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Estrogens facilitate memory processing through membrane mediated mechanisms and alterations in spine density

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Abstract

Estrogens exert sustained, genomically mediated effects on memory throughout the female life cycle, but here we review new studies documenting rapid effects of estradiol on memory, which are exerted through membrane-mediated mechanisms. Use of recognition memory tasks in rats, shows that estrogens enhance memory consolidation within one hour. 17 α -estradiol is more potent than 17 β -estradiol, and the dose response relationship between estrogens and memory is an inverted U shape. Use of specific estrogen receptor (ER) agonists suggests mediation by an ER β -like membrane receptor. Enhanced memory is associated with increased spine density and altered noradrenergic activity in the medial prefrontal cortex and hippocampus within 30 min. of administration. The environmental chemical, bisphenol-A, rapidly antagonizes enhancements in memory in both sexes possibly through actions on spines. Thus, estradiol and related compounds exert rapid alterations in cognition through non-genomic mechanisms, a finding which may provide a basis for better understanding and treating memory impairments.

1. Introduction

Only 40 years have elapsed since the brain, specifically the hypothalamus, rather than the pituitary, was recognized as the site for estrogen's regulation of ovulation and sexual behavior [26,42]. An even shorter time, approximately 15 years, has passed since neuroendocrine research, driven by advances in molecular biology and technology, established that estradiol also impacts higher order brain functions such as mood and psychiatric disorders [90, 95], bonding and affiliation [12], and the focus of this review, cognitive function [6,9,18]. Cognitive effects of estradiol are mediated by actions of estrogens at sites and/or neural systems in the cerebral cortex, basal forebrain, hippocampus and striatum that are responsible for complex, higher order neural function. Thus, it is now recognized that estrogens not only regulate homeostatic/house-keeping functions of the CNS but also influence the intellectual/cognitive realms of life.

Estrogen's influence on learning and memory, similar to its reproductive effects occur through binding to classical nuclear receptors. These receptors, which were first isolated and

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characterized from the uterus, were subsequently found in many brain sites (see Figure 1 and [51] for review). It is now known that there are two nuclear estrogen receptors, estrogen receptor alpha (ER α) and estrogen receptor beta (ER β) [54]. Both receptors are ligand dependent transcription factors and through interactions at specific sites on DNA, initiate a cascade of intra-cellular reactions culminating in unique physiological responses within estrogen target tissues including the uterus, breast, osteoclasts and CNS (See [79] for a detailed description of genomic mechanisms of estrogen action in the CNS and for information concerning some non-ligand-dependent actions). Genomic actions in the nucleus by hormones result in long lasting and sustained effects on neural function. Thus, it is likely that genomic alterations by estradiol sub-serve developmental hormone effects, which exert life-long actions in programming sex-dependent functions and sex differences in neural functions. In adults, genomic effects underlie changes in neural function that occur during the menstrual and estrous cycles, pregnancy, menopause and aging.

Another recent advance in understanding mechanism(s) for estradiol's mediation of physiological and neural function is that receptor(s) for estrogens are present outside of the cell nucleus in membranes of cells in the periphery and the CNS [See Figure 2 and [6,82,111]. Moreover, these receptors are present in the medial prefrontal cortex (PFC) and the hippocampus, areas which are not directly involved in neuroendocrine regulation. In peripheral target tissues, the breasts, osteoclasts and epithelial cells of the cardiovascular system, the estrogen membrane receptors are similar or identical to classically identified ER α and β receptors, and they mediate rapid hormonal effects (sec to min) through signal transduction pathways (See Figure 1 and [59] for review of early work on the topic). Thus, estrogen effects can be rapid and short-lived as well as delayed in onset and long lasting.

The focus of this review is to present results of current studies demonstrating that estradiol exerts rapid enhancements of memory that may be mediated through actions at membrane receptors. First we provide a short background on methods for assessing cognitive function, i.e. learning and memory. Then studies demonstrating estradiol's enhancements of cognition through classic, genomic mechanisms are provided in order to provide background for recent studies, which show rapid effects of various estrogens and specific estrogen agonists on recognition memory. Possible mechanisms underlying the cognitive changes are discussed, including the role of rapid increases in spine density in the medial PFC and hippocampus, as well as, a brief consideration of signaling cascades that may initiate the estradiol-dependent changes. We also provide evidence that the environmental estrogen, bisphenol-A, rapidly impairs memory consolidation through effects at the membrane and dendritic spines. We close with a consideration of how rapid signaling by estrogen may impact cognitive function in animals and humans. Thus, this review will provide evidence that both 17 α - and 17 β -estradiol initiate a rich repertoire of neural inter-actions that regulate physiology and cognition.

2. Assessing cognitive effects of estrogens

A substantial literature has demonstrated that gonadal hormones, mainly estradiol, influence cognition function during development, at adulthood and during aging [33,64]. However, it is critical to note at the onset of this review that estradiol does not enhance all aspects of cognition [18,33,64]. This observation is not surprising since cognition represents a complex, multidimensional set of intellectual functions whose component processes are subserved by specific, yet interrelated, brain regions. Moreover, cognition itself consists of several components. First, there is the process of learning or acquiring information. Then the information must be consolidated and stored. Finally, the memory or information must be retrieved when needed. This process can be illustrated through the playing of card games. First, the rules for playing Bridge or Poker must be learned or acquired (acquisition). Then

cards played during a current game must consolidated and stored. Finally, memory of the played cards must be retrieved at the appropriate time during the game. Experimental rats undergo a similar process when they learn and then perform various cognitive tasks. An important point to emphasize is that estradiol may not enhance every aspect of this cognitive process.

Spatial memory tasks have been used in many learning and memory studies, including those utilizing estradiol, and these tasks have both learning and memory components. The radial arm maze and the water maze rely on rats making relationships between cues in the environment and a reinforcement site, hence their designation as spatial learning. The reinforcement site could be food hidden in a cup (radial maze) or a submerged platform on which to escape (water maze). For the radial arm maze, rats must learn that there is food at the end of the arm and that it is not replaced once they visit the arm and eat it (acquisition). In contrast, after acquiring the task and during testing, rats must remember the arms visited using the spatial cues in order to complete the trials efficiently and obtain their food (memory). Thus, the acquisition phase for the task is different from the memory-testing phase. In the water maze, rats must also learn where the escape platform is located using spatial cues (acquisition) and then use these spatial cues to find the platform during retention testing (memory). Memory tasks usually rely on different reinforcements (positive rewards such as food or water or aversive stimuli such as shocks or swimming) for the learning phase. Tasks can also measure memory using visual or olfactory cues instead of spatial cues. The results of research, using a variety of learning and memory tasks, show that estradiol enhances cognition [18,33,64]. The balance of studies show estrogen dependent enhancements in performance during the acquisition phase [16,71,72], but when subjects have acquired (learned) how to complete/solve the tasks, estradiol no longer appears to enhance performance [27,71] and may even impair spatial memory [See 18 for further discussion]. When memory is assessed in spatial and non-spatial tasks (using and remembering within trial information), consistent enhancements by estradiol are seen [reviewed in 64]. Thus, it is critical to consider which component(s) of cognition are being measured in studies assessing effects of estrogens.

Another important consideration for assessing hormonal effects on cognition is that context and/or experience can influence performance, and these factors may account, in part, for some discrepancies in the literature. For example, stress encountered during task performance may interfere with or override estrogen enhancements of some spatial tasks [4,21,34] and could be even more critical when rapid hormonal effects are assessed. Furthermore, extensive handling, housing conditions, or environmental enrichment can lessen enhancing effects in some tasks [35,64]. Thus, an extensive literature demonstrates that estrogens enhance cognitive function, but these enhancements may be small and may not occur under all testing conditions or in every study (18, 64-66).

2.1 Use of recognition memory tasks

In order to mitigate the influence of environmental, consolidation and retrieval variables in assessing hormonal effects on memory, our laboratory and others (35,36) have adopted the use of recognition memory tasks [23,24]. Recognition tasks do not require use of positive (food or water) or negative (shock or fear of drowning) rewards which may inadvertently influence performance. Hormones are known to affect motivation for, or salience of, these stimuli (18). Recognition memory tasks instead rely on the observation that rats are curious, seek novelty and explore their environment. Rats will readily explore new or novel objects and are more likely to explore a new object than one previously explored a few hours earlier. When delay periods are interspersed between first exploring an object and when subjects are presented that same, known object again and a new object, memory for the known object can be assessed. In addition, recognition memory tasks require minimal learning which

allows for measuring hormone effects on memory, not both learning and memory. It should also be emphasized that previously described spatial memory tasks can also be configured to measure only memory. In addition, performance on recognition memory tasks may be influenced by environmental and psychological performance parameters such as anxiety and motor activity. The contribution of these parameters to estrogen effects can be assessed within the recognition tasks (See 3a below) and by the use of other tasks such as an open field for activity and an elevated plus maze for anxiety measures. Thus, hormonal effects on cognition must be interpreted with a careful regard for both the task employed and the experimental design.

Recognition memory tests are conducted as depicted in Figure 3. Rats spend three minutes exploring two identical objects on an open field in the sampling or training trial (T1). After inter-trial delays of 1 to 4 h, the subject is returned to the field for testing in the recognition/retention trial (T2). As shown in the bottom portion of Figure 3, one of the identical objects can be replaced with a new object, which is termed the object recognition (OR) task or one object can be moved to a new location, which is termed the object placement (OP) task. In both versions of the task, the time spent exploring the new object/ location and old object/ location is noted. Spending significantly more time exploring at the new object/location vs. the old object/location indicates that the rat significantly discriminates between old and new configurations, i.e. remembers the old object/location. If subjects spend the same amount of time exploring the new object/ location and old object/location, then poor memory is exhibited. Recognition memory results can also be reported using the exploration ratio (time exploring new/time exploring old + new) where ratios of 0.5 indicate chance performance (poor memory) and ratios higher than 0.5 indicate that subjects remember and significantly discriminate between the objects or locations. Ratios less than 0.5 indicate perseverative behavior. Perseveration is rare in young adult rats, but is often present in aged rats and mice [8,64].

Both versions of the recognition tasks are working memory tests which require an intact hippocampus. However, OP is a spatial task that, although primarily dependent on an intact hippocampus [8] and/or fornix [22], also requires PFC input [23]. On the other hand, OR is less dependent on the hippocampus, is not considered a spatial memory task, and requires PFC activity [23]. Lesioning of the hippocampus impairs memory in the OP task, but causes lesser effects in the OR task [8,87]. For example, Broadbent et al [8] showed that damage to 30-50% of the dorsal hippocampus caused spatial memory impairments, while lesions of 75-100% were required to impair object memory. The cognitive load for spatial memory is also considered greater than for non-spatial memory [25]. This difference may be attributed to the fact that objects can be encoded and discriminated through multiple sensory modalities (visual or tactile) using cues such as size, shape, color and texture of objects, while discrimination of location involves abstract categorizations and use of cues and cognitive maps. The differences between the two recognition tasks are important for understanding how estrogens mediate rapid enhancements in memory.

3. Enhancement of cognitive function by estrogens

3. a. Sub-chronic and chronic treatments

The performance of several learning and memory tasks respond to estrogens when they are administered for a few days (sub-chronically) or for a few weeks (chronically). In addition, ovariectomy (Ovx) results in impaired performance of the same tasks. For example, rats showed impaired OR at 1 week following Ovx, and OP declined at 4 weeks following Ovx [122]. However, most studies have assessed effects of administering estrogens to Ovx subjects. When given to Ovx rats or mice for a few days, estradiol enhances water maze [106], object recognition and placement [49, 61,107] and T-maze tasks [39]. Longer

treatments are required to enhance eight arm radial maze and conditioning (trace eyeblink and fear) tasks in Ovx rats [15,43,56,64,65]. Aged female rodents, who have low levels of estradiol, also show impaired memory as compared to 2–4-month-old young rats on a number of tasks including the spatial memory dependent Y and T mazes, radial arm maze, Morris water maze and water radial arm maze [see 68]. Estrogen treatments are effective in enhancing memory in aged rats and mice [72 and see 33 for review].

Figure 1, Inset A, shows results of treatment with 17β -estradiol for two days at a dose of $50\mu\text{g/kg}$ and assessment of memory 48 hours later. Treated subjects significantly discriminated in both OR and OP, and the exploration ratio was higher following treatment, approximately 0.70 in both tasks following estradiol treatment but only 0.5 in vehicle treated rats. It was possible that enhanced memory by estradiol occurred through enhancements of activity and/or exploration. However, 17β -estradiol did not affect initial exploration of the objects in T1 (data not shown) and, under the same treatment conditions, did not affect activity on an open field (49). In addition, anxiety, which could affect performance, was not affected on an elevated plus maze by this treatment (40).

Thus, a substantial literature has shown that estradiol supports cognitive function in rodents by exerting long term effects on neural function. It is beyond the scope of the current review, but estradiol also exerts positive effects on cognitive function in human females over their life span [108,120]. As with rodents, this literature is inconsistent, and careful attention must be paid to the methodologies employed. The predominant mechanism believed responsible for estrogen's chronic influence on cognition in humans and in animal models is genomic, i.e. estradiol binds to two types of receptors, $\text{ER}\alpha$ and $\text{ER}\beta$, and the complexes act as nuclear transcription factors by binding to estrogen response elements (ERE) on DNA and stimulating transcription of specific genes whose proteins then alter neural functions including the enhancement of memory (Figure 1). As indicated earlier, it was surprising to many neuroendocrinologists when non-nuclear receptors for estradiol were identified in many estrogen target tissues including the brain. This discovery initiated new hypotheses regarding mechanisms for estrogen effects on neural function (Figure 2 and see other reviews in this issue) and brought about new approaches for research including our work assessing possible rapid effects of estrogens on memory.

3b. Acute Estradiol treatments-Preliminary Evidence for rapid effects on memory

Evidence accumulated during the 1990's that receptor(s) for estrogens were present outside of the cell nucleus in membranes of cell bodies, axons, spines, presynaptic terminals, and near post-synaptic neurotransmitter receptors [6,82,111] in a number of brain regions including the medial PFC and the hippocampus. In peripheral estrogen target tissues like the breasts and vascular epithelial cells, it had already been shown that receptors similar or identical to classically identified $\text{ER}\alpha$ and β receptors mediated rapid hormonal effects (sec to min) through signal transduction pathways (See figure and [59] for review and details). Thus, our lab began investigating whether estrogens might more rapidly activate learning and memory processes than in previous studies where injections of 17β -estradiol or Silastic capsules, which release constant amounts of the hormone, were given for days or weeks. It should be noted that estradiol is present in two enantiomeric forms, 17α - or 17β -estradiol depending on the orientation of the hydrogen at carbon 17 of the molecule. For binding to nuclear receptors, 17α -estradiol has a two-fold lower affinity than 17β -estradiol for $\text{ER}\alpha$ and a ten-fold lower affinity for $\text{ER}\beta$ (54). 17β -estradiol has been traditionally considered to be the biologically active form of the hormone. The synthetic estrogen diethylstilbestrol (DES) has a higher affinity for both receptors than 17β -estradiol, approximately five-fold for $\text{ER}\alpha$ and three-fold for $\text{ER}\beta$ (54). Less information is available concerning binding of estrogens to neural membrane receptors, but 17α -estradiol has a higher affinity than 17β -

estradiol for some membrane-related estrogen receptors (120) which is opposite to its binding affinities for classical nuclear receptors.

In our initial studies, 15 $\mu\text{g/kg}$ of 17 α - or 17 β -estradiol was administered to Ovx subjects 30 min before subjects explored the objects in T1. A 4 h inter-trial delay was subsequently given, and then the rats were tested in the T2 recognition/retention trial. Figure 2 Inset A shows that Ovx rats did not significantly discriminate between the objects, but both 17 α - and 17 β -estradiol treated subjects significantly discriminated between the old and the new objects, spending about three times longer exploring the new object than the old object (no differences were seen in T1, data not shown). Behavioral efficacy of both estradiol enantiomers was expected because both have an affinity for putative membrane receptors (120). In the same pre-T1 paradigm, 15 $\mu\text{g/kg}$ of diethylstilbestrol enhances recognition memory in Ovx rats (Fig. 4A, DES Pre-T1). DES was effective for place memory at the same dose, but a higher dose of 17 α -E, 60 $\mu\text{g/kg}$, was required for enhancing place as compared to object memory (70). The reasons for differences in potency of various estrogens on recognition memory are unclear, but dose-response curves appear related to both the specific estrogen used and the memory task employed (See section 5). Thus, a variety of estrogens rapidly enhance recognition memory, 4.5 h after injection. Moreover, effective doses, 15 $\mu\text{g/kg}$, were much lower than in studies where estrogens were given chronically, 50 $\mu\text{g/kg/day}$ [49]. Supporting these novel behavioral findings, Rhodes and Frye reported that inhibitory avoidance, a task relying on conditioned learning, was also enhanced by a similar dose (10 μg) of 17 β -estradiol within 4 h (99).

The dose and time parameters for rapid estrogenic enhancements of recognition memory suggested that the effects were not mediated via classical nuclear receptors. In addition, the involvement of classical receptors seems unlikely because 17 α -E₂ binds to nuclear receptors only at very high doses [54,120]. On the other hand, 4.5 h could allow sufficient time for nuclear receptor activation and a high dose of 17 α -estradiol was required for enhancing place memory. Moreover, it could also be argued that estradiol might be indirectly altering memory by affecting non-mnemonic factors such as motivation, motor or sensory processes since it was given before the sample/acquisition trial. While several lines of evidence argue against these possibilities (see [70] for details), we still adopted new procedures for analyzing estrogenic effects on memory (see section 4).

4. Post training treatments for assessing rapid effects of estradiol on memory

Hormonal enhancements in memory function need to be integrated with extant theories on the mechanisms for learning and memory. At the beginning of the 20th century, the laboratory of Muller and Pilzecher advanced the theory of memory consolidation (see [80] for discussion). It was hypothesized that newly acquired information or memories (short-term memory) were consolidated into long term memory. Thus, as shown in Figure 4, subjects first acquire or learn information. This knowledge is then consolidated or stored within specific brain areas, and finally, when appropriate, the stored information is retrieved for use. The exact nature of these processes still remain unclear, but experiments by McGaugh and colleagues (reviewed in [80]) provide empirical evidence for this theory using what has come to be called a post-training protocol for assessing memory. In this protocol, drugs or hormones are given after subjects acquire information such as the location of a platform in a water maze or after subjects have explored objects in recognition tasks (the T1 Trial). This paradigm is based on the idea that following a training, learning or sampling trial, the new information requires consolidation and that drugs or hormones could influence memory storage processes during the immediate period following training. Indeed, rats receiving injections of “memory enhancing drugs” like amphetamine or noradrenergic

agonists during this period showed better retention of what they had learned [80]. An important additional finding was that the effectiveness of post-training treatments was time-limited; only treatments given within 1-2 h after training/sampling trials were effective; treatments given later were ineffective. Thus, a window for consolidation of memory exists within which drugs can enhance or impair the process (shown in Figure 4 as the period for consolidation of memory). An additional important advantage of post training protocols is that consolidation enhancements after immediate, but not delayed, post-trial injections rule out the possibility that drug or hormonal enhancing effects derive from non-mnemonic effects during learning [91]. This is because the agents are given after the learning trial and thus are unable to influence the learning process. We adopted the post-training paradigm in order to 1) assess effects on memory more rapidly and 2) eliminate possible non-mnemonic effects of estrogens.

In a series of experiments, various estrogens were given immediately after the sample trial, and recognition memory was tested 2-4 h later. When 15 μ g of DES was given immediately following the sample trial and recognition/retention was tested 4 h later, DES treated subjects discriminated between old and new objects whereas Ovx rats did not (Figure 4 inset B). If DES injections were delayed until 2 h after the sample trial, then subjects did not significantly discriminate (Figure 4 inset C). A similar pattern was seen when 30 μ g/kg of 17 β -estradiol was given immediately following T1 and object placement memory tested, i.e. estradiol was required to be given within 2h of the sampling trial in order to enhance place memory [70]. When the dose of 17 β -estradiol is lowered to 20 μ g/kg, treatment is required within 45 min in order to enhance place memory (47). Studies in the Frick laboratory showed that post sample trial enhancements in OR in OVX mice were still present 48 h following 17 β -estradiol-cyclodextrin administration [28,60]. Cyclodextrin leads to rapid metabolism of estradiol and indicates that the hormone need not be present at the time of memory enhancements. Moreover, 17 β -estradiol-cyclodextrin applied directly to the dorsal hippocampus enhanced object memory suggesting that estrogenic activation within the hippocampus is sufficient to enhance memory for objects in a post sample trial paradigm (28,60).

The pattern of enhancements for recognition memory is similar to the results of Packard and colleagues who investigated effects of estradiol on spatial memory using the Morris water maze task [91]. Immediately following the last training trial, Ovx rats received IP injections of 200 μ g/kg of 17 β -estradiol-cyclodextrin. In this case, retention was tested 24 h later, and estradiol treated rats exhibited better memory for the hidden platform. If estradiol injections were delayed for 2 h after training, then the hormone was not effective 24 h later. Interestingly, enhanced memory was also exhibited when castrated male rats received the same 17 β -estradiol treatment and after intra-hippocampal administration to either sex [91]. Using another learning task, inhibitory avoidance, Rhodes and Frye [99] found that 10 μ g of 17 β -estradiol immediately, but not 1, 2 or 3 h posttraining, increased crossover latencies as compared to vehicle treatment. Taken together, results show that estrogens rapidly enhance consolidation of memory and that the process takes between 45 to 120 min depending on the estrogen dose and the time delay in retention testing.

Therefore, evidence shows that estradiol enhances memory consolidation in a variety of learning and memory tasks within approximately an hour. Further, the characteristics of performance enhancements are consistent with augmentation of mnemonic processes through membrane associated estrogen receptors.

5. Dose-response relationships for rapid estradiol effects

A series of experiments determined the dose-response (D-R) curve for α and β -estradiol enhancements in object and place discrimination. Rapid effects of estrogens in biological and toxicological studies have been shown to exhibit inverted U dose-response curves [1,10,127]. Moreover, hormones, drugs, environmental chemicals and neurotransmitters also show inverted U dose-response curves in post-training injection paradigms. Thus, it was important to determine whether the dose-response curve between estrogens and recognition memory showed this relationship. Based on our previous work [70], we tested doses of 17β -estradiol from 10-60 $\mu\text{g/kg}$ for enhancing object placement. The D-R curve was an inverted U with 20 $\mu\text{g/kg}$ enhancing place memory, and both higher and lower doses were ineffective (Figure 2B). A similar inverted U curve was found for object memory, but a lower dose was effective, 5 $\mu\text{g/kg}$, than for place memory [47]. Analysis of 17α -estradiol also showed inverted U curves and a different sensitivity for each of the tasks. Importantly, 17α -estradiol was more potent than 17β -estradiol which is consistent with other membrane mediated functions. The effective dose of 17α -estradiol for place memory was 5 $\mu\text{g/kg}$ and for object memory was 1 $\mu\text{g/kg}$. This inverted U dose-response relationship may also explain some negative experimental findings when only one dose of estradiol was used. Using only one dose may have led to the erroneous conclusion that estradiol is ineffective in enhancing memory. Thus, memory consolidation, like other physiological processes, shows an inverted U relationship with concentration of both 17α -estradiol and 17β -estradiol.

6. Which estrogen receptor mediates memory effects, ER α or ER β ?

To determine which estrogen receptor(s) might be responsible for EB's enhancing effects in the recognition memory tasks, we first tested selective agonists for ER α (PPT [46]) and ER β (C-19 [123]; DPN [84]) under the same sub-chronic treatment regimen as with estradiol (dosing for two days and memory tested 48 h after last dose). The two ER β agonists, C-19 and DPN, enhanced object and place memory, but the ER α agonist did not (Figure 1 Inset B). These results are consistent with those of Rissman [101] who showed that estrogen-treated mice with a knock-out of ER β did not learn the Morris water maze task and of Liu et al. [62] who showed that estrogen treatment to ER α knock-out, but not ER β knock-out, mice improved hippocampal-dependent Y-maze performance. Also, Walf et al. [119] reported that DPN administration to wild type, but not ER β knock-out mice, enhanced both OR and OP performance. Other experiments in which estradiol and ER-specific agonists were given to OVX rats also indicate the importance of ER β . The ER β agonist, WAY-200070, but not the ER α agonist PPT, given for two days, enhanced spatial memory on the radial arm maze [62]. However, a recent study tested acquisition and memory in a delayed matching-to-position (DMT) T-maze task after chronic treatments [43]. Estradiol, PPT and DPN all enhanced acquisition/learning as compared to OVX females; however, none of these treatments altered memory in tests with long inter-trial delays following the acquisition trials. In this case, treatment was chronic, not sub chronic, as acquisition was tested beginning 10 days after initiation of treatments. Thus, it is possible that the longer duration of treatment led to loss of receptor selectivity of the agonists. In addition, the two estrogen receptors have been shown to influence the expression of one another [102], so it is possible that the chronic treatments led to changes in receptor expression, which resulted in ER α activation of memory (see Rissman [102] for further discussion). In sum, a preponderance of the literature indicates that estrogenic enhancements of recognition memory in a sub-chronic paradigm rely on activation of ER β .

We then examined possible rapid effects of the ER agonists in the post training paradigm. Like estradiol, drugs were given immediately following the sample trial and retention was tested 4 h later (post training paradigm). For OR, the exploration ratio for DPN was

significantly higher than for the vehicle-treated group while in the PPT-treated group it was not (Figure 2 Inset C). The same was true for the OP task, exploration ratios of PPT and vehicle treated groups were not significantly different from each other but were significantly higher for the DPN treated group (49). Thus, acute treatments with the ER β -specific agonist, DPN, but not the ER α -specific agonist, PPT, enhanced both object and place recognition. Similar memory enhancing effects by DPN, but not PPT, were obtained by Rhodes and Frye [100] in a post training treatment paradigm in the water maze task. However, Frye's group has recently reported that when the agonists are given post T1 and memory tested 24 h later, both PPT and DPN enhanced OP [36]. Furthermore, PPT, but not DPN, enhances OR [100]. Differences in agonist doses do not appear to account for the differences in effects (1 vs. 0.9 mg/kg), but the differences in inter-trial delay lengths (4 h vs. 24 h) may be important. The additional 20 h may allow for receptor interactions or other unknown processes to occur. Recently Phan et al. [98] showed that PPT and DPN both enhanced OR and OP within 40 min. Since the doses were higher than other studies, 2.5 mg/kg, receptor selectivity may have been compromised. On the other hand, Mitra et al. [83] reported differences in the distribution of ER β in rats vs. mice, which may account for the behavioral differences in Phan et al. [98] as compared to other studies.

Currently, the identity of the neural membrane receptor which mediates rapid effects of estrogens on memory is unknown (indicated as ER? In Figure 2, Inset C). Possible mediation at a G protein-coupled receptor 30 (GPR30) does not seem likely because 17 α -estradiol activates OR memory at 5 ug/kg but shows little competitive binding to GPR30 in the micromolar range (see 48 further discussion). Collectively, our behavioral data indicate that both rapid and sub chronic effects of estradiol on memory function in rats are mediated through ERs, most likely ER β ; however, not all studies are in agreement. Thus, the role of currently described estrogens receptors in enhancing memory function requires further investigation.

7. Xenoestrogens rapidly antagonize estrogen-dependent memory

Xenoestrogens include chemicals which are naturally present in the environment, such as phytoestrogens in whole grains, soy and clover, as well as synthetic compounds like bisphenol A (BPA) and DES. Some xenoestrogens, like phytoestrogens and BPA, are “weak” estrogens because they bind poorly to classic estrogen nuclear receptors, and thus are not potent in causing estrogenic effects [120] while others, like DES, bind to classic receptors and are more potent than estradiol [70]. However, chronic exposure to “weak” estrogens may lead to disruption of physiologic functions or mimicking of estrogen's effects [120]. For example, we found that chronic ingestion (5 weeks) of phytoestrogens present in rat chow increased uterine weight and enhanced recognition memory in OVX, adult rats [67]. BPA and DES cause alterations in estrogen's normal effects, especially during developmental periods [115]. However, it is now clear that some xenoestrogens can bind to membrane receptors and rapidly activate signaling pathways, even at very low concentrations ([120], Figure 4 Inset A, B). Thus, BPA has a powerful impact on neural systems at low doses and appears to be a mixed agonist/antagonist for both estrogen and androgen receptors [124]. BPA can, depending on the dose and the presence or absence of circulating gonadal hormones, mimic or block gonadal hormone function. Most studies show that BPA alters neural systems during vulnerable periods of development and that exposure leads to impairments in behaviors including memory in adulthood [115]. Based on our work showing that very low doses of estrogens or chronic exposure to phytoestrogens enhanced memory and the demonstration that BPA rapidly antagonizes increases in spine synapse density by estrogens and androgens in the medial PFC and hippocampus [45,74], we investigated the effects of low doses of BPA given in the post sample trial on recognition memory in female and male rats.

When 40 µg/kg of BPA is given to gonadally intact males immediately following a sample trial, object and place memory is impaired 2-4 hours later (Figure 5A and [20]). In cycling rats, BPA, given immediately after the sample trial, impaired object memory during proestrous but not on other days of the estrous cycle [48]. Therefore, BPA rapidly impairs hormone dependent recognition memory in gonadally intact male and female rats. However, BPA does not appear to act as an agonist in females because doses from 1 to 400 µg did not affect recognition memory in OVX females when it was given immediately after the sample trial (48). BPA (40 µg/kg given 30 min before the sample trial) blocked recognition memory enhancements in OvX females that received 17β-E₂ immediately after the sample trial (Figure 5B). Thus, BPA appears to rapidly antagonize estrogen and/or androgen effects on recognition memory, but given alone it does not enhance memory.

8. Estradiol effects on dendritic spines

Given that dendritic spines, located within the hippocampus and medial PFC have been linked to learning and memory, we have been investigating a possible role for dendritic spines in estrogen's enhancements in memory. However, we first provide some background for these studies.

8a. Plasticity of dendritic spines

Most neurons have dendrites that are covered extensively by dendritic spines, which serve to increase the surface area for neurotransmission [89]. Dendritic spines were first described in 1888 by Ramon y Cajal in cerebellar purkinje cells (reviewed [37]). In addition to identifying spines on many different neurons, Ramon Y Cajal also described three different types of spines and noted that the density of dendritic spines increased with increased innervation. Pyramidal neurons, which are found in the hippocampus and cortex, are particularly rich in spines [89,116] See Figure 6 for examples of golgi impregnated pyramidal cells in the medial PFC and CA1 area of the hippocampus and Figure 7 for an enlargement of a secondary basal dendrite in CA1 with spines. Several subtypes of dendritic spines are recognized with their classification depending on the author, but generally dendritic spines consist of a protrusion and either have a bulbous termination (mushroom spines) or not. One may also distinguish between thin spines with a smaller head and stubby spines that lack any terminal enlargement and extremely thin filapodia, which may be the precursor to mature spines [9,113,116].

Ramon Y Cajal [37] first suggested that the function of dendritic spines was to increase the surface area for communication. Although the localization of synapses on dendritic spines was shown by electron microscopy much later, it is now readily accepted that dendritic spines increase the surface area for synaptic contact. Neonatal dendrites are largely devoid of spines, and spine density increases with age until a critical level is reached in the adult rat, which neuroscientists initially assumed remained static. It has become increasingly evident that the opposite is true, and it appears that the dendritic spine is a critically important mediator of neural plasticity (reviewed by Urbanska et al. [113]). Indeed, the number and type of dendritic spines fluctuate in response to many different types of stimuli. Classic studies in neural plasticity have shown that dendritic spines are sensitive to alterations in connectivity. Mice raised in total darkness have decreased dendritic spine density on pyramidal cells in layer V of the primary visual cortex that can be reversed after only a few days of life in normal lighting [113]. In hippocampal granule cells, there is a decrease in dendritic spine density with deafferentation that can be reversed with reinnervation [92].

These early, classic studies were followed by experiments clearly demonstrating that dendritic spines are sensitive to many different types of manipulation. Cocaine increases dendrite spine density in the PFC and nucleus accumbens [104] of adult rats, and cocaine

injection in dams increases dendritic spine density in the medial PFC, hippocampus and striatum of 21 day old offspring [31]. While stimulants have been shown to increase dendritic spine density, morphine has been demonstrated to decrease dendritic spine density in the cortex and nucleus accumbens of adult rats [103]. Stress causes sex dependent changes in dendritic spine density in the hippocampus [14,58] and decreases in spine density in the PFC of rats [7]. With aging, significant alterations in neuronal structure are observed in both male and female rats [7, 38,73,77,117,121] and monkeys [19,112]. In both species, aging corresponds to a decline in dendritic spines in the apical and basal trees of pyramidal cells in layers III and V of the cortex. Dumitriu [19] found that there was a 33% decrease in spines on pyramidal cells that was accompanied by a 32% decrease in axospinous synapses in layer III pyramidal cells of area 46 of the monkey. Although most of these studies were done in males, Wallace et al. [121] demonstrated that there was a 16% decrease in dendritic spine density on the apical branch of pyramidal cells in the medial PFC in aged female Fischer 344 rats as compared to young female rats. A 16% decrease in dendritic spine density was also seen on apical branches of CA1 pyramidal cells in female aged Fischer 344 rats but no decreases in dendritic spine density on basal dendrites were seen in CA1 or either branch of CA3 cells [73]. The decreases observed in these studies are accompanied by impairments in recognition memory and decreased levels of circulating estradiol, which supports the importance of estradiol in memory processes. Thus, alterations in dendritic spine density are found in the hippocampus and the PFC following different stimuli and across the lifespan in mammals.

8b. Functions of dendritic spines in learning and memory

There is increasing evidence that the mechanism(s) underlying learning and memory involve dendritic remodeling. In the hippocampus, which is critical to short term memory, the acquisition of new memories in a conditioning paradigm is associated with increases in dendritic spine density in the CA1 region in adult male rats [50,56] and female rats [5]. In addition, evidence shows that existing spines undergo structural alterations that result in LTP [50,56]. It is in the hippocampus, where the functional implications of alterations in spine density are best understood because increases in dendritic spine density and/or spine sizes are associated with long term potentiation (Muller et al, 2000, LTP [81,86] and decreases in number and size are associated with long term depression [3]. Taken together it appears that memory formation requires alterations in dendritic spines that then lead to alterations in LTP [78].

8c. Relationship between chronic changes in estrogens and spines

It has over twenty years since it was shown that chronic changes in estradiol alter dendritic spine density (See [79]). In the present review, we focus on recent studies showing acute effects of estrogen on dendritic spines and memory. However, we will first review the chronic studies on estrogen-induced changes in spine density. It is important to note that in these studies the amount of estrogen administered, the isomer used and the length of time that the animals were OvX prior to estrogen treatment, as well as diet ([67] and see below) greatly impact the results. Even given these variables, it is abundantly clear that dendritic spines are extremely responsive to changes in circulating estrogen.

Fluctuations of approximately 30% occur in dendritic spine density on neurons in the ventromedial hypothalamic nucleus (VMN) [30] and pyramidal cells in the CA1 region of the hippocampus (CA1) during the 4-5 day estrous cycle of the rat [125]. In both regions, dendritic spine density is greatest when estrogen levels are highest. There is a decrease in dendritic spine density in the VMN [30], CA1 [41,122] and the medial PFC [122] following OvX. Administration of estradiol to OvX rats restores the levels of dendritic spines in the VMN [11,30], CA1 [41] and the amygdala [17]. Similar results have been obtained in the

monkey for CA1 [45,91] and the PFC, where estradiol has been shown to increase both dendritic spine [10] and spine synapse density [44].

In addition, we have also demonstrated that phytoestrogens, found in soy meal [93] and used in laboratory chow fed to rats, influence both spine density and memory. Ovx rats, that have little circulating estrogens, were fed a high phytoestrogen diet and had a 48% increase in spine density in CA1 and performed better on an object placement task compared to rats on a low phytoestrogen diet [67]. In the same study, rats that received the diet low in phytoestrogens had 79% of the spine density present in the high phytoestrogen diet in the PFC.

Pregnant rats, that have high levels of circulating estrogens, have greater levels of dendritic spines in both apical and basal branches of pyramidal cells in CA1 and the medial PFC, 20 days after parturition when compared to virgin females [53]. Recently we have shown that pregnancy results in an increase in dendritic spine density of 16% in the medial preoptic area when compared to age matched virgins [32 and see 52 for review].

Thus, a broad range of studies have shown that chronic changes, days to months, in circulating estrogens are accompanied by alterations in dendritic spine density and in cognitive function in the few studies which have investigated both spines and cognition. However, as indicated previously (section 3) it has been demonstrated, by our group and others, that estrogen facilitation of memory can occur within minutes and that the doses, as well as the active estrogens, are not consistent with genomic mediation through classic estrogen receptors.

8d. Relationship of acute changes in estrogens to spines and spine synapses

The demonstration that estrogen is capable of enhancing memory in as little as 4.5h [70] led to a study in which the effects of both estradiol isomers on pyramidal spine synaptic density (PSSD) in cells in CA1 were assessed [74]. In Ovx rats given injections of either 17 α - or 17 β -estradiol, there was a dose dependent increase in PSSD in CA1 within 30 minutes. For 17 β -estradiol, a 15 μ g/kg dose had no effect at either 30 minutes or 4.5h, whereas 45 μ g/kg increased PSSD by 44% as compared to control at 30 minutes and by 24% at 4.5h. When 45 μ g/kg of 17 α -estradiol was administered, the increase in PSSD was 62% after 30 minutes and even greater when the lower dose, 15 μ g/kg, was used. A greater effect with the lower dose supports a U shaped dose response to estrogens [120] and that both 17 α - and 17 β -estradiol promote differential increases in dendritic spine synapses that may be important to the process of memory formation. Lastly, it appears that 17 α -estradiol may be more potent at increasing dendritic spine synapses than 17 β -estradiol. Whether 17 α -estradiol increases the density of the spines has not been assessed, but based on the greater ability of 17 α -as compared to 17 β -estradiol to rapidly enhance memory and synapses, it can be predicted that spines would be increased by 17 α -estradiol in the PFC and CA1.

PPT, an agonist for ER α and DPN, an agonist for ER β , respectively, have been shown to differentially affect dendritic spine density on pyramidal cells in CA1 in the mouse [98]. Within 40 minutes of administration, PPT increased dendritic spine density in the stratum radiatum and lacunosum-moleculare whereas DPN decreased spine density in lacunosum-moleculare. In the rat arcuate nucleus, an area involved in reproductive function, administration of 50 μ g of estradiol benzoate resulted in a large increase in dendritic spines after 4h that persisted through 48h [113]. Moreover, in this study the effects of estradiol on spinogenesis were blocked when mGluR1a was blocked, indicating that the effects of estradiol were mediated by a membrane receptor. Thus, results in a few studies have shown that estradiol or estrogen agonists can rapidly alter dendritic spine density in brain areas.

8e. Relationship of acute changes in estrogens to spines and memory

In order to determine whether estrogen dependent increases in spines in the medial PFC and hippocampus contribute to the rapid enhancements in recognition memory by estrogens, Ovx female rats were given a recognition memory sample trial and an immediate injection of 20 µg/kg 17β-estradiol. Subjects were sacrificed 30 min after the T1 trial and spine density measured. At this time interval and dose, 17β-estradiol enhances place, but not object, memory consolidation [47]. In pyramidal cells in CA1 and the medial PFC, spine density increased 30 minutes after estradiol administration (Figure 7B). In CA1, 17β-estradiol increased basal spine density by 29% compared to control but did not alter apical spine density. In the PFC, 17β-estradiol administration resulted in a 16% increase in apical and a 27% increase in basal spine density as compared to control after 30 minutes. Thus, estradiol leads to a rapid increase in spine density in both areas known to be important for memory function and at a time when memory consolidation is known to occur.

Consistent with these *in vivo* results, Srivastava et al. [109] demonstrated that administration of WAY-0700, an ERβ agonist, induced an increase in spinogenesis, as well as an increase in spine size within 30 minutes in cortical cell cultures. A concomitant increase in PSD-95 was also observed in these cells after 30 minute exposure to WAY-0700.

8e. Effects of Bisphenol-A on dendritic spines

The synthetic non-steroidal compound, bisphenol-A (BPA) has mixed estrogen agonist/antagonist properties and androgen antagonist properties (See section 7). As shown in Figure 5, we found that 40 µg/kg BPA impaired recognition memory in male rats and blocked estrogen enhancements in memory in Ovx females. Thus, spine density was measured in BPA treated males and females. Spine density was measured in males 30-40 min after BPA injection which occurred immediately following the completion of a sample trial for recognition memory (Figure 7C). BPA treated males showed small, but significant, decreases in spine density in both the PFC and CA1 [20]. In CA1, BPA decreased dendritic spine density by 10% in apical dendrites and by 9% in basal dendrites as compared to control, and BPA also decreased PSD 95 in the hippocampus. In the medial PFC, there was a decrease of 23% on apical dendrites and 26% on basal dendrites as compared to control. With these conditions, no changes in spine density in CA3 pyramidal cells were noted.

In females, BPA also altered estradiol induced increases in dendritic spine density. Density was assessed in Ovx female rats by treating with 40 µg/kg of BPA, waiting 30 min, giving a T1 sample trial followed immediately by injection of 20 µg/kg of estradiol and then sacrificing the subjects 30 minutes and 4 h later [48]. After 30 min, estradiol increased basal (29% above control), but not apical spine density in CA1. The combination of BPA and estradiol increased spine density in both apical (25% above control) and basal dendrites (48% above control). In the PFC, estradiol induced a 16% increase in apical and a 27% increase in basal dendritic spine density after 30 min. Co-administration of BPA with E2 also significantly increased apical (24% above control) and basal (33% above control). Therefore, acute BPA does not affect E2-induced spine density in the PFC, but further elevates spine density in CA1 at 30 min post T1. At 4 h post T1 in CA1, estradiol alone did not affect apical spine density, but BPA+ E2 together increased it by 12%. In contrast, basal spine density was significantly increased by E2 (12% above control), but there were no differences in spine density between vehicle-treated and BPA+E2 treated subjects. Thus, at 4 h after treatment, BPA suppressed E2-induced increases in spine density in CA1 basal dendrites but it further enhanced the E2-induced increases in spine density in the apical dendrites. In the PFC, basal spine density was significantly increased by both E2 (33% above control) and BPA+E2 (44% above control,) but the groups were not different from each other, and no effects were found on the apical dendrites in the PFC. Thus, a mixed

pattern of BPA effects was found in areas mediating memory function, and the changes were time dependent. BPA did block estrogen dependent increases in spine density in basal CA1 dendrites 4 h later. Because CA1 is critical for memory (8), this block may be an important contributor to impairments in memory consolidation by BPA.

In contrast to these results in spines, when spine synapses are measured in CA1, MacLusky et al. [75] found that simultaneous administration of 40 ug/kg of BPA and 60 ug/kg of 17 β -estradiol led to an approximately 7% decrease in synapses as compared to estradiol alone 30 min later. Thus, changes in synapses may occur earlier than changes in spines or responses to BPA may differ depending on whether BPA is present in the system before estradiol. Overall, BPA treatments to male and female rats rapidly impair memory and alter spine expression in the PFC and CA1, but whether there are causal relationships between the variables requires further investigation.

8f. Downstream regulation of signaling pathways by estradiol

Plasticity of dendritic spines includes both the generation of “new” spines and the process of maturation from thin filipodial projections to more complex structures that can make synapses (reviewed by [126]). This gradual process requires the mobilization of many proteins, especially the reorganization of actin (reviewed in [96]), which is highly concentrated in dendritic spines [81] and actin associated proteins which extend into the post synaptic density (reviewed by [29]). Estrogen has been shown to stimulate the assembly of actin as well as associated proteins important to spine plasticity [105;109]. Moreover estrogen has been shown to increase many other proteins concentrated in dendritic spines. When 1 μ g of 17 β estradiol is administered to Ovx C57BL/6J mice for 5days both spinophilin and PSD 95, two a presynaptic proteins, which correspond to mature spines, are increased in the dorsal hippocampus [61]. In rats chronic estradiol increases in spinophilin have been demonstrated in the hippocampus [65] and arcuatenucleus [13].

Our studies suggest several alternatives for estrogenic effects on spines including the generation of new spines, rapid maturation of spines or an activation of extant spines. Support for the last two possibilities comes from studies which show that monoaminergic activity is substantially and rapidly altered in both the hippocampus and frontal cortex following estradiol administration (Table 1). Ovx females underwent a sample trial for recognition memory, were immediately injected with vehicle or 17 β -estradiol and then sacrificed 30 min later. Activity of monoamine containing systems was indexed by measuring metabolites. The noradrenergic metabolite, MHPG, was increased by approximately 100% in the medial PFC and decreased in CA1 and DG by approximately 30% following estradiol treatment. The metabolites of dopamine and serotonin were not altered in these areas. Monoamines, especially norepinephrine in the medial PFC and hippocampus contribute to genomic mediation of memory by estradiol [49,66,71]. Chronic estradiol treatments also result in differential changes in various monoaminergic systems in the areas and appear dependent on dose and duration of treatment (66). This preliminary evidence links rapid effects of estradiol on memory to norepinephrine containing neural systems, but further studies on dopaminergic and serotonergic systems are clearly necessary to understand the relevance of the current findings to memory consolidation.

9. Possible functions for rapid enhancements in memory by estrogens

Whether these newly described rapid changes in memory elicited by estrogens have functional relevance for organisms is currently unclear. However, it could be imagined that ingestion of naturally occurring phytoestrogens in clover or soy plants might have been beneficial to our ancestor hunter-gatherers or to grazing animals today. Ingestion of these plants might quickly enhance remembrance of the location of these food sources which are

rich in protein. While we have shown that chronic exposure to phytoestrogens in food enhances place memory and increases spines [67], acute effects have not yet been investigated and should be undertaken. At present, BPA is still found in many plastic containers in the USA and, therefore, one can envision the negative effects of BPA on cognition that occur with the ingestion of water from plastic bottles after exercise. We have shown that an acute dose of BPA impairs recognition memory in male and female rats [20,48], and Parducz et al. [91] showed that BPA blocks estrogen's induction of spine synapses in non-human primates. Another positive role for rapid actions of hormones on memory is related to stress. Stress is known to cause peripheral release and central synthesis of gonadal hormones, estradiol in females and testosterone (which can be rapidly aromatized to estradiol) in males [63]. Thus, in the classic stress situation of the lioness pursuing the antelope across the savannah, estrogens may promote memory of the location of an attack or details of its overall circumstance. A similar effect might be envisioned for people in cities, for example enhancing memory for the locations of traffic jams or dangerous locals. While release of corticosterone during acute stress is associated with memory enhancements, a possible role for estrogens in this process has not yet been investigated. Finally, it can be speculated that activation of estrogen signaling mechanisms may facilitate genomic responses and thus nuclear crosstalk may serve as a "priming" mechanism for long term changes associated with classic receptor mechanisms. Support for this concept is the observation that following long term Ovx, larger doses of estradiol and longer treatment times are necessary to activate responses [79].

10. Discussion and Conclusions

Overall, these results show that estrogens and related compounds rapidly influence recognition memory. Behavioral evidence is strong that membrane receptors mediate this response because estradiol effects are rapid and key brain areas responsible for memory, the medial PFC and hippocampus, are activated. Moreover, 17 α -estradiol is more potent than 17 β -estradiol, and responses are inverted U shapes. What remains unclear is the type of estrogen membrane receptor involved. For both object and place memory, our work supports involvement of an ER β -like receptor, but results in other labs and for other kinds of memory are not as clear. Whether rapid alterations in dendritic spines underlie estrogenic enhancements in memory also remains somewhat unclear. 17 β -estradiol administration increases spine density in the medial PFC and hippocampus within 30 min, a time course concomitant with estrogen's enhancement of recognition memory consolidation. In addition, BPA, an estrogen and androgen receptor antagonist impairs recognition memory and alters expression of spines by estrogen. However, the acute effects on spines are complex with both the dose and duration of treatments critical for the outcome of the responses. Of interest is that in some cases dendritic spine density is greater 30 minutes after estradiol administration than 4h after estradiol. It is possible that the high spine density seen at 30 minutes represents increased filipodial or immature processes that mature into different subtypes of spines at 4h. This process has been suggested to occur during development [113]. Thus, a more detailed study assessing the change in different spine subtypes over time after estradiol administration would help to clarify the role of spines in memory consolidation processes. Another consideration for future studies is the role, if any, of spines in the memory process itself independent of hormonal influences. In males, BPA induced decreases in spines were found only when subjects underwent a sample trial for recognition memory; if the sample trial was not given, BPA did not alter spines [20]. In addition, spine density was higher in the memory tested than memory untested males.

In conclusion, we suggest that the ability of estradiol to activate a diverse set of physiological functions through membrane signaling should now also include memory processes. Thus, estradiol is responsible for mediating changes in memory, which are both

delayed in onset and sustained (genomically mediated) as well as those, which are rapid and short, lived (membrane mediated). On the other hand, it may be that the membrane receptors provide a more rapid way to alter protein synthesis and that both chronic and acute estrogen may ultimately exert their effects through a common mechanism (reviewed by [40 and 120]).

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Figure 1.

Schematic of genomic mechanism for estrogenic responses and effects of estradiol and receptor agonists on recognition memory.

Circulating estrogens enter the cell nucleus where they bind to two types of receptors, ERα and ERβ, and the complex acts as a nuclear transcription factor by binding to an estrogen response element (ERE) on DNA and stimulating transcription of specific genes whose proteins then alter neural functions including the enhancement of memory.

A: Estradiol enhances memory. EB was given at 50ug/kg for 2 days and object recognition and placement tested 48 hours later, $P < 0.001$.

B: Effects of specific ER agonists on object recognition. ERα specific agonist, PPT, and ERβ specific agonists, C19 and DPN, were given as in Inset A. Only ERβ agonists enhanced memory, $p < 0.05$. Data from [49].

ESTROGENS RAPIDLY ENHANCE MEMORY THRU ACTIONS AT MEMBRANE RECEPTORS

Figure 2.

Schematic for non-genomic mechanism for estrogenic responses and effects of estrogens, estrogen receptor agonists and D-R relationship.

Circulating estrogens bind at cell membrane receptors to initiate rapid “non -genomic” mechanisms at the membrane surface and thereby bypass the nuclear EREs and instead activate intracellular signaling cascades and immediately early genes which initiate rapid memory enhancements. Membrane ER α and βs have been identified as well as others not as well described (ER?).

A. 17α and β-estradiol enhance memory. When given 30 min prior to a sample trial, both isomers of estradiol enhance object recognition memory 4 h later. Data from [70].

B. Dose-response curve for 17β-estradiol and object placement. Given immediately following the sample trial and retention testing 4 h later, the D-R curve is an inverted U with 20ug/kg the only effective dose. Data from [47]. C. Effect of ER agonists on object recognition. Given immediately following the sample trial and retention testing 4 h later, only the ERβ agonist, DPN, enhanced memory. Data from [49].

Abbreviations: ERK, extracellular signal-regulated kinase; CREB, cAMP response element binding protein; CRE, cAMP response element. Data from[49].

Recognition Memory Tasks

Figure 3.
Depiction of recognition memory tasks.
A rat is shown on the open field with objects.

Figure 4.

Timeline for memory consolidation and effects of DES given before and after sample trial on consolidation.

Work by McGaugh [80] has shown that memories are consolidated during the first 1-2 hours following a sample or training trial. Drug or hormone treatments can enhance or impair memory during this window for treatment even when retention is tested up to 48 hours later. If treatments are given later than 2 h post T1, they do not affect memory consolidation.

A. DES, 15 ug/kg, was given 30 min before T1, and object recognition was enhanced 4 h later.

B. DES, 15 ug/kg, was given immediately after T1, and object recognition was enhanced 4 h later.

C. DES, 15 ug/kg, was given 2h after T1, and object recognition was not enhanced 4 h later.

** $P < 0.01$. Data from [70]

Figure 5.

BPA impairs recognition memory in male and female rats.

A. Male rats. BPA was given immediately following T1 at 40 ug/kg and Object and Place recognition was tested 4 h later and expressed as % time with new object/place. * $P < 0.05$. Data from Eilam-Stock and Luine, unpublished.

B. Female Rats. BPA was given 30 min before T1 at 40 ug/kg, estradiol was given immediately following T1 at 5 ug/kg for object memory and 20 ug/kg for place memory, retention was tested 4 h after T1. ** $P < 0.01$. Data from [48].

Figure 6.

Golgi impregnated pyramidal cells in the brain of an adult female rat.

A. Medial prefrontal cortex. Cells are shown at 10x magnification. Layer II/III pyramidal cells are used in our analyses. Adapted from [68].

B. CA1 of the hippocampus. The photomicrograph is at 40x magnification. Frankfurt, unpublished.

Figure 7.

Rapid effects of 17 β -estradiol and BPA on spine density

A. Golgi impregnated secondary basal dendrite from a CA1 neuron illustrating dendritic spines (arrows). Scale bar = 20 μ m. Adapted from [68].

B. Estradiol rapidly increases spine density in adult female rats. OVX female rats were given a recognition memory sample trial and then were immediately injected with vehicle or 20 μ g/kg 17 β -estradiol. Subjects were sacrificed 30 min after the T1 trial and spine was density measured. * $P < 0.05$, ** $P < 0.01$. Data from [48].

C. BPA rapidly decreases spine density in adult male rats.

Males were given a recognition memory sample trial and then were immediately injected with vehicle or 40 μ g/kg BPA. Subjects were sacrificed 30-40 min after the T1 trial. * $P < 0.05$, ** $P < 0.01$. CA3 data unpublished by Restrepo and Luine, other areas from [20].

Table 1Effect of 17 β -Estradiol on monoaminergic metabolites

METABOLITE	TREATMENT	mPFC	CA1	DG
MHPG	Control	3.11 $\pm$ 0.26	18.45 $\pm$ 2.64	3.34 $\pm$ 0.35
	17 β -E ₂	6.38 $\pm$ 0.41 ***	12.63 $\pm$ 0.69 *	2.17 $\pm$ 0.22 **
HVA	Control	5.30 $\pm$ 0.44	0.12 $\pm$ 0.02	0.09 $\pm$ 0.01
	17 β -E ₂	5.83 $\pm$ 0.55	0.12 $\pm$ 0.02	0.06 $\pm$ 0.01
5-HIAA	Control	4.09 $\pm$ 0.32	2.06 $\pm$ 0.21	2.17 $\pm$ 0.22
	17 β -E ₂	4.90 $\pm$ 0.29	2.20 $\pm$ 0.24	1.95 $\pm$ 0.31

Entries are the average $\pm$ SEM for control (vehicle)-treated (n=6) and 20 μ g/kg 17 β -Estradiol-treated Ovx rats that were sacrificed 30 post T1 sample trial. MHPG, 3-methoxy-4-hydroxyphenolglycol, norepinephrine metabolite; HVA, homovanillic acid, dopamine metabolite; 5-HIAA 5-hydroxyindole acetic acid, serotonin metabolite.

Data from [47].

*
P < 0.058

**
P < 0.05

P < 0.001.