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Reviews

Synthesis and impact of neuroestradiol on hippocampal neuronal networks

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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.

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

Abbreviations

AI, aromatase inhibitors; CCK, cholecystokinin; E2, estradiol; ER, estrogen receptor; GNRH, gonadotropin-releasing hormone; IN, inhibitory neuron; LTP, long-term potentiation; nE2, neurostradiol; PV, parvalbumin; PYR, pyramidal neuron; GABA, γ-aminobutiric acid.

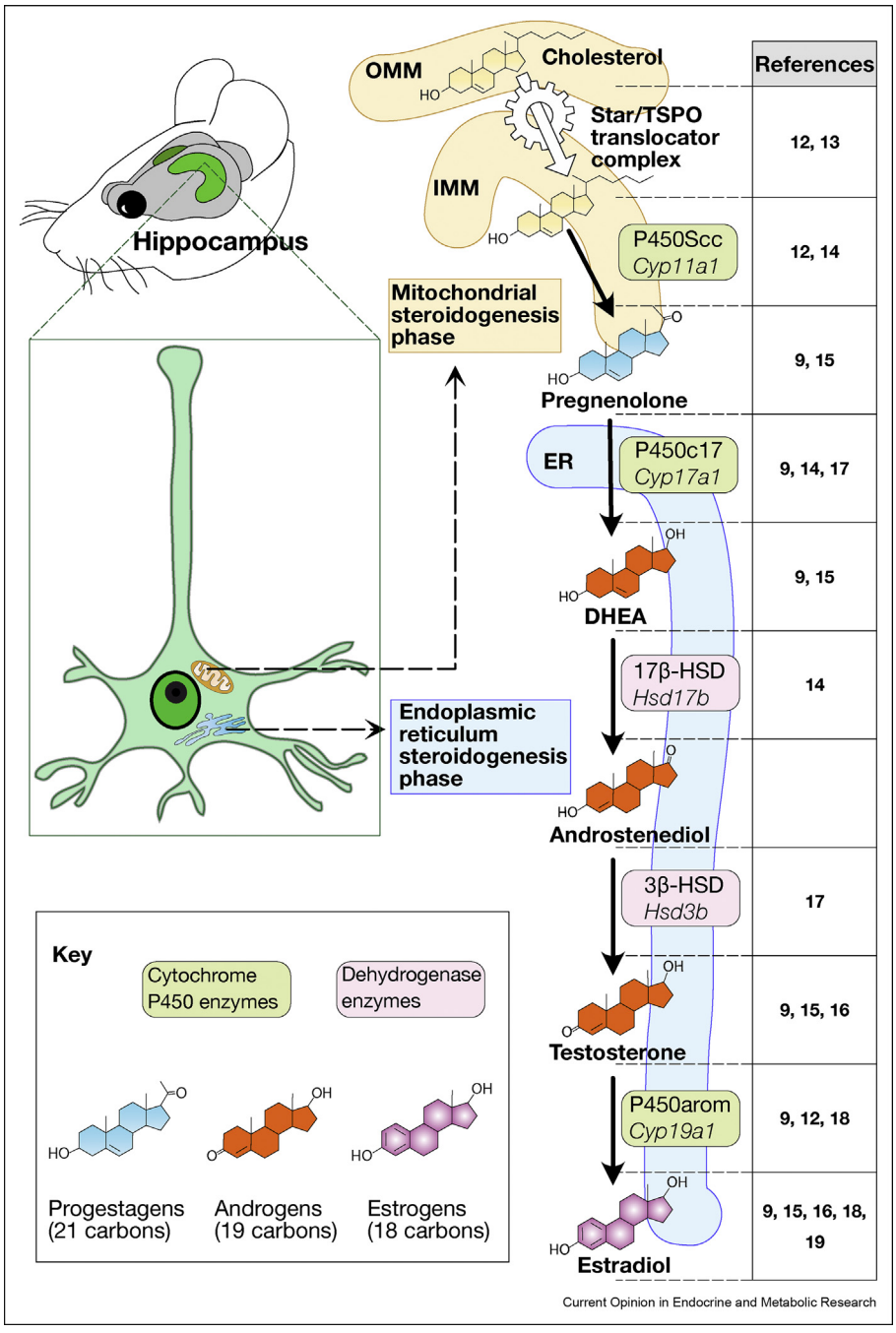
The source matters

The study of estradiol (E2) synthesis within the hippocampus, a brain region with a critical role in spatial navigation and memory, is rooted in several aspects of clinical and basic research: (i) the well-described sex effects in brain development [1] and adult cognitive functions [2]; (ii) the interest in the cognitive effects of hormonal replacement therapy [3]; (iii) the fact that estradiol synthesis blockers commonly used in clinics for breast cancer treatment have been associated to cognitive deficits [4]; (iv) numerous basic research studies documenting the impact of E2 in the regulation of important physiological processes underlying cognition such as neurogenesis [5], neurotransmission and synaptic plasticity [6–8]. Since the pioneering studies on the effects of gonad-derived E2 in hippocampal synaptic structure and physiology, much progress has been done to elucidate the mechanisms used by peripheral hormones to regulate brain function. In addition, the synthesis of E2 within the hippocampus, neuroestradiol (nE2), is now widely documented [*9,**10]. Despite sharing cellular receptors, the estrogen receptors (ERs), nE2, and peripheral E2 independently regulate brain function [11]. This highlights the importance of defining the hippocampal cellular types involved in estradiol action, including those that act as sources, that is, the ones possessing the enzymatic machinery to produce nE2 and those that act as targets, that is, the ones expressing ERs. Here, we review the role of nE2 in hippocampal function. We pay special attention to the current knowledge about the cell types involved in producing and sensing nE2 and the regulatory role of nE2 in the activity of the hippocampal network. Since sex differences likely exist, we review pieces of evidence that suggest that nE2 also regulates critical aspects of male brain function.

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

Figure 1



Synthesis of estradiol (E2) in the hippocampus. The StAR/TSPO complex initiates estradiol synthesis by translocating cholesterol from the outer (**OMM**) to the inner mitochondrial membrane (**IMM**). The IMM enzyme **P450Scc** (cytochrome P450 cholesterol side-chain cleavage enzyme, gene *Cyp11a1*) converts cholesterol in pregnenolone, which is exported to the endoplasmic reticulum, where it enters in the multistep route to render E2. The image depicts a rodent hippocampal neuron, but E2 synthesis takes place also in glial cells. All protein complexes and enzymes involved in E2 synthesis have been detected by immunohistochemistry, Western blotting, in situ hybridization, RT-PCR as reported in the indicated references. **P450c17**: cytochrome P450 17α-hydroxylase; **17β-HSD**: 17β-hydroxysteroid dehydrogenase; **3β-HSD**: 3β-hydroxysteroid dehydrogenase; **P450arom**: cytochrome P450 aromatase. Steroid measurements were performed mainly by RIA, ELISA and mass spectrometry.

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

decrease, the expression increases again around puberty [22]. Despite robust evidence of neuronal and glial expression in the hippocampus, the identity of neuronal subtypes involved in nE2 synthesis has received relatively less attention. In the healthy hippocampus, aromatase is expressed in the main hippocampal subdivisions, namely, dentate gyrus, CA3 and CA1 [23]. The expression of aromatase in CA1 excitatory neurons is suggested by the accumulation of aromatase mRNA and protein immunostaining in the pyramidal cell layer [9,10]. However, multiple cell types populate the CA1 pyramidal layer, including different subtypes of inhibitory neurons (IN) that use ɣ-aminobutiric acid (GABA) as a neurotransmitter. Indeed, aromatase is expressed in hippocampal IN of the human hippocampus [24]. Moreover, CA1 pyramidal (PYR) neurons are not a homogenous population and can be subdivided anatomically and functionally along proximo-distal and deep−superficial axes [25]. Since different excitatory and inhibitory neuronal populations have specific roles in controlling hippocampal activity and cognitive functions [26], accurate determination of aromatase expression in molecular, neurochemical, and anatomically defined neuron populations will surely advance our understanding of nE2 actions in the hippocampus.

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 suggest 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 also 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]. 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].

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 ERs 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 [33], 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.

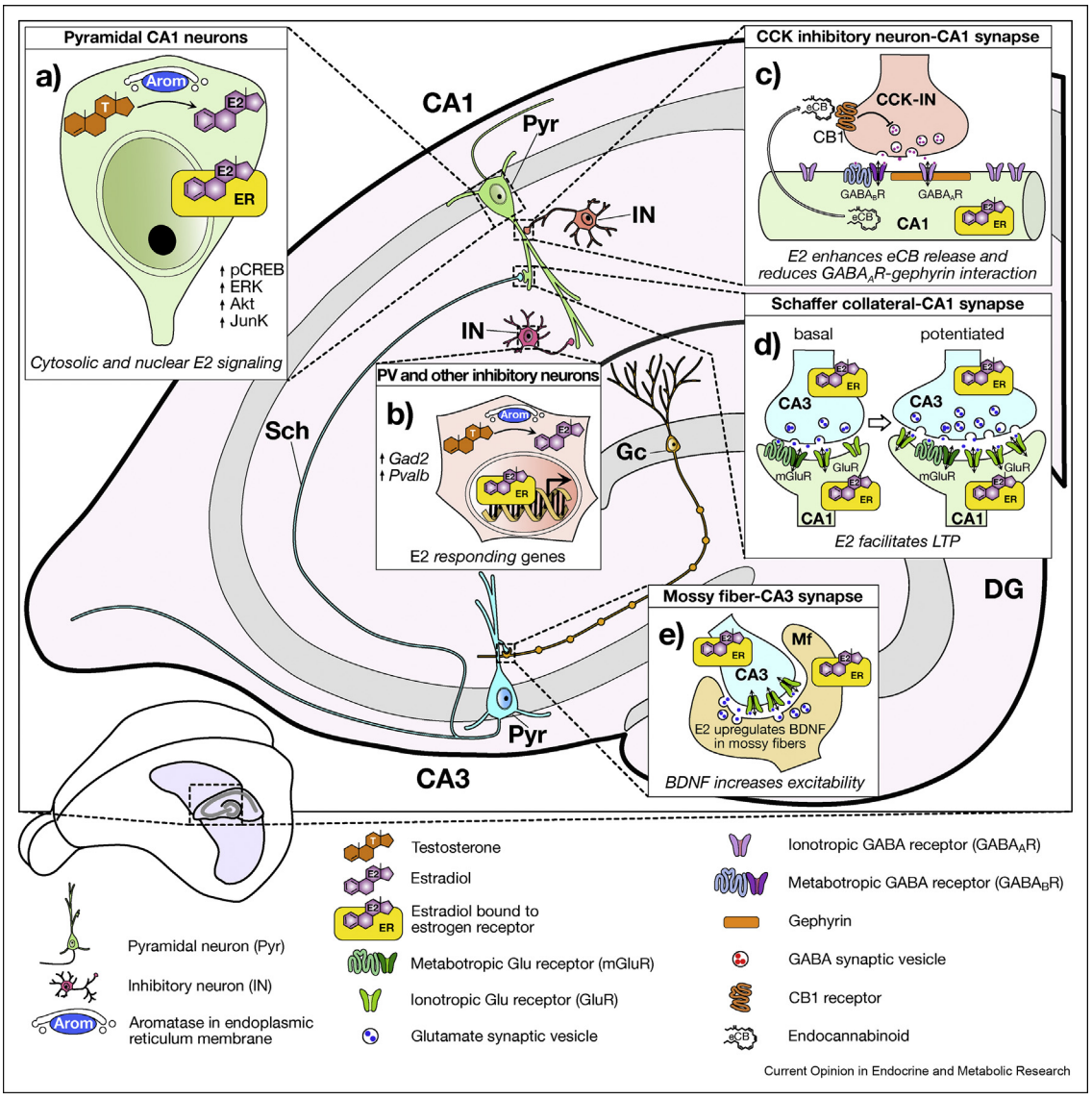
Of note, the vast majority of studies have been focused on the plasticity of excitatory synapses of CA1 PYR neurons but similar phenomena may also be present in excitatory synapses of GABAergic cells [**59]. Moreover, IN are extremely diverse and nE2 may differentially affect the intrinsic and synaptic 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 ɣ-oscillations, which have a critical role in information binding. Ovariectomy decreases ɣ-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

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 post-synaptic 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 (GABA$_A$R) for the scaffold protein gephyrin and the density of post-synaptic GABA$_A$R. 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 glutamartergic 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).

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

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 chromosomic 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.

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