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# Oestradiol as a neuromodulator of learning and memory

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## Abstract

Although hormones such as glucocorticoids have been broadly accepted in recent decades as general neuromodulators of memory processes, sex steroid hormones such as the potent oestrogen 17β-oestradiol have been less well recognized by the scientific community in this capacity. The predominance of female subjects in studies of oestradiol and memory, and the general (but erroneous) perception that oestrogens are 'female' hormones, have probably prevented oestradiol from being more widely considered as a key memory modulator in both sexes. Indeed, although considerable evidence supports a crucial role for oestradiol in regulating learning and memory in females, a growing body of literature indicates a similar role in males. This Review discusses the mechanisms of oestradiol signalling and provides an overview of oestradiol's effects on spatial, object-recognition, social and fear memories. Although the primary focus is on data collected in females, effects of oestradiol on memory in males will be discussed, as will sex differences in the molecular mechanisms that regulate oestrogenic modulation of memory, which may have important implications for the development of future cognitive therapeutics.

## ToC blurb

Sex steroid hormones such as the potent oestrogen 17β-oestradiol ($E_2$) have only recently started to be acknowledged as important neuromodulators. Taxier, Gross and Frick review $E_2$ signalling in the brain and its effects on different types of memory.

## Introduction

Oestrogens, androgens and progestogens are most commonly associated with their roles in reproduction, despite their involvement in a broad range of physiological and neural functions. One particular realm in which these sex-steroid hormones exert wide-ranging effects is that of learning and memory. In particular, considerable effort has been devoted to

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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understanding how 17β-oestradiol ($E_2$), the most potent and prevalent circulating oestrogen, regulates the function of the hippocampus, a bilateral medial temporal lobe structure with extensive connections to numerous cortical and subcortical brain regions. Although the hippocampus is not the only brain region important for memory, damage or dysfunction to this structure leads to deficits in the formation and retention of numerous types of memories, including those used for spatial navigation, recognizing objects and conspecifics, and recalling fear-associated contexts. However, roles for sex-steroids in regulating the function of mnemonic brain regions like the hippocampus have rarely been considered by scientists other than neuroendocrinologists.

In the early 1990s, a series of papers describing the effects of oestrogens on neuronal morphology and plasticity in the hippocampus was paradigm shifting for the field of neuroendocrinology[1–5]. These publications demonstrated that dendritic spine density on CA1 pyramidal neurons in the hippocampus fluctuates across the reproductive (oestrous) cycle of female rats, and is increased in bilaterally ovariectomized **[G]** female rats by exogenous $E_2$ treatment[3]. Other contemporaneous work showed that $E_2$ potentiates hippocampal excitatory synaptic plasticity[5–7] and protects hippocampal neurons from drug-induced excitoxicity[8]. Collectively, this body of work unlocked a new research frontier that has broadened understanding of how $E_2$ regulates hippocampal function to modulate memory. Since their publication, these findings have inspired much research examining actions of $E_2$ and other sex-steroid hormones in non-reproductive, pro-cognitive brain regions including the hippocampus and prefrontal cortex (PFC). A subset of this newer research has unveiled very rapid actions of $E_2$ on molecular and cellular mechanisms in the hippocampus, PFC, amygdala and other regions that facilitate memory-consolidation processes.

Although the myriad effects of oestrogens such as $E_2$ on hippocampal function have been intensely studied by neuroendocrinologists for nearly three decades, oestrogens have yet to be as widely accepted in neuroscience as general neuromodulators of cognitive processes. As oestrogens exert numerous effects in multiple cognitive brain areas in both sexes[9,10,11–13], elucidating how these hormones regulate memory could promote better mental health outcomes in people of all genders by identifying potential therapeutic targets for neurodegenerative diseases, mood disorders and other conditions in which memory dysfunction features prominently. Effective treatments for memory loss are sorely lacking, highlighting an urgent need to characterize the neural mechanisms underlying memory formation, including those mediated by oestrogens. Moreover, such information will greatly inform our general understanding of how memories are formed. Thus, more broadly considering oestrogens and related sex-steroid hormones as important neuromodulators of memory formation will provide fundamental insights into the neurobiology of memory and new avenues for drug development.

The goal of this Review is to highlight the importance of $E_2$ as a critical modulator of synaptic plasticity and memory circuitry. Although other sex-steroid hormones can regulate memory, the influence of $E_2$ on learning and memory is the most extensively documented and thus $E_2$ is the focus of this Review. The discussion will centre upon the effects of $E_2$ in rats and mice because their size and short lifespans afford convenient mammalian systems in

which to explore molecular mechanisms of $E_2$ action in the brain, and thus the preponderance of data are from these species. However, non-human primate studies (reviewed in[11–13]) have also provided insights about oestrogenic regulation of memory and neuronal morphology that corroborate findings from rodent studies, highlighting the translational potential of this work. Here, we first outline molecular mechanisms underlying $E_2$ signalling in the rodent brain, including both classical and non-classical signalling. Next, we describe several forms of learning and memory modulated by $E_2$ in rodents, and the mechanisms thus far identified that underlie this mediation. Although findings from female rodents are the primary focus, data from males are also discussed, as are sex differences in the neural mechanisms through which $E_2$ influences memory. Last, we conclude by discussing the broad health implications of considering $E_2$ as a neuromodulator, and speculate about next steps necessary to further advance knowledge about oestrogenic regulation of memory. We hope readers will be convinced of the importance of $E_2$ as a general neuromodulator that, like stress hormones and growth factors, should be considered in mainstream models of the neurobiological mechanisms underlying memory.

## Oestradiol signalling

Unlike neurotransmitters, which are stored in vesicles after synthesis for later release, steroid hormones are generated in response to a stimulus and released immediately. Sex-steroid biosynthesis begins with the catabolism of cholesterol to progesterone, which is then broken down into the androgens testosterone and androstenedione, which are subsequently converted to the oestrogens $E_2$ and oestrone, respectively[14,15]. In this synthesis pathway, $E_2$ is generated from testosterone via the enzyme aromatase. The primary sources of sex steroids in both sexes are the gonads, which synthesize progestogens, androgens and oestrogens in varying sex-dependent amounts (with more androgens in males, and more progestogens and oestrogens in females). Hormones synthesized in the gonads can exert paracrine effects on adjacent cells in the gonads, or endocrine effects on distant tissues via the bloodstream[16]. However, other tissues synthesize sex-steroid hormones[14], including adipose tissue, the adrenal glands and numerous brain areas, including the hippocampus[16–19] (Box 1). In the brain, steroids including $E_2$ exert rapid paracrine or synaptocrine effects on neighbouring cells[20,21].

In female mammals, circulating $E_2$ is synthesized by the ovaries, which release fluctuating levels into the bloodstream as part of the reproductive cycle. During the human menstrual cycle and rodent oestrous cycle, oestrogen levels rise to promote follicle maturation in advance of ovulation, peak to stimulate ovulation and then return to baseline as the degenerating follicle secretes progesterone in preparation for implantation of a fertilized egg. In mice and rats, the oestrous cycle consists of four approximately 12–24-hour long stages: proestrus, oestrus, metoestrus and dioestrus[22,23]. $E_2$ levels surge during proestrus and remain high through early oestrus, after which they plunge in late oestrus and remain low throughout metoestrus, rising again during late dioestrus. These fluctuations are reflected in the hippocampus, where $E_2$ levels are substantially higher during proestrus than during other phases[24], as is CA1 dendritic spine density[2] and neurogenesis[25]. Interestingly, hippocampal $E_2$ levels in ovariectomized rats are similar to those of ovary-intact rats in dioestrus and metoestrus[24], reflecting hippocampal $E_2$ synthesis in the absence of the ovaries. As

discussed in Box 1, de novo hippocampal $E_2$ synthesis is activity-dependent and crucial for rapid synaptic plasticity and memory formation. Oestrogen receptor (ER) expression is also influenced by the oestrous cycle[26].

## Classical and non-classical signalling.

Early research demonstrated that sex-steroid receptors act as transcription factors, dimerizing and translocating to the nucleus upon ligand activation to bind hormone response elements **[G]** and regulate gene transcription[27]. $E_2$ binds two ER subtypes, ERα and ERβ, to accomplish this 'classical' mechanism of steroid hormone action, resulting in slow transcriptional changes that become evident within hours to days[28]. However, data from the 1960s revealed that $E_2$ could increase cellular concentrations of cyclic AMP (cAMP) in uterine tissue within seconds[29], a time course too rapid for classical genomic signalling. Similarly rapid effects on the order of seconds to minutes were later found in the brain, where $E_2$ altered neuronal activity in the preoptic area of the hypothalamus[30]. More recent studies have characterized myriad 'non-classical', membrane-initiated mechanisms through which oestrogen rapidly triggers cell signalling and epigenetic processes to produce downstream alterations in gene expression, local protein synthesis, actin polymerization, synaptic physiology and dendritic spine morphology[31–35].

Non-classical oestrogen signalling is initiated at the cell membrane and influences cellular processes through the activation of downstream second messenger pathways. The identities of the ERs responsible for this signalling have been controversial, as the early characterization of ERα and ERβ as purely nuclear receptors seemed to exclude a role in membrane-initiated signalling. However, technological advances have since permitted observation of these receptors at the cell membrane[36–40], and studies using overexpression or deletion of ERα and ERβ demonstrated that these classical receptors are indeed responsible for many of the defining effects of rapid $E_2$ action, including increased cAMP response-element binding protein (CREB) phosphorylation and activation of extracellular signal-regulated kinase–mitogen activated protein kinase (ERK–MAPK) signalling[38,41,42]. Evidence of the classical ERs (that is, ERα and ERβ) in dendritic spines and axon terminals of neurons[43–45] suggests that these receptors could be directly involved in synaptic signalling.

How classical ERs initiate non-classical oestrogen signaling is still not thoroughly understood. The field continues to struggle with technical limitations, such as insufficient antibody validation[46], in studying the subcellular localization of ERs, and many questions remain about the nature of the interaction between classical ERs and the cell membrane. The ERα and ERβ structures do not contain prototypical transmembrane domains[47,48], indicating that these receptors are probably not fully inserted into the membrane. However, ERs undergo post-translational lipidation[49–51] and associate with caveolins **[G]**[52,53]. Both of these factors contribute to the ability of ERs to localize to and associate with the cell membrane[50,53–55].

In addition, how ERα and ERβ, which do not have inherent kinase activity, can interact with and activate downstream second messengers is unclear. One explanation is that ERs localized to the membrane co-opt the signalling machinery of other membrane receptors. At

the membrane, ERα and ERβ can physically interact with and activate metabotropic glutamate receptors (mGluRs) independent of glutamate release, resulting in rapid ERK and CREB phosphorylation[56,57]. Functional coupling of ERs to mGluRs is observed throughout the nervous system and seems to contribute to the effects of $E_2$ on memory consolidation, motivated behaviour and sexual receptivity **[G]**[57–59]. ERs also interact with receptor tyrosine kinases at the membrane, activating signalling at the insulin-like growth factor receptor 1 and the neurotrophin receptor TRKB through both rapid and gene-expression-dependent mechanisms[60–63]. Activation of these receptors subsequently engages neuroprotective and plasticity-related signalling cascades[62–66]. The integration of ERs into a signalling complex with other receptors, G proteins and kinases at the cell membrane is facilitated by multiple scaffold and adaptor proteins. These include: caveolins, which facilitate ER interaction with mGluRs as well as membrane association[50,53,55]; p130CAS, which couples ERs to kinases such as SRC and phosphoinositide 3-kinase (PI3K)[67]; and striatin, which binds caveolins and G proteins at the membrane[68].

Non-classical $E_2$ signalling also occurs through recently identified membrane-localized ERs. Although multiple putative ERs exist, the most well defined and studied is G protein-coupled oestrogen receptor (GPER; also known as GPR30). Characterized in the early 2000s, GPER is a 7-transmembrane-domain G-protein-coupled receptor activated by oestrogens[69,70]. It most frequently interacts with $G_{\alpha/s}$ subunits, leading to increased levels of cAMP and activation of the ERK, protein kinase A (PKA) and PI3K signalling pathways[69–72]. GPER is highly expressed in the brain, including in forebrain regions such as cortex, hippocampus and hypothalamus[73,74], and pharmacological targeting of GPER has implicated it in mediating oestrogen effects on cognitive[75–78], social[79] and reproductive behaviours[80,81]. Given the considerable overlap of GPER and classical ER expression in regions like the hippocampus and PFC, as well as similarities in the outcomes of their activation, an intriguing question is whether these ER subtypes use parallel or distinct mechanisms to modulate neuronal function and behaviour. Current evidence suggests that the answer to this question is complex, with classical ERs and GPER often producing similar or complementary outcomes but through distinct molecular mechanisms[78,82].

### Intracellular cascades.

$E_2$ activation of membrane-localized receptors triggers numerous intracellular signalling events that ultimately influence synaptic plasticity in cognitive brain regions. These downstream cellular and molecular mechanisms have been best elucidated in the hippocampus (Fig. 1). Activation of membrane-localized ERs in cultured hippocampal neurons leads to rapid calcium influx[83,84] and activation of phospholipase C and adenylyl cyclase signalling via G protein activation[56,85]. In turn, these events activate small GTPases like RHOA[35,82] and a host of kinase pathways, including the ERK–MAPK[56,84,86–89], PI3K–AKT[62,89–92], PKA[93–95], protein kinase C (PKC)[56,96] and JUN N-terminal kinase (JNK)[78] pathways. The activation of these second messenger cascades influences processes that regulate synaptic structure and function, such as local protein synthesis, actin polymerization, ion channel dynamics and gene expression.

$E_2$ activation of ERK and AKT stimulates local protein translation by activating mechanistic target of rapamycin (mTOR) signalling and promoting the phosphorylation of translational initiation factors such as 4E-binding protein 1 (4E-BP1)[32,82,97,98], and this new protein synthesis is required for $E_2$-induced spinogenesis in the hippocampus[99]. Recruitment of actin-remodelling pathways also contributes to $E_2$-induced structural plasticity: $E_2$ activation of RHOA elicits phosphorylation of LIM kinase (LIMK) and its substrate, the actin-binding protein cofilin, to promote actin polymerization[35,100–103]. LIMK–cofilin activation and enhanced actin polymerization contribute to $E_2$ effects on spine structure and synaptic transmission[35,100,103]. Moreover, although membrane-initiated $E_2$ signalling is often referred to as 'non-genomic' to differentiate from the classical effects of $E_2$, the intracellular signalling cascades initiated by surface ERs ultimately influence gene expression. $E_2$ activation of ERK signalling rapidly induces phosphorylation of the transcription factor CREB[56,83,88,104] and produces epigenetic modifications such as histone acetylation[31,105,106], both of which increase expression of genes with neurotrophic effects, such as *Bdnf*[106].

### Synaptic function.

$E_2$-induced changes in intracellular signalling ultimately alter synaptic function. In the hippocampus, the general result of $E_2$ exposure is a rapid increase in excitatory neurotransmission. Applying $E_2$ to hippocampal slices increases intrinsic excitability of CA1 pyramidal neurons[107,108], enhances baseline excitatory neurotransmission[5,6,35,109–111] and enhances long-term potentiation (LTP) at CA3–CA1 synapses[35,109,111–114].

The effects of $E_2$ on synaptic function correlate with increases in dendritic spine density[5,110,113,114] and result primarily from regulation of ionotropic glutamate receptors. In hippocampal neurons, $E_2$ influences the phosphorylation and subcellular trafficking of both AMPA receptors (AMPARs) and NMDA receptors (NMDARs)[112,115–120]. AMPAR-mediated excitatory postsynaptic potentials can be positively modulated by $E_2$ [6,35]; however, many of the effects of $E_2$ on synaptic plasticity rely on NMDAR-dependent mechanisms[5,109,110,120,121]. In particular, NMDARs containing the NR2B subunit are required for $E_2$ enhancement of LTP[120,121]. $E_2$ can also increase hippocampal excitability by suppressing inhibitory GABAergic signalling; however, this mechanism varies by sex[122,123].

In sum, considerable data supports that the rapid cell-signalling mechanisms resulting from activation of surface-localized ERs enhance the multiple forms of synaptic plasticity thought to be cellular substrates for learning and memory. As we discuss below, the activation of intracellular signaling, and the resulting effects on neuronal structure and function, are necessary for the beneficial effects of $E_2$ on memory in female rodents.

## E2 modulation of memory in females

The numerous classical and non-classical effects of $E_2$ on brain function provide abundant opportunities for this hormone to influence learning and memory. Copious data supporting a modulatory influence of $E_2$ on hippocampal plasticity has led most rodent researchers to focus on forms of learning and memory mediated by the hippocampus, such as spatial- and object-based memories. However, oestrogens also regulate other types of learning and

memory, including social learning, social discrimination, and fear memory mediated by the amygdala, perirhinal cortex, prefrontal cortex, and other regions. Thus, the sections below will focus not only on the hippocampus, but also on these brain regions as well.

Studies of ER-null mice[114,124–126] or viral vectors to modulate ERα expression[127] have suggested key roles for ERα and ERβ in various forms of memory. However, pharmacological manipulations are the most common method of interrogating roles for $E_2$ and ERs in memory processes (Table 1). Although the compounds used afford better temporal specificity than genetic manipulations, they lack absolute specificity for one ER over the other. However, most can be used at doses that promote preferential binding to a single ER. Ongoing synthesis of more potent and selective ER compounds will undoubtedly help pinpoint discrete functions of specific ERs[128].

Most studies examining the neuromodulatory role of $E_2$ have been conducted in bilaterally ovariectomized female rodents, although some reports have examined memory in naturally cycling females or in males (with or without gonadectomy **[G]**). Although the widespread use of ovariectomized females has limitations that require cautious considerations about extrapolating effects to the natural cycle, this model has permitted more systematic investigations of oestrogen actions than is possible in the presence of daily ovarian hormone fluctuations. Much of this work has used young rodents (2–3 months old), but a rich literature also exists on the mnemonic effects of $E_2$ in middle-aged rodents (for example, 14–19 months old) and aged rodents (older than 20 months)[129,130] (Box 1). Besides animal age, there are several other experimental variables that influence behavioural and mechanistic outcomes. Timing of administration relative to testing, length of administration (for example, chronic (that is, over several days) or acute (that is, a single dose)) and dose can vary across studies, making direct comparisons across studies challenging. Moreover, when $E_2$ is administered systemically, the site of $E_2$ action is often unknown. Indeed, given that $E_2$ is also synthesized in the brain (Box 2), differentiating the effects of brain-derived $E_2$ from systemically administered $E_2$, or from gonadal $E_2$ in ovary-intact rodents, poses a unique challenge. In addition, age at ovariectomy and the duration between ovariectomy and first $E_2$ treatment have also been demonstrated to affect oestrogenic regulation of memory[129,130]. The age at testing and duration of oestrogen deprivation are particularly important, as ER expression in the rodent hippocampus declines not only with age, but also several months after bilateral ovariectomy[131,132]. Combined, these data highlight the importance of experimental parameters when designing studies or interpreting data. The sections below mirror the preponderance of literature using young ovariectomized rodents (but see Box 2 and reviews[129,133–135] for additional information relevant to ageing female and male rodents). As a comprehensive synthesis of all types of memory modulated by $E_2$ is beyond the scope of this Review, the text below focuses on selected forms of memory and behavioural tasks that have proved especially useful to pinpoint the neural mechanisms underlying $E_2$-facilitated memory.

## Spatial and object memory

The tasks most commonly used to assess hippocampal-dependent spatial and object memory include the Morris water maze (MWM), the radial arm maze (RAM), and object recognition

(OR) and object placement (OP; also known as object location) tasks (Fig. 2). In the MWM and RAM, animals use extra-maze cues to traverse a round or wheel-shaped maze to escape from water or locate a food or water reward. Memory in these tasks is typically measured across multiple days, enabling within- and between-subject performance to be assessed over time. Whereas both the MWM and RAM involve explicit motivational stimuli to compel performance, the OR and OP tasks rely on the animal's inherent proclivity for novelty. That is, mice and rats will spend more time investigating a novel object, or a familiar object in a novel location, than a previously investigated object in a familiar location. One advantage of these single-trial learning tasks is that they permit learning assessment while minimizing appetitive or aversive confounds. Moreover, the rapidity with which object tasks are learned allows effects of acute hormone administration to be associated with specific neural events and discrete phases of memory formation (including acquisition **[G]**, consolidation **[G]** and retention **[G]**).

### Morris water maze.

Studies using the MWM to test oestrogenic regulation of spatial reference memory present conflicting data on the role of $E_2$ in this form of memory. For example, chronic systemic $E_2$ given to ovariectomized rats or high $E_2$ levels during the rat oestrous cycle can impair spatial learning and memory[136–138], whereas high $E_2$ levels in cycling mice were associated with enhanced spatial learning[139], and ovariectomized rats given systemic $E_2$ 72 and 48 hours before testing exhibited better memory for a hidden platform[140]. Moreover, high plasma $E_2$ in ovary-intact female meadow voles correlated with longer latency to locate the escape platform during acquisition trials, whereas lower plasma $E_2$ was associated with better learning[141]. Given the varied reported effects of $E_2$ level on spatial MWM learning in different rodent studies, $E_2$ dose and species may determine how $E_2$ affects spatial memory in this task.

Although few studies have examined the acute effects of intracranially administered $E_2$ on MWM performance, existing research suggests beneficial effects. For example, $E_2$ infusion into the dorsal hippocampus of young ovariectomized rats immediately, but not 3 hours, after spatial MWM training facilitates retention 24 hours later, indicating that $E_2$ specifically enhances spatial memory consolidation[142].

### Radial arm maze.

The RAM allows researchers to disambiguate effects of $E_2$ on spatial reference memory **[G]** and working memory (Fig. 2). Broadly, chronic systemic $E_2$ injections in ovariectomized rats enhance spatial working memory in the RAM, an effect that manifests most prominently after several days of $E_2$ administration[143–145]. Consistent with this, chronic systemic $E_2$ treatment in rats rescues ovariectomy-induced deficits in working memory, but not reference memory[146]. Similarly, intracerebroventricular $E_2$ infusion reduces working memory errors in ovariectomized rats[147]. As such, $E_2$ appears to facilitate spatial working memory, but not spatial reference memory, in the RAM among young ovariectomized rodents.

Many brain regions, including the PFC, are required to coordinate the effects of $E_2$ in the RAM, although the effects of $E_2$ in these brain regions differ by dose. For example, spatial

working memory in ovariectomized rats was facilitated by infusion of a high, but not a low, dose of $E_2$ into the PFC, and infusion of a low, but not high, dose into the dorsal hippocampus[148]. Thus, effects of $E_2$ on spatial working memory in the RAM may depend on both dose and brain region[148].

### Single-trial learning.

It is difficult to disaggregate the rapid non-classical effects of $E_2$ from its long-term classical effects in tasks such as the MWM and RAM, because learning in these tasks requires multiple trials across several days. Moreover, both tasks involve motivational components (for example, escape stress and thirst or hunger) to compel performance, which may alter how the brain responds to $E_2$. By contrast, single-trial learning tasks such as OR and OP afford more precise assessment of the neural mechanisms through which $E_2$ facilitates memory formation. As illustrated below, findings from studies using these tasks to assess the effects of $E_2$ on spatial and recognition memory are considerably more consistent than those from maze studies.

As in the MWM and RAM, memory in the OR and OP tasks is negatively affected by ovariectomy[149,150]. However, unlike in the MWM and RAM, exogenous $E_2$ consistently improves spatial and object recognition memory in the object tasks. For example, systemic $E_2$ given immediately post-training enhances memory consolidation in the OR and OP tasks among young ovariectomized rats and mice.[151–153]. However, systemic $E_2$ does not enhance memory when administered 2 hours after training[152], suggesting that the window in which $E_2$ exerts pro-cognitive effects on OR and OP memory consolidation is time-limited and probably dependent on non-classical mechanisms.

$E_2$ infused immediately, but not 3 hours, post-training into the dorsal hippocampus similarly enhances memory consolidation in the OR and OP tasks, indicating that the dorsal hippocampus has a crucial role in mediating the rapid effects of $E_2$ on memory consolidation[57,87,154,155]. The perirhinal cortex also plays a part, as $E_2$ infused immediately post-training into the perirhinal cortex of ovariectomized rats enhanced their preference for a novel object over a familiar object compared with vehicle-treated controls[156]. However, perirhinal $E_2$ infusions also impaired object memory consolidation in a delayed-non-match-to-sample task **[G]**, complicating the role of this brain region in mediating the influence of $E_2$ on OR memory[156,157]. Overall, these data suggest that $E_2$ generally enhances OR and OP memory consolidation in young ovariectomized rodents when it is administered either pre-training or immediately post-training.

### Molecular mechanisms.

The memory-enhancing effects of $E_2$ in the hippocampus involve numerous cellular and molecular events in female rodents. Several cell-signalling cascades are rapidly activated in response to $E_2$, including the ERK, mTOR and PI3K–AKT pathways; activity in these pathways is required for $E_2$ to enhance memory consolidation in the OR and OP tasks among ovariectomized mice[57,87,91,97,158]. Within the dorsal hippocampus, these signalling cascades are initiated by $E_2$ binding to hippocampal ERα and ERβ, but interestingly, not to GPER[57,78]. Indeed, although GPER activation in the dorsal hippocampus enhances OR and

OP memory consolidation in ovariectomized females, these effects are independent of $E_2$ signalling[78].

The ability of $E_2$ to activate dorsal hippocampal cell signalling is closely tied to its ability to mediate epigenetic processes associated with facilitated memory. ERK activation is necessary for $E_2$ to increase acetylation of histone H3 within the dorsal hippocampus of ovariectomized mice[105,106]. Dorsal hippocampal $E_2$ infusion also decreases levels of histone deacetylases 2 and 3 in the hippocampus of ovariectomized mice, thereby providing a mechanism for increased H3 acetylation in the $E_2$-treated hippocampus[105,106]. Importantly, infusion of a histone acetylation inhibitor prevents $E_2$ from enhancing OR memory consolidation in ovariectomized mice, demonstrating an crucial role for histone acetylation in the mnemonic effects of $E_2$[31,105].

In ovariectomized females, systemic $E_2$[159] and intrahippocampally administered $E_2$ increase the dendritic spine density of CA1 pyramidal neurons, an effect dependent on ERK and mTOR signalling[99]. Consistent with these data, elevated $E_2$ robustly influences hippocampal synaptic plasticity by facilitating LTP[121,160,161], which correlates with enhanced memory in hippocampus-dependent tasks[120,162].

Although much remains to be learned, researchers are well on their way towards characterizing the neurobiological mechanisms through which $E_2$ modulates spatial and object memory consolidation.

## Social cognition

The term ‘social cognition’ encompasses various processes, but perhaps the most well studied in animals is social recognition. Memory for conspecifics and past social interactions allows for the formation of meaningful relationships and selection of appropriate behavioural responses in future social interactions, which are crucial abilities in social species[163]. Social discrimination is heavily influenced by neuroendocrine mechanisms; however, much of this research has focused on the neuropeptides oxytocin and vasopressin, so only more recently have sex-steroid hormones become appreciated as modulators of social memory[164].

Ovary-intact female mice trained on a habituation–dishabituation task (Fig. 2) during proestrus show better discrimination of a novel conspecific 24 hours later relative to females trained in dioestrus, suggesting that elevated $E_2$ during training may improve long-term social recognition[165]. Other studies specifically testing effects of exogenous $E_2$ on social recognition in ovariectomized rodents have found that systemic $E_2$ given before training improves social memory in a habituation–dishabituation task[166–168].

Many of these studies use chronic $E_2$ replacement before training and then test memory at extended timepoints, suggesting that classical actions of $E_2$ are involved. Indeed, $E_2$ action at ERα increases transcription of the oxytocin receptor in the medial amygdala (MeA)[169], a region where oxytocin signalling is crucial for social recognition memory[170]. However, rapid membrane-initiated $E_2$ signalling also contributes substantially to these effects. In a modified social discrimination task where acquisition and recall occur within 40 minutes of

drug administration, systemic $E_2$ enhanced social recognition memory in ovariectomized rats[33]. The relatively short period between training and testing makes it unlikely that classical $E_2$ mechanisms have a role in this effect, thereby implicating membrane-initiated signalling as the key mediator. Furthermore, rapid $E_2$-induced CA1 plasticity correlates with this memory enhancement; within 40 minutes of systemic or intracranial administration, $E_2$ increased CA1 dendritic spine density in ovariectomized mice, which was surprisingly associated with reduced AMPAR-mediated signalling[33,119]. This change in excitatory signalling may reflect the generation of new silent synapses **[G]**.

The effects of $E_2$ on social recognition memory are mediated by both classical receptors and GPER. ERα plays a particularly important role, with several studies reporting that ERα-null or ERα-deficient female rodents show impaired social recognition memory[165,169,171,172]. Systemic injection of the ERα-selective agonist PPT (propyl pyrazole triol) improved memory among ovariectomized mice in a social discrimination task within 40 minutes, suggesting that rapid action of membrane-localized ERα is important for this effect[173]. The GPER agonist G1 induces similarly rapid improvements in social recognition memory when systemically injected in ovariectomized mice[77]. However, whether ERα and GPER initiate similar cellular and molecular mechanisms to facilitate social recognition memory is unclear. Interestingly, the facilitative effects of ERα and GPER on social recognition do not seem to extend fully to ERβ. Social recognition memory in female ERβ-null mice is either unaffected or only modestly impaired[165,169,171], and treatment with the ERβ-selective agonist DPN (diarylpropionitrile) in ovariectomized mice either impairs or does not improve social recognition memory[173].

Similar to spatial and object recognition memory, the hippocampus is a critical locus of $E_2$ action for social recognition memory. Infusions of $E_2$, PPT or G1 directly into the dorsal hippocampus of ovariectomized mice induce a rapid enhancement of social recognition memory that is correlated with increased CA1 spine density and modulation of excitatory neurotransmission[119,174]. However, $E_2$ also modulates the function of other brain regions crucial for social recognition memory. For example, knockdown of ERα in the MeA of ovariectomized female rats abolishes social recognition memory[172], and infusion of ERα and GPER agonists into the MeA of ovariectomized mice rapidly enhances social recognition[175]. An integrated understanding of how $E_2$ works across multiple brain regions, receptor subtypes and signalling mechanisms to influence social cognition and recognition behaviour remains an area ripe for future research.

## Fear memory

The study of fear conditioning and its underlying circuitry and mechanisms is a cornerstone of the learning and memory field. Nevertheless, despite its prominence, researchers have until relatively recently largely ignored how sex-steroid hormones such as $E_2$ affect fear learning. Existing work reveals an interesting, and at times contradictory, role for $E_2$ in modulating both the acquisition and extinction **[G]** of fear memory.

### Fear acquisition.

Studies examining how $E_2$ modulates fear acquisition report both enhancing and impairing effects. For example, chronic systemic $E_2$ enhanced cued and context-dependent fear conditioning in ovariectomized mice[176,177], and fear-potentiated startle in ovariectomized rats[178], suggesting that $E_2$ enhances acquisition of fear learning. However, other findings reveal impaired contextual fear conditioning **[G]** (CFC) in proestrus rats relative to males or rats in oestrus[179]. Similarly, ovary-intact or ovariectomized rats given $E_2$ for 2 days before CFC froze less than did males or untreated ovariectomized rats[180], suggesting $E_2$ may impair fear learning.

These seemingly contradictory findings probably result from differences in experimental approach, with dose and timing of $E_2$ administration as important variables. High-dose systemic $E_2$ days or weeks before CFC enhanced acquisition in ovariectomized mice, with lower doses of $E_2$ having no effect[181,182]. By contrast, when $E_2$ was injected into ovariectomized rats 30 minutes before CFC training, high doses impaired acquisition, whereas a low dose enhanced learning[183]. These results suggest important differences in how $E_2$ dose and treatment duration interact to impact fear learning, but further research is needed to more clearly define these relationships.

### Fear extinction.

Research on the role of $E_2$ in modulating fear extinction paints a clearer picture than does the fear acquisition literature, with evidence from passive avoidance tasks[184], conditioned taste aversion[185] and both cued and conditioned fear conditioning[186–188] supporting the conclusion that $E_2$ improves extinction learning. In ovary-intact female rats, fear-extinction training during proestrus produces more rapid learning across trials[186] and reduced freezing during extinction recall testing[187] compared with extinction during metoestrus. $E_2$ seems to be both necessary and sufficient for these effects, as systemic $E_2$ given to metoestrus or ovariectomized rats enhances extinction learning[186,187], whereas systemic blockade of ERs in proestrus rats impairs extinction recall[187]. Similarly, reducing circulating $E_2$ via hormonal contraceptives impairs extinction recall in cycling female rats[189].

As with fear acquisition, the influence of $E_2$ on extinction is dose-dependent. In cycling female rats, $E_2$ exhibits an inverted-U dose–response effect on extinction learning, such that rats with very low (untreated metoestrus) or very high (proestrus or metoestrus given high dose $E_2$) levels of $E_2$ exhibited poor extinction recall, and rats with moderate $E_2$ levels (untreated proestrus or metoestrus given low dose $E_2$) extinguished well[190].

ERβ is an important mediator of the effects of $E_2$ on fear extinction. Systemic injection of the ERβ-selective agonist DPN before extinction training improves extinction learning in ovariectomized or metoestrus rats, whereas the ERα-selective agonist PPT has no effect[186,188]. Infusion of DPN into the dorsal hippocampus of ovariectomized rats enhanced contextual fear extinction learning, suggesting that this brain region is an important locus for ERβ activity in this paradigm[186]. However, $E_2$ has more pervasive actions in the neurocircuitry of fear extinction beyond the hippocampus. The amygdala, a central region in fear extinction, shows widespread expression of ER subtypes[16] and structural and synaptic

plasticity following $E_2$ treatment[191,192]. The infralimbic cortex (IL) sends excitatory projections that regulate amygdala subregions during extinction[193], and is also sensitive to oestrogens[194]. $E_2$ seems to modulate the activity and connectivity of these regions during extinction recall; studies using FOS **[G]** as a marker of neuronal activity report that metoestrus rats treated with $E_2$ displayed less amygdalar activation[188,195] and greater IL activation[188] following extinction recall than did untreated controls. Both the oestrous-cycle phase and exogenous $E_2$ treatment influence the activity of IL–amygdala projections in female rats[195,196], suggesting that $E_2$ increases IL-driven activation of an inhibitory circuitry in the amygdala that reduces fear responses.

### Fear generalization.

In addition to fear acquisition and extinction, $E_2$ also influences fear generalization **[G]**. Ovariectomized rats treated with systemic $E_2$ show increased generalization of fear to neutral contexts relative to untreated controls[197]. Similar to extinction, this effect seems to depend primarily on hippocampal ERβ signalling, as infusion of the ERβ-selective agonist DPN, but not ERα-selective PPT, into the hippocampus of ovariectomized rats increases fear generalization[198].

That $E_2$ can enhance both fear expression (via acquisition and generalization) as well as extinction may at first seem contradictory, but is not surprising given that these processes share certain underlying molecular mechanisms. Moreover, fear acquisition, generalization and extinction all require new learning. Thus, the effects of $E_2$ on all aspects of fear learning highlight its actions as a general promoter of learning and memory.

Together with findings from the spatial, object and social memory tasks discussed above, the fear memory data suggest that $E_2$ is uniquely positioned as a general neuromodulator that broadly facilitates new learning of many sorts.

## E2 modulation of memory in males

Although oestrogens are often (incorrectly) considered ‘female’ hormones, accumulating research indicates a primary role for $E_2$ in enhancing cognition in male rodents. Indeed, $E_2$ levels in the male rat hippocampus are higher than in that of cycling females[199,200]. Emerging findings, reviewed below, imply that $E_2$ may influence memory via distinct molecular mechanisms in males and females. Why might these differences matter if the beneficial effects on memory are similar in both sexes? From a drug discovery standpoint, these data suggest the potential need to develop sex-specific treatments for memory dysfunction in humans. Treatments that target mechanisms through which $E_2$ modulates memory in one sex (for example, ERK in females), but not in the other, are bound for failure in half the population. Additional research to determine which oestrogenic mechanisms are sex-specific and which are common to both sexes will substantially advance the development of effective memory therapeutics for both men and women.

### Sex differences in hippocampal $E_2$ signalling.

In the hippocampus, $E_2$ acts as a neuromodulator in both males and females, but an emerging literature has found notable sex differences in how $E_2$ influences hippocampal

neurotransmission and the molecular underpinnings of these effects. In both sexes, $E_2$ potentiates excitatory neurotransmission in the hippocampus[6,35,111], and although some molecules and pathways, such as SRC, ERK–MAPK, calcium/calmodulin-dependent protein kinase II (CAMKII) and TRKB signalling, contribute to these effects in both sexes[35,201,202], others are sex-specific. For example, potentiation of excitatory post-synaptic currents and LTP in the hippocampus depend on PKA in female rats, but not in males[202]. The sexes also differ in the ER subtypes that mediate neural excitation, as hippocampal glutamatergic neurotransmission is regulated presynaptically by ERβ in females and ERα in males, and postsynaptically by GPER in females and ERβ in males[111]. Other work has similarly shown differential contributions of ERα versus ERβ in facilitating LTP between males and females[35,201]. Differing roles of ER subtypes in modulating excitatory signalling may arise from sex differences in subcellular ER localization, which in turn could lead to sex-specific recruitment of signalling kinases by $E_2$[167,201].

Unlike excitatory signalling, inhibitory hippocampal neurotransmission seems to be modulated by $E_2$ only in females and through a sex-specific mechanism. In the ovariectomized female rat hippocampus, ERα activates post-synaptic mGluR1 signalling to stimulate endocannabinoid release, which inhibits presynaptic GABAergic terminals[122]. The functional coupling of ERs with mGluR subtypes has previously been shown to occur only in females[56] and further investigation has found that although ER–mGluR complexes can exist in both sexes, $E_2$ only activates mGluR-dependent signalling in females[123].

A similar sex difference has been found with locally synthesized $E_2$ in the hippocampus. Although male and female rodents both express aromatase and synthesize $E_2$ in the hippocampus[17,200,203], systemic aromatase inhibition reduces hippocampal synapse number and severely impairs LTP induction in female mice, but has no effect on synapse number and only modestly reduces LTP in males[204]. Similarly, aromatase-null female mice were shown to have reduced hippocampal spine density compared with wild-type female mice, whereas spine density was unaffected in male aromatase-null mice[205]. However, in a forebrain-specific aromatase-null mouse, both males and females exhibited reduced hippocampal spine density[206]. Understanding the cellular and molecular consequences of local oestrogen synthesis in the male hippocampus will require further investigation[207].

### Oestrogenic modulation of cognition in males.

Despite differences in the mechanisms of $E_2$ signalling in cognition-related regions of the brain, $E_2$ exerts similar mnemonic benefits in males and females. Systemic injection or dorsal hippocampal infusion of $E_2$ given immediately post-training enhances memory consolidation in the OR and OP tasks in gonad-intact and gonadectomized male rats and mice[208,209]. Notably, sex differences in the mechanisms that drive the beneficial effects of $E_2$ on these tasks have been found: in contrast to its central importance to the memory-enhancing effects of $E_2$ in ovariectomized mice, dorsal hippocampal ERK phosphorylation is not necessary for $E_2$ to facilitate spatial and object memory consolidation in male mice, regardless of gonadal status[209]. In the MWM, gonad-intact adult male rats and aged male mice treated with systemic $E_2$ immediately post-training exhibited enhanced spatial memory compared with vehicle-treated males[210,211]. Gonad-intact adult males receiving chronic $E_2$

also made fewer working memory errors in the RAM than did vehicle-treated controls[212]. Local synthesis of oestrogens is also important for memory processes in male rodents, as aromatase-null male mice exhibit deficits in social recognition[213]. Furthermore, acute oral aromatase inhibition increases errors in a working memory task in male rats[214], and acute intrahippocampal aromatase inhibition prevents object and spatial memory consolidation in gonadectomized male mice[215]. As such, the data thus far suggest that hippocampally-synthesized $E_2$ is important for memory formation in male rodents, as has been observed in female rodents. Although much more remains to be learned, these selected findings support a beneficial effect of $E_2$ on memory formation in male rodents.

## Conclusions and future directions

This Review has illustrated some of the myriad ways in which $E_2$, acting in several brain regions via multiple ERs, can facilitate numerous forms of learning and memory (Fig. 3). Three decades of research have demonstrated that $E_2$ is a potent regulator of the neural mechanisms that are critically important for memory formation, including cell signalling, gene expression, protein synthesis, extrinsic and intrinsic excitability, dendritic spine morphology and neurogenesis. Thus, researchers studying the neurobiology of learning and memory should recognize oestrogens as essential neuromodulators akin to other well-accepted modulatory factors such as glucocorticoids and growth factors.

Widespread acceptance of oestrogenic neuromodulation may have been historically slow to take hold because of the strong association between oestrogens and female reproduction, which has led to the erroneous perceptions that oestrogens are ‘female’ hormones and that cyclic oestrogen fluctuations in females confound experimental outcomes due to increased variability. In fact, oestrogens are important modulators of memory and neural function in both sexes[216,217]. Depending on the behaviour being assessed, ovary-intact females do not necessarily exhibit more behavioural variability than males[218], which argues for greater consideration of the modulatory influence of oestrogens in females and in males. Future progress on this issue may stem from the US National Institutes of Health’s 2016 policy requiring vertebrate animal researchers to consider sex as a biological variable[219–221], as more explicit comparisons between the sexes could lead to new insights about hormonal regulation of cognition. Although some investigators were initially resistant to this policy, attitudes towards the requirement are improving[222]. Greater appreciation of oestrogens as neuromodulators that exert wide-ranging effects in all individuals — not just females — is crucial for the advancement of both basic and clinical science.

To further advance knowledge about oestrogenic modulation in both sexes, future studies should exploit newer technologies including single-cell sequencing, ‘omics’-level analyses, and targeted genetic manipulations to pinpoint crucial molecules and cellular processes that underlie oestrogenic facilitation of learning and memory. For example, a multiplexed CRISPR–Cas9 gene-editing approach could be used to simultaneously target multiple oestrogen-responsive genes implicated in neurodegenerative disease to understand their role in memory function[223]. Future research must also evolve from a focus on oestrogenic effects in individual brain regions to addressing how oestrogens concurrently influence multiple brain areas within memory circuits. Recent work using multiplexed chemogenetic silencing

has shown that the memory-enhancing effects of $E_2$ in the dorsal hippocampus requires concurrent activity of the dorsal hippocampus and PFC[155]. Thus, determining how brain regions interact synergistically to support oestrogen-mediated memory processes is a crucial next step for the field. As part of this approach, researchers should also consider cell-type specificity, as memory-modulating effects of $E_2$ on cell types such as inhibitory neurons and glial cells have been overshadowed by a predominant focus on excitatory neurons.

By leveraging new technologies and asking circuit-level and cell-type-specific questions in both males and females, scientists will discover fundamental new insights into the ways in which $E_2$-induced modulation of brain function influences memory formation. Given the dearth of effective treatments for memory dysfunction in various disorders, this information could provide valuable new avenues for therapeutic development that benefits both sexes.

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## Glossary terms

**Ovariectomized**
Ovariectomy involves surgical removal of the ovaries to eliminate ovarian hormone cycling. Subjects that have undergone ovariectomy are considered ovariectomized.

**Hormone response elements**
Short DNA sequence within the promoter region of a gene that binds a hormone receptor complex to enable gene transcription.

**Caveolins**
Integral membrane proteins that form functional microdomains of receptors and their associated signaling proteins at the plasma membrane.

**Sexual receptivity**
A positive state of responsivity towards the initiation of sexual behaviour by another individual. Often indicated by a species-specific mating posture.

**Gonadectomy**
Surgical removal of the gonads (ovaries or testes); because ovariectomy is the preferred term for females, gonadectomy is most commonly used for males.

**Acquisition**
A process through which information is learned through physical or sensory interaction with environmental stimuli.

**Consolidation**
Process through which learned information is encoded and stored to form a memory that can be recalled at a later time.

**Retention**
Storage of acquired and consolidated information that enables subsequent recall or retrieval of the information.

**Spatial reference memory**
Memory for locations that do not change over time — for example, the layout of buildings on a college campus — used for navigating through an environment.

**Spatial working memory**
Memory for locations that change over time — for example, the locations of your keys or your car in your campus parking lot.

**Delayed non-match-to-sample task**
Test of memory for items that differ from an initial stimulus array, assessed at some delay after original stimulus presentation.

**Silent synapses**
Immature synapses containing few AMPA receptors, which could allow for greater synaptic potentiation and learning facilitation upon interaction with a training stimulus.

**Extinction**
Process whereby a learned association between two stimuli (for example, shock occurs in context A) becomes unlearned through repetitive exposure to one stimulus (context) without the other (shock).

**Contextual fear conditioning**
Model of fear learning in which repeated exposure to footshocks in one context eventually elicits fear (freezing) to the context in the absence of shock.

**FOS**
An immediate early gene and transcription factor that is activated rapidly and transiently in response to neuronal activity, leading to expression of memory-related genes.

**Generalization**
Process whereby a stimulus–response association learned in one context (for example, a stimulus induces fear) becomes transferred to another similar context.

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**Box 1 |**

### A role for hippocampally synthesized oestrogens in memory

Although the gonads are a primary source of oestrogens in both sexes, oestrogens are synthesized in numerous tissues, including the brain. Of most relevance to learning and memory, the enzyme aromatase, which converts testosterone to 17β-oestradiol ($E_2$), is widely expressed in the brain and has been shown to produce $E_2$ locally in regions such as the hypothalamus and hippocampus[17,18,200,237,238]. In the hippocampus, neuron-derived de novo $E_2$ supports multiple aspects of synaptic plasticity, including synaptogenesis and long-term potentiation (LTP)[19,204,239,240]. In hippocampal cultures from female rats, pharmacologically blocking de novo $E_2$ synthesis with the aromatase inhibitor letrozole results in reduced spine density, decreased expression of synaptic proteins, and impaired LTP[19,204,239].

Interestingly, neural $E_2$ synthesis seems to be regulated by neuronal activity. For example, activation of NMDA receptors in cultured hippocampal neurons or in hippocampal slices increased $E_2$ synthesis[200,203], and exposure to a learning stimulus in ovariectomized mice increased de novo $E_2$ synthesis in the hippocampus, an effect blocked by letrozole[226]. In vivo, systemic letrozole treatment decreases CA1 dendritic spine density and levels of hippocampal synaptic proteins in both ovary-intact and ovariectomized females[240], demonstrating that neuron-derived $E_2$ contributes to hippocampal synaptic plasticity regardless of other sources of the hormone.

The central importance of de novo hippocampal $E_2$ synthesis in both sexes is illustrated by studies in which letrozole infusion into the dorsal hippocampus of gonadectomized male or female mice impaired consolidation of spatial and object recognition memory[215,226]. Similarly, hippocampal implants of the aromatase inhibitor ATD impaired spatial memory in male zebra finches[241], suggesting that the importance of hippocampal $E_2$ synthesis is conserved across species. Supporting this conclusion are recent data showing oral letrozole treatment impaired spatial working memory and reduced hippocampal intrinsic excitability in male and female marmosets[228].

Aromatase outside of the hippocampus also seems to be important for memory consolidation, as infusion of letrozole into the perirhinal cortex of gonad-intact male mice impaired short- and long-term memory in an object placement memory task[224]. In humans, letrozole is an authority-approved treatment for oestrogen receptor (ER)-positive breast cancer; however, women taking letrozole may experience a number of adverse side effects that affect memory[242]. For example, women taking letrozole for ER-positive breast cancer exhibited episodic memory deficits, supporting the idea that brain-synthesized oestrogens are crucial in memory processes[227].

**Box 2 |**

## The effects of oestradiol in ageing rodents

Ageing female rodents are frequently used to examine the effects of oestrogen deprivation and replacement during the transition to reproductive senescence (see refs [133,134,243] for recent reviews). In humans, menopause onset coincides with follicular depletion, whereas oestropause, the menopausal parallel in rodents, is driven by alterations in the function of the hypothalamus–pituitary–gonad axis[244,245]. Given this difference, researchers should be cautious when interpreting studies using rodent models of reproductive ageing. Nevertheless, the ageing rodent model has proved useful in understanding the effects of oestrogen deprivation, timing of hormone therapy administration and age-associated alterations in responsivity to 17β-oestradiol ($E_2$).

Broadly, $E_2$ deprivation as a consequence of ageing or artificial long-term ovarian hormone depletion lowers sensitivity to the memory-enhancing effects of $E_2$. In spatial memory tasks such as the Morris water maze (MWM), systemic $E_2$ administration typically enhances memory in middle-aged (14–19-month-old), but not aged (20-month-old or older), ovariectomized female rodents [246–249]. However, $E_2$ can still facilitate memory in aged females under certain experimental conditions. Chronic, high doses of $E_2$ can elicit better memory in the MWM in aged ovariectomized female rats[250] and in aged female mice[251], and chronic $E_2$ administration over 3 weeks enhanced object memory in aged ovariectomized female mice[252].

Age and duration of oestrogen deprivation also modulate the effects of systemic $E_2$ treatment on spatial reference and working memory. For example, chronic systemic $E_2$ treatment enhanced spatial working memory in middle-aged ovariectomized rats in a water-motivated radial arm maze (RAM) task[253]. However, daily systemic injections of $E_2$ given to ageing female mice ovariectomized at 17.5 months did not affect spatial reference or working memory in a water-motivated RAM task, whereas intermittent $E_2$ treatment mimicking normal hormonal cycling impaired spatial reference and working memory[254]. As with the MWM, chronic $E_2$ treatment enhanced spatial reference memory in aged ovariectomized rats, but only when rats were primed with additional acute injections of $E_2$[255]. These data suggest not only an age-related loss of responsivity to the memory-enhancing effects of $E_2$, but also a detrimental effect of $E_2$ on memory in aged rodents. Supporting the idea that the duration of hormone loss affects responsivity to $E_2$, systemic $E_2$ implants administered to 12- and 17-month-old mice immediately after ovariectomy enhanced spatial working memory in a RAM[256], whereas such $E_2$ treatment given to 17-month-olds 5 months after ovariectomy no longer enhanced spatial working memory. These data support the well-accepted notion that the ageing brain is less responsive to $E_2$ after long periods of oestrogen deprivation.

As in the maze tasks, ageing female rodents or rodents subjected to delayed oestrogen replacement post-ovariectomy exhibit diminished sensitivity to the beneficial effects of $E_2$ in object memory consolidation. For example, rats treated with systemic $E_2$ 9 or 15 months post-ovariectomy exhibited intact memory for a previously seen object[91]. However, increasing the treatment delay to 19 months post-ovariectomy eliminated the

memory-facilitating effects of $E_2$[162], consistent with other findings that $E_2$ typically enhances object recognition and object placement memory in middle-aged, but not aged, female rodents[248,257]. Combined, these data suggest that $E_2$ is less efficacious when given to aged rodents, especially when post-ovariectomy $E_2$ treatment is delayed.

Several potential mechanisms may underlie decreased sensitivity to $E_2$ in older rodents. For example, $E_2$-induced hippocampal transcription is reduced in aged female rodents[258], and several studies suggest that the expression of oestrogen receptors ERα and ERβ diminishes with age[131,259,260]. The precise mechanisms underlying decreased responsivity to $E_2$ in advanced age remain to be determined.

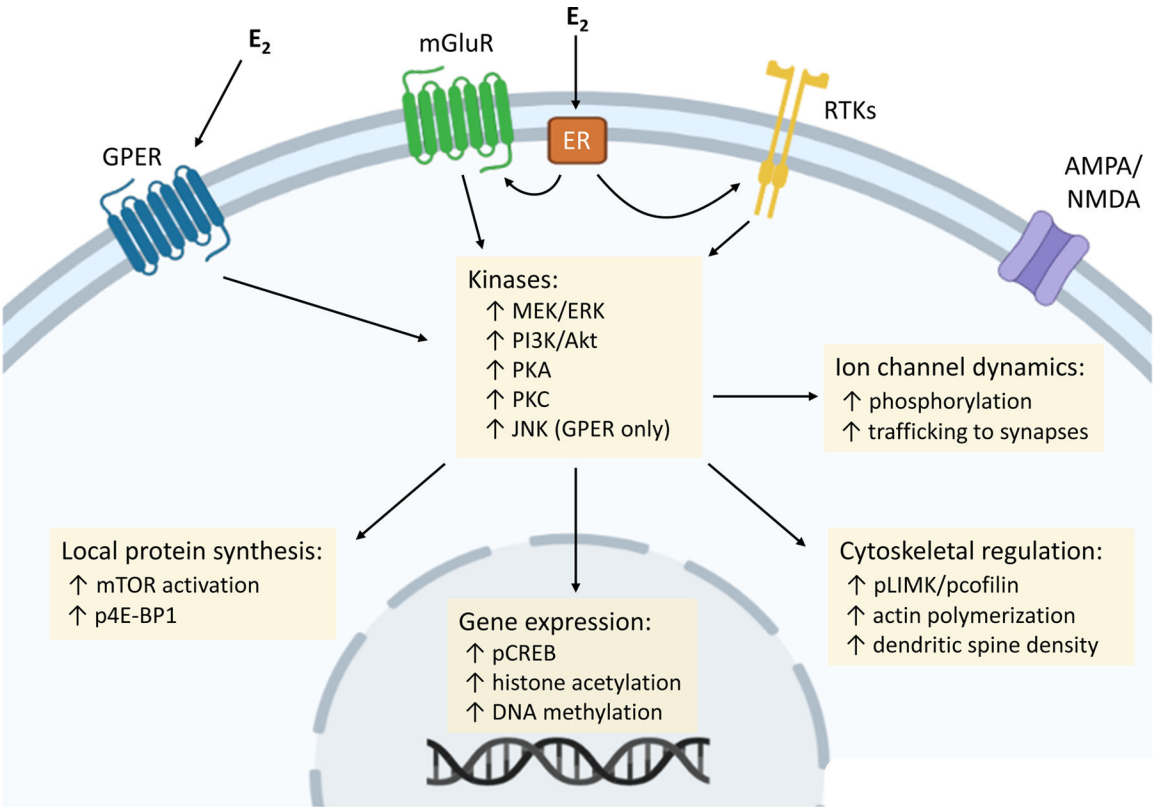


**Fig. 1 |. Membrane-initiated oestrogen signalling and downstream intracellular events.**

Intracellular processes are initiated by 17β-oestradiol ($E_2$) binding to the G protein-coupled oestrogen receptor (GPER), or functional interaction between the canonical oestrogen receptors (ERα and ERβ) and other receptors located at the membrane (such as metabotropic glutamate receptors (mGluRs)). Several kinase cascades, key for the memory-enhancing effects of $E_2$, rapidly increase activity in response to membrane-initiated signalling events. ERα and ERβ seem to trigger similar kinases, whereas GPER activates distinct signalling pathways such as JUN N-terminal kinase (JNK). In turn, kinase activity facilitates additional regulatory processes, including protein synthesis, ion channel phosphorylation and trafficking, gene expression, and cytoskeletal regulation. AMPAR, AMPA receptor; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase kinase; mTOR, mechanistic target of rapamycin; NMDAR, NMDA receptor; p4E-BP, phosphorylated 4E-binding protein 1; pCREB, phosphorylated cAMP response-element binding protein; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; pLIMK, phosphorylated LIM kinase; RTK, receptor tyrosine kinase

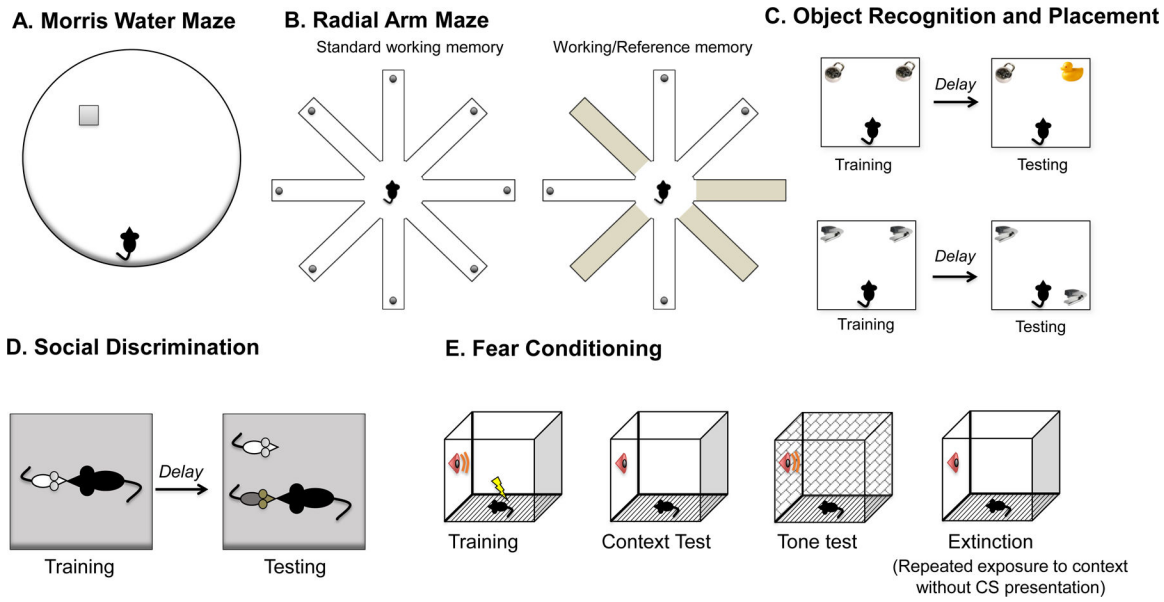


**Fig. 2 |. Behavioural approaches to studying oestrogenic effects on memory.**

**a** | The Morris water maze tests a rodent's ability to navigate in space and remember the location of spatial cues in the environment[229,230]. Rodents are placed in a large round pool of water made opaque with nontoxic paint or powdered milk, and must use extramaze spatial cues to navigate to a platform hidden just below the surface of the water[229,230]. Measures of performance include time to reach the platform, distance swum and swim speed. During probe trials in which the hidden platform is inaccessible to subjects for a portion of, or throughout, the trial, memory for the platform location can be assessed by measuring the number of times that animals cross the platform location and/or the time spent in close proximity to the location of the platform. **b** | The radial arm maze also tests spatial navigation abilities. Food- or water-restricted subjects traverse a wheel-shaped maze in which they must retrieve food or water rewards from the ends of 8 or 12 long arms that radiate from a round central platform[231,232]. Rewards are not replaced, so animals should visit each arm only once. In the standard version, all arms are baited to measure working memory, which is memory for information that changes from trial to trial. In a common variation, half of the arms are baited to measure working memory and half are never baited to measure reference memory, or memory for information that does not change from trial to trial. **c** | Object recognition and object placement tasks typically consist of a training trial, during which animals explore objects in an open field for 5–20 minutes, followed by a delay (minutes to days) and a single testing phase, during which a new object is introduced (object recognition) or a training object is moved (object placement)[233,234]. Because rodents are drawn to novelty, animals that remember the identity and location of the training objects will spend more time than chance with a novel of moved object during testing. **d** | Social recognition can be tested using a habituation–dishabituation task or a social discrimination task. In habituation–dishabituation, subjects are repeatedly exposed to the same stimulus conspecific, leading to a decrease in investigative behavior (habituation) as the stimulus animal becomes known. Animals are then exposed to a novel conspecific, and, if social recognition memory is intact, investigative behaviour returns (dishabituation). In social

discrimination, animals undergo a test trial where they are simultaneously exposed to a familiar conspecific, seen previously in habituation trials, and a novel conspecific. Increased investigative behaviour of the novel conspecific is indicative of intact social recognition memory. **e |** Fear memory is most commonly studied using classical conditioning paradigms that pair a stimulus (a cue or context) with a foot shock, generating a learned association that leads to fear responses (such as freezing) upon subsequent presentations of the conditioned stimulus (CS). Cued and contextual fear conditioning differ in their underlying neurocircuitry, with cued fear being independent of hippocampal function, and contextual fear requiring hippocampal input[235]. Extinction can be tested with repeated presentation of the context in the absence of CS presentation[236]. Figure adapted with permission from Frick and Fortress (2015)[236].

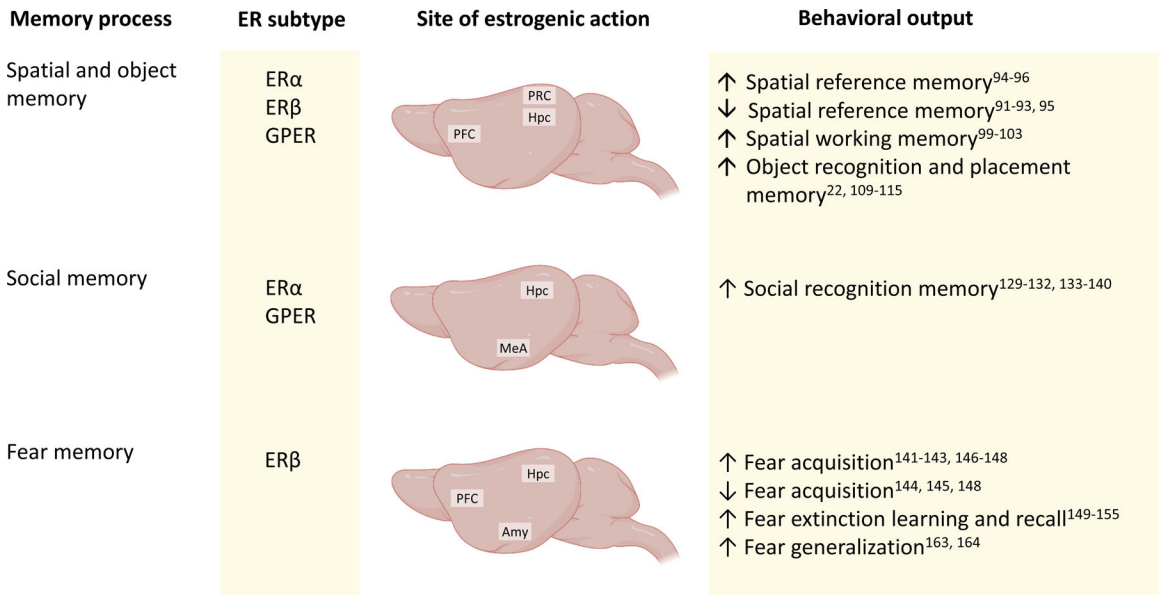


**Fig. 3 |. Summary of oestrogenic actions on memory processes.**

This schematic shows the receptors and brain regions involved in the effects of 17β-oestradiol ($E_2$) on memory processes, and the behavioural output of the actions of $E_2$ in young female rodents. Spatial reference memory[142], working memory[148] and object memory[57,87,154,155,173] are facilitated by $E_2$ (but see discussion in main text), and are largely dependent on the hippocampus (HPC) and prefrontal cortex (PFC). The perirhinal cortex (PRC) is also involved in $E_2$-mediated object recognition memory[156,157]. Social recognition memory is facilitated by oestrogen receptor-α (ERα) and G protein-coupled oestrogen receptor (GPER) signalling in the HPC and medial amygdala (MeA)[119,172–174]. Fear acquisition, fear extinction learning and recall, and fear generalization are regulated by ERβ in the PFC, amygdala (Amy), and HPC[186,188,195,196,198].

**Table 1.**

Compounds used to demonstrate a role for specific estrogen receptors in memory

| Target | Action | Drug | Chemical Name | References |
|---|---|---|---|---|
| ERα | Agonist | • PPT (propyl pyrazole triol) | • 4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol | 29, 125, 150, 177, 197 |
| | Antagonist | • MPP dihydrochloride | • 1,3-*Bis*(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1*H*-pyrazole dihydrochloride | 209, 210 |
| ERβ | Agonist | • DPN (Diarylpropionitrile)<br>• WAY200070 (Benzoxazole)<br>• ERB 041<br>• ISP358–2 | • 2,3-*bis*(4-Hydroxyphenyl)-propionitrile<br>• 7-Bromo-2-(4-hydroxyphenyl)-1,3-benzoxazol-5-ol<br>• 7-Ethenyl-2-(3-fluoro-4-hydroxyphenyl)-5-benzoxazolol<br>• 4-[*trans*-4-(hydroxymethyl)cyclohexyl]phenol | 29, 99, 125, 150, 177, 197 |
| | Antagonist | • PHTPP | • 4-[2-Phenyl-5,7-*bis*(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidin-3-yl]phenol | 125, 209, 210 |
| ERα & ERβ | Antagonist | • ICI 182,780 (Fulvestrant) | • 7α,17β-[9-[(4,4,5,5,5-Pentafluoropentyl)sulfinyl]nonyl]estra-1,3,5(10)-triene-3,17-diol | 62, 175, 197 |
| GPER | Agonist | • G1 | • (1-[4-(6-bromobenzo[1,3]dioxol-5yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethenone) | 51, 53, 79, 148, 150, |
| | Antagonist | • G15 | • (3a*S**,4*R**,9b*R**)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3*H*-cyclopenta[*c*]quinoline | 50, 53, 197 |
| Aromatase | Enzymatic inhibitor | • Letrozole<br>• Anastrozole<br>• ATD | • 4,4'-(1*H*-1,2,4-Triazol-1-ylmethylene)*bis*benzonitrile<br>• α1,α1,α3,α3-Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-benzenediacetonitrile<br>• 1,4,6-androstatriene-3,17-dione | 194–200 |