Hypotheses

Histone modifications proposed to regulate sexual differentiation of brain and behavior

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Expression of sexually dimorphic behaviors critical for reproduction depends on the organizational actions of steroid hormones on the developing brain. We offer the new hypothesis that transcriptional activities in brain regions executing these sexually dimorphic behaviors are modulated by estrogen-induced modifications of histone proteins. Specifically, in preoptic nerve cells responsible for facilitating male sexual behavior in rodents, gene expression is fostered by increased histone acetylation and reduced methylation (Me), and, that the opposite set of histone modifications will be found in females. Conversely, in ventromedial hypothalamic neurons that are responsible for coordinating female sexual behavior, transcriptional activities in genetic females are fostered by increased histone acetylation and reduced Me, and, further, that the opposite set of histone modifications will be found in males. Thus, these epigenetic events will guarantee that effects of sex hormone exposure during the neonatal critical period will be translated into lasting sex differences in adult reproductive behaviors.

Keywords:
androgens; chromatin remodeling; estrogens; histone modification; hypothalamus; sex behavior

Introduction

Our hypothesis, newly presented here, that during development estrogen-bound estrogen receptors (ERs) induce sexually dimorphic covalent modifications of histone tails, is based on broad knowledge of ERs and their transcriptional activity, which involves modifications of chromatin structure through association with coregulator proteins with histone acetyltransferase (HAT) or histone methyltransferase (HMT) activities. By inducing differential histone modifications in a region- and sex-dependent manner, estrogen-bound ERs may be able to create a specific "histone code" for the set of genes that facilitate development of male sexual behaviors in medial preoptic area (MPOA) neurons and another "code" for the genes necessary for the expression of female sexual behaviors in ventromedial hypothalamic (VMH) neurons.

Historical background

The sexual differentiation of a mammalian embryo starts with the expression of the *SRY* gene, which is located on the sex-determining region of the Y chromosome and drives the formation of testes from the bipotential gonads [1]. The testes in turn produce a peptide, anti-Mullerian hormone (AMH) that causes the atrophy of the female gonads and accessory reproductive tissues. In the absence of the *SRY* gene and AMH, XX females develop ovaries and female

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Abbreviations:
AVPV, anteroventral periventricular nucleus; **E2,** estradiol; **ER,** estrogen receptor; **HDAC,** histone deacetylase; **MPOA,** medial preoptic area; **PR,** progesterone receptor; **SDN-POA,** sexually dimorphic nucleus of the preoptic area; **VMH,** ventromedial hypothalamus.

sexual characteristics [2]. In the last days of gestation in rodents the testes begin to produce testosterone [3], which stimulates the formation of the internal and external organs of reproduction [4], and at the same time has a profound effect on the development of sexually dimorphic brain.

During the perinatal period the hormonal milieus of male and female brains differ significantly. In developing male rodents, testosterone secreted from the testes around the time of birth, freely enters the brain [5]. In contrast, female brains are relatively devoid of sex steroid influence during this period due to quiescent ovaries and high levels of circulating α-fetoprotein, which is produced by the embryo and potently binds and sequesters maternal estradiol (E2) in the fetal circulation [6]. The organizational/activational hypothesis, proposed by Phoenix *et al.* [7] in the landmark 1959 paper, states that during development testosterone and its metabolites act to permanently establish (*i.e.* ''organize'') male neural circuits, which are then activated by gonadal steroids in adulthood to produce male-specific behaviors [7]. On the other hand, in the absence of hormonal influence a female-typical brain is developed. Later it was shown that E2, one of the metabolites of testosterone, could masculinize the brain to a similar extent as testosterone [8, 9], leading to the aromatization hypothesis [10]. The discovery that sexually dimorphic brain regions express high levels of P450 aromatase [11], the enzyme that converts testosterone to E2, and high concentrations of ERs supported this hypothesis [12, 13].

Estrogens are gonadal steroids that exert their biological effects by binding to intracellular receptors that are members of the nuclear receptor superfamily of transcription factors [14, 15]. There are two isoforms of the ER (ERα [16] and ERβ [17]), both of which can bind estrogens with high affinity [17, 18]. ERs bound to estrogens can dimerize [19, 20] and translocate to the nucleus where they bind to hormone-responsive elements (*e.g.* the high affinity estrogen-responsive element) in the promoter regions of target genes and alter the rate of transcription [21]. Transcriptional coregulators, which physically associate with the receptors [22, 23], control the interaction between nuclear receptors and the basic transcriptional machinery. In addition, nuclear receptor coregulators can induce chromatin remodeling through their intrinsic enzymatic activity by placing or removing acetyl and methyl groups on histone proteins, major constituents of chromatin [23–25].

The basic structural unit of chromatin is the nucleosome, which comprises 147 base pairs of DNA wrapped around a core of eight histones (two H2A, H2B, H3, and H4 histones). The N-terminal tails of core histones protrude from the nucleosomes and are subject to a wide range of post-translational modifications of specific amino acid side chains [26], including acetylation of lysines, methylation (Me) of lysines and arginines, and phosphorylation of serines and threonines. These modifications of histone tails disrupt or strengthen interactions between the nucleosome and DNA, thus regulating the access of transcription factors, such as ERs, to the *cis*-acting elements on the target gene promoters [27]. It is now well accepted that hyperacetylated histones H3 and H4 are mostly associated with activated gene transcription, while deacetylation results in gene repression [28, 29]. On the other hand, histone Me appears to have multiple, sometimes opposing effects on chromatin function. Among these, Me of H3K9 and H3K27 is mostly associated with repressed chromatin and gene silencing, whereas Me of H3K4 often leads to permissive chromatin structure [30]. Compared with other post-translational modifications, lysine Me of histone tails is also considered a long-lasting mark [31] found on chromatin regions that are silenced over the long term, inactive X chromosome being one example.

Recently it has been proposed that distinct stimuli (or combination of stimuli) may elicit specific sequences and combinations of histone modifications, establishing the so-called ''histone code'', which in turn determines the transcriptional profile of response genes associated with it [32, 33]. Multiple studies have demonstrated that proper epigenetic control of gene expression requires the cooperation of histone modifications and disruption of these processes can lead to abnormal gene expression profiles seen in human cancers.

Hypotheses

Based on these data we hypothesize that to support the development of dimorphic sexual behavior, ER signaling may induce differential chromatin remodeling in the brain areas that are involved in the expression of these behaviors, *i.e.* MPOA in males and VMH in females (Fig. 1). Specifically, by inducing histone acetylation at the promoters of target genes in male rodent brain, estrogen can increase the expression of genes that facilitate development of male sexual behavior, while Me of histones at the promoters of genes critical for female-type behaviors will lead to their suppression. These molecular events will result in the fine-tuning of neural circuits in MPOA necessary for the expression of male sexual behaviors. In the absence of estrogen in the female rodent brain the pattern of histone modifications is most likely the opposite of that found in male brain. Promoters of genes in the VMH involved in the expression of female sexual behaviors will be associated with acetylated histones and exhibit increased transcriptional activity. On the other hand, genes necessary for masculinization of behaviors will be turned off as a result of association with methylated histones.

These differential processes are presumably induced by association of ligand-bound ERs with various transcriptional coregulators that can catalyze covalent histone modifications on the promoters of target genes and alter their rate of transcription. Such mechanisms of transcriptional regulation employed by ERs have been described in neuronal as well as non-neuronal systems [34–37], and a large number of cofactors involved in ER-mediated transactivation have already been identified [22–24, 38–40]. Among them, nuclear receptor coactivators, SRC and CPB, as well as the corepressor NCoR exhibit sexually dimorphic pattern of expression [41–43], and are necessary for the development of sexually dimorphic behaviors in adulthood [42–44]. These data directly support our hypothesis and emphasize the importance of chromatin remodeling in the development of dimorphic brain and behavior.

The most exquisite test of our hypothesis would be to use chromatin immunoprecipitation (ChIP) for specific,

Hypotheses

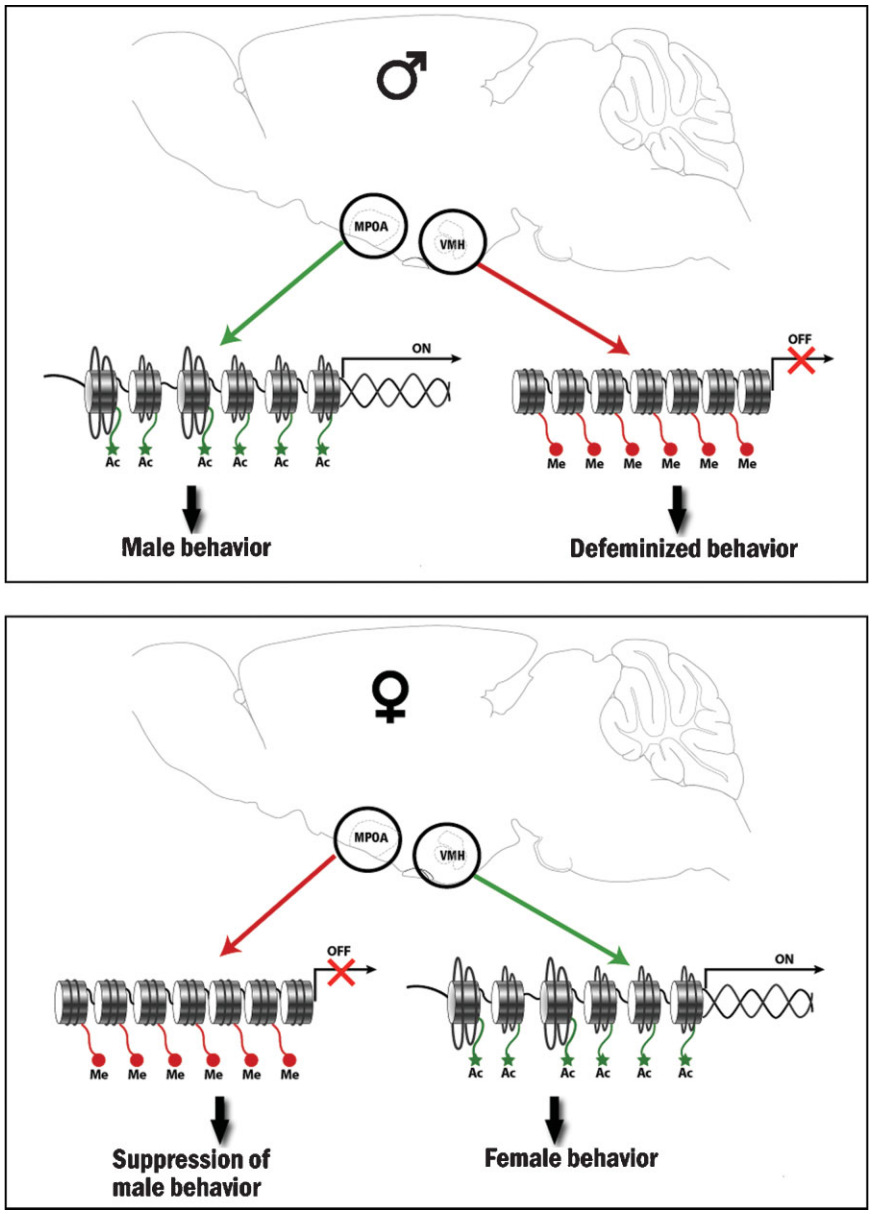

Figure 1. Cartoon sketched to illustrate in the simplest possible terms the types of histone modifications we hypothesize. For clarity, they are illustrated in their most extreme possible form. Top panel: In the MPOA of the male, we predict that a larger number of histone tails would be acetylated (Ac) and methylation (Me) would be minimized. As a result, behaviorally relevant transcriptional activities would be heightened, and the initiation of male-typical behaviors would be facilitated. By comparison, in the VMH, Me would predominate, and female-typical behavior would be suppressed. Bottom panel: In contrast, in the female, in the MPOA, Me would be widespread with the consequence of suppressing transcription-dependant male behavior. In the VMH, acetylation would predominate, thus facilitating a range of behaviorally relevant transcriptional events and permitting the initiation of female-typical behaviors.

behaviorally relevant genes in the VMH and in the MPOA (Fig. 2). For example, histones governing access to the progesterone receptor (PR) promoter in the VMH of the female brain should be more heavily acetylated and less heavily methylated than in the VMH of the male brain. This is because estrogens induce PR and, as a result, the crucial, lordotic sex behavior in the female but not in the male rodents. Conversely, MPOA neurons must be turned on by dopamine (DA) in the male rodent brain for male sex behavior to occur. Thus, we would predict that in POA neurons of the male brain, histones governing the promoter of the D1 DA receptor would be more heavily acetylated and less heavily methylated, than would be true in the female brain.

Our hypothesis suggests that taking into account the number of nuclear receptor coregulators and the number of possible histone modifications that they can induce, estrogen binding to its receptor may result in a nearly limitless combination of activated or repressed genes, creating the developmental framework that will lead to the expression of complex mammalian behaviors in the adulthood in a sex-specific manner.

Appropriate histone modifications result in sexually dimorphic behaviors

Perinatal exposure to steroid hormones has profound effects on the expression of sexually dimorphic behaviors in adulthood. Behaviors that serve a reproductive function are considered the best examples of sexually dimorphic behaviors and include proceptive and receptive behaviors in female rodents as well as appetitive and copulatory behaviors for male rodents. The neural substrates that are important for the execution of these behaviors have been mapped out and include the MPOA, a hypothalamic nucleus that is critical for the expression of male sexual behavior, and the VMH, a nucleus that is essential for the display of estrogen-induced lordosis, the receptive posture of female rodents [45, 46].

In both sexes, the expression of reproductive behaviors is hormone dependent and can be abolished by gonadectomy in adulthood [7, 47–49]. Although there are some emerging data that describe the importance of genetic sex in the development of sex differences in brain morphology and behavior [50], a large body of evidence supports the critical role of the hormonal milieu during the perinatal period of development in the expression of sexual behaviors in adulthood, particularly in mammals. In male rodents the perinatally released testosterone and its

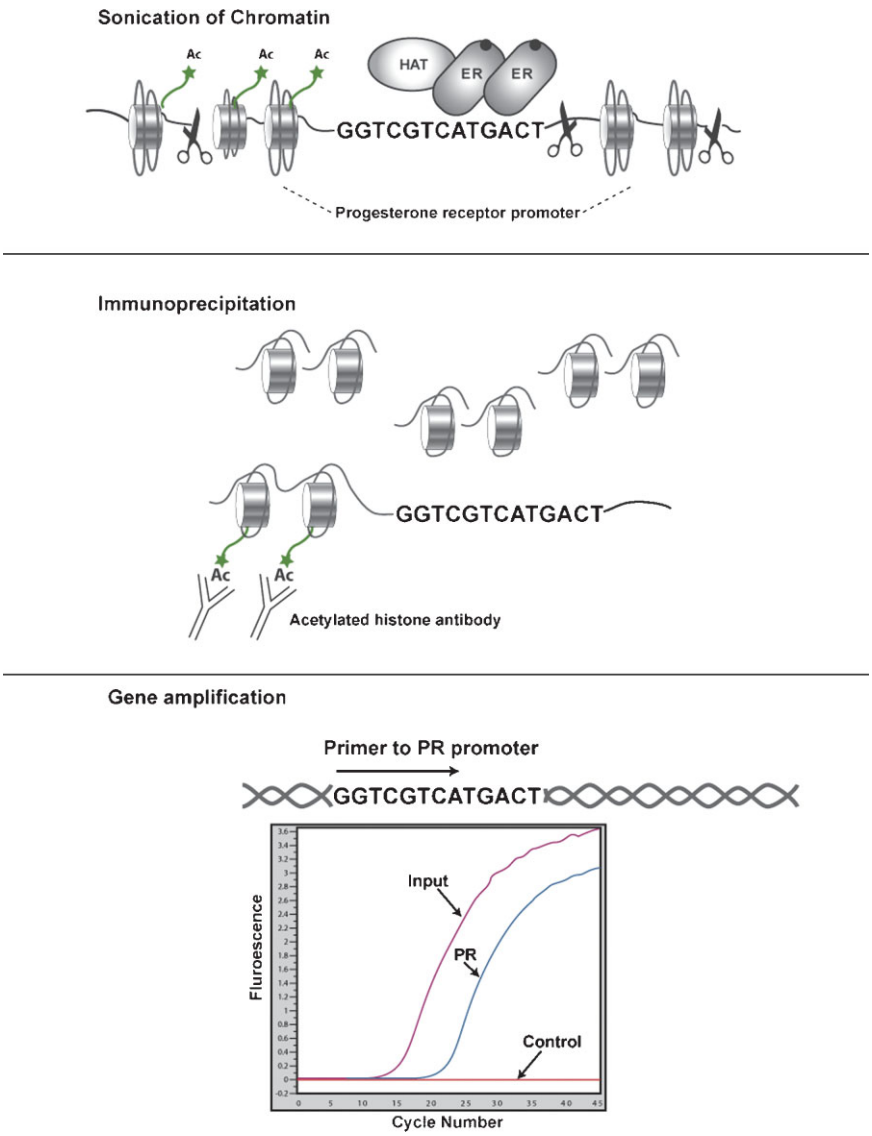

Figure 2. The type of hypothesis illustrated in Fig. 1 predicts increased transcription, in the VMH, for genes that participate in the facilitation of female reproductive behavior. Sketched here is the means for testing that prediction with respect to one crucial gene encoding the progesterone receptor (PR). Following the isolation of chromatin, the assay (ChIP) gains its specificity from two sources: First, the immunoprecipitation uses an antibody specific for a particular form of histone modification (middle panel). Then, the use of a primer specific to the PR promoter is used for PCR (bottom panel). We predict that the type of estrogen administration that facilitates PR transcription and, as a consequence, female reproductive behavior would result in acetylation of histones associated with the PR promoter.

metabolite-estrogen ''masculinize'' brain morphology and male-typical behaviors [51–53]. The time window during which steroid hormones are able to induce changes that lead to the sex differences in adulthood is very restricted and is termed a ''critical period''. Manipulations of hormone levels during this time period by gonadectomy or exogenous hormone treatments as well as modulation of aromatase activity can reverse sex-typical phenotype and result in males that display lordosis or females that exhibit male-typical sex behaviors in adulthood [7, 47, 48, 53, 54].

Perinatal hormone exposure is necessary not only to ''masculinize'' but also to ''defeminize,'' *i.e.* to reduce the ability to display female sexual behavior. Rather than being a ''default'' state, defeminization is also believed to comprise active developmental processes that can be dissociated from ''masculinization'' at the cellular and molecular level [55]. This distinction became most evident from the analysis of sexual behaviors of ERα- and ERβ-knockout mice. Although these mice are not suitable for differentiating the ''organizational'' and ''activational'' effects of estrogen, comparison of male and female sexual behaviors in adulthood led to the distinction that ERα signaling is necessary for the masculinization and ERβ is necessary for the defeminization of behavior [56–59].

It is evident that the ''organization'' of sex-typical behaviors following hormonal exposure during development is not absolute and has variable effect on different components of male and female motivational and sexual behaviors, suggesting the complexity of the developmental programming. These complex outcomes and persistent effects of relatively brief hormonal exposure can be easily explained by epigenetic regulation of gene expression. This mechanism allows cells to maintain repressed or activated transcription states long after the initial signal is terminated by using acetylated or methylated histone proteins as ''tags'' for the association of transcriptional activators and repressors with the promoters of specific genes [60–62]. Thus, the genes that are necessary for the expression of various components of male sexual behaviors can be turned on and genes that foster the development of female sexual behaviors can be turned off in the brains of males as a result of a singe brief exposure to E2 during development. Further, this pattern of gene expression may be able to persist through adulthood without any additional signal, as long as histone proteins associated with the regulatory regions of target genes retain post-translational modifications induced by E2.

Among these genes may be the classical target gene of ER-PR, which itself is a transcription factor of the nuclear receptor superfamily [15] and has been shown to be important for normal reproductive behavior in male rats [63] and the formation of the sexually dimorphic nucleus of the preoptic area (SDN-POA) [64]. Interestingly, the pattern of sex difference in the expression and transcriptional regulation of the PR in the MPOA and VMN during development

can not be explained just by steroid hormone exposure, suggesting that additional factors, possibly chromatin-modifying coregulators, may be modulating ER transcriptional activity [65].

Other gene products that may be differentially regulated by estrogen-induced epigenetic factors could include COX-2, an enzyme involved in the prostaglandin-E2 (PGE2) synthesis, which in turn has been shown to masculinize sexual behavior without a defeminizing effect [66, 67].

In addition, preliminary results from our group indicate that neonatal expression of connexin-36, a major constituent of neuronal gap junctions is sexually dimorphic, with higher levels of mRNA found in mediobasal and preoptic areas of hypothalamus and amygdala of male mice (Westberg, Devidze, and Pfaff, unpublished data). Although the importance of these differences in the development of sexually dimorphic behaviors has not been addressed, it is plausible that gap junction channels, which modulate neuronal activity by synchronizing large neuronal ensembles, exert profound effects on the development and fine-tuning of circuits underlying dimorphic sexual behaviors. Thus, the examination of the influence of hormonal exposure and the chromatin remodeling on the transcriptional regulation of Cnx-36 gene during critical period of development, is an interesting avenue of future research. Moreover, analyses of gene expression profiles following ChIP with antibodies for ERs, their coregulators and acetylated and methylated histones (ChIP on Chip) will provide valuable information about the molecular pathways employed by estrogen and identify largely unknown genes that are necessary for the development of sexually dimorphic brain and behaviors as well as help decipher the ''histone code'' associated with this process.

Same histone modifications result in sexually dimorphic brain morphology

Exposure to gonadal steroid hormones during the developmental ''critical period'' not only determines the expression of sexually dimorphic behaviors in adulthood but also permanently changes cytoarchitecture of the brain. Several dimorphic brain structures have been described in a number of species. Although sex differences are mostly found in nuclei that are involved in the execution of sexually dimorphic behaviors, such as MPOA and VMH, the direct link between the structure and function has not been clearly demonstrated [68].

The sex differences found in dimorphic brain structures are not uniform in nature: some result from disproportion in cell numbers and some arise from the differences in synaptic connections or neuropil density; dimorphisms in chemical characteristics of cells and arborization have also been described [52, 69–73]. Moreover, most dimorphic nuclei described in rodent species are larger in males with the exception of anteroventral periventricular nucleus (AVPV), which is larger in females [70]. Notably the difference in AVPV between the sexes is revealed in the density of cells as well as in their chemical characteristics: females have many more dopaminergic [74] and about ten times more kisspeptin-expressing neurons [71, 75], which project to and stimulate gonadotropin-releasing hormone (GnRH) neurons triggering the luteinizing hormone (LH) surge [76, 77]. Thus, the higher number of kisspeptin neurons in the female AVPV may have direct physiological consequences and explain the sex difference in the induction of LH surges, as males do not show this neuroendocrine response.

Regardless of the brain region and cellular compartment in which sex differences are found, they all result from perinatal exposure to the steroid hormones: testosterone converted into E2 and signaling through ERs drives the formation of sexually dimorphic brain structures. Experimental manipulations of hormone levels in neonatal animals, such as castration of males and treatment of females with E2 or testosterone propionate can eliminate these differences [69, 78–80]. Studies done using selective agonists of ERα or ERβ, as well as mice with genetic ablation of ERα and ERβ and the aromatase genes also confirmed that estrogen-mediated activation of ERs is a critical step for the dimorphic brain development [81, 82]. However, in male mice with a mutation that renders androgen receptors hypofunctional (Tfm mutation) the sex differences in the size of posterior bed nucleus of the stria terminalis (pBNST) is also reduced, suggesting that androgen receptor signaling itself is important for the development of sex difference in this nucleus [83].

It has been hypothesized that the control of cell numbers by apoptosis is the mechanism by which sex differences in brain morphology are achieved [84]. Supporting this hypothesis are the studies demonstrating dimorphism in the expression of pro- and anti-apoptotic proteins, Bax and Bcl-2, respectively, in structurally dimorphic brain regions [85, 86]. Moreover, both overexpression of anti-apoptotic proteins or deletion of pro-apoptotic genes in brain have been shown to eliminate structural sex differences [87, 88].

Sexually dimorphic expression of pro- and anti-apoptotic genes and apoptotic cell death is regulated by the same stimulus, E2, and evidence suggests that this regulation is on the transcriptional level [89]. In fact it has been demonstrated that the human Bcl-2 gene contains the sequence of estrogen-response elements, and that estrogen can inhibit apoptosis in human breast cancer MCF-7 cells by inducing transcription of Bcl-2 [90]. Thus, it is plausible that estrogen can directly regulate Bcl-2 transcription, and thereby also control cell death by apoptosis in neurons.

Paradoxically, estrogens regulate apoptotic genes with opposing patterns of expression in brain regions that exhibit opposing morphology between sexes, *i.e.* SDN-POA, which is larger in males, and AVPV that is larger in females. The question remains as to how this is achieved? One mechanism by which estrogens may be able to accomplish such dual regulation is through chromatin remodeling. By recruiting histone-modifying enzymes, which will place permissive acetyl groups or repressive methyl marks on the promoters of Bcl-2 and Bax, respectively, estrogen can selectively activate transcription of the former and repress the latter in the SDN-POA of male pups. On the other hand, if the histone modifications associated with pro- and anti-apoptotic gene promoters in AVPV are opposite to those found in SDN-POA

(methylated histones on Bcl-2 promoter and acetylated histones on Bax promoter), *e.g.* due to variations in coregulator availability, the reverse structural dimorphism will be achieved. Thus, recruitment by ERs of coactivators and corepressors with different histone modifying enzymatic activity will enable these nuclear transcription factors to differentially induce or repress pro- and anti-apoptotic genes in different brain regions of the two sexes that will lead to the development of sexually dimorphic structures.

Histone modifications are already associated with functional changes in the nervous system

Our hypothesis that implicates chemical modifications of histones in a set of central nervous system (CNS) processes has precedents. Although the cancer field has always been the stronghold of chromatin biology, in recent years a great deal of attention has been devoted to the study of epigenetics in the nervous system. Recent evidence indicates that within the CNS neurons employ epigenetic modifications to translate external stimuli into long-lasting functional and morphological changes that produce both physiological and pathological behaviors. For example, histone modifications, specifically histone acetylation, have been shown to play an important role in memory consolidation in a variety of paradigms as well as synaptic plasticity [91, 92]. Chromatin remodeling has also been implicated in the development of pathological conditions such as depression and addiction [93, 94] and has been shown to be induced by acute and chronic stress [95]. Furthermore, epigenetic dysregulation, specifically reduced histone acetylation, is a common theme in neurodegenerative and neurodevelopmental disorders [96, 97]. Based on these evidence, histone deacetylase (HDAC) inhibitors are now considered as valid drug targets for the treatment of a number of neurological and psychiatric disorders [96].

In line with our hypothesis that estrogen may induce sexually dimorphic changes in histone acetylation and Me, leading to the development of sexually dimorphic behaviors, is the recent study demonstrating sex differences in both acetylation (H3K9/14Ac) and Me (H3K9Me3) levels of histone H3 in cortex and hippocampus [98]. The authors also determined that H3 acetylation was regulated by prenatal exposure to testosterone; however, H3 Me was dimorphic regardless of hormonal milieu. Interestingly, these differences were only found in cortex/hippocampus and not in POA/hypothalamus, the area that exhibits dimorphic morphology and is involved in the control of dimorphic sex behaviors. In this regard, it needs to be considered that the authors only analyzed modifications affecting three residues on one of the histone proteins. It is plausible and expected that more complex combinatorial changes in chromatin structure will be necessary for the development of many components of sexually dimorphic behavior. It is also quite possible that estrogen induces significant changes in the acetylation and/or Me of histones associated with the promoters of specific genes without affecting the global levels of these modifications. If this is the case, the type of analyses described by Tsai *et al.* [98] is not sufficient to detect these changes and more detailed examination of target genes will be necessary.

Another piece of solid evidence that epigenetic regulation is important for the development of a sexually dimorphic bed nucleus of the stria terminalis (BNST) formation came from a study in which injection of HDAC inhibitor valproic acid (VPA) on the day of birth blocked masculinization of this nucleus [99] in males as well as in hormone-treated females. These data suggest that histone deacetylation is critical for the development of this sexually dimorphic brain region. However, the specific histone modifications that were affected by VPA treatment and/or specific genes regulated by these modifications were not described.

These studies, although preliminary, open a window onto the new emerging field of epigenetic regulation of neural functions and will certainly advance our understanding of developmental processes underlying brain formation and complex mammalian behaviors.

Outlook: Our new transcriptional interpretation of a classic hypothesis portends a new generation of experimental work

Few scientific hypotheses have stood the test of time as well as the ''organizational/activational'' hypothesis proposed by Phoenix and colleagues. Fifty years later, it is well established that the developing mammalian brain is a bipotential organ, which can assume either a male or female sex identity. The process that will determine the social and reproductive fate of the animal takes place within a very short developmental time period, generally around the time of birth, and is driven by estrogens, and to lesser extent androgens. During this developmental time window estrogen binding to its receptors initiates a cascade of molecular and cellular changes that permanently ''organize'' male brains and allows the expression of male-typical behaviors in adulthood, while suppressing female-specific behaviors. Very little, however, is known about the nature of the effector molecules and the downstream processes that underlie these permanent changes in the brain.

Testing our histone-code-based hypothesis will require systematic analysis of histone modifications in different regions of developing brain and, with the availability of new research tools, this is within reach. More important and challenging tasks would be identifying effector genes that are turned on and off by these epigenetic changes and elucidating downstream molecular and cellular mechanisms underlying hormone-induced sex differences.

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References

1. **Koopman P**, **Munsterberg A**, **Capel B**, **Vivian N**, *et al*. 1990. Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature* **348**: 450–2.

2. **Sinclair AH**, **Berta P**, **Palmer MS**, **Hawkins JR**, *et al*. 1990. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* **346**: 240–4.
3. **Weisz J**, **Ward IL.** 1980. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology* **106**: 306–16.
4. **Wilson JD**, **Griffin JE**, **George FW.** 1980. Sexual differentiation: early hormone synthesis and action. *Biol Reprod* **22**: 9–17.
5. **Rhoda J**, **Corbier P**, **Roffi J.** 1984. Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: aromatization of testosterone to 17 beta-estradiol. *Endocrinology* **114**: 1754–60.
6. **Andrews GK**, **Dziadek M**, **Tamaoki T.** 1982. Expression and methylation of the mouse alpha-fetoprotein gene in embryonic, adult, and neoplastic tissues. *J Biol Chem* **257**: 5148–53.
7. **Phoenix CH**, **Goy RW**, **Gerall AA**, **Young WC.** 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* **65**: 369–82.
8. **Feder HH**, **Whalen RE.** 1965. Feminine behavior in neonatally castrated and estrogen-treated male rats. *Science* **147**: 306–7.
9. **Booth JE.** 1977. Sexual behaviour of neonatally castrated rats injected during infancy with oestrogen and dihydrotestosterone. *J Endocrinol* **72**: 135–41.
10. **MacLusky NJ**, **Naftolin F.** 1981. Sexual differentiation of the central nervous system. *Science* **211**: 1294–302.
11. **Reddy VV**, **Naftolin F**, **Ryan KJ.** 1974. Conversion of androstenedione to estrone by neural tissues from fetal and neonatal rats. *Endocrinology* **94**: 117–21.
12. **Pfaff D**, **Keiner M.** 1973. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J Comp Neurol* **151**: 121–58.
13. **Shughrue PJ**, **Lane MV**, **Merchenthaler I.** 1997. Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* **388**: 507–25.
14. **Tsai MJ**, **O'Malley BW.** 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem* **63**: 451–86.
15. **Mangelsdorf DJ**, **Thummel C**, **Beato M**, **Herrlich P**, *et al*. 1995. The nuclear receptor superfamily: the second decade. *Cell* **83**: 835–9.
16. **Green S**, **Walter P**, **Kumar V**, **Krust A**, *et al*. 1986. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature* **320**: 134–9.
17. **Kuiper GG**, **Carlsson B**, **Grandien K**, **Enmark E**, *et al*. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* **138**: 863–70.
18. **Hewitt SC**, **Korach KS.** 2002. Estrogen receptors: structure, mechanisms and function. *Rev Endocr Metab Disord* **3**: 193–200.
19. **Kuntz MA**, **Shapiro DJ.** 1997. Dimerizing the estrogen receptor DNA binding domain enhances binding to estrogen response elements. *J Biol Chem* **272**: 27949–56.
20. **Kumar V**, **Chambon P.** 1988. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* **55**: 145–56.
21. **Beato M**, **Sanchez-Pacheco A.** 1996. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocr Rev* **17**: 587–609.
22. **Onate SA**, **Tsai SY**, **Tsai MJ**, **O'Malley BW.** 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**: 1354–7.
23. **McKenna NJ**, **Xu J**, **Nawaz Z**, **Tsai SY**, *et al*. 1999. Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *J Steroid Biochem Mol Biol* **69**: 3–12.
24. **Bannister AJ**, **Kouzarides T.** 1996. The CBP co-activator is a histone acetyltransferase. *Nature* **384**: 641–3.
25. **Spencer TE**, **Jenster G**, **Burcin MM**, **Allis CD**, *et al*. 1997. Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* **389**: 194–8.
26. **Kouzarides T.** 2007. Chromatin modifications and their function. *Cell* **128**: 693–705.
27. **Kim MY**, **Hsiao SJ**, **Kraus WL.** 2001. A role for coactivators and histone acetylation in estrogen receptor alpha-mediated transcription initiation. *EMBO J* **20**: 6084–94.
28. **Utley RT**, **Ikeda K**, **Grant PA**, **Cote J**, *et al*. 1998. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. *Nature* **394**: 498–502.
29. **Turner BM.** 2000. Histone acetylation and an epigenetic code. *BioEssays* **22**: 836–45.
30. **Goll MG**, **Bestor TH.** 2002. Histone modification and replacement in chromatin activation. *Genes Dev* **16**: 1739–42.
31. **Margueron R**, **Trojer P**, **Reinberg D.** 2005. The key to development: interpreting the histone code? *Curr Opin Genet Dev* **15**: 163–76.
32. **Strahl BD**, **Allis CD.** 2000. The language of covalent histone modifications. *Nature* **403**: 41–5.
33. **Jenuwein T**, **Allis CD.** 2001. Translating the histone code. *Science* **293**: 1074–80.
34. **Molenda HA**, **Griffin AL**, **Auger AP**, **McCarthy MM**, *et al*. 2002. Nuclear receptor coactivators modulate hormone-dependent gene expression in brain and female reproductive behavior in rats. *Endocrinology* **143**: 436–44.
35. **Perillo B**, **Ombra MN**, **Bertoni A**, **Cuozzo C**, *et al*. 2008. DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression. *Science* **319**: 202–6.
36. **Ellison-Zelski SJ**, **Solodin NM**, **Alarid ET.** 2009. Repression of ESR1 through actions of estrogen receptor alpha and Sin3A at the proximal promoter. *Mol Cell Biol* **29**: 4949–58.
37. **Kim H**, **Heo K**, **Kim JH**, **Kim K**, *et al*. 2009. Requirement of histone methyltransferase SMYD3 for estrogen receptor-mediated transcription. *J Biol Chem* **284**: 19867–77.
38. **Belandia B**, **Orford RL**, **Hurst HC**, **Parker MG.** 2002. Targeting of SWI/SNF chromatin remodelling complexes to estrogen-responsive genes. *EMBO J* **21**: 4094–103.
39. **McKenna NJ**, **O'Malley BW.** 2002. Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **108**: 465–74.
40. **Belandia B**, **Parker MG.** 2003. Nuclear receptors: a rendezvous for chromatin remodeling factors. *Cell* **114**: 277–80.
41. **Misiti S**, **Koibuchi N**, **Bei M**, **Farsetti A**, *et al*. 1999. Expression of steroid receptor coactivator-1 mRNA in the developing mouse embryo: a possible role in olfactory epithelium development. *Endocrinology* **140**: 1957–60.
42. **Auger AP**, **Perrot-Sinal TS**, **Auger CJ**, **Ekas LA**, *et al*. 2002. Expression of the nuclear receptor coactivator, cAMP response element-binding protein, is sexually dimorphic and modulates sexual differentiation of neonatal rat brain. *Endocrinology* **143**: 3009–16.
43. **Jessen HM**, **Kolodkin MH**, **Bychowski ME**, **Auger CJ**, *et al*. 2010. The nuclear receptor corepressor has organizational effects within the developing amygdala on juvenile social play and anxiety-like behavior. *Endocrinology* **151**: 1212–20.
44. **Auger AP**, **Tetel MJ**, **McCarthy MM.** 2000. Steroid receptor coactivator-1 (SRC-1) mediates the development of sex-specific brain morphology and behavior. *Proc Natl Acad Sci USA* **97**: 7551–5.
45. **Pfaff DW**, **Sakuma Y.** 1979. Facilitation of the lordosis reflex of female rats from the ventromedial nucleus of the hypothalamus. *J Physiol* **288**: 189–202.
46. **Larsson K**, **Heimer L.** 1964. Mating behaviour of male rats after lesions in the preoptic area. *Nature* **202**: 413–4.
47. **Beach FA**, **Noble RG**, **Orndoff RK.** 1969. Effects of perinatal androgen treatment on responses of male rats to gonadal hormones in adulthood. *J Comp Physiol Psychol* **68**: 490–7.
48. **Baum MJ.** 1979. A comparison of the effects of methyltrienolone (R 1881) and 5 alpha-dihydrotestosterone on sexual behavior of castrated male rats. *Horm Behav* **13**: 165–74.
49. **Olster DH**, **Blaustein JD.** 1988. Progesterone facilitation of lordosis in male and female Sprague-Dawley rats following priming with estradiol pulses. *Horm Behav* **22**: 294–304.
50. **Arnold AP.** 2009. The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Horm Behav* **55**: 570–8.
51. **Pfaff DW**, **Zigmond RE.** 1971. Neonatal androgen effects on sexual and non-sexual behavior of adult rats tested under various hormone regimes. *Neuroendocrinology* **7**: 129–45.
52. **Wu MV**, **Manoli DS**, **Fraser EJ**, **Coats JK**, *et al*. 2009. Estrogen masculinizes neural pathways and sex-specific behaviors. *Cell* **139**: 61–72.
53. **Morris JA**, **Jordan CL**, **Breedlove SM.** 2004. Sexual differentiation of the vertebrate nervous system. *Nat Neurosci* **7**: 1034–9.
54. **McEwen BS.** 1983. Gonadal steroid influences on brain development and sexual differentiation. *Int Rev Physiol* **27**: 99–145.
55. **McCarthy MM**, **Wright CL**, **Schwarz JM.** 2009. New tricks by an old dogma: mechanisms of the organizational/activational hypothesis of steroid-mediated sexual differentiation of brain and behavior. *Horm Behav* **55**: 655–65.
56. **Rissman EF**, **Wersinger SR**, **Taylor JA**, **Lubahn DB.** 1997. Estrogen receptor function as revealed by knockout studies: neuroendocrine and behavioral aspects. *Horm Behav* **31**: 232–43.
57. **Ogawa S**, **Washburn TF**, **Taylor J**, **Lubahn DB**, *et al*. 1998. Modifications of testosterone-dependent behaviors by estrogen receptor-

alpha gene disruption in male mice. *Endocrinology* **139**: 5058–69.
58. **Ogawa S**, **Chan J**, **Chester AE**, **Gustafsson JA**, *et al*. 1999. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. *Proc Natl Acad Sci USA* **96**: 12887–92.
59. **Kudwa AE**, **Bodo C**, **Gustafsson JA**, **Rissman EF.** 2005. A previously uncharacterized role for estrogen receptor beta: defeminization of male brain and behavior. *Proc Natl Acad Sci USA* **102**: 4608–12.
60. **Cavalli G**, **Paro R.** 1998. Chromo-domain proteins: linking chromatin structure to epigenetic regulation. *Curr Opin Cell Biol* **10**: 354–60.
61. **Cao R**, **Wang L**, **Wang H**, **Xia L**, *et al*. 2002. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**: 1039–43.
62. **Felsenfeld G**, **Groudine M.** 2003. Controlling the double helix. *Nature* **421**: 448–53.
63. **Lonstein JS**, **Quadros PS**, **Wagner CK.** 2001. Effects of neonatal RU486 on adult sexual, parental, and fearful behaviors in rats. *Behav Neurosci* **115**: 58–70.
64. **Quadros PS**, **Lopez V**, **De Vries GJ**, **Chung WC**, *et al*. 2002. Progesterone receptors and the sexual differentiation of the medial preoptic nucleus. *J Neurobiol* **51**: 24–32.
65. **Quadros PS**, **Wagner CK.** 2008. Regulation of progesterone receptor expression by estradiol is dependent on age, sex and region in the rat brain. *Endocrinology* **149**: 3054–61.
66. **Amateau SK**, **McCarthy MM.** 2004. Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nat Neurosci* **7**: 643–50.
67. **Todd BJ**, **Schwarz JM**, **McCarthy MM.** 2005. Prostaglandin-E2: a point of divergence in estradiol-mediated sexual differentiation. *Horm Behav* **48**: 512–21.
68. **de Vries GJ**, **Sodersten P.** 2009. Sex differences in the brain: the relation between structure and function. *Horm Behav* **55**: 589–96.
69. **Gorski RA**, **Gordon JH**, **Shryne JE**, **Southam AM.** 1978. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res* **148**: 333–46.
70. **Bleier R**, **Byne W**, **Siggelkow I.** 1982. Cytoarchitectonic sexual dimorphisms of the medial preoptic and anterior hypothalamic areas in guinea pig, rat, hamster, and mouse. *J Comp Neurol* **212**: 118–30.
71. **Kauffman AS**, **Gottsch ML**, **Roa J**, **Byquist AC**, *et al*. 2007. Sexual differentiation of Kiss1 gene expression in the brain of the rat. *Endocrinology* **148**: 1774–83.
72. **Madeira MD**, **Ferreira-Silva L**, **Paula-Barbosa MM.** 2001. Influence of sex and estrus cycle on the sexual dimorphisms of the hypothalamic ventromedial nucleus: stereological evaluation and Golgi study. *J Comp Neurol* **432**: 329–45.
73. **Amateau SK**, **McCarthy MM.** 2002. A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin-E2. *J Neurosci* **22**: 8586–96.
74. **Simerly RB**, **Swanson LW**, **Handa RJ**, **Gorski RA.** 1985. Influence of perinatal androgen on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral periventricular nucleus of the rat. *Neuroendocrinology* **40**: 501–10.
75. **Clarkson J**, **Herbison AE.** 2006. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. *Endocrinology* **147**: 5817–25.
76. **Terasawa E**, **Wiegand SJ**, **Bridson WE.** 1980. A role for medial preoptic nucleus on afternoon of proestrus in female rats. *Am J Physiol* **238**: E533–9.
77. **Wiegand SJ**, **Terasawa E**, **Bridson WE.** 1978. Persistent estrus and blockade of progesterone-induced LH release follows lesions which do not damage the suprachiasmatic nucleus. *Endocrinology* **102**: 1645–8.
78. **Dohler KD**, **Coquelin A**, **Davis F**, **Hines M**, *et al*. 1984. Pre- and postnatal influence of testosterone propionate and diethylstilbestrol on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. *Brain Res* **302**: 291–5.
79. **Guillamon A**, **Segovia S**, **Del Abril A.** 1988. Early effects of gonadal steroids on the neuron number in the medial posterior region and the lateral division of the bed nucleus of the stria terminalis in the rat. *Brain Res Dev Brain Res* **44**: 281–90.
80. **Davis EC**, **Shryne JE**, **Gorski RA.** 1996. Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. *Neuroendocrinology* **63**: 142–8.
81. **Tsukahara S**, **Watai K**, **Kuroda Y**, **Ozawa T**, *et al*. 2007. Elimination of sex differences in the size of the principal nucleus of the bed nucleus of the stria terminalis (BSTp) in estrogen receptor-α and -β and aromatase knockout mice, Yokohama, Japan.
82. **Patchev AV**, **Gotz F**, **Rohde W.** 2004. Differential role of estrogen receptor isoforms in sex-specific brain organization. *FASEB J* **18**: 1568–70.
83. **Durazzo A**, **Morris JA**, **Breedlove SM**, **Jordan CL.** 2007. Effects of the testicular feminization mutation (tfm) of the androgen receptor gene on BSTMPM volume and morphology in rats. *Neurosci Lett* **419**: 168–71.
84. **Tsukahara S.** 2009. Sex differences and the roles of sex steroids in apoptosis of sexually dimorphic nuclei of the preoptic area in postnatal rats. *J Neuroendocrinol* **21**: 370–6.
85. **Tsukahara S**, **Kakeyama M**, **Toyofuku Y.** 2006. Sex differences in the level of Bcl-2 family proteins and caspase-3 activation in the sexually dimorphic nuclei of the preoptic area in postnatal rats. *J Neurobiol* **66**: 1411–9.
86. **Chung WC**, **Swaab DF**, **De Vries GJ.** 2000. Apoptosis during sexual differentiation of the bed nucleus of the stria terminalis in the rat brain. *J Neurobiol* **43**: 234–43.
87. **Gotsiridze T**, **Kang N**, **Jacob D**, **Forger NG.** 2007. Development of sex differences in the principal nucleus of the bed nucleus of the stria terminalis of mice: role of Bax-dependent cell death. *Dev Neurobiol* **67**: 355–62.
88. **Forger NG**, **Rosen GJ**, **Waters EM**, **Jacob D**, *et al*. 2004. Deletion of Bax eliminates sex differences in the mouse forebrain. *Proc Natl Acad Sci USA* **101**: 13666–71.
89. **Tsukahara S**, **Hojo R**, **Kuroda Y**, **Fujimaki H.** 2008. Estrogen modulates Bcl-2 family protein expression in the sexually dimorphic nucleus of the preoptic area of postnatal rats. *Neurosci Lett* **432**: 58–63.
90. **Perillo B**, **Sasso A**, **Abbondanza C**, **Palumbo G.** 2000. 17beta-estradiol inhibits apoptosis in MCF-7 cells, inducing bcl-2 expression *via* two estrogen-responsive elements present in the coding sequence. *Mol Cell Biol* **20**: 2890–901.
91. **Levenson JM**, **O'Riordan KJ**, **Brown KD**, **Trinh MA**, *et al*. 2004. Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem* **279**: 40545–59.
92. **Vecsey CG**, **Hawk JD**, **Lattal KM**, **Stein JM**, *et al*. 2007. Histone deacetylase inhibitors enhance memory and synaptic plasticity *via* CREB:CBP-dependent transcriptional activation. *J Neurosci* **27**: 6128–40.
93. **Kumar A**, **Choi KH**, **Renthal W**, **Tsankova NM**, *et al*. 2005. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48**: 303–14.
94. **Tsankova NM**, **Berton O**, **Renthal W**, **Kumar A**, *et al*. 2006. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci* **9**: 519–25.
95. **Hunter RG**, **McCarthy KJ**, **Milne TA**, **Pfaff DW**, *et al*. 2009. Regulation of hippocampal H3 histone methylation by acute and chronic stress. *Proc Natl Acad Sci USA* **106**: 20912–7.
96. **Abel T**, **Zukin RS.** 2008. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Curr Opin Pharmacol* **8**: 57–64.
97. **Ricobaraza A**, **Cuadrado-Tejedor M**, **Perez-Mediavilla A**, **Frechilla D**, *et al*. 2009. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. *Neuropsychopharmacology* **34**: 1721–32.
98. **Tsai HW**, **Grant PA**, **Rissman EF.** 2009. Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics* **4**: 47–53.
99. **Murray EK**, **Hien A**, **De Vries GJ**, **Forger NG.** 2009. Epigenetic control of sexual differentiation of the bed nucleus of the stria terminalis. *Endocrinology* **150**: 4241–7.