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Membrane Estrogen Receptor Signaling Impacts the Reward Circuitry of the Female Brain to Influence Motivated Behaviors

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Abstract

Within the adult female, estrogen signaling is well-described as an integral component of the physiologically significant hypothalamic-pituitary-gonadal axis. In rodents, the timing of ovulation is intrinsically entwined with the display of sexual receptivity. For decades, the importance of estradiol activating intracellular estrogen receptors within the hypothalamus and midbrain/spinal cord lordosis circuits has been appreciated. These signaling pathways primarily account for the ability of the female to reproduce. Yet, often overlooked is that the desire to reproduce is also tightly regulated by estrogen receptor signaling. This lack of emphasis can be attributed to an absence of nuclear estrogen receptors in brain regions associated with reward, such as the nucleus accumbens, which are associated with motivated behaviors. This review outlines how membrane-localized estrogen receptors affect metabotropic glutamate receptor signaling within the rodent nucleus accumbens. In addition, we discuss how, as estrogens drive increased motivation for reproduction, they also produce the untoward side effect of heightening female vulnerability to drug addiction.

Keywords

Estrogen Receptors; Drug Addiction; Sex; mGluR; Brain

Estrogen regulation of sexual behavior

The actions of estradiol on brain function and behavior have been studied for many years [1]. Within the adult female, estrogen signaling plays an essential role in reproduction. Primarily synthesized in the ovaries, estradiol synthesis and its subsequent signaling is a principal component regulating the hypothalamus-pituitary-gonadal (HPG) axis [2–5]. Even

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before the identification of estrogen receptor alpha (ER α) [6,7], estradiol was demonstrated to accumulate in brain regions involved with reproduction [8,9]. Cloning of the receptor further promulgated the hypothesis that the principal responsibility of estrogen signaling within the brain is to regulate female reproduction, as its distribution is primarily found in the rodent ventromedial hypothalamus, medial preoptic area and central gray region of the midbrain, all essential for the display of lordosis [10].

Coordinating the ability to display lordosis with ovulation is essential for rodent reproduction. But the ability to procreate is a process dissociable from the desire to have sex. Often underappreciated is that estradiol not only synchronizes the ability to display lordosis with ovulation, but, in parallel, also drives the desire to copulate (Figure 1). Simply, sexual behavior is a motivated behavior. Like most motivated behaviors, it requires the integration of sensorimotor circuitry and information about reinforcement in order to properly evaluate a situation, determine its salience, and execute the appropriate behavior [11–13]. Failure to recognize that estrogens coordinate the desire to copulate with both ovulation and the ability to display lordosis is most likely due to the limited nuclear estrogen receptor expression in reward-related regions of the brain [8,14,15]. Even when the necessity of estrogen to impact sexual drive has been considered, often it has been theorized that estradiol modifies the motivational system indirectly, through the estrogen receptor-expressing hypothalamic regions [16]. And notably, while in humans the desire and ability to engage in sex has been divorced from ovulation, women report heightened sexual drive following the rise in circulating estrogens around the time of ovulation [17,18].

Estradiol regulates the female motivational system

Over the last several years it has become increasingly apparent that the female motivational system is both exquisitely and directly sensitive to estradiol, and that this system plays an important role in sex behavior [19]. Similar to what has been reported in males [20,21], sexual behavior drives dopamine release in the nucleus accumbens [22,23] and this dopamine signal is reinforcing [24]. Accordingly, females who engage in paced mating behavior develop a conditioned place preference [25] and will work for access to a mate [26]. Inhibiting signaling in this brain region blocks the reinforcement properties of sex [27], and lesions of the nucleus accumbens impacts sexual reward in female rats [28–31].

Estradiol regulates both the activity and the structure of the nucleus accumbens in order to accommodate these changes when the animal is in a position to copulate. The nucleus accumbens is responsible for much of the complex processing needed for motivated behaviors. Current models posit that glutamatergic afferents from regions including the hippocampus, prefrontal cortex, and amygdala provide signals needed for prediction, dopaminergic inputs from the ventral tegmental area provide reinforcement information, and GABAergic inputs engage in action selection and subsequent motor output [32–34]. With the motivational circuitry system largely devoid of nuclear estrogen receptor signaling, the mechanism by which estradiol supports plasticity in the nucleus accumbens is just now being fully elucidated.

In addition to estradiol binding intracellular estrogen receptors that act as ligand-gated transcription factors, estrogens can also have immediate effects on cellular function [35]. For decades, the mechanism(s) behind these rapid effects of estrogens remained controversial. Since at least the 1980s, some have theorized that ER α (and upon its discovery, estrogen receptor beta, ER β [36]) also acts at the cellular membrane. In some of the first studies to support this hypothesis, estrogen receptors were found to translocate to the membrane of *Xenopus* oocytes [37,38]. Since then, membrane-localized estrogen receptors have been reported in various cell types and across multiple brain regions [39–41]. Some of the most compelling evidence for classical estrogen receptor involvement in rapid estradiol signaling came from experiments using estrogen receptor knockout mice in which ER α and/or ER β (dependent on brain region) were found responsible for the rapid activation of the MAPK/ERK signaling pathway [42].

Estradiol signals through ER/mGluR complexes at the membrane

Mechanistically, membrane-localized ER α and ER β were found to activate G-protein signaling [43], though current evidence suggests this is not through direct interactions with G-proteins. (This should not be confused with the estrogen-sensitive GPCR, GPR30/GPER [44].) Evidence from our lab and others shows that membrane-associated ER α and ER β functionally couple to various group I and II metabotropic glutamate receptors (mGluRs) in order to activate second messenger signaling [40,45–48]. Through this mechanism, estradiol can activate mGluR signaling in the absence of glutamate. The particular estrogen receptor-mGluR (ER/mGluR) pairing is cell-type specific, but more than one pairing can occur within the same cell [45,49]. Interestingly, while ER/mGluR signaling is initiated at the membrane, it too can regulate gene expression and long-term changes to neuronal function [50]. Because ER/mGluR signaling is hypothesized to be directly responsible for altering the female motivational circuit, details regarding its mechanism of action are described below. This is not to imply that ER/mGluR signaling is limited to just this neuronal system. In fact, classical lordosis behavior driven by estrogen modulation of hypothalamic function also has an essential ER/mGluR component [51].

In order for ER α and ER β to be trafficked to the surface membrane, palmitoylation of the receptor must occur. Palmitoylation is a reversible lipid modification that controls membrane tethering of otherwise cytosolic proteins [52–54]. Palmitoyl acyltransferases (PATs) are responsible for adding the 16-carbon fatty acid palmitate to target proteins, which increases the hydrophobicity of the protein to facilitate association with lipid membranes and lipophilic proteins. Palmitoylation of a single site on ER α and ER β is required for membrane-initiated estrogen signaling [55–57]. Furthermore, two PATs, DHHC7 and DHHC21, are essential for estrogen receptor trafficking and membrane localization [58]. Knockdown of either of these PATs eliminates ER α and ER β mGluR signaling in neurons [57]. The reversible nature of the palmitoyl group addition sets up the possibility for palmitoylation-depalmitoylation estrogen receptor cycling to dynamically modulate the membrane signaling responses [59]. Whether this also plays a role with β -arrestin-dependent cycling of ER α /mGluR1a in and out of neuronal membranes [60] remains to be seen.

At the plasma membrane, estrogen receptors must interact with caveolin proteins in order to signal to mGluRs. Caveolins are small integral membrane proteins that spatially organize signaling proteins, including mGluRs, into functional microdomains. In peripheral tissue, caveolins form large oligomers – caveolae – that create invaginations in the plasma membrane. Caveolae are not observed in the brain, but caveolin proteins are still expressed. Modifications to caveolin expression and/or activity interfere with ER/mGluR signaling [47,49,61]. There are three isoforms of caveolin, and all of these are expressed in the brain [49]. Interestingly, different caveolin isoforms produce different ER/mGluR interactions: CAV1 is necessary for ER α pairing with group I mGluRs, whereas CAV3 is necessary for ER α and ER β coupling to group II mGluRs [49]. Hence, CAV1 and CAV3 organize unique signaling microdomains that link membrane-associated ERs to specific mGluR partners in order to produce divergent effects. Furthermore, caveolin proteins appear to play a dual role in the context of ER/mGluR signaling in that not only do they confer signaling specificity through protein-protein interactions, but, as with palmitoylation, they also likely facilitate trafficking of ERs to the plasma membrane [61,62]. This dynamic interplay of various signaling partners can also be seen following the mutation of the ER α palmitoylation site, interfering with the ability of the estrogen receptor to associate with CAV1 [55]. Figure 2 provides such an example.

This functional coupling of ERs with mGluRs provides an avenue through which estradiol can exert far-reaching effects. Importantly, this relationship between estrogen receptors and mGluRs appears unique to females across many brain regions, and appears to exist even in the absence of ER activation. Co-immunoprecipitation studies have indicated a physical association between estrogen receptors and mGluRs [51]. The transactivation hypothesis is further supported by the fact in cultured neurons generated from female rat pups, the estrogen receptor antagonist, ICI 182,780, will attenuate DHPG-induced group I mGluR activation, which leads to increased CREB phosphorylation (Figure 3). Furthermore, disrupting ER α interacting with group I mGluRs via use of a dominant-negative CAV1 construct eliminates the effect. Additional potential off-target effects of ICI 182,780 were ruled out using male-derived cultures, which lack ER/mGluR coupling [45]. In this preparation, ICI 182,780 had no effect on DHPG-induced CREB phosphorylation.

Estradiol alters the dendritic structure of nucleus accumbens neurons

One of the most striking effects of ER/mGluR signaling is the change of functional circuitry of the nucleus accumbens. This can be seen through structural changes in medium spiny neurons (MSNs), the predominant neuronal subtype in this brain region. MSNs are the output neurons of the nucleus accumbens, and integrate multiple inputs [63,64], processing these signals in order to influence motor and cognitive behaviors through projections to other brain regions [65,66]. Repeated exposure to psychostimulants enhances both glutamatergic and dopaminergic input to the nucleus accumbens [32,67]. MSNs are aptly named for the high density of spines lining their dendrites. These spines receive glutamatergic inputs on the spine heads, and dopamine inputs on the spine necks. The high density of spines indicate the vast number of inputs MSNs receive, and changes in spine density are an essential component of synaptic plasticity [68–73].

Estradiol injections in adult ovariectomized rats decrease MSN dendritic spine density in the core region of the nucleus accumbens, indicating a decrease in putative glutamatergic input [74,75]. Pre-treatment with an mGluR5 antagonist, MPEP, eliminates the estradiol-induced change, indicating that this estradiol effect on structure is mediated by ER α /mGluR5 [75]. In contrast, estradiol increases the spine densities on nucleus accumbens shell MSNs through an ER α /mGluR1a mechanism [75]. Estradiol increasing dendritic spines via mGluR1a has been demonstrated/hypothesized in various other brain regions, including the hippocampus and hypothalamus [74,76–79]. Through direct mGluR receptor activation, mGluR5 signaling will decrease MSN spine densities throughout the nucleus accumbens, whereas mGluR1a activation results in increased spine densities [80]. Additionally, follow-up experiments determined that ER/mGluR changes in MSN spine densities required mGluR-mediated endocannabinoid release and activation of CB1 receptors [81]. Current experiments are examining which of the specific glutamatergic afferents into the nucleus accumbens are strengthened/weakened following ER/mGluR signaling, ultimately producing increased sexual drive.

Influence of estradiol on sexual motivation generalizes to other motivated behaviors

It is important to note that the neurobiological circuitry driving different motivated behaviors is not distinct from behavior to behavior. Hence, estradiol promotion of lordosis through increased sexual drive impacts other motivated behaviors. And while increased motivation to copulate during ovulation is biologically advantageous, enhanced responsiveness to other potentially rewarding stimuli is not necessarily so. This is most readily observed in models of drug addiction, as drugs of abuse exert their effects through co-opting the brain's natural reward system. Interactions between hormones and responses to drugs of abuse are revealed in part by the sex differences observed between men and women in addiction. For example, 16–17% of drug users progress to an addicted state [82], but women appear more likely to reach this addicted state than men [83,84]. Additionally, women begin psychostimulant use at younger ages, have increased acute responses, more rapidly escalate use, and progress to addiction faster [85–87]. This is especially evident with psychostimulants, but is also seen with opioids, nicotine, alcohol, and marijuana [88,89]. Women also report that psychostimulants produce greater subjective effects when estradiol levels are elevated, due either to natural hormonal cycles or to exogenous administration [86,90–92]. These effects have been reproduced in rodent studies, where ovariectomy eliminated sex differences in models of addiction, and estradiol replacement restored those differences [89,93–95].

One of the consequences of neuronal plasticity in the motivational system is that sex behavior and exposure to drugs of abuse cross-sensitize. This also solidifies the notion that the motivational pathways for reproduction and drugs of abuse are interrelated [96]. For example, prior sexual experience produces increased dopamine release in the nucleus accumbens during subsequent mating [97] and increases drug-induced locomotor responsiveness [98]. The reverse has also been demonstrated; that is, previous exposure to drugs of abuse enhances motivation to engage in sex behavior [99].

ER/mGluR signaling influences responses to drugs of abuse

Modulation of neurotransmission systems in the nucleus accumbens by drugs of abuse produces long-lasting changes in MSN excitability and structure; these changes are seen as the neurobiological basis for drug addiction [100–102]. Thus, it follows that estradiol-induced plasticity within the female nucleus accumbens could impact the effects of drugs of abuse. Much of our current work in females has focused on the role ER/mGluR signaling plays in regulating various aspects of drug addiction. This work builds on a growing body of evidence linking group I mGluRs (especially mGluR5) to responses to drugs of abuse [103]. We find that estradiol replacement facilitates cocaine-induced locomotor sensitization in ovariectomized female rats in an mGluR5-dependent manner [104]. This ER α /mGluR5 mediated effect also relies on endocannabinoid signaling and CB1 receptor activation [81].

Estradiol not only affects locomotor responses to cocaine; interactions between ER/mGluR-induced plasticity and the immense reinforcing properties of drugs of abuse also lead to increased vulnerability to addiction and relapse. In order to explore the effects of estradiol within a model more closely related to addiction, we examined the effects of estradiol and their dependence on mGluR activation using an extended access self-administration paradigm. Estradiol increased cocaine intake in female rats undergoing self-administration; this effect was abolished by the mGluR5 antagonist, MPEP [105].

To illustrate the effects of estradiol-induced plasticity on relapse potential, we performed a conditioned place preference (CPP) study using three groups of ovariectomized female mice (Figure 4). After showing no initial place preference, all animals underwent a four-day training period in which they received a daily injection of either cocaine or saline, and were then restricted to one side of the chamber or the other. Following the training period, all mice displayed a strong preference for the cocaine-associated side of the chamber. Animals then underwent five days of extinction training in which they no longer received cocaine. On the sixth day, the preference for the cocaine-associated side of the chamber was eliminated. Finally, animals were subjected to a reinstatement trial in which they were given a low dose of cocaine or saline prior to being placed back in the CPP chamber. This concentration of cocaine was previously found not to induce reinstatement of CPP in male mice. Saline-treated animals that were administered estradiol 24 hours prior to the reinstatement trial exhibited no reinstatement. Oil-treated animals that were administered the sub-threshold dose of cocaine also did not reinstate. However, estradiol-treated animals given this same dose of cocaine exhibited a robust reinstatement of CPP, suggesting that estrogen potentiation of cocaine action [106–108], increases the likelihood for drug-relapse behavior. Notably, while estradiol has been found to directly increase striatal dopamine release [109], we have yet to find conditions where estradiol, in the absence of other trigger events, will induce relapse behavior on its own.

Summary and conclusions

Within adult females, estrogen receptor signaling plays an essential role in reproduction. For most species, females will only display sexual receptivity during ovulation. This has led to the false presumption that these animals have sex in order to reproduce. Further, when

compared to women who will engage in intercourse outside periods of fertility, it was presumed that humans were one of the few species to engage in sex for pleasure. We now believe the converse is closer to the truth. That is, for most species, estradiol not only ties ovulation with the ability to engage in sexual intercourse, but also synchronizes ovulation with the motivation to engage in sex. In contrast, humans, who comprehend the consequences of intercourse, are one of the few species that (at times) engage in sex for reproduction. This misunderstanding regarding the role of motivation in sex behavior can be at least partially attributed to the lack of nuclear estrogen receptors in the reward circuitry of the brain, leading to a lack of evidence for estradiol impacting the motivational system. We now know that in females, both ER α and ER β functionally couple to various group I and II mGluRs across multiple brain regions, including the nucleus accumbens. One of the functional consequences of ER/mGluR signaling in the nucleus accumbens is alteration of neurotransmission, at least partially due to changes in dendritic connectivity. Details regarding how the estradiol-induced changes in dendritic structure ultimately impact sex drive are still forthcoming, but we do know that another functional consequence of ER/mGluR signaling in this brain region is that estradiol produces periods of heightened vulnerability to drug abuse.

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Figure 1.

In females, estrogen receptor signaling coordinates multiple pathways to enable reproduction. These pathways time ovulation with both the ability and desire to copulate. By altering the brain reward system, estrogen receptor signaling also impacts other motivated behaviors, such as vulnerability to drugs of abuse.

Figure 2. Mutation of the ERα palmitoylation site prevents CAV1 association and membrane localization. (A) HEK293 cells transfected with ERα or palmitoylation-null ERα (ERα Mut) along with CAV1. Western blot revealed that elimination of the ERα palmitoylation site reduced CAV1-ERα co-IP without affecting overall expression of either ERα or CAV1. Untransfected HEK293 cells were run in lane two (i.e. negative control). (B) Membrane (MF) and cytosolic (CF) fractions of HEK293 cells transfected with ERα or ERα Mut indicate the necessity of ERα palmitoylation for membrane localization. Flotillin (Fltn) was used as membrane fraction control.

Figure 3.

Transactivation of mGluRs by estrogen receptors. (A) Model of ER α physical association with group I mGluRs via CAV1. (B) Administration of the estrogen receptor antagonist ICI 182,780 (ICI) attenuates DHPG-mediated group I mGluR-dependent CREB phosphorylation (pCREB) in cultured neurons from female rat pups. Transfecting a separate population of neurons with a dominant-negative form of CAV1 tagged with EGFP (EGFP-dnCAV1), eliminates the effect of ICI. (C) ICI has no effect on DHPG-mediated group I mGluR-dependent pCREB in cultures from male rat pups. Group data is the average fluorescent intensity within the nucleus of pyramidal hippocampal neurons across two coverslips. The findings were replicated twice using different cultures.

Figure 4.

Estradiol facilitates reinstatement of cocaine-mediated conditioned place preference, a model of drug relapse behavior. Three groups of ovariectomized mice ($n=5-6/\text{group}$) underwent cocaine-mediated conditioned place preference (CPP) and extinction, followed by a test for reinstatement of CPP. Before conditioning (baseline) there was no preference for either side of the chamber. Following two pairings of cocaine (7.5 mg/kg, s.c.) with a particular side of the chamber, all animals displayed CPP (cocaine preference). Following six days of access to both sides of the chamber with no drug pairing, all three groups of animals extinguished CPP (extinction). The following day, only animals that received estradiol (2.5 μg , s.c.) 24 hours before, and a subthreshold dose of cocaine (5 mg/kg) immediately prior to testing, reinstated CPP (reinstatement). Animals receiving either estradiol or cocaine alone did not exhibit reinstatement.