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The epigenetics of estrogen

Epigenetic regulation of hormone-induced memory enhancement

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Epigenetic processes have been implicated in everything from cell proliferation to maternal behavior. Epigenetic alterations, including histone alterations and DNA methylation, have also been shown to play critical roles in the formation of some types of memory, and in the modulatory effects that factors, such as stress, drugs of abuse and environmental stimulation, have on the brain and memory function. Recently, we demonstrated that the ability of the sex-steroid hormone 17β-estradiol (E_2) to enhance memory formation is dependent on histone acetylation and DNA methylation, a finding that has important implications for understanding how hormones influence cognition in adulthood and aging. In this article, we provide an overview of the literature demonstrating that epigenetic processes and E_2 influence memory, describe our findings indicating that epigenetic alterations regulate E_2-induced memory enhancement, and discuss directions for future work on the epigenetics of estrogen.

Key words: histone acetylation, DNA methylation, estradiol, cognition, hippocampus

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An increasing body of evidence demonstrates a critical role of epigenetic processes in mediating complex psychological processes like learning and memory. Both histone alterations (e.g., acetylation, phosphorylation, methylation) and DNA methylation appear to play important roles in long-term memory formation, and recent work suggests that these epigenetic processes work synergistically to regulate memory.[1] In addition, factors that modulate memory formation, such as stress, drugs of abuse, depression and environmental stimulation, have been reported to influence the brain and cognitive function via epigenetic mechanisms,[2-5] suggesting that epigenetic alterations are critical for both basic memory formation and the modulatory influences of environmental experience and hormones. Recently, my lab has shown that the ability of sex-steroid hormones, specifically the potent estrogen 17β-estradiol (E_2), to enhance memory also depends on epigenetic mechanisms.[6] This finding has important implications for understanding how sex-steroid hormones affect cognitive function in development, adulthood and aging, and it will be argued here that epigenetic alterations are critically important in mediating the effects of hormones on cognition. The sections that follow provide a brief overview of how epigenetic processes and E_2 independently influence memory, and then discuss the roles that epigenetic alterations play in regulating E_2-induced memory enhancement.

The Epigenetics of Memory

Studies exploring the epigenetics of memory (reviewed in refs. 7–9) have focused primarily on the hippocampus, a bilateral medial temporal lobe structure necessary for various memories, including those for spatial, relational and contextual information.[10-12] This structure is exceedingly vulnerable to the detrimental effects of aging and Alzheimer's disease[13,14] and, therefore, has been the subject of considerable efforts to understand the neurobiology of

learning and memory. The first, and most well studied, epigenetic alteration implicated in hippocampal memory is histone acetylation. A growing body of evidence suggests that increased histone acetylation enhances hippocampal long-term memory. Much of this evidence comes from studies showing that histone deacetylase inhibitors (HDACis) such as trichostatin-A and sodium butyrate increase histone H3 and H4 acetylation in the hippocampus, and enhance several types of hippocampal-dependent memories, including contextual fear conditioning, object recognition and spatial memory in rodents.[6,15-18] HDACis also reverse hippocampal memory deficits in mouse models of Alzheimer's disease[19,20] and traumatic brain injury.[21] Conversely, overexpression of specific HDACs, like HDAC2 and HDAC3, impairs several types of hippocampal memory, reduces hippocampal synaptic plasticity and suppresses the expression of plasticity-related genes in the hippocampus; however, many of these deficits can be reversed by HDACis.[22,23] Histone acetyltransferase (HAT) enzymes also play an important role in mediating hippocampal memory, as demonstrated by the fact that mice with mutations of HATs such as p300,[24] CBP[25-27] or PCAF[28,29] exhibit impaired hippocampal memory and synaptic plasticity. Collectively, these data support the notion that increasing histone acetylation (e.g., by inhibiting HDAC activity) enhances hippocampal memory, whereas reducing histone acetylation (e.g., by inhibiting HAT activity) impairs hippocampal memory.

Hippocampal learning itself also regulates epigenetic processes. For example, contextual fear conditioning, a paradigm in which rodents learn to associate a particular testing context with the delivery of a mild footshock, significantly increases the acetylation, phosphorylation and trimethylation of histone H3.[15,30,31] The learning-induced alterations in acetylation and phosphorylation are dependent on activation of both NMDA receptors and the cell signaling molecule extracellular signal-regulated kinase (ERK).[15,30] The ERK-dependence of learning-induced changes in H3 acetylation is significant because phosphorylation of the p42 isoform of ERK in the hippocampus is critical for long-term hippocampal memory formation.[32] ERK activation leads to gene transcription, primarily through the transcription factor cAMP response element-binding protein (CREB), which interacts with HATs to promote transcription.[33] Thus, factors that alter ERK activation should also affect histone acetylation. Indeed, the ability of E_2 to increase dorsal hippocampal histone H3 acetylation is dependent on dorsal hippocampal ERK activation.[6] In preliminary work, we have also shown that a HAT inhibitor can prevent E_2 from increasing expression of a specific DNA methyltransferase (DNMT) enzyme,[34] suggesting an interaction between histone acetylation and DNA methylation. Such interactions have been previously reported with respect to contextual fear conditioning, which increases mRNA expression of the de novo methyltransferases DNMT3A and DNMT3B (but not of the maintenance methyltransferase DNMT1).[35,36] Fear conditioning learning also increases methylation of the memory suppressor gene *protein phosphatase 1* (*PP1*), while decreasing methylation of the memory promoting gene *reelin*.[35] Evidence that DNA methylation is necessary for hippocampal learning comes from studies showing that the DNMT inhibitors 5-aza-deoxycytidine (5-AZA) and zebularine block contextual fear memory and hippocampal long-term potentiation.[35,36] These inhibitors also prevent the increase in histone H3 acetylation induced by contextual fear conditioning training or cell signaling activators,[1,36] suggesting that DNA methylation regulates histone acetylation. Together, the aforementioned data strongly support the conclusion that both histone alterations and DNA methylation are necessary for long-term hippocampal memory formation and have provided some tantalizing glimpses into how these processes may work together to regulate memory.

Estradiol and Memory

Estrogens such as E_2 are synthesized and secreted by both sexes, although levels are considerably higher in young reproductively mature females than in males. In females, the primary sources of E_2 are the ovaries, but E_2 is also synthesized in the hippocampus.[37,38] Estrogen receptors (ERs) are located throughout hippocampal neurons, including the nucleus, dendritic spines and axon terminals of pyramidal neurons,[39] the primary excitatory neurons of the hippocampus. The hippocampus is exceptionally sensitive to levels of E_2 in circulation; elevated levels of E_2 during the natural reproductive cycle or after exogenous administration significantly increase hippocampal synaptogenesis, neurogenesis and synaptic plasticity.[40-44] Exogenous E_2 also enhances several types of hippocampal-dependent memory in young ovariectomized rodents, including spatial memory (reviewed in ref. 45), trace eyeblink conditioning,[46] and object recognition memory.[47-50] Although some of the effects of E_2 on memory and the hippocampus are likely due to traditional "genomic" mechanisms, whereby the intracellular estrogen-ER complex acts as nuclear transcription factor by binding to an estrogen response element on DNA, the presence of ERs in dendrites and axons suggests a mechanism for E_2 to initiate more rapid mechanisms of action.

Indeed, E_2 can rapidly activate intracellular signaling cascades critical for memory, including ERK/MAPK and phosphatidylinositol-3-kinase/Akt (PI3K/Akt).[51-53] In vitro, E_2 can induce hippocampal ERK phosphorylation within 10 min, an effect blocked by inhibitors of the enzyme MAP kinase kinase (MEK), the exclusive upstream activator of ERK.[52] MEK inhibitors also block E_2-induced increases in hippocampal synaptophysin levels, glutamate release and CA1 spine synapses.[53,54] In vivo, we have found that infusions of E_2 directly into the dorsal hippocampus increase dorsal hippocampal p42-ERK phosphorylation within 5 min of infusion.[6,55] Further, we demonstrated that ERK activation is necessary for E_2 to enhance memory formation.[6,55,56] In this series of studies, memory was tested in ovariectomized female mice trained to recognize objects in a novel object recognition task (**Fig. 1A**) that tests the ability of an animal to recognize an object they have seen before. Mice first accumulate 30 sec exploring two identical objects in an open arena. Immediately after this training (i.e., post-training), mice are treated with E_2 either systemically or directly

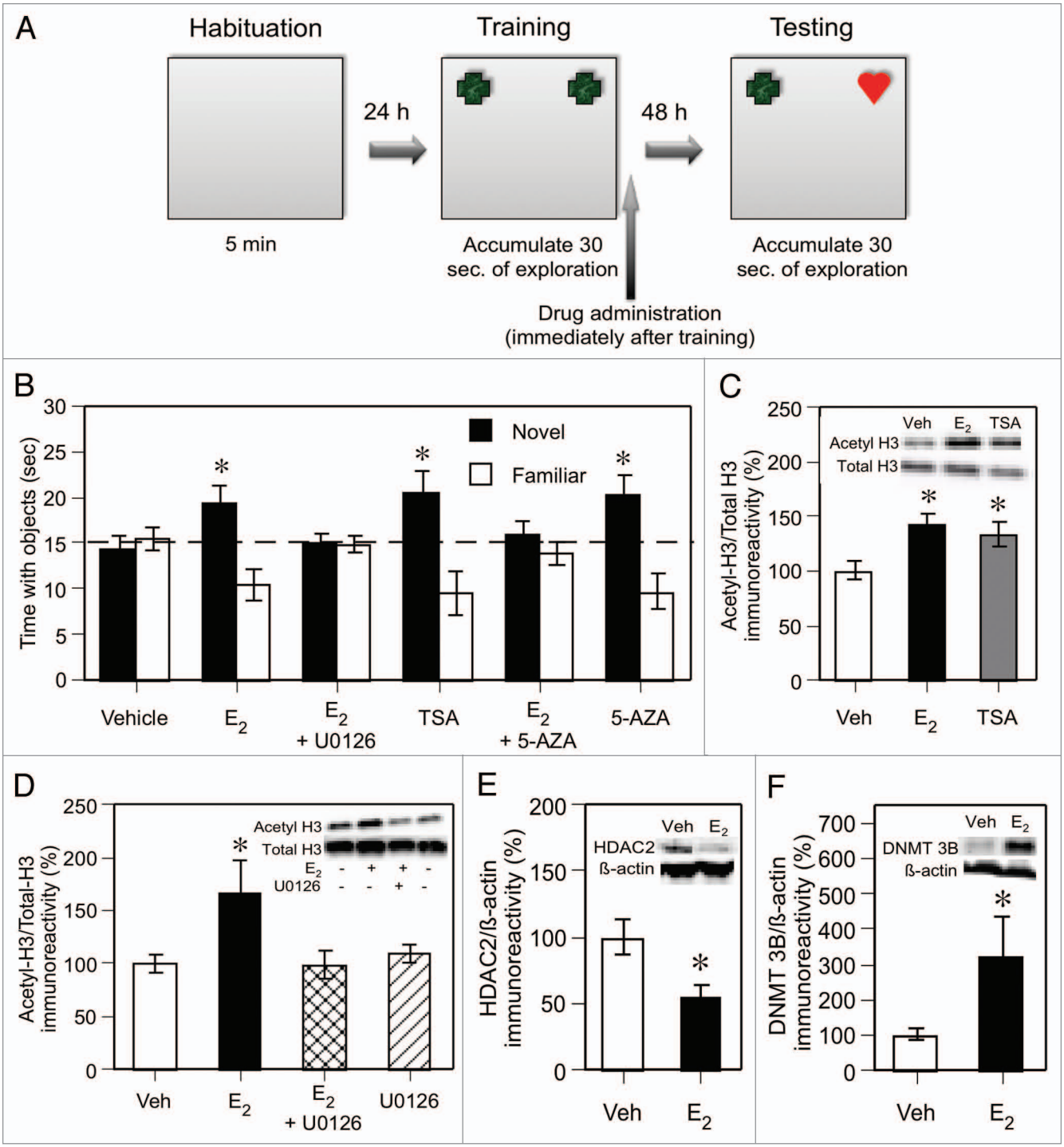

Figure 1. (A) Schematic diagram illustrating the novel object recognition testing protocol. Mice are first habituated to an empty testing arena located in a dimly lit room for 5 min to habituate them to the testing apparatus. Twenty-four hours later, they are exposed to two identical objects placed near the corners of the arena and allowed to accumulate 30 sec exploring the objects. Drugs and hormones are infused into the dorsal hippocampus immediately after training. Forty-eight hours later, mice are allowed to accumulate 30 sec exploring a novel object and one identical to those they explored during training. Mice that remember the familiar object will spend more time than chance (15 sec) exploring the novel object. (B) Mice receiving bilateral dorsal hippocampal infusions of E_2 (5 μg/side), the HDACi TSA (16.5 mM/side) or the DNMT inhibitor 5-AZA (100 μg/side) spent significantly more time than chance with the novel object (*$p < 0.05$ relative to chance), demonstrating memory for the familiar object. Vehicle-treated mice spent similar amounts of time with both objects, indicating no memory for the familiar object. The MEK inhibitor U0126 (0.5 μg/side) and 5-AZA completely blocked E_2's ability to enhance object recognition. (C) Dorsal hippocampal infusion of E_2 or TSA significantly increased dorsal hippocampal histone H3 (lys 14) acetylation relative to Vehicle (Veh; *$p < 0.05$) 30 min after infusion. (D) Dorsal hippocampal infusion of U0126 completely prevented E_2 from increasing dorsal hippocampal histone H3 (lys 9,14) acetylation (*$p < 0.05$ E_2 relative to Veh). (E) Dorsal hippocampal infusion of E_2 significantly decreased HDAC2 protein levels relative to Vehicle 4 h after infusion (*$p < 0.05$). (F) Dorsal hippocampal infusion of E_2 significantly increased DNMT3B protein levels relative to Vehicle 4 h after infusion (*$p < 0.05$). (B–F) adapted from reference 6.

into the dorsal hippocampus. Retention is tested 48 h later by presenting mice with one novel and one familiar (identical to training) object. Because mice are inherently drawn to novelty, those who remember the familiar object spend more time than chance (15 sec) exploring the novel object. Lesions or pharmacological inactivations of the hippocampus impair object recognition in this task, demonstrating hippocampal involvement in this type of memory.[55,57,58] The administration of a rapidly metabolized form of E_2 immediately post-training has allowed us to pinpoint effects of E_2 to the memory consolidation phase of memory processing; because E_2 is not in the circulation during training or testing, its specific effects on memory consolidation can be examined in the absence of non-mnemonic performance factors (e.g., motivation, anxiety) that could influence object exploration.[59] We, and others, have shown that E_2 administered immediately post-training significantly enhances novel object recognition in young female rats and mice when injected systemically[48,50,55,60-62] or infused directly into the dorsal hippocampus[6,55,56] (**Fig. 1B**). As mentioned above, we have also found that dorsal hippocampal E_2 infusion significantly increases phosphorylation of p42-ERK in the dorsal hippocampus within 5 min of infusion.[6,55] Further, concurrent dorsal hippocampal infusion of the MEK inhibitor U0126 completely blocks E_2's ability to increase p42-ERK phosphorylation and enhance object recognition (**Fig. 1B**), demonstrating that dorsal hippocampal ERK activation is necessary for E_2 to enhance object memory consolidation.[6,55,56] Subsequent work has also demonstrated that kinases upstream from ERK, such as PI3K and PKA, are also critically necessary for E_2 to influence memory consolidation.[56,61]

The Epigenetics of Estradiol-Induced Memory Enhancement

Given the importance of ERK to hippocampal memory consolidation,[32] the E_2-induced facilitation of memory consolidation,[6] and the learning-induced increase in histone H3 acetylation,[15] we wondered whether ERK-driven epigenetic alterations might play an important role in the memory-enhancing effects of E_2. We first wanted to demonstrate that novel object recognition, as tested by our protocol, was affected by epigenetic alterations. Therefore, we infused the HDACi trichostatin-A (TSA) into the dorsal hippocampus of young ovariectomized mice immediately after training and found that TSA enhanced object recognition[6] (**Fig. 1B**). TSA also significantly increased acetylation of histones H3 (**Fig. 1C**) and H4 in the dorsal hippocampus 30 min after infusion.[6] In contrast to this more general effect on histone acetylation, dorsal hippocampal infusion of E_2 significantly increased acetylation of histone H3 (**Fig. 1C**), but not histone H4, 30 min after infusion.[6] This relative specificity is consistent with the effects of contextual fear conditioning on histone acetylation, which also affects histone H3 rather than H4,[15] suggesting that histone H3, but not H4, regulates hippocampal memory consolidation. As with object recognition, dorsal hippocampal infusion of the MEK inhibitor U0126 completely blocked the E_2-induced increase in histone H3 acetylation[6] (**Fig. 1D**), demonstrating that ERK activation in the dorsal hippocampus is necessary for E_2 to influence histone acetylation. Interestingly, dorsal hippocampal infusion of E_2 also significantly decreased dorsal hippocampal expression of HDAC2 4 h after infusion[6] (**Fig. 1E**), suggesting that E_2 represses the translation of proteins that are detrimental to hippocampal memory. This effect was specific to HDAC2, as HDAC1 expression was not affected by E_2.[6] We next examined the involvement of DNA methylation in the mnemonic effects of E_2. We first assessed whether dorsal hippocampal infusion of E_2 could affect the expression of the maintenance methyltransferase DNMT1 and the de novo methyltransferases DNMT3A and DNMT3B. We found that E_2 significantly increased DNMT3A and DNMT3B mRNA in the dorsal hippocampus 45 min after infusion, and increased DNMT3B protein 4 h after infusion[6] (**Fig. 1F**), suggesting that DNA methylation might play a role in the memory-enhancing effects of E_2. In support of this notion, we also found that dorsal hippocampal infusion of the DNMT inhibitor 5-AZA prevented E_2 from enhancing object recognition[6] (**Fig. 1B**), demonstrating that DNA methylation is necessary for E_2 to enhance memory. Interestingly, 5-AZA enhanced object recognition when given alone[6] (**Fig. 1B**), indicating that object recognition itself depends on DNA methylation. Perhaps in the absence of E_2, 5-AZA prevents the methylation of memory promoter genes like *reelin*, which should theoretically enhance memory formation. The fact that 5-AZA blocked the mnemonic effects of E_2 suggests that E_2 enhances memory, at least in part, by methylating genes. Some logical candidate genes for this methylation would be memory repressors such as *HDAC2*, *HDAC3* and *PP1*. Methylation patterns in these, and other, genes will need to be examined in future studies.

Conclusions and Future Directions

In sum, the data on the epigenetics of E_2-induced memory enhancement thus far suggest that both histone H3 acetylation and DNA methylation are critically important for this hormone to enhance object recognition memory. Because this work is in its infancy, much more research will be necessary to elucidate the fundamental epigenetic events through which estradiol, and other sex-steroid hormones, influence cognitive processes. For example, effects of E_2 on the methylation and expression of specific genes must be examined, as should the involvement of histones H2A and H2B, specific HDACs and HATs, and other histone alterations (e.g., phosphorylation) in the mnemonic effects of E_2. Our preliminary data suggest synergy between histone acetylation and DNA methylation in the modulation of memory, so future work should explore these interactions more thoroughly. The role of epigenetic factors in age-related sex-steroid hormone loss, as occurs in menopause, should also be explored, as epigenetic treatments (e.g., HDAC inhibitors) might provide to menopausal women the cognitive benefits of hormone replacement therapy without the harmful side effects (e.g., cancer, heart disease, stroke) common to such therapy.[63]

Importantly, however, the data on epigenetics, E_2 and memory reveal that

epigenetic processes are crucial to the ability of hormones to influence memory processes. If this finding generalizes to other sex-steroid hormones, then this research may have far reaching implications for understanding the neurobiological origin of sex differences in cognition, the modulatory effects of sex steroid hormones on the brain and cognitive function in adulthood, and the role that declining sex steroid hormone levels during menopause play in age-related cognitive decline. The elucidation of the epigenetic code will undoubtedly revolutionize the study of cognition, and provide new avenues for the development of treatments to reduce neurodegenerative and psychiatric disorders. Modulatory factors, such as hormones, will likely play a major role in fine-tuning the epigenetic code, opening the door to exciting new areas of research and drug development.

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