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Hippocampus-based behavioral, structural, and molecular dynamics across the estrous cycle

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Abstract

The activity of neurons in the rodent hippocampus contributes to diverse behaviors, with the activity of ventral hippocampal neurons affecting behaviors related to anxiety and emotion regulation, and the activity of dorsal hippocampal neurons affecting performance in learning- and memory-related tasks. Hippocampal cells also express receptors for ovarian hormones, estrogen and progesterone, and are therefore affected by physiological fluctuations of those hormones that occur over the rodent estrous cycle. In this review, we discuss the effects of cycling ovarian hormones on hippocampal physiology. Starting with behavior, we explore the role of the estrous cycle in regulating hippocampus-dependent behaviors. We go on to detail the cellular mechanisms through which cycling estrogen and progesterone, through changes in the structural and functional properties of hippocampal neurons, may be eliciting these changes in behavior. Then, providing a basis for these cellular changes, we outline the epigenetic, chromatin regulatory mechanisms through which ovarian hormones, by binding to their receptors, can affect the regulation of behavior- and synaptic plasticity-related genes in hippocampal neurons. We also highlight an unconventional role that chromatin dynamics may have in regulating neuronal function across the estrous cycle, including in sex hormone-driven X chromosome plasticity and hormonally-induced epigenetic priming. Finally, we discuss directions for future studies and the translational value of the rodent estrous cycle for understanding the effects of the human menstrual cycle on hippocampal physiology and brain disease risk.

Keywords

Hippocampus; emotion regulation; learning and memory; estrous cycle; ovarian hormones; estrogen; progesterone; steroid hormone receptors; epigenetic; chromatin

Introduction

The rodent estrous cycle is characterized by cyclic fluctuations in hormones released by the hypothalamus, pituitary, and ovaries over a 4–5 day period through the coordinated function of the hypothalamic-pituitary-gonadal (HPG) axis in females. While essential

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for the maintenance of reproductive function, these hormones, particularly the ovarian hormones estrogen and progesterone, have profound effects in diverse organs of the body, including the brain where they act as potent neuromodulators[1,2]. The estrous cycle in rodents has a hormonal profile similar to that of the human menstrual cycle (Figure 1A), and therefore provides a translational system to study the biological mechanisms underlying the neurobehavioral effects of ovarian hormones. Although the rodent estrous cycle is shorter and has four hormonally distinct stages (proestrus, estrus, metestrus, and diestrus), the human follicular phase is mimicked by the proestrus phase (marked by high estrogen and low progesterone) while the luteal phase is mimicked by the early diestrus phase (marked by low estrogen and high progesterone) (Figure 1A). In general, the proestrus phase is the only estrous cycle phase in rodents marked by high estrogen levels, while progesterone peaks in both the estrus and the diestrus phases (Figure 1A).

Initial studies on the neurobehavioral effects of ovarian hormones were focused on brain regions that are dense in sex hormone receptor expression such as the hypothalamus, where estrogen and progesterone regulate sexual behavior[3,4], energy balance[5,6], temperature[7,8], and maintenance of the HPG axis[9,10]. However, estrogen and progesterone receptors are expressed in neurons and glial cells distributed throughout the brain[11–13], and therefore affect numerous other brain regions including the hippocampus[14,15]. The hippocampus is well characterized for its role in learning and spatial memory, though recent studies have demonstrated that the hippocampus can be divided into two functionally distinct subregions in rodents along the dorsoventral axis[16,17] (Figure 1B; anteroposterior axis in humans). The rodent dorsal hippocampus (posterior in humans) plays an important role in learning and spatial memory tasks, while the ventral hippocampus (anterior in humans) is involved in anxiety and emotion regulation (Figure 1B). Illustrating this behavioral distinction, either lesion or optogenetic activation of ventral hippocampal neurons in rodents affects anxiety-related behavior without affecting memory[18–22], while the same manipulations of the dorsal hippocampus affect spatial memory without affecting anxiety indices[19,20,23–25]. Distinctions between the ventral and dorsal hippocampus are also apparent at the molecular level, as cells in these two rodent brain regions exhibit divergent transcriptomes[26–29], proteomes[27], and epigenomes[28,30] at baseline or in response to environmental stimuli including stress and environmental enrichment. Importantly, estrogen and progesterone receptors are expressed in both the dorsal and ventral hippocampus[11,13], and therefore ovarian hormones can have concomitant effects on the molecular and structural characteristics of both regions and the behaviors associated with their respective function.

In this review, we highlight the role of cycling ovarian hormones in dynamically regulating hippocampal physiology over the estrous cycle. We describe the impact of these hormone fluctuations on the hippocampus at multiple levels of analysis, including their effects on hippocampus-dependent behavioral phenotypes, as well as their underlying cellular effects on hippocampal neurons including changes in dendritic spine density, hippocampal neurogenesis, and long-term potentiation. Since, at a fundamental level, all of these effects are initiated by the ovarian hormone-induced receptor signaling and molecular changes in the hippocampus, we detail what was recently discovered regarding the epigenetic, gene regulatory mechanisms underlying these effects. Lastly, we identify unanswered questions about the effects of ovarian hormones on the hippocampus and discuss the translational

value of the rodent estrous cycle for understanding the effects of the human menstrual cycle on hippocampal physiology and brain disease risk.

1. The estrous cycle modulates hippocampus-dependent behaviors

Given the expression of ovarian hormone receptors across the hippocampus, the estrous cycle can affect hippocampus-dependent behaviors, including dorsal hippocampus-dependent indices of learning and memory as well as ventral hippocampus-dependent indices of anxiety, as we will discuss in the following section.

1.1. The effect of the estrous cycle on emotion regulation—

The activity of the rodent ventral hippocampus plays a direct role in modulating anxiety-related behaviors[16] and these behaviors are altered as a function of the estrous cycle stage. In rats, for example, multiple studies demonstrated that high-estrogenic proestrus females exhibit lower anxiety-related behavior than low-estrogenic females[31–34]. Corroborating this finding, we recently found that the same phenomenon is observable in mice across multiple anxiety paradigms including the open field, light-dark box, and elevated plus maze (Figure 1B). Across all behavioral tests, high-estrogenic proestrus females consistently exhibit lower anxiety indices compared to low-estrogenic diestrus females[35]. The protective effect of the proestrus state on measures of anxiety-related behaviors strongly implies an anxiolytic-like effect of estradiol as the primary driver of these estrous cycle-dependent effects. Supporting this hypothesis, both systemic and, importantly, intra-hippocampal administration of estradiol in adult low-estrogenic[33,36] or ovariectomized[37,38] (OVX) female rodents decreases their anxiety-related behaviors. The observation that hippocampal estrogen is sufficient to drive these estrous cycle-dependent behaviors strongly supports the notion that cycling ovarian hormones impact indices of anxiety behavior through their effects on the hippocampus.

Although much of the available evidence indicates cycling estrogen levels are the primary determinant of the estrous cycle's effects on anxiety-related behaviors, progesterone and its metabolites are likely involved as well. While progesterone itself can have direct actions in the brain by binding progesterone receptors, it is important to note that progesterone also acts as a precursor to other neuroactive steroids, including allopregnanolone[39]. Allopregnanolone is an allosteric modulator of the GABA-A receptor that has been extensively studied in rodents for its anxiolytic[40–42] and antidepressant[41,43]-like effects. An intravenous formulation of allopregnanolone (brexanolone) is also currently prescribed in humans for postpartum depression treatment[44–46]. Interestingly, estrogen, progesterone, and allopregnanolone can all be locally synthesized in the rodent brain[47,48]. While levels of locally-synthesized estrogen and progesterone tend to correspond to systemic levels over the estrous cycle[49], allopregnanolone levels show more complex, locally determined regulation. For example, allopregnanolone levels in rats peak in the hippocampus during proestrus[50], while they are simultaneously at their nadir in the hypothalamus at this phase[51]. Therefore, while the estrous cycle modulates the levels of allopregnanolone, it does so in a locally controlled manner which results in hippocampal levels peaking during proestrus, where allopregnanolone presumably acts additively to the anxiolytic-like effects of rising hippocampal estrogen levels[41].

1.2. The effect of the estrous cycle on learning and memory—The effect of the estrous cycle on learning and memory has been evaluated primarily using three paradigms including the Morris water maze (Figure 1B), the novel object recognition test, and the novel object placement test, all of which are thought to involve the dorsal hippocampus, likely to different extents. While the dorsal hippocampus is known to be essential in spatial memory tasks such as Morris water maze and novel object placement, it is still debated whether this region is required, or only involved, in object memory tasks such as novel object recognition (we refer the readers to a recent review on this topic[52]).

Studies examining the impact of the estrous cycle on spatial memory performance in the Morris water maze have observed complex, species and test stage-specific effects[2]. In rats, it was observed that proestrus females exhibit reduced performance compared to estrus females[53], while another study found that the estrous cycle has no effect on performance, except for an interaction effect with a stressor[54]. Notably, these studies both accounted for the estrous cycle stage on the day of testing only, and therefore were addressing the estrous cycle effects on memory retrieval. In contrast, a study in mice recorded the estrous cycle stage just prior to training, and found that both proestrus and metestrus females demonstrated improved performance compared to estrus mice[55]. Additional studies will be necessary to clarify whether there are differential effects of the estrous cycle on acquisition and retrieval in the Morris water maze.

In the novel object recognition task, a test which assesses memory of the identity of objects, studies in both mice and rats have noted improved performance during the proestrus phase compared to either the estrus or diestrus phases[56–58]. Similarly, in the novel object placement task, a test which involves both object identity and spatial memory, proestrus rats outperform diestrus rats[58,59]. In these latter paradigms, studies accounted for the estrous cycle before training and performed the test later that day. These results indicate a role for estrogen in improving performance in novel object recognition and placement. Interestingly, an increase in novel object placement performance in rats has also been observed in estrus compared to diestrus[59], and, in one study, in estrus compared to all other phases[60], indicating a possible effect of the estrus-associated peak in progesterone (Figure 1A) on performance in this task.

The effect of the estrous cycle on learning and memory-associated tasks is notably more complex than its effects on anxiety indices. The discrepancy of results across paradigms has been suggested to arise, in part, due to a shift in learning strategy across the estrous cycle[61]. For example, in spatial navigation tasks, proestrus rats were observed to prefer a “place” strategy in which they remembered the area of the maze where a reward was found, while estrus rats were biased toward a “response” strategy in which they remembered the action they took (e.g. making a right turn) to get the reward[61]. Another factor to consider is that, relative to the human menstrual cycle, the rodent estrous cycle is very short (Figure 1A), with a change in phase occurring daily in most cases. While this is not a complication for basic anxiety-related behavioral tests, which are short in duration and can be performed in one session, learning and memory tasks require multiple trials, often administered across multiple days, making it difficult to parse the effects of a particular estrous cycle phase on the outcome of the test[62]. To address this, studies have used modified protocols which

allow for training and testing to occur on the same day[55–60,62], though even in these cases training and testing can be separated by several hours. In certain phases, such as proestrus, this length of time can correspond with markedly different hormonal profiles (Figure 1A). Other studies have employed strategies such as separating training and testing by one estrous cycle[63], or by analyzing behavioral data based on the different combinations of a female's estrous cycle stage across training and testing days[64,65]. While further studies will be needed to accurately assess the role of the physiological estrous cycle on object and spatial memory, studies utilizing ovariectomy and hormone replacement have consistently demonstrated that estradiol or specific ERα and ERβ agonists, when administered directly to the dorsal hippocampus, reliably improve performance in the Morris water maze[66], object recognition[67–70], and object placement[67,70] tasks in adult female rodents (we refer the reader to several reviews on this topic[2,62,71,72]).

Lastly, while we discussed the behavioral distinction between the ventral and dorsal hippocampus, it is interesting to note that fear conditioning, which has both an anxiety and learning component[73,74], also shows changes over the estrous cycle. Interestingly, in both context fear conditioning and fear potentiated startle tests, it has been shown that rats trained in diestrus but tested in proestrus exhibited reductions in fear learning[64,65]. One explanation for this phenomenon is that the fear memories formed during diestrus, in contrast to those formed during proestrus, are state-dependent, requiring the diestrus phase for appropriate retrieval[64]. Another possibility is that testing during proestrus obscures fear learning due to estrogen-induced generalization[65], as studies in adult OVX rats have demonstrated that administration of estradiol, either systemically[75] or directly to the dorsal hippocampus[76], increases fear generalization to an unpaired context.

Estrous cycle effects have also been observed in fear extinction paradigms. In contextual fear conditioning, for example, it was found that diestrus rats undergo extinction more rapidly than proestrus rats[63]. However, in cued fear conditioning, multiple studies have shown that female rats in the proestrus phase undergo extinction more quickly than rats at the other, low estrogenic phases of the estrous cycle[63,77,78]. Estrogen is implicated in driving this effect, as administration of estrogen to metestrus females before or just after extinction training reduced their freezing behaviors during recall[79]. Together with the findings from single-trial anxiety tests discussed earlier, these findings further support an anxiolytic-like effect of estrogen.

To summarize, the estrous cycle affects hippocampus-dependent behaviors including ventral hippocampus-dependent anxiety indices and performance in dorsal hippocampus-dependent learning and memory tasks. Experiments leveraging hormone administration demonstrate that estrogen acts as an anxiolytic, and the bulk of evidence indicates that estrogen also facilitates improvements in learning and memory tasks. Importantly, the observation that intra-hippocampal manipulations of ovarian hormones in rodents can affect indices of anxiety[37,41] and learning and memory[66–70,76] suggests that the cycling ovarian hormones impact hippocampus-dependent behaviors through their local actions on hippocampal cells. Since behavioral changes follow from changes in the activity of neural circuits, we will focus in the next section on the cellular mechanisms through which ovarian hormones affect hippocampal physiology.

2. The hippocampus undergoes structural and functional changes over the estrous cycle

On a circuit-level, signals enter the hippocampus from the entorhinal cortex through the perforant pathway (Figure 2A), where they are transmitted across synapses with CA1 pyramidal neurons[80]. The estrous cycle can affect the excitability of this circuit by changing the cellular morphology and the functional output of the neurons involved, as discussed in this section.

2.1. The effect of the estrous cycle on hippocampal dendritic spine density

—As an example, the dendrites of hippocampal CA1 pyramidal neurons can vary in the number of spines along their surface (Figure 2B). These dendritic spines are structural elements that exhibit dynamic regulation and are key determinants of neuronal excitability[81]. Notably, spine loss and formation have been associated with hippocampus-dependent behavioral phenotypes. For example, hippocampal dendritic spine density in the mouse CA1 region is decreased following chronic stress and rescued by antidepressant treatment, and these changes in spine density are strongly correlated with the induction and subsequent rescue of anxiety- and depression-related behavioral phenotypes[82]. Further, dendritic spine density increases in the rat hippocampus following spatial learning[83] and CA1 spine density is associated with memory task performance[84].

Seminal studies by Woolley & McEwen demonstrated that both spine and synapse density in CA1 pyramidal neurons are higher in proestrus compared to estrus rats[85,86] (Figure 2B), which implicates the physiological rise and drop in estrogen in this phenomenon. Interestingly, using hormone treatment of OVX females, Woolley & McEwen found that while estradiol drives this increase in spines and synapses, the dissipation of estradiol is not sufficient to explain the rapid decrease in spine density occurring over 24 hours in the proestrus-estrus transition[87]. They find, in fact, that while co-administration with progesterone initially augments estradiol's effects by further increasing spine density, this is followed by a more rapid decline in spine density which more closely mimics the spine density phenotype (and hormonal pattern) of cycling females transitioning from proestrus to estrus[87]. Testing this in a physiological context, they found that administration of a progesterone receptor antagonist in cycling females leads to an extension of the proestrus-induced increase in CA1 spine density[87]. These experiments demonstrate the complexity of the estrous cycle, as replacements of single hormones in OVX females are insufficient to reproduce the phenotypic pattern observed in the physiological cycle, where fluctuating hormones act in concert to elicit specifically timed effects.

While these earlier studies focused on the dorsal hippocampus, we recently found the same pattern of dendritic spine density fluctuations in the mouse ventral hippocampus over the estrous cycle, with higher spine density in proestrus compared to diestrus mice (Figure 2B)[35]. Considering the functional distinction across the dorsoventral hippocampal axis, the varying dendritic spine density in the ventral hippocampus as a function of fluctuating estrogen levels is likely to be important for anxiety-related phenotypes rather than for memory. Dendritic spines therefore represent a cellular substrate which is responsive to ovarian hormones[72] and can provide a link between hormonal rhythms and

the electrophysiological changes that would be required for hormones to alter hippocampus-dependent behaviors.

2.2. The effect of the estrous cycle on the functional output of hippocampal neurons—Work focused on hippocampal electrophysiology has demonstrated the ability of the estrous cycle and ovarian hormones to alter measures of long-term potentiation (LTP), or the persistent strengthening of a synapse following high-frequency neuronal stimulation. It was shown, for instance, that proestrus rats exhibit enhanced measures of LTP in the CA1 region compared to estrus and diestrus rats[88,89]. Estradiol has been the focus of many of these studies, as it has been shown in vivo[90] in rats and in ex vivo slices[91,92] to potentiate excitatory postsynaptic potentials, and to have diverse effects on glutamate signaling[91,93]. Fewer studies have investigated the effect of progesterone on hippocampal LTP, though it appears to decrease LTP in the CA1 through a GABA-related mechanism[94] which could be due to direct effects of progesterone or through one of its metabolites such as allopregnanolone. In addition to LTP, a recent study measuring local field potentials (LFPs) recorded from the rat hippocampus and medial prefrontal cortex found changes in LFP synchrony between these two brain regions occurring over the estrous cycle[95]. Importantly, LFP synchrony in these regions has been demonstrated in other studies to be a critical factor in the expression of hippocampus-dependent behaviors[22,96,97].

2.3. The effect of the estrous cycle on hippocampal neurogenesis—Another cellular mechanism which has important implications for hippocampal physiology is dentate gyrus neurogenesis. The proliferation of new neurons in the rodent dentate gyrus (Figure 2A) occurs throughout adulthood[98], with important implications for both learning[99–101]- and anxiety-related[99,102,103] phenotypes. In rats, it was shown that during the proestrus phase there is an increase in proliferating cells in the dentate gyrus compared to rats in the estrus or diestrus phases[104]. Further, there is a significant decrease in hippocampal neurogenesis in adult female rats shortly after OVX which can be rescued with estrogen treatment[104]. While, again, most studies have focused on the role of estradiol[72], progesterone administration in male[105,106] and OVX female[107] rats has been shown to increase hippocampal neurogenesis in vivo. Interestingly, progesterone seems to antagonize estrogen's effects on neurogenesis when they are co-administered to OVX rats[107], which could play a regulatory role in the context of intact cycling females, similar to what was observed with changes in dendritic spines[87]. In mice, however, a study examining the effects of the estrous cycle on dentate gyrus neurogenesis did not find an effect, indicating potential species-specific effects of ovarian hormones on this process[108].

Taken together, studies have demonstrated effects of the estrous cycle on hippocampal cell structure and function, including changes in dendritic spine and synapse density in both the dorsal and ventral hippocampus as well as changes in dentate gyrus neurogenesis. These cellular changes are accompanied by changes in hippocampal LTP, indicating that these cellular effects have functional consequences on neuronal activity. By altering the activity of hippocampal circuits, these cellular mechanisms represent a substrate through which fluctuating ovarian hormones can have direct effects on hippocampus-dependent behavioral outcomes.

3. Molecular mechanisms underlying sex hormone-dependent changes in hippocampal structure and function

Behavioral and structural changes across the estrous cycle described above have been well-known in the field of neuroendocrinology for decades now. However, molecular mechanisms that underlie these brain and behavioral dynamics have only been described in the last couple of years, representing an important and exciting new development in the field[35,36]. In this section, we will discuss recently-revealed epigenetic mechanisms through which cycling ovarian hormones affect hippocampal physiology, and the efforts that have been made to link these mechanisms to steroid hormone receptor mechanisms. We start with describing estrogen and progesterone receptor signaling and their downstream effects including changes in gene expression.

3.1. Ovarian hormone receptor mechanisms—

Upstream of structural and functional changes in the hippocampus, any direct effects of the estrous cycle on hippocampal physiology depend on the binding of ovarian hormones to their cognate receptors expressed in hippocampal cells (Figure 3A). There are a variety of these receptors expressed in the neurons and glia of the hippocampus, including estrogen receptor alpha (ERα) and beta (ERβ)[15], as well as progesterone receptor isoforms PRa and PRb[11]. Upon binding estrogen or progesterone, these intracellular hormone receptors enter the nucleus where they act as transcription factors, regulating the expression of genes by binding to estrogen response elements (EREs) or progesterone response elements (PREs) throughout the genome[109,110] (Figure 3A). In addition to these genomic effects, sex steroid receptors such as ERα and ERβ can also integrate into the membrane and trigger rapid effects through the activation of various kinase pathways[111–113] (Figure 3A). Several other membrane-receptors have also been identified which bind sex steroids and act through similar rapid mechanisms, including G-protein coupled estrogen receptor[114] (GPER), progesterone receptor membrane associated components[11](PGRMCs) and the seven transmembrane domain progesterone receptors[11] (referred to broadly as mPRs) (Figure 3A). In addition to rapid cellular effects, activation of these membrane-associated receptors can also cause indirect genomic effects which result from kinase pathways activating other transcription factors, such as Egr1[115] (Figure 3A).

3.2. Dynamic neuronal gene expression across the estrous cycle—

Since the actions of ovarian hormones in the hippocampus are likely to result in large part from changes in gene expression, studies have explored whether the estrous cycle regulates the hippocampal transcriptome. We and others have recently demonstrated significant alterations in hippocampal gene expression that occur over the rodent estrous cycle[35,116,117]. Importantly, in the mouse ventral hippocampus, we observed cyclic expression of more than a hundred genes implicated in neuronal excitability, synaptic plasticity, and anxiety-related behaviors[35], linking hormone-dependent gene expression to the observed structural, functional and behavioral phenotypes across the cycle. To more clearly represent the connection between gene expression dynamics and dynamic structural and behavioral alterations across the estrous cycle, we select two genes as examples. One example is the gene *Dlk1* (encoding Delta like non-canonical notch ligand 1) implicated in anxiety-related behavior[118]. *Dlk1* was identified as a transcriptional marker of the ventral hippocampus[119]

and its deletion in male mice is sufficient to induce increased anxiety indices[118]. In females, though, we see the expression of this gene naturally cycling in the ventral hippocampus, reaching low levels in the low-estrogenic diestrus phase when we observe higher anxiety-like behavior[35]. We also observed changes in expression of genes related to dendritic spine regulation, including our second example the *Ptprt* gene (encoding Protein tyrosine phosphatase, receptor type T)[120]. The functional role of *Ptprt* in dendritic spine regulation was established in cultured hippocampal neurons, in which an overexpression of *Ptprt* increases dendritic spine density and the number of synapses, while knockdown of *Ptprt* results in a decreased number of dendritic spines and reduced synapse formation[120]. Consistent with this, across the estrous cycle, *Ptprt* reaches its highest expression levels as estrogen levels peak during proestrus, corresponding to the peak in dendritic spine density observed during this phase[35]. In sum, cycling ovarian hormones affect hippocampal expression of genes implicated in structural[120] and behavioral[118] changes that we and others have observed over the estrous cycle[35], providing a link between the molecular actions of ovarian hormones and their dynamic effects at the structural and behavioral level. It is of critical interest, though, to understand molecular mechanisms that underlie cyclic changes in gene expression induced by ovarian hormones, which will be addressed in the following section.

3.3. Dynamic epigenetic regulation in hippocampal neurons across the estrous cycle—

We recently implicated an epigenetic mechanism, the dynamic re-organization of chromatin, in neuronal gene regulation across the estrous cycle[35,36]. Chromatin is the dynamic structure, including DNA and its associated proteins (mostly histones), which can undergo conformation changes through various mechanisms to regulate gene expression[121] (Figure 3B). Accessible or open chromatin structure within gene regulatory regions, such as promoters and enhancers, promotes the binding of transcription factors and gene activation, while closed chromatin configuration is not permissive for transcription[121]. In addition to local accessibility (1D chromatin, Figure 3B), chromatin conformation includes complex three-dimensional (3D) arrangements[122] (3D chromatin, Figure 3B). Features of 3D chromatin that can affect gene expression include chromosome compartments, chromatin (CTCF) loops, and enhancer-promoter interactions, which allow interactions of genes with their distant cis-regulatory elements regulating transcription[122].

In dividing cancer cell lines, it has been known for a long time that changes in chromatin are an important feature of the genomic effects of ovarian hormones[123–125]. However, we recently demonstrated that features of both 1D and 3D chromatin are dynamically regulated by the estrous cycle in postmitotic neurons of the ventral hippocampus[35,36]. To profile changes in 1D chromatin (Figure 3B) we performed the Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq), a procedure that involves sequencing of DNA enriched from accessible chromatin[126], on ventral hippocampal neurons from proestrus and diestrus female mice. Strikingly, we found that approximately 30% of the profiled genomic regions change chromatin accessibility across the estrous cycle and these regions are enriched nearby genes critical for neuronal function[35]. For example, pathway analysis indicated estrous cycle-driven chromatin changes occurring near genes involved in synaptic plasticity, and, importantly, serotonergic signaling, which has been strongly linked

to anxiety-related behaviors in rodents[127,128]. The two genes mentioned previously, *Dlk1* and *Ptprt*, both show increased chromatin accessibility in their putative regulatory regions during proestrus, when expression of these genes is high, compared to diestrus, when their expression is low[35]. The rising of estrogen, therefore, promotes chromatin opening and transcription in a dynamic fashion, for these genes.

To profile changes in 3D chromatin (Figure 3B), we performed the unbiased chromosome conformation capture (Hi-C) assay, which involves sequencing DNA regions that are proximal in physical space[129], on ventral hippocampal neurons from these same groups. We demonstrated that the estrous cycle can dynamically alter 3D chromatin at all levels of organization and impact gene expression[36]. For instance, we observed that anxiety-related genes such as *Htr2c* (encoding the serotonin receptor 2c)[130], and *Adcyap1* (encoding pituitary adenylate cyclase activating polypeptide)[131] have differential 3D interactions in ventral hippocampal neurons between diestrus and proestrus females which are associated with changes in gene expression[36]. Proestrus-specific 3D chromatin changes are partially recreated by estrogen replacement in OVX female mice[36], confirming the role of estrogen fluctuation in the dynamic chromatin organization across the estrous cycle.

Together, these findings indicate that sex hormone-driven chromatin dynamics regulates gene expression in ventral hippocampal neurons, underlying synaptic and behavioral plasticity across the estrous cycle.

3.4. Chromatin dynamics as a broader feature of the “cycling neuron”—

As described above, chromatin (re)organization is a dynamic process that is typically involved in transcriptional regulation across organs and cell types including hippocampal neurons[35,36,121]. Dynamic chromatin regulation is with no doubt critically important for the regulation of female brain function since, in addition to changes in chromatin organization (as demonstrated by ATAC-seq and Hi-C), we also observed changes in the *expression* of chromatin remodeling factors (identified by RNA-seq), across the estrous cycle[35,36]. However, there are many genes that exhibit cycling chromatin states without changes in gene expression, suggesting that chromatin reorganization is involved in other regulatory functions in the female brain, beyond the control of transcriptional initiation. As an example, there are more than 2,000 differential enhancer-promoter (E-P) interactions across the estrous cycle, but only 10% of differentially expressed genes exhibit changes in E-P interactions[36]. In the epigenetics field there is a phenomenon of ‘epigenetic priming’, described in the areas of learning[132] and immunological memory[133], where epigenomic changes were shown to precede changes in gene expression requiring another stimulus to be expressed. Accordingly, we propose the model of “hormonal epigenetic priming” where cycling hormones alter the neuronal epigenome but the epigenomic changes are not necessarily translated into gene expression changes unless there is another physiological (or pathological) stimulus. Importantly, this molecular priming is consistent with female reproductive physiology where many events across the reproductive cycle including thickening of the uterus wall and likely brain structural changes are preparatory rather than functionally relevant events. Notably, the estrous cycle-dependent E-P interactions are enriched for genes involved in neurological disorders and serotonergic transmission and anxiety, providing a possible molecular basis for hormonally-driven, female-specific

vulnerability for certain brain disorders such as anxiety and depression, which may require another risk factor (or stimulus) such as stress to be expressed.

Finally, we would like to highlight the unusually high dynamics of the 3D genome organization of the X-chromosome across the estrous cycle[36]. We find that ~16% of the X-chromosome shows changes in Hi-C compartmental signal, typically associated with transcriptional activity[122], in ventral hippocampal neurons of proestrus vs. diestrus female mice[36]. These chromatin changes are partially associated with gene expression changes. We describe for the first time the estrous cycle-dependent "X-escapee genes" in these neurons, which are X-linked genes that appear to be biallelically expressed in only one stage of the estrous cycle (e.g. the above mentioned *Htr2c* gene). In fact, our findings strongly indicate that the inactive X (Xi) chromosome undergoes a partial decondensation and increase in volume during the high estrogenic phase of the cycle[36]. This is important because hormone-induced Xi volume change may have a global effect on cellular environment, affecting not only X-linked genes but also the expression and function of autosomal genes[134].

In sum, intensive chromatin dynamics appears to be an important feature of "cycling hippocampal neurons", undergoing cyclical exposure to ovarian hormones, which extends beyond immediate, local effects on gene regulation. Phenomena such as hormone-induced epigenetic priming and X-chromosome plasticity are very intriguing, and are possibly involved in cellular memory associated with cycling events. Further research in this area is warranted as it may offer radically new mechanistic insights into female-specific neuronal function with critical implications for female physiology and disease risk.

3.5. Linking epigenetic changes to receptor mechanisms across the estrous cycle—

As mentioned previously, sex hormones can elicit their effects through either membrane or nuclear receptors, which, in both cases, are likely to lead to changes in gene expression (Figure 3A). Though more mechanistic studies will be required to fully delineate the contribution of these two pathways to the chromatin and gene regulation by ovarian hormones in hippocampal neurons across the cycle, analysis of ATAC-seq and Hi-C datasets give some preliminary indications about which pathways are at work.

Our ATAC-seq data indicate the involvement of membrane-bound estrogen signaling in the regulation of chromatin accessibility and gene expression across the estrous cycle (Figure 3A). Motif analysis of genomic regions specifically open in proestrus (closed in diestrus) showed the enrichment for binding sites of Egr1 and several other transcription factors, but not the enrichment of EREs, implicating the membrane ER in estrogen-induced chromatin opening and gene expression (Figure 3B). We were able to confirm the localization of ERβ to the membrane in ventral hippocampal neurons[35] (Figure 3A). Interestingly, the expression of Egr1 is responsive to estrogen level changes, reaching its highest expression in proestrus when we see increased accessibility of Egr1 binding sites and increased expression of genes enriched for the Egr1 motif[35]. Indeed, previously mentioned example genes, *Dlk1* and *Ptprt*, with elevated expression during proestrus, contain Egr1 binding sites within their putative regulatory regions which gain chromatin accessibility during the high-estrogenic proestrus phase. The model here implies that rising estrogen levels during proestrus would activate membrane-bound ERs, leading to downstream activation and binding of Egr1, chromatin

opening and transcriptional activation of the Egr1 target genes such as *Dlk1* and *Ptprt* (Figure 3A). Since immediate early gene products such as Egr1 were recently revealed as initiators of chromatin opening in neurons[135,136], we propose that Egr1 is a candidate estrogen-dependent regulator of chromatin and gene expression in the ventral hippocampus across the estrous cycle. Egr1 was also suggested as a regulator of the estrous cycle-driven gene expression in the prefrontal cortex[137], indicating a potential shared mechanism for the effects of cycling ovarian hormones on chromatin and gene regulation across brain regions.

Intriguingly, motif analysis of Hi-C data identified the enrichment of EREs in estrous cycle-dependent 3D chromatin changes consistently, across all levels of higher order chromatin organization[36] (Figure 3B). We also find ERα localizing in the nucleus of ventral hippocampal neurons, indicating that nuclear ERα is involved in mediating estrogen-driven changes in 3D genome organization[36] (Figure 3A), consistent with earlier studies performed in breast cancer cells[123,124]. Together, these analyses suggest a cooperative role of membrane-bound and nuclear ER signaling in estrogen's effects on chromatin organization in ventral hippocampal neurons (Figures 3A–B).

The work discussed here provides a framework for understanding the molecular mechanisms underlying the dynamic effects of the estrous cycle on the hippocampus-based structural and behavioral phenotypes. In the ventral hippocampus, for example, our data supports a model in which estrogen, acting through both nuclear and membrane pathways, triggers a neuronal transcriptional program during proestrus that affects genes implicated in the cellular and behavioral phenotypes associated with the proestrus state (Figure 3). This transcriptional program involves altered levels and activity of transcription factors, such as Egr1 and ERs, which can affect transcription by binding gene enhancer and promoter regions and through initiating changes in 1D and 3D chromatin organization. While the studies discussed so far provide important insights into the mechanisms through which cycling ovarian hormones affect the hippocampus across the estrous cycle, there are still many questions that need to be addressed. We will discuss these questions in the next section.

4. Future directions

Despite significant recent progress, many details surrounding the actions of cycling ovarian hormones in the hippocampus are still unknown and below we provide several future directions for the field.

First, we need to provide a stronger functional link between hormone-induced chromatin dynamics and structural and behavioral changes across the estrous cycle. We can do this: i) by using a classic hormone replacement approach (with which we already confirmed estrogen's role in neuronal 3D genome organization and anxiety-related behavior[36]), or ii) by genetically manipulating our candidate hormone-driven chromatin regulators (e.g. Egr1), as a novel approach to model the estrous cycle stage-specific transcriptional program(s).

Second, while described genomics assays were performed with cell type-specific resolution, using purified neurons[35,36], leveraging single-cell sequencing methods will help us further dissect cellular subtypes and neuronal clusters in the hippocampus that are responsive to

cycling hormones and possible drivers of cyclic changes in chromatin and behavior across the estrous cycle.

Further, to determine the relative contributions of different hormone receptor isoforms, and the contributions of nuclear or membrane-bound pathways, future studies should take advantage of specific hormone receptor agonists and antagonists, receptor knockout animal models, as well as BSA-bound hormones that are impermeable to the cell membrane, coupled with behavioral, cellular, and molecular assays.

Additionally, much of the work described here has focused on the role of estradiol, and future studies should aim to further elucidate the role of progesterone and its metabolites, solely and in combination with estrogen, on hippocampal function. Of note, in the cases where it is necessary to use OVX females and administer hormones, researchers should always be aware of ovariectomy-induced adaptations and dose-dependent effects of hormones and work within the naturalistic context of the estrous cycle whenever possible.

Last but not least, furthering our studies on the above-discussed hormonal epigenetic priming and hormone-induced plasticity of the X chromosome may lead to exciting new discoveries with important implications for both basic neuroscience and clinical research.

In sum, the findings described here establish an exciting new field of dynamic, sex hormone-driven gene regulation in the female brain, with important implications for women's health as we will discuss in the following section.

5. The translational value of the estrous cycle studies for understanding human brain physiology and disease risk

As mentioned previously, hormonal profiles of the estrous and menstrual cycle are very similar (Figure 1A). In general, these hormonal cycles have the same role in the human and the mouse reproductive function. It is, therefore, not surprising that, regardless of stark differences in complexity, fluctuating ovarian hormones induce comparable changes in brain structure and behavior between humans and mice. For instance, multiple studies have reported changes in hippocampal gray matter across the menstrual cycle in humans[138–141], with gray matter increase typically following increased estrogen levels[140]. In addition, both changes in emotion regulation[142,143] and cognitive function[142] were reported as a function of the menstrual cycle stage. There is also strong clinical evidence supporting the role of fluctuating ovarian hormones in increased female risk for anxiety and depression disorders, and we refer the reader to the recent review article on this topic[144]. Overall, comparable sex hormone-induced brain dynamics between mice and humans, indicates that, indeed, studying the estrous cycle can help us understand the molecular mechanisms underlying the dynamic regulation of hippocampal physiology by fluctuating ovarian hormones in humans. Most importantly, considering the comparable physiology, a mouse model could, in this case, have a rare constructive validity for studying the increased female risk for depression and anxiety disorders induced by naturally-cycling ovarian hormones. As such, combined mechanistic studies of the estrous cycle in mice with translational studies of hormonally-induced risk in humans is likely to reveal novel sex-specific targets for treatment.

Conclusion

Here, we discussed dynamic changes in hippocampal physiology occurring over the rodent estrous cycle. Cycling ovarian hormones impact hippocampus-dependent behaviors, including indices of anxiety and performance in learning and memory tasks. Changes in hippocampal structure and function, including dendritic spine density and LTP, have been observed over the estrous cycle, providing a cellular mechanism through which cyclical estrogen and progesterone can impact hippocampus-dependent behaviors. Importantly, recent work has implicated epigenetic mechanisms, namely dynamic chromatin changes, in the hippocampus-based structural and behavioral effects of ovarian hormones. Future studies will yield translationally-relevant insights into the dynamic regulation of hippocampal physiology by fluctuating ovarian hormones with important implications for the health of women and other menstruating individuals across the gender.

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Data availability statement:

Data sharing is not applicable to this article since no new data were created or analyzed in this study.

References

1. Marrocco J & McEwen BS Sex in the brain: hormones and sex differences. Dialogues Clin Neurosci. 2016;18:373–383. [PubMed: 28179809]
2. Taxier LR, Gross KS & Frick KM Oestradiol as a neuromodulator of learning and memory. Nat Rev Neurosci. 2020;21:535–550. [PubMed: 32879508]
3. Boling JL & Blandau RJ The estrogen-progesterone induction of mating responses in the spayed female rat. Endocrinology. 1939;25:359–364.
4. Edwards DA Induction of estrus in female mice: estrogen-progesterone interactions. Hormones and Behavior. 1970;1:299–304.
5. Gao Q et al. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. Nat Med. 2007;13:89–94. [PubMed: 17195839]
6. Olofsson LE, Pierce AA & Xu AW Functional requirement of AgRP and NPY neurons in ovarian cycle-dependent regulation of food intake. Proceedings of the National Academy of Sciences. 2009;106:15932–15937.
7. Zhang Z et al. Estrogen-sensitive medial preoptic area neurons coordinate torpor in mice. Nature Communications. 2020;11:6378.
8. van Veen JE et al. Hypothalamic oestrogen receptor alpha establishes a sexually dimorphic regulatory node of energy expenditure. Nature Metabolism. 2020;2:351–363.
9. Couse JF, Yates MM, Walker VR & Korach KS Characterization of the Hypothalamic-Pituitary-Gonadal Axis in Estrogen Receptor (ER) Null Mice Reveals Hypergonadism and Endocrine Sex Reversal in Females Lacking ERα But Not ERβ. Molecular Endocrinology. 2003;17:1039–1053. [PubMed: 12624116]
10. Vadakkadath Meethal S & Atwood CS The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. Cell Mol Life Sci. 2005;62:257–270. [PubMed: 15723162]

11. Brinton RD et al. Progesterone receptors: form and function in brain. Front Neuroendocrinol. 2008;29:313–339. [PubMed: 18374402]
12. Garcia-Segura LM, Naftolin F, Hutchison JB, Azcoitia I & Chowen JA Role of astroglia in estrogen regulation of synaptic plasticity and brain repair. J Neurobiol. 1999;40:574–584. [PubMed: 10453057]
13. Mitra SW et al. Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. Endocrinology. 2003;144:2055–2067. [PubMed: 12697714]
14. Mitterling KL et al. Cellular and subcellular localization of estrogen and progestin receptor immunoreactivities in the mouse hippocampus. J Comp Neurol. 2010;518:2729–2743. [PubMed: 20506473]
15. McEwen B et al. Tracking the estrogen receptor in neurons: Implications for estrogen-induced synapse formation. Proceedings of the National Academy of Sciences. 2001;98:7093–7100.
16. Fanselow MS & Dong HW Are the dorsal and ventral hippocampus functionally distinct structures? Neuron. 2010;65:7–19. [PubMed: 20152109]
17. Sunkin SM et al. Allen Brain Atlas: an integrated spatio-temporal portal for exploring the central nervous system. Nucleic Acids Res. 2013;41:D996–d1008. [PubMed: 23193282]
18. Bannerman DM et al. Ventral hippocampal lesions affect anxiety but not spatial learning. Behav Brain Res. 2003;139:197–213. [PubMed: 12642189]
19. Kheirbek MA et al. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. Neuron. 2013;77:955–968. [PubMed: 23473324]
20. Trivedi MA & Coover GD Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. Neurobiology of Learning and Memory. 2004;81:172–184. [PubMed: 15082019]
21. Jimenez JC et al. Anxiety Cells in a Hippocampal-Hypothalamic Circuit. Neuron. 2018;97:670–683.e676. [PubMed: 29397273]
22. Padilla-Coreano N et al. Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. Neuron. 2016;89:857–866. [PubMed: 26853301]
23. Aggleton JP, Hunt PR & Rawlins JN The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. Behav Brain Res. 1986;19:133–146. [PubMed: 3964405]
24. Clark RE, Broadbent NJ & Squire LR Impaired remote spatial memory after hippocampal lesions despite extensive training beginning early in life. Hippocampus. 2005;15:340–346. [PubMed: 15744736]
25. Duva CA et al. Disruption of spatial but not object-recognition memory by neurotoxic lesions of the dorsal hippocampus in rats. Behavioral Neuroscience. 1997;111:1184–1196. [PubMed: 9438788]
26. Lee AR, Kim JH, Cho E, Kim M & Park M Dorsal and Ventral Hippocampus Differentiate in Functional Pathways and Differentially Associate with Neurological Disease-Related Genes during Postnatal Development. Front Mol Neurosci. 2017;10:331. [PubMed: 29085281]
27. Floriou-Servou A et al. Distinct Proteomic, Transcriptomic, and Epigenetic Stress Responses in Dorsal and Ventral Hippocampus. Biol Psychiatry. 2018;84:531–541. [PubMed: 29605177]
28. Zhang T-Y et al. Environmental enrichment increases transcriptional and epigenetic differentiation between mouse dorsal and ventral dentate gyrus. Nature Communications. 2018;9:298.
29. Cembrowski MS, Wang L, Sugino K, Shields BC & Spruston N Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. Elife. 2016;5:e14997. [PubMed: 27113915]
30. Caradonna SG et al. Corticosterone induces discrete epigenetic signatures in the dorsal and ventral hippocampus that depend upon sex and genotype: focus on methylated Nr3c1 gene. Translational Psychiatry. 2022;12:109. [PubMed: 35296634]
31. Frye CA, Petralia SM & Rhodes ME Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. Pharmacol Biochem Behav. 2000;67:587–596. [PubMed: 11164090]
32. Gouveia A Jr. et al. Influence of the estrous cycle on the behavior of rats in the elevated T-maze. Behav Processes. 2004;67:167–171. [PubMed: 15240054]

33. Marcondes FK, Miguel KJ, Melo LL & Spadari-Bratfisch RC Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav. 2001;74:435–440. [PubMed: 11790402]
34. Mora S, Dussaubat N & Díaz-Véliz G Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology. 1996;21:609–620. [PubMed: 9044444]
35. Jaric I, Rocks D, Greally JM, Suzuki M & Kundakovic M Chromatin organization in the female mouse brain fluctuates across the oestrous cycle. Nature Communications. 2019;10:2851.
36. Rocks D et al. Sex-specific multi-level 3D genome dynamics in the mouse brain. Nature Communications. 2022;13:3438.
37. Walf AA & Frye CA A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. Neuropsychopharmacology. 2006;31:1097–1111. [PubMed: 16554740]
38. Walf AA & Frye CA ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. Neuropsychopharmacology. 2005;30:1598–1609. [PubMed: 15798780]
39. Dong E et al. Brain 5alpha-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci. 2001;98:2849–2854. [PubMed: 11226329]
40. Bitran D, Shiekh M & McLeod M Anxiolytic Effect of Progesterone is Mediated by the Neurosteroid Allopregnanolone at Brain GABAA Receptors. Journal of Neuroendocrinology. 1995;7:171–177. [PubMed: 7606242]
41. Frye CA & Walf AA Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Horm Behav. 2002;41:306–315. [PubMed: 11971664]
42. Yoshizawa K, Okumura A, Nakashima K, Sato T & Higashi T Role of allopregnanolone biosynthesis in acute stress-induced anxiety-like behaviors in mice. Synapse. 2017;71:e21978.
43. Almeida FB, Nin MS & Barros HMT The role of allopregnanolone in depressive-like behaviors: Focus on neurotrophic proteins. Neurobiol Stress. 2020;12:100218. [PubMed: 32435667]
44. Walton N & Maguire J Allopregnanolone-based treatments for postpartum depression: Why/how do they work? Neurobiology of Stress. 2019;11:100198. [PubMed: 31709278]
45. Meltzer-Brody S & Kanes SJ Allopregnanolone in postpartum depression: Role in pathophysiology and treatment. Neurobiology of Stress. 2020;12:100212. [PubMed: 32435663]
46. Kanes S et al. Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. Lancet. 2017;390:480–489. [PubMed: 28619476]
47. Diotel N et al. Steroid Transport, Local Synthesis, and Signaling within the Brain: Roles in Neurogenesis, Neuroprotection, and Sexual Behaviors. Frontiers in Neuroscience. 2018;12:84. [PubMed: 29515356]
48. Tsutsui K, Ukena K, Usui M, Sakamoto H & Takase M Novel brain function: biosynthesis and actions of neurosteroids in neurons. Neuroscience Research. 2000;36:261–273. [PubMed: 10771104]
49. Kato A et al. Female hippocampal estrogens have a significant correlation with cyclic fluctuation of hippocampal spines. Frontiers in Neural Circuits. 2013;7:149. [PubMed: 24151456]
50. Palumbo MA et al. Allopregnanolone concentration in hippocampus of prepubertal rats and female rats throughout estrous cycle. Journal of Endocrinological Investigation. 1995;18:853–856. [PubMed: 8778157]
51. Genazzani AR et al. Evidence for a role for the neurosteroid allopregnanolone in the modulation of reproductive function in female rats. Eur J Endocrinol. 1995;133:375–380. [PubMed: 7581957]
52. Chao OY, Nikolaus S, Yang Y-M & Huston JP Neuronal circuitry for recognition memory of object and place in rodent models. Neuroscience & Biobehavioral Reviews. 2022;141:104855. [PubMed: 36089106]
53. Warren SG & Juraska JM Spatial and nonspatial learning across the rat estrous cycle. Behav Neurosci. 1997;111:259–266. [PubMed: 9106666]

54. Rubinow MJ, Arseneau LM, Beverly JL & Juraska JM Effect of the estrous cycle on water maze acquisition depends on the temperature of the water. Behav Neurosci. 2004;118:863–868. [PubMed: 15301613]
55. Frick KM & Berger-Sweeney J Spatial reference memory and neocortical neurochemistry vary with the estrous cycle in C57BL/6 mice. Behav Neurosci. 2001;115:229–237. [PubMed: 11256446]
56. Walf AA, Rhodes ME & Frye CA Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. Neurobiol Learn Mem. 2006;86:35–46. [PubMed: 16529958]
57. Walf AA, Koonce C, Manley K & Frye CA Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behav Brain Res. 2009;196:254–260. [PubMed: 18926853]
58. Paris JJ & Frye CA Estrous cycle, pregnancy, and parity enhance performance of rats in object recognition or object placement tasks. Reproduction. 2008;136:105–115. [PubMed: 18390689]
59. Frye CA, Duffy CK & Walf AA Estrogens and progestins enhance spatial learning of intact and ovariectomized rats in the object placement task. Neurobiol Learn Mem. 2007;88:208–216. [PubMed: 17507257]
60. Sutcliffe JS, Marshall KM & Neill JC Influence of gender on working and spatial memory in the novel object recognition task in the rat. Behav Brain Res. 2007;177:117–125. [PubMed: 17123641]
61. Korol DL, Malin EL, Borden KA, Busby RA & Couper-Leo J Shifts in preferred learning strategy across the estrous cycle in female rats. Horm Behav. 2004;45:330–338. [PubMed: 15109907]
62. Tuscher JJ, Fortress AM, Kim J & Frick KM Regulation of object recognition and object placement by ovarian sex steroid hormones. Behav Brain Res. 2015;285:140–157. [PubMed: 25131507]
63. Blume SR et al. Sex- and Estrus-Dependent Differences in Rat Basolateral Amygdala. J Neurosci. 2017;37:10567–10586. [PubMed: 28954870]
64. Blair RS et al. Estrous cycle contributes to state-dependent contextual fear in female rats. Psychoneuroendocrinology. 2022;141:105776. [PubMed: 35489312]
65. Carvalho MC, Genaro K, Leite-Panissi CRA & Lovick TA Influence of estrous cycle stage on acquisition and expression of fear conditioning in female rats. Physiol Behav. 2021;234:113372. [PubMed: 33647267]
66. Packard MG & Teather LA Intra-hippocampal estradiol infusion enhances memory in ovariectomized rats. Neuroreport. 1997;8:3009–3013. [PubMed: 9331907]
67. Boulware MI, Heisler JD & Frick KM The memory-enhancing effects of hippocampal estrogen receptor activation involve metabotropic glutamate receptor signaling. J Neurosci. 2013;33:15184–15194. [PubMed: 24048848]
68. Fernandez SM et al. Estradiol-induced enhancement of object memory consolidation involves hippocampal extracellular signal-regulated kinase activation and membrane-bound estrogen receptors. J Neurosci. 2008;28:8660–8667. [PubMed: 18753366]
69. Pereira LM, Bastos CP, de Souza JM, Ribeiro FM & Pereira GS Estradiol enhances object recognition memory in Swiss female mice by activating hippocampal estrogen receptor α. Neurobiol Learn Mem. 2014;114:1–9. [PubMed: 24726465]
70. Tuscher JJ, Taxier LR, Schalk JC, Haertel JM & Frick KM Chemogenetic Suppression of Medial Prefrontal-Dorsal Hippocampal Interactions Prevents Estrogenic Enhancement of Memory Consolidation in Female Mice. eNeuro. 2019;6:ENEURO.0451–0418.
71. Frick KM, Kim J, Tuscher JJ & Fortress AM Sex steroid hormones matter for learning and memory: estrogenic regulation of hippocampal function in male and female rodents. Learn Mem. 2015;22:472–493. [PubMed: 26286657]
72. Sheppard PAS, Choleris E & Galea LAM Structural plasticity of the hippocampus in response to estrogens in female rodents. Molecular Brain. 2019;12:22. [PubMed: 30885239]
73. Myers KM & Davis M Mechanisms of fear extinction. Mol Psychiatry. 2007;12:120–150. [PubMed: 17160066]

74. Maeng LY & Milad MR Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. Horm Behav. 2015;76:106–117. [PubMed: 25888456]
75. Adkins JM, Lynch JF 3rd, Hagerdorn P, Esterhuizen M & Jasnow AM Anterior cingulate cortex and dorsal hippocampal glutamate receptors mediate generalized fear in female rats. Psychoneuroendocrinology. 2019;107:109–118. [PubMed: 31125757]
76. Lynch JF 3rd, Winiecki P, Vanderhoof T, Riccio DC & Jasnow AM Hippocampal cytosolic estrogen receptors regulate fear generalization in females. Neurobiol Learn Mem. 2016;130:83–92. [PubMed: 26851128]
77. Milad MR, Igoe SA, Lebron-Milad K & Novales JE Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience. 2009;164:887–895. [PubMed: 19761818]
78. Rey CD, Lipps J & Shansky RM Dopamine D1 receptor activation rescues extinction impairments in low-estrogen female rats and induces cortical layer-specific activation changes in prefrontal-amygdala circuits. Neuropsychopharmacology. 2014;39:1282–1289. [PubMed: 24343528]
79. Zeidan MA et al. Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol Psychiatry. 2011;70:920–927. [PubMed: 21762880]
80. Basu J & Siegelbaum SA The Corticohippocampal Circuit, Synaptic Plasticity, and Memory. Cold Spring Harb Perspect Biol. 2015;7:a021733. [PubMed: 26525152]
81. Sala C & Segal M Dendritic Spines: The Locus of Structural and Functional Plasticity. Physiological Reviews. 2014;94:141–188. [PubMed: 24382885]
82. Wang G et al. Systematic correlation between spine plasticity and the anxiety/depression-like phenotype induced by corticosterone in mice. Neuroreport. 2013;24:682–687. [PubMed: 23839258]
83. Moser MB, Trommald M & Andersen P An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci. 1994;91:12673–12675. [PubMed: 7809099]
84. Eilam-Stock T, Serrano P, Frankfurt M & Luine V Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behav Neurosci. 2012;126:175–185. [PubMed: 22004261]
85. Woolley CS & McEwen BS Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci. 1992;12:2549–2554. [PubMed: 1613547]
86. Woolley CS, Gould E, Frankfurt M & McEwen BS Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J Neurosci. 1990;10:4035–4039. [PubMed: 2269895]
87. Woolley CS & McEwen BS Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. J Comp Neurol. 1993;336:293–306. [PubMed: 8245220]
88. Warren SG, Humphreys AG, Juraska JM & Greenough WT LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. Brain Res. 1995;703:26–30. [PubMed: 8719612]
89. Good M, Day M & Muir JL Cyclical changes in endogenous levels of oestrogen modulate the induction of LTD and LTP in the hippocampal CA1 region. Eur J Neurosci. 1999;11:4476–4480. [PubMed: 10594677]
90. Córdoba Montoya DA & Carrer HF Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. Brain Res. 1997;778:430–438. [PubMed: 9459564]
91. Smejkalova T & Woolley CS Estradiol Acutely Potentiates Hippocampal Excitatory Synaptic Transmission through a Presynaptic Mechanism. The Journal of Neuroscience. 2010;30:16137–16148. [PubMed: 21123560]
92. Foy MR et al. 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. J Neurophysiol. 1999;81:925–929. [PubMed: 10036289]
93. Tada H et al. Estrous Cycle-Dependent Phasic Changes in the Stoichiometry of Hippocampal Synaptic AMPA Receptors in Rats. PLOS ONE. 2015;10:e0131359. [PubMed: 26121335]
94. Foy MR, Akopian G & Thompson RF Progesterone regulation of synaptic transmission and plasticity in rodent hippocampus. Learn Mem. 2008;15:820–822. [PubMed: 18984562]

95. Schoepfer KJ, Xu Y, Wilber AA, Wu W & Kabbaj M Sex differences and effects of the estrous stage on hippocampal-prefrontal theta communications. Physiol Rep. 2020;8:e14646. [PubMed: 33230976]
96. Adhikari A, Topiwala MA & Gordon JA Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron. 2010;65:257–269. [PubMed: 20152131]
97. Padilla-Coreano N et al. Hippocampal-Prefrontal Theta Transmission Regulates Avoidance Behavior. Neuron. 2019;104:601–610.e604. [PubMed: 31521441]
98. Bordiuk OL, Smith K, Morin PJ & Semënov MV Cell Proliferation and Neurogenesis in Adult Mouse Brain. PLOS ONE. 2014;9:e111453. [PubMed: 25375658]
99. Li Y-D et al. Hypothalamic modulation of adult hippocampal neurogenesis in mice confers activity-dependent regulation of memory and anxiety-like behavior. Nature Neuroscience. 2022;25:630–645. [PubMed: 35524139]
100. Berdugo-Vega G et al. Increasing neurogenesis refines hippocampal activity rejuvenating navigational learning strategies and contextual memory throughout life. Nature Communications. 2020;11:135.
101. Alam MJ et al. Adult Neurogenesis Conserves Hippocampal Memory Capacity. The Journal of Neuroscience. 2018;38:6854–6863. [PubMed: 29986876]
102. Revest JM et al. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Molecular Psychiatry. 2009;14:959–967. [PubMed: 19255582]
103. Hill AS, Sahay A & Hen R Increasing Adult Hippocampal Neurogenesis is Sufficient to Reduce Anxiety and Depression-Like Behaviors. Neuropsychopharmacology. 2015;40:2368–2378. [PubMed: 25833129]
104. Tanapat P, Hastings NB, Reeves AJ & Gould E Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci. 1999;19:5792–5801. [PubMed: 10407020]
105. Montes P et al. Progesterone treatment in rats after severe global cerebral ischemia promotes hippocampal dentate gyrus neurogenesis and functional recovery. Neurological Research. 2019;41:429–436. [PubMed: 30762490]
106. Barha CK, Ishrat T, Epp JR, Galea LA & Stein DG Progesterone treatment normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus after traumatic brain injury. Exp Neurol. 2011;231:72–81. [PubMed: 21684276]
107. Bali N et al. Differential responses of progesterone receptor membrane component-1 (Pgrmc1) and the classical progesterone receptor (Pgr) to 17β-estradiol and progesterone in hippocampal subregions that support synaptic remodeling and neurogenesis. Endocrinology. 2012;153:759–769. [PubMed: 22147012]
108. Lagace DC, Fischer SJ & Eisch AJ Gender and endogenous levels of estradiol do not influence adult hippocampal neurogenesis in mice. Hippocampus. 2007;17:175–180. [PubMed: 17286277]
109. Yi P et al. The effects of estrogen-responsive element- and ligand-induced structural changes on the recruitment of cofactors and transcriptional responses by ER alpha and ER beta. Mol Endocrinol. 2002;16:674–693. [PubMed: 11923465]
110. Jacobsen BM & Horwitz KB Progesterone receptors, their isoforms and progesterone regulated transcription. Mol Cell Endocrinol. 2012;357:18–29. [PubMed: 21952082]
111. Levin ER Plasma membrane estrogen receptors. Trends Endocrinol Metab. 2009;20:477–482. [PubMed: 19783454]
112. Sellers K, Raval P & Srivastava DP Molecular signature of rapid estrogen regulation of synaptic connectivity and cognition. Front Neuroendocrinol. 2015;36:72–89. [PubMed: 25159586]
113. Srivastava DP et al. Rapid estrogen signaling in the brain: implications for the fine-tuning of neuronal circuitry. J Neurosci. 2011;31:16056–16063. [PubMed: 22072656]
114. Funakoshi T, Yanai A, Shinoda K, Kawano MM & Mizukami Y G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. Biochemical and Biophysical Research Communications. 2006;346:904–910. [PubMed: 16780796]
115. Chen CC, Lee WR & Safe S Egr-1 is activated by 17beta-estradiol in MCF-7 cells by mitogen-activated protein kinase-dependent phosphorylation of ELK-1. J Cell Biochem. 2004;93:1063–1074. [PubMed: 15449318]

116. DiCarlo LM, Vied C & Nowakowski RS The stability of the transcriptome during the estrous cycle in four regions of the mouse brain. J Comp Neurol. 2017;525:3360–3387. [PubMed: 28685836]
117. Iqbal J et al. Estradiol Alters Hippocampal Gene Expression during the Estrous Cycle. Endocr Res. 2020;45:84–101. [PubMed: 31608702]
118. García-Gutiérrez MS, Navarrete F, Laborda J & Manzanares J Deletion of Dlk1 increases the vulnerability to developing anxiety-like behaviors and ethanol consumption in mice. Biochem Pharmacol. 2018;158:37–44. [PubMed: 30268817]
119. Dong H-W, Swanson LW, Chen L, Fanselow MS & Toga AW Genomic-anatomic evidence for distinct functional domains in hippocampal field CA1. Proceedings of the National Academy of Sciences. 2009;106:11794–11799.
120. Lim SH et al. Synapse formation regulated by protein tyrosine phosphatase receptor T through interaction with cell adhesion molecules and Fyn. Embo j. 2009;28:3564–3578. [PubMed: 19816407]
121. Bell O, Tiwari VK, Thomä NH & Schübeler D Determinants and dynamics of genome accessibility. Nature Reviews Genetics. 2011;12:554–564.
122. Rowley MJ & Corces VG Organizational principles of 3D genome architecture. Nature Reviews Genetics. 2018;19:789–800.
123. Le Dily F & Beato M Signaling by Steroid Hormones in the 3D Nuclear Space. Int J Mol Sci. 2018;19:306. [PubMed: 29360755]
124. Le Dily F et al. Hormone-control regions mediate steroid receptor-dependent genome organization. Genome Res. 2019;29:29–39. [PubMed: 30552103]
125. Magnani L & Lupien M Chromatin and epigenetic determinants of estrogen receptor alpha (ESR1) signaling. Mol Cell Endocrinol. 2014;382:633–641. [PubMed: 23684889]
126. Buenrostro JD, Wu B, Chang HY & Greenleaf WJ ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. Curr Protoc Mol Biol. 2015;109:21.29.21–21.29.29.
127. Holmes A, Yang RJ, Lesch K-P, Crawley JN & Murphy DL Mice Lacking the Serotonin Transporter Exhibit 5-HT1A Receptor-Mediated Abnormalities in Tests for Anxiety-like Behavior. Neuropsychopharmacology. 2003;28:2077–2088. [PubMed: 12968128]
128. Nishitani N et al. Manipulation of dorsal raphe serotonergic neurons modulates active coping to inescapable stress and anxiety-related behaviors in mice and rats. Neuropsychopharmacology. 2019;44:721–732. [PubMed: 30377380]
129. van Berkum NL et al. Hi-C: a method to study the three-dimensional architecture of genomes. J Vis Exp. 2010;39:1869.
130. Heisler LK, Zhou L, Bajwa P, Hsu J & Tecott LH Serotonin 5-HT(2C) receptors regulate anxiety-like behavior. Genes Brain Behav. 2007;6:491–496. [PubMed: 17451451]
131. Ressler KJ et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature. 2011;470:492–497. [PubMed: 21350482]
132. Marco A et al. Mapping the epigenomic and transcriptomic interplay during memory formation and recall in the hippocampal engram ensemble. Nat Neurosci. 2020;23:1606–1617. [PubMed: 33020654]
133. Bevington SL et al. Inducible chromatin priming is associated with the establishment of immunological memory in T cells. Embo j. 2016;35:515–535. [PubMed: 26796577]
134. Wijchers PJ & Festenstein RJ Epigenetic regulation of autosomal gene expression by sex chromosomes. Trends Genet. 2011;27:132–140. [PubMed: 21334089]
135. Su Y et al. Neuronal activity modifies the chromatin accessibility landscape in the adult brain. Nature Neuroscience. 2017;20:476–483. [PubMed: 28166220]
136. Vierbuchen T et al. AP-1 Transcription Factors and the BAF Complex Mediate Signal-Dependent Enhancer Selection. Molecular Cell. 2017;68:1067–1082.e1012. [PubMed: 29272704]
137. Duclot F & Kabbaj M The estrous cycle surpasses sex differences in regulating the transcriptome in the rat medial prefrontal cortex and reveals an underlying role of early growth response 1. Genome Biol. 2015;16:256. [PubMed: 26628058]

138. Protopopescu X et al. Hippocampal structural changes across the menstrual cycle. Hippocampus. 2008;18:985–988. [PubMed: 18767068]

139. Lisofsky N et al. Hippocampal volume and functional connectivity changes during the female menstrual cycle. NeuroImage. 2015;118:154–162. [PubMed: 26057590]

140. Barth C et al. In-vivo Dynamics of the Human Hippocampus across the Menstrual Cycle. Scientific Reports. 2016;6:32833. [PubMed: 27713470]

141. Dubol M et al. Neuroimaging the menstrual cycle: A multimodal systematic review. Frontiers in Neuroendocrinology. 2021;60:100878. [PubMed: 33098847]

142. Sundström Poromaa I & Gingnell M Menstrual cycle influence on cognitive function and emotion processing-from a reproductive perspective. Front Neurosci. 2014;8:380. [PubMed: 25505380]

143. Albert K, Pruessner J & Newhouse P Estradiol levels modulate brain activity and negative responses to psychosocial stress across the menstrual cycle. Psychoneuroendocrinology. 2015;59:14–24. [PubMed: 26123902]

144. Kundakovic M & Rocks D Sex hormone fluctuation and increased female risk for depression and anxiety disorders: From clinical evidence to molecular mechanisms. Frontiers in Neuroendocrinology. 2022;66:101010. [PubMed: 35716803]

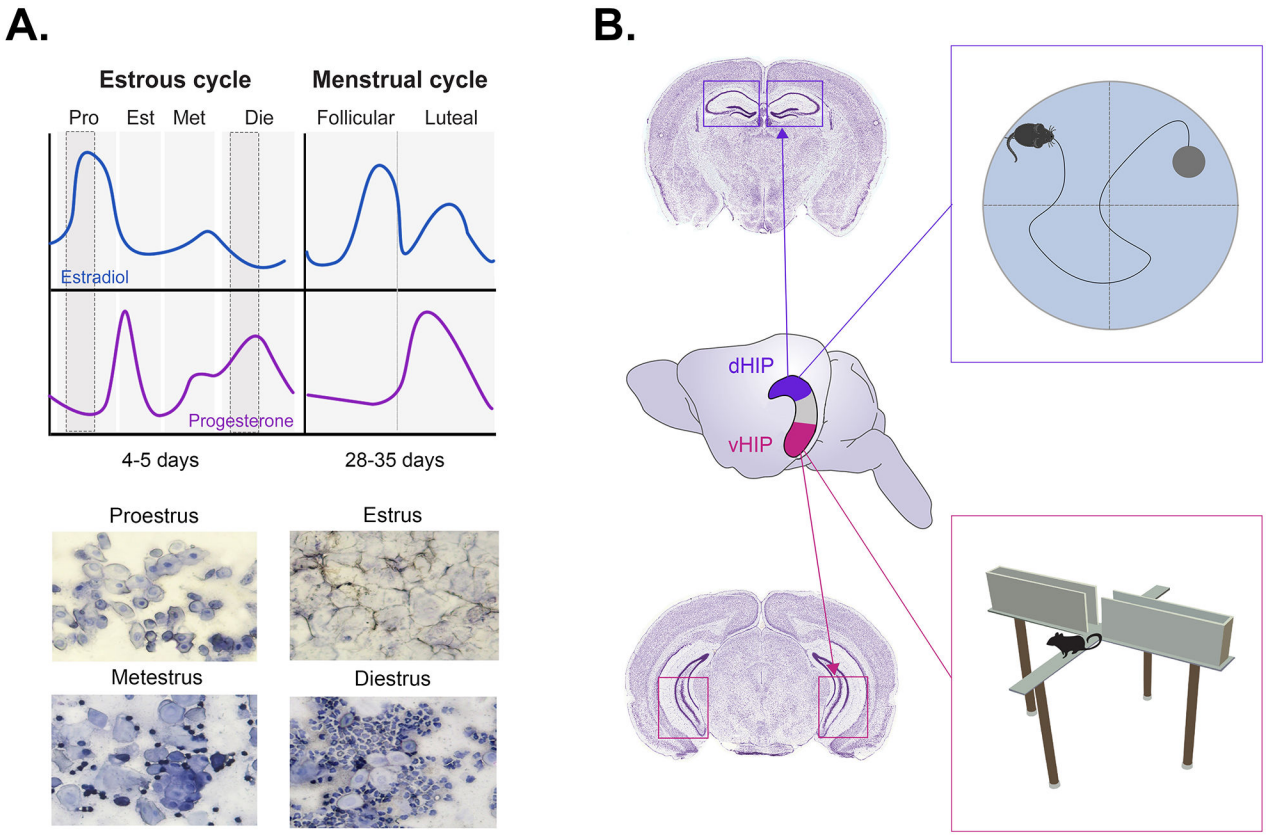

Figure 1. Fluctuating ovarian hormones and hippocampal function.

A. Ovarian hormone fluctuations occuring across the mouse estrous cycle (left) and human menstrual cycle (right). Shaded are the proestrus and early diestrus phases, which mimic the human follicular and luteal phases, respectively. The corresponding vaginal cytology used to determine each estrous cycle phase is shown below (adapted from Jaric et al.[35]). **B.** The hippocampus has two functionally distinct subregions, with the rodent dorsal hippocampus (dHIP) affecting behavior in learning and memory tasks such as the depicted Morris water maze, and the ventral hippocampus (vHIP) affecting anxiety-related behaviors measured in tests which include the elevated plus maze (shown below). Coronal brain sections showing the dHIP and vHIP (boxed) are also depicted (adapted from Sunkin et al.[17]). Pro, proestrus; Est, estrus; Met, metestrus; Die, diestrus.

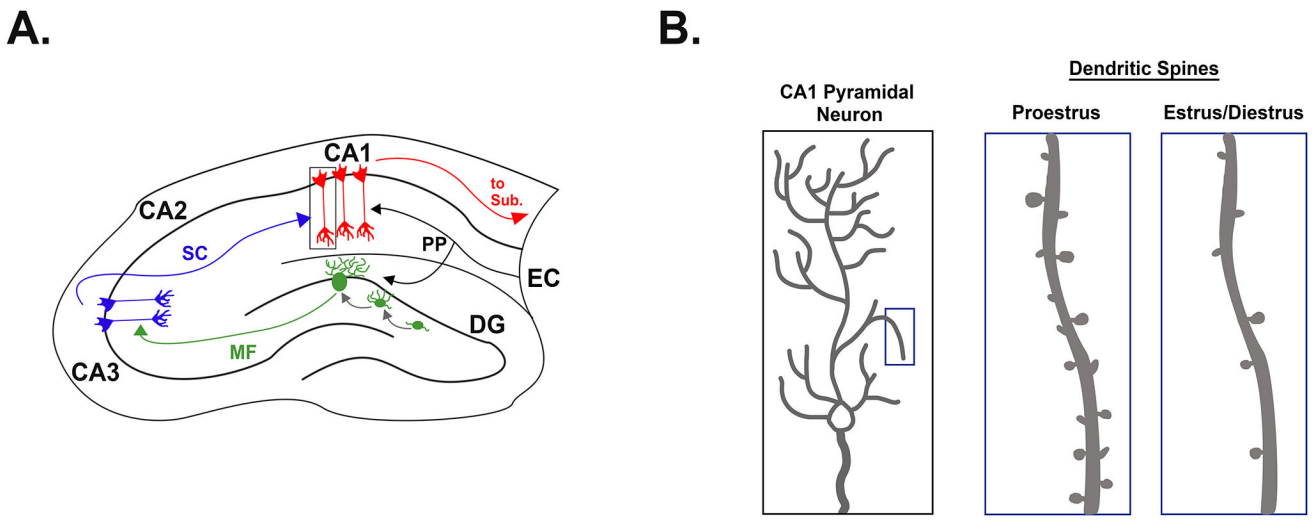

Figure 2. Circuit and structure of the hippocampus and hippocampal neurons.

A. A schematic of hippocampal circuitry is depicted. Signals enter from the entorhinal cortex (EC) through the perforant pathway (PP) where they synapse with CA1 pyramidal neurons and dentate gyrus (DG) granule neurons, which undergo turnover via neurogenesis. DG neurons project to CA3 neurons via mossy fibers (MF), and CA3 neurons project to CA1 via the Schaffer collateral (SC). CA1 neurons project to other brain regions, including the subiculum (Sub.). **B.** A single CA1 pyramidal neuron is depicted, with an inset focusing on a dendrite. Work in both the dorsal[78, 79] and ventral[35] hippocampus demonstrated rapid changes in the density of dendritic spines on these neurons occurring between the high-estrogenic proestrus and lower-estrogenic estrus and diestrus phases of the estrous cycle.

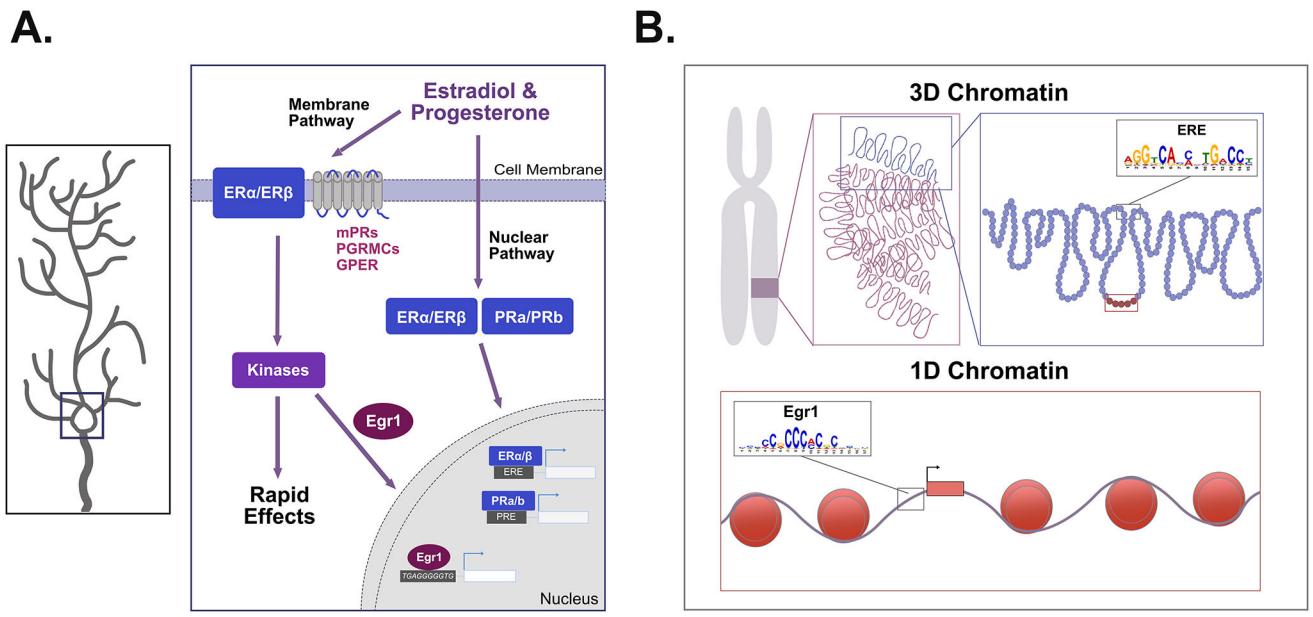

Figure 3. Molecular mechanisms underlying ovarian hormone actions in the hippocampus.

A. A schematic showing, in the cell body of hippocampal neurons, ovarian hormones acting through membrane or intra-cellular receptors, whose actions converge in the nucleus where both pathways can elicit genomic effects. **B.** In the nucleus, chromatin acquires 3D structure that includes chromatin loops. Differential loops and other 3D chromatin interactions over the estrous cycle exhibit enrichment of EREs[36] (implicating the nuclear ER in 3D chromatin regulation). On the level of 1D chromatin, regions which gain accessibility during the proestrus phase are enriched for Egr1 binding sites (implicating the membrane ER) and are proximal to genes important for neuronal function[35].