Title: Brain estrogen production and the encoding of recent experience

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Abstract: The vertebrate central nervous system integrates cognition and behavior, and it also acts as both a source and target for steroid hormones like estrogens. Recent exploration of brain estrogen production in the context of learning and memory has revealed several common themes. First, across vertebrates, the enzyme that synthesizes estrogens is expressed in brain regions that are characterized by elevated neural plasticity and is also integral to the acquisition, consolidation, and retrieval of recent experiences. Second, measurement and manipulation of estrogens reveal that the period following recent sensory experience is linked to estrogenic signaling in brain circuits underlying both spatial and vocal learning. Local brain estrogen production within cognitive circuits may therefore be important for the acquisition and/or consolidation of memories, and new directions testing these ideas will be discussed.

Introduction

Historically, steroid hormones were thought to be produced exclusively in peripheral endocrine glands and to influence vertebrate behavior through long-term (hours to days) regulation of gene expression. In the case of estrogens, these ‘classical’ effects are mediated in the brain via the nuclear steroid receptors, estrogen receptor α (ER α) and ER β . It is now clear that the brain itself is also a key site of steroid hormone synthesis and action [1]. Brain-derived steroids provide a local source of neuromodulators that can act upon neural circuits at rapid timescales akin to classical neurotransmitters (seconds to minutes) [2]. While the rapid effects of steroid hormones are often studied in the context of sexual behavior [3], the role of neurosteroids in behaviors and neural systems beyond reproduction has only recently received attention. One area in particular has been understanding how estrogen signaling may enhance or otherwise alter cognition on momentary timescales. While there are a host of hormones that modulate learning and memory [4,5], the potent endogenous estrogen 17 β -estradiol (E2) has a clear influence on cognition and neural plasticity [6-8]. As such, this review will concentrate on the role of locally-synthesized brain E2 in learning and memory.

Focusing on recent findings, we evaluate three fundamental aspects of E2 and cognition: 1) the expression of estrogen synthase (aromatase) in brain regions critical for memory consolidation; 2) how measurement and manipulation of relatively rapid E2 synthesis relates to encoding recent experience; and 3) whether learning and post-learning epochs are associated with periods of E2 production and/or suppression. For the purposes of this review, we define the following terms:

Learning: active process of acquiring new information through experience.

Memory: stored information and/or consolidation of new information from a learning experience/event

Cognition: an active, sensory-dependent process that encompasses both a learning event (e.g. training) and the subsequent consolidation of the memory about that event (e.g. post-training), which can be recruited in future contexts.

Recent experience: a discrete window of time including both a potential learning event and the ~2-hour period that follows immediately after the learning event.

Encoding: the active process of memory consolidation of a recent learning event.

Does the role of E2 in brain regions associated with cognition depend on the local availability of aromatase, as well as membrane estrogen receptors, within these same regions?

Estradiol appears to influence learning and memory across a diverse group of species, including: nematodes [9], songbirds [10], rodents [6], and nonhuman [11] and human [12] primates. One interesting observation supporting the proposed role of acute neuroestrogen signaling in cognition is the presence of aromatase (estrogen synthase) in brain regions critical for memory encoding, consolidation, and recall among vertebrates. Aromatase expression is conserved across several functionally homologous neural structures in vertebrates [13]. **Figure 1** presents for the first time a cross-species comparison of aromatase expression in three brain regions that facilitate distinct types of memory: 1) fear memory consolidation and social recognition (*amygdala* [14]); 2) spatial navigation and novel object recognition (*hippocampus* [8,15]); and 3) vocal communication learning, and language acquisition (*auditory cortex/forebrain* [8]). Neuronal

aromatase is enriched in these canonical ‘memory’ regions in mammals and their functionally similar regions in nonmammalian species; we present representatives showing this in human (*Homo sapiens*) and nonhuman primates (*Maca mulatta*), rodents (*Mus musculus*), birds (*Taeniopygia guttata*), reptiles (*Aspidoscelis uniparens*), and fish (*Porichthys notatus*). While aromatase is found in the brain of amphibians [16-18], the spatial resolution and region specificity are less clear and difficult to resolve for present purposes. Of note, at present, there is a paucity of direct evidence for the presence of aromatase in mouse hippocampus [19,20], which may be explained by the promoter used to identify its presence. A recent finding in *Xenopus* provides intriguing evidence that there may be multiple splice variants for brain-specific aromatase [18]. Therefore, the absence of evidence for aromatase in mouse hippocampus (as well as the auditory cortex) may be due to antibody specificity. In contrast to mice, aromatase is reliably found in rat dorsal hippocampus [21].

Insert Figure 1 approximately here.

Figure 1 – Aromatase is typically expressed in brain regions crucial for cognition among vertebrates. Aromatase expression is abundantly expressed within the hippocampus, auditory cortex/forebrain, and amygdala of several representative species across a wide range of classes. Black stripped-filled brain regions indicate no reported presence of aromatase, whereas maroon-filled brain regions indicate detectable presence of aromatase as assessed through various techniques. Briefly, 1) **hippocampus** - humans: [22,23]; rhesus macaques: [24]; mice: not seen in hippocampus [19,20]; but see [25]; birds: [26,27]; reptiles (medial cortex): [28,29]; fish (dorsolateral telencephalon): [30,31]; 2) **auditory cortex/forebrain** – humans: [32,33]; rhesus

macaques: [24]; birds (caudomedial nidopallium; NCM [34]): [26,27]; reptiles (anterior dorsal ventricular ridge; ADVR [34]): [28]; fish (posterior portion of the ventral telencephalon; Vp): [30,35,36]; 3) **amygdala** - humans: [37]; rhesus macaques: [38]; mice: [19]; birds (nucleus taenia; TnA): [27]; reptiles: [28,29,39,40]; fish (supracommissural nucleus of the ventral telencephalon; Vs [41,42]): [30,36].

While the presence of aromatase demonstrates the capability for local E2 synthesis, acute changes in neurophysiology and behavior typically depend on membrane-bound ERs present within these same aromatase-expressing brain regions. In addition to membrane-trafficked versions of the classical nuclear ERs (ER α and ER β), there are also several membrane-bound estrogen receptors (mERs) that rapidly modulate E2-dependent behaviors [43] and neurophysiology [44], including: mERs associated with a membrane glutamate receptor (mGluR), Gq-coupled mER (Gq-ER), GPER1 (formerly GPR30), and ER-X [as reviewed in 45]. These cognate mERs are typically co-expressed in aromatase-enriched brain regions associated with the encoding of recent experience. For example, both aromatase and GPER1 are found in the hippocampus, nucleus taeniae of the amygdala (TnA), and the caudomedial nidopallium (NCM; functionally homologous to mammalian secondary auditory cortex) of adult and developing male songbirds [46]. Regions such as NCM and hippocampus are necessary for auditory and spatial memory consolidation, respectively, across the lifespan [47-49].

In sum, the molecular machinery necessary to both synthesize and respond to local E2 fluctuations are found within neural structures critical for memory consolidation and encoding. It

is therefore important to consider the functional significance of aromatase expression and its relationship to learning.

What is the relationship between fluctuating brain E2 levels and the acquisition vs. consolidation of recent experience?

In addition to the strong overlap of aromatase expression in functionally homologous brain regions across diverse taxa, there is ample evidence to suggest that acute neuroestrogen synthesis actively influences learning and memory. Local E2 production is implicated in learning and memory across a broad range of species, including humans, non-human primates, songbirds, rodents, and nematodes [6,8,9,45]. Research has primarily focused on hippocampal-dependent memory and E2, and mounting evidence indicates that exogenous E2 enhances hippocampal-dependent memory consolidation (which may reflect endogenous fluctuations during and after learning). For example, E2 infused into the dorsal hippocampus of adult female mice within a critical 2 hour window following a training event caused an enhancement in subsequent recognition memory performance [45]. In addition to an E2-dependent enhancement, systemic and local inhibition of aromatase activity impairs spatial and auditory memory consolidation in songbirds, as well as long-term potentiation (LTP) in rodents [10,48,50]. Therefore, exogenous manipulation of E2 availability impacts the encoding of recent experience in spatial memory tasks. However, it is less clear if pharmacologically induced changes in local E2 levels reflect physiological changes of neuroestrogen production in non-manipulated animals.

Understanding the molecular mechanisms of learning and memory has been dominated by approaches that manipulate the neurochemistry and activity of cognitive circuits. Recent approaches now allow the measurement of the on-line activity and neurochemical state of cognitive circuits. Relevant to the current topic, *in vivo* central E2 measurements have provided direct information about physiological changes in local steroid environments, and have been successfully adapted for songbirds [51], quail [52], rats [53], and nonhuman primates [54]. Studies using *in vivo* microdialysis, as well as brain content assays of macroarea homogenates, have revealed that E2 synthesis is elevated following recent learning events [51,55]. Specifically, E2 levels are elevated within 60 mins subsequent to spatial navigation and vocal communication training [51,55]. This timeframe parallels the critical window for pharmacological effects on enhancing or impairing memory consolidation by administering E2 or inhibiting aromatase, respectively [8,10,45]. One functional consequence of post-learning elevations in brain E2 may be the rapid enhancement of synaptogenesis in critical cognitive structures such the hippocampus and prefrontal cortex [56]. Thus, E2 appears to be dynamically upregulated immediately after learning events, and these increases are likely important for dendritic spine alterations and modulations of synaptic strength. In this way, modifying the strength of functional synaptic connections between neurons is a key candidate mechanism for E2 altering higher cognitive function, such as learning and memory.

A competing hypothesis – is the enhanced memory consolidation mediated by the suppression of E2 synthesis during a learning event vs. a rebound increase in E2 after training?

Work in rodents and songbirds has led to the idea that rapid post-training E2 elevations are cognitively enhancing. However, recent findings in rodents and songbirds highlight the intriguing possibility that dynamic *suppression* of E2 synthesis during a learning event may be a critical component of memory formation/consolidation [57]. In adult rats, systemic treatment with an aromatase inhibitor prior to and during a spatial learning task actually improves working memory in subsequent tests [58]. Furthermore, E2 levels are suppressed in the auditory forebrain of juvenile songbirds during a song learning event [51], and this suppression during tutoring is followed by a subsequent post-training elevation in E2. These findings that E2 is suppressed during a training event and subsequently elevated may explain similar observations that E2 is elevated post-training in other vertebrates [51,55]. Together, these observations lead to the hypothesis that E2 levels are “rebounding” from neuroestrogen suppression during a learning event. Therefore it is important to clarify the functional role of reduced neural E2 production in the acquisition of sensory experience, in songbirds, rats and other model systems. In particular, key future research directions include understanding the acute control mechanisms for *in vivo* brain aromatase activity (such as calcium-dependent phosphorylation of the enzyme [3]), as well as improving our temporal resolution for the fluctuations in neuroestradiol during and following discrete learning events.

While suppressing E2 could facilitate learning, elevated E2 may actually interfere with the encoding of recent experience. In corvids [59] and finches [49], exogenous E2 interferes with hippocampal-dependent spatial memory, which is consistent with recent findings in the prefrontal cortex in aged nonhuman primates [11]. Thus, it may be that the plasticity-enhancing effects of E2 may be deleterious to the faithful initial encoding of a novel sensory stimulus [57].

As such, it remains important to consider the balance between potential cognitively-enhancing, as well as –impairing roles for brain-derived E2 in the encoding and consolidation of recent experience. This is especially important when considering the timing of fluctuations in local E2 levels in higher cognitive circuits.

Conclusions and future directions

Thus far, we have presented work illustrating the largely conserved expression of aromatase in brain regions associated with learning and memory, proposed functional roles for E2 synthesis within these regions as it relates to memory consolidation, and suggested an alternative possibility that local suppression of E2 may be an important modulator for experience encoding. It is clear that more work is needed to further clarify the pluripotent mechanisms by which brain E2 signaling contributes to learning and memory.

The study of estrogen signaling in learning & memory has been largely focused on spatial navigation and object recognition memory in adult animals within the hippocampus. It will be interesting and necessary to expand the study of acute E2 production in cognition to include: 1) novel memory types (e.g. sensory: auditory and olfactory [48,60]); 2) ages across the lifespan (e.g. critical periods early in development, especially in relation to sensorimotor learning); 3) aromatase-enriched regions outside of the hippocampus (e.g. medial amygdala), and 4) areas of the brain in which neurophysiological signatures of experiential learning can be readily accessed. Broadening the range of research initiatives (i.e., across neural structures, age, memory-type, and

species) is now necessary to build a generalized understanding of E2's role in cognition. Moreover, there is little information about fluctuating steroid levels in oft studied brain regions involved in cognition. For example, we now have the opportunity to determine *in vivo* changes in central E2 levels during and following training in regions such as the hippocampus.

Other burgeoning areas of steroid-mediated learning and memory include E2's apparent effect on epigenetic alterations. Epigenetic mechanisms, namely histone acetylation and DNA methylation, appear to mediate several aspects of learning and memory, and recent evidence suggests that E2's enhancement of memory consolidation relies on local chromatin modifications [61]. While there is no direct evidence for rapid neural aromatization regulating epigenetics, future studies should begin testing the effect of aromatase inhibitors on subsequent epigenetic changes and memory retrieval.

Another exciting prospect for future work is neuroestrogens' potential role in facilitating critical period plasticity for sensorimotor learning. HVC (proper name; functionally similar to Broca's area) is a requisite telencephalic sensorimotor nucleus for vocal learning, and integrates both auditory input and vocal output in songbirds. During development, rapid dendritic spine remodeling occurs within HVC immediately after initial tutoring experience, and the amount of spine remodeling post-tutoring is a strong predictor for vocal development and model imitation [62]. E2 is required for both the development of the sensorimotor circuit (including HVC) and for proper tutor song imitation. Therefore, acute fluctuations in brain-derived E2 may facilitate memory consolidation during development in estrogen-sensitive forebrain regions (such as

NCM), which project to and modulate downstream auditory representations in HVC [63]. It is interesting to note that a role for E2 in vocal communication learning has been recently implicated in human infants, as well [64].

Research on the role of brain-derived estrogens in learning and memory has just begun. Expanding the research spotlight to include novel structures, behaviors, species, now presents an exciting jumping off point to explore the way that rapid changes in brain estrogen fluctuations regulate the encoding of recent experience.

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ANNOTATED REF's:

****Chao, et al 2015 *Dev Neurobio*:** This study revealed that E2 levels are suppressed in the auditory forebrain during tutoring for both male and female juvenile zebra finches. Post-tutoring, E2 “rebounds” and is significantly elevated, relative to baseline. This timeline is in contrast to adult zebra finches that demonstrated rapidly elevated E2 during song presentation, which return to baseline levels following cessation of the playback.

****Bailey 2013 *Endo*:** In this study, researchers demonstrate that shunting aromatase activity in the hippocampus of adult zebra finches hinders spatial memory acquisition and subsequent retrieval using a novel food caching task. Aromatase inhibition impaired memory consolidation and retrieval to similar levels as those subjects with lesioned hippocampi.

****Boulware et al 2013 *J Neurosci*:** This set of experiments provides evidence that one route of action for the E2-dependent enhancement of novel object recognition is via metabotropic glutamate receptors in the dorsal hippocampus (DH) of female mice. Additionally, their findings suggest the E2-dependent action for E2 is through membrane ER/mGluR1 coupling within the DH.

***Wermke et al 2014 *Bio Letters*:** Levels of free serum estradiol (but not testosterone) were positively correlated with an early index for future language skills in human infants (4 – 8 weeks old). These results reflect similar findings in songbirds, such that circulating E2 is significantly elevated during critical periods for sensorimotor learning.

*** Tabatadze et al., 2014 *PLoS One*:** This paper found pervasive expression of long-form aromatase mRNA in the brain of male and female rats, including key cognitive regions such as

440 dorsal hippocampus and amygdala. Moreover, this group also found brain region-specific effects
441 of sex and gonadal hormones on aromatase expression.

442
443 *Rensel et al., 2015 *H&B*: In scrub jays, E2 implants appear to interfere with a hippocampal-
444 dependent spatial memory tasks (food caching), consistent with the idea that in some contexts
445 estrogens can be cognitively impairing.
446

446 **Conflict of Interest Statement**

447 Nothing declared.

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