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Rapid effects on memory consolidation and spine morphology by estradiol in female and male rodents

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Abstract

Rapid, neurosteroid-like effects of estrogens on memory consolidation during recognition memory tasks in both male and female rodents are described. We discuss how these mnemonic changes are related to rapid estrogenic effects on dendritic spine density, the distribution of spine types and the expression of PSD95 and GluA2 within spines in the hippocampus and medial prefrontal cortex, two areas critical for learning and memory. Overall, these data lead to the conclusion that estrogens are capable of exerting rapid and potent influences on memory and spine morphology in both sexes. The demonstration of estrogenic effects in males, which are used in the majority of memory studies, may provide a model for better understanding how hormone dependent changes in signaling pathways mediating memory and spinogenesis are coordinated to promote memory consolidation.

Keywords

AMPA receptors; Estradiol; Dendritic spines; Hippocampus; Memory consolidation; Recognition memory

Introduction

The principal ovarian estrogen, estradiol, is implicated in long term regulation of cognition (Luine, 2014), mood (Altemus, 2017) and some psychiatric disorders (Bangasser and Valentino, 2014). Effects on these neural functions have been found following changes in circulating gonadal hormones including after ovariectomy, over the menstrual and estrus cycle, during menopause or estrapause and after administration of hormones to gonadectomized subjects. These estrogenic effects are manifested over days or months. More recently, estrogens have been shown to affect neural functions, including learning and memory, within minutes, providing support for the idea that they act as neurosteroids (Luine

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and Frankfurt, 2012). Within minutes following estrogen and/or receptor agonist treatments female rats and mice show enhanced performance on memory tasks such as recognition memory (Inagaki et al., 2012; Inagaki et al., 2010; Jacome et al., 2016; Luine et al., 2003; Phan et al., 2012; Phan et al., 2015; Walf et al., 2006). Moreover, current studies show that estrogen's action is not limited to females but also affects cognition in males (Jacome et al., 2016). The goal of this review is to describe findings of rapid, neurosteroid-like effects of estrogens on memory in male and female rodents. First, a short description of memory testing is provided. Then, we show how these behavioral changes are related to rapid estrogenic effects on dendritic spine density, the distribution of spine types and the trafficking of PSD95 and GluA2 within spines in the hippocampus and medial prefrontal cortex (mPFC), two areas critical for learning and memory. Overall, these data lead to the conclusion that estrogens are capable of exerting rapid and potent influences on memory in both sexes.

Recognition memory tasks

Many neuroscientists have recently adopted recognition memory tasks to investigate memory processes and to determine possible effects of drugs and hormones on memory (Ennaceur and Meliani, 1992). Most memory tasks utilize positive (food or water) or negative (shock or possibility of drowning) reinforcements, which can confound results. However, recognition tasks utilize the innate, novelty seeking and exploratory nature of rodents and thereby largely eliminate many confounding influences found in other tasks. Rodents readily explore new or novel objects without any reinforcements and will likely explore a new object or an object in a new location more than one previously explored. This propensity can be used to test memory by instituting delay periods of 1-48 h between the first exploration of an object (training trial, T1) and presentation of the same, known object and a new object or the same, known object in a new location in the retention trial (T2) (Luine, 2015; Tuscher et al., 2015). Figure 1 depicts a rat on an open field during T1 and T2 trials. Thus, either non-spatial memory (object recognition, OR, bottom left of Fig 1) or spatial memory (object placement, OP, or place recognition, bottom right of Fig 1) can be assessed. More time spent with the new object or with a familiar object at a new location indicates the formation or consolidation of memory for the old object or old location during the inter-trial interval. Data in the T2 retention trial is often presented as the time spent exploring old and new objects or locations or as the exploration ratio (time exploring new object/time exploring both objects) with ratios greater than 0.5 showing that subjects remember the old object/old location. For a detailed description of these tasks and references supporting their use, please see previous reviews (Luine, 2015; Tuscher et al., 2015).

Recognition memory tasks require input from the hippocampus. OP is a spatial memory task that, although primarily dependent on an intact hippocampus (Broadbent et al., 2010) and/or fornix (Ennaceur and Aggleton, 1994), also requires mPFC input (Ennaceur et al., 1997). On the other hand, OR is less dependent on the hippocampus, is not considered a spatial memory task, and requires prefrontal activity (Ennaceur et al., 1997). Damage to the hippocampus impairs memory in the OP task, but results in fewer deficits in the OR task (Broadbent et al., 2010; Mumby et al., 2002). However, more recent studies suggest a

greater role for the hippocampus in OR than was previously thought (Cohen et al., 2013; Cohen and Stackman, 2015).

Chronic, genomic effects of estrogens on memory

In assessing effects of drugs or hormones using recognition memory tasks, agents to be tested can be given before the training trial (Pre-T1 injection) or after the training trial (Post T1 injection) which is known as a post-training protocol (See Fig. 2, top). Classically, most studies using estrogens gave treatments before T1, then gave T1 trials, an inter-trial delay and then tested for effects in the recognition trial. On the left side of Fig. 2, results of a Pre-T1 treatment experiment is shown (Jacome et al., 2010). Estradiol benzoate (EB) was given daily for two days. On the third day, T1 followed by a four hour inter-trial delay was given, and then recognition memory tested (T2). Ovariectomized (OVX), vehicle treated rats did not significantly differentiate between objects or between locations as indicated by chance exploration ratios, 0.50. On the other hand, EB treated, OVX rats showed exploration ratios of approximately 0.70 indicating that they spent more time at the new object or new location. These results are consistent with previous studies over the past twenty years showing that estrogens or estrogen receptor agonists given chronically, days to weeks, enhance performance of memory tasks and that these effects rely on genomic activations of specific neurochemicals in areas of the brain mediating learning and memory (see Luine, 2014 ; Luine 2015 or Tuscher et al, 2015, for further examples).

Rapid enhancements in memory tasks – Pre-training estradiol treatments

During the 1990's, evidence accumulated that receptor(s) for estrogens were also present outside of the cell nucleus in membranes of cell bodies and in axons, spines, presynaptic terminals, and near post-synaptic neurotransmitter receptors (See Almey et al, 2015 for review). Brain regions mediating reproductive function such as the hypothalamus as well as regions mediating cognition, including the mPFC and the hippocampus, were shown to contain non-nuclear receptors. Thus, we and others began investigating whether estrogens might more rapidly activate sex behavior or learning and memory than in previous studies where injections of EB or Silastic capsules containing 17 β -estradiol (E₂), were given for several days or weeks.

In our initial studies, we gave E₂ to OVX rats 30 min before T1, and the T2 recognition/retention trial was given 4 h later (Luine et al., 2003). As in previous experiments, OVX rats did not significantly discriminate between the objects, but E₂ treated subjects significantly discriminated between the old and the new objects or between the old and new locations. This was the first demonstration of rapid enhancements, 4.5 h, in performance of recognition memory tasks by estradiol. Diethylstilbestrol, a highly potent synthetic estrogen, also enhanced OR and OP in the same pre-T1 paradigm (Luine et al., 2003). The effective doses after acute treatments were much lower than in studies where estrogens were given chronically, 50 ug/kg/day (Jacome et al., 2010) vs one 15 ug/kg dose (Luine et al., 2003). In similar experiments with OVX mice, Phan et al (2011) gave the estrogen receptor α agonist, PPT, and the estrogen receptor β agonist, DPN, to OVX mice 15 min before T1 and tested recognition memory 40 min later. With this shorter treatment interval than in our studies,

PPT facilitated OR and OP while DPN did not affect OR but slightly facilitated OP. More recently, using the same paradigm, this group injected E₂, PPT and DPN directly into the hippocampus and tested recognition memory (Phan et al., 2015). As with the peripheral injections, E₂ and PPT enhanced OR and OP whereas DPN only enhanced OP. Thus, subcutaneous (SC) or intra-hippocampal injection of estrogens 15-30 min prior to T1 enhances recognition memory in OVX rodents within minutes to hours.

Post training trial injections rapidly enhance memory consolidation

In subsequent studies, we and others utilized post training protocols, post T1 injections (Fig. 2), as advanced by McGaugh and colleagues (McGaugh, 1989), for assessing memory. Post T1 treatments are given after subjects acquire information such as searching for a submerged platform in water maze tasks or having explored objects in recognition tasks. Following the acquisition or training trial, new information requires consolidation, and drugs or hormones can influence memory storage processes during the immediate period following training. Studies by McGaugh's group (1989) showed that rats receiving amphetamine or noradrenergic agonists post T1 had better T2 retention. Moreover, the post-training injections were time dependent. Only treatments given within 1-2 h following the training/sampling trials enhanced memory, and those given later in the inter-trial delay did not. The important attribute of post training protocols is that enhancements after immediate, but not delayed, post-trial injections show that the enhancing effects on memory do not derive from non-mnemonic effects such as psychological performance variables or learning since the treatments are given after the learning trial. Several labs, using post-training injections in recognition memory tasks, found that E₂ enhances consolidation of memory in OVX rodents when given immediately following T1 but not when given between 45 min to 2 h after T1 (Avila et al., 2017; Fernandez et al., 2008; Inagaki et al., 2012; Inagaki et al., 2010; Jacome et al., 2010; Lewis et al., 2008; Luine et al., 2003; Packard, 1998). The length of time required for memory consolidation varied depending on the dose of E₂ and the inter-trial delay between T1 and T2.

Fig. 2, lower right, shows that SC E₂ enhances OR and OP in OVX rats 4 h later when treatments are given immediately following the training trial (Inagaki et al., 2010). OVX, vehicle treated females did not discriminate between objects or places while post T1 E₂ injections increased the exploration ratio to approximately 0.7. Diethylstilbestrol and 16 α -iodo-estradiol, two synthetic estrogens, also enhance recognition memory when given in the same post-training injection paradigm (Luine et al., 2003). Thus, this data combined with the timed injection experiments after T1 described above, show that estrogens enhance consolidation of memory. The dose-response curves for post T1 estrogen effects are inverted U curves which is consistent with other membrane mediated effects (Baldi and Bucherelli, 2005; Inagaki et al., 2010). Other studies show that immediate, post-sample trial enhancements of OR in OVX mice are present 48 h following E₂ (See Frick, 2012). In addition, 17 β -estradiol-cyclodextrin (does not pass through cell membranes) applied directly to the dorsal hippocampus immediately following T1 enhances object memory consolidation (Fernandez et al., 2008; Lewis et al., 2008) or place memory consolidation (Boulware et al., 2013) suggesting that estrogenic activation within the hippocampus is sufficient to enhance memory for objects or places in a post sample trial paradigm and that inter-actions of E₂ at

the membrane are sufficient. In addition, the synthetic chemical, bisphenol-A (BPA), which is added to plastics and other materials and is a mixed agonist/antagonist for estrogens, interferes with recognition memory consolidation. BPA, given SC at 40 µg/kg (below EPA safe limit), acutely blocks enhancements in OR and OP memory in estradiol treated, OVX female rats when it is given 30 min before T1 and E₂ is given immediately following T1 (Inagaki et al., 2012). Packard et al. (1998) reported that post training injections of estradiol, either IP or intra-hippocampal, enhanced Morris water maze performance 24 h later, and inhibitory avoidance, a task relying on conditioned learning, was also enhanced by E₂ within 4 h in a post train protocol (Rhodes and Frye, 2004).

Thus, recent studies show that estrogens can rapidly enhance consolidation of memory in recognition memory tasks as well as in spatial memory dependent water mazes and inhibitory avoidance. The rapid effects of estrogens have opened new areas for investigation and are consistent with information that the hippocampus may synthesize estradiol *de novo* (see section below). Additionally, It has now been shown that estrogens initiate female rodent sexual behavior, avian male sexual displays, nutrient ingestion, and social behaviors within minutes of peripheral injection (Micevych et al., 2015).

Rapid enhancements of memory consolidation by estradiol: males

Gonadal hormones also influence cognition in males. Castration of rats impairs consolidation of memory on both OR and OP tasks (Aubele et al., 2008; Luine, 2015; Tuscher et al., 2015) and on working memory during acquisition of the radial arm maze (Daniel et al., 2003). Few studies, however, have examined males for rapid hormonal effects. Thus, we recently investigated rapid influences of gonadal hormones in castrated male rats (Jacome et al., 2016) and, as shown in Figure 3, both E₂ and testosterone enhance place recognition in males. Castrated males (ORX) receiving 750 µg/kg of testosterone (left panel) or 20 µg/kg of E₂ (right panel) immediately following a training trial for OP and tested 2 h later, spend more time exploring at the new location than the old location while vehicle treated, ORX males, spend the same amount of time exploring at the old and new location. In addition, similar to females, BPA rapidly interferes with memory consolidation in males. When BPA (40 µg/kg) is injected SC to gonadally intact male rats immediately following a training trial, consolidation of memory is impaired when OR or OP is tested 2 h later (Eilam-Stock et al., 2012). Whether BPA impairments of memory consolidation are dependent on actions at estrogen or androgen receptors is not known since BPA can antagonize at both receptors (Eilam-Stock et al., 2012). Thus, a limited number of studies suggest that recognition memory consolidation is rapidly enhanced in males by gonadal hormones, but further verification of the effects is warranted. The demonstration of rapid hormonal effects in male subjects is important because most studies and information on mechanisms of memory have been conducted in this sex, and thus, the ability to investigate gonadal hormone action in males may advance our understanding of how estrogen enhances memory.

Acute Estradiol increases spine density in both female and male rats

Increasing evidence shows that dendritic remodeling is a general mechanism underlying learning and memory. The acquisition of new memories in a conditioning paradigm is associated with increases in dendritic spine density in the CA1 region of the hippocampus (CA1) in both male (Jedlicka et al., 2008; Leuner et al., 2003) and female rats (Beltran-Campos et al., 2011). In addition, existing spines undergo structural alterations that result in long term potentiation (LTP) (Bourne and Harris, 2008; Morgado-Bernal, 2011). The functional implications of the alterations in spine density are best understood in the hippocampus because increases in dendritic spine density and/or spine sizes are associated with increases in LTP (Muller et al., 2000). Overall, memories appear to require alterations in dendritic spines that then alter LTP (Matsuzaki et al., 2011). In relation to estrogen effects, Vedder et al, 2013 reported that treatment with E₂ for two days enhanced OR memory with the same time course as increases in CA1 LTP magnitude and that both effects required an increase in NMDA receptors. With respect to hippocampal spines and memory, it is notable that chronic treatment with E₂ was shown to increase hippocampal dendritic spine density 25 years ago (See McEwen and Alves, 1999 for review) and that it is now clear that dendritic spines are extremely responsive to both acute and chronic changes in circulating estrogen.

We assessed effects of acute E₂ treatments on dendritic spine density in pyramidal cells in CA1 and the mPFC (Figure 4). In OVX female rats, E₂ increased dendritic spine density in apical and basal dendrites in the mPFC and in basal dendrites in CA1 at 30 min or 4 h following E₂ (Inagaki et al., 2012) but did not alter spine density in the dentate gyrus at either time interval (Luine, 2015). E₂ also increased spine density in the ventromedial nucleus, a brain region mediating lordosis, at 4 h but not at 30 min, post E₂ (Frankfurt and Luine, 2015). Increased CA1 spine density was found within 40 min following E₂ (Phan et al., 2015) administration to OVX mice. In addition MacLusky et al. 2005, reported a dose dependent increase in CA1 postsynaptic density protein (PSD95) within 30 minutes when E₂ was given to OVX rats. Agonists for both ER α and ER β have also been shown to alter spine density in CA1. In OVX mice, PPT, an ER α agonist increased dendritic spine density in CA1 within one hour, whereas DPN, an ER β agonist, decreased spine density (Phan et al., 2011).

More recently, we reported in OVX mice that direct intra-hippocampal infusion of 17 β -estradiol-cyclodextrin increased dendritic spine density in both apical and basal dendrites of pyramidal cells in CA1 within 30 minutes, and the increased dendritic spine density was still present 2h post injection (Tuscher et al., 2016a). No changes in spine density were observed in either the dentate gyrus or the ventromedial nucleus of the hypothalamus following intra-hippocampal injection; however, increased dendritic spine density was observed after 2 h, but not 30 min, in the mPFC. Since increased spine density in CA1 precedes the increase in the mPFC (Tuscher et al., 2016a), estradiol mediated activity in the dorsal hippocampus may drive mPFC spinogenesis. More importantly, in the same study, we found that hippocampal infusion of inhibitors of extracellular signal-regulated kinase (ERK) or mammalian target of rapamycin (mTOR) blocked the estradiol-dependent increases in spines (Tuscher et al., 2016a). Since intrahippocampal blockade of this cell-signaling pathway also blocks

recognition memory consolidation (Fortress et al., 2013), the results provide preliminary evidence that increases in spines play an important role in estrogens enhancement of memory in females.

We investigated whether E₂ might affect spine density in males and found that estrogenic effects on spine density in ORX male rats after SC injection of E₂ was similar to effects in OVX female rats. E₂ administration resulted in an increase in dendritic spine density within 30 minutes and up to 2 h later on the apical branches of CA1 pyramidal cells (Jacome et al., 2016), but it had no effect on dendritic spine density in the dentate gyrus. Whether estrogenic effects on spines are present in male mice has not been investigated. BPA effects on dendritic spine density in male rats show a similar pattern to that of females. Within 30–40 min following BPA, dendritic spine density decreased in CA1 and mPFC in male rats (Eilam-Stock et al., 2012). Consistent with these spine changes, BPA has also been shown to block estrogen and testosterone dependent formation of dendritic spine synapses in male rats (Leranth et al., 2008; MacLusky et al., 2005a) and to decrease the post synaptic density protein (PSD95) in the hippocampus of intact male rats (Eilam-Stock et al., 2012). In another study, application of PPT, an ER α agonist, or estradiol (Murakami et al., 2006) to hippocampal slices from male rats increased dendritic spine density within 2 hours, but the ER β agonist, DPN, had no effect. Thus, acute estrogens appear to exert similar effects in both sexes, rapidly increasing spine density in brain areas important for memory function; however, further confirmatory studies are warranted in males.

Role of neurally derived Estradiol on hippocampal function

The source of estrogens which maintain cognitive function and spine stability in the hippocampus of female rodents has been assumed, until recently, to be ovarian. However, many studies suggest that estradiol maybe acting as a neurosteroid, a steroid synthesized in the brain which acts locally, to modulate neuronal excitability by rapid non-genomic actions. In support of this idea, levels of estradiol in the hippocampus are higher than in the circulation over the rat estrous cycle. For example, 1.0 ± 0.4 nM vs 0.017 ± 0.005 nM at estrus, and, also, estradiol levels in the hippocampus are maintained after OVX, 0.7 ± 0.01 nM (Kato et al., 2013). However, Kato et al. (2013) also showed that hippocampal estradiol levels fluctuate over the estrus cycle suggesting that ovarian hormone secretion may also contribute to hippocampal levels. Estradiol is present in the male hippocampus, and levels are two to eight-fold higher than in females, depending on the day of the estrous cycle (Kato et al., 2013). Whether hippocampal estrogens impact spines or cognitive function is currently unclear. In cultures of mouse hippocampus, inhibition of the final step in estradiol synthesis by letrozole inhibits synaptogenesis and decreases the number of spine synapses in neurons of females but not males (Prange-Kiel et al., 2006; Vierk et al., 2012). In contrast, Murakami et al (2018) found that estrogens or androgens increased CA1 spine density in cultured male hippocampal slices 1 h post administration. In terms of cognition, Frick and colleagues (Tuscher et al., 2016b) recently showed that inhibition of local estrogen synthesis in the hippocampus *in vivo* by letrozole impaired hippocampal memory consolidation in OVX female mice, a result congruent with letrozole's ability to inhibit hippocampal spinogenesis. Importantly, aromatase inhibition did not prevent exogenous E₂ from enhancing memory which suggests both ovarian and hippocampal estradiol synthesis may

contribute to hippocampal memory formation. In relation to males, Saldanha and colleagues reported that inhibition of aromatase in the hippocampus by ATD in male songbirds impaired spatial memory performance in a food finding task (Bailey and Saldanha, 2015). However, peripheral, chronic letrozole administration did not alter spatial learning and memory on the Morris water maze test in either female or male rats (Vierk et al, 2015). Clearly, more research is necessary to determine whether, or the extent to which, centrally synthesized gonadal hormones contribute to hippocampal function(s).

Estrogen induced spinogenesis in CA1: potential mechanisms

While it is accepted that E₂ promotes increased dendritic spine plasticity in brain areas implicated in memory, the molecular mechanisms underlying plasticity are only beginning to emerge. The discovery that mERs could functionally couple to the metabotropic glutamate receptors, mGluR1 and 2, in the hippocampus (Boulware et al., 2005) provided the first indication of how estrogen signaling might alter spinogenesis. Since then, Mermelstein's group has documented the interaction between estrogen receptors and metabotropic glutamate receptors and their impact on drug addiction. Results show that mGluR5 activity is required for estrogen's enhancing of cocaine-induced locomotor behavior sensitization and regulation of dendritic spines in the striatum and core of the nucleus accumbens (Tonn Eisinger et al., 2018). Further, studies also demonstrate coupling of ER α or β to other mGluRs in the hypothalamus. Micevych's group has shown that the ability of E₂ to rapidly activate lordosis and increase dendritic spines in the arcuate nucleus is directly linked to mGluR1 receptors (Micevych and Christensen, 2012). Associations of mERs with ionotropic glutamate receptors (iGluRs) have also been identified: AMPA receptors GluR1 in the prefrontal cortex (Khan et al., 2013), GluR2 in hippocampus (Avila et al., 2017) and NMDA receptors NR1 in cortex (Sellers et al., 2015a) and NR2 in the hippocampus have all been shown to be activated by mERs. Most of these associations with NMDA receptors have been tied to modulation of spine density, memory formation or other behaviors rapidly modulated by estrogens, but further studies are required to establish the connections. Interactions of mERs with neurotrophin receptors, especially BDNF, may also regulate spinogenesis (Luine and Frankfurt, 2013), but participation of hippocampal G-protein coupled estrogen receptor (GPER1) in E₂-dependent memory has recently been ruled out (Kim et al., 2016). The ability of mERs to interact with a variety of membrane receptors provides an array of signaling pathways which could underlie neurosteroid action of estrogens. For further discussion of signaling pathways and NMDA receptors involved in estrogen- dependent memory enhancement and spinogenesis, see Choleris et al, (this volume) and Frick et al, (this volume).

Most studies, including our own, have utilized Golgi impregnation to measure effects of estrogens on total spine density in the hippocampus. However, as first described by Ramon Y Cajal, spines are heterogeneous. Although spine subtype classification varies among researchers, dendritic spines generally consist of a protrusion and may, in the case of mushroom spines, have a bulbous termination. One may also distinguish between stubby spines and thin spines with a smaller head, which lack any terminal enlargement and have extremely thin filopodia. Mushroom spines serve as active neurotransmission sites in CA1 and are mainly excitatory, glutamatergic and contain PSD95, a synaptic marker.

In an attempt to better understand mechanisms underlying estrogenic effects on memory and spines, we applied Simultaneous Golgi-Cox Laser Scanning Confocal Microscopy and Immunohistochemistry (GC-IHC) to the brains of castrated males who received E₂ at 30 and 120 min before sacrifice (Avila et al., 2017). This dose and time enhances place memory and spine density in CA1 (Avila et al., 2017). Four spine types (stubby, filopodial, long thin and mushroom) were identified by Golgi impregnation. Filopodia and stubby spines are immature while long-thin and mushroom spines are considered mature and have larger spines heads than immature spines (Bourne and Harris, 2007; Rochefort and Konnerth, 2012). PSD95 and the AMPA receptor, GluA2, in the spines were evaluated by IHC (Figures 4 and 5). We chose GluA2, which is located within the synaptic membrane, because of its importance for memory consolidation (Migues et al., 2010). Fig. 4 shows that stubby and filopodial spines did not change after estradiol, but long-thin spines decreased in density at 30 min, and mushroom spines increased in density at 120 min. Further, the density of mushroom spines, which showed co-localization of GluA2/PSD-95, increased three-fold between 30 and 120 min following estradiol treatment while other spine types were not altered (Fig. 5). These results are the first demonstration of a time dependent shift in the density of various spine types in CA1 following E₂ and suggest that E₂ enhances the transformation of spines into mature, synapse containing spines, i.e. GluA2/PSD-95 mushroom spines.

While only a few studies have measured spine types following estrogen treatment, similar, but more rapid, changes were reported in cultured cortical neurons where increased synaptogenesis and levels of PSD-95 were found 30 min following E₂ (Sellers et al., 2015a). Rapid, time dependent changes in spines following E₂ were also found in the arcuate nucleus of the hypothalamus of OVX rats. Stubby spines were not affected, but at 4 h post E₂, filopodial spines increased and then returned to normal at 20 h post E₂ while mushroom spines increased at 20 h and remained at high levels through 48 h (Christensen et al., 2011; Micevych and Christensen, 2012). Thus, this study and our own study in males suggest that E₂ promotes the maturation of thin spines to mushroom spines which in turn may underlie behavioral or memory enhancements. Consistent with our results and the role of mushroom spines in memory, increased mushroom spine density in CA1 produced by water maze training is prevented by ovariectomy (Beltran-Campos et al., 2011). Few *in vivo* studies of estrogen effects have been conducted in the hippocampus in either sex. Nonetheless, our results in males may be consistent with ex-vivo findings in females showing that AMPA currents are rapidly reduced by E₂ in female prefrontal cortex (Sellers et al., 2015b) and CA1 (Phan et al., 2015). In females, the rapidly induced spines are initially silent synapses whereas in males, a similar functional outcome may be achieved by a physical reduction in spines. These sex differences in effects of E₂ on AMPA may relate to a newly described category, latent sex differences, wherein different molecular mechanisms converge to the same functional endpoint in males versus females (Oberlander and Woolley, 2016)).

Our findings are consistent with other findings in males. A similar pattern in spine turnover was found following induction of LTP. Decreases in small thin spines in the first 30 min were followed by an increase in PSD area at 2 h (Bourne and Harris, 2011). Since both LTP and E₂ promote memory formation, spine trafficking may be integral to the process. Our results suggest that E₂ activates the P13- GluA2/PKM ξ pathway resulting in the rapid

growth of spines, particularly long thin spines to mushroom spines, and the expression of GluA2 receptors in the spines (Frick, 2015). Based on current knowledge of synaptic dynamics in the hippocampus (Bourne and Harris, 2007; Bourne and Harris, 2008), these rapid changes initiated by E₂ may provide an important substrate for enhanced memory. Since E₂ and other estrogenic neurosteroids activate several other membrane linked signaling pathways to enhance memory consolidation (See above and Sheppard et al, 2017), it will be critical to determine how all of these rapid events are coordinated in order to result in formation of new spines and enhanced memory consolidation. In addition, whether neurosteroidal effects of E₂ contribute to women's mental health issues needs to be addressed since these actions represent a novel approach for therapeutic intervention.

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Highlights

Rapid enhancements in memory consolidation by estrogens are reviewed

Estradiol enhances recognition memory in male and female rodents

Estradiol increases dendritic spine density in male and female rodents

Rapid increases in GluA2/PDS95 mushroom spines may mediate enhanced memory consolidation.

Recognition Memory Tasks

Figure 1.

Schematic of recognition memory task protocols

A rat is depicted during the training trial (T1) where two identical objects are explored, typically for 3-5 min, or until a set amount of exploration time is obtained. An inter-trial delay of 1-24 h is given, and then, the retention or testing trial (T2) is given (usually 3-5 min duration). If one identical object is replaced with a new object, left bottom, object recognition (OR) is tested. If one of the objects is moved to a new location, right bottom, place recognition or, also known as the object placement task (OP), is tested. Reprinted from Luine, 2015 by permission.

Figure 2.

Effects of estradiol on recognition memory in Pre and Post T1 configurations.

Shown at the top is a time line for recognition memory testing. Agents can be given hours to days prior to the training trial (Pre-T1 injections) immediately post-training (Post-T1 injections). Post-training injections, immediately after T1, test effects of agents on memory consolidation. The retention trial is given 1-24 h after T1.

The lower left panel depicts Pre T1 injections of estradiol. OVX rats received either vehicle or 50 µg/kg of estradiol for two days and on the third day, a training trial followed 4 h later by the retention trial. Entries are exploration ratios (new/total exploration) ± S.E.M. for vehicle and estradiol treated subjects. Dotted line at 0.5 indicates spending the same amount of time exploring new and old objects or locations. ***P < 0.001 (paired t-test within each group of old vs new. Figure reprinted with permission Neurobiology of Learning and memory, Jacome et al, (2010).

The lower right panel depicts effects of estradiol given in a post T1 paradigm. Treatments were given immediately following T1. For OR, OVX rats received vehicle or 5 µg of estradiol and for OP, 20 µg of estradiol was given. Data is presented and analyzed as in Pre T1 figure. Estradiol significantly increased the ratio, ** P < 0.01 in both object and place recognition. Data redrawn from Inagaki et al, 2010.

Figure 3.

Acute T or E₂ treatment to castrated (ORX) males enhances OP

Time spent exploring objects at old and new locations $\pm$ S.E.M is shown for ORX males given vehicle (Veh), E₂ at 20 μ g/kg or testosterone (T) at 750 μ g/kg immediately following the training trial, T1, and tested for retention 2 h later. After both hormonal treatments, subjects spent significantly more time exploring the new vs the old location during the retention trial, T2. Data analyzed by paired t-test where * $P < 0.05$. Figure reprinted from Jacome et al, 2016 by permission.

Figure 4.

Acute estrogen increases spine density in female rats.

A. Spine density is shown in areas of brain from Ovx rats given corn oil or 20 μg/kg of estradiol immediately following T1 tests and sacrificed 30 min later. Entries are means ± S.E.M. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$. PFC and CA1 data from Inagaki et al. (2012) and DG data from Luine (2015).

B. Representative photomicrograph from pyramidal neurons of CA1 30 min after acute vehicle (control). Arrows denote spines on the secondary basal dendrites. X100 under oil.

C. Representative photomicrograph from pyramidal neurons of CA1 30 min after acute estradiol. Arrows denote spines on the secondary basal dendrites. X100 under oil. Figures B and C adapted from Inagaki et al., 2012.

A. Spine Density (spines/um)

B. GluA2/PSD95 (voxels/um)

Figure 5.

Effects of estradiol on distribution of spines in CA1.

A. Estradiol treatment decreases the density of long-thin spines and increases mushroom spines. Stubby (A) and filopodia (B) spines did not change as a consequence of E2 treatment. The mature spines, long-thin (C) and mushroom (D) both show an overall effect of treatment (long thin; $F_{(2,14)} = 3.85$, $p = 0.046$; mushroom $F_{(2,15)} = 4.38$, $p = 0.03$ and a significant Tukey post-hoc test (long thin, vehicle vs E₂ 30 min, $p = 0.04$; mushroom, vehicle vs. E₂ 120 min, $p = 0.03$). Figure reprinted from Avila et al, 2017 by permission.

B. Estradiol increases GluA2/PSD95 colocalization levels within mushroom spines. GluA2/PSD95 co-localization levels (voxels/um) within stubby (A), filopodia (B), and long-thin (C) spines did not significantly change following E₂ treatment. GluA2/PSD95 co-localization levels within mushroom spines shows an overall significant effect of treatment ($F_{(2,14)} = 4.78$, $p = 0.026$) and a significant Tukey post-hoc test (E₂ 30 min vs E₂ 120, $*p = 0.036$). Figures reprinted from Avila et al, 2017 by permission.

Figure 6.

Photomicrograph of Simultaneous Golgi-Cox Laser Scanning Confocal Microscopy and Immunohistochemistry in hippocampus

Hippocampal CA1 spines captured at 100× magnification. Estradiol increases co-localization of GluA2 and PSD95 within Mushroom spines. Representative dendrites from E30 min (A) and E120 min (B). Yellow voxels indicate co-localized GluA2 and PSD95 within Mushroom spines. All carats indicate mushroom spines. Red carats indicate mushroom spines that do not contain co-localized GluA2 and PSD95. Blue carats indicate mushroom spines that contain co-localized GluA2 and PSD95. Note the increased density of blue carats in lower panel as compared to upper panel (quantitation of photomicrograph is shown in Figure 5). Scale bar = 5 μ m. Figure reprinted from Avila et al, 2017 by permission.