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Sex as a Biological Variable in Neuroendocrinology

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Abstract

The National Institutes of Health (NIH) policy requiring consideration of sex as a biological variable (SABV) was introduced to address long-standing sex biases in biomedical research and to improve the generalizability of findings. Although this has increased the inclusion of females, many studies still treat sex differences descriptively, without explaining the biological processes that produce variability. This review focuses on whether fluctuations in ovarian hormones, particularly estradiol, can account for this variability in a systematic, state-dependent way rather than as unstructured variability. Literature was collected through a systematic methodology on PubMed Central with a focus on peer-reviewed studies on estradiol's effects on neural plasticity, circuit function, and behavior in mammalian models, and findings were considered across hippocampal, corticolimbic, and mesolimbic systems with attention to differences in hormone state, task design, and behavioral measures. Outcomes vary with circuit engagement and task demands: in the hippocampus, estradiol is most closely linked to memory consolidation; in corticolimbic circuits it modulates fear extinction and regulation; and in mesolimbic systems, it influences motivation and reward sensitivity. Apparent inconsistencies across studies are better explained by differences in how tasks recruit neural systems and may be traced to differences in experimental design and the neural systems being tested. This interpretation is limited by reliance on rodent models, variability in estrous cycle staging, and the use of ovariectomized designs that simplify endogenous hormonal dynamics. In addition, focusing on estradiol limits consideration of interacting hormones such as progesterone, which may alter or counter some of the effects described. Overall, the evidence suggests that hormone-linked variability resembles organized modulation of neural systems, and considering hormonal state in this way allows SABV to move beyond group comparisons toward a more mechanistic understanding of brain–behavior relationships.

Keywords: Estradiol; Sex as a Biological Variable (SABV); Neuroplasticity; Ovarian Hormones; Hippocampus; Fear Extinction; Mesolimbic Dopamine; State-Dependent Modulation

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Introduction

In 2016, the National Institutes of Health implemented a policy requiring applicants to account for sex as a biological variable (SABV) in research design (National Institutes of Health, 2015). This mandate emerged in response to a longstanding bias in biomedical research toward male subjects, particularly in animal studies and clinical trials, which limited the generalizability of findings and obscured biologically relevant sources of variation (Beery & Zucker, 2011; Clayton & Collins, 2014). Incorporating sex as a biological variable has since been recognized as essential for improving rigor, reproducibility, and translational validity in research (Clayton, 2016).

This policy reflects a change in how biological variability is conceptualized. Historically, variability, particularly in females, was often treated as a confounding factor to be minimized, leading to the exclusion of females from many experimental designs. The insufficient study of females in research has contributed to the incomplete understanding of disease mechanisms and, in some cases, harmful therapeutic outcomes that disproportionately affect women (Beery & Zucker, 2011). In contrast, SABV recognizes that variability may reflect meaningful biological processes rather than confounds. However, a challenge remains in moving beyond the identification of sex differences toward understanding the mechanisms that generate variability. In many cases, SABV has been applied in a primarily descriptive manner, demonstrating differences between males and females without resolving the underlying mechanisms that produce them (Shansky & Murphy, 2021; Joel & McCarthy, 2016).

Addressing this limitation requires a foundation capable of linking biological variability to underlying mechanisms. Neuroendocrinology provides a tractable approach, as hormonal fluctuations offer a structured and temporally dynamic source of modulation across neural systems.

Estradiol as a Model of State-Dependent Neural Modulation

Within neuroendocrinology, ovarian hormones provide a biologically grounded model for examining how internal physiological state shapes brain function. Fluctuations in ovarian hormones are not random, but are regulated by the hypothalamic–pituitary–gonadal (HPG) axis (Moenter & Starrett, 2024). The hypothalamus releases gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), driving ovarian hormone production. Estradiol then feeds back onto both hypothalamic and pituitary systems, producing tightly regulated, cyclical patterns of hormone release. These dynamics provide a basis for how internal physiological state can influence brain function across time and support the interpretation of sex as a state-dependent variable rather than a fixed categorical distinction.

While both rodents and humans exhibit cyclical ovarian hormone fluctuations, the estrous and menstrual cycles differ in structure and timescale. Rodents undergo a 4–5 day estrous cycle characterized by brief, tightly timed hormonal peaks, and the human menstrual cycle occurs over approximately 28 days with more prolonged hormonal phases (Becker et al., 2005; Kundakovic & Rocks, 2022) (Table 1). Estrous cycles do not include menstruation, and phases are defined based on reproductive receptivity rather than endometrial shedding. These differences are

important when translating findings across species, given that the timing and duration of estradiol exposure vary substantially between models.

Estrous cycle in rodents

Phase	Estradiol level	Progesterone level	Timing (approximate)
Proestrus	High	Brief surge in late proestrus	Late day 3-4
Estrus	Rapid decline	Low	Day 1 (post-ovulation; ovulation occurs late proestrus)
Metestrus	Low	Low-moderate	Day 2
Diestrus	Low (baseline)	Low	Day 3

Menstrual cycle

Phase	Estradiol level	Progesterone level	Timing (approximate)
Follicular (early-mid)	Low –> rising	Low	Days 1-10
Late follicular/ pre-ovulatory	High	Low	Days 11-14
Ovulation	Brief drop	Low	Day 14
Luteal	Moderate	High	Days 15-26
Late luteal	Falling	Falling	Days 26-28

Table 1. Temporal dynamics of estradiol and progesterone across the rodent estrous cycle and human menstrual cycle. Estradiol has a pre-ovulatory peak, while progesterone rises following ovulation, producing distinct but comparable patterns of hormonal fluctuation across species. In rodents, these changes occur over a 4–5 day estrous cycle, and in humans they unfold across an approximately 28-day menstrual cycle. Hormone levels and timing are schematic and may vary across individuals and experimental conditions.

This thesis is situated within this background and focuses on the role of ovarian hormones, particularly 17β-estradiol, the primary and most biologically active form of estrogen in the brain (commonly referred to as E2, estradiol, or estrogen), in shaping neural plasticity and behavior (Fiocchetti et al., 2012). Estradiol's effects extend beyond reproduction, and it has the capacity to impact synaptic structure, neuronal excitability, intracellular signaling, and gene expression across multiple brain regions, including the hippocampus, corticolimbic circuits, and mesolimbic systems (Woolley & McEwen, 1992; Frick et al., 2018; Yoest et al., 2018). These effects may influence learning and memory, emotional regulation, and motivated behavior.

Estradiol Signaling Mechanisms in the Brain

17β-estradiol (E2) is a steroid hormone synthesized primarily in the ovaries and through the aromatization of testosterone (Taxier et al., 2020). In addition to its role in reproductive physiology, estradiol is widely expressed in the brain, where it functions as a neuroactive steroid capable of modulating synaptic transmission, intracellular signaling, and gene expression.

Estradiol acts through three primary receptor classes: estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and the G protein–coupled estrogen receptor (GPER) (Bendis et al., 2024). ERα and ERβ can function both as nuclear transcription factors and as membrane-associated receptors, while GPER primarily mediates rapid, membrane-initiated signaling. These receptors are broadly expressed throughout the central nervous system and contribute to the regulation of neuronal signaling across multiple brain regions.

Estradiol signaling can be broadly categorized into classical genomic and non-classical membrane-initiated mechanisms. In classical genomic signaling (Figure 1), estradiol diffuses across the cell membrane and binds intracellular estrogen receptors, which then dimerize and translocate to the nucleus to regulate gene transcription via estrogen response elements (Bendis et al., 2024). This mechanism operates on slower timescales and supports longer-term changes in cellular function.

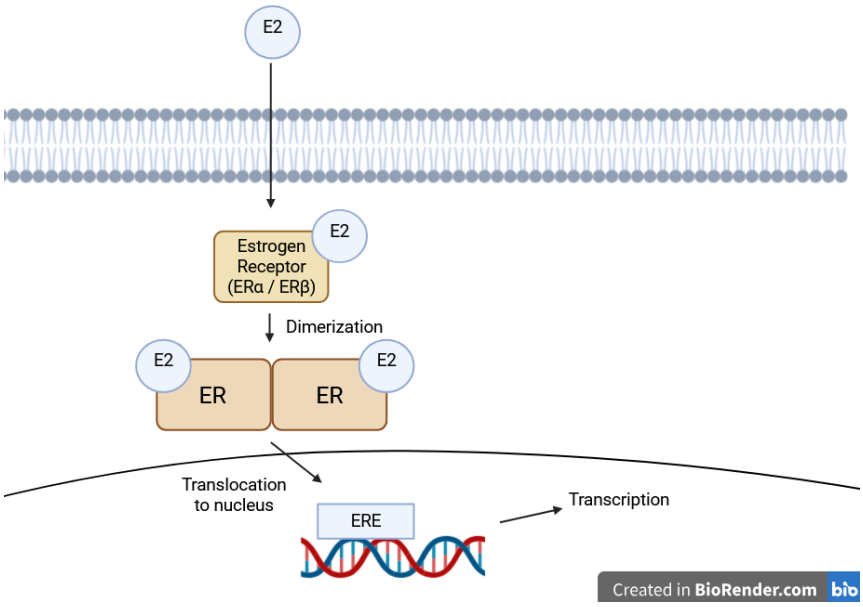

Figure 1. Classical (genomic) estradiol signaling. Estradiol (E2) diffuses across the plasma membrane and binds intracellular estrogen receptors (ERα/ERβ), forming ligand-bound complexes that dimerize and translocate to the nucleus. These dimers bind estrogen response elements (EREs) on DNA to regulate gene expression. This pathway shows the slower, transcription-dependent action of estradiol (hours–days), which distinguishes it from rapid, non-classical signaling pathways. Adapted from: Koszegi and Cheong (2022).

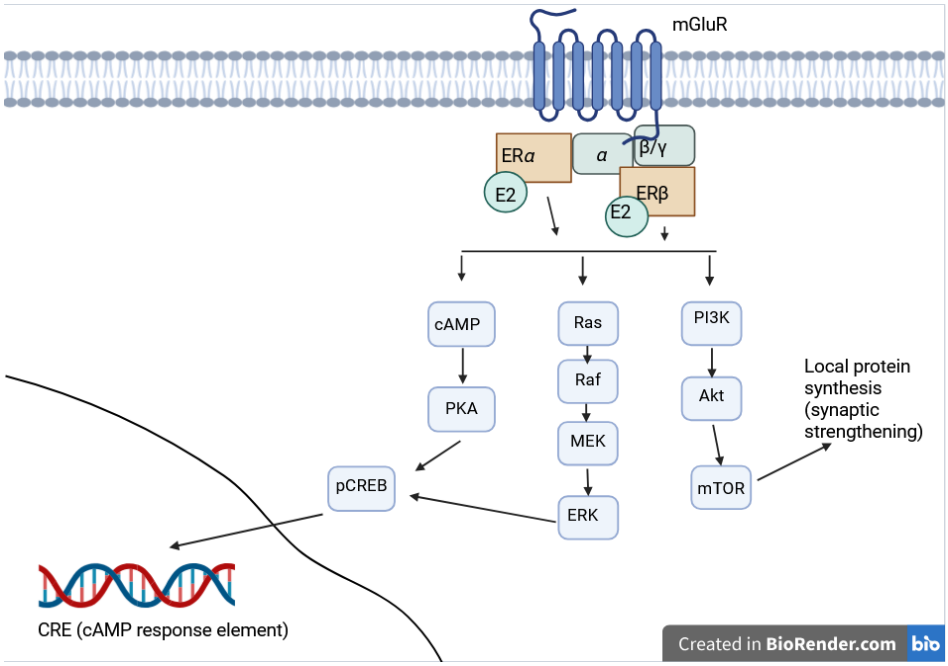

Figure 2. mGluR-ER coupling as a non-classical signaling pathway. Estradiol (E2) activates membrane-associated estrogen receptors (ERα/ERβ), which functionally couple with metabotropic glutamate receptors (mGluRs) to initiate intracellular signaling pathways. This coupling enables activation of ERK/MAPK, cAMP/PKA, and PI3K/Akt signaling cascades. ERK and PKA signaling converge on phosphorylation of CREB, which translocates to the nucleus and binds cAMP response elements (CRE) to regulate gene transcription. In parallel, PI3K/Akt signaling activates mTOR, promoting local protein synthesis associated with synaptic strengthening. These pathways support glutamate-independent modulation of synaptic plasticity. Adapted from: Frick et al. (2015).

Estradiol can also act through rapid, membrane-initiated signaling pathways. Membrane-associated estrogen receptors activate intracellular kinase cascades, including MAPK/ERK and PI3K/Akt signaling pathways, allowing estradiol to influence neuronal activity on timescales relevant to synaptic plasticity and behavior (Taxier et al., 2020). Although often described as ‘non-genomic,’ membrane-initiated signaling ultimately converges on transcriptional and epigenetic mechanisms that influence long-term cellular function.

A distinct form of membrane-initiated signaling involves functional coupling between estrogen receptors and metabotropic glutamate receptors (mGluRs) (Meitzen & Mermelstein, 2011). Through this interaction, estradiol can engage intracellular signaling pathways typically associated with glutamatergic neurotransmission, but doesn't require glutamate binding (Figure 2). This positions estradiol as a regulator of existing signaling networks.

In addition to ERα- and ERβ-mediated signaling, G protein–coupled estrogen receptors (GPERs) provide another pathway for rapid estradiol action (Brailoiu et al., 2007). Activation of GPER can engage second messenger systems and kinase signaling pathways similar to those activated by membrane-associated ERs. However, the functional role of GPER in mediating estradiol-dependent effects on synaptic plasticity and behavior remains less well defined compared to classical estrogen receptor pathways.

These mechanisms show that estradiol signaling is not limited to a single pathway, and instead operates through multiple interacting systems that enable both rapid and long-term modulation of neural function. These pathways provide a basis for understanding how fluctuations in estradiol levels can produce context-dependent effects across different neural circuits.

Despite extensive evidence for estradiol's influence, findings across studies have often appeared inconsistent, particularly at the behavioral level. To address these inconsistencies, this thesis examines whether variability associated with ovarian hormone fluctuations reflects structured, state-dependent modulation of neural systems. Rather than assuming uniform effects of estradiol across contexts, this work considers how differences in experimental design, circuit

engagement, and the timing of hormonal state relative to behavioral testing may impact observed outcomes.

However, translating these conceptual insights into empirical findings presents substantial methodological challenges, particularly when studying naturally cycling individuals.

Methodological and Physiological Considerations in Studying the Estrous Cycle

Challenges associated with studying naturally cycling females have historically contributed to their exclusion from research under the assumption that hormonal fluctuations introduce excessive variability. However, with the incorporation of sex as a biological variable, these should be addressed rather than avoided. Main considerations include:

Cycle monitoring and timing: Female rodents exhibit short estrous cycles spanning 4–5 days, requiring precise staging (typically through daily vaginal cytology) to link results with hormonal state (Becker et al., 2005). Because individuals cannot be synchronized to specific phases, studies often require large sample sizes or flexible scheduling.

Within-subject vs. between-subject designs: Within-subject designs increase statistical power by testing the same individuals across multiple cycle stages, but may introduce order or learning effects. Between-subject designs avoid repeated testing but require careful control of individual variability and accurate cycle staging. Each approach involves trade-offs that must be accounted for in experimental design.

Estrous stage classification: Inconsistent staging or grouping of cycle phases can obscure hormone-dependent effects. Transitional phases, such as early estrus, may not resemble the same

hormonal conditions as more stable phases, and differences in how studies define “high” and “low” hormone states contribute to variability across findings (Becker et al., 2005).

Endocrine Dynamics and Physiological Context: Hormone levels vary not only across the estrous cycle but also within it. For example, estradiol exhibits diurnal fluctuations during proestrus, with peak levels occurring prior to ovulation. In addition to these endogenous rhythms, ovarian hormone signaling interacts with stress-responsive systems through bidirectional regulation of the hypothalamic–pituitary–adrenal (HPA) and HPG axes (Domes et al., 2024). While a detailed discussion of HPA axis function is beyond the scope of this review, chronic or severe stress has been associated with inhibition of HPG axis activity, whereas acute stress can produce variable effects on gonadal steroid secretion, including increases in estradiol under certain conditions. These interactions show that hormonal state is shaped not only by intrinsic cycle dynamics but also by a larger physiological context. Behavioral paradigms that involve stress may engage both endocrine and neural systems simultaneously, which complicates the attribution of observed effects to hormonal state alone. For a more comprehensive discussion of HPA–HPG interactions in the context of stress, see Domes et al., 2024.

Experimental Modeling Approaches: Ovariectomized (OVX) models with hormone replacement provide experimental control over hormone exposure and are widely used to establish causal effects. However, these models do not fully replicate the dynamic endocrine environment of intact cycling systems, as they lack endogenous hormonal rhythms and interactions (Koebele & Bimonte-Nelson, 2016). They are often best interpreted as complementary mechanistic models rather than direct representations of natural physiology.

Assumed Variability: The long-standing assumption that females are inherently more variable than males has been challenged. Meta-analytic evidence indicates that females are not more variable across many physiological and behavioral measures and that variability alone does not indicate hormone sensitivity unless endocrine state is explicitly measured (Becker et al., 2016).

Scope and Organization

Given these conceptual and methodological considerations, this thesis is structured to examine estradiol-dependent modulation across multiple levels of analysis. Hormonal effects on the brain can be broadly categorized as organizational or activational. Organizational effects occur during developmental periods and produce long-lasting structural changes in neural circuitry, whereas activational effects arise from fluctuations in hormone levels in adolescence and adulthood and act on already-established circuits (Arnold & Breedlove, 1985). The present thesis focuses on activational effects of estradiol in the mature mammalian brain, where hormone-dependent modulation operates on timescales directly relevant to cognition, emotion, and motivated behavior.

For clarity, the term “female” is used here in a biological and neuroendocrine context, referring to organisms possessing chromosomal and gonadal features that enable ovarian estradiol production (Bhargava et al., 2021). Considerations of gender identity, intersex variation, or transgender physiologies are important but beyond the scope of this review (Clayton, 2016).

To examine how estradiol modulates brain function, this thesis is organized across three major functional systems: chapter 3 focuses on hippocampal plasticity and learning and memory; chapter 4 addresses corticolimbic circuits involved in emotion and stress, with a particular emphasis on fear learning and extinction processes; and chapter 5 examines mesolimbic

dopaminergic systems and the role of estradiol in motivation, reward processing, and vulnerability to substance use disorders.

Methodologically, this thesis uses a structured narrative review to synthesize findings across species, neural systems, and behavioral paradigms. A narrative approach is better suited than quantitative meta-analysis for identifying mechanistic patterns and resolving apparent inconsistencies across heterogeneous experimental designs.

By integrating findings across these domains, this thesis aims to clarify how hormone-dependent variability reflects structured modulation of neural systems, providing a basis for interpreting sex as a biological variable in neuroscience research.

Methodology

Literature Search Strategy

Database searches were conducted using PubMed Central (PMC), with supplementary searches performed in Google Scholar for citation tracking and identification of additional relevant literature.

Search terms were not applied as a single fixed query but were combined and refined iteratively across searches to capture relevant literature spanning molecular, circuit, and behavioral levels. Multiple complementary searches were used to ensure coverage across hippocampal, corticolimbic, and mesolimbic systems, as well as across mechanistic and behavioral domains.

In addition to database searches, relevant studies were identified through backward and forward citation tracking of articles. Reference lists of foundational and highly cited studies were examined to locate earlier work, and Google Scholar was used to identify more recent studies citing these articles. This process was applied iteratively to ensure comprehensive coverage of influential literature.

Search Terminologies

Main Medical Subject Headings (MeSH) included “Estradiol,” “Estrogens,” “Estrogen Receptors,” “Ovariectomy,” and “Sex Characteristics.” Additional keywords included “E2,” “estrous cycle,” and “ovarian hormones.” Chapter-specific MeSH and keyword terms were used to target relevant neural systems and behavioral domains:

Chapter 3: hippocampus; synaptic plasticity; long-term potentiation; dendritic spines; neurogenesis; glutamate receptors; NMDA receptors; AMPA receptors; learning; memory; memory consolidation

Chapter 4: amygdala; prefrontal cortex; limbic system; fear; fear conditioning; fear extinction; anxiety; stress, physiological; stress, psychological

Chapter 5: mesolimbic system; nucleus accumbens; ventral tegmental area; striatum; dopamine; reward; motivation; reinforcement; substance-related disorders; cocaine; drug self-administration

These terms were used in multiple combinations to identify studies examining estradiol-dependent effects across levels of analysis.

Study Selection and Evaluation

Studies were evaluated at the level of title, abstract, and full text for relevance to estradiol signaling in neural systems. Rather than relying on a single fixed search output, literature was assessed across multiple searches to identify studies that met conceptual and methodological criteria.

Approximately 200-300 articles were examined in detail at the full-text level. Application of the inclusion and exclusion criteria resulted in a final set of 118 studies included in the qualitative synthesis. Because searches were conducted iteratively and refined throughout the

review process, these counts are reported as approximate and are intended to reflect the scale of literature engagement rather than a fixed systematic sampling.

Eligibility Criteria

Inclusion criteria

Studies were eligible for inclusion if they:

- Examined estradiol or ovarian hormone signaling in the brain
- Investigated neuroplasticity, neural circuitry, or behavior related to learning and memory, emotional or stress regulation, or motivation and reward processing
- Used mammalian models, including rodents and humans
- Were peer-reviewed primary research articles or scholarly review papers
- Were available in English
- Provided sufficient methodological detail to allow interpretation of hormone-dependent neural or behavioral outcomes

Exclusion criteria

Studies were excluded if they:

- Focused exclusively on neurosteroids synthesized locally in the brain
- Included only male samples
- Examined progesterone or other hormones without addressing interactions with estradiol
- Used non-mammalian species
- Were not published in English
- Did not directly relate to neuroplasticity, neural circuitry, or behavior

- Were not accessible through open access or author-provided versions

This review focuses on activational hormone mechanisms; therefore, studies of organizational effects were excluded unless necessary for mechanistic context.

Hippocampal Plasticity and Learning & Memory

The hippocampus is a major structure in the vertebrate brain involved in learning and memory consolidation, and one of the most well-studied systems for relating synaptic plasticity to behavior (Stepan et al., 2015). Synaptic plasticity is modulated by hormonal signals such as 17β-estradiol, but despite the evidence that estradiol affects hippocampal plasticity, its effects on learning and memory have been inconsistent across studies. Many of these inconsistencies likely arise from differences in the specific circuits, mechanisms, and behavioral paradigms employed across studies.

Es**tradiol-related structural plasticity in CA1**

The hippocampal formation is composed of the dentate gyrus (DG), hippocampal subfields CA1/CA2/CA3, and associated regions including the entorhinal cortex (Stepan et al., 2015). The organization of these is a trisynaptic circuit composed of excitatory cells: the entorhinal cortex inputs terminate in DG; DG cells project to CA3; CA3 neurons project to CA1; the CA1 projects back toward the entorhinal cortex. Hippocampus-dependent tasks are reliant on the information flow through DG-CA3-CA1 outputs. CA1 is a main output node that integrates CA3 and entorhinal inputs.

The entorhinal cortex (EC) consists of six layers, with layers II and III projecting to the DG and hippocampus, but layer II is most associated with the trisynaptic circuit (Stepan et al., 2015). The EC-hippocampal connections are important for spatial memories including memory formation and consolidation. The dentate gyrus is a subfield of the hippocampus that only receives direct inputs from the entorhinal cortex. Its role is associated with separating information into distinct and specific details to ensure that new memories are encoded separately from similar, previously stored memories.

At the level of neuronal synapses, estradiol has been shown to mediate changes of hippocampal synapse density in rats. The Woolley & McEwen (1992) study shows a decrease in density of synapses on dendritic spines with low levels of estradiol, and an increase in this synapse density with high levels. Between the proestrus and estrus phases of their cycle, a 32% decrease in CA1 dendritic spine synapse density was seen within 24 hours. They hypothesized that due to this significant degree in changes of synaptic plasticity, the circulating levels of estradiol in rodents may impact hippocampal function.

Their study has inspired decades of research on this topic. A later study by Woolley (1998) showed that estradiol-induced changes in the hippocampus may, indeed, have functional effects: dendritic spines formed under periods of high estradiol are suggested to represent a specialized subpopulation of synapses that are predominantly NMDA receptor–mediated, which are thought to increase the potential for synaptic plasticity. This is consistent with the idea that estradiol transiently increases the capacity for synaptic potentiation. This idea is further supported by evidence that 17β-estradiol can improve hippocampal memory consolidation in female rodents through epigenetic mechanisms and multiple-cell signaling (Taxier et al., 2020). In the hippocampus, these mechanisms converge on synaptic and intracellular processes that regulate neuronal plasticity on behaviorally relevant timescales.

Estradiol effects on glutamatergic synaptic transmission

One way that estradiol impacts hippocampal plasticity is through modulation of glutamatergic signaling at CA3–CA1 synapses. Glutamate is the principal excitatory neurotransmitter in the brain and acts through NMDA and AMPA receptors, which play complementary roles in synaptic plasticity. NMDA receptors permit calcium influx that initiates intracellular signaling cascades, whereas AMPA receptors mediate fast excitatory transmission

and determine the strength of postsynaptic responses (Zhang et al., 2015). Estradiol enhances NMDA receptor–mediated signaling and increases the excitability of CA1 pyramidal neurons, thereby increasing postsynaptic responsiveness to excitatory input (Carrer et al., 2003; Woolley et al., 1997; Kumar & Foster, 2002). These effects are associated with enhanced long-term potentiation (LTP) at CA3–CA1 synapses, a form of activity-dependent synaptic strengthening considered a cellular substrate for learning and memory (Bi et al., 2000; Smith & McMahon, 2005; Vedder et al., 2012). Estradiol-induced effects in both LTP and object recognition memory require these NMDA receptors (Vedder et al., 2012).

Although the exact contribution of estradiol to AMPA receptor trafficking is less well defined, its facilitation of NMDA-dependent signaling suggests that estradiol promotes synaptic conditions permitting subsequent AMPAR insertion during potentiation (Smith & McMahon, 2005). While AMPA receptor insertion increases responsiveness to glutamate and stabilizes synaptic connections, it does not sustain the elevated synaptic plasticity observed during potentiation. Instead, Smith & McMahon (2005) suggest that increased LTP magnitude occurs when NMDA receptor transmission is elevated relative to AMPA receptor transmission, and that subsequent increases in AMPAR-mediated signaling restore this balance and normalize LTP magnitude. This indicates that estradiol-dependent hippocampal plasticity reflects a transient shift toward NMDA-dominant signaling, followed by AMPAR-mediated stabilization of synaptic strength.

Rapid intracellular signaling pathways

Rapid estradiol signaling in the hippocampus is mediated in part through functional coupling between membrane-associated estrogen receptors and metabotropic glutamate receptors

(mGluRs) (Figure 2). This interaction enables estradiol to engage intracellular signaling pathways on behaviorally relevant timescales. This mechanism suggests that estradiol modulates ongoing glutamatergic signaling rather than acting as an independent activator of intracellular cascades (Boulware et al., 2013).

One intracellular signaling pathway is the ERK/MAPK cascade, which has been particularly well characterized (Bozon et al., 2003; Kelly et al., 2003). In the dorsal hippocampus, estradiol-dependent enhancement of object recognition memory consolidation requires ERK/MAPK signaling, as pharmacological inhibition of MEK prevents these behavioral effects (Kuroki et al., 2000; Boulware et al., 2013). These findings indicate that ERK activation is necessary for estradiol-induced improvements in hippocampus-dependent memory.

Estradiol-induced activation of PI3K/Akt signaling in the dorsal hippocampus functions upstream of ERK and is similarly required for memory enhancement. Inhibition of PI3K blocks both ERK activation and estradiol-dependent improvements in object recognition memory, indicating coordinated signaling rather than independent pathway activation (Fan et al., 2010; Fortress et al., 2013).

Estradiol regulation of mTOR-dependent protein synthesis and transcriptional regulation

Downstream of kinase signaling, estradiol activates the mammalian target of rapamycin (mTOR) pathway, which regulates protein synthesis needed for long-term synaptic plasticity. Estradiol increases the phosphorylation of downstream mTOR targets in the dorsal hippocampus (Hoeffer & Klann, 2010), and the inhibition of mTOR signaling prevents estradiol from improving object recognition memory consolidation (Dash et al., 2006; Myskiw et al., 2008). This suggests that protein synthesis is necessary for estradiol-induced memory enhancement.

ERK signaling also influences transcriptional regulation through phosphorylation of the transcription factor CREB (Boulware et al., 2005). Activated CREB regulates transcription of genes associated with synaptic plasticity, which links kinase signaling to longer-term molecular processes that stabilize memory-related synaptic changes.

These findings support a coordinated signaling model in which PI3K activation functions upstream of ERK, converging on mTOR-dependent protein synthesis and CREB-mediated transcription to stabilize synaptic changes underlying memory consolidation.

Dorsal vs. Ventral Hippocampus: Memory and Emotion

Despite this evidence, linking estradiol's modulating capacity to observable memory changes has been complex. The early attempts to correlate estrous fluctuations or hormonal treatments with performance on standard tasks that are hippocampal-dependent (such as mazes) produced mixed results (Woolley, 1998). This discrepancy suggests that either the tasks were not sensitive to subtle estradiol effects, or that these effects may only manifest under specific conditions or in specific hippocampal subregions. These ideas have led to researchers considering moderating factors, such as which part of the hippocampus is engaged by particular tasks.

The hippocampus can be divided into two parts along the dorsoventral axis (in rodents) and the anteroposterior axis (in humans) (Fanselow & Dong, 2010). The ventral hippocampus (anterior in humans) is important for emotions such as anxiety and fear, and the dorsal hippocampus (posterior in humans) is important for spatial memory and learning tasks. This is supported anatomically, as well: the dorsal hippocampus is connected with cortical areas for memory processing such as the entorhinal cortex, whereas the ventral hippocampus projects to

regions involved in emotional processing, such as the amygdala, nucleus accumbens, and hypothalamus (Kheirbek & Hen, 2010).

This distinction is important for interpreting behavioral findings, as tasks that preferentially engage dorsal hippocampal circuits are more likely to show estradiol-dependent effects on memory, and tasks engaging ventral hippocampal networks may reflect the effects on affective or stress-related processes instead.

Spatial Memory Tasks (Morris water maze)

The Morris water maze (MWM) is a test of spatial navigation and referential memory, which are heavily reliant on the dorsal hippocampus. Rodents are placed in a water tank that is filled with opaque water and must rely on distal cues in the testing room to find a hidden platform. This task is typically conducted over multiple days, with 4–6 days of training followed by a probe trial 1–2 days later, meaning that animals may experience substantial fluctuations in hormonal state across the course of testing. In naturally cycling females, this can span a large portion of the estrous cycle, introducing variability in estradiol levels across training, consolidation, and retrieval phases. Studies using this task have shown contradictory effects: for example, in rats, high E2 levels during the estrous cycle or chronically administered systemic E2 have been suggested to negatively impact their performance in the MWM, but in mice high E2 increased performance in the MWM (Taxier et al., 2020).

One explanation for this inconsistency in the MWM is that rats and mice don't navigate the water maze in the same way. Frick et al. (2000) found that with a one-day water maze protocol, rats relied on an allocentric spatial strategy to find the platform, but mice relied less on spatial cues and more on procedural strategies. Because the MWM is intended to assess hippocampal-dependent spatial navigation through the use of distal cues, the reliance on

non-spatial or procedural strategies lessens the task's sensitivity to hippocampal function. Mice also showed more wall-hugging behavior (thigmotaxis), which could have obscured hippocampal-dependent spatial learning. Thigmotaxis is often interpreted as an anxiety-related or non-goal-directed behavior, which can further interfere with the accurate measurement of spatial memory performance. Therefore, even if estradiol has the capacity to improve hippocampal function in mice, their navigation strategy might not be able to show these results as clearly as in rats. This suggests that variability in task performance may show differences in strategy use rather than true differences in hippocampal-dependent memory.

Overall, the most commonly used test of spatial learning and memory may lack the sensitivity that is necessary to detect cognitive changes caused by estradiol-related synaptic changes. This suggests that alternative tasks are likely better aligned with measuring these hormonal effects.

Object Recognition and Object Placement Tasks

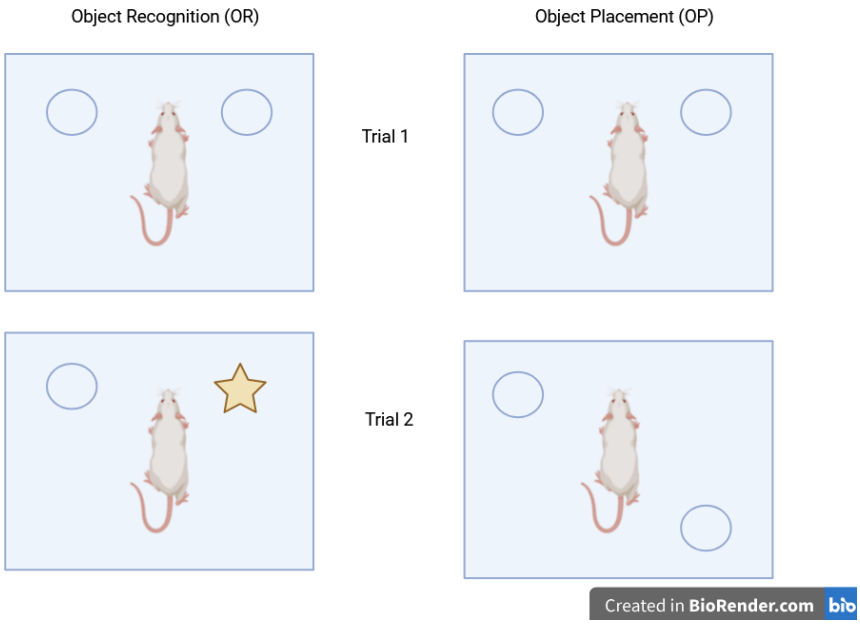

Figure 3. Object Recognition (OR) and Object Placement (OP) paradigms. OR and OP paradigms are measures of hippocampal learning in rodents. OR tasks involve placing two identical objects in an enclosure with a rodent, which is later replaced with a new object. Exploration of the new object implies adequate recognition memory in rodents, as they've retained knowledge of the familiar object. OP paradigms involve placing a rodent in an enclosure with two identical objects, then moving one of them to a different location of the space. Rodents with sufficient spatial memory will exhibit more exploration of the moved object.

Single-trial object memory-based tests have been able to more clearly show estradiol-related changes on hippocampal learning. Object placement (OP), which requires the detection of a change in spatial configuration, preferentially engages dorsal hippocampal circuits. Object recognition (OR) primarily engages the dorsal hippocampus when memory consolidation/contextual processing is needed (Figure 3). As opposed to many usages of the MWM (or other spatial maze paradigms), OR and OP use a single learning exposure and a short retention interval, which lessens the likelihood of animals transitioning between estrous stages during testing. This is especially important considering the rapid fluctuations in ovarian hormones during the 4-day rodent estrous cycle.

In gonadally intact female rats, some studies report improved performance on OR and OP tasks during high-hormone phases of the estrous cycle, particularly proestrus, compared to low-hormone phases (Luine et al., 2003). However, these effects are not always consistently observed across studies. In contrast, studies that manipulate hormone levels through ovariectomy more reliably show estradiol's role in memory. Ovariectomized rats and mice typically show impaired performance on OR and OP tasks, while post-training administration of 17β-estradiol restores or improves 24-hour memory retention (Fernandez et al., 2008; Tuscher et al., 2015).

These findings suggest that estradiol improves hippocampal-dependent memory when the task design is aligned with the functional specialization of the dorsal hippocampus and the temporal dynamics of hormone signaling. This explains why the estrous cycle impacts are more consistent in object-based tasks than multi-day spatial maze tasks.

Overall, estradiol-dependent memory improvement is shown most clearly when certain conditions are met, including engagement of dorsal hippocampal circuits, alignment with the early post-training consolidation window, and use of tasks that isolate memory consolidation rather than strategy or acquisition.

Conclusion

The hippocampal literature suggests that estradiol's effects are best understood in relation to how behavioral tasks engage plasticity mechanisms, rather than overall changes in memory performance. Across studies, effects are most consistently observed under conditions that depend on rapid synaptic modification, aligning with evidence that estradiol modulates CA1 spine dynamics, NMDA receptor–dependent signaling, and processes associated with memory consolidation.

This perspective frames variation in findings as resembling differences in experimental design, species-specific strategies, and regional engagement along the dorsal–ventral axis. Importantly, these effects occur within a broader system in which hippocampal outputs contribute to contextual and affective processing, particularly through ventral hippocampal projections.

Extending this idea suggests that if estradiol influences plasticity in hippocampal circuits under specific task conditions, similar state-dependent effects may emerge in corticolimbic systems that rely on related mechanisms to support emotional learning and regulation.

Emotion and Stress Circuits

Biological sex is a major risk factor for anxiety- and stress-related disorders, with women showing approximately twice the lifetime prevalence of many anxiety disorders and greater symptom severity in several conditions, including PTSD (McLean et al., 2011; Maeng & Milad, 2015). Importantly, this increased vulnerability is not constant across the lifespan, but varies across reproductive stages associated with changes in ovarian hormone dynamics, including puberty, menstrual cycling, the postpartum period, and perimenopause (Kundakovic & Rocks, 2022). These patterns suggest that fluctuations in ovarian hormones contribute to emotional vulnerability, while also interacting with psychosocial factors such as stress exposure, gendered social context, and environmental adversity, rather than replacing them as explanatory frameworks (Kundakovic & Rocks, 2022; Ter Horst et al., 2009).

Although this chapter is centered on anxiety- and stress-related phenotypes, it uses fear conditioning and extinction as the primary mechanistic model. This approach is justified because impaired fear inhibition, poor extinction, and exaggerated generalization are core features of anxiety disorders and PTSD (Rauch et al., 2006), making fear extinction paradigms a tractable way to study the neural mechanisms that underlie pathological threat response.

Circuitry

Anxiety and fear arise from overlapping corticolimbic circuitry that integrates threat detection, contextual processing, and top-down regulation of emotional responses (Graham & Milad, 2013; McEwen et al., 2015). Although these processes are often grouped together, they represent distinct modes of threat processing. Fear typically involves discrete, learned responses to specific stimuli, and anxiety is characterized by sustained and context-dependent responses to

uncertain or diffuse threats. Despite these differences, both rely on a conserved network centered on the amygdala, hippocampus, and medial prefrontal cortex (mPFC), suggesting that hormonal modulation of this shared system can influence multiple emotional phenotypes simultaneously (Schiller & Delgado, 2010; Graham & Milad, 2013; McEwen et al., 2015).

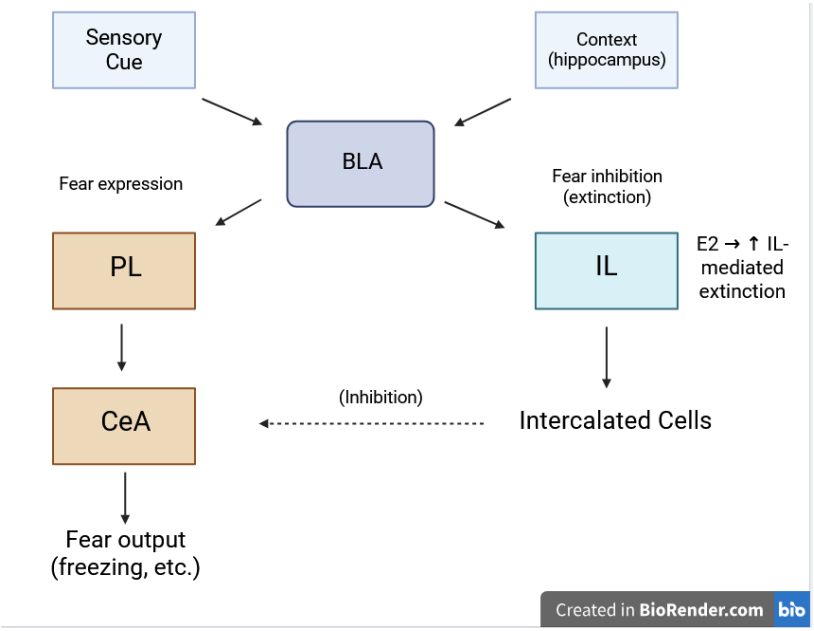

Figure 4. Circuit mechanisms of estradiol modulation of fear expression and extinction. Inputs from sensory cues and context converge on the basolateral amygdala (BLA). Prelimbic (PL) pathways support fear expression, while infralimbic (IL) pathways suppress fear via inhibitory control over central amygdala (CeA) output. Estradiol enhances IL-dependent plasticity, promoting extinction recall.

Within this network, the amygdala serves as a central node for threat detection and behavioral output. However, amygdala activity alone does not determine behavioral output, and instead reflects integration within a broader corticolimbic network (Figure 4). The basolateral amygdala (BLA) integrates sensory and contextual information related to threat, while the central amygdala (CeA) drives downstream defensive responses through projections to brainstem and hypothalamic regions (Amano et al., 2010; Likhtik et al., 2008). Activity within the amygdala is

bidirectionally regulated by the medial prefrontal cortex. Specifically, the prelimbic (PL) cortex promotes fear expression, whereas the infralimbic (IL) cortex is required for fear extinction and the suppression of conditioned fear responses (Sotres-Bayon & Quirk, 2010). The infralimbic cortex exerts this effect through projections to inhibitory intercalated cells within the amygdala, which suppress central amygdala output and reduce fear expression (Amano et al., 2010). In contrast, prelimbic projections preferentially engage excitatory amygdala circuits that support fear expression (Burgos-Robles et al., 2009). This organization establishes a dynamic balance in which prefrontal subregions exert opposing control over amygdala-driven behavior.

Although this circuitry is sufficient to explain phasic fear responses, anxiety-like states involve additional network components that support sustained and anticipatory threat processing. The ventral hippocampus, which is more strongly connected to emotion-related regions than the dorsal hippocampus, plays a key role in anxiety-like behavior by influencing both amygdala activity and prefrontal regulation (Kheirbek & Hen, 2010). Through its connections with both the amygdala and prefrontal cortex, the hippocampus enables discrimination between safe and threatening environments and regulates the specificity of fear memories. These findings strengthen the view that sustained anxiety-like states are not simply “more fear,” but involve partially distinct network dynamics layered onto a shared corticolimbic architecture.

Human neuroimaging studies provide converging evidence for a conserved functional organization of these circuits. For example, the ventromedial prefrontal cortex (vmPFC) and ventral anterior cingulate cortex are associated with fear extinction and inhibitory control, whereas the dorsal anterior cingulate cortex (dACC) and dorsal prefrontal regions are associated with fear expression and threat responding (Milad & Quirk, 2012). These regions are considered functional homologs of the rodent infralimbic and prelimbic cortices, respectively. While these

comparisons reflect functional rather than anatomical homology, the convergence between rodent and human findings supports the existence of a conserved system for regulating emotional behavior.

Cellular and Molecular Mechanisms

Fear extinction relies on intracellular signaling pathways previously described, including NMDA receptor–dependent transmission and downstream ERK/MAPK and PI3K/Akt signaling, which support extinction memory consolidation. In extinction circuits, ERK activation is required for consolidation, as inhibition of ERK in the amygdala impairs extinction recall, and extinction learning is associated with increased ERK phosphorylation (Lu et al., 2001; Herry et al., 2006). Similarly, PI3K/Akt signaling contributes to extinction in a region-specific manner, and NMDA receptor activation is necessary for long-term extinction memory (Chen et al., 2005; Kritman & Maroun, 2013). These pathways converge on CREB-dependent transcription of plasticity-related genes, which link synaptic activity to longer-term changes in circuit function (Carbone & Handa, 2013).

While these intracellular mechanisms are necessary for extinction, the extent to which estradiol directly engages these pathways within extinction circuits is incompletely characterized. Given that estradiol is known to modulate ERK/MAPK, PI3K/Akt, and NMDA receptor–dependent signaling in other brain regions, it is likely that these same pathways contribute to estradiol-dependent effects on extinction, but their precise roles appear to be region- and context-dependent rather than uniformly expressed across circuits.

Instead, estradiol's effects on extinction can be understood in terms of how it alters the relative engagement of prefrontal–amygdala circuits. Using activity-dependent markers such as

c-Fos expression to quantify neuronal activation, it has been observed that estradiol produces selective changes in activity across these regions rather than uniformly increasing activation (Maeng et al., 2016). Within the amygdala, estradiol is associated with shifts in activity consistent with reduced output from amygdala networks, while within the medial prefrontal cortex it biases activity toward the infralimbic cortex relative to the prelimbic cortex. Because the infralimbic cortex is involved in suppressing amygdala output and the prelimbic cortex is associated with maintaining amygdala-driven activity, this relative shift reflects a change in the balance of circuit dynamics rather than an overall increase in activity within any single region.

A more specific mechanism of this effect has been seen at the level of synaptic plasticity within the infralimbic cortex. Estradiol regulates the plasticity state of infralimbic glutamatergic synapses, such that high endogenous estradiol is associated with a state that favors synaptic potentiation, whereas low estradiol impairs this capacity (Galvin & Ninan, 2014). The activation of ERβ restores this potentiation by enhancing GluN2B-containing NMDA receptor–mediated transmission, which suggests that estradiol facilitates extinction by enabling NMDA-dependent synaptic strengthening within infralimbic circuits. Because synaptic potentiation in the infralimbic cortex strengthens its influence over downstream amygdala targets, this provides a direct cellular mechanism linking estradiol to changes in circuit-level regulation.

Behavioral Paradigms

Understanding estradiol's effects on emotional behavior requires careful consideration of behavioral paradigms, as different tasks isolate distinct components of fear processing and recruit different neural circuits (Shanazz et al., 2022). In fear conditioning paradigms, animals learn an association between a conditioned stimulus (CS), such as a previously neutral cue such as a tone,

and an unconditioned stimulus (US), which is an aversive stimulus such as a mild footshock. In cued fear conditioning, the CS predicts the US, and learning depends mostly on the lateral and basolateral amygdala (Kim & Cho, 2017). In contextual fear conditioning, the "context" refers to the environment in which the aversive stimulus is experienced, which requires encoding and later recognition of that environment and engages the hippocampus in addition to the amygdala (Fanselow & Dong, 2010).

After acquisition, fear extinction involves the repeated presentation of the CS or re-exposure to the context without the US, which forms a new inhibitory memory that suppresses fear expression rather than erasing the original association. These paradigms usually use freezing as the primary behavioral measure in rodents, but freezing does not capture the full range of defensive behaviors and may miss sex differences in strategies such as darting or avoidance (Shanazz et al., 2022).

Estradiol's effects on acquisition across fear conditioning paradigms are mixed. In cued acquisition, many studies report no behavioral sex differences when freezing is the primary readout (Milad et al., 2009; Clark et al., 2019), but others report either reduced or increased fear conditioning in females (Greiner et al., 2019; Baran et al., 2010). In contextual acquisition, findings are also inconsistent: some studies saw stronger conditioning in females, and others saw no sex differences (Keiser et al., 2017; Urien et al., 2021). Females can also show impairments when there is little time to learn the context before shock, suggesting that differences may reflect context encoding rather than fear learning itself (Rudy & O'Reilly, 2001; Wiltgen et al., 2001).

The clearest estradiol-related effects appear in cued fear extinction and extinction recall. In naturally cycling female rats, extinction during proestrus led to lower freezing during recall

than extinction during metestrus, which indicates better extinction retention under high-estradiol conditions (Milad et al., 2009). Furthermore, estradiol administered immediately after extinction training improves extinction memory consolidation in rats, but delayed administration does not (Zeidan et al., 2011). In humans, higher estradiol levels are associated with lower skin conductance responses (signifying reduced fear responses) during extinction recall and greater extinction retention (Zeidan et al., 2011), and women using hormonal contraceptives show poorer extinction recall than naturally cycling women (Graham & Milad, 2013). These findings suggest that estradiol does not consistently affect fear learning itself, but more reliably regulates extinction retention and recall.

For anxiety-like behavioral tests, naturally cycling females show phase-dependent differences in anxiety-like behavior (Kundakovic & Rocks, 2022). During the proestrus phase, females tend to exhibit reduced anxiety-like behavior compared to low-estradiol phases. For example, proestrous rodents spend more time exploring open or brightly lit areas (interpreted as reduced anxiety) than diestrus rodents, which is an effect observed across multiple tests including the open field, elevated plus maze, and light–dark box (Jaric et al., 2019).

However, some experimental paradigms show that very high or acutely administered estradiol can increase certain fear- or anxiety-related outcomes, particularly in non-physiological conditions such as ovariectomy followed by hormone replacement (Graham & Milad, 2013). These findings are interpreted as reflecting dose-, timing-, and endocrine-history-dependent effects rather than a single directional influence of estradiol. Because these paradigms do not isolate specific phases of learning such as acquisition, extinction, or recall, they are less suited for identifying the mechanisms through which estradiol regulates learned fear, but they still provide valuable insights.

Contextual fear conditioning is more difficult to interpret than cued conditioning because it depends not just on fear learning, but also on how the context (the environment where the aversive stimulus occurs) is encoded, retrieved, and distinguished from other environments, which adds variability due to its reliance on hippocampal processing (Fanselow & Dong, 2010). For example, in naturally cycling females, proestrus is associated with lower contextual fear conditioning in some studies, whereas others report no estrous cycle effect (Cushman et al., 2014; Keiser et al., 2017).

This variability likely reflects differences in how contextual information is represented rather than differences in fear learning itself. For example, females can show greater fear generalization to neutral contexts than males, meaning they respond with fear in environments that resemble, but are not identical to, the training context (Asok et al., 2019; Keiser et al., 2017). Similarly, in those studies they found that estradiol administration prior to training can increase generalization to neutral contexts in ovariectomized females. These findings suggest that contextual paradigms are capturing both fear learning and hippocampal-dependent memory processes, which makes them harder to interpret than cued extinction paradigms when trying to identify a single consistent effect of estradiol. In addition, many fear conditioning paradigms are conducted across multiple days, which complicates interpretation in naturally cycling females, as hormonal state can shift between training, consolidation, and testing phases. These temporal changes in estradiol levels can differentially influence neural processing at each stage, making it difficult to isolate a single, stable effect of estradiol on behavior.

Conclusion

In corticolimbic systems, estradiol's effects are most apparent in conditions that require modification of previously learned responses, rather than in the initial encoding of fear. Across studies, the most consistent findings are observed in extinction paradigms, where estradiol is associated with improved extinction recall under specific hormonal conditions, consistent with its influence on fear circuitry.

Differences in behavioral outcomes reflect variation in how paradigms distinguish between acquisition, expression, and extinction, as well as differences in endocrine context, including hormone timing and experimental design. Under conditions that deviate from endogenous hormonal dynamics, such as ovariectomy with hormone replacement, estradiol can produce effects that do not fully align with those observed in naturally cycling animals.

These findings indicate that estradiol's influence on emotional behavior depends on the interaction between hormone state and the specific processes being measured. Because these processes contribute to how stimuli acquire and maintain salience, this provides a basis for examining estradiol's role in mesolimbic systems involved in motivation and reward.

Motivation and Reward-Related Plasticity

United States survey data pooled from 2022-2024 found that 17.9% of adults and 8.3% of adolescents (ages 12-17) met the criteria for substance use disorder (SUD) (SAMHSA, 2025). Adult men had increased odds of SUD than women (odds ratio of 1.6), but among adolescents it was higher in girls (odds ratio of 0.7 for boys). This difference results from social as well as biological factors. Cross-national data show that within many cultures, men have easier access to drugs than women, and when access is equalized this gender difference seems to disappear (McHugh et al., 2018). There are also differences in drugs of choice for men and women, which can also be historically explained; prescriptions and marketing techniques of opiates and select psychostimulants targeted toward women led to higher rates of usage (Becker et al., 2012). Therefore, it is not enough to cite overall prevalence– one must examine how sex and gender influence patterns of use and progression to addiction. For example, in some contexts and for certain substances, women show 'telescoping' patterns with faster escalation from initial use to problematic use. These differences in patterns of usage are further supported by reviews showing that although men have historically shown more SUD prevalence, rising rates in women suggest a strong role of gender-related sociocultural impacts (McHugh et al., 2018). These epidemiological patterns show the need to examine how sociocultural influences and biological mechanisms interact to shape differential progression through the addiction cycle.

The Addiction Cycle: Binge, Withdrawal, Craving

Addiction is commonly modeled as a self-reinforcing cycle that consists of three stages (Koob & Volkow, 2010; Volkow et al., 2019). In the first stage of binge/intoxication, drug taking is driven by positive reinforcement. The user takes the drugs for their euphoric effects, which

depend on rapid dopamine surges in the mesolimbic pathway (ventral tegmental area→ nucleus accumbens) (Koob & Volkow, 2010). Faster routes of administration, like injection, cause faster dopamine increases and thus a stronger subjective reward. Drug-related cues and behaviors then become linked through learning, and with repeated exposure the brain uses habit circuitry even in this early stage.

Stopping drug use, whether voluntary or involuntary, leads to the second stage in this cycle: withdrawal. When the drug is absent in the person's system, they experience a negative emotional state which encourages drug use through negative reinforcement (taking the drug to relieve distress). Koob & Volkow (2010) describe this as motivational withdrawal (an anti-reward stress state) that sustains a person's addiction rather than physical symptoms alone. Adaptations in the amygdala and other stress-related circuits amplify this effect, making the drug taking habit revolve around avoiding low moods rather than achieving pleasure. However, a detailed discussion of the biobehavioral mechanisms of addiction is beyond the scope of this chapter.

The final stage, preoccupation/anticipation (craving), occurs during prolonged abstinence. Stressors or learned cues (such as a smoker smelling cigarette smoke) can cause intense cravings and relapse. Neurocircuitry differs by the trigger experiences: cue/drug-induced relapse relies on frontal cortex and ventral striatum circuits, and stress-induced relapse involves amygdala pathways (Shalev, 2002). This stage involves a large network that underlies cue-based motivation and impaired control, involving brain regions such as the orbitofrontal/anterior cingulate cortex, dorsal striatum, basolateral amygdala, hippocampus, insula, and more.

Throughout this cycle, there is a shift from impulsive drug use to compulsive drug-seeking. As stated, the early use is driven by positive reinforcement (euphoric feelings), and the later use is driven by the relief from withdrawal and distress (negative reinforcement) (Koob

& Le Moal, 2008). In the brain, this shift is associated with the recruitment of limbic (ventral striatum) circuits to habit-oriented (dorsal striatum and orbitofrontal) circuits as SUD develops.

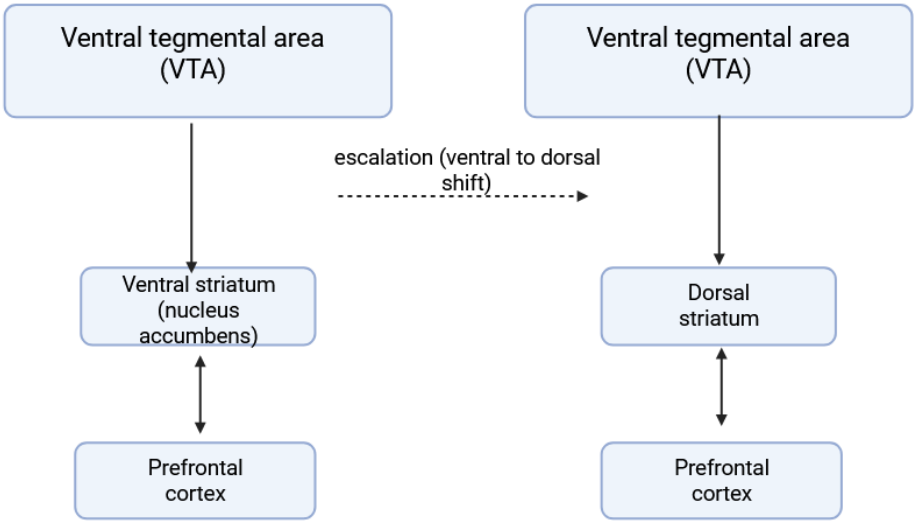

Figure 4. Ventral-to-dorsal striatal shift in addiction: circuit-level transition from reward-driven to habit-based drug seeking. Dopaminergic projections from the ventral tegmental area (VTA) initially engage the ventral striatum (nucleus accumbens) to mediate reward and incentive salience. With repeated drug exposure, activity shifts toward dorsal striatal circuits associated with habitual and compulsive behavior alongside continued interactions with prefrontal cortical regions.

Neural Circuitry of Motivation and Addiction

The striatum, which includes ventral and dorsal areas, is a central component of the brain's motivation circuitry (Yoest et al., 2014). Dopamine neurons in the ventral tegmental area (VTA) project to the ventral striatum (nucleus accumbens, NAc), which supports the attribution of incentive salience ("wanting") to rewards and associated predictive cues. On the other hand, dopamine input to the dorsal striatum from the substantia nigra controls the execution and automatization of learned actions and habits. In the context of addiction, ventral striatum

dopamine is likely to be involved when a smoker craves a cigarette upon seeing one, whereas dorsal striatum dopamine would be involved in the process of lighting a cigarette reflexively under a condition in which they'd normally use one (such as after a meal).

Addictive drugs impact this system by producing rapid and supraphysiological dopamine transients. Natural rewards will use dopamine in a manner shaped by feedback and context, whereas drugs of abuse increase dopamine pharmacologically independent of physiological signals. This repeated and supraphysiological activation causes pathological synapse plasticity: for example, drug-associated cues become hypersalient (disproportionately attention-grabbing and motivationally significant), corticostriatal learning will shift toward habit, and prefrontal control of behaviors weakens (Volkow et al., 2019). Dopamine neurons that had once fired for drug *reward* begin to fire in response to drug *cues*, which motivates someone toward the drug before it is taken. Drug taking then becomes a compulsive and cue-driven process, explaining the cravings and relapses seen in addiction, which have been shown in both animal models and human imaging studies (Koob & Volkow, 2010; Volkow et al., 2019).

Estradiol Increases Mesolimbic Dopamine

In females, estradiol acts rapidly in the striatum through membrane-associated estrogen receptors, specifically ERα and ERβ localized at the cell membrane, to modulate dopamine release (Yoest et al., 2014). These receptors are functionally coupled to metabotropic glutamate receptors (mGluRs). In females, estradiol application selectively reduces Ca^{2+} currents in GABAergic medium spiny neurons via G-protein–coupled signaling, indicating an indirect modulation of Ca^{2+} channel activity that decreases local GABA release and disinhibits dopamine terminals. Acute estradiol application or high-estrogen cycle phases also create larger phasic DA

transients in the NAc (in other words, these hormonal states increase the magnitude of sub-second dopamine spikes) (Yoest et al., 2018; Yoest et al., 2014). These dopaminergic effects occur in distinct subregions of the nucleus accumbens (Xu et al., 2020). The NAc shell is mostly associated with reward valuation and the initial reinforcing effects of drugs, and the NAc core is more involved in cue-driven responding and action–outcome associations (West & Carelli, 2016). Increases in dopamine signaling by estradiol may, then, differentially increase reward sensitivity in the shell while strengthening cue-related processing in the core, although direct subregion-specific evidence remains limited.

At the same time, estradiol engages intracellular kinase signaling pathways. As discussed previously (Figure 2), membrane-associated ERα coupled to mGluRs can activate signaling cascades such as MAPK and related intracellular pathways, leading to increased CREB phosphorylation (Yoest et al., 2018). These pathways are associated with synaptic modifications, including increases in dendritic spine density and enhanced glutamatergic input onto neurons in the nucleus accumbens (Tonn Eisinger et al., 2018).

Excitatory synapses in this region rely on both AMPA and NMDA receptors (Wolf, 2010; Kalivas & Volkow, 2005). In addiction models, repeated drug exposure induces the formation of “silent synapses,” which contain NMDA receptors but lack functional AMPA receptors, rendering them weak at baseline. Over time, these synapses are strengthened through the insertion of AMPA receptors into the postsynaptic membrane, increasing synaptic efficacy and contributing to long-term circuit remodeling associated with addiction. Dopamine signaling (particularly through D1-class receptors) plays a major role in facilitating this AMPA receptor insertion and synaptic strengthening (Wolf, 2010).

Although estradiol has not been directly shown to drive these specific synaptic transitions, it enhances dopaminergic signaling within mesolimbic circuits and activates intracellular pathways that overlap with those involved in synaptic plasticity. Through these converging mechanisms, estradiol may bias the system toward greater activity-dependent strengthening of glutamatergic inputs, potentially amplifying the neural adaptations that support drug-associated learning and the persistence of drug-seeking behavior.

These glutamatergic changes are not uniform across addictive substances. For example, increases in AMPAR expression seen following cocaine exposure are not consistently seen in amphetamine-sensitized animals, which suggests that different drugs engage distinct forms of synaptic plasticity within the nucleus accumbens (Nelson et al., 2009). This implies that estradiol's effects on glutamatergic signaling likely interact with drug-specific plasticity mechanisms rather than affecting a single, uniform pathway of synaptic strengthening.

Addiction Paradigms Across the Stages of Addiction

Animal models of addiction have been used to discern the behavioral components of the binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (relapse) stages. In the binge/intoxication stage, paradigms are designed to assess acute drug reward, reinforcement, and motivation to obtain the drug (Shippenberg & Koob, 2002). Although the addiction cycle provides a behavioral framework, the mechanistic focus of this chapter is on mesolimbic and striatal processes underlying reinforcement; later stages recruit additional circuits and are discussed only for context. Classical models of positive reinforcement include intravenous or intracranial self-administration and conditioned place preference. In these paradigms, escalation

of drug use is often operationalized as the rate at which animals increase intake following the initial exposure (Hu et al., 2003).

Many studies show that females show increased escalation-related behaviors during this stage. For example, estradiol quickens the acquisition of cocaine self-administration in OVX females, with rodents treated with estradiol prior to testing acquiring cocaine taking faster than untreated OVX controls (Hu et al., 2003; Hu & Becker, 2009). This facilitation appears to be constrained to physiological estradiol levels, as supraphysiological hormone replacement can produce exaggerated behavioral effects, such as increased acquisition rates, increased drug intake, or heightened responding that do not reflect patterns observed in naturally cycling females. Furthermore, sex differences in self-administration are typically most pronounced under extended access conditions (Quigley et al., 2021), which suggests that environmental structure and opportunity for escalation affects the expression of these differences.

The withdrawal/negative affect stage is characterized by behavioral and physiological responses to drug cessation, including increased anxiety-like behavior, hyperalgesia (more pain sensitivity), and elevated reward thresholds (more drug intake needed to feel reward) (Flores et al., 2020). Drug-seeking during this stage is driven by negative reinforcement, as animals increase effort to obtain the drug to alleviate these withdrawal states. This stage is less extensively studied in cocaine models with respect to hormonal modulation compared to other substances, and is often described using alternative terminology such as: abstinence, drug-free period, incubation, or sensitization.

Studies using nicotine models suggest that ovarian hormones differentially affect withdrawal-related affective states. In female rodents, one study focusing on nicotine withdrawal

showed that in both intact and OVX female rats, estradiol was positively correlated with the amount of anxiety-like behaviors displayed (Flores et al., 2020). Although these findings are from nicotine models, they suggest potential mechanisms that might generalize to other addictive substances. These findings suggest that hormonal state can impact the negative affective component of withdrawal and potentially influence relapse vulnerability via negative reinforcement mechanisms.

The preoccupation/anticipation (relapse) stage introduces another layer of complexity in interpreting sex differences. While many studies report that females exhibit greater cue-induced reinstatement or drug-seeking behavior, these measures likely do not reflect a single underlying process and may reflect stable circuit differences and cumulative plasticity effects, which are beyond the scope of this review. In a study by Perry et al., cue-induced reinstatement was separated into early versus sustained phases of responding, with initial responding defined as behavior during the first five minutes following cue presentation and sustained responding measured across the remainder of the two-hour session. The authors found that males and females showed equivalent responding during this initial phase of cue exposure, which they interpreted as similar levels of cue-induced craving (Perry et al., 2013). However, sex differences were seen when behavior was measured across the full two-hour test, with females showing greater overall responding. The authors suggest that this later-emerging difference might not reflect increased craving per se, but instead could arise from other processes such as conditioned responding to the drug-paired cue or differences in cognitive and behavioral strategy. The initial response to a drug-associated cue might reflect rapid cue-triggered “wanting,” indexed by how quickly animals respond following cue presentation. In contrast, behavior measured over longer

periods reflects additional processes, including learned cue associations and sustained responding over time.

Although estrous cycle stage was monitored, the Perry et al. (2013) study did not find cycle-dependent effects on motivation for cocaine, suggesting that hormonal influences may not be uniformly expressed across all measures of drug-seeking behavior in this paradigm. In the same study, females were more likely to develop a cocaine preference, indicating greater vulnerability to transitioning into addiction-like behavior. These findings suggest that sex differences depend on which component of behavior is being measured.

Conclusion

In mesolimbic systems, estradiol's effects are most clearly understood when reward-related behavior is considered across its component processes. This pattern suggests that estradiol does not produce a general increase in reward sensitivity, but instead influences how motivationally relevant stimuli are processed under specific conditions, likely through its modulation of dopaminergic signaling and striatal function. Differences across paradigms therefore resemble variation in task structure and behavioral metrics rather than inconsistent hormonal effects.

Considered alongside findings from hippocampal and corticolimbic systems, these results support a broader interpretation in which estradiol's effects depend on the interaction between hormone state, circuit engagement, and task demands. This convergence provides a way for understanding how hormonal state contributes to variation in brain–behavior relationships, which is addressed in the following discussion.

Discussion

The findings presented across hippocampal, corticolimbic, and mesolimbic systems converge on a common conclusion: estradiol does not uniformly improve or impair neural and behavioral function, but instead regulates plasticity in a state-dependent and circuit-specific manner. Variability observed across learning, fear regulation, and reward paradigms is therefore best interpreted as reflecting differences in circuit engagement, temporal dynamics, and behavioral processes rather than inconsistency in estradiol's effects. Estradiol acts as a regulator of how and when specific neural systems contribute to behavior (Woolley & McEwen, 1992; Woolley, 1998; Frick et al., 2015; Taxier et al., 2020).

Across domains, estradiol engages a shared set of intracellular signaling pathways, including ERK/MAPK, PI3K/Akt, and CREB-dependent processes that support synaptic plasticity (Kuroki et al., 2000; Boulware et al., 2013; Fan et al., 2010; Fortress et al., 2013; Hoeffer & Klann, 2010). However, the behavioral effects of these mechanisms depend on where and when they are expressed, as well as how experimental paradigms isolate specific cognitive processes. In the dorsal hippocampus, estradiol supports rapid consolidation processes that operate within temporally constrained windows after learning (Frick et al., 2015), which are most clearly observed in tasks that isolate single-trial memory formation, such as object recognition and object placement. In contrast, multi-day spatial tasks such as the Morris water maze produce more variable outcomes, likely reflecting additional influences of strategy selection, stress exposure, procedural learning components, and day-to-day fluctuations in hormonal state across training and testing sessions (Rubinow et al., 2004; Frick et al., 2000). In corticolimbic circuits,

particularly infralimbic–amygdala pathways, similar mechanisms support extinction consolidation and retrieval, contributing to the regulation of conditioned fear (Milad et al., 2010; Graham & Milad, 2013), with estradiol showing more consistent effects on extinction than acquisition. In mesolimbic systems, estradiol modulates dopaminergic signaling and structural plasticity within the nucleus accumbens, shaping motivational salience and reinforcement learning (Yoest et al., 2014; Yoest et al., 2018).

These circuit- and paradigm-dependent effects are more accurately characterized as changes in cognitive and affective strategy rather than changes in overall capacity, as estradiol appears to shift how neural systems are recruited to solve a task. In spatial paradigms, estradiol may bias the relative contribution of hippocampal versus striatal systems without necessarily altering baseline performance (Frick et al., 2000). In fear paradigms, differences in behavior may reflect altered contextual processing or generalization rather than changes in fear learning itself (Asok et al., 2019; Keiser et al., 2017). In reward-related tasks, estradiol may influence the salience of cues or the motivational value of outcomes, shaping patterns of reinforcement and escalation (Yoest et al., 2014; Yoest et al., 2018). Together, these findings indicate that estradiol reorganizes circuit engagement in a task-dependent manner, such that behavioral outcomes depend on how hormonally biased strategies align with task demands.

These effects occur within a broader neuroendocrine context in which multiple hormones interact across time. Fluctuations in estradiol are regulated by the hypothalamic–pituitary–gonadal (HPG) axis, producing temporally structured changes in hormone levels that influence neural plasticity and circuit function (Moenter & Starrett, 2024). Progesterone, which fluctuates alongside estradiol, can produce effects that differ from or interact with those of estradiol at both neural and behavioral levels. For example, in the mesolimbic

system discussed in Chapter 5, progesterone and estradiol produce largely opposing effects on mesolimbic dopamine (Paula et al., 2021; Lynch & Sofuoglu, 2010). Estradiol increases mesolimbic dopamine-mediated motivation and plasticity that may enhance the impact of drug-related cues, whereas progesterone appears to restore inhibitory tone and rebalance circuit activity following estradiol-driven excitation (Yoest et al., 2014; Yoest et al., 2018).

Because these hormones co-vary across the estrous cycle, isolating the effects of estradiol in intact systems presents a persistent experimental challenge. This raises an important interpretive question: are observed effects driven by estradiol itself, or by concurrent changes in other hormones such as progesterone? Ovariectomized (OVX) models with controlled estradiol replacement address this issue by eliminating endogenous hormonal co-variation and allowing precise control over hormone exposure (Koebele & Bimonte-Nelson, 2016). However, these models also remove the endogenous temporal dynamics generated by the HPG axis and eliminate interactions between estradiol and other ovarian hormones, such as progesterone, testosterone, inhibin, and relaxin. In addition, exogenous hormone administration may produce circulating levels and temporal profiles that differ from physiological conditions depending on dosing strategy (Taxier et al., 2020; Frick et al., 2015; Ström et al., 2008). As a result, OVX paradigms are best interpreted as complementary mechanistic models that capture the effects of controlled estradiol exposure, but do not fully represent the dynamic endocrine environment of naturally cycling individuals.

At a broader level, it is important to recognize that estradiol signaling represents an exceptionally complex biological system that resists simple mechanistic reduction. In vivo, multiple receptor populations, intracellular signaling pathways, and interacting hormonal systems are engaged simultaneously, often with region-specific and time-dependent effects that cannot be

cleanly separated. These processes unfold within dynamically changing physiological contexts, where even small differences in timing, dose, or circuit engagement can produce divergent outcomes.

The National Institutes of Health mandate to incorporate sex as a biological variable (SABV) has substantially increased the inclusion of both sexes in preclinical and clinical research; however, its implementation remains a subject of ongoing debate (Clayton, 2015). Critics have noted that SABV is often applied descriptively, emphasizing sex differences without resolving underlying mechanisms, and that incorporating hormonal factors into experimental design can increase complexity, including larger sample sizes, more precise staging of endocrine state, and greater resource demands (Clayton, 2015; Shansky, 2019; Joel & McCarthy, 2016). At the same time, treating sex as a static categorical variable risks obscuring the dynamic endocrine processes that alter neural function (Becker et al., 2016). The findings synthesized in this thesis support a complementary perspective in which SABV is most informative when applied mechanistically. Rather than focusing solely on differences between males and females, incorporating hormone-dependent variability allows for the identification of when and how specific neural circuits are engaged. In this context, estradiol provides a tractable model for demonstrating how temporally structured biological signals organize plasticity and behavior across hippocampal, corticolimbic, and mesolimbic systems. From this perspective, the increased complexity associated with incorporating hormonal state reflects the structured nature of endocrine modulation rather than unnecessary variability.

The use of a structured narrative review approach was therefore essential for addressing the questions posed in this thesis. While quantitative meta-analyses are well-suited for estimating overall effect sizes, they are less effective in contexts where outcomes depend on differences in

experimental design, circuit engagement, and temporal dynamics. In the case of estradiol, variability across studies reflects meaningful differences in task structure, species, hormonal state, and level of analysis. Aggregating these findings into a single effect size risks obscuring the mechanisms that generate this variability. A narrative synthesis allows for the integration of evidence across domains, making it possible to identify patterns that would not be apparent through statistical aggregation alone. By prioritizing mechanistic interpretation over effect size estimation, this approach aligns with the central goal of the thesis: to explain when and how estradiol's effects emerge across levels of analysis.

Future work should build on these findings by more precisely resolving how hormone-dependent changes in neural function unfold across time and circuit levels. Approaches that combine within-subject designs across estrous or menstrual cycles with circuit-specific manipulations will be necessary for linking endocrine state to discrete neural computations. Integrating these methods with high-resolution measures of neural activity and behavior may clarify how estradiol-dependent changes in plasticity translate into changes in strategy, learning, and decision-making. Extending this work to human populations, particularly across developmental and reproductive transitions, will be critical for understanding how these mechanisms contribute to variability in cognition, emotion, and vulnerability to neuropsychiatric disorders.

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Appendix: Abbreviations

A

AMPA — α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR — α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

B

BLA — Basolateral amygdala

C

CA1 — Cornu Ammonis 1 (hippocampal subfield)

CA2 — Cornu Ammonis 2 (hippocampal subfield)

CA3 — Cornu Ammonis 3 (hippocampal subfield)

CeA — Central amygdala

CREB — cAMP response element-binding protein

CS — Conditioned stimulus

D

DA — Dopamine

dACC — Dorsal anterior cingulate cortex

DG — Dentate gyrus

dHPC — Dorsal hippocampus

E

E2 — 17β-estradiol

EC — Entorhinal cortex

ER — Estrogen receptor

ERα — Estrogen receptor alpha

ERβ — Estrogen receptor beta

ERK — Extracellular signal-regulated kinase

ERE — Estrogen response element

F

FSH — Follicle-stimulating hormone

G

GnRH — Gonadotropin-releasing hormone

GPER — G Protein-Coupled Estrogen Receptor 1

H

HPA axis — Hypothalamic–pituitary–adrenal axis

HPG axis — Hypothalamic–pituitary–gonadal axis

I

IL — Infralimbic cortex

L

LH — Luteinizing hormone

LTP — Long-term potentiation

M

MAPK — Mitogen-activated protein kinase

mER — Membrane estrogen receptor

MeSH — Medical Subject Headings

mGluR — Metabotropic glutamate receptor

mPFC — Medial prefrontal cortex

mTOR — Mechanistic target of rapamycin

MWM — Morris water maze

N

NAc — Nucleus accumbens

NIH — National Institutes of Health

NMDA — N-methyl-D-aspartate

NMDAR — N-methyl-D-aspartate receptor

O

OP — Object placement task

OR — Object recognition task

OVX — Ovariectomized

P

PI3K — Phosphoinositide 3-kinase

PL — Prelimbic cortex

PMC — PubMed Central

PKA — Protein kinase A

PTSD — Post-traumatic stress disorder

S

SABV — Sex as a Biological Variable

SUD — Substance use disorder

U

US — Unconditioned stimulus

V

vHPC — Ventral hippocampus

vmPFC — Ventromedial prefrontal cortex

VTA — Ventral tegmental area