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Mechanisms of Action of Estrogen in the Brain: Insights from Human Neuroimaging and Psychopharmacologic Studies

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Abstract

Use of estrogen therapy in the perimenopausal and postmenopausal periods has been shown in several clinical trials to help women maintain a premenopausal level of cognitive function. What is not yet fully understood is how the neurobiological effects of estrogen contribute to these cognitive effects. This review explores data from two related bodies of human literature that provide compelling evidence in support of the biological plausibility that estrogen treatment can benefit cognition. The first half of the literature review focuses on studies from the estrogen neuroimaging literature, and the second half focuses on pharmacologic challenge studies assessing estrogen-neurotransmitter interactions. We integrate these two bodies of literature by focusing on the neurophysiologic underpinnings of estrogen effects on cognition and linking these clinical studies to preclinical studies. The focus on verbal memory is important because it is a cognitive function that has been shown to change with estrogen treatment and predict Alzheimer's disease risk but is not addressed by preclinical studies. Overall, we conclude that estrogen interacts with cholinergic and serotonergic systems to affect hippocampal and frontal cortical brain areas and thereby enhance memory, particularly at the retrieval stage.

Keywords

Estrogen; brain; cognition; psychopharmacology; neuroimaging

Several epidemiologic studies found that use of estrogen in the perimenopausal and postmenopausal periods helps women to maintain a premenopausal level of cognitive function, particularly verbal memory,^{1–5} though other studies show no benefit^{6–10} or show harm.^{11,12} One way to better understand these conflicting results is to understand more about the biological plausibility that estrogen could enhance brain function. We propose that understanding the effects of estrogen on the neurobiology of the brain is necessary to interpret clinical trial findings and to more effectively design future studies. This review paper will focus on two experimental methodologies in humans that will allow us to

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examine the neurobiological effects of estrogen. First, we will consider the effects of estrogen on brain function by reviewing the functional neuroimaging literature. Second, we will consider the psychopharmacologic effects of estrogen on different neurotransmitter systems by reviewing human studies that used pharmacologic challenge methods. We propose that estrogen interacts with cholinergic and serotonergic neurotransmitter systems to alter the function of the hippocampus and prefrontal cortex and thereby influence memory, particularly at the retrieval stage of memory processing.

Neuroimaging, Estrogen, and Brain Functioning

Three types of neuroimaging have been used to understand the neural targets of estrogen: positron emission tomography (PET), single photon emission tomography (SPECT), and functional magnetic resonance imaging (fMRI). PET neuroimaging is an invasive nuclear imaging technique involving the administration of a radiolabeled ligand on a biologically active substance such as water (O-15 water) or glucose (18F-FDG) to measure blood flow and glucose metabolism, respectively. The PET scanner detects pairs of gamma rays that are indirectly emitted from positrons in those ligands. PET provides better spatial resolution and higher detection sensitivity than do other methods. SPECT (or SPET) is similar to PET in that both require use of a radiotracer, but the radiotracers in SPECT are longer lasting, the method is less expensive, and the resolution of SPECT is poor compared with that of PET. In contrast, fMRI is a noninvasive neuro-imaging technique that measures changes in the magnetic properties of hemoglobin as oxygen is used in relation to neural activity. The changes seen on fMRI scans are changes in cerebral blood flow. Heightened changes are commonly referred to as “activations.” A benefit of PET over fMRI in studying the effects of estrogen on the hippocampus and frontal cortex is that fMRI is susceptible to air and bone artifact in medial temporal brain regions and air artifact in orbital and polar regions of frontal cortex.¹³ A benefit of fMRI over PET is that multiple tasks can be administered in a single session without concern about exposure to excessive radiation. In this way, fMRI allows for greater experimental control over contrasts of interest, particularly those involving the comparison of a task of interest (e.g., verbal recall of unique words studied only once) versus a control task (e.g., verbal recall of frequently repeated and well-memorized words). Through such a comparison, this experimental approach allows analysis of memory while controlling for such factors as visual and word processing.

Many studies have shown that estrogen therapy benefits memory for words, paragraphs, or other verbal materials on standardized tests.^{2,3,5,14,15} For this reason, imaging studies of verbal episodic memory tasks are of particular interest. Episodic memory tasks involve the encoding and retrieval of specific episodes or events over a delay of several minutes (e.g., 15 or 20 minutes) and are largely dependent on hippocampal function¹⁶ though the prefrontal cortex also plays a role, with left prefrontal cortex more involved in the encoding of items—particularly in the processing of the meaning of items—and right prefrontal cortex more involved in the retrieval of items.¹⁷ Early declines in episodic memory are clinically relevant because they are associated with an increased risk of Alzheimer's disease.¹⁸ Neuroimaging assessments can be conducted during encoding phases (e.g., while women are learning word lists) and during retrieval (e.g., while women are recognizing words as previously studied or new). This capacity allows us to better understand whether the behavioral effects observed in

clinical trials are due to estrogen effects on brain structures during encoding and/or retrieval. Behavioral measures alone cannot address this issue.

Working memory tasks involve encoding and retrieval of information over very short delays (e.g., seconds) and rely on prefrontal function.¹⁶ An example of working memory task is remembering a phone number that directory assistance has just provided for only as long as needed to dial the number. In general, estrogen with or without progesterone does not alter performance on standardized tests of working memory, such as digit span,^{5,15,19,20} though there are exceptions as studies show benefits with estrogen alone¹⁴ and detrimental effects with conjugated equine estrogens plus medroxy-progesterone acetate.²¹ Evidence from experimental laboratory tests of working memory (as distinguished from standardized clinical tests) suggests that estrogen affects the manipulation of items in working memory though not the passive maintenance of items.²² We focus our review on studies of estrogen effects during the encoding and retrieval phases of episodic memory tasks and during the encoding phase of working memory tasks, as these studies are relevant for understanding the relative impact of estrogen at the encoding versus retrieval stages of episodic memory.

Estrogen Enhances Hippocampal Function and Prefrontal Function During Memory Tasks

The first study to examine the effects of estrogen on episodic memory and hippocampal function involved a sample of 32 older women (mean age = 66 years) participating in the Baltimore Longitudinal Study on Aging.²³ Participants completed O-15 water PET imaging while resting and while performing verbal and figural memory tasks. Patterns of regional cerebral blood flow in women taking hormone therapy (HT)—either estrogen alone or estrogen plus progesterone—were compared with patterns in women not taking HT. Participants studied abstract figures and abstract words outside of the PET scanner and 30 minutes later underwent neuro-imaging assessments as they completed a recognition test for the figures and words. Differences in brain activation patterns were evident between those on and off of HT in areas that mediate memory, including the inferior frontal cortex and the parahippocampal gyrus. Importantly, although the two groups were similar in overall verbal knowledge, age, and education, the HT group performed better on standardized tests of delayed verbal and figural memory. This was the first demonstration that estrogen enhanced hippocampal and prefrontal function in women during the retrieval stage of episodic memory tasks.

In a subsequent study, a subset of the same cohort was followed prospectively from the first to third year of the longitudinal study to examine the effects of HT on the rate of change in brain function over that 2-year time interval. Over time, women receiving HT ($n = 12$) showed increased blood flow to the right hippocampus, right entorhinal cortex, right posterior parahippocampal cortex, other temporal structures, and inferior frontal cortex compared with that of nonusers ($n = 16$).²⁴ Analyses within condition (i.e., rest, verbal memory, figural memory) demonstrated that the hippocampal effect was evident during verbal recognition, whereas the para-hippocampal effect was evident during figural recognition. During rest, the right middle/superior temporal gyrus, right inferior temporal

gyrus, and left middle temporal gyrus were more active over time in users compared with that in nonusers. HT users continued to show better memory performance on standardized tests. In both the cross-sectional and longitudinal studies, the large majority of women had initiated hormone therapy early in the menopausal transition and continued on therapy.

A recent fMRI study investigated patterns of brain activation during the retrieval stage of a figural episodic memory task.²⁵ Participants included women (mean age = 58.5 years) receiving conjugated equine estrogens (CEEs; $n = 10$), estradiol ($n = 4$), or no hormone therapy ($n = 9$). Compared with no use, estrogen use was associated with increased activation in the right hippocampus during retrieval of previously (i.e., 15 to 40 minutes earlier) and repeatedly studied (i.e., 30 times) line drawings compared with that for novel line drawings. The fMRI findings revealed no significant functional difference of one hormone preparation versus the other. On behavioral measures, compared with women receiving no HT, women receiving CEEs performed significantly worse on a standardized test of verbal memory, whereas those on estradiol performed significantly better. Thus, whereas the behavioral data indicated that estradiol was superior to CEEs in its effects on verbal memory, the fMRI data during a figural memory task revealed no parallel neural differences. There are no clinical trials to date directly contrasting the effects of CEEs and estradiol on memory. In randomized trials, CEEs have been shown to reverse the negative effects of pharmacologic ovarian suppression on verbal memory¹⁵ and to enhance verbal memory.²⁶ Furthermore, the majority of women in the two PET studies reviewed above used CEEs as hormone therapy, and hormone therapy was associated with both enhanced memory and enhanced hippocampal and prefrontal activation in those two studies.^{23,24} On the other hand, CEEs in conjunction with medroxyprogesterone acetate (MPA) has been shown to have a negative effect on verbal memory¹¹ or a near significant ($p = 0.06$) negative effect on verbal memory in clinical trials.²⁷

A small fMRI study, but one done in the context of a randomized clinical trial, contrasted brain activation patterns during retrieval of words in five women randomized to receive transdermal estradiol (0.05 mg/day) compared with that in six women randomized to receive placebo (mean age = 51 years).²⁰ Neuroimaging was completed before and after treatment during the recognition phase of a verbal memory task. Compared with placebo, estrogen enhanced activation in the prefrontal cortex, in Brodmann area 11, an area involved in higher-order executive functions such as planning and strategizing. Differences were also evident in somatosensory cortex, but there were no differences in hippocampal function. Estrogen also affected activation in several prefrontal regions (Brodmann area 9) and anterior and posterior cingulate during performance of a spatial working memory task. On a standardized verbal memory test administered outside the scanner, estrogen treatment did not enhance learning but did decrease the number of executive errors associated with frontal dysfunction (e.g., impulsive errors and errors in updating of working memory). This study demonstrated that estrogen affected prefrontal areas during both the retrieval stage of an episodic verbal memory task and a spatial working memory task. This study also demonstrated that estrogen might enhance verbal memory in part by enhancing executive functions, such as control, updating of items in working memory, and impulsivity.

A larger but shorter-term clinical trial investigated the effects of estrogen on brain function measured by fMRI during verbal and spatial working memory tasks.²⁸ The verbal information probed in the verbal working memory test was abstract letter combinations (e.g., MIJ) rather than the type of verbal information tested in clinical trials. The spatial working memory task, like the tests used in clinical trials, involved abstract geometric forms. Women were randomized to receive CEEs (1.25 mg/day) or placebo for 21 days, and then, after a 14-day washout, crossed over to the other treatment condition for 21 days. Estrogen treatment did not affect performance on the tests. Estrogen increased activation in the right superior frontal gyrus during retrieval. Estrogen did not increase activation in any area during encoding. An examination of activation in the superior frontal gyrus as a function of the three-way interaction among (a) treatment, (b) task (encoding versus retrieval), and (c) cerebral hemisphere (right versus left) collapsed across working memory tasks revealed that estrogen increased activation in the left hemisphere during encoding and decreased activation in the right hemisphere during retrieval. During storage of verbal material (e.g., between encoding and retrieval a few seconds later), estrogen increased activation in the inferior parietal lobule. During storage of nonverbal material, estrogen decreased activation in that region. In summary, this working memory study suggests that estrogen enhances prefrontal activation during encoding of abstract verbal and spatial material.

In summary, all of the episodic memory studies reviewed above examined the effects of estrogen at retrieval but not encoding. There is considerable evidence from PET and fMRI studies that estrogen therapy enhances episodic memory at the retrieval stage by altering functioning of the hippocampus and regions of prefrontal cortex. Two PET studies ($n = 32$ and $n = 28$)^{23,24} found an effect in both brain areas, and one fMRI study ($n = 23$)²⁵ found evidence that estrogen enhances activation of the right hippocampus during verbal retrieval. A small fMRI study ($n = 11$)²⁰ found an effect of estrogen on prefrontal, but not hippocampal, activation during verbal retrieval. The variable results with fMRI studies may reflect the lower sensitivity of fMRI versus PET due to air artifact. Insights into the effects of estrogen on the encoding stage of memory processing is gained from a working memory study where estrogen enhanced left prefrontal activation during encoding.²⁸ To better understand the clinical trial findings, studies are needed to examine the relative effects of estrogen at the encoding versus retrieval stage in the same sample and in relation to behavioral performance.

Estrogen Enhances Function in Brain Regions Affected by Alzheimer's Disease

PET studies during a resting state are important in understanding how estrogen alters function of brain areas showing hypometabolism in Alzheimer's disease, including posterior cingulate cortex, temporal cortex, and parietal cortex. A pair of related PET studies examined 2-year longitudinal changes in glucose metabolism during rest in estrogen users versus nonusers. The first study was a pilot study and involved four women on estrogen therapy (mean age = 66 years) and eight non-users (mean age = 72 years). At year 1, there were no differences in metabolism in *a priori* regions of interest, including lateral temporal regions, inferior parietal cortex, and posterior cingulate cortex. Two-year follow-up data

again indicated no baseline differences in brain function, but significant differences were evident over time such that women receiving estrogen had an increase in metabolism in the lateral temporal regions, whereas the women not receiving estrogen showed no change.²⁹ In a subsequent study, this sample size was increased to 11 users and 9 nonusers, and results supported previous findings in posterior cingulate cortex, but not lateral temporal regions.³⁰ This pair of investigations demonstrates that estrogen therapy is associated with reliably enhanced neural activity in posterior cingulate cortex, a region showing early decreases in metabolism in Alzheimer patients and in healthy individuals with a genetic risk factor for Alzheimer's disease.³¹ Effects in other brain regions were not robust.

Another observational PET study demonstrated that the resting level of glucose metabolism in frontal and temporal areas in older estrogen nonusers ($n = 5$; mean age = 75 years) is intermediate between that of women with Alzheimer's disease ($n = 13$; mean age = 76 years) and that of women receiving estrogen therapy ($n = 8$; mean age = 71 years).³² A later study contrasted glucose metabolism in 15 estrogen users, 15 nonusers, and 10 women receiving treatment with the antiestrogen intervention, tamoxifen (mean age ≈ 66 years). The estrogen users showed greater metabolism in the inferior frontal cortex and temporal cortex compared with that in the nonusers, and the tamoxifen group showed decreased activation in the left superior frontal gyrus, left medial frontal gyrus, left postcentral gyrus, right superior frontal gyrus, and right medial frontal gyrus.³³

In summary, these resting PET studies provide evidence that estrogen helps to preserve function in brain areas affected by Alzheimer's disease. In this way, the studies suggest that estrogen helps to maintain a pattern of brain function associated with a lower risk of Alzheimer's disease. Again, however, the results must be interpreted with caution because the sample sizes are quite small and the results are based on observational data. Furthermore, these studies included samples of older postmenopausal women, and there is a need for resting studies in younger postmenopausal women. Finally, there is suggestion that antiestrogen therapies used for breast cancer are associated with a pattern of brain function that suggests vulnerability for Alzheimer's disease.

Estrogen Reverses Cognitive Deficits After Hormone Suppression

An elegant model that has been used to study the effects of ovarian steroid hormones on memory and mood involves pharmacologic suppression of ovarian hormones with the gonadotropin-releasing hormone (GnRH) agonist analogue leuprolide acetate.³⁴ An early study investigated the effects of leuprolide acetate treatment on cognitive function in 19 premenopausal women.¹⁵ Participants performed cognitive tests prior to hormonal suppression, during hormonal suppression with leuprolide acetate, and after randomization to leuprolide acetate plus placebo or add-back estrogen. Results demonstrated a decrease in verbal memory (e.g., story recall) from prechallenge to postchallenge (for similar findings, see Ref. 35) and a reversal of this memory impairment after treatment with estradiol but not placebo. These findings suggested that endogenous estradiol levels help to maintain verbal memory, hormone suppression leads to verbal memory deficits, and estrogen reverses those deficits. These findings concur with other clinical trial findings, where estrogen reversed

verbal memory deficits after surgical menopause.¹⁴ Subjective memory complaints also increase with leuprolide treatment.³⁶

Studies using leuprolide acetate as a model of transient menopause have also demonstrated effects on executive function, such as mental flexibility and working memory, as well as changes in the prefrontal areas that contribute to performance on executive function tests. For example, PET was used in conjunction with leuprolide acetate to understand the effects of hormone suppression on performance on a modified version of the Wisconsin Card Sorting Task (WCST), a test requiring abstract reasoning, problem solving, working memory, strategizing, and mental shifting.³⁷ Eleven young women (mean age = 36 years), five with menstrual-related mood disorder and six without the disorder, performed the WCST during ovarian hormone suppression with leuprolide acetate alone; leuprolide acetate plus estrogen; and leuprolide acetate plus progesterone. Though there were no significant changes in task performance, the typical pattern of neural activity during the task—activation in dorsolateral prefrontal cortex, inferior parietal cortex, and inferior temporal cortex—was attenuated during leuprolide-alone treatment. Treatment with estrogen or progesterone reversed this effect. Notably, the only difference between the estrogen add-back and progesterone add-back groups was greater left hippocampal activation during the estrogen add-back condition.

Ovarian hormone suppression with leuprolide acetate also leads to impairments in working memory.³⁸ Twenty-five young women (mean age = 37 years) performed a cognitive test battery, including measures of verbal memory, working memory, and attention, during baseline and during treatment with leuprolide acetate (e.g., 4 weeks). Their performance was contrasted with a group of 25 young women (mean age = 26 years) who received no treatment but who performed the tests 4 weeks apart. Ovarian hormone suppression was associated with reductions in some measures of working memory, but not with a reduction in verbal memory as measured by a list-learning task. The reduction in working memory was associated with changes in estradiol, but not progesterone.

In summary, studies using leuprolide acetate as a model of ovarian hormone suppression demonstrate behavioral deficits in verbal memory and working memory and functional deficits on neuroimaging during performance of executive tasks. These studies underscore the importance of endogenous hormones in cognitive function.

Estrogen Interacts With Neurotransmitter Systems to Influence Cognition in Animals

Another investigational focus that has been shown to translate from preclinical to clinical studies centers on functional interactions between estrogen and several neurotransmitter systems. The implications of these interactions are that estrogen affects cognitive processes that are supported by specific neurotransmitter systems. Estrogen has been shown to modify cholinergic,³⁹ serotonergic,⁴⁰ dopaminergic,⁴¹ GABAergic,⁴² and glutamatergic⁴³ function. These interactions in humans have been recently examined in studies in which estrogen and cholinergic and serotonergic systems were experimentally manipulated. We discuss the specific effects of estrogen on cholinergic and serotonergic systems by describing the bodies

of cellular and animal literature, then presenting the human data. First, we begin with the cholinergic system.

Many lines of preclinical evidence support the view that estrogen effects on cholinergic function are critically important in understanding the effects of estrogen on cognition. In brief, loss of estrogen after ovariectomy has been shown to decrease high-affinity choline uptake, choline acetyltransferase (ChAT) activity, and ChAT mRNA levels.^{44–47} Cholinergic fiber density in the dorsolateral prefrontal cortex decreased 2 years after ovariectomy in surgically menopausal versus intact monkeys, and estrogen treatment prevented this decrease.⁴⁸ After only 1 month of estrogen treatment, cholinergic fiber density can be restored in ovariectomized monkeys.⁴⁹ Estrogen appears to modulate attention performance in primates through its interaction with the cholinergic system.^{50,51} After ovariectomy, monkeys were impaired on an attention task, and estrogen but not placebo improved performance.⁵⁰ In a second step, monkeys underwent a pharmacologic challenge with scopolamine, an anticholinergic (i.e., antimuscarinic) compound. Estrogen attenuated scopolamine-induced behavioral declines in the attention task but not on the memory task. The finding that estrogen affects cholinergic circuits in the frontal lobe and hippocampus is important because these areas support cognitive functioning, and estrogen may enhance attention and memory through cholinergic systems. Further consideration of the role of the cholinergic system in modulating cognitive functioning in humans should provide insights into the effects of estrogen in clinical trials.

Estrogen and Acetylcholine Interact to Influence Cognition in Humans

Only a handful of clinical studies have examined the interaction of estrogen and the cholinergic system in relation to cognitive performance. Two methodological approaches have been useful in understanding these interactions: short-term estrogen treatment with acute cholinergic challenge^{52,53} and quantification of cholinergic receptors in association with long-term estrogen treatment.⁵⁴ The two acute cholinergic challenge studies^{52,53} investigated the influence of estrogen on two subtypes of cholinergic systems: the nicotinic system with the antinicotinic drug mecamylamine, and the muscarinic system with the antimuscarinic drug scopolamine. Cholinergic depletion is a useful model of cognitive aging because these deficits model those seen with advanced age and dementia.⁵⁵ Each of these two studies will be reviewed in turn.

In the first study, postmenopausal women ages 50 years and older were randomly assigned to receive either oral 17 β -estradiol (1 mg/day) or placebo for 3 months.⁵² Women then participated in each of five cholinergic challenges, each on a separate day, including 2.5 μ g/kg scopolamine, 5.0 μ g/kg scopolamine, 10 mg mecamylamine, 20 mg mecamylamine, or placebo. As this was the first study assessing the effects of the estrogen-cholinergic interaction on cognition, two doses of both antimuscarinic and antinicotinic challenges were investigated. A broad battery of cognitive tests was used on each challenge day to assess a broad spectrum of cognitive domains including perceptual-motor speed, attention, and verbal and nonverbal episodic memory. After this first phase of estrogen treatment and cholinergic challenge, women crossed over to the alternate treatment (i.e., estrogen or placebo) for another 3 months and then completed another 5 challenge days.

As predicted, the anticholinergic challenges impaired performance across all domains of cognition. Interestingly, 3 months of estradiol treatment reversed this impairment on lower-level perceptual tasks and speeded tasks. Although the effects of estrogen treatment on verbal and nonverbal memory were in the expected direction, these effects were not significant. These findings indicate that estrogen mediates cognition through the cholinergic system. However, it was thought that further study and perhaps an increased dose of estradiol might be necessary to observe the estrogen-cholinergic interaction on verbal memory and other higher-order tasks.

In a second study, the estradiol dose was increased to 2 mg per day for 3 months.⁵³ In addition, women were stratified by age to test the critical period hypothesis, which, as reviewed elsewhere in this issue of *Seminars in Reproductive Medicine*, suggests that there is a critical time after the menopause transition during which estrogen treatment will be beneficial, and after this time, estrogen treatment will confer no effect or negative cognitive effects.^{56–59} Two groups of postmenopausal women were enrolled: one group ages 50 to 62 years (mean age = 55 years) and another group ages 70 to 81 years (mean age = 74 years). Participants randomly received oral estradiol (2 mg/day) or placebo for 3 months. They then participated in 3 challenge days. On each day they received 2.5 µg/kg scopolamine, 20 mg mecamylamine, or placebo. The cognitive battery was the same as that used in the first cholinergic challenge study.⁵²

As in the first study, anticholinergic drugs impaired cognition. Estrogen at the 2 mg/day dose reversed the drug-induced impairment on verbal memory for younger postmenopausal women but not for older post-menopausal women. The effects on perceptual and speed tasks seen with the 1 mg/day dose were not observed with the higher dose of estrogen. Thus, two challenge studies demonstrate that dose and age are important determinants of the efficacy of estrogen in mitigating against cognitive dysfunction associated with cholinergic loss.

A second experimental approach that has been helpful in understanding the interaction between estrogen and the cholinergic system focuses on cholinergic markers in long-term users of estrogen therapy. A study using SPECT demonstrated a relationship between long-term estrogen use and muscarinic (m) receptors.⁵⁴ The study used a ligand with a high affinity for m₁/m₄ receptors in three groups: younger premenopausal women; older postmenopausal women who were long-term estrogen users; and older postmenopausal women who never used estrogen. Muscarinic receptor density was higher in younger versus older women. Older women who were estrogen users had high muscarinic receptor density in brain areas that have been shown in other studies to be critical for cognition: the hippocampus, striatum, lateral frontal cortex, and thalamus. In addition, plasma estradiol levels positively correlated with the magnitude of muscarinic receptor binding in the hippocampus and temporal cortex, key areas for memory processing. Furthermore, there was a trend toward better performance on tests of executive function for the estrogen users compared with that of the non-users, but there was no relationship between level of cognitive performance and muscarinic receptor levels.

In summary, though the number of empirical articles examining the interaction of estrogen and the cholinergic system in humans is small, the findings from these studies support

preclinical studies and suggest that estrogen affects cognition, at least in part, by interacting with the cholinergic system. One notable area of convergence between the preclinical and clinical studies is the role of cholinergic function in modulating the age-specific effects of estrogen on memory. Both preclinical and clinical studies suggest that estrogen reverses scopolamine-induced impairments in memory in younger, but not older, postmenopausal women.⁶⁰ Similarly, low doses of estrogen appear to reverse scopolamine-induced deficits in attention in both humans and non-human primates.

Estrogen and Serotonin Interact to Influence Brain Function in Humans

Preclinical and clinical studies also provide important contributions to our understanding of the serotonergic system in mediating estrogen effects on cognition. Neurons that produce serotonin are found in 10 main nuclei in the midbrain and hindbrain regions.⁴⁰ Neurons from these nuclei project to cortical areas that are involved in cognition as well as mood regulation. Estrogen modulates expression of genes that code for tryptophan hydroxylase, the serotonin transporter, and the 5-HT_{1a} autoreceptor.⁴⁰ In addition, estrogen may affect genes that code for accessory proteins involved in the expression of these serotonin receptors, other molecules that are involved in serotonin degradation, or any of the 14 serotonin genes.⁴⁰ Thus, there are numerous sites for interactions of estrogen and serotonin. Studies in postmenopausal women demonstrate enhanced serotonergic tone among users of hormone therapy compared with that in nonusers.⁶¹

An experimental approach for understanding the interactive effects of estrogen and serotonin on cognition is the tryptophan depletion procedure. This pharmacologic challenge procedure results in a rapid reduction of brain tryptophan that in turn results in a reduction of serotonin. In young adults, the challenge produces adverse mood effects only in those with a history of mood disorders; however, impairments in memory are a reliable result of the challenge.⁶² Thus, serotonin is a key neurotransmitter for memory performance.

Tryptophan depletion challenges have been performed in conjunction with estrogen treatment to better understand the interactive effects of serotonin and estrogen on mood and cognition.

Nineteen menopausal women participated in tryptophan depletion procedures before and after 8 weeks of transdermal open-label estrogen treatment.⁶³ Women completed both the depletion procedure as well as a sham procedure before and after estrogen treatment. As expected, verbal memory decreased significantly after the tryptophan depletion challenge, but estrogen treatment buffered this effect. Spatial learning did not decrease significantly, and estrogen had no influence on spatial learning. In addition, estrogen enhanced mood on the day after the depletion challenge. Thus, this study provides experimental support for the benefit of the effect of estrogen on serotonergic function in relation to both memory and mood cognition.

Additional evidence suggesting that estrogen effects on memory may be mediated in part by serotonin comes from a recent SPECT study.⁶⁴ This SPECT study used the selective 5-HT(2A) receptor ligand (123)I-5-I-R91150 to study differences in serotonin receptor

availability between a group of 17 long-term oophorectomized estrogen users (mean age = 62 years) and a group of 17 postmenopausal women who were not using estrogen (mean age = 65 years). In the hippocampus, 5-HT(2A) receptor availability was decreased in estrogen users compared with that in nonusers. Decreased 5-HT(2A) receptor availability in the hippocampus was associated with better verbal memory, general memory, and delayed recall. Additionally, decreased 5-HT(2A) receptor availability in the right superior temporal lobe was associated with better verbal memory. These results were interpreted as suggesting that estrogen leads to increased serotonergic transmission, which in turn leads to downregulation of postsynaptic serotonin receptor activity, and this cumulative effect is associated with improved memory.

PET and the radioligand [18F]deuteroaltanserin, which binds to the 5-HT(2A) receptor, was used to understand the influence of estrogen on serotonin receptor binding in relation to cognitive performance.⁶⁵ Ten postmenopausal women (mean age = 54.5 years) completed cognitive assessments and PET scans before and after treatment with transdermal estradiol (mean dose at baseline and posttreatment were 0.08 mg and 0.09 mg, respectively) for an average of 10 weeks. Receptor binding significantly increased in right frontal cortex, including prefrontal cortex (Brodmann areas 9 and 47), as well as anterior cingulate. Behavioral improvements were observed on category fluency (a measure of executive function) and Trails A (a measure of psychomotor speed), but not on measures of mood or verbal memory. Changes in estradiol correlated significantly with changes in right prefrontal function.

To summarize, preclinical studies demonstrate that estrogen can interact with systems that affect serotonin synthesis, receptor production, or degradation. Pharmacologic challenge results demonstrate that estrogen ameliorates deficits in verbal memory after serotonin depletion. Neuroimaging studies suggest that estrogen effects on verbal memory are related to serotonin receptors in the hippocampus. Additional data suggests that estrogen alters serotonin binding in the prefrontal cortex. Thus, though estrogen effects on serotonin are commonly considered to be important for mood, these effects also seem critical to cognitive performance in humans.

Conclusions

The aim of this review was to inform our understanding of the clinical trial literature by considering neuroimaging and pharmacologic challenge studies in humans. These methodological approaches are critical to understanding the mechanisms through which estrogen alters cognitive function. They are especially important in understanding estrogen effects on verbal memory, a cognitive ability that cannot be well understood through animal studies. Furthermore, these methods are important for understanding estrogen effects on neural and neurotransmitter systems in younger women who are performing well on cognitive tests and therefore have a restricted range in which their performance in clinical trials can improve. Depletion methods impair cognitive performance even in younger women, and the role of estrogen can be more fully evaluated. Similarly, neuroimaging studies in younger postmenopausal women are helpful in identifying biological evidence of neuroprotection, even in the absence of behavioral change.

Several important conclusions can be drawn from this review. First, in younger postmenopausal women, depletion of estrogen,¹⁵ acetylcholine,⁵³ and serotonin⁶⁶ each led to deficits in episodic memory, and estrogen reverses these effects. These findings support the view that the initiation of estrogen treatment in younger women is associated with neural protection. Second, challenge studies also help us understand the clinical finding that the initiation of estrogen by older women has neutral or detrimental effects. Evidence from pharmacologic challenge studies demonstrates that estrogen reverses memory impairment after cholinergic depletion in young but not older women.⁵³ Thus, this age-dependency in clinical studies may be due to age-dependency in estrogen-cholinergic interactions. Third, it is not feasible to conduct a randomized trial to determine whether the continued use of estrogen from early in the menopause to later in life is associated with the cognitive benefits or risks. However, neuroimaging studies suggest that hippocampal and prefrontal cortex function are enhanced in long-term users of estrogen and that this may relate to increased serotonergic activity. Fourth, there is an interesting convergence of the findings of the effects of estrogen on cholinergic and serotonergic systems. Estrogen produced similar results in two very different challenge procedures. To explain these similarities, we may look to the common neuroanatomy that underlies these effects, and in particular to the hippocampus. Fifth, neuroimaging findings suggest that estrogen enhances episodic memory at the retrieval stage through effects on the hippocampus and prefrontal cortex. PET and fMRI studies of episodic memory retrieval reviewed earlier also primarily found estrogen effects in right hemispheric structures, consistent with the idea that the right hemisphere is differentially involved in memory retrieval. Evidence from an fMRI investigation of working memory study also suggests that estrogen alters functioning of left prefrontal cortex during encoding. In general, these studies represent a successful translation of preclinical findings of enhanced neural plasticity and memory with estrogen to human studies.

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