Memory and Epigenetics: Role of Estrogen

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Introduction

Evidence that hormones could regulate behavior was first demonstrated in 1849 when Arnold Berthold showed that castration and reimplantation of the testes in roosters affected normal male development, sexual desire, and aggression. However, only in the last 30 years has it been demonstrated that sex steroid hormones are poised to mediate memory formation. Some of the first evidence supporting this notion came from the discovery of estrogen receptors in the dorsal hippocampus and the entorhinal cortex (Loy et al., 1988; Maggi et al., 1989) and from seminal work demonstrating that spine density in the CA1 region of the hippocampus could be regulated by estrogens, including the potent 17β-estradiol (E_2), and progesterone in female rats (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992, 1993). In the nearly three decades since the initial demonstration that ovarian hormones regulate CA1 dendritic spine density, preclinical research has provided evidence that E_2 can enhance learning and memory in adults of a variety of species, including songbirds, rodents, nonhuman primates, and humans (for reviews see Maki, 2012; Hammond and Gibbs, 2011; Frick, 2009, 2012; Schlinger and Remage-Healey, 2012; Bimonte-Nelson et al., 2010). Recent work has demonstrated that E_2 employs numerous cellular and molecular mechanisms, including epigenetic processes, to influence memory formation, and these epigenetic mechanisms will constitute the focus of this review. A general overview of the beneficial effects of E_2 on memory will first be described below, followed by a discussion of the epigenetic mechanisms through which E_2 exerts these effects.

The Role of E_2 in Cognitive Function

Estradiol and Cognitive Function in Humans

Clinical studies investigating the estrogenic regulation of memory have focused largely on the effects of estrogens in the context of aging (i.e., as it relates to menopause), or changes in performance in cognitive tasks across the menstrual cycle. With respect to aging, numerous studies have shown that ovarian hormone loss due to natural or surgical menopause impairs various aspects of cognitive function, including verbal and spatial memory (Sherwin and Henry, 2008) (Fig. 1). Menopausal women are also at increased risk of Alzheimer's disease relative to men, even when accounting for women's longer lifespans (Dye et al., 2012; Launer et al., 1999). Indeed, longer periods of lifetime estrogen exposure are significantly and negatively correlated with the likelihood of developing Alzheimer's disease in women (Fox et al., 2013). The deleterious effects of menopause on cognition and risk of Alzheimer's disease can be reduced by E_2 therapy (Rocca et al., 2007, 2011), suggesting not only that E_2 loss at menopause contributes to memory dysfunction, but also that maintaining E_2 levels at menopause may help prevent Alzheimer's disease and reduce age-related cognitive decline.

Evidence also indicates that E_2 fluctuations across the month-long menstrual cycle in premenopausal women impacts certain aspects of cognitive function. For example, women experiencing high E_2 levels during the midluteal phase of the cycle perform better in tests of verbal fluency, fine motor, and perceptual speed than women experiencing low E_2 levels during the menstrual

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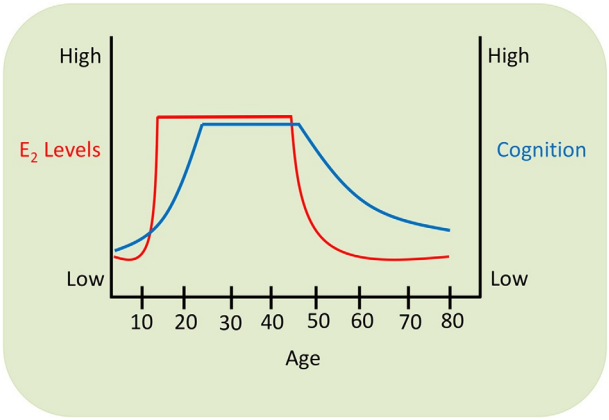

Fig. 1 Schematic illustration of changes in estradiol (E_2) levels and cognitive abilities across the life span in women. E_2 levels surge upwards at puberty and remain elevated (although fluctuating across the menstrual cycle) during the reproductive years. In the early 50's, E_2 levels drop substantially at menopause. Cognitive function reaches adult levels in the early-mid 20s and then remains relatively stable until middle-age, at which point certain aspects of cognitive function (e.g., episodic memory, divided attention, spatial navigation) decline gradually with age.

phase (Hampson, 1990; Maki et al., 2002). Some evidence suggests that visual memory is also poorer during the menstrual phase (Phillips and Sherwin, 1992). High levels of E_2 do not always correlate with better performance on cognitive tasks, however, as women's performance appears to be best in tasks in which men traditionally outperform females (e.g., those related to spatial ability) when E_2 levels are low (Hampson, 1990; Maki et al., 2002).

In addition to its role in regulating normal cognitive function, E_2 has also been implicated in several disorders that disproportionately affect women relative to men, including Alzheimer's disease, posttraumatic stress disorder (PTSD), and certain aspects of addiction. Although beyond the scope of this article, reviews on the role of E_2 in Alzheimer's disease (Dye et al., 2012), PTSD (Albert et al., 2015; Milad et al., 2010), traumatic brain injury (Asl et al., 2013; Day et al., 2013b), and substance abuse (Becker, 2016) provide important insights into the ways in which sex-steroid hormones may impact disorders characterized by memory dysregulation, and suggest directions for future research to better understand underlying neural mechanisms.

Animal Models Demonstrate the Importance of E_2 in Cognitive Function

Basic scientific research conducted in animal models is critical for mechanistic investigations into the role of E_2 in cognitive function. E_2-mediated actions in brain regions that support cognition, such as the hippocampus, are complex and impacted by numerous factors, including dose, duration of treatment, age, length of ovarian hormone deprivation prior to treatment, type of cognitive task, timing of administration relative to testing, task difficulty, and reproductive history (Acosta et al., 2009, 2010; Luine, 2014; Daniel, 2006; Frick, 2009). However, some generalizations can be drawn, particularly among studies utilizing rodent models. The majority of rodent studies support the conclusion that E_2 facilitates learning and memory in behavioral tasks that require the hippocampus (Packard and Teather, 1997; Fader et al., 1998; Daniel et al., 1999; Luine et al., 2003; Walf et al., 2008; Fernandez et al., 2008; Lewis et al., 2008; Zhao et al., 2010; Daniel, 2006; Fan et al., 2010); see (Daniel, 2006; Tuscher et al., 2015) for review. For example, young adult female rodents treated with exogenous E_2 exhibit enhanced spatial memory in the object placement, Morris water maze, radial arm maze, and T-maze tasks (Sandstrom and Williams, 2004; Luine et al., 1998; Fader et al., 1998, 1999; Daniel et al., 1997; Bowman et al., 2002; Bimonte et al., 2002). E_2 can also facilitate memory in a number of nonspatial tasks, as demonstrated in object recognition (Fernandez et al., 2008; Fortress et al., 2013; Boulware et al., 2013), social recognition (Phan et al., 2012), inhibitory avoidance (Singh et al., 1994; Rhodes and Frye, 2004), fear conditioning (Lebron-Milad and Milad, 2012; Chang et al., 2009; Barha et al., 2010; Zeidan et al., 2011; Milad et al., 2010), and trace eyeblink conditioning (Leuner et al., 2004). Collectively, these studies provide evidence that E_2 treatment can benefit hippocampal memory in numerous behavioral tasks. The molecular mechanisms through which E_2 exerts these beneficial effects will be discussed in greater detail below.

Estrogen Receptor Expression and Cell-Signaling Mechanisms

Protein synthesis is an essential component of memory formation, and E_2 regulates the synthesis of new proteins through at least two different estrogen receptor (ER)-mediated mechanisms: the classical genomic pathway and the rapid nonclassical activation of cell-signaling pathways. Many of the brain regions that support memory formation express classical intracellular ERs (ERα and ERβ), which are found within the cytoplasm and nucleus of the cell. Both ERα and ERβ have their own distinct patterns of expression in the cerebral cortex, basal forebrain, amygdala, prefrontal cortex, and hippocampus in a variety of species, including mouse, rat, nonhuman primates, and humans (Gillies and McArthur, 2010). The classical "genomic" action of E_2 is initiated once the hormone diffuses through the target cell's outer membrane and binds ERα or ERβ within the cytoplasm (Nelson, 2000). Once the E_2-ER complex is formed, it translocates to the nucleus, where it binds to estrogen response elements on the DNA. Here, the complex acts

as a transcription factor, and can initiate the transcription of E_2-sensitive genes that help maintain the neural circuitry that ultimately influences behavior (Heldring et al., 2007; Jensen, 1962). Changes in gene expression elicited by such nuclear ER-hormone interactions via the genomic mode of action occur slowly (on the scale of hours—days) and are thought to yield long lasting changes.

E_2 can also influence cell function in a nonclassical manner by binding to membrane-associated ERs (mERs; e.g., GPER, Gq-mER; Srivastava and Evans, 2013), classical ERs located near the membrane (Boulware et al., 2005, 2013), or by interacting with neurotransmitter receptors (e.g., mGluRs, NMDARs; Lewis et al., 2008; Boulware et al., 2005, 2013) to rapidly activate intracellular signaling pathways on the order of seconds to minutes (Gillies and McArthur, 2010). Although these mechanisms are often referred to as "nongenomic," this designation should not be taken literally, as activation of mERs can ultimately influence gene transcription. Rather, it should be thought of as way to distinguish between the effects of mERs and classical nuclear ER activation.

Work in our own lab has focused on the cell-signaling pathways through which E_2 regulates function in the dorsal hippocampus (DH) and medial prefrontal cortex, brain regions critical for learning and memory and whose function is compromised in various neuropsychiatric disorders and during aging (Small et al., 2011; Maillet and Rajah, 2013; Sampath et al., 2017). Rapid activation of cell-signaling cascades in these regions allows for acute modulation of cellular function in response to an experience (i.e., learning). Several pathways previously identified in studies focused on memory modulation in male rodents, such as extracellular signal-regulated kinase/mitogen activated protein kinase (ERK/MAPK), phosphatidylinositol 3-kinase (PI3K), protein kinase A (PKA), calcium calmodulin kinase II (CaMKII), and mammalian target of rapamycin (mTOR) (Adams and Sweatt, 2002; Horwood et al., 2006; Impey et al., 1998; Silva et al., 1992; Hoeffer and Klann, 2010) are also regulated by E_2 in ovariectomized females. E_2-mediated activation of these pathways has been linked to changes in neuronal excitability (Woolley, 2007), long-term potentiation (LTP) (Smith and McMahon, 2005), spinogenesis (Tuscher et al., 2016a), and memory enhancement (Fernandez et al., 2008; Fan et al., 2010; Fortress et al., 2013) in ovariectomized females. Specifically, E_2 infusion directly into the DH rapidly activates cell-signaling cascades like ERK, PI3K, PKA and mTOR within 5 min of DH infusion (Fernandez et al., 2008; Fan et al., 2010; Fortress et al., 2013). Further, activation of ERK, PI3K, PKA, and mTOR are all required for the beneficial mnemonic effects of E_2 in hippocampus-dependent tasks, as pharmacological inhibition of any of these pathways prevents the memory-enhancing effects of E_2 in ovariectomized female mice (Fernandez et al., 2008; Fan et al., 2010; Fortress et al., 2013) (Fig. 2). As will be discussed below, E_2-mediated activation of certain pathways, such as ERK, can also alter gene transcription by regulating downstream epigenetic modifications such as DNA methylation and histone acetylation. Before addressing these data, the next section will first review the major epigenetic mechanisms that regulate hippocampal memory.

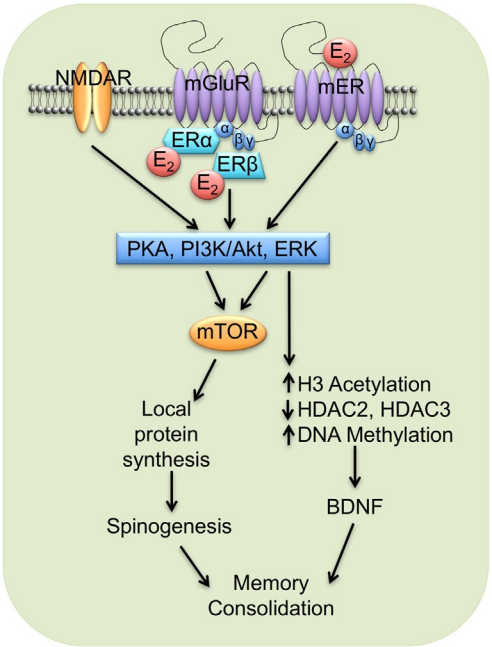

Fig. 2 Schematic representation of the cellular mechanisms through which E_2 regulates memory in ovariectomized mice. In the dorsal hippocampus, E_2 interacts with intracellular ERs (ERα and ERβ), mERs, and neurotransmitter receptors to rapidly activate PKA, PI3K/Akt, ERK, and mTOR cell signaling, which then triggers spinogenesis via local protein synthesis. Similarly, phosphorylation of ERK can enhance gene transcription via alterations in H3 acetylation, HDAC protein levels, and DNA methylation. These changes all contribute to the memory-enhancing effects of acute dorsal hippocampal E_2 infusion on memory consolidation. Abbreviations: *NMDAR*, *N*-methyl-D-aspartate receptor; *mGluR*, metabotropic glutamate receptor; *mER*, membrane estrogen receptor, *PKA*, protein kinase A; *PI3K*, phosphatidylinositol 3-kinase; *mTOR*, mammalian target of rapamycin; *H3*, histone 3; *HDAC*, histone deacetylase; *BDNF*, brain derived neurotrophic factor.

Epigenetic Modifications Related to Synaptic Plasticity and Memory

The term "epigenetics" refers to the idea that the expression of genes can be regulated "above the gene" by altering accessibility to genes rather than the genetic code itself (Crick, 1984; Holliday, 1999). Chromosomes are comprised of DNA tightly coiled around nucleosome subunits. Each nucleosome consists of an octamer containing eight histone (H) proteins, two each of H2A, H2B, H3, and H4 (Fig. 3). Each histone protein has a N-terminal tail that can be altered through posttranslational modifications such as acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation (Rothbart and Strahl, 2014) (Fig. 3). Chromatin is formed from the supercoiling of adjacent nucleosomes which are connected by the linker protein H1 (Mazzio and Soliman, 2012). The ability of a certain segment of chromatin to be modified depends on the posttranslational modification state of its histone tail, which controls how tightly wound the chromatin structure is and, therefore, whether the 3D structure is permissive to binding factors that regulate transcription. Each major modification and its individual role in memory are reviewed elsewhere (Fortress and Frick, 2014). Histone acetylation, phosphorylation, and methylation, along with DNA methylation, are summarized briefly below.

Histone modifications

Histone modifications can alter accessibility of DNA to the transcriptional machinery required for regulating the expression of genes, including those relevant to neuroplasticity and cognition. Histones are primarily modified at the N-terminal portion of their tails that extends beyond the nucleosome. The histone tail can interact with regulatory proteins, as well as neighboring histones and the DNA itself (Tsankova et al., 2007; Marmorstein, 2001). A number of alterations are possible including methylation, phosphorylation, ubiquitination, SUMOylation, and acetylation. Of these, acetylation is perhaps the most well characterized in the neuroepigenetic literature. Acetylation is carried out by histone acetyltransferase (HAT) enzymes, which facilitate the transfer of an acetyl group from acetyl-CoA to lysine residues present on histone tails. In the majority of cases, acetylation of histone tails yields a transcriptionally active state, conferring open euchromatin that is accessible to transcriptional machinery (Tsankova et al., 2007). Conversely, hypoacetylation generally has the opposite effect, largely because replacement of hydrogen with an acetyl group reduces the net positive charge of the histone, reducing its interaction with the negatively charged DNA backbone and increasing the likelihood of nucleosome displacement (Carrozza et al., 2003). This displacement opens up the chromatin, making it readily accessible to transcriptional machinery and regulatory factors that can influence transcription and, thereby, regulate gene expression.

As mentioned earlier, activation of signal transduction pathways can facilitate epigenetic changes such as histone acetylation, which may be a mechanism contributing to the precision and the stability of a memory. One example is the ERK/MAPK signaling pathway, which stimulates CREB binding protein (CBP), a transcriptional coactivator with intrinsic HAT activity (Ait-Si-Ali et al., 1999). Evidence that histone acetylation can influence learning was first demonstrated in rodents using the conditioned taste aversion paradigm. Using this task, mice that developed a conditioned taste aversion had increased ERK/MAPK activation and histone acetyltransferase activity in the insular cortex 48 h later (Swank and Sweatt, 2001). Further evidence for the role of histone acetylation in memory was determined in mice with reduced levels of CBP ($cbp^{+/-}$ mice), which exhibit impaired long-lasting-LTP, as well as deficits in hippocampus-dependent object recognition memory and contextual fear conditioning (Alarcón et al., 2004). Similar behavioral results were obtained in mice with an inducible dominant-negative system to turn off intrinsic CBP-mediated HAT activity. Interestingly, a histone deacetylase inhibitor rescued memory deficits in these mice, suggesting that reduced histone acetylation could underlie memory deficits in numerous disorders (Korzus et al., 2004). Indeed, mice overexpressing the histone deacetylase HDAC2, which removes acetyl groups from histones, have poor hippocampus-dependent spatial memory and reduced

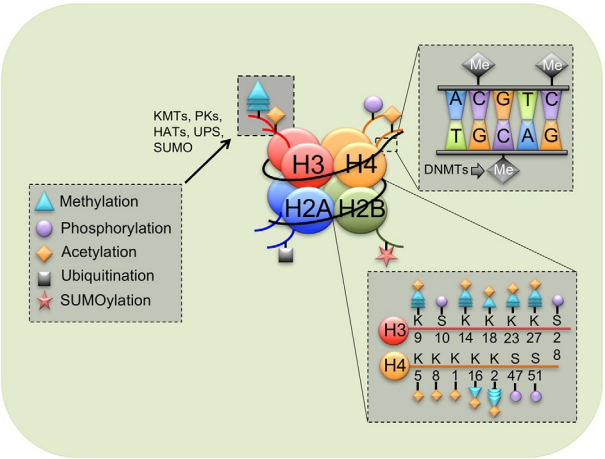

Fig. 3 Diagrammatic illustration of the nucleosome and common epigenetic alterations. The histone octamer consists of two each of histones H2A, H2B, H3, and H4. Abbreviations: *K*, lysine; *S*, serine; *KMTs*, lysine methyltransferases; *PKs*, protein kinases; *HATs*, histone acetyltransferases; *UPS*, ubiquitin proteasome system; *SUMO*, small ubiquitin-like modifier; *Me*, methyl group; *DNMTs*, DNA methyltransferases. Adapted from Fortress, A. M. and Frick, K. M. (2014). Epigenetic regulation of estrogen-dependent memory, *Frontiers in Neuroendocrinology*, **35**(4), 530–549.

synaptic plasticity (Guan et al., 2009). The behavioral deficits induced by overexpressing HDAC2 were reversed by administering an HDAC2 inhibitor, suggesting that memory disorders accompanied by elevated HDAC2 levels could be treated by HDAC2 inhibitors (Guan et al., 2009). Cellular mechanisms responsible for histone acetylation in the hippocampus were determined using contextual fear conditioning in rats. In a series of studies, it was demonstrated that ERK/MAPK signaling, acting upstream of mitogen and stress-activated protein kinase 1 (MSK1), is necessary for the acetylation of H3 (Levenson et al., 2004; Chwang et al., 2007). This evidence collectively suggests that rapid cell-signaling events can lead to changes in epigenetic modifications, such as histone acetylation, to regulate gene transcription and memory.

In addition to histone acetylation, histone phosphorylation is another posttranslational modification poised to regulate memory formation. Protein Serine/Threonine phosphatase 1 (PP1), which dephosphorylates proteins, has been implicated in hippocampal memory impairment and reduced plasticity in rodents (Genoux et al., 2002). In general, phosphorylation is associated with the activation of proteins responsible for a sequence of events that promotes memory and, therefore, interfering with phosphorylation processes could disrupt memory formation. In support of this notion, mice with inducible deficiency of forebrain PP1 demonstrated enhanced long-term memory in the object recognition, object placement, and Morris water maze tasks. This enhanced memory performance coincided with an increase in total phosphorylation of histone H3 on Serine 10 (phospho-H3S10) in addition to increased phospho-H3S10 at the CREB promoter (Koshibu et al., 2009). In addition to its role in regulating histone acetylation, ERK/MAPK signaling can also mediate histone phosphorylation. Activation of the upstream ERK/MAPK regulators PKC and PKA, as well as contextual fear conditioning training, all increased phospho-H3S10 in the hippocampus of rats (Chwang et al., 2006). Further, inhibition of ERK/MAPK prevented phosphorylation of H3S10, suggesting that ERK/MAPK signaling is necessary for H3S10 phosphorylation and contextual fear memory consolidation (Chwang et al., 2006). Notably, H3S10 phosphorylation is also increased in the hippocampus following both retrieval of a fear memory and the phosphorylation of ERK/MAPK (Besnard et al., 2014). Although site-specific dephosphorylation on histone tails has not been demonstrated as a cause of memory impairment, these studies collectively suggest that histone phosphorylation contributes to memory formation.

Whereas histone acetylation and histone phosphorylation are associated with a permissive transcriptional state, histone methylation can be transcriptionally permissive or repressive depending on the residue and number of sites affected. The most commonly examined residue is lysine, which can be mono-(me), di-(me2), or tri-methylated (me3) (Ng et al., 2009). Methyl groups are added to residues by histone (lysine) methyltransferases, which are specific to the residue they modify (Black et al., 2012) and are removed by lysine demethylases. For example, unmethylated lysine9 on H3 (H3K9) is converted to H3K9me1 and H3K9me2 by the G9a methyltransferase, but only the MLL methyltransferase can convert H3K4 to H3K4me3 (Jarome and Lubin, 2013). H3K4me3 and H3K9me2, associated with transcriptional activation and repression, respectively, are both increased in the hippocampus following contextual fear conditioning (Gupta et al., 2010). In support of the role of H3K9me2 in memory formation, inhibition of the G9a methyltransferase with BIX01294 impairs contextual fear memory formation (Gupta-Agarwal et al., 2012). The importance of the H3K4 MLL methyltransferase in memory has been demonstrated in two independent studies in which mice deficient in *Mll1* exhibited selective impairments in hippocampus-dependent contextual fear memory (Gupta et al., 2010), and mice deficient in *Mll2* displayed impaired hippocampus-dependent object recognition and object placement memory (Kerimoglu et al., 2013). Although these studies suggest that methylation can affect histones in a site-specific manner, future studies will need to investigate the extent to which demethylation is site-specific.

DNA methylation

DNA methylation occurs when a methyl group is added to cytosine residues predominantly within cytosine-guanine (CpG, where 'p' refers to the phosphodiester bond) dinucleotides by a methyl donor (e.g., *S*-adenosyl methionine; SAM). CpG dense areas are referred to as CpG islands, (Mastroeni et al., 2011). DNA methylation typically represses transcriptional activity when it occurs within the promoter region of a gene, although this is not always the case (Chahrour et al., 2008). The addition of methyl groups results in the recruitment of corepressor complexes to the DNA, inducing a repressive state by conferring a state of tightly packed heterochromatin that prevents binding of transcription factors and molecules necessary for the initiation of transcription (Tsankova et al., 2007). This often includes the recruitment of histone deacetylases (HDACs), which can act concurrently to affect the acetylation state of neighboring histones, further compacting the chromatin and reducing transcriptional activity. The addition of methyl groups occurs with the aid of DNA methyltransferases or DNMTs (Fig. 3), of which at least two types have been identified. Maintenance methylation, which preserves DNA methylation patterns throughout cellular replication, is catalyzed by DNMT1 (Chouliaras et al., 2010). De novo methyltransferases DNMT3a and DNMT3b are responsible for methylation throughout development regardless of previous methylation status (Okano et al., 1999). DNMT2 is not involved in DNA methylation, but instead plays a role in the methylation of aspartic acid transfer RNA (Chouliaras et al., 2010). Together, these enzymes influence transcription and the expression of a number of genes that are critical for neuroplasticity and memory formation (Miller and Sweatt, 2007; Day et al., 2013a). The dysregulation of these enzymes can lead to detrimental changes in gene expression, which may impair neuronal function and contribute to any number of neuropsychiatric disorders.

Early demonstration for the role of DNA methylation in memory came from evidence showing that contextual fear conditioning increased *Dnmt3a* and *Dnmt3b* mRNA in the hippocampus of rats 30 min after training, and that infusion of a DNMT inhibitor into the hippocampus impaired consolidation of contextual fear memory (Miller and Sweatt, 2007). Interestingly, the increase in methylation was at least partially due to hypermethylation of the memory suppressing *PP1* gene (resulting in a net decrease in expression of *PP1*). The persistence of contextual fear memories were later determined to be maintained through DNA methylation in the dorsomedial prefrontal cortex, a brain region important for long-term memory storage (Miller et al., 2010). Additional

evidence for the role of DNA methylation in regulating memory consolidation came from work linking contextual fear memory with expression of brain derived neurotrophic factor (BDNF), a neurotrophin well known to promote synaptic plasticity and memory (Cowansage et al., 2010). The *Bdnf* gene is unique in that it has multiple exons that can be independently and differentially regulated. Contextual fear conditioning increased the mRNA expression of exon IV, which was associated with hypomethylation at CpG islands corresponding to exon IV (Lubin et al., 2008). Similarly, treatment with a DNMT inhibitor was sufficient to impair memory, increase methylation of the *Bdnf* gene, and decrease *Bdnf* gene transcription (Lubin et al., 2008). These findings support the conclusion that manipulation of DNA methylation is yet another method of regulating memory.

Estrogenic regulation of histone modifications and effects on memory

As mentioned earlier in this chapter, the ability of a dorsal hippocampal E_2 infusion to enhance hippocampal memory consolidation depends on the rapid activation of numerous cell-signaling cascades, including ERK/MAPK, in the hippocampus (Fernandez et al., 2008; Fortress et al., 2013). Activation of ERK in the hippocampus leads to an increase in the acetylation and phosphorylation of the histone protein H3, and these increases are associated with memory formation (Levenson et al., 2004; Chwang et al., 2006). Thus, our laboratory reasoned that E_2 might enhance memory by triggering ERK-mediated histone alterations. Indeed, direct infusion of E_2 into the DH increases HAT activity in the DH within 15 min and increases DH H3K9,14 acetylation within 30 min (Zhao et al., 2010, 2012). However, E_2 infusion into the DH does not affect acetylation of H2BK12 or H4K12 (Zhao et al., 2010, 2012), suggesting an effect specific to H3K9,14. Additionally, DH infusion of an ERK inhibitor impaired memory and blocked the E_2-induced increase in H3K9,14 acetylation (Zhao et al., 2010), suggesting that H3 acetylation is critical for estrogenic regulation of hippocampal memory consolidation. To determine the extent to which histone acetylation is necessary for E_2 to enhance memory, the HAT inhibitor garcinol was coinfused with E_2. Garcinol prevented DH-infused E_2 from elevating H3K9,14 acetylation and enhancing object memory consolidation (Zhao et al., 2012), demonstrating that H3 acetylation is necessary for the estrogenic enhancement of object memory formation.

E_2 may regulate histone acetylation in the long term by altering the expression of HDAC proteins. Four hours after DH infusion, E_2 significantly decreases levels of HDAC2 and HDAC3 protein (Zhao et al., 2012; Fortress et al., 2014), both of which have been implicated as negative regulators of hippocampus-dependent memory (Guan et al., 2009; McQuown et al., 2011; Graff et al., 2012). E_2-induced downregulation of HDAC2 protein expression was blocked by garcinol (Zhao et al., 2012), suggesting that HAT activity regulates expression of this memory repressing HDAC. Collectively, these findings suggest that E_2 may exert its beneficial effects on memory by increasing H3 acetylation and reducing the expression of negative regulators of memory such as HDAC2 and HDAC3 (Fig. 2).

Many of the specific gene targets affected by E_2-mediated H3 acetylation remain to be defined, although genes involved in neuroplasticity are likely candidates. One such example is the neurotrophin BDNF, which has an established role in regulating spine density, synaptic plasticity, and memory (Scharfman et al., 2003; Heldt et al., 2007; Spencer et al., 2008; Luine and Frankfurt, 2013). Recent evidence demonstrates that E_2 infusion directly into the DH specifically increases H3 acetylation at *Bdnf* promoters pII and pIV 30 min after infusion in both young and middle-aged ovariectomized mice (Fortress et al., 2014). These alterations in H3 acetylation at pII and pIV precede a significant increase in BDNF and pro-BDNF protein levels that occurs in the DH 4 and 6 h after infusion. Thus, one potential mechanism through which E_2 exerts its beneficial mnemonic effects is via epigenetic regulation of BDNF in the hippocampus. However, many genes are involved in memory formation, so additional research is needed to elucidate how histone alterations may impact the expression of other genes that contribute to the beneficial effects of E_2 on memory.

Estrogenic regulation of DNA methylation and effects on memory

DNA methylation can also be modified by E_2 treatment. For example, infusion of E_2 directly into the DH significantly increases *Dnmt3a* and *Dnmt3b* mRNA levels 45 min later, however, only DNMT3b protein levels were significantly elevated 4 h later (Zhao et al., 2010). Protein and mRNA levels of the maintenance enzyme DNMT1 remained unaffected after direct DH infusion of E_2. Interestingly, E_2-mediated increases in DNMT3b protein were blocked by coadministration of the HAT inhibitor garcinol (Zhao et al., 2012), suggesting that histone acetylation is required for E_2-mediated changes in DNMT3b protein. This finding is consistent with other research demonstrating that interactions between DNA methylation and histone acetylation are critical for modulating cognition and neuroplasticity (Miller et al., 2008). The E_2-mediated enhancement of object memory consolidation is also blocked by a DH infusion of the DNMT inhibitor 5-aza-2′-deoxycytidine (Zhao et al., 2010), suggesting that DNA methylation is also critical for the memory-enhancing effects of E_2 (Fig. 2). However, the specific genes and CpG sites methylated by E_2 in the DH remain unknown and this is an area ripe for future research.

Gaps in Knowledge and Implications for Future Studies

Understanding the mechanisms through which E_2 can orchestrate epigenetic processes to regulate memory is important for understanding cognitive function in both health and disease. Thus far, these mechanisms have only begun to be elucidated as outlined in this chapter. The extent to which E_2 regulates other posttranslational modifications such as histone phosphorylation, ubiquitination, methylation, or SUMOylation in the hippocampus through various cell-signaling mechanisms remains to be identified. Further, only very few gene targets important for memory whose expression is epigenetically regulated by E_2 have been identified to date. Thus, understanding which epigenetic modifications are regulated by E_2 and the downstream transcriptional

and translational consequences of these modifications will be critical next steps. Finally, understanding the functional implications of estrogenic regulation of neuroepigenetics will be imperative to better understanding the etiology of disease and improving the design of drugs for reducing memory dysfunction in the future.

It's also important to consider that it is very unlikely that the epigenetic effects of E_2 on memory are restricted to the hippocampus. Parallel evidence for estrogenic regulation of epigenetic processes exists in other brain regions outside of the hippocampus, including the amygdala and the prefrontal cortex. For example, both the prefrontal cortex and the amygdala are actively being investigated for sensitivity to epigenetic modifications in neuropsychiatric disorders, including PTSD (Pizzimenti and Lattal, 2015). E_2 facilitates extinction learning following fear conditioning, which corresponds to decreased activation in the amygdala and increased activation in the ventromedial prefrontal cortex in rats (Zeidan et al., 2011). Although a necessary role for E_2 in regulating epigenetic mechanisms in the fear memory circuitry has not been tested, a necessary role of histone acetylation in fear memory consolidation was demonstrated using direct infusion of the HAT inhibitor garcinol into the lateral amygdala, which impaired fear memory consolidation in rats (Maddox et al., 2013). Mice that were in the metestrus phase of the estrous cycle (low estrogen state) or were ovariectomized exhibited increased *Hdac4* mRNA in the amygdala following cued fear conditioning, suggesting a potential mechanism through which low levels of E_2 could impair processing of cues related to PTSD (Maddox et al., 2017). These findings suggest that E_2 may promote fear memory consolidation by increasing histone acetylation in the other brain areas, however direct evidence has not been provided.

Finally, the role of de novo neurosteroidogenesis in regulating the epigenetic processes that govern memory formation remains unknown and is an area of increasing clinical relevance. All sex steroid hormones, including E_2, are synthesized in the brain, including the hippocampus, and suppressing endogenous E_2 synthesis with aromatase inhibitors prevents memory formation in female mice and male song birds (Bailey et al., 2013; Tuscher et al., 2016b). Similarly, clinical studies suggest that aromatase inhibition may negatively impact cognitive function in human females. Aromatase inhibitors such as letrozole are used to treat hormone-receptor positive forms of breast cancer (Geisler et al., 2002; Puddefoot et al., 2002), and some findings suggest that such treatments compromise working memory, concentration, and performance in verbal and visual memory tasks (Collins et al., 2009; Bender et al., 2007, 2015). Epigenetic regulation of the aromatase enzyme and downstream epigenetic changes in E_2-mediated memory in response to aromatase inhibitors have yet to be identified, but may prove to be important given increasing evidence that aromatase inhibition leads to memory impairment in females.

Summary

The past 30 years has provided exciting new information about the molecular mechanisms underlying memory formation and dysfunction. In particular, neuroepigenetic studies have illuminated the complexities of gene regulation and revealed a multitude of ways in which epigenetic processes can alter behavior without changing the genetic code. Combined with behavioral neuroendocrinology, neuroepigenetics is advancing our understanding of how hormones regulate the epigenetic processes that influence behavior. Such regulation could explain how environmental, chromosomal, and psychological factors determine individual responses to specific situations. Although information on E_2-mediated epigenetic alterations in the brain remains limited, there is ample potential to explore how sex-steroid hormones modulate behavior and disease in numerous brain regions with implications across a spectrum of disorders. It is our hope that this is where the future will take us and other investigators.

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