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Sex, epilepsy, and epigenetics

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Abstract

Epilepsy refers to a heterogeneous group of disorders that are associated with a wide range of pathogenic mechanisms, seizure manifestations, comorbidity profiles, and therapeutic responses. These characteristics are all influenced quite significantly by sex. As with other conditions exhibiting such patterns, sex differences in epilepsy are thought to arise—at the most fundamental level—from the “organizational” and “activational” effects of sex hormones as well as from the direct actions of the sex chromosomes. However, our understanding of the specific molecular, cellular, and network level processes responsible for mediating sex differences in epilepsy remains limited. Because increasing evidence suggests that epigenetic mechanisms are involved both in

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epilepsy and in brain sexual dimorphism, we make the case here that analyzing epigenetic regulation will provide novel insights into the basis for sex differences in epilepsy.

Keywords

chromatin; DNA methylation; epigenetic; epilepsy; histone; non-coding RNA

Introduction

Epilepsy refers to a broad spectrum of disease states, including both genetic and acquired disorders, which can be associated with varying pathogenic mechanisms, seizure patterns and frequencies, comorbid conditions (e.g., autism and other neurodevelopmental and neuropsychiatric disorders), therapeutic responses and toxicities (e.g., alterations in sex hormone metabolism), and clinical outcomes. Clinical observations and translational research efforts suggest that these features are all influenced to a significant degree by sex (Veliskova and Desantis, 2013)[Perucca, Camfield, et al., in this issue][Scheffer and Gecz, in this issue][Savic and Engel, in this issue][McCarthy and Kight, in this issue][Moshe and Giorgi, in this issue][Galanopoulou and Akman, in this issue][Jones et al., in this issue][vanLuijtelaa et al., in this issue][Scharfman, in this issue][Harden and Koppel, in this issue][Reddy, in this issue][Perucca, Tomson, et al., in this issue][Pitkanen, in this issue]. For example, epidemiological and genetic studies have revealed that some syndromes are more common in females, including those which are thought to be X chromosome-linked (e.g., Aicardi syndrome, Rett syndrome [RS], and protocadherin 19-related infantile epileptic encephalopathy) as well as those that show gender differences due to different genetic and non-genetic factors (e.g., juvenile myoclonic epilepsy). Other syndromes are more common in males (e.g., Ohtahara syndrome, infantile spasms [IS], Lennox–Gastaut syndrome, Landau–Kleffner syndrome, and febrile seizures), with males exhibiting an overall incidence of seizures and prevalence of epilepsy slightly greater than that of females. Additional studies have demonstrated sex-specific patterns of seizure susceptibility, laterality, and generalization; brain regional dysfunction between ictal periods; and seizure-associated neuronal injury in epileptic disorders, such as temporal lobe epilepsy (TLE) (Veliskova and Desantis, 2013)[Perucca, Camfield, et al., in this issue][Scheffer and Gecz, in this issue][Savic and Engel, in this issue][McCarthy and Kight, in this issue][Moshe and Giorgi, in this issue][Galanopoulou and Akman, in this issue][Jones et al., in this issue][vanLuijtelaa et al., in this issue][Scharfman, in this issue][Harden and Koppel, in this issue][Reddy, in this issue][Perucca, Tomson, et al., in this issue][Pitkanen, in this issue]. There is also emerging evidence that common pathological features in epilepsy syndromes are linked with sex differences, such as subtle distinctions in white matter associated with hippocampal sclerosis that can be identified in patients with TLE with diffusion tensor imaging (Oguz et al., 2013). Studying the mechanisms responsible for these selective differences is of great interest for better understanding the onset and progression of epilepsy and for uncovering novel, more effective, and personalized strategies for diagnosis, prevention, and therapy.

As with other conditions exhibiting such patterns, sex differences in epilepsy are thought to arise from the effects of sex hormones. Indeed, many important studies have focused on dissecting the complex and multidimensional influences of androgens, estrogens, and progesterone (along with glucocorticoids and mineralocorticoids) on the process of epileptogenesis and the expression of seizure disorders, including their “organizational” roles during developmental programming and “activational”, or acute, effects later in life, which can include modulating neuronal excitability and cell death (Frye, 2008; Veliskova and Desantis, 2013). Their mechanisms can include both canonical genomic functions as well as non-genomic activities. These hormones can even be synthesized locally within the nervous system (i.e., neurosteroids), highlighting their extremely high degree of integration into neurobiological processes (Reddy and Rogawski, 2012)[Reddy, in this issue]. In addition, sex differences in epilepsy are also likely to arise because of the direct effects of sex chromosomes (McCarthy and Arnold, 2011). In fact, recent evidence has shown that cell type- and region-specific gene expression profiles in brain and associated behavioral phenotypes can be sexually dimorphic independent from the effects of gonadal sex hormones. These observations demonstrate that the complement of sex chromosomes and the genes they encode, such as the sex determining region Y (*SRY*) gene, mediate sex differences directly, which represents a paradigm shift away from the classic hormonal milieu model (McCarthy and Arnold, 2011). This insight is specifically relevant for epilepsy as several sex chromosome complement modulated genes are linked with neuronal excitability and neurotransmitter signaling, and many sex chromosome complement modulated behaviors (i.e., social interaction, aggression, anxiety, feeding, habit formation, learning, nociception, circadian rhythms, and visuospatial attention) share underlying modules of neural circuitry with epilepsy and its comorbid conditions (Cox et al., 2014; McCarthy and Arnold, 2011; Seney et al., 2013). Embracing this novel perspective on the basis for sex differences has, in turn, raised important questions about how these hormonal and genetic factors function separately and interactively.

In this review, we introduce the principal epigenetic regulatory mechanisms and discuss how these processes are now emerging as prime mechanisms responsible for integrating hormonal and genetic influences—along with environmental stimuli—at the molecular, cellular, and neural network levels (McCarthy and Nugent, 2013; Qureshi and Mehler, 2010b). Furthermore, we highlight the rapidly expanding body of evidence, which suggests that epigenetic factors and mechanisms (and their deregulation) serve as key players in the pathogenesis of epileptic disorders and the process of epileptogenesis (Hwang et al., 2013; Qureshi and Mehler, 2010a). We believe that, because epigenetic processes are so intimately involved in sexual dimorphism and in epilepsy, analyzing epigenetic regulation will provide novel, clinically relevant, and potentially actionable insights into the basis for sex differences in epilepsy.

Principal epigenetic mechanisms

Epigenetic mechanisms are essentially those cellular processes that regulate the structure and function of the genome in response to interoceptive and environmental stimuli. These mechanisms are responsible for storing, accessing, and selectively utilizing genetic information in a biological context-dependent manner (e.g., during development and cellular

differentiation (Tollervey and Lunyak, 2012)). More specifically, epigenetic processes act at a biophysical and biochemical level to promote the execution of genomic programs, such as transcriptional regulation, long term gene silencing, transposable element activity, genomic imprinting, X-chromosome inactivation (XCI), DNA replication and repair, and the maintenance of genomic stability. Because of their roles in these critical functions, epigenetic factors and mechanisms are linked to most, if not all, physiological processes and to nearly every major class of disease (Portela and Esteller, 2010). In particular, recent studies have begun to define how the differential deployment of epigenetic mechanisms underlies brain development and aging, neural cell identity and diversity, synaptic and neural network connectivity and activity-dependent plasticity, and homeostatic and stress responses; and, in turn, primary or secondary deregulation of epigenetic processes is increasingly being implicated in the pathophysiology of nervous system diseases, including epilepsy (see below) (Mehler, 2008; Portela and Esteller, 2010; Qureshi and Mehler, 2012).

The foremost epigenetic mechanisms include DNA methylation (and hydroxymethylation), histone protein post-translational modifications (PTMs) and higher-order chromatin remodeling, and non-coding RNA (ncRNA) regulation. These multilayered processes are highly interconnected and exert their regulatory effects through coordinate actions.

DNA methylation describes the covalent modification of carbon atoms at the 5-position in the cytosine aromatic ring, which leads to the formation of 5-methylcytosine (5mC) (Mehler, 2008; Portela and Esteller, 2010; Qureshi and Mehler, 2012). 5mC can be found associated with CpG dinucleotides in gene regulatory regions (i.e., promoters) as well as other genomic sites (particularly in neurons). This epigenetic “mark” is generally thought to promote transcriptional silencing of methylated regions. Mechanistically, several factors can bind to methylated regions (e.g., methyl-CpG-binding domain [MBD] proteins), and these “readers” of methylation marks recruit additional modulatory factors to methylated sites. Many of these effector proteins play roles in transcriptional regulation and chromatin modifications. The methylation reaction is catalyzed by members of the DNA methyltransferase (DNMT) family of enzymes, including those responsible for *de novo* methylation events and for the maintenance of methylation. In addition, profiles of 5mC are dynamic and can be subject to active methylation-demethylation cycles. Specifically, 5mC can be oxidized into 5-hydroxymethylcytosine (5hmC) and other derivatives, which have distinct but still poorly characterized functions that are nevertheless clearly important in brain. Members of the ten-eleven translocation (TET) family of enzymes catalyze these oxidation reactions.

Chromatin is responsible for the compaction of DNA within the cell nucleus (Mehler, 2008; Portela and Esteller, 2010; Qureshi and Mehler, 2012). Dynamic changes in chromatin states into more or less open configurations modulate the accessibility of specific DNA sequences to other nuclear factors, such as those involved in transcription and DNA replication and repair. These alterations in chromatin can occur over multiple hierarchical levels. Histone protein PTMs refer to covalent modifications of histone proteins, which comprise nucleosomes, the basic structural units of chromatin. Histone PTMs can include, but are not limited to, acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. Particular modifications are linked with specific functionalities, such as transcriptional

activation, repression, and poising. These diverse and interactive histone marks are thought to constitute a combinatorial histone “code” at individual genomic sites (Jenuwein and Allis, 2001). PTMs are elaborated by site-selective histone modifying enzymes, or “writers” of these epigenetic marks, including histone deacetylases (HDACs), acetyltransferases (HATs), demethylases (HDMs), methyltransferases (HMTs), and many other enzymatic classes. Higher-order chromatin structures refer to additional interrelated layers of chromatin organization that can exist, ranging from assemblies of nucleosomes to entire chromosomes. Chromatin reorganization is mediated by chromatin remodeling factors—typically acting via macromolecular complexes, which contain proteins having the ability to recognize, or “read”, existing chromatin states (e.g., bromodomain, chromodomain, and plant homeodomain) as well as those with the capacity to “write” and “erase” epigenetic marks. These include, for example, the SWI/SNF, Polycomb and Trithorax, and RE1-silencing transcription factor (REST) and CoREST/RCor protein complexes (Euskirchen et al., 2012; Qureshi et al., 2010a; Schuettengruber et al., 2011; Schwartz and Pirrotta, 2013).

ncRNAs are novel factors with key regulatory roles that can be functionally linked with DNA methylation and chromatin modifications (Mehler, 2008; Portela and Esteller, 2010; Qureshi and Mehler, 2012). The Encyclopedia of DNA Elements (ENCODE) Project reported that, while less than 2% of the human genome codes for protein, nearly the entire genome is transcribed into RNA (Bernstein et al., 2012). Thus, the majority of the genome gives rise to ncRNAs, sometimes called “dark matter” RNAs because their existence and functions were largely unknown prior to the advent of high-resolution deep sequencing technologies. Now, it is evident that the human nuclear (and mitochondrial) genome harbors tens of thousands of these ncRNA genes, which are expressed in highly cell- and tissue-specific patterns and particularly abundant within neural cells (Derrien et al., 2012; Kapranov et al., 2010). Classes of ncRNAs are typically designated as being short (or small) ncRNAs or long ncRNAs (lncRNAs) based on their size, with lncRNAs defined as those greater than 200 nucleotides in length. These classes can be divided further based on a diverse range of salient features, such as their genomic contexts, structural characteristics, specific biogenesis pathways, mechanisms of action, interacting partners, and biological roles (Cech and Steitz, 2014; Mercer and Mattick, 2013). Amongst the most important classes of short ncRNAs are microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), and PIWI-interacting RNAs (piRNAs). miRNAs are the best characterized. They are generally thought to be involved in post-transcriptional regulation of gene networks through RNA-induced silencing complex (RISC) activation. However, they can also mediate genomic site-specific chromatin remodeling events (Hawkins and Morris, 2008). lncRNAs are more heterogeneous and versatile than their small ncRNA counterparts. For example, because of their relatively large size, lncRNAs can engage in sequence-specific interactions with other DNA and RNA molecules and simultaneously in conformational interactions with other molecular partners (Mercer and Mattick, 2013; St Laurent and Wahlestedt, 2007). Accordingly, lncRNAs have a complex and increasingly broad spectrum of activities that is still being uncovered, including roles in recruiting non-selective transcriptional and epigenetic (e.g., chromatin remodeling complexes) factors to specific genomic loci, forming nuclear bodies, sequestering miRNAs (i.e., miRNA sponge activity), modulating nuclear-

cytoplasmic transport of proteins, and controlling local protein synthesis at synapses (Mercer and Mattick, 2013; Qureshi et al., 2010b).

Epigenetic mechanisms and sex differences in brain

It is clear that epigenetic mechanisms are key mediators of brain form and function, and emerging data has begun to define the roles played by these processes in promoting sexual dimorphism in brain (and body) (Qureshi and Mehler, 2010b). Specifically, epigenetic factors and mechanisms seem to act as integrators of genetic and hormonal influences—along with environmental stimuli—to establish and maintain sex differences in gene expression and, thus, underlie dissemblance at molecular, cellular, regional, and neural network levels. Here, we provide selected examples supporting this view.

The most prominent illustration is provided by XCI, which refers to the random inactivation of one of the two X chromosomes in females, a seminal form of dosage compensation. The X inactivation-specific transcript (*XIST*) is a lncRNA derived from the X-inactivation center of the future inactive X chromosome (Xi) that is primarily responsible for orchestrating XCI (Froberg et al., 2013; Lee and Bartolomei, 2013). Simply, *XIST* coats the Xi and recruits silencing chromatin-remodeling complexes, leading to the formation of a heterochromatic and transcriptionally inactive state. These complexes include the Polycomb repressive complex 2 (PRC2), which decorates the Xi with repressive histone H3 lysine 27 trimethylation (H3K27me3) marks. Several additional lncRNAs (i.e., *JPX*, *FTX*, *TSIX*, and *RepA*) also play crucial roles in XCI by modulating *XIST* expression and function through various mechanisms. XCI is particularly relevant for neural development and functioning, through the effects of XCI mosaicism on cellular diversity and topography, left/right brain asymmetry, and the expression of Xlinked traits (Wu et al., 2014). For example, one intriguing study (Wu et al., 2014) recently showed that inhibitory interneurons in the cerebral cortex exhibit a high degree of intermixing of XCI mosaic cells, suggesting that a heterozygous defect in an X-linked gene would perturb all cortical circuits equally. By contrast, excitatory neurons display significantly less intermixing of XCI mosaic cells, implying that a heterozygous defect in an X-linked gene could yield columnar, regional, or lateralized dysfunction of cortical circuits.

Another example is genomic imprinting, a process that silences genes in a parent-of-origin-dependent fashion in order to promote monoallelic gene expression (Lee and Bartolomei, 2013). Most imprinted genes are found in genomic clusters that contain paternally and maternally expressed imprinted genes, including protein-coding genes and multiple classes of ncRNAs (e.g., miRNAs, snoRNAs, and lncRNAs). The purpose of imprinting is not well understood, however, it seems to be important for biological processes such as growth and development. Perturbations in imprinting are in turn associated with several diseases, which include the neurodevelopmental disorders, Prader-Willi and Angelman syndromes. Like XCI, imprinting is mediated by the coordinated action of multiple epigenetic factors, such as *cis*-acting lncRNAs, DNMTs, histone modifying enzymes, and chromatin remodeling complexes (i.e., PRC2). Imprinting exhibits complex parentally biased brain region-, developmental stage-, cell type-, and gene isoform-selective patterns (Gregg et al., 2010a; Gregg et al., 2010b). Furthermore, these profiles can exhibit sex differences. For example, a

study performed utilizing mouse brain tissue identified sex-specific parent-of-origin allelic effects for 347 autosomal genes, including 150 genes in females and 48 genes in males within the sexually dimorphic pre-optic area (POA) of the hypothalamus (Gregg et al., 2010a). These findings strongly suggest that imprinted gene expression contributes to sex differences in brain and behavior.

In concert, a number of epigenetic factors are expressed—and corresponding epigenetic marks are elaborated—in sex-specific patterns in brain regions well known to be dimorphic, such as the hypothalamus, as well as other regions. Moreover, these factors and mechanisms are sensitive to, and integrated with, sex steroid hormone pathways. For example, the expression (and function) of the X chromosome encoded epigenetic factors—methyl-CpG-binding protein 2 (*MECP2*), histone lysine-specific demethylase 6A (*KDM6A/UTX*), and *XIST* (and related ncRNAs)—is sexually dimorphic in the brain. Similarly, the Y chromosome encoded gonadal sex determining gene, *SRY*, which can act as a circular lncRNA with miRNA sponge activity (Hansen et al., 2013), is expressed in selective regional patterns in the male brain. Sexual differences in the expression of epigenetic factors can arise as a result of sex chromosome effects as in the above examples, but the expression of epigenetic factors is also sensitive to sex hormone exposure. For instance, one study showed that intra-hippocampal estradiol infusion in 12-week-old female mice preferentially increases DNMT3a and DNMT3b and HDAC1 expression and decreases HDAC2 expression (Zhao et al., 2010). These observations suggest that epigenetic regulators in brain are deployed in a sex-specific manner, consistent with other evidence from expression quantitative trait loci analyses revealing sex-biased gene regulatory architectures in human brain (Trabzuni et al., 2013). In addition, several autosomally encoded factors with epigenetic functions exhibit such sex-biased modulation, including those involved in DNA methylation (e.g., methylenetetrahydrofolate reductase [*MTHFR*]), chromatin remodeling (e.g., SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 [*SMARCA5*]), and ncRNA regulation (e.g., brain cytoplasmic RNA 1 [*BCYRN1*]).

Correspondingly, sex differences in profiles of DNA methylation and histone modifications are also found in the brain. One recent genome-wide study demonstrated that the adult human prefrontal cortex displays a profoundly sex-specific DNA methylation pattern with 8,357 CpG sites showing significantly differential methylation (Xu et al., 2013). Another study performed in mice brain tissue found sex differences in specific histone marks (i.e., H3K9ac, H3K14ac, and H3K9me3) associated with cortical and hippocampal development, which are sensitive to prenatal treatment with testosterone, suggesting that these marks mediate the organizational effects of sex hormones on brain (Tsai et al., 2009). Indeed, another study showed that the masculinizing effects of testosterone on the sexually dimorphic principal nucleus of the bed nucleus of the stria terminalis (BNSTp) are mediated by histone acetylation (Murray et al., 2009). Further studies have revealed that many miRNAs, including those encoded on autosomes, are expressed in a sex specific manner in various regions of the human and murine brains during development and adult life (Koturbash et al., 2010; Ziats and Rennert, 2013). A number of these are also sensitive to

sex steroid hormones in terms of their expression, in an age and brain region-dependent fashion, particularly within the hippocampus (Pak et al., 2013).

Moreover, epigenetic processes are implicated in sex hormone signaling through a complex and emerging set of mechanisms that includes modulating—and being modulated by—key members of these pathways, regulating sex hormone responsive genes in concert with transcription factors and various co-activators and corepressors, and other processes (Gagnidze et al., 2013; Westberry et al., 2010). Steroid hormone receptor expression can be regulated in a cell, tissue, and sex-specific manner by DNA methylation, histone/chromatin marks, and ncRNAs. In turn, the effects of steroid hormones are mediated by steroid hormone receptors that orchestrate the recruitment of several transcriptional and epigenetic regulators to target genes. Table 1 provides representative examples illustrating the interplay between estrogen signaling and epigenetic mechanisms. Amongst the most interesting examples of sex hormone-epigenetic crosstalk are the roles played by ncRNAs. For example, the steroid receptor activator RNA (*SRA*) is a ncRNA that serves as a key co-activator of several sex hormone receptors (Colley and Leedman, 2011). Further, one recent study showed that two lncRNAs, *PRNCRI/PCAT8* and *PCGEMI*, bind to the androgen receptor and strongly induce androgen-receptor-mediated gene activation programs, both in a ligand-dependent and a ligand-independent manner (Yang et al., 2013). Another intriguing observation that may potentially be relevant is the recent report that a single piRNA is responsible for primary sex determination in the silkworm (Kiuchi et al., 2014).

Epigenetic mechanisms and epilepsy

We have previously suggested several non-mutually exclusive paradigms for describing how epigenetic factors and processes might be linked with neurological disease states (Qureshi and Mehler, 2013). These include the following: (i) mutations in genes encoding epigenetic factors cause disease, (ii) genetic variation in genes encoding epigenetic factors modify disease risk, (iii) the expression and function of epigenetic factors—and the corresponding elaboration of epigenetic marks—are abnormal and target disease-associated genomic loci, gene products, and cellular pathways. Here, we provide selected examples within this framework, which convincingly demonstrate that epigenetic mechanisms are important in the pathogenesis of epilepsy.

First and foremost, a number of studies have shown that mutations in genes encoding factors from across the spectrum of different epigenetic mechanisms are responsible for causing human epileptic disorders. Point mutations, deletions, and insertions in the *MECP2* gene represent the primary causes of Rett syndrome (RS), a dominant X-linked disorder associated with infantile spasms (IS) in girls or other epileptic encephalopathies. This example is the most well known, however, mutations in genes encoding additional members of the MBD protein family, including *MBD5* and *MBD6*, are similarly linked to neurodevelopmental disorders and to epileptic phenotypes of varying severity (Cukier et al., 2012). These observations clearly link the disruption of DNA methylation-related regulatory processes with the development of epilepsy. Histone modification enzymes and higher order chromatin remodeling factors are also connected with epileptic disorders. For example, mutations in the SET domain bifurcated 1 (*SETDB1*) gene, which encodes a protein

containing an MBD and acts as a HMT, are implicated in epilepsy (Cukier et al., 2012). Mutations of the nuclear receptor-binding SET domain containing protein (*NSD1*) gene, which also encodes a HMT, are diagnostic of Sotos syndrome, an overgrowth syndrome associated with febrile seizures and childhood and adult epilepsy (Nicita et al., 2012; Turkmen et al., 2003). Furthermore, mutations in the SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a member 2 (*SMARCA2*) gene, which encodes an ATP-dependent chromatin remodeling enzyme, cause the Nicolaides-Baraitser syndrome, which is associated with epilepsy (Wolff et al., 2012). Mutations in the chromodomain helicase DNA binding protein 2 (*CHD2*) gene are linked with epileptic encephalopathies, including a form that is reminiscent of Dravet syndrome (Carvill et al., 2013; Suls et al., 2013). Mutations in the alpha thalassemia/mental retardation syndrome X-linked (*ATRX*) gene, which also encodes a chromatin-remodeling enzyme of the SWI/SNF family, cause alpha thalassemia and intellectual and developmental disabilities (Ratnakumar and Bernstein, 2013). This is one of an increasing number of X-linked intellectual disability (XLID) disorders associated with epilepsy that are linked to mutations in histone modification and chromatin remodeling genes including, for instance, the histone lysine-specific demethylase 5C and 6A (*KDM5C* and *KDM6A/UTX*) genes (Jensen et al., 2005; Lindgren et al., 2013). Intriguingly, mutations in *KDM5C* disrupt the activity of REST, a master transcriptional and epigenetic regulator of neural genes including those involved in neural development, synaptic transmission, and neuronal excitability, suggesting an important role for REST in the development of the epileptic phenotype (Tahiliani et al., 2007). Mutations in the prickly homolog 1 (*PRICKLE1*) gene, which encodes a protein involved in nuclear-cytoplasmic trafficking of REST, produce a form of progressive myoclonic epilepsy that is similar to Unverricht-Lundborg disease in its clinical presentation, further implicating deregulated REST activity as a final common pathway that leads to epilepsy (Bassuk et al., 2008). In addition to mutations in factors involved in DNA methylation and histone/chromatin remodeling, mutations affecting ncRNAs are also implicated in epilepsy (though these have not been studied systematically). For example, one recent study showed that selective disruption of the *BX118339* lncRNA gene by a *de novo* balanced translocation was responsible for a case of infantile spasms (Vandeweyer et al., 2012). Parallel analyses in animal models further demonstrate that mutations in epigenetic factors lead to epilepsy. These additional genes encode, for example, factors involved in histone deacetylation (e.g., *Hdac4*) (Rajan et al., 2009).

Second, emerging data suggests that variations in genes encoding epigenetic factors modify vulnerability to human epileptic disorders. For example, polymorphisms of the bromodomain-containing protein 2 (*BRD2*) gene confer susceptibility to common forms of juvenile myoclonic epilepsy and to photoparoxysmal responses (Lorenz et al., 2006; Pal et al., 2003). Furthermore, mice heterozygous for a null mutation in *Brd2* (*Brd2*^{+/-}) exhibit sex-specific decreases in seizure thresholds, with males having decreased clonic seizure thresholds and females decreased tonic-clonic seizure thresholds in response to flurothyl (Velisek et al., 2011). These *Brd2*^{+/-} females also display spontaneous seizure activity on long-term EEG/video recording. In addition, these *Brd2*^{+/-} mice show corresponding disruptions in the GABAergic system, including decreased numbers of GABAergic neurons in the cortex, striatum, and substantia nigra, pars reticulata as well as regional changes in the

levels of the GABA producing enzyme, glutamate decarboxylase 67. These findings provide evidence for the existence of complex interconnections between epigenetic mechanisms and sex-specific vulnerabilities to epileptic disorders and to seizures.

Third, several lines of evidence support the conclusion that epigenetic factors and mechanisms are functionally deregulated in epilepsy, including analyses performed utilizing human specimens as well as many different animal models (Crowe et al., 2011; Huang et al., 2012; Hwang et al., 2013; Kobow and Blumcke, 2012; Kobow et al., 2009; Kobow et al., 2013; Machnes et al., 2013; Miller-Delaney et al., 2012; Park et al., 2014; Ryley Parrish et al., 2013; Sng et al., 2006; Taniura et al., 2006; Tsankova et al., 2004; Williams-Karnesky et al., 2013; Zhu et al., 2012). One of the most persuasive studies found that, in a rat model of TLE, increased levels of DNA methylation in the hippocampus are associated with epileptogenesis and that adenosine, an endogenous anticonvulsant and anti-epileptogenic factor, exerts these effects via inhibition of DNA methylation (Williams-Karnesky et al., 2013). These studies also reveal that epigenetic processes unequivocally target genomic loci, gene products, and cellular pathways previously associated with the onset and progression of epilepsy. For example, a seminal study examined hippocampal subregions from human TLE patients and found increased levels of DNA methylation in the reelin (*RELN*) gene promoter that were highly correlated with granule cell dispersion, an important pathological feature of TLE (Kobow et al., 2009). Related studies have shown increased levels of DNMT expression (i.e., DNMT1 and DNMT3A) in human TLE neocortical specimens (Zhu et al., 2012). Genome-wide DNA methylation profiling in hippocampal tissue from a rat model of chronic TLE further revealed differential methylation at 2,573 loci, including 1,452 hypermethylated and 1,121 hypomethylated loci, corresponding with deregulated expression of genes involved in epilepsy-linked pathways (i.e., cytoskeleton organization, immune response and inflammation, neuronal development and differentiation, cell adhesion, calcium signaling, DNA binding and transcription, programmed cell death, and synaptic transmission) as well as epigenetic regulation (Kobow et al., 2013). Another examination of genome-wide DNA methylation profiling in hippocampal tissue from a mouse model of status epilepticus with and without preconditioning revealed complementary profiles of DNA methylation changes (mostly hypomethylation) associated with 321 gene loci (Miller-Delaney et al., 2012). Parallel studies reveal histone-modifying enzymes (i.e., HDAC2) are deregulated in human TLE neuropathological specimens and in hippocampal tissue from acute and chronic TLE animal models (Huang et al., 2012). Moreover, various alterations in histone PTMs associated with H3, H4, and H2A.X have also been identified in epilepsy models (Crowe et al., 2011; Sng et al., 2006; Taniura et al., 2006; Tsankova et al., 2004).

Moreover, ncRNAs are also deregulated in and involved in the pathogenesis of epilepsy through various mechanisms (Henshall, 2014; Jimenez-Mateos et al., 2011; Jimenez-Mateos et al., 2012; McKiernan et al., 2012a; McKiernan et al., 2012b; Sano et al., 2012). For example, one recent study showed that *miR-128* modulates neuronal excitability and that reducing *miR-128* expression in mice causes severe seizures and death at 2 to 3 months of age (Tan et al., 2013). Another study demonstrated that *miR-134* is upregulated in human and animal models of TLE and suggested that silencing this miRNA exerts anticonvulsant, neuroprotective, and potentially even anti-epileptogenic effects (Jimenez-Mateos et al.,

2012). Complementary analyses have also implicated other classes of ncRNAs, including lncRNAs, in epilepsy (Qureshi et al., 2010b).

Conclusion

We have highlighted how epigenetic mechanisms, in a complex regulatory network that involves sex chromosomes and their crosstalk with autosomes and with sex steroid hormones, promote sex differences in neural gene expression and function. We have also illustrated how epigenetic regulation is intimately involved in the pathogenesis of epilepsy. These overall observations strongly suggest that the biased deployment of epigenetic factors and mechanisms in males and females underlies sex differences associated with risk, onset, and progression of epileptic disorders. Further investigation is necessary to unravel the complexities of these processes and to account for the additional roles played by epigenetics in sex differences in epilepsy associated with early-life stress, nutrition, metabolism, immunology, circadian rhythms, and numerous other seminal parameters.

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Research highlights

- Epigenetic regulation mediates brain sexual dimorphism and epilepsy pathogenesis.
- Epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNAs.
- Analyzing epigenetic (de)regulation will provide novel insights into sex differences in epilepsy.

Table 1

Epigenetic mechanisms and factors regulate estrogen signaling	Estrogen signaling modulates epigenetic mechanisms
<ul style="list-style-type: none"> DNA methylation regulates ERβ expression in cerebral cortex during aging (Westberry et al., 2011) DNA methylation regulates ERα expression in cerebral cortex during development (Westberry et al., 2010) DNA methylation regulates ERα expression in cerebral cortex following ischemic injury in females (Westberry et al., 2008) DNA methylation regulates ERα expression in breast cancer (Keen et al., 2004; Prabhu et al., 2012; Wei et al., 2012) [pRb2/p130-E2F4/5-HDAC1-SUV39H1-p300] and [pRb2/p130-E2F4/5-HDAC1-SUV39H1-DNMT1] epigenetic complexes mediate ERα transcription (Macaluso et al., 2003) HDAC1 negatively regulates ERα (Kawai et al., 2003) DNA methyltransferase and histone deacetylase inhibition activates ERα in ERα-negative breast cancer cells (Yang et al., 2001) Valproic acid restores ERα and anti-estrogen sensitivity in ERα-negative breast cancer cells (Fortunati et al., 2010) The HDAC inhibitor, entinostat activates ERα and sensitizes ERα-negative breast cancer to letrozole (Sabnis et al., 2011) <i>Let-7</i> regulates ERα signaling in breast cancer and breast cancer stem cells (Sun et al., 2013; Zhao et al., 2011) 21 microRNAs (e.g., <i>miR-18a</i>, <i>miR-18b</i>, <i>miR-193b</i>, <i>miR-206</i> and <i>miR-302c</i>) regulate estrogen receptor signaling in breast cancer cell lines (Leivonen et al., 2009) The SRA lncRNA and translated protein modulate ER transcriptional activity (Chooniedass-Kothari et al., 2010; Lanz et al., 1999) The histone methyltransferase, SMYD2, regulates ERα function via site-specific protein methylation (Zhang et al., 2013) 	<ul style="list-style-type: none"> Estradiol regulates sex-specific DNA methylation patterns in the developing preoptic area (Kurian et al., 2010) Estrogen-activated transcription requires TIP48/Reptin- and H2A.Z-mediated chromatin remodeling (Dalvai et al., 2013) Estrogen induces recruitment of ERα and histone acetylation in the <i>Kiss1</i> promoter region and enhances chromatin loop formation of the <i>Kiss1</i> promoter and enhancer regions in mouse brain, resulting in an increase in <i>Kiss1</i> expression (Tomikawa et al., 2012) ERα and Sin3A act at the proximal promoter of <i>ESR1</i> to repress its expression (Ellison-Zelski et al., 2009) Estradiol alters microRNA levels in brain in an age- and brain region-dependent manner (Rao et al., 2013) Estrogen modulates microRNA expression and processing in other tissues (Bhat-Nakshatri et al., 2009; Chu et al., 2014; Edvardsson et al., 2013; Klinge, 2009; Pinho et al., 2013; Queiros et al., 2013; Yamagata et al., 2009) Estrogen modulates the expression of MALAT-1, HOTAIR, and other lncRNAs (Bhan et al., 2013; Qiu et al., 2014a; Qiu et al., 2014b; Zhao et al., 2014)