Synthesis and impact of neuroestradiol on hippocampal neuronal networks
Íñigo Azcoitia¹, Alicia Hernández-Vivanco², Nuria Cano-Adamuz² and Pablo Méndez²

Abstract
The production of estradiol within the brain, that is, neuroestradiol (nE2), is widely documented. nE2 deeply impacts adult brain physiology and synaptic plasticity. In the hippocampus, a region of the brain essential for cognitive function, multiple cellular sources, and targets of nE2 have been identified. The impact of estradiol in excitatory and inhibitory neurotransmission suggests a role for regulated nE2 synthesis in the coordination of the activity of different cellular elements of hippocampal network. Here, we review the role of nE2 in the physiology of the hippocampal circuits taking into account the cellular heterogeneity of the hippocampus. We aspire at expanding the consideration of neuron-derived estradiol as a neuromodulator of hippocampal network activities underlying cognition.

Addresses
¹ Departamento de Biología Celular, Universidad Complutense de Madrid, C José Antonio Nováis 12, 28040, Madrid, Spain
² Instituto Cajal (CSIC), Av Dr. Arce 37, 28002, Madrid, Spain

Keywords
Aromatase, Excitatory neurons, Estradiol, Estrogen, Hippocampus, Inhibitory neurons, Neurosteroids.

Sources of nE2 in the hippocampus
E2 synthesis is a multistep process initiated with the translocation of cholesterol from the outer to the inner mitochondrial membrane and continued in the endoplasmatic reticulum, where pregnenolone is transformed to E2. The expression of the multiple enzymes involved in E2 synthesis has been documented in the hippocampus (Figure 1 and refs therein [12–14,9,15–19]). E2 accumulation at concentrations that exceed that of plasma several-fold has been observed in situ [*18]. Moreover, the administration of aromatase inhibitors (AI) directly into the brain of female mice lacking the
ovaries — the main source of peripheral E2 — or the use of aromatase conditional knock-out from forebrain neurons, have further demonstrated the impact of nE2 synthesis in the hippocampus [10,20,21].

Aromatase mRNA and protein are expressed in the embryonic hippocampus and its expression peaks around birth, coinciding with the critical period for sex differentiation of the brain. After a subsequent
differentially regulate E2 synthesis. Alternatively, increases from cellular and extracellular sources may collectively define neuron populations will surely advance our expression in molecular, neurochemical, and anatomical terms.

NMDA receptors in hippocampal slices increase Ca²⁺ and nE2 production [9,31]. This suggests that Ca²⁺ signaling pathways involving phosphorylation and dephosphorylation of intracellular proteins [32]. Calcium-dependent control of hippocampal nE2 suggests a potential coupling of nE2 synthesis and network activity that could explain the increase of nE2 concentrations observed after learning [33]. In this same line, epileptic hippocampal activity following administration of the glutamate receptor agonist kainate has been shown to induce estradiol production in the hippocampus [34]. Finally, nE2 biological action may be regulated by mechanisms preventing or promoting aromatase degradation and the catabolism of nE2 [35], although the expression of responsible enzymes in the hippocampus is poorly documented [36].

**Regulation of nE2 synthesis**

Estradiol is synthesized on-demand in the ovaries and secreted into the blood in response to signals from the hypothalamic—pituitary—gonadal axis. Due to its lipophilic nature, E2 is difficult to mobilize, which suggests the importance of regulatory mechanisms in aromatase expression and activity. Transcriptional regulation of aromatase gene is achieved by transcription factor binding and activation of multiple response elements contained in tissue and cell type-specific promoters [27]. Additionally, nE2 production is controlled by substrate availability in both male and female hippocampal neurons [28]. Peptidergic control of aromatase by gonadotropin-releasing hormone (GNRH) has been documented. Due to the cyclic nature of GNRH secretion, this mechanism has been proposed to mediate the regulation of hippocampal excitatory synapses and the associated cognitive effects during the estrous cycle [29].

Brain intrinsic mechanisms may additionally control nE2 synthesis. The pioneering work of Balthazart [30] on the rapid modulation of nE2 synthesis in the brain, suggests post-translational control of aromatase through phosphorylation. Aromatase is negatively regulated by the release of intracellular calcium (Ca²⁺), as is the case in dispersed cultured rodent hippocampal neurons [31]. On the other hand, activation of Ca²⁺ permeable NMDA receptors in hippocampal slices increases Ca²⁺ and nE2 production [9,31]. This suggests that Ca²⁺ increases from cellular and extracellular sources may differentially regulate E2 synthesis. Alternatively, aromatase regulation may differ between different hippocampal neuronal populations expressing aromatase, such as excitatory and inhibitory neurons. Neuronal Ca²⁺ concentrations are firmly controlled by action potential and synaptic activity and regulate different signaling pathways involving phosphorylation and dephosphorylation of intracellular proteins [32].

**Targets of nE2: ERs in excitatory and inhibitory neurons**

The actions of nE2 are mediated by binding to different types of cellular ER. ERα and ERβ are ligand-activated transcription factors and are potent regulators of gene expression. GPER, a membrane-bound ER, is a G-protein coupled receptor that controls intracellular signaling [37,38]. In addition to their nuclear function, ERα and ERβ exist in membrane-anchored forms that activate intracellular signaling cascades by interaction with receptor tyrosine kinases and G-protein coupled receptor [38]. ERα, ERβ and GPER are expressed in the hippocampus [38].

Information about the involvement of ER receptors in nE2 actions is scarce and much of the information we have about the potential mechanisms is suggested by experiments that use exogenous E2 applications [39–41]. The effects of aromatase loss of function are recovered by *in vitro* and *in vivo* exogenous administration of E2 [10,21]. Besides, pharmacological or genetic suppression of aromatase increases ERβ expression and reduces ERα in the hippocampus [10,21]. These lines of evidence suggest the implication of ERs on nE2 action in the hippocampus. Much of the research of nE2 in the hippocampus has been focused on excitatory synapse structure and plasticity, in particular in long-term potentiation (LTP), a form of activity-dependent modification of synapse strength with a critical role in learning and memory [42]. These studies report that acute application of an ERα antagonist blocks CA3-CA1 synapse LTP in female mice [8] while both ERα and ERβ antagonists block LTP in male rats [43,44], mimicking the effects of long term *in vivo* treatment of female mice with AI [8,21]. Interestingly, preventing membrane localization of ERα also block LTP in female mice [8]. Although the acute application of AI or ER
antagonists may have different effects on LTP compared with prolonged ones [8,21], these experiments suggest a role for ERα and ERβ in nE2 regulation of female and male synaptic plasticity. These studies have prompted the investigation of ER involvement in learning and memory. Intra-hippocampal administration of ERα antagonists reduces ovariectomized female mice performance on memory for object recognition (both ERα and ERβ antagonists) and spatial location (ERβ antagonist) [45]. These results, together with the reported increase in nE2 concentration detected after training in these memory tests [35], suggest that binding to ERα and ERβ mediate nE2 actions on hippocampal-dependent learning and memory.

The role of ERs on nE2 regulation of inhibitory neurons is relatively less characterized. In principle, nE2 may exert presynaptic effects on inhibition through ERα and ERβ since the expression of both receptors has been documented in inhibitory neurons [46–48]. The Gad 2 promoter has an estrogen response element that drives transcription upon activated ER binding, a potential mechanism for nE2 regulation of GABA synthesis [49]. On the other hand, postsynaptic actions of E2 through ERα on inhibition have been reported [*50]. E2 suppresses GABAergic neurotransmission through ERα mediated activation of retrograde endocannabinoid signaling [51]. Interestingly, ERα and exogenous E2 effects in inhibition seem to converge on a particular subtype of CA1 inhibitory neurons expressing the marker Cholecystokinin (CCK) and modulated by endocannabinoids [52].

**nE2 function on the hippocampus: a network view**

The different hippocampal subdivisions are made of a large variety of neuronal cell types whose coordinated action gives rise to different forms of network activity underlying cognitive functions, including spatial navigation and learning and memory [26,53]. As mentioned above, the most studied aspect of nE2 impact on the hippocampus is CA1 excitatory synaptic function, plasticity and structure. In a nutshell, these studies show that nE2 supports different aspects of CA1 excitatory synapse structure, function and intracellular signaling pathways linked to synaptic plasticity in the female rodent hippocampus (reviewed in Ref. [54]). In addition, E2 has been shown to influence the excitability of hippocampal neurons [55] and decrease neuronal inhibition [51]. nE2 may simultaneously increase CA1 excitatory drive and decrease inhibitory synapse activity and promote in this way plasticity and memory (Figure 2). In line with this view, recent reports confirm that brain aromatase activity, altered through the direct hippocampal infusion of AI [33] or genetic down regulation [10,56] is critical for learning and memory. nE2 regulation of memory processes must be dependent on its ability to modulate the activity of hippocampal regions that participate in specific forms of learning. These include CA1, as mentioned before, but other regions such as DG and CA3, with roles in pattern separation and completion and whose function is also regulated by E2 [57,58], must be considered in order to fully explain nE2 in specific aspects of learning and memory.

Of note, the vast majority of studies have been focused on the plasticity of excitatory synapses of CA1 PYR neurons but similar phenomena may also be present in excitatory synapses of GABAergic cells [**59]. Moreover, IN are extremely diverse and nE2 may differentially affect the intrinsic and synaptic excitability of IN subpopulations. CCK + IN are not the only subtype of IN that are sensitive to nE2. Fluctuating E2 levels regulate parvalbumin (PV) levels in hippocampal INs and modulate cortical PV + IN during social interactions in female rats [46,47]. These two types of IN show an alternating mode of function during mice locomotion and are differently entrained by sharp-wave ripples, rapid hippocampal oscillations with a prominent role in memory consolidation [60]. PV activity increases at locomotion onset and during sharp-wave ripples while CCK IN shows the opposite responses [61]. By impacting the function of these INs, nE2 may regulate network activity associated with different behavioral states (quietness, locomotion, sleep, and so on).

Oscillatory activity in the hippocampus is essential for communication with other brain regions. nE2 regulation of cognitive processes involves interactions between the hippocampus and other brain regions [**62]. However, very little is known about the impact of E2 on hippocampal network oscillations that emerge from the complex but temporally coordinated activity of large groups of neurons, both excitatory and inhibitory. A recent study addressed the role of E2 γ-oscillations, which have a critical role in information binding. Ovariectomy decreases γ-oscillations power when rats are exposed to a novel environment, an effect that is prevented by treatment with E2 [63]. The role of nE2 in driving pathological oscillations has been investigated in the context of epilepsy with interesting results: intracerebral applications of AI reduce intense oscillations associated with epileptic seizures [34]. Although the role of nE2 in regulating oscillatory behavior is far from being understood, these studies highlight the importance of analyzing behaviorally-driven network activity to understand nE2 modulation of hippocampal circuits.

**Sex effects in nE2 function**

The mammalian hippocampus has prevalent yet poorly understood sex differences that range from sexual dimorphisms to graded differences and affect different aspects of inhibitory and excitatory neuronal function [64,65]. In addition, E2 actions in the hippocampus
often show sex effects. E2 activation of the cAMP response element binding protein is only observed in female hippocampal neurons [40]; different ER isoforms are implicated in presynaptic and postsynaptic effects of E2 in excitatory synapses of male and female rodents [41]; inhibitory neurotransmission is reduced by ERα activation only in females [66]. On top of this, several reports point to female-specific effects of nE2 in mice [8,21]. Interestingly, nE2 effects on memory have also been demonstrated to be sex-dependent: AI blocked memory in intact females but not in males [33,67]. In contrast, conditional knock out of aromatase genes in excitatory forebrain neurons reduces memory performance of both males and females [10]. Mammalian sex

Figure 2

Nuclear and synaptic estradiol (E2) effects in the hippocampal trisynaptic circuit. E2 is synthetized in hippocampal pyramidal neurons (Pyr) and GABAergic inhibitory neurons (INs). Both classic and membrane-anchored estrogen receptors (ERs) are present in neuronal somata and pre- and postsynaptic terminals. (a) Nuclear ER modulates gene expression while membrane ER activates signaling intermediates of the MAPK, JunK or the PI3K pathways, including the phosphorylation of the cAMP response element binding protein (CREB). (b) Nuclear E2 signaling is also present in inhibitory neurons expressing ER: the Gad2 (glutamate descarboxilase) gene promoter has an E2 response element and the Pvalb gene (parvalbumin) is positively modulated by E2. (c) to (e) correspond to different examples of E2 contribution to synaptic transmission. (c) E2 reduces CA1 pyramidal neurons inhibition, decreasing the affinity of GABAergic ionotropic receptors (GABAAR) for the scaffold protein gephyrin and the density of postsynaptic GABAAR. Besides, E2 enhances the release of endocannabinoids (eCB) that bind to GABAergic presynaptic CB1 receptors, reducing the exocytosis of synaptic vesicles. (d) E2 contributes to glutamatergic plasticity (long-term potentiation, LTP) through a mechanism dependent on ER and probably by the synthesis of the steroid in the synapse. (e) The synapse between mossy fibers (Mf) and CA3 pyramidal thorny excrescences is also modulated by E2, in this case through the regulation of BDNF (brain-derived neurotrophic factor) in dentate granule cells (Gc).
differences may arise from two different origins: the different complement of sex chromosomes in male (XY) and female (XX) cells and the different hormonal milieu determined by the presence of male or female gonads at late embryonic development, puberty and adulthood [68]. While the female-specific effects of E2 on cAMP response element binding protein signaling is determined by perinatal hormonal milieu [69], whether nE2 actions on the hippocampus depends on sex chromosomes or gonads secretions acting around birth, during puberty, or in adulthood is currently unknown. Understanding the origin of sex differences will surely help to reconcile apparent discrepancies in sex dependency of nE2 effects.

Conclusions and future perspectives
It is increasingly clear that nE2 production affects different regions and cell types involved in information processing and storage in the hippocampus. While many studies have unraveled the importance of nE2 in some of the basic mechanisms of memory, such as neurotransmission and plasticity, hippocampal function relays on the complex interaction between different cell types whose coordinated activity supports specific aspects of hippocampal function. The use of conditional approaches to modify estrogen production and interfere with ER function in a cell type-specific manner is highly needed. Also, transcriptomic approaches will be invaluable to determine cell type-specific responses to nE2. Sex differences in nE2 regulation of hippocampal neurons through the life span may be important to understand sex bias in neurodevelopment and neurodegenerative diseases. Research on nE2 action on the hippocampus will be beneficial for women’s health since it aims to understand the central effects of aromatase inhibitors widely used for breast cancer treatment and the role of brain estradiol synthesis in the postmenopausal brain. This is particularly important in the context of the protracted life expectancy of the aging global population that progressively increases the prevalence of menopause in women’s lifetime. The study of the chromosomal and gonadal origins of sex differences will help us to understand sexual differentiation of the healthy and diseased brain and may have implications for sex determination and gender identity.

Conflict of interest statement
Nothing declared.

Acknowledgements
This work was supported by grants PID2020-112824GB-100 (to PM) funded by MCIN/AEI/ 10.13039/501100011033. N.C.A is supported by the PhD fellowship PRE2018-084857 funded by MCIN/AEI/ 10.13039/501100011033 by “ESF Investing in your future” and Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (to IA).

References
Papers of particular interest, published within the period of review, have been highlighted as:
* of special interest
** of outstanding interest


Exhaustive description of nE2 synthesis in the hippocampus and its regulation by glutamate receptors. Ultrastructural imaging of pre and postsynaptic expression of aromatase protein.

10. Lu Y, Sareddy GR, Wang J, Wang R, Li Y, Dong Y, Zhang Q, et al.: Steroidogenic factor-1 expression was deleted from excitatory forebrain neurons. This cell type-specific approach unveils a critical role for neuroestrogen in synaptic structure, plasticity and hippocampal-dependent learning in male and female mice.

15. Caruso D, Pesaresi M, Abbiati F, Calabrese D, Giatti S, Garcia-Segura LM, Melcangi RC: Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain,


This study shows that a clinically relevant treatment with aromatase inhibitors impairs long term potentiation of excitatory neurotransmission in the hippocampus. The effects observed in intact and ovarioctomized females and, to a lesser extent, in males, suggesting the role of neuroestradiol in regulating basic mechanisms of learning and memory.


The interest of this study is two-fold. First it shows that, in female ovarioctomized mice, intrahippocampal infusions of aromatase inhibitors prevents the increase in hippocampal E2 levels and decrease performance in object recognition and location tests. Second, the authors show that E2 infusion recovers memory performance, suggesting a role of de novo hippocampal E2 synthesis in memory consolidation.


A comprehensive review of E2 actions on the brain mechanism of learning and memory, from molecules and synapses to behavior.


This study addresses E2 and selective ER modulator raloxifene regulation of gamma oscillations, a fundamental form of network activity organized by inhibitory neurons and involved in information processing in the hippocampus. The authors show that ovariectomy suppresses and E2 and raloxifene increases gamma-oscillations during a spatial memory task.


Together with ref. 47, this work describes a mechanism by which estradiol rapidly suppresses inhibition in hippocampal principal neurons through mGluR and endocannabinoids. Interestingly, this works shows sex differences in E2 regulation of the endocannabinoid system, a potent regulator of synaptic transmission in the hippocampus.

