Rapid increases in immature synapses parallel estrogen-induced hippocampal learning enhancements

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Dramatic increases in hippocampal spine synapse density are known to occur within minutes of estrogen exposure. Until now, it has been assumed that enhanced spinogenesis increased excitatory input received by the CA1 pyramidal neurons, but how this facilitated learning and memory was unclear. Delivery of 17β-estradiol or an estrogen receptor (ER)-α (but not ER-β) agonist into the dorsal hippocampus rapidly improved general discrimination learning in female mice. The same treatments increased CA1 dendritic spines in hippocampal sections over a time course consistent with the learning acquisition phase. Surprisingly, estrogen-activated spinogenesis was associated with a decrease in CA1 hippocampal excitatory input, rapidly and transiently reducing CA1 AMPA activity via a mechanism likely reflecting AMPA receptor internalization and creation of silent or immature synapses. We propose that estrogens promote hippocampally mediated learning via a mechanism resembling some of the broad features of normal development, an initial overproduction of functionally immature connections being subsequently "pruned" by experience.

immature synapse | short-term memory | structural plasticity | synaptic plasticity | signal-to-noise ratio

Estradiol rapidly and dramatically increases hippocampal dendritic spine and synapse density within minutes of application (1–4). There is a strong correlative association between estrogen-induced spinogenesis and improvements in cognition (5); however, the relationship of these structural changes to estrogen-induced alterations in hippocampal function is unclear. Our laboratory recently reported that the density of hippocampal CA1 pyramidal dendritic spines increases very rapidly after systemic treatment with 17β-estradiol or estrogen receptor (ER) -selective agonists in ovariectomized female mice, changes that are paralleled by learning enhancements (2, 3). Estrogen-induced rapid structural changes are substantial, increasing spine density by 30–50% within 15–40 min of hormone application (1–3, 6). As a result, adult rodents can experience the addition of thousands of CA1 synapses within a span of minutes after exposure to estradiol. These effects of estrogens reproduce the changes occurring during the 4-d estrous cycle of female rodents, which include the induction of CA1 spines (7).

How these processes contribute to the behavioral changes observed after estradiol treatment is not understood. Estradiol enhances excitatory neurotransmission throughout the hippocampus (8–10), and activates BDNF signaling in the mossy fiber system (11). Dendritic spines turn over more rapidly in the hippocampus than in the neocortex (12), particularly in the case of estradiol-induced spines (13). Such rapid, transient, and apparently indiscriminate increases in excitatory synapse formation would seem, at first sight, to be more likely to interfere with preexisting brain circuits and impair normal information processing than to enhance cognitive function.

How then, does enhancement of spine formation lead to improved cognitive function? To address this question, we focused specifically on the effects of estradiol in the dorsal CA1 hippocampus, as a site mediating the rapid improving effects of estrogens on learning. Estradiol application, via a mechanism involving ERα, rapidly increases dendritic spine density in the CA1 pyramidal stratum oriens and stratum radiatum subregions. However, contrary to our initial assumptions based on previous work in this field, increased spinogenesis did not result in increased CA1 excitatory input. Rather, estrogen-induced formation of hippocampal spines was associated with a decrease in the ability of CA1 neurons to respond to AMPA receptor (AMPAR) activation, resulting in decreased excitatory input to CA1 neurons. This appears to be the result of AMPAR internalization from the synaptic membrane (6). Taken together, our data suggest that estrogens induce formation of "silent" or immature synapses that act as a substrate for the storage of new memories (14, 15). This finding explains estrogens' ability to rapidly improve learning without precipitating uncontrolled activation of the hippocampal circuitry. These effects of estradiol on CA1 pyramidal neurons phenotypically resemble the structural and functional properties of neurons during development, when neurons with higher levels of spine density and higher numbers of silent/immature synapses are present before the activity dependent refinement of neural circuitry.

Significance

Estrogens are known to rapidly affect learning and memory, but how they do so is not well understood. Here we identify the hippocampus as a brain structure responsible for rapid estradiol-induced improvement of general discrimination learning in female mice, which coincides with a substantial increase in hippocampal dendritic spines, the postsynaptic sites of excitatory synapses. Until now, it had been assumed that estradiol-induced increases in hippocampal synapses would result in increased excitatory input into the neuron. Surprisingly, we find the opposite: increases in hippocampal spines are associated with a decrease in the excitatory glutamatergic input. This finding suggests that estradiol improves learning through inducing the formation of silent or immature synapses, which provide the substrate for storing new memories.

Results

Intrahippocampal 17β-Estradiol Rapidly Improves Learning. To determine the role of the hippocampus in mediating estrogens' rapid effects on learning (2), ovariectomized CD1 mice were fitted with bilateral guide cannulae aimed at the dorsal anterior hippocampus. Mice were microinjected with 17β-estradiol (25, 50, or 100 nM) or the vehicle control [artificial cerebrospinal fluid (aCSF) + 0.02% ethanol] in a between-subjects design. After 15 min, animals were trained on learning tasks modified to examine estrogens' rapid effects on different types of memory (3): spatial learning (object placement), item recognition (object recognition), and conspecific recognition (social recognition) (Fig. 1B). These tasks rely on the natural tendency of mice to sniff novel or displaced stimuli more than familiar stimuli. Because systemic estrogen administration previously induced learning and memory enhancements, we used tasks with reduced learning opportunities (Fig. 1A) such that vehicle controls do not display discrimination of the novel stimulus at test (3). Effects of treatment on investigation durations and other behavioral measures were recorded and are discussed below.

Object placement, object recognition, and social recognition performances were all improved within 40 min of intrahippocampal 17β-estradiol administration. Fifty nanomolars of 17β-estradiol improved object placement and object recognition above vehicle controls (Fig. 1C). In addition, mice receiving 50 nM 17β-estradiol were capable of distinguishing the displaced and novel stimulus in all three learning paradigms (investigation percent at test was higher than at habituation) (Fig. 1C). Twenty-five nanomolars and 100 nM 17β-estradiol did not affect learning and memory performance, demonstrating that 17β-estradiol has an inverse U-shaped dose–response curve, consistent with literature on estrogenic effects (3).

Therefore, infusing 50 nM of 17β-estradiol into the hippocampus enhances general learning and memory.

Learning Enhancements Are Mediated by ERα. To identify the estrogen receptor responsible for these learning enhancements, we microinfused 50, 100, or 150 nM of ERα-selective agonist PPT [1,3,5-Tris(4-hydroxyphenyl)-4-propyl-1H- pyrazole] or ERβ-selective agonist DPN [2,3-bis(4-hydroxyphenyl)-propionitrile; or vehicle (aCSF + 0.02% ethanol) into the hippocampus and tested mice on the learning paradigms described above. Higher doses were used for these selective agonists compared with 17β-estradiol because PPT and DPN have a ~50% binding affinity to ERα and ERβ, respectively, relative to estradiol (16, 17). One-hundred nanomolars PPT improved social recognition above vehicle controls (Fig. 1D), and groups administered 100 nM or 150 nM PPT were able to successfully identify the novel and displaced stimuli in all three learning tests (Fig. 1D). In contrast, the ERβ agonist DPN improved performance only on the object placement task (100 nM DPN) (Fig. 1E), and did not improve object or social recognition (Fig. 1E). Therefore, the learning and memory effects of 17β-estradiol are replicated by intrahippocampal delivery of the ERα agonist PPT.

Effects Are Not Secondary to Changes in Other Behaviors. We performed an ethological analysis on the mice during the learning paradigms, recording behaviors listed in Table S1. Intrahippocampal delivery of 17β-estradiol or ER agonists did not generally affect other behaviors recorded during the task [Fig. S1, total investigation durations (novel + familiar stimulus investigation durations) and Figs. S2 and S3]. Hence, the learning enhancements reported cannot be explained by changes such as increased interest in stimuli or enhanced activity. The one exception is the higher
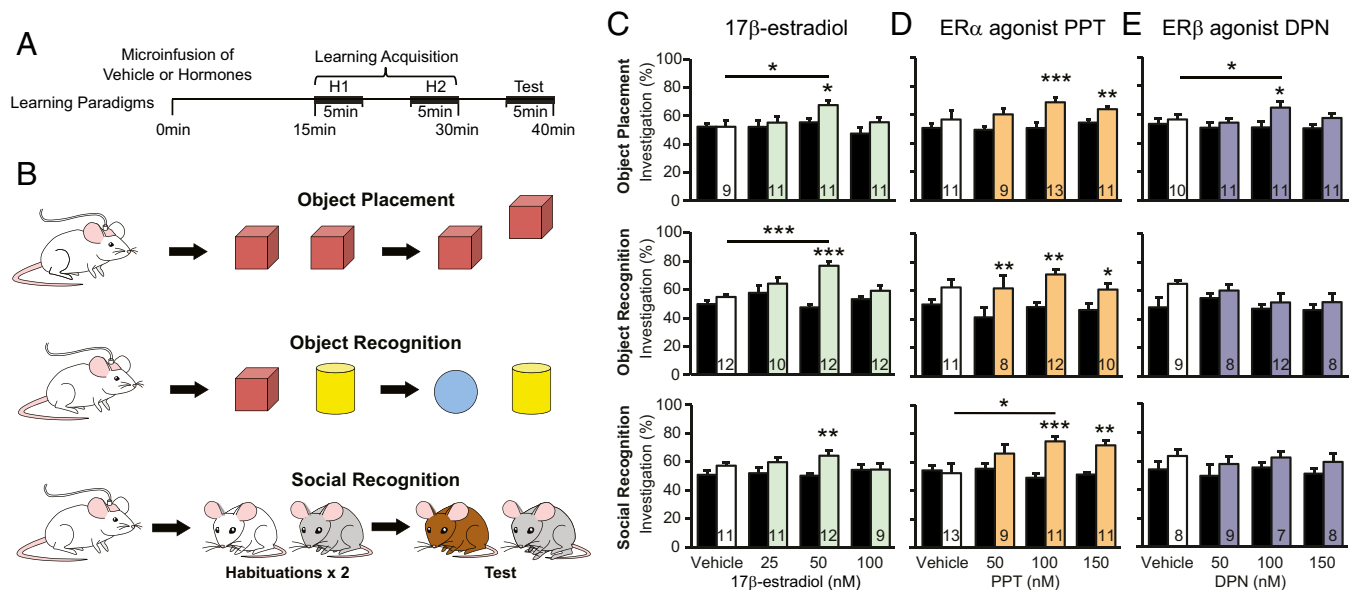

Fig. 1. Intrahippocampal 17β-estradiol and ERα agonist PPT (but not ERβ agonist DPN) rapidly improve general learning. (A) Learning paradigm timeline. "H" indicates habituation. (B) Behavioral paradigms, each performed with experimentally naïve mice. (C–E) Percent investigation (PI) scores of mice during object placement (Top), object recognition (Middle), or social recognition (Bottom) learning paradigms. Black bars are PI at habituation (average of H1 and H2), white/colored bars the PI at test and contain group numbers, mean ± SEM. (C) Object placement and recognition: Microinfusion of 50 nM 17β-estradiol into the hippocampus improves object placement and object recognition learning within 40 min of administration ($F_{3, 38} = 3.14$, $P < 0.05$ and $F_{3, 42} = 9.56$, $P < 0.001$, post hoc $q = 3.87$, df = 18, $P < 0.05$ and $q = 7.13$, df = 22, $P < 0.001$, respectively). Groups treated with 50 nM of 17β-estradiol also demonstrate recognition of the displaced or novel object during test (PI at test is significantly higher than habituation; $t = −2.55$, df = 10, $P < 0.05$ and $t = −8.28$, df = 11, $P < 0.05$). Social recognition: Mice receiving 50 nM of 17β-estradiol discriminate the novel individual during the social recognition task ($t = 3.38$, df = 11, $P < 0.01$). (D) Object placement: Mice receiving 100 nM and 150 nM of ERα agonist PPT distinguish the displaced object ($t = 5.79$, df = 12, $P < 0.001$ and $t = 3.30$, df = 10, $P < 0.01$). Object recognition: Administering 50 nM, 100 nM, or 150 nM PPT result in successful discrimination of the novel object (50 nM, $t = 3.88$, df = 7, $P < 0.01$; 100 nM, $t = 4.16$, df = 11, $P < 0.01$; 150 nM, $t = 2.90$, df = 9, $P < 0.05$). Social recognition: 100 nM of PPT improves social recognition learning ($F_{3, 40} = 3.13$, $P < 0.05$, post hoc $q = 3.92$, df = 22, $P < 0.05$). Groups administered 100 nM and 150 nM PPT demonstrate discrimination of the novel individual at test ($t = 5.18$, df = 10, $P < 0.001$ and $t = 4.39$, df = 10, $P < 0.01$). (E) Object placement: 100 nM of DPN significantly improves object placement learning ($F_{3, 38} = 4.01$, $P < 0.05$, post hoc $q = 3.66$, df = 21, $P < 0.05$). This group also distinguishes the displaced object at test ($t = 2.67$, df = 9, $P < 0.05$). Object and social recognition: DPN does not improve object or social recognition learning. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

total investigation duration detected in the 17β-estradiol 50-nM group during the object recognition paradigm (Fig. S1). However, it is unlikely that this higher investigation duration indicates an enhanced interest in objects that then leads to a facilitation of object recognition learning, because this effect was not found in the object placement paradigm, in PPT experiments, or during systemic experiments (2, 3). Therefore, we conclude that intra-hippocampal delivery of 17β-estradiol, via ERα, rapidly and specifically enhances learning and memory.

17β-Estradiol Rapidly Increases CA1 Spine Density. To determine the cellular effects of estradiol on CA1 pyramidal neurons, the same doses of 17β-estradiol used in the learning tasks were bath-applied to hippocampal sections from experimentally naïve mice. CA1 pyramidal neurons from regions corresponding to our cannula placements (Fig. S4) were whole-cell patch-clamped and electrophysiological measures were obtained (data below), filled with biocytin, then visualized with diaminobenzidine (DAB) staining to reveal their structure. Dendritic spine density increased within the stratum oriens and stratum radiatum after 20–30 min of treatment with 50 nM of 17β-estradiol (Fig. 2 *B* and *C*). This timeframe coincides with the training sessions of the learning experiments. We did not detect changes in dendritic spine length (Fig. 2*D*); however, this may be because our analyses were completed on 2D images. The lack of significance in the stratum lacunosum-moleculare may be because of the greater experimental variance observed in this stratum: previous reports have detected estrogen-induced spine changes within this hippocampal subregion (4).

Rapid Increases in CA1 Spine Density Are Mediated by ERα. We bath-applied the same doses of ERα agonist PPT or ERβ agonist DPN (50, 100, or 150 nM) used in the learning tasks, or vehicle (aCSF + 0.02% ethanol), onto hippocampal sections. Twenty to 30 min of exposure to 100 nM or 150 nM of ERα agonist PPT increased stratum oriens and stratum radiatum spine density (Fig. 2 *B* and *C*). Again, changes within the lacunosum-moleculare did not reach statistical significance. In contrast, there were no rapid effects of ERβ agonist DPN on spine density within any CA1 hippocampal subregion examined (Fig. 2 *B* and *C*). Consistent with the effects of 17β-estradiol, neither PPT nor DPN resulted in a discernable change in dendritic spine length (Fig. 2*D*).

17β-Estradiol Decreases CA1 AMPA Miniature Excitatory Postsynaptic Current Frequency. What are the consequences of rapid increases in hippocampal dendritic spine density for CA1 pyramidal neuron connectivity? Estrogens rapidly enhance hippocampal excitability, in part via presynaptic changes in CA3 Schaffer collateral terminals, which synapse onto CA1 (8–10). However, because we were interested in CA1 pyramidal neuron changes that accompany CA1 dendritic spine changes, we investigated estradiol effects on miniature excitatory postsynaptic currents (mEPSCs) resulting from spontaneous vesicular release of neurotransmitters. The same doses of 17β-estradiol used in learning and dendritic spine experiments were bath applied onto hippocampal sections for 15–20 min (Fig. 3*B*), coinciding with the onset of learning acquisition during our behavioral experiments (Fig. 1*A*).

Surprisingly, 17β-estradiol decreased CA1 pyramidal mEPSC frequency in neurons with increased spine density. Fifteen to 20 min of treatment with 50 nM 17β-estradiol significantly reduced mEPSC frequency in CA1 neurons, whereas 25 nM or 100 nM 17β-estradiol did not have an effect (Fig. 3 *C* and *E* and Fig. S5*A*). Because our recordings were obtained at −70 mV, these mEPSC responses are not NMDA receptor (NMDAR) mediated. Addition of the AMPA/kainate receptor antagonist NBQX consistently abolished recorded mEPSCs (Fig. S5*B*). 17β-Estradiol affected mEPSC frequency but not amplitude (Fig. 3 *D* and *F*).

17β-Estradiol Decreases CA1 Responses to AMPA. If the estradiol-induced decrease in mEPSC frequency resulted from a post-synaptic mechanism, this may be because of the internalization of AMPARs (6, 18) or the movement of synaptic AMPARs to perisynaptic sites. To investigate these possibilities, we examined membrane potential responses of CA1 pyramidal neurons to bath application of (S)-AMPA (Fig. 4 *A–C* and Fig. S6). This approach allowed us to examine the effects of estradiol on the ability of a CA1 pyramidal neuron to depolarize upon AMPAR activation. Internalization of AMPAR should result in a decreased response to the AMPA agonist, whereas the movement of receptors from postsynaptic sites to perisynaptic sites should not affect CA1 responses to bath-applied AMPA. Because 17β-estradiol is reported to affect CA1 pyramidal NMDAR subunit composition and function over longer periods of exposure (24–48 h), we also examined whether estradiol might alter CA1 responses to NMDA in
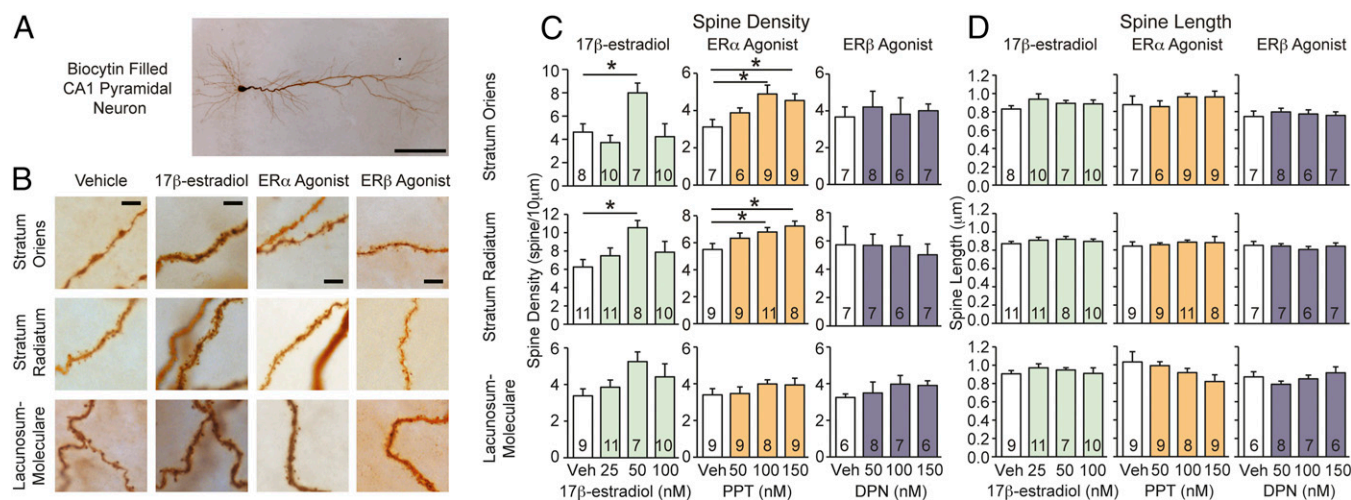

Fig. 2. 17β-Estradiol and ERα agonist PPT (but not ERβ agonist DPN) rapidly increase CA1 dendritic spines. (A) Biocytin filled CA1 pyramidal neurons. (Scale bar, 100 μm.) (B) Images of CA1 dendrites from slices treated with 20–30 min of vehicle, 50 nM 17β-estradiol, 100 nM ERα agonist PPT, or 100 nM ERβ agonist DPN. (Scale bars, 5 μm.) (C) Measures of spine density. 17β-Estradiol: 50 nM 17β-estradiol increases spine density in the stratum oriens and stratum radiatum (stratum oriens: $F_{3, 31} = 4.35$, $P < 0.05$; post hoc $q = 3.59$, $df = 13$ $P < 0.05$ and stratum radiatum, $F_{3, 36} = 3.36$, $P < 0.05$; post hoc $q = 4.43$, $df = 17$, $P < 0.05$). Spine density in the lacunosum-moleculare is not statistically significant ($F_{3, 33} = 2.00$, $P = 0.13$). ERα agonist PPT: 100 nM or 150 nM of ERα agonist PPT increases spine density in the stratum oriens and stratum radiatum (stratum oriens, $F_{3,27} = 3.68$, $P < 0.05$; post hoc 100 nM: $q = 4.39$, $df = 14$, $P < 0.05$, 150 nM: $q = 3.52$, $df = 14$, $P < 0.05$ and stratum radiatum, $F_{3, 34} = 3.13$, $P < 0.05$; post hoc 100 nM $q = 3.55$, $df = 18$, $P < 0.05$, 150 nM: $q = 4.10$, $df = 15$, $P < 0.05$). ERβ agonist DPN: Did not affect spine density. (D) None of the treatments affect average spine length; mean ± SEM. *$P < 0.05$.
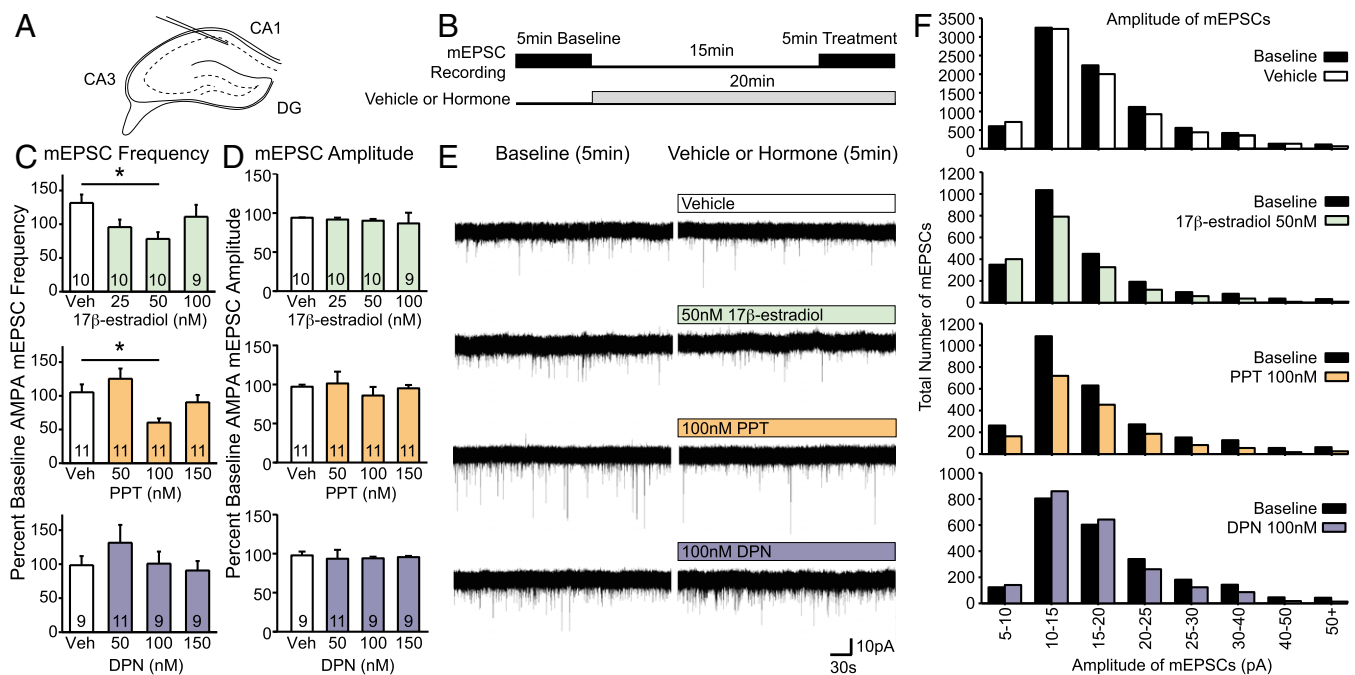
Fig. 3. Rapid effects of 17β-estradiol and estrogen receptor agonists on CA1 pyramidal neuron mEPSCs. (A) Location of patched neurons corresponds to location of bilateral cannulas in behavioral experiments. (B) Schematic of treatment protocols. (C and D) Percent baseline of mEPSC frequency and amplitude; mean ± SEM. (C) Fifteen to 20 min of 50 nM 17β-estradiol or 100 nM ERα agonist PPT reduces CA1 mEPSC frequency (Estradiol: $F_{3, 35} = 3.18$, $P < 0.05$; post hoc $q = 4.20$, df = 18, $P < 0.05$; PPT: $F_{3, 40} = 5.72$, $P < 0.01$; post hoc $q = 3.93$, df = 20, $P < 0.05$). ERβ agonist DPN does not affect mEPSC frequency. (D) Treatments do not affect mEPSC amplitude. (E) Five-minute mEPSC traces of baseline (Left) and traces after 15–20 min of vehicle, 50 nM 17β-estradiol, 100 nM PPT, or 100nM DPN application (Right). White and colored boxes indicate the presence of vehicle or hormones during the recording. (F) Total number of mEPSCs for all neurons recorded within a treatment group. 17β-Estradiol and PPT appear to decrease mEPSCs across all amplitudes fairly equally. *$P < 0.05$.

a separate group of neurons (Fig. 4 D–F) (19, 20). Membrane potentials were recorded from CA1 pyramidal neurons during bath application of subthreshold concentrations (i.e., concentrations that did not produce action potential firing) of (S)-AMPA or NMDA to elicit a 5- to 7-mV amplitude depolarization on average. Consistent with our mEPSC results, 15–20 min of 50 nM 17β-estradiol (Fig. 4 B and C) decreased the amplitude of AMPAR-mediated depolarization. In contrast, 17β-estradiol did not affect CA1 responses to NMDA (Fig. 4 E and F).

ERα Agonist PPT Decreases CA1 AMPAR Responses. All of 17β-estradiol's effects on the electrophysiological properties of CA1 pyramidal neurons were replicated by ERα agonist PPT. Bath application of 100 nM PPT increased CA1 dendritic spine density (Fig. 2C), and decreased mEPSC frequency after 15–20 min of treatment (Fig. 3C and Fig. S5A). Similarly, PPT at 100 nM and 150 nM also reduced the membrane depolarization amplitude elicited by (S)-AMPA agonist (Fig. 4 B and C and Fig. S6). In contrast, the ERβ agonist DPN did not affect mEPSC frequency (Fig. 3C and Fig. S5A), nor did it affect AMPA-induced membrane depolarizations (Fig. 4 B and C and Fig. S6). None of the treatments affected NMDAR-mediated membrane depolarizations (Fig. 4 E and F).

Decreased AMPAR Function Is Time-Dependent and Transient. In a subset of neurons, a 15-min aCSF washout was applied after treatment with 17β-estradiol or PPT. This process resulted in a partial recovery of AMPA-induced membrane depolarizations. The average recovery for effective doses of 17β-estradiol and PPT was 89–93% of baseline (average effect of 17β-estradiol or PPT was 60–79% of baseline). The equivalent of a washout measure for vehicle controls was 99–105% of baseline (Fig. S7). Interestingly, estrogen effects on AMPA neurotransmission are first detected after 15 min of treatment (Fig. S8). When 5- to 10-min time points were examined during pilot studies, no effect of 17β-estradiol, PPT, or DPN could be detected.

Discussion

Estrogens, via ERα, Enhance Learning and Affect Hippocampal Connectivity. 17β-Estradiol delivered directly to the dorsal CA1 hippocampus facilitates object placement, object recognition, and social recognition learning within 40 min of administration (Fig. 1). Experiments using estrogen receptor agonists reveal these effects are mimicked by ERα activation. Conversely, ERβ activation enhances performance only in the object placement task. Thus, 17β-estradiol, via an ERα mechanism, rapidly facilitates general learning on discrimination tasks, whereas ERβ effects appear limited to spatial tasks. The same doses of 17β-estradiol and ERα agonist PPT that enhanced learning also increased dendritic spine density within the stratum oriens and stratum radiatum of CA1 pyramidal neurons after 20–30 min (Fig. 2). Interestingly, the ERβ-mediated spatial learning enhancements are not accompanied by rapid, large-scale changes in CA1 spine density. Although 17β-estradiol and PPT structurally increased the density of hippocampal spines, functionally the CA1 hippocampal excitatory input was decreased in those same neurons. After 15–20 min of application, 17β-estradiol and PPT attenuated both AMPA mEPSC frequency and AMPA induced membrane depolarization in CA1 pyramidal neurons (Figs. 3 and 4 and Figs. S5 and S6). Thus, surprisingly, estrogen-mediated increases in hippocampal spine density do not result in increased hippocampal excitatory input.

Estrogen-Induced Immature Synapses and Learning and Memory. 17β-Estradiol and ERα agonist PPT increased spine density while decreasing AMPAR responses. Dendritic spines with no or low numbers of synaptic AMPARs are silent or immature spines, and are considered "learning spines"; structures capable of long-term potentiation through the rapid insertion of AMPARs (14). Thus, they are thought to be the sites where new memories can be stored. In contrast, mature synapses or "memory spines" with high levels of AMPARs, are not capable of long-term potentiation (14), and are thought to be the sites where previously
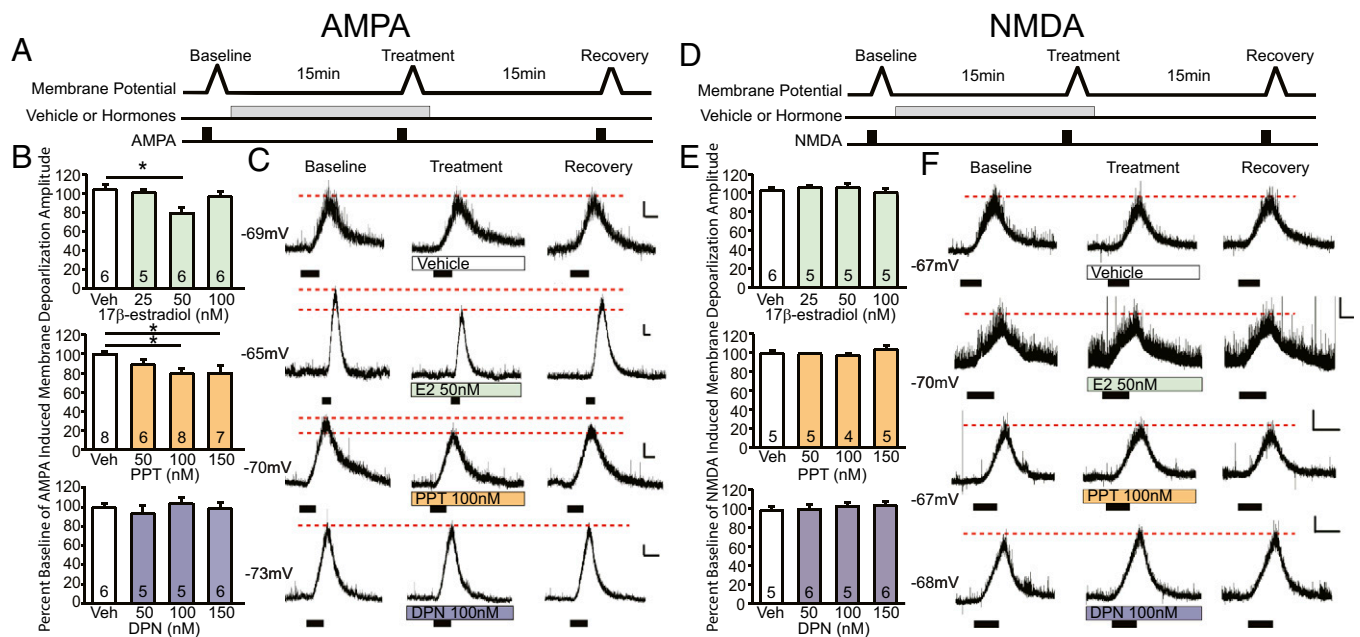
Fig. 4. Rapid effects of estrogens and receptor agonists on CA1 AMPA- or NMDA-induced membrane depolarization. (*A* and *D*) Treatment protocol for (S)-AMPA or NMDA induced depolarization measures. (*B* and *E*) Percent baseline of membrane depolarization amplitude elicited by (S)-AMPA or NMDA; mean ± SEM. (*B*) Fifteen to 20 min of 50 nM 17β-estradiol decreases AMPA-mediated membrane potential amplitudes ($F_{3, 19} = 4.05$, $P < 0.05$, post hoc $q = 4.45$, df = 8, $P < 0.05$). One-hundred nanomolars or 150 nM of PPT also decreases AMPA-mediated membrane depolarization ($F_{3, 25} = 3.33$, $P < 0.05$, post hoc for 100 nM $q = 3.91$, df = 14, $P < 0.05$, and for 150 nM $q = 3.73$, df = 13, $P < 0.05$). DPN does not affect AMPA membrane depolarization amplitude. (*E*) Hormone treatments do not affect NMDA induced membrane depolarization. (*C* and *F*) Membrane potential traces. Black boxes indicate length and duration of (S)-AMPA or NMDA agonist application. [Scale bars, 2 mV (vertical axis) and 1 min (horizontal axis).] *$P < 0.05$.

stored memories are maintained. 17β-Estradiol and ERα agonist PPT thus appear to induce the formation of learning spines in dorsal hippocampal CA1 pyramidal neurons, which likely have corresponding presynaptic inputs (6). Although the occurrence of silent/immature excitatory synapses is low in adult hippocampal and cortical neurons, they are found at high frequencies during development, correspond with the opening of critical periods, and are thought to allow a dramatic degree of plasticity for the activity dependent refinement of neural circuits (15). Thus, it seems as though estradiol, through ERα activation, rapidly promotes a hippocampal neuronal phenotype that is structurally and functionally similar to developmental neurons.

Based on our findings, we propose the following mechanism of action for estrogens' rapid enhancement of hippocampal learning. During behavioral training, mice intrahippocampally treated with 17β-estradiol or PPT experience a rapid increase in hippocampal "silent/immature" synapses containing relatively few AMPARs. We speculate that upon neural activity induced by learning, a subset of these spines would be potentiated and stabilized through AMPAR insertion into the synapse (14, 21), followed by pruning of unused synapses. Behaviorally, this hypothesis could be tested using transgenic mice that allow the labeling of neurons involved in a specific learning event.

This proposed mechanism of estrogen-enhanced learning is supported by in vitro findings in cultured cortical neurons and the two-step estrogen plasticity model, which reported estrogen-treated neurons have an enhanced AMPA response following activation of NMDARs (6). Although in contrast to the present findings, estradiol-induction of dendritic spines in cortical neurons appears to be ERβ-dependent (22). We demonstrate for the first time (to our knowledge) that the rapid effects of estrogens, which include increased dendritic spines and AMPAR internalization in the hippocampus, occur in parallel with behavioral learning enhancements.

Different Effects of ERα and ERβ Activation. We report different roles for ERα and ERβ in rapid estrogen-mediated learning enhancement. ERα activation replicated all of the effects of

17β-estradiol on learning, dendritic spines, and CA1 responses to AMPAR activation. ERβ activation however, did not increase spine density, nor did it affect CA1 pyramidal neuron responses to AMPA or NMDA, and enhanced only object placement learning. Our data suggest the existence of distinct nongenomic mechanisms of action for ERα and ERβ. Interestingly, we also recently demonstrated that the rapid learning and hippocampal dendritic spine effects of G protein-coupled estrogen receptor (GPER) activation are more comparable to those of ERα than ERβ (23).

In female rat hippocampal sections, ERβ activation has been shown to increase presynaptic vesicular glutamate release from the Schaffer collaterals onto CA1 neurons, whereas ERα or GPER activation had no effect (9). Hence the intrahippocampal delivery of ERβ agonist DPN may enhance object placement performance via presynaptic mechanisms. This finding would suggest that ERβ activation rapidly facilitates memory formation through increasing the activity-dependent stimulation received by CA1 pyramidal neurons that is required for synapse strengthening. Therefore, ERα and ERβ may rapidly affect hippocampal learning through separate pre- and postsynaptic mechanisms, respectively.

Estrogens Rapidly Affect CA1 AMPAR but Not NMDAR Responses. Although 17β-estradiol and the ERα agonist PPT attenuated AMPAR responses, none of the estrogen treatments affected NMDAR-mediated membrane depolarizations. This finding is in agreement with experiments demonstrating rapid AMPAR cycling into and out of the synapse (within minutes), while NMDARs do not display the same rapid turnover (24). Because these changes occur after ~15 min of estrogen application, they likely result from changes in cell-signaling cascades (25), rather than a direct effect of estrogens on AMPARs (Fig. S8). Our findings are consistent with our learning effects, and with other data demonstrating estrogens begin to affect sexual behavior and aggression ~15 min after administration (26, 27).

In the CA3 region of the hippocampus, metabotropic glutamate receptor activation combined with GPER activation was

recently shown to reduce AMPAR subunit GluA1 and induce long-term depression (18). Although there are differences in AMPAR plasticity between the CA3 and CA1 (18), similar mechanisms may underlie the effects we find with ERα in CA1.

AMPAR Internalization and Learning and Memory. Curiously, our findings demonstrate that estrogens not only induce the formation of new CA1 hippocampal dendritic spines, but they also appear to cause the internalization of surface AMPAR at preexisting synapses. There are two, nonmutually exclusive reasons why this could lead to enhanced learning and memory. First, our data suggest a conversion of some mature contacts to immature or silent type synapses, promoting circuit rewiring that facilitates learning by providing increased synaptic sites for information storage. As a consequence, the rapid effects of estrogens via ERα may cause weakening of some previously established memories (28) and facilitate their overwriting or updating if activated by a stimulus or a learning event (29). Second, the decrease in AMPA responses we observe can lead to an increase in the signal-to-noise ratio that improves learning and memory. Noise within the nervous system presents challenges for information processing, and is suggested to be a major source of behavioral variability (30). Because the transmission of neuronal signals occurs nonlinearly, small fluctuations, such as spontaneous vesicular release, can significantly influence neuronal function (30, 31). As we have shown, estrogens rapidly decrease hippocampal mEPSC frequency, and thus may reduce hippocampal noise to aid information processing. This interpretation suggests that the rapid effects of estrogens on learning are not dependent on increases in spine density per se, and would be replicated by decreased spontaneous neurotransmitter release. We consider the former hypothesis more likely given the strong correlation between estrogen-induced spine changes and behavior (5), the importance of silent/immature synapses for plasticity and neural circuit establishment (15), and the ability of silent/immature spines to undergo synaptic potentiation to store and maintain memories (14, 21).

Conclusions

To our knowledge, these results demonstrate for the first time that estrogen action within the hippocampus rapidly improves general discrimination learning, which is associated with a decrease in AMPA-mediated CA1 pyramidal excitatory input, despite the addition of new dendritic spines. Our data support the idea that estrogens rapidly and nongenomically increase the formation of silent or immature synapses within the CA1 hippocampus, via ERα activation. The formation and existence of immature or silent synapses has been proposed to be important for the establishment and modification of neural circuits (15). We propose that rapid ERα-mediated increases in immature or silent synapses phenotypically resemble the structural and functional aspects of developmental hippocampal neurons, promoting plasticity and facilitating learning.

Methods

Subjects. Young adult female CD1 mice (*Mus musculus*) were ovariectomized and implanted with bilateral cannulae in the anterior dorsal hippocampus for learning experiments. Pregnant female CD1 mice were purchased, and female pups 19- to 32-d-old were used for dendritic spine and electrophysiology experiments. Experiments were conducted in accordance with the Canadian Council on Animal Care, approved by University of Guelph's Animal Care and Use Committee. See *SI Methods* for details.

Dendritic Spine and Electrophysiology Analysis. Electrophysiological recordings were obtained from CA1 pyramidal neurons, a subset of which were filled with biocytin, then visualized using DAB. See *SI Methods* for details.

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