affect a second LTP-related pathway, involving the GTPases Rac and Cdc42 and their effector p21-activated kinase, which may explain why its acute effects are reversible. Finally, these authors note that “...ovariectomy depressed RhoA activity, spine cytoskeletal plasticity, and LTP, whereas brief infusions of estrogen rescued plasticity, suggesting that the deficits in plasticity arise from acute, as well as genomic, consequences of hormone loss” (108).

D. Estrogen Actions on Synapse Formation Across Species

While the seminal studies on spine induction in the hippocampus were performed in rats, there have been more recent studies showing that related processes occur in mice as well as in monkeys. In mice, we have seen that estrogen and estrogen-inducible progesterin receptors are found in the hippocampus in the same nongenomic and genomic sites as in the rat (142). A Golgi impregnation study showed that estrogen treatment had no overall effect on spine synapse density in the CA1 region of the dorsal mouse hippocampus, but caused an increase in the number of spines with mushroom shapes that are associated with high synaptic efficacy and strength (116). Radioimmunocytochemistry and silver-enhanced immunocytochemistry were used to show that the immunoreactivity for postsynaptic markers (PSD-95 and spinophilin) and a presynaptic marker (synaptophysin) was enhanced by estrogen treatment throughout all fields of the dorsal hippocampus. Moreover, estrogen treatment enhanced performance on the object-placement test, a measure of spatial episodic memory that is dependent on the hippocampus (116). Taken together, the morphological and radioimmunocytochemistry results suggest a previously uncharacterized role of estrogen in synaptic structural plasticity that, consistent with the literature in rat hippocampus, may be interpreted as a facilitation of the spine-maturation process and may be associated with enhancement of hippocampus-dependent memory.

The same signaling pathways activated by estrogen in the rat hippocampus are also triggered in the female mouse hippocampus. In female C57BL/6 mice, activation of Akt, LIMK, and the tropomyosin-related kinase B receptor (TrkB) neurotrophin receptor is increased in dorsal hippocampus during proestrus when estradiol levels peak (204). Estrus cycle stage also modulates expression of the pre- and postsynaptic markers synaptophysin and PSD-95. Despite these changes in synaptic markers, cycle phase does not regulate spatial memory performance in the object placement test. Interestingly, this type of spatial memory is sensitive to the complete absence of ovarian hormones. Together these results suggest that ovarian estradiol and progesterone through activation of specific signaling pathways modulate actin remodeling, synapse formation, and ultimately cell excitability.

Because the TrkB receptor that binds to the brain-derived neurotrophic factor (BDNF) is important for the synaptic plasticity underlying hippocampal-dependent learning and memory, the precise location of TrkB activation is likely important for its specific actions. An electron microscopic study revealed that phosphorylated-TrkB (pTrkB), the activated form of TrkB, is localized primarily to axons and axon terminals, but is also present in dendritic shafts and spines of hippocampal neurons (202). There was also abundant pTrkB in microglial profiles identified using immunofluorescence and confocal microscopy. In high estradiol states in proestrus females, pTrkB in axons and glial cells are more abundant in the hippocampal CA1 stratum radiatum than in low estradiol states (estrus and diestrus females and males). Thus it would appear that presynaptic TrkB is positioned to modulate estradiol-mediated and BDNF-dependent synaptic plasticity, and they also suggest that there may be a novel role for TrkB in microglial function in the neuroimmune system.

E. Interactive Effects of Progestin on Synapse Formation and Withdrawal

Progestin can interact with the effects of estrogen to exert a biphasic effect on synapse number in the hippocampal CA1. After estrogen increases spine density, subsequent progesterin administration further boosts spine density for 6 h, but this increase is followed by a rapid reduction in spines (238). By 18 h after progesterin administration, spine density is decreased to levels observed after ovariectomy. Similar to estrogen, progesterin activates mitogen-activated protein (MAP) kinase and Akt signaling (62, 138, 198). However, work in a mammary tumor cell line suggested that a direct interaction between PR and ER may be necessary for progesterin to induce MAP kinase signaling (138). These receptor interactions may underlie the initial increase in synapse number detected after progesterin treatment. After a prolonged period following progesterin administration, ER levels drop and other PR actions can facilitate the withdrawal of spines and reduction in synapse number. Together, estrogen and progesterone act together to promote both the induction and ultimate retraction of spines through nongenomic mechanisms as well as genomic actions leading to PR expression. While the mechanism for synapse downregulation is presently unknown, it is likely to involve novel nongenomic actions based on the predominant nonnuclear localization of PR in rodents.
F. Roles of Different Estrogen Receptor Types

There are two primary nuclear/nonnuclear estrogen receptors, ER α and ER β, and they both contribute to signaling and synaptic plasticity. ER α and β knockout mice and wild-type littermates were used to investigate their respective roles in estradiol effects on hippocampal plasticity and learning (201). After 6 h of estradiol administration, there was increased immunoreactivity for phosphorylated Akt throughout the hippocampal formation, and after 48 h of estradiol treatment, there was increased immunoreactivity for the pTrkB receptor. Estradiol administration in ER α and ER β knockout mice did not result in increased phosphorylation of Akt and TrkB. However, there were different effects of estradiol in ER α and β knockout mice upon PSD-95 protein levels and BDNF mRNA. Overall, it thus appears that estradiol acts through both ER α and β, albeit somewhat differently, throughout the hippocampal formation, and the different actions of 6 and 48 h of estradiol suggest that multiple estrogen signaling mechanisms participate in hippocampal synaptic structural and functional plasticity.

One of the advantages of using mouse models is the possibility to study alleles of common genes, such as is the case with the BDNF polymorphism and the influence of this polymorphism on estrogen actions related to cognitive function and affective behavior. The effects of the common human variant BDNF Val66Met were examined in cycling female mice (203). Mice homozygous for the BDNF Met variant had increased anxiety behavior, impaired memory, and increased expression of BDNF and its receptor TrkB in the hippocampal formation. The magnitude of fluctuation in spatial memory, hippocampal Akt phosphorylation, and PSD-95 protein expression across the estrous cycle was also significantly altered in BDNF Met homozygous mice. The BDNF Val66Met variant should therefore be considered as one of the candidates that mediates genetic differences in ovarian steroid-related behavioral changes and disorders.

IV. INTERACTIVE EFFECTS OF ESTROGEN AND AGING

A. Introduction

The findings reviewed above provide ample support for a prominent role for estradiol and multiple ERs in the synaptic underpinnings of cognitive functions mediated by the hippocampus and PFC. In addition, as discussed above, there are age-related alterations in ERs that vary across regions and species. Thus there is a critical convergence of endocrinology and brain aging with respect to the role played by estradiol and ERs in cognitive functions such as memory and learning. It is widely recognized that cognitive functions mediated by the medial temporal lobe and PFC are highly vulnerable to aging in rodents, monkeys, and humans (145), yet the cause of the heightened vulnerability of these regions is not well understood. It is of particular importance to women’s health to determine the degree to which alterations in circulating estradiol and other endocrine events associated with menopause mediate age-related decline, since women in the developed world can expect to live one-third of their lives after the menopausal transition. The aged female rhesus monkey has proven to be a highly valuable model for the investigation of the synaptic events that are sensitive to both estradiol and aging in an effort to tease out the particular role of estradiol in age-related synaptic alterations that affect cognitive performance. Below, we review these findings and their translational relevance to cognitive aging in women.

B. Menopause in Women and Its Association With Cognitive Functions

Menopause is characterized by the permanent cessation of menstrual cycles and the loss of ovarian hormone production, signaling the end of a woman’s reproductive phase. The menopausal transition is accompanied by changes in hormonal levels, including ovarian ones. While widely variable across women, estradiol starts to decrease 2 yr prior to the final menstrual period and reaches barely detectable levels 2 yr after the final period (21). Women undergoing the menopausal transition often report memory problems, among other symptoms. It has become increasingly evident that an interaction exists between age-related cognitive decline and endocrine changes (67, 78).

Menopause can occur naturally, or in cases of medical necessity, can also be induced surgically. Removal of ovaries in women, referred to as oophorectomy, is associated with an increased risk in cognitive impairment and dementia later in life (181, 190). Indeed, estrogen replacement in these women can improve cognitive functions (79, 170). In contrast, natural menopause in women has a smaller magnitude of effect on cognitive skills. A cross-sectional study involving 326 women found no significant differences across different menopausal statuses (early and late menopausal transition, and early and late postmenopause) in episodic verbal memory as measured by a supraspan word list recall task, although their performance was not compared directly with that of premenopausal women (80). In the Study of Women’s Health Across the Nation (SWAN) study, 2,362 women (42–52 yr old) were enrolled and their cognitive performance was followed for 4 yr (60). While verbal memory (East Boston Memory Test scores) improved with time in premenopausal and postmenopausal women, this enhancement was not observed in women undergoing early or late perimenopause, suggesting that the ability to learn may be temporarily compromised during the menopausal transition (60). Mild deficits in concentration...
and processing speed are also observed in women undergoing the menopausal transition (106). More recently, the effect of menopause on verbal memory was assessed in a 14-yr, longitudinal, population-based study involving 403 women from the Penn Ovarian Aging Study cohort (36). As all women were recruited when they were premenopausal, verbal memory scores (from the Buschke Selective Reminding Test) could be precisely followed through, and compared across, individual phases of the menopausal transition. Independent of the effects of normal aging, delayed verbal recall declined early in the menopausal transition while immediate recall declined late in the transition, although the magnitudes of change for both were modest (36). Collectively, it appears the abrupt loss of estrogen with oophorectomy has more severe consequences to cognitive functions than the gradual decline of estrogen seen in natural menopause (21).

Menopause induced surgically can also have different physiological consequences from that occurring naturally. In women, serum levels of estradiol and testosterone are lower in oophorectomized women compared with those who have undergone natural menopause (107). In addition, recent work has shown that ER β expression in the monkey hippocampal subiculum is upregulated in natural menopause, while ovariectomy is associated with increased aromatase (enzyme required for estrogen synthesis) expression without any changes in ER β levels (84). These studies and others suggest that the morphological and molecular consequences of menopause may vary depending on the different trajectories of endocrine and physiological changes that occur with natural versus surgical menopause.

C. Clinical Trials Examining the Effects of Hormone Replacement Therapy on Cognition in Postmenopausal Women

Clinical studies that tested the efficacy of hormone replacement therapy in protecting cognitive functions have produced inconsistent findings compared with those from animal models (8, 191). The largest trial ever undertaken was the Women’s Health Initiative Memory Study (WHIMS), a randomized, double-blind, controlled clinical trial, which tested the effects of estrogen plus progesterin on the incidence of dementia and mild cognitive impairment in 4,532 postmenopausal women (194). This trial concluded that not only did hormone therapy fail to improve cognitive function, but it increased the risk for probable dementia in postmenopausal women aged 65 years and older (194).

This WHIMS study has a major caveat that may partly explain its findings that are contrasting from other trials in women and from work in animal models. The women enrolled in the WHIMS clinical trial were on average 72 yr old at the time of hormone therapy initiation (194), ~15 yr after menopause. This study and other work in animal models have led to a “critical period” hypothesis, which proposes that there is a narrow window of opportunity in which hormone therapy can be initiated for it to be beneficial (53, 125, 191, 251). Indeed, initiation of hormone replacement therapy during perimenopause or shortly after menopause can have beneficial effects on cognitive function. In a cross-sectional study involving 428 subjects, women receiving hormone replacement therapy before the age of 56 scored higher on a test of global cognitive function (the Mini-Mental State Examination; MMSE) and had better attention compared with those who started therapy after 56 yr of age (124). This same study revealed that women who started therapy after the age of 56 performed worse on the MMSE compared with those who never used hormone therapy (124), suggesting that late initiation of hormone therapy can have detrimental effects. In another study, women who initiated hormone replacement therapy at a younger age (mean age of 48.8) and terminated treatment after an average of 5.2 yr, scored higher on the MMSE and exhibited better mental flexibility compared with those who never received hormone replacement or those who continued treatment for a longer duration (average of 14.30 yr) (130).

With regard to more specific cognitive functions, verbal memory has received particular attention and has been assessed in postmenopausal women receiving hormone replacement therapy. A longitudinal study that enrolled 727 elderly women showed that a subgroup of 81 women who had initiated hormone therapy around the time of menopause scored higher than untreated women on tests of verbal memory and fluency (88). When follow-up tests were administered a few years later, verbal scores had improved in the past-hormone-users, while declined in the never-users (88). This improvement in verbal memory with hormone replacement therapy has also been observed in women who underwent oophorectomy and received estrogen treatment soon after the surgery (219).

It is important to note that other studies in women have found inconclusive or negative effects of hormone replacement on cognitive function, even when the treatment was initiated soon after menopause (97, 126). Factors other than the timing of treatment initiation, such as the hormone formulation, treatment schedule, and administration route (oral versus transdermal), likely also contributed to and confounded the clinical outcomes. One critical consideration is the effect of the synthetic progestin (medroxyprogesterone acetate; MPA) that was often given in combination with estrogen, such as in the WHIMS. Unlike endogenous progesterone, MPA can antagonize the neuroprotective effects of estradiol in hippocampal cell cultures (153). Potential detrimental effects of the combination formulation (conjugated equine estrogens plus MPA) on verbal memory have been observed in
postmenopausal women, even when treatment was started shortly after menopause (average age, 52 yr old) (126).

A greater consensus is seen in conclusions derived from reports where hormone replacement was initiated later in life relative to the age of menopause. Many of these show that hormone therapy is ineffective or detrimental to cognitive function (37, 89, 178, 194, 241), further supporting the “critical period” hypothesis for its effectiveness.

In addition to the wide-ranging effects exerted by different hormone treatments, the brain itself has the capacity to synthesize small amounts of estradiol “on demand” through aromatase, which is present in cortical and hippocampal neurons (85, 242). Evidence from models of ischemia suggests that traumatic challenges can activate the aromatase system, promoting extragonadal estradiol synthesis that is critical for neuroprotection (132). This same capacity of local synthesis of estrogen in the brain is likely called into play during menopause when circulating estrogen levels fall dramatically. Finally, there may be endocrine factors other than circulating estradiol that impact the symptoms associated with the menopausal transition, including effects on cognition. Recent data from both women and rhesus monkeys highlight the importance of adrenal steroids during the menopausal transition, which can vary greatly across individuals, have estrogenic bioactivity, and are also responsive to hormone treatments (28, 113, 131). Whether changes in circulating adrenal steroid levels in the face of decreasing estradiol affect synaptic health or cognition is not known at this time, but it warrants investigation.

D. Nonhuman Primates as a Model of Menopause

Many factors can influence cognitive outcomes of hormone replacement therapy in women. Other than the various formulations, schedules, and routes of the therapy regimen, participants may have had variable levels of educational attainment, socioeconomic status, and diet; some may also have had undiagnosed medical conditions or prodromal neurodegenerative disorders. The use of an animal model allows investigation of the effects of hormone treatment on cognitive function while controlling for, or avoiding altogether, some of these complex variables. Female macaque monkeys are valuable models of menopause, because their reproductive physiology and patterns of endocrine senescence closely resemble those of women (56, 129, 152, 221, 234). For example, premenopausal monkeys have 28-day menstrual cycles with ovarian hormone fluctuations similar to those of women (55). This is in contrast to the 4-day estrous cycles rodents experience. And most importantly, monkeys undergo a low-estrogen menopause in a manner similar to women (55, 152, 221).

In addition to these similarities in reproductive physiology, macaques are also ideal for studying menopause-related cognitive symptoms. Their brain anatomy, neuronal structures, and neuronal gene expression closely resemble those of women (72, 120, 168). Furthermore, their cognitive functions can be assessed using the same or similar tests that assess the rich repertoire of cognitive capacities in humans (149, 207). In contrast to humans, macaques do not develop the neuropathological symptoms of Alzheimer’s disease (48, 105, 165). Therefore, menopause-related cognitive symptoms can be assessed in macaques without the confounding factors inherent to neurodegenerative diseases.

E. Effects of Menopause in the Monkey Hippocampus

The menopause-related cognitive impairment seen in women is also evident in female nonhuman primates. Compared with premenopausal monkeys, naturally peri/postmenopausal female monkeys exhibit deficits in recognition memory (70, 180), a function reliant on the integrity of medial temporal lobe structures that include the hippocampal formation and perirhinal cortex (141, 175, 206). This menopause-related impairment is significant even after accounting for the effect of chronological age (70, 180). In surgically menopausal aged ovariectomized monkeys, cyclic estradiol replacement consisting of a single injection every 3 wk, a regimen closely mimicking the natural fluctuations of estrogen, modestly improves recognition memory scores (176).

The dentate gyrus (DG) is a subregion of the hippocampus that has received the most attention with regards to memory decline in normal aging. The DG receives perforant path input from the entorhinal cortex (FIGURE 2), and this projection is now thought to be one of the most vulnerable to the effects of normal, nonpathological aging (146, 233, 246). Indeed, energy metabolism in the DG, but not the other hippocampal subregions, declines with normal aging and is correlated with worse recognition memory scores (200).

In the rhesus monkey, hippocampal volume and neuronal numbers remain stable with chronological age (23, 104, 127, 166, 174, 186, 187). However, in the monkey DG, natural menopause results in a lower density of perforated synapse spines, which is correlated with worse recognition memory (70). The menopause-related decrease in perforated synapse spines is significant after accounting for the effect of age, and no other synaptic parameters examined, including total synapse density or synapse size are different across groups designated by age or menses status (70). Perforated synapses are an important class of synapses for memory functions (50, 51) and are morphologically characterized by a discontinuity in the postsynaptic density (a
hole, slit, or complete segmentation in the postsynaptic density plate) (52). These synapses are often present on large mushroom spines and are proposed to be a structural correlate of synaptic efficacy (52, 61, 213). In fact, they have an exceptionally high level of glutamate AMPA receptors (45, 71), a correlate of synaptic strength (81, 103). This study suggests that the decrease in estrogen that accompanies menopause may impact memory, in part, by the selective loss of this highly efficient and exceptionally strong synaptic subtype. Interestingly, the synaptic dynamics in PFC are quite different from the hippocampus, with the highly plastic thin spines representing the primary target of estradiol.

In a related study in the monkey DG, types of synaptic connections have been compared between young and aged, premenopausal and naturally postmenopausal monkeys (69). Normal aging resulted in an increase in boutons making no synaptic contact (nonsynaptic boutons; NSBs) and a loss of boutons forming multiple synaptic contacts (MSBs) (FIGURE 5A). MSBs are presynaptic boutons that are synaptically connected to more than one dendritic shaft or spine and can act as a morphological substrate to enhance coupling between presynaptic and postsynaptic neurons (49, 74, 212, 245). While natural menopause did not cause a further increase in NSBs or a further decline in total MSBs, it was associated with a loss of a distinct class of synapses. Compared with aged premenopausal monkeys, postmenopausal monkeys exhibited a lower incidence of MSBs forming perforated synapses (69) (FIGURE 5A). MSBs forming perforated synapses may represent a special class of synaptic connection that possesses the strongest synaptic strength and the ability to simultaneously depolarize multiple postsynaps.

**FIGURE 5.** Natural menopause is associated with a selective loss of complex and strong synaptic connections in the monkey hippocampus. A: schematic diagrams illustrating shifts in the types of synaptic connections in the monkey dentate gyrus with aging and menopause. Aging results in an increase in nonsynaptic boutons (NSB) and a decrease in multiple-synaptic boutons (MSB). While menopause does not result in changes in the proportions of NSBs or MSBs, it is associated with a selective decrease in MSBs forming one or more perforated synapses, a type of connection that possesses strong synaptic strength and the ability to simultaneously activate multiple postsynaptic neurons. Arrows indicate potential shifts in bouton subtypes with aging and menopause. Blue, axonal bouton; red, postsynaptic density; yellow, dendritic spine. B: a diagram illustrating the relationships between types of synaptic connections in the monkey dentate gyrus and recognition memory performance. Recognition memory scores positively correlate with the incidence of MSBs forming one or more perforated synapses and inversely correlate with NSB frequency. Serial sections from the monkey dentate gyrus were reconstructed using the free software developed by Dr. Kristen Harris and her laboratory. Reconstruct, version 1.1.0.0; http://synapses.clm.utexas.edu. Diagrams and 3-dimensional reconstructions were made based on findings from Hara et al. (69).
apical neurons. Indeed, the prevalence of this class of synapses positively correlates with recognition memory scores in monkeys (69) (FIGURE 5B). Together, these studies suggest that one way estrogen exerts its procognitive effects may be via preservation of this special class of DG synapses that are associated with better memory.

**F. Effects of Aging and Estrogen in the Monkey PFC**

1. **Nonhuman primate models mimicking natural estrogen cycles**

The prefrontal cortex is critical for executive functions, including working memory and cognitive set-shifting (7, 43, 72). The causes of menopause-associated cognitive symptoms in women are likely to be multifactorial; however, estrogen deficiency is believed to be one of the culprits for deficits in cognitive functions observed by the PFC (100). In fact, postmenopausal women receiving hormone replacement therapy outperform women receiving no treatment on tests of executive functioning (100). Some of these PFC-reliant cognitive functions are also restored by hormone replacement therapy in nonhuman primates. For example, in aged ovariectomized female monkeys, the cyclic estradiol regimen that closely mimics the natural fluctuations of estrogen in premenopausal monkeys reverses the age-related impairment in working memory (176). Estrogen replacement also improves executive functions in middle-aged ovariectomized monkeys, as measured by performance on a set-shifting task (220).

Improvements in cognitive function with cyclic estradiol treatment are accompanied by alterations in neuronal morphology in dorsolateral PFC area 46, a cortical region strongly implicated in working memory (43, 122). The effects of estrogen on area 46 pyramidal neurons were experimentally tested in a cohort of young and aged ovariectomized female monkeys receiving cyclic unopposed estradiol or vehicle injections (67). In the delayed response test of visuospatial working memory, young monkeys receiving cyclic estradiol or vehicle, and aged monkeys receiving cyclic estradiol perform equally well (67, 176). Reconstructions of pyramidal neurons in PFC area 46 revealed that cyclic estradiol increases the density of the highly plastic small thin spines in both young and aged monkeys (67) (FIGURE 6). Although young vehicle-treated monkeys exhibit lower spine density, their total dendritic length and branch numbers are increased such that the total number of spines per neuron is equivalent to the young monkeys treated with estradiol (67) (FIGURE 6). This dendritic expansion may be a compensatory mechanism that protects cognitive function in young monkeys experiencing an abrupt loss of estrogen. In contrast, only vehicle-treated aged ovariectomized monkeys exhibit significant working memory impairment, and this deficit occurs concomitantly with a dramatic decrease in area 46 spine density in the absence of a compensatory extension of dendritic arborization (67) (FIGURE 6). Indeed, the combined effects of aging and estrogen deprivation result not only in decreased spine density, but also a significant shift towards larger spines such that aged ovariectomized vehicle-treated monkeys are left with a mere one-third of small plastic thin dendritic spines compared with what is observed in young estradiol-treated monkeys (67, 99) (FIGURE 6).

As discussed above, estrogen has many sites of action, and there are likely to be multiple mechanisms and pathways that underlie its beneficial effects on cognition in aged monkeys. One such example was explored in the same behaviorally characterized young and aged ovariectomized monkeys. Specifically, the synaptic distribution of ERα in area 46 has been examined with electron microscopy (222). While ERα levels remain stable across age and hormone treatment groups, the abundance of ERα within the spine postsynaptic density (PSD) correlates with working memory performance, exclusively in aged monkeys receiving cyclic estradiol (222) (FIGURE 7). Hence, the cognitive benefits observed in aged monkeys with cyclic estradiol may be mediated, in part, by the activation of ERα in a synaptic domain coupled to signaling cascades involved in spine/synapse formation and stabilization (205).

2. **Nonhuman primate models mimicking clinically relevant hormone treatments**

In contrast to the unopposed cyclic estradiol regimen that produced procognitive effects in rhesus monkeys, the hormone replacement therapy used in women in the WHIMS trial consisted of estrogenic compounds combined with a progestin, in a continuous (daily) regimen. Therefore, hormone treatments modeled after those used in clinical settings were tested in aged ovariectomized monkeys. Treatments that included continuous unopposed estrogen, continuous estrogen opposed with cyclic or continuous progestin, or cyclic estrogen opposed by cyclic progestrone, all failed to improve PFC-reliant working memory in aged ovariectomized monkeys (11). Consistent with these findings, these same treatments also failed to restore dendritic spine synapses on area 46 neurons that had been observed with cyclic unopposed estrogen treatment (159) (FIGURE 8). Young ovariectomized monkeys also showed parallel results; continuous estrogen with or without progesterone resulted in area 46 spine density and morphology that were indistinguishable from those receiving vehicle alone (249). Homeostatic mechanisms likely take effect with chronic estrogen administration, during which its levels remain high, leading to desensitization of mechanisms involved in spinogenesis. Indeed, there is evidence in the rodent literature that continuous treatment of estrogen can lead to decreased levels of ER mRNA and protein in several brain areas (14, 17, 20, 196). Different formulations of hormone treatment also differentially affect circulating ad-
renal steroids that have estrogenic activity through ERs, and these effects are age dependent. For example, both unopposed estrogen and estrogen combined with progesterone decrease circulating dehydroepiandrosterone sulfate (DHEAS) in young ovariectomized monkeys (28). In aged monkeys, however, unopposed estrogen has no effect on DHEAS levels and combined estrogen plus progesterone increases DHEAS (28).

Together, these studies suggest that the specific formulation of hormones (estrogen alone versus a combination of estrogen and progesterone) and the treatment schedule (continuous versus cyclic) are both key factors for estrogen to exert its positive effects on cognitive and synaptic health. These data provide evidence that the standard forms of hormone replacement therapy commonly prescribed to women may not provide the same cognitive and synaptic benefits as formulations that mimic the natural menstrual cycle.

**G. Estrogen Effects on Metabolism and Mitochondria: Implications for Cognition and Menopause**

Among the many functions estrogen has on the brain, it also improves cerebral metabolic rate and blood flow (34, 189). In a double-blind trial that enrolled 52 peri- and postmenopausal women, estrogen therapy increased PFC blood oxygenation levels during tests of verbal and spatial working memory, and this increase was associated with fewer errors (94).

In addition, estrogen exerts many antioxidant effects. Estradiol increases the expression of the antiapoptotic protein Bcl-2 (46, 154). This estrogen-induced increase in Bcl-2 enhances the capacity for mitochondria to sequester cytosolic calcium, imparting more resistance to neurons from the harmful effects of glutamate-induced excitotoxicity (19,
154). Furthermore, a single injection of estradiol in adult ovariectomized rats upregulates mitochondrial proteins that include key metabolic enzymes such as pyruvate dehydrogenase, aconitase, and ATP-synthase (155). These increased mitochondrial protein expressions translate to enhanced mitochondrial efficiency as measured by increased respiratory control and improved enzymatic activities (155). Importantly, estradiol reduces formation of reactive oxygen species (ROS) (155), which damage DNA, RNA, proteins, and lipids, and can induce cell death. These antioxidant effects of estrogen are noteworthy in light of strong evidence that mitochondrial dysfunction and damage accrued from oxidative stress precede, and may be causative for, neurodegenerative disorders such as Alzheimer’s disease (96).

Some of the estrogen effects on metabolism are likely mediated by ER $\beta$. Estradiol can bind to ER $\beta$ that are localized to the mitochondrial matrix (244). It can then impact the expression of mitochondrial DNA genes that encode 13 proteins that are components of the electron transport chain and oxidative phosphorylation (197). Both ER $\alpha$ and ER $\beta$ also regulate many nuclear genes involved in mitochondrial electron transport and ROS pathways (157).

Working memory reliant on the dorsolateral PFC area 46 (in humans and nonhuman primates) is susceptible to decline with aging and menopause (10, 43, 176, 180), and this may be partly due to the high energy requirement to sustain this function. Mitochondria supply the energy required for neurons, and often accumulate at synapses, where energy demands are especially high (13, 25). Mitochondria are also structurally dynamic and continually undergo fusion and fission events (248) (FIGURE 8). A recent study in rhesus monkeys revealed that estrogen treatment alters mitochondrial morphology in area 46 (73). Specifically, cyclic estradiol treatment in aged ovariectomized monkeys decreases the incidence of presynaptic donut-shaped mitochondria (73), a form that is associated with oxidative stress and ROS production (3, 119) (FIGURE 8). Notably, presynaptic boutons harboring donut-shaped mitochondria are inversely correlated with working memory performance in
monkeys and form abnormally small synaptic contacts (73) (FIGURE 8). Collectively, these studies suggest that cyclic estrogen treatment after surgical menopause may restore working memory, in part, by promoting mitochondrial and synaptic health in Area 46.

V. CONCLUSIONS AND FUTURE DIRECTIONS

Over the last two decades, it has become clear that estradiol exerts effects on the brain that go well beyond the regulation of reproductive behaviors mediated by the hypothalamus. In fact, brain regions such as the hippocampus and PFC contain all of the key ERs present throughout the body and are highly responsive to estradiol, as are the cognitive behaviors these regions mediate. In addition, along with the classical genomic effects mediated by activation of ER α and ER β, there are membrane-bound ER α, ER β, and GPER1 that can mediate rapid nongenomic effects, and all three receptors are present in excitatory synapses in the hippocampus and PFC.

While we know less about progesterone and its effects on these cortical regions, it is clear that it also has rapid, nongenomic effects and the cerebral cortex is a key target. In addition, adrenal steroids can activate ERs, particularly when circulating estradiol is low and adrenal steroids increase such as during the menopausal transition (28), yet we know virtually nothing about how such interactions might affect hippocampal or neocortical synaptic transmission. Finally, cortical neurons can synthesize their own estradiol and it is synaptically active, yet the role of this system and its relationship to circulating estradiol remains poorly understood. Clearly, we must now incorporate the actions of this critically important sex steroid into our conceptual frameworks for the synaptic organization of cerebral cortex and cognitive behavior along with its direct role in the regulation of female reproductive behavior.

In addition, male rodents and primates also express ER α, ER β, and GPER1, but little is known about potential sex differences in the abundance and distributions of these receptors. Most studies that attempted to explore sex effects in these receptors found no significant differences between...
males and females, but it is unclear whether these negative findings are due to limited sample size or statistical power. It is also worth noting here that androgens can exert their effects on androgen receptors present in the forebrain of rodents and primates. There is currently limited knowledge on how androgens such as testosterone may interact with the effects of estrogen on synaptic plasticity. Furthermore, only a few studies thus far have explored sex differences in cognitive aging altogether. Therefore, understanding the roles of these hormones, their receptors, and their interactions would shed light on potential sex-related differences in the trajectories of cognitive decline.

Estradiol is a powerful mediator of multiple signaling cascades in the brain related to synaptic signaling, actin polymerization with respect to spine dynamics, protein synthesis, calcium balance, energy metabolism, and neuronal survival. These actions are presumably mediated by synaptic and nuclear ERα, ERβ, and GPER1. These receptors have different subcellular localization, have various actions (genomic and/or nongenomic), and are linked to unique second messenger pathways that can oppose one another. It is only recently that studies have emerged showing estrogen-induced neuroprotective effects that are mediated selectively by distinct estrogen receptors. Given the wide-ranging effects estrogen has on the body, including effects on female reproductive cancers, it is critical to delineate the actions of each of these receptors and their interactions. Such information will be critical for development of better interventions for cognitive symptoms experienced in menopause that are devoid of adverse effects on peripheral organs. This objective is further complicated by the fact that synaptic ERs and responses to estrogen are altered with age and such alterations vary across brain regions and species. While it seems clear that estrogen depletion can affect these brain regions and the cognitive processes they mediate, such effects are highly variable in women and we do not know the source of such variability. The hormone treatments that have been used thus far for women concentrate largely on circulating estrogen and progesterone levels rather than on receptor dynamics and synaptic regulation, and this focus must shift if we are to develop more successful treatments.

One clear reflection of this problem has emerged from the nonhuman primate model; while cyclical unopposed estrogen treatments led to better synaptic health and cognitive performance in ovariectomized females, chronic estrogen or a combination of estrogen with progesterone did not. Our current hypothesis is that this reflects the important role of cyclical estrogen with respect to receptor dynamics that would be disrupted by chronic levels of estrogen or estrogen combined with progesterone.

Another issue that requires further attention is the differential effects of natural versus surgical menopause. Recent literature suggests that the abrupt loss of estrogens induced by oophorectomy/ovariectomy in humans and nonhuman primates produces a more severe consequence in cognitive and synaptic health than what are seen with the gradual loss of estrogens with natural menopause. In addition, differences in estrogen receptor and aromatase levels have been observed in naturally versus surgically menopausal monkeys. These differences warrant investigation of distinct treatment regimens that are most efficacious for each condition.

In summary, a more detailed appreciation of the signaling cascades activated by estrogen, the distinct role of classical and nonclassical actions for each receptor, and age- and menopause-related alterations of such processes will be required to develop interventions for menopausal women that are both more effective and more easily tailored to individual variations.

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