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Transgenerational neuroendocrine disruption of reproduction

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Abstract

Exposure to endocrine disrupting chemicals (EDCs) is associated with dysfunctions of metabolism, energy balance, thyroid function and reproduction, and an increased risk of endocrine cancers. These multifactorial disorders can be ‘programmed’ through molecular epigenetic changes induced by exposure to EDCs early in life, the expression of which may not manifest until adulthood. In some cases, EDCs have detrimental effects on subsequent generations, which indicates that traits for disease predisposition may be passed to future generations by nongenomic inheritance. This Review discusses current understanding of the epigenetic mechanisms that underlie sexual differentiation of reproductive neuroendocrine systems in mammals and summarizes the literature on transgenerational epigenetic effects of representative EDCs: vinclozolin, diethylstilbesterol, bisphenol A and polychlorinated biphenyls. The article differentiates between context-dependent epigenetic transgenerational changes—namely, those that require environmental exposure, either via the EDC itself or through behavioral or physiological differences in parents—and germline-dependent epigenetic mechanisms. These processes, albeit discrete, are not mutually exclusive and can involve similar molecular mechanisms including DNA methylation and histone modifications and may predispose exposed individuals to transgenerational disruption of reproductive processes. New insights stress the crucial need to develop a clear understanding of how EDCs may program the epigenome of exposed individuals and their descendants.

Introduction

Scientists, the general public, policy makers and the media have focused on the effect of environmental contaminants on human health and ecosystems. Deformities and reproductive anomalies in fish and amphibians have captured the headlines, as have reports on early puberty in young girls. Whether or not to ban bisphenol A (BPA), a compound used in the production of plastics and materials used as food containers, has been the subject of much debate by regulatory agencies throughout Europe and North America. All of these scenarios have something in common—they are attributed at least in part to environmental endocrine disrupting chemicals (EDCs).

EDCs are compounds that interfere with the ability of endocrine systems to maintain homeostasis (Box 1).^{1,2} These compounds have entered the environment from industrial

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waste, contaminated water systems, the widespread use of chemical pest control products and many other sources. From the air, soil and water, EDCs enter the food chain. Because some of these compounds are lipophilic and persistent, they have the potential to bioaccumulate and become a part of a plant's or animal's body burden. Concentrations of such EDCs can, therefore, biomagnify as they work their way up the food chain. Top predators, for example, eagles, seals, polar bears and humans, have amongst the highest concentrations of these bioaccumulative EDCs in their bodies.

Box 1

Definition of an endocrine disrupting chemical

A working group at the US Environmental Protection Agency defined an endocrine disrupting chemical (EDC) as “an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes”.⁸⁰ A number of synthetic compounds have potential endocrine disrupting activity, including, but not limited to, pesticides (dichlorodiphenyltrichloroethane [DDT], chlorpyrifos, methoxychlor), industrial chemicals and by-products (polychlorinated biphenols [PCBs], dioxins), fungicides (vinclozolin), plastics (bisphenol A) and plasticizers (phthalates). Additionally, numerous naturally occurring potential EDCs exist, such as phytoestrogens (genistein, daidzein and coumestrol) found in soy, alfalfa and clover. Although some synthetic EDCs are now banned in different parts of the world, they can persist in soil and water and remain in the ecosystem for years or decades.

In the USA, more than 80,000 chemicals are registered with the Environmental Protection Agency, some of which are known or potential EDCs. Although not all of these compounds are still in commercial use, it remains imperative that we act now to develop an understanding of how these compounds affect human and wildlife populations in both the short term and the long term.^{3,4}

Endocrine systems maintain homeostasis through the release of hormones from glands that, in turn, are regulated by feedback circuits. When perturbed, hormonal systems that control reproduction, stress, metabolism, energy balance, growth and electrolyte balance cannot function properly, and dysregulation and disease result. The timing of exposure is critically important in determining the effects of EDC exposures. Although no life stage is immune to EDC effects, the developing organism is particularly vulnerable. The concept of the ‘fetal (developmental) basis of adult disease’ has been proposed⁵ to explain how the prenatal and early postnatal periods of development are highly sensitive to environmental perturbations, including those related to exposure to EDCs.⁶ Such exposures, even at very low levels, may ‘reprogram’ the developing organism through irreversible changes in gene expression and predispose it for dysfunction much later in life.

Research in the past decade has provided compelling evidence for the biological plausibility of links between early life exposures to EDCs and an increased propensity to develop adult endocrine disorders, including endocrine cancers, infertility, type 2 diabetes mellitus, obesity or thyroid disease, although considerable controversy remains. Mechanistic molecular studies have begun to unravel how EDCs may act. Among the most exciting but disturbing discoveries is the finding that the effects of EDCs are not limited to an exposed individual but can be transmitted to future generations.

As will be discussed in this Review, effects of EDCs on the germline (sperm, ova) may reprogram molecular epigenetic processes such as DNA methylation and histone modifications, among others (Box 2). These developmental programming changes can, in some cases, be heritable via germline transmission to subsequent generation and, thereby, initiate a transgenerational cascade. The actions of EDCs on the brain, behavior and hormone concentrations or actions in exposed parents, especially the mother, might also change maternal behavior and in turn modify epigenetic programming of offspring in a germline-independent manner, as elaborated upon below. Some animal and human populations are geographically constrained due to a variety of factors that may be beyond the individuals' control. For example, in humans, socioeconomic, job or family circumstances may limit geographic mobility. Wildlife might also be constrained owing to their social systems and availability (or lack) of a suitable habitat or resources. If people and animals reside in contaminated environments, such constraints may also cause many generations of offspring to have continued exposure to the contaminated environment.^{7–9} In these situations, epigenetic alterations that cross generations are again germline-independent, as they relate to the social or physical environment of the family or litter and have been referred to by David Crews as 'context-dependent epigenetic modification or change'.¹⁰ Understanding the molecular mechanisms involved in both germline-dependent and context-dependent EDC-related epigenetic reprogramming is more important than ever in order to enable humans to intervene now to break the cycle of transgenerational transmission.

Box 2

History of epigenetics

The origin of epigenetics begins with epigenesis, a theory attributed first to Aristotle who proposed that development is a gradual process in which a simple precursor becomes increasingly complex. This theory contrasted with preformationism, the (incorrect) belief that organisms arose from fully-formed, pre-existing miniature beings. The theory of epigenesis was championed by the British physician William Harvey and later by Caspar Friedrich Wolff, the eponymous German embryologist who also described the Wolffian ducts that are precursors to male reproductive organs. The concept of epigenetics is first credited to Erasmus Darwin⁸¹ and in wide use in psychology in the works of William James, James Mark Baldwin, and Zing-Yang Kuo in the late 1890s to the early 1930s. Conrad Waddington coined the phrase 'epigenetic landscape' as a metaphor for how gene–environment interactions are similar to a ball rolling downhill with fates being established in the deepening grooves. More recently, the concept of gene–environment interactions has gone beyond mutation to more subtle modifications of gene expression, including DNA methylation and histone regulation.

Hypothalamic sexual differentiation

The central neuroendocrine systems control reproductive function through the release of the hypothalamic (gonadotropin-releasing hormone), pituitary (luteinizing hormone [LH], follicle-stimulating hormone) and gonadal steroid (estradiol, progesterone and testosterone) hormones (Figure 1). Reproductive neuroendocrine systems are sexually dimorphic and accommodate obvious sex differences in gametogenesis, steroidogenesis and sexual behavior. The control of these processes takes place in a common hypothalamic–limbic neural network that develops in a sexually dimorphic manner. This complex circuitry is organized in a sex-specific manner by gonadal steroid hormones during a critical period of development—usually late in fetal development and soon after birth in mammals—and results in the capacity for male-typical or female-typical reproductive physiology and

behavior in adulthood (Box 3). In reference to brain sexual differentiation, the term organization refers to developmental changes in the nervous system that must occur early in life to enable proper functioning later in life (for example, adulthood).

Box 3

Sexual differentiation and sexual dimorphisms of the hypothalamus

The central nervous systems of developing male and female fetuses are remarkably similar in the early periods of gestation. During embryogenesis, exposures to hormones cause changes in neuronal phenotype, cell survival and the structure of brain regions in a sex-specific manner. The fetal male testis produces large quantities of testosterone, which is metabolized to estradiol through actions of the enzyme p450 aromatase.^{11,12} These hormonal exposures are necessary for normal brain masculinization. By contrast, the female ovary produces much lower levels of these gonadal steroid hormones. Additionally, in rodents, the female brain is further protected from maternal estrogens by the α -fetoprotein.¹⁴ This phenomenon results in brain feminization.

The sexually dimorphic nucleus of the preoptic area (SDN-POA) was one of the first regions of the brain that was shown to be sexually dimorphic in structure. As first identified by Roger Gorski in rats,⁸² the SDN-POA of males is approximately four times larger than in females. Its size is correlated with masculine sexual behavior. The anteroventral periventricular nucleus is another important sexually dimorphic nucleus in the preoptic area.⁸³ Its larger size and greater number of estrogen receptors in females are correlated with the ability of females, but not males, to respond to estradiol positive feedback and to cause the hypothalamic gonadotropin-releasing hormone neurons to drive ovulation.

In males, circulating testosterone is important for masculinization of reproductive organs but is also important for the masculinization of the brain primarily through its conversion to estradiol catalyzed by the aromatase enzyme.^{11,12} Synthesis of the aromatase enzyme in the fetal brain¹³ enables estradiol to act upon its neural estrogen receptors, ER α and ER β , to cause masculinization. By contrast, the female brain has much lower exposure to testosterone or estradiol. In rodents, the female brain is further protected from maternal estrogens by circulating α -fetoprotein in the fetus,¹⁴ enabling the development of a female-typical pattern of brain organization.

The consequences of the organizational effects of steroids are beginning to be understood at the molecular level through studies in animal models. For example, gene and protein expression of steroid hormone receptors, neurotransmitters, and neuropeptides and their receptors in the hypothalamus differ considerably between males and females, even shortly after birth. Male and female brains are also morphologically and anatomically different. In rats, the sexually dimorphic nucleus of the preoptic area is larger in males than in females,^{15,16} and the anteroventral periventricular nucleus is larger in females than in males (Box 3).¹⁷ These sexual dimorphisms are correlated with the ability of the adult organism to behave in a sex-typical manner and to coordinate reproductive physiological processes with the social, physiological, nutritional and geographical environment.

Whereas the hypothalamus is organized early in life, activation of the sex-typical physiology and behavior must occur before an organism attains adult reproductive competency.¹⁸ This activational process typically occurs during puberty, when the developing adrenals and gonads begin to secrete increased levels of steroid hormones, particularly estrogens (estradiol), progestins (progesterone) and androgens (testosterone and dihydrotestosterone). The consequence of organization in early life, followed by activation in later life, is the

ability of the organism to regulate reproductive physiology and behavior in a manner that is appropriate to the sex of the animal and that enables it to respond to its environment in a manner that maximizes the likelihood of reproductive success.

The hypothalamic–pituitary–gonadal axis both produces and responds to reproductive hormones and is, therefore, vulnerable to EDCs that mimic or perturb hormonally-sensitive pathways (Figure 1). Exposure to environmental EDCs, particularly during critical periods of development that include the organizational and activational periods, may interfere with normal neuroendocrine processes.

The remainder of this Review will discuss the epigenetic processes by which normal hypothalamic–pituitary–gonadal function develops, consider the molecular mechanisms by which EDCs may perturb these processes and review evidence for transgenerational epigenetic transmission of these disrupted traits to offspring. Considerable evidence shows that exposure to EDCs results in epigenetic changes both *in vivo* and *in vitro*;¹⁹ however, our discussion will focus on four compounds for which not only epigenetic but also transgenerational effects have been evidenced in mammals *in vivo*: vinclozolin, diethylstilbesterol, BPA and polychlorinated biphenyls (PCBs).

Epigenetics and the hypothalamus

Developmental programming of the hypothalamus in a sexually differentiated manner involves changes in the transcriptome or the regulatory machinery of mRNA transcription.²⁰ These processes involve epigenetic changes, which do not cause mutations to the DNA nucleotide sequences but rather involve the ability of genes to be expressed or repressed through non-genomic modifications. Epigenetic changes may be heritable by germline transmission or context-dependent inheritance.¹⁰ Both of these processes, albeit independent, can be accomplished by the same molecular epigenetic mechanisms such as DNA methylation, histone modifications, chromatin remodeling and regulation via microRNAs.

DNA methylation is a modification of the DNA molecule in which a methyl group is covalently added to the cytosine of a CpG dinucleotide, which is a cytosine next to a guanine connected by a phosphodiester bond on a DNA strand (Figure 2). In the regulatory regions of genes, CpG dinucleotides are sometimes found in clusters called CpG islands. Generally, an increase of DNA methylation is associated with a decrease in gene expression, whereas a decrease in methylation is associated with an increase in gene expression. DNA methylation is a relatively stable event involved in long-term developmental processes such as tissue-specific gene expression, gene imprinting and X chromosome inactivation.²¹ Thus, DNA methylation is a plausible mechanism by which the hypothalamus is organized through sex steroid hormones.^{22,23} Furthermore, DNA methylation of estrogen receptor α ²⁴ and the progesterone receptor²² are sexually dimorphic in the hypothalamus of neonatal rats, and female rats can be masculinized by neonatal treatment with estradiol.

Histone proteins aid in packaging the DNA into the nucleus and help regulate interactions of transcription factors with the DNA (Figure 2). Modifications to histones include acetylation, phosphorylation, ubiquitination and methylation, which lead to a relaxed (transcriptionally active) or condensed (transcriptionally repressed) chromatin state. For example, when histones are acetylated at lysines, a positive charge is removed from the histone tail, which leads to relaxation of the interaction between the DNA and the histone and increases the likelihood of transcription factor binding.

DNA methylation and histone modifications act in concert to regulate gene expression. When CpG sites are methylated, methyl-binding proteins bind to the DNA molecule and

recruit histone deacetylases, which remove acetyl groups from the tails of histones (usually H3 and H4). Certain histone modifications, such as an increase in acetylation, can lead to a remodeling of the chromatin such that it becomes fixed in an 'open state', leading to a transcriptionally active gene via the recruitment of two enzymes (SWI/SNF) to the nucleosome.²¹ Evidence from the past few years in rodents suggests that histone modifications are sexually dimorphic in several brain regions²⁵ and, specifically, have a role in the masculinization of the bed nucleus of the stria terminalis, a region of the hypothalamus that is larger in males than in females. Treatment of neonatal males and androgenized female mice with the histone deacetylase inhibitor valproic acid resulted in a feminized bed nucleus of the stria terminalis in adulthood.²⁶ Taken together, these data suggest that histone modifications may play an important part in sexual differentiation of the brain.

Context-dependent epigenetic mechanisms are molecular changes that require the exposure of the organism to a behavioral, hormonal or social context or the presence of a causative agent (such as an EDC); for the trait to be perpetuated the exposure must occur in each generation (Figure 3).¹⁰ For example, variations in maternal behavior and/or maternal stress in rodents program the developing fetal brain through alterations in epigenetic regulation of the hippocampal glucocorticoid receptor. Female offspring whose brains have been reprogrammed go on to exhibit the same types of maternal behaviors and to, thereby, reprogram their own offspring.²⁷ These processes are not mediated by classical Mendelian inheritance, nor are they passed via the germline, as the epigenetic trait is also passed on to crossfostered offspring. Rather, the context of the animal's environment—its mother's hormones and behavior—caused the reprogramming. This example illustrates the point that both germline and context-dependent epigenetic modifications can be transmitted to further generations, but these processes differ in the sense that germline traits will 'breed true', whereas context-dependent traits require the continued exposure of each individual to the environmental stimulus. Context-dependent transgenerational epigenetic effects have also been investigated in relation to altered reproductive strategies in female rats²⁸ and the transmission of polycystic ovary syndrome.²⁹

EDCs and epigenetic transmission

Accumulating evidence shows that the epigenetic effects of EDCs on DNA methylation in the germline result in transgenerational inheritance. During mammalian development, a wave of demethylation and subsequent remethylation of the germ cells occurs—a time when alterations to the epigenome are most likely to result in germline transmission (Figure 4).³⁰ Very early in embryonic development, before sex determination, the germ cells migrate down the genital ridge to the bipotential and undifferentiated gonad. Once in the genital ridge, the DNA in the germ cells is demethylated. At the onset of sex determination, the germ cell DNA is remethylated in a sex-specific manner. Imprinted genes are those that are not demethylated during this time and retain the methylation pattern of their maternal or paternal genome, which results in monoallelic expression of the imprinted gene. Germ cells undergo vast changes in methylation patterns during early development, which is why DNA methylation has been the focus of many investigations and has been proposed as a major mechanism for transgenerational epigenetic effects.³¹

The mechanisms by which steroid hormones are involved in sexual differentiation of the brain and body are due in part to their actions as transcription factors that modulate expression of target genes during those critical developmental periods when methylation status is being established. EDCs act on sex-steroid hormone receptors; therefore, developmental exposures to EDCs in parents may interfere with remethylation of the DNA in their germ cells and lead to altered programming of the genome in subsequent

generations. The following sections will review the current literature on epigenetic mechanisms and the transgenerational effects of developmental EDC exposures. This concept requires an appreciation that exposure of a pregnant female (F0 generation) will directly expose her fetuses (F1 generation) and the germ cells of the fetuses that will later develop into the F2 generation. Thus, the first truly unexposed generation is the F3 generation (Table 1; Figure 5). Only a limited number of studies have investigated the molecular mechanisms underlying the transgenerational effects of EDCs, thus, we speculate, when possible, upon the mechanisms that may underlie the transgenerational effects described below.

Vinclozolin

Transgenerational epigenetic effects of the antiandrogenic EDC vinclozolin may be the most comprehensively studied to date (Table 1). Vinclozolin is a dicarboximide fungicide widely used in agriculture, most widely in the viniculture industry. As with many EDCs, embryonic exposure to vinclozolin has long-term consequences on reproductive behavior and physiology in the F1 generation of rats and mice.³² In the past 5 years, studies focusing on the transgenerational effects of embryonic exposure to vinclozolin and the epigenetic mechanisms underlying these alterations have been conducted.

Michael Skinner's laboratory was the first to report epigenetic, transgenerational effects of vinclozolin in the rat,³³ and this model has recently been extended to the mouse.³⁴ Skinner and colleagues found that embryonic exposure of F1 fetuses to a high dose of vinclozolin (100 mg/kg) from embryonic days 8–14 increased apoptosis of germ cells in the testes and decreased sperm motility and concentration in adulthood, an effect that was carried forward for at least four generations (F4) in male rats. These physiological effects were accompanied by alterations in DNA methylation in the testes of young (postnatal day 6) F1 males and the sperm of F2 and F3 adults,³³ which suggests that epigenetic mechanisms may underlie the reduced fertility phenotype.

Follow-up studies from the same laboratory confirmed and extended these initial findings.^{35–37} Altered blood chemistry, an increase in kidney, testis and immune diseases and increased tumor incidence were observed in four generations (F1–F4) of males descended from the original fetally-exposed F1 male rats.³⁸ In females, increased uterine hemorrhage and anemia during pregnancy, glomerular abnormalities, altered blood chemistry and an increase in tumor incidence in three generations (F1–F3) were reported.³⁹ Microarray analysis of testes from male F1–F3 fetuses at embryonic day 16 has identified 196 genes affected by the original exposure, the majority of which were downregulated. Interestingly, expression of several genes involved in the regulation of DNA methylation, including DNA methyltransferases, was decreased in all three generations.³⁶

In mice, Stouder and Paolo-Giacobino showed that vinclozolin (50 mg/kg exposure on embryonic days 10–18) decreased sperm motility in two generations of male mice but motility recovered to the level of controls by the third generation (F1 = 56%, F2 = 90%, F3 = 100%).³⁴ An increase in methylation of paternally imprinted genes and decreased methylation of maternally imprinted genes in the sperm of the F1 and F2 males was also observed, although most of the methylation patterns on imprinted genes had returned to the level of control animals by the F3 generation.³⁴ Environmental conditions may be integral to the induction of the transgenerational epigenetic effects, as these findings were not replicated in two other laboratories (Table 1),^{40,41} possibly due to experimental differences.⁴²

Do the transgenerational epigenetic effects of vinclozolin have an evolutionary context? When tested for mate preference, female F3 descendents of vinclozolin-exposed or vehicle-

exposed rats spent significantly more time with the F3 vehicle-lineage male than the F3 vinclozolin-lineage males (Figure 6; Table 1).⁷ Importantly, the males in this experiment were young and healthy and had not yet developed the reduced fertility and disease phenotype. Thus, we postulate that females were discriminating against these males on the basis of something other than a decrease in fertility, such as pheromones, major histocompatibility proteins, vocalizations or behavioral differences. Interestingly, males showed no preference between untreated or treated females,⁷ which is consistent with the well-known concept in evolutionary biology and mate choice theory that the parent with the greatest investment (the female rat) has a stronger need to discriminate more carefully when choosing a partner.

The findings of Crews *et al.*⁷ are also important because they showed, for the first time, that the transgenerational effects of vinclozolin are manifested as reproductive behavioral changes. Since that report, F3 vinclozolin-lineage male and female rats were also found to differ from corresponding F3 vehicle descendants in anxiety-like behaviors and in gene expression in the hippocampus and amygdala,⁴³ brain regions involved in learning, memory, and anxiety and fear responsiveness. Taken together, these data suggest that perinatal exposure to vinclozolin may alter the hypothalamolimbic system in subsequent generations of rats, providing a mechanistic basis for the behavioral alterations. Current work is underway to investigate expression of genes, and epigenetic markers in the hypothalamus of these rats. Also important for the field is the future study of more environmentally relevant doses of vinclozolin for their potential epigenetic transgenerational actions.

Diethylstilbesterol

Transgenerational epigenetic effects of the estrogenic EDC diethylstilbesterol (DES) began with observations of rare vaginal clear-cell carcinomas and reproductive tract abnormalities in young women whose mothers had been prescribed DES in a misguided effort to avert miscarriage (Table 1). These observations provided the first evidence for developmental programming or the fetal basis of adult disease caused by exogenous estrogens in humans.⁴⁴ A potent estrogenic pharmaceutical, DES not only failed to reduce miscarriage risk, but it also exposed the developing daughters and sons to high levels of prenatal estrogens and predisposed them to adult diseases.⁴⁵ Animal studies have replicated many of these effects of prenatal DES treatment and have begun to reveal the molecular mechanisms by which DES programs developing tissues.⁴⁶ The case of DES is important because it is one of the first to link fetal exposure to a hormonally active compound with the latent development of disease years or even decades after the insult. This example in humans has laid the groundwork for much of the work on the effect of other EDC exposures to the fetus.

Epidemiological studies have also found that DES is associated with a small but significant increase in birth defects in grandchildren of women given DES during pregnancy. The granddaughters reported an increase in heart conditions,⁴⁷ slight but significant differences in their menstrual cycles and a reduction in live births when compared with women whose grandmothers were not exposed to DES.⁴⁸ Another study found an increase in the risk of hypospadias in the sons of women who were exposed to DES *in utero*,⁴⁹ and there was the single clinical observation that the 15-year-old granddaughter of a woman who took DES while she was pregnant developed a small-cell carcinoma of the ovary.⁵⁰ Although the sample sizes in the last two studies were exceedingly small (28 and 1, respectively), they provide preliminary evidence for the potential of transgenerational effects of EDCs in the human population.

Rodent studies have revealed transgenerational epigenetic effects of DES (Table 1). DES exposure of the F1 generation during embryonic and early postnatal development at a range of dosages had adverse consequences in the F2 generations. In F2 males, the incidence of

proliferative lesions of the testes was increased, and serum estradiol concentration was reduced.⁵¹ In F2 females, the incidence of uterine adenocarcinomas⁵² and other tumors of the reproductive tract was increased in a subset of the dosage groups.⁵³ Exposure of newborn (postnatal day 1–5) F1 female mice to 2 µg of DES led to demethylation of the estrogen-sensitive gene lactotransferrin promoter at two CpG dinucleotides (–464 and –454) in the uterus,⁵⁴ and to upregulation of uterine expression of lactotransferrin in both F1 female mice and their F2 female offspring.⁵⁵ Female F1 animals exposed to the same dosing paradigm had decreased methylation of exon 4 of the *Fos* gene on postnatal day 5 and increased *Fos* mRNA expression in the uterus in adulthood.⁵⁶ Additionally, mice exposed gestationally to 10 µg/kg of DES from embryonic days 9–16 resulted in increased methylation of the homeobox A10 promoter and intron and increased Hox-A10 protein expression in the postnatal uterus (day 14).⁵⁷

Treatment of newborn, female and male mice on post-natal days 1–5 with 3 µg per pup per day of DES changed total DNA methylation in reproductive tissues.^{58,59} DES also altered expression of the enzymes that regulate DNA methylation (Table 1). For example, expression of DNA methyltransferases 1, 3a and 3b was increased in the epididymis, and DNA methyltransferases 1 and 3b were increased in the uterus of mice exposed to DES compared with animals exposed to the vehicle control.^{58,59} Finally, in rats treated with 1 mg/kg DES on postnatal days 10–12, the exposure decreased global histone trimethylation at lysine 27 in the uterus on postnatal day 12⁶⁰ and altered the expression of several estrogen-sensitive genes in the uterus of the neonatal and adult exposed animals.⁶¹ Although most of these studies have only been conducted in tissues from F1 individuals, they provide insight into the potential targets for molecular epigenetic modifications that merit further study in subsequent generations in rodents.

Bisphenol A

BPA is a monomer used in the plastics industry that has estrogenic properties and has also been proposed to have effects on androgen and thyroid systems (Box 4).⁶² Although extensively studied as an endocrine disruptor in the F1 generation in rodents (Table 1),⁶³ only a few studies have focused on its effects in multiple generations. Salian *et al.* found that fertility was compromised in three generations of male rats when the pregnant or lactating dam was exposed to 1.2 or 2.4 µg/kg of BPA per day from gestational day 12 until postnatal day 21. Furthermore, immunohistochemistry revealed that androgen receptor, ERβ,⁶⁴ steroid receptor co-regulator 1 and nuclear co-repressor protein expression⁶⁵ were decreased in the testes of three generations of these rats (F1–F3). Interestingly, steroid receptor co-regulators are also histone acetyltransferases and histone deacetylases.²¹ These results suggest that early life exposure to BPA or other steroid receptor mimics may result in long-term alterations to histone modifications and chromatin remodeling in a transgenerational manner.

Box 4

The controversy over bisphenol A

There is considerable dispute in the scientific community about the endocrine disrupting properties of bisphenol A (BPA), a synthetic monomer that is used in the plastics industry. Originally synthesized in the 1930s, BPA is used in the production of plastics. When it was discovered that the presence of BPA caused estrogen-dependent cells to proliferate and for oocytes in mice exposed to BPA to have aneuploidy, endocrinologists began to investigate the endocrine-disrupting properties of BPA.⁶³ Very low-dose detrimental effects of BPA were reported on endocrine organs, including prostate, mammary gland, reproductive tract and neuroendocrine tissues.³ These data continue to

be challenged;⁸⁴ nevertheless, the weight of evidence supports a need to be concerned about low-dose BPA exposures⁸⁵ and to invoke the precautionary principle when new chemicals are introduced into household and food products.

Early life exposure to BPA can cause long-term epigenetic changes. For example, BPA decreased DNA methylation of phosphodiesterase type 4 variant 4, an enzyme involved in the breakdown of cyclic AMP, in the prostate of rats,⁶⁶ altered DNA methylation in the fore-brain of mice⁶⁷ and shifted coat color by decreasing DNA methylation of the agouti gene in viable, yellow, agouti *A^{vy}* mice.⁶⁸ The agouti gene in mice encodes a gene involved in coat coloration, with color ranging from yellow to black depending upon the level of DNA methylation, and thus enables a visual determination of epigenetic modifications.⁶⁹ Finally, exposure to 10 µg/kg BPA on embryonic days 14–18 resulted in altered maternal behavior, as F0 and F1 dams spent less time nursing and more time away from their pups.⁷⁰ As mentioned above, differences in maternal behavior are transferred to subsequent generations via a context-dependent epigenetic mechanism in rats.^{27,28} Although more research is necessary, these data provide supportive evidence that maternal behavior may be regulated by an epigenetic mechanism in mice and that EDCs can disrupt this behavioral parameter. They also raise the provocative possibility for the interactions of context-dependent and germline-dependent epigenetic modifications in a highly complex manner.²⁰

Polychlorinated biphenyls

PCBs are persistent contaminants used in a variety of industrial applications as lubricants, in carbonless paper, in electric transformers, and for many other purposes, from the 1930s until the 1970s, at which time they were banned because of their carcinogenic properties. Depending upon their structures, PCBs can interact with various receptor and enzyme systems in the body including thyroid, reproductive, and neurotransmitter systems (Table 1).

Prenatal PCB exposure has detrimental physiological and behavioral effects on neuroendocrine systems of F1 female rats.^{71–74} Effects have also been reported in the F2 generation, and effects in F3 males and females are in the process of being studied. In F2 females whose grandmothers were injected during pregnancy (gestational days 16 and 18) with 0.1, 1 or 10 mg/kg of a mixture of PCBs, the LH preovulatory surge was attenuated.⁷⁴ Additionally, these animals had lower serum progesterone concentrations on proestrus at the anticipated time of the preovulatory gonadotropin-releasing hormone/LH surge, slightly lower uterine weights,⁷⁴ and preliminary data suggest that androgen receptor mRNA expression is reduced in the preoptic area of F2 males and females on postnatal day 1.⁶ Specific epigenetic effects were not investigated; however, other reports have shown that early life exposures to PCBs alter expression of DNA methyltransferases 1 in the hypothalamus⁷⁵ and DNA methyltransferases 1, 3a and 3b in the liver.^{75,76} The doses of PCBs used in the studies described above were chosen to estimate levels in human body burden and are probably ecologically relevant. Together, these findings show that PCBs interfere with DNA methylation machinery in the rat. Finally, numerous studies have shown that PCBs can have long-term effects on gene expression after developmental exposure,^{77,78} providing evidence that epigenetic mechanisms may be involved in the persistent effects of perinatal PCB exposure.

Conclusions

The study of EDCs has yielded new information regarding the importance of the perinatal environment to long-term outcomes of disease states and human health. Research in the past decade has begun to illuminate the potential molecular mechanisms underlying the programming of the epigenome, both during normal development as well as when it is

disrupted. We are only beginning to understand how acute exposure to EDCs may result in long-term epigenetic effects on the developing individual and on subsequent generations through germline-dependent or context-dependent mechanisms of transmission. Although more research is necessary to fully understand the mechanisms and processes involved, policymakers and regulators must be proactive in preventing future exposures from environmental EDCs. This action is imperative if we are to break the cycle of transgenerational transmission. Furthermore, by identifying the molecular epigenetic mechanisms of transmission, we may be able to reverse adverse changes in DNA methylation and histone modifications through interventions targeted to these biological pathways.

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References

1. Weybridge, UK, Report No. EUR 17549, Environment and Climate Research Programme, DG XXI. European Commission; Brussels: 1996.
2. Kavlock RJ, Ankley GT. A perspective on the risk assessment process for endocrine-disruptive effects on wildlife and human health. *Risk Anal.* 1996; 16:731–739. [PubMed: 8972105]
3. Diamanti-Kandarakis E, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev.* 2009; 30:293–342. [PubMed: 19502515]
4. Koistinen J, Stenman O, Haahti H, Suonperä M, Paasivirta J. Polychlorinated diphenyl ethers, dibenzo-p-dioxins, dibenzofurans and biphenyls in seals and sediment from the Gulf of Finland. *Chemosphere.* 1997; 35:1249–1269. [PubMed: 9308160]
5. Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol.* 2002; 31:1235–1239. [PubMed: 12540728]
6. Gore AC. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Front Neuroendocrinol.* 2008; 29:358–374. [PubMed: 18394690]
7. Crews D, et al. Transgenerational epigenetic imprints on mate preference. *Proc Natl Acad Sci USA.* 2007; 104:5942–5946. [PubMed: 17389367]
8. Crews D, Willingham E, Skipper JK. Endocrine disruptors: present issues, future directions. *Q Rev Biol.* 2000; 75:243–260. [PubMed: 11008698]
9. Wingfield JC. Comparative endocrinology, environment and global change. *Gen Comp Endocrinol.* 2008; 157:207–216. [PubMed: 18558405]
10. Crews D. Epigenetics and its implications for behavioral neuroendocrinology. *Front Neuroendocrinol.* 2008; 29:344–357. [PubMed: 18358518]
11. Cornil CA, Ball GF, Balthazart J. Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? *Brain Res.* 2006; 1126:2–26. [PubMed: 16978590]
12. Juntti SA, et al. The androgen receptor governs the execution, but not programming, of male sexual and territorial behaviors. *Neuron.* 2010; 66:260–272. [PubMed: 20435002]
13. Lephart ED, et al. Brain aromatase cytochrome P-450 messenger RNA levels and enzyme activity during prenatal and perinatal development in the rat. *Brain Res Mol Brain Res.* 1992; 16:187–192. [PubMed: 1337928]
14. Bakker J, et al. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. *Nat Neurosci.* 2006; 9:220–226. [PubMed: 16388309]
15. Davis EC, Popper P, Gorski RA. The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. *Brain Res.* 1996; 734:10–18. [PubMed: 8896803]
16. Davis EC, Shryne JE, Gorski RA. A revised critical period for the sexual differentiation of the sexually dimorphic nucleus of the preoptic area in the rat. *Neuroendocrinology.* 1995; 62:579–585. [PubMed: 8751283]

17. Davis EC, Shryne JE, Gorski RA. Structural sexual dimorphisms in the anteroventral periventricular nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. *Neuroendocrinology*. 1996; 63:142–148. [PubMed: 9053778]
18. Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*. 1959; 65:369–382. [PubMed: 14432658]
19. Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr*. 2009; 21:243–251. [PubMed: 19663042]
20. Crews D. Epigenetic modifications of brain and behavior: theory and practice. *Horm Behav*. 10.1016/j.yhbeh.2010.07.001
21. Bolander, FF. *Molecular Endocrinology*. 3. Elsevier Academic Press; San Diego: 2004.
22. McCarthy MM, et al. The epigenetics of sex differences in the brain. *J Neurosci*. 2009; 29:12815–12823. [PubMed: 19828794]
23. Westberry JM, Trout AL, Wilson ME. Epigenetic regulation of estrogen receptor alpha gene expression in the mouse cortex during early postnatal development. *Endocrinology*. 2010; 151:731–740. [PubMed: 19966177]
24. Kurian JR, Olesen KM, Auger AP. Sex differences in epigenetic regulation of the estrogen receptor-alpha promoter within the developing preoptic area. *Endocrinology*. 2010; 151:2297–2305. [PubMed: 20237133]
25. Tsai HW, Grant PA, Rissman EF. Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics*. 2009; 4:47–53. [PubMed: 19029819]
26. Murray EK, Hien A, de Vries GJ, Forger NG. Epigenetic control of sexual differentiation of the bed nucleus of the stria terminalis. *Endocrinology*. 2009; 150:4241–4247. [PubMed: 19497973]
27. Champagne FA. Epigenetic mechanisms and the transgenerational effects of maternal care. *Front Neuroendocrinol*. 2008; 29:386–397. [PubMed: 18462782]
28. Cameron NM, et al. Epigenetic programming of phenotypic variations in reproductive strategies in the rat through maternal care. *J Neuroendocrinol*. 2008; 20:795–801. [PubMed: 18513204]
29. Homburg R. Androgen circle of polycystic ovary syndrome. *Hum Reprod*. 2009; 24:1548–1555. [PubMed: 19279033]
30. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science*. 2001; 293:1089–1093. [PubMed: 11498579]
31. Jablonka E, Raz G. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q Rev Biol*. 2009; 84:131–176. [PubMed: 19606595]
32. Gray LE, et al. Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum Reprod Update*. 2001; 7:248–264. [PubMed: 11392371]
33. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*. 2005; 308:1466–1469. [PubMed: 15933200]
34. Stouder C, Paoloni-Giacobino A. Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction*. 2010; 139:373–379. [PubMed: 19887539]
35. Anway MD, Memon MA, Uzumcu M, Skinner MK. Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. *J Androl*. 2006; 27:868–879. [PubMed: 16837734]
36. Anway MD, Rekow SS, Skinner MK. Transgenerational epigenetic programming of the embryonic testis transcriptome. *Genomics*. 2008; 91:30–40. [PubMed: 18042343]
37. Anway MD, Rekow SS, Skinner MK. Comparative anti-androgenic actions of vinclozolin and flutamide on transgenerational adult onset disease and spermatogenesis. *Reprod Toxicol*. 2008; 26:100–106. [PubMed: 18762243]
38. Anway MD, Leathers C, Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. *Endocrinology*. 2006; 147:5515–5523. [PubMed: 16973726]
39. Nilsson EE, Anway MD, Stanfield J, Skinner MK. Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction*. 2008; 135:713–721. [PubMed: 18304984]

40. Inawaka K, et al. Maternal exposure to anti-androgenic compounds, vinclozolin, flutamide and procymidone, has no effects on spermatogenesis and DNA methylation in male rats of subsequent generations. *Toxicol Appl Pharmacol.* 2009; 237:178–187. [PubMed: 19303894]
41. Schneider S, Kaufmann W, Buesen R, van Ravenzwaay B. Vinclozolin—the lack of a transgenerational effect after oral maternal exposure during organogenesis. *Reprod Toxicol.* 2008; 25:352–360. [PubMed: 18485663]
42. Gore AC. Neuroendocrine targets of endocrine disruptors. *Hormones (Athens).* 2010; 9:16–27. [PubMed: 20363718]
43. Skinner MK, Anway MD, Savenkova MI, Gore AC, Crews D. Transgenerational epigenetic programming of the brain transcriptome and anxiety behavior. *PLoS ONE.* 2008; 3:e3745. [PubMed: 19015723]
44. Herbst AL. The current status of the DES-exposed population. *Obstet Gynecol Annu.* 1981; 10:267–278. [PubMed: 7024873]
45. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med.* 1971; 284:878–881. [PubMed: 5549830]
46. McLachlan JA, Newbold RR. Estrogens and development. *Environ Health Perspect.* 1987; 75:25–27. [PubMed: 3319561]
47. Titus-Ernstoff L, et al. Birth defects in the sons and daughters of women who were exposed *in utero* to diethylstilbestrol (DES). *Int J Androl.* 2010; 33:377–384. [PubMed: 20002218]
48. Titus-Ernstoff L, et al. Menstrual and reproductive characteristics of women whose mothers were exposed *in utero* to diethylstilbestrol (DES). *Int J Epidemiol.* 2006; 35:862–868. [PubMed: 16723367]
49. Klip H, Burger CW, de Kraker J, van Leeuwen FE. Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. *Hum Reprod.* 2001; 16:2451–2458. [PubMed: 11679537]
50. Blatt J, Van Le L, Weiner T, Sailer S. Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J Pediatr Hematol Oncol.* 2003; 25:635–636. [PubMed: 12902917]
51. Newbold RR, et al. Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis.* 2000; 21:1355–1363. [PubMed: 10874014]
52. Newbold RR, et al. Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis.* 1998; 19:1655–1663. [PubMed: 9771938]
53. Walker BE, Haven MI. Intensity of multigenerational carcinogenesis from diethylstilbestrol in mice. *Carcinogenesis.* 1997; 18:791–793. [PubMed: 9111216]
54. Li S, et al. Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res.* 1997; 57:4356–4359. [PubMed: 9331098]
55. Newbold RR, Padilla-Banks E, Jefferson WN. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology.* 2006; 147 (Suppl):S11–S17. [PubMed: 16690809]
56. Li S, et al. Promoter CpG methylation of Hox-a10 and Hox-a11 in mouse uterus not altered upon neonatal diethylstilbestrol exposure. *Mol Carcinog.* 2001; 32:213–219. [PubMed: 11746833]
57. Bromer JG, Wu J, Zhou Y, Taylor HS. Hypermethylation of homeobox A10 by *in utero* diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming. *Endocrinology.* 2009; 150:3376–3382. [PubMed: 19299448]
58. Sato K, et al. Neonatal exposure to diethylstilbestrol alters the expression of DNA methyltransferases and methylation of genomic DNA in the epididymis of mice. *Endocr J.* 2006; 53:331–337. [PubMed: 16714842]
59. Sato K, et al. Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in the mouse uterus. *Endocr J.* 2009; 56:131–139. [PubMed: 18997445]

60. Bredfeldt TG, et al. Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. *Mol Endocrinol*. 2010; 24:993–1006. [PubMed: 20351197]
61. Greathouse KL, et al. Identification of uterine leiomyoma genes developmentally reprogrammed by neonatal exposure to diethylstilbestrol. *Reprod Sci*. 2008; 15:765–778. [PubMed: 19017814]
62. Pearce EN, Braverman LE. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab*. 2009; 23:801–813. [PubMed: 19942155]
63. Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. *Endocr Rev*. 2009; 30:75–95. [PubMed: 19074586]
64. Salian S, Doshi T, Vanage G. Perinatal exposure of rats to bisphenol A affects the fertility of male offspring. *Life Sci*. 2009; 85:742–752. [PubMed: 19837096]
65. Salian S, Doshi T, Vanage G. Impairment in protein expression profile of testicular steroid receptor coregulators in male rat offspring perinatally exposed to bisphenol A. *Life Sci*. 2009; 85:11–18. [PubMed: 19379760]
66. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res*. 2006; 66:5624–5632. [PubMed: 16740699]
67. Yaoi T, et al. Genome-wide analysis of epigenomic alterations in fetal mouse forebrain after exposure to low doses of bisphenol A. *Biochem Biophys Res Commun*. 2008; 376:563–567. [PubMed: 18804091]
68. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc Natl Acad Sci USA*. 2007; 104:13056–13061. [PubMed: 17670942]
69. Rosenfeld CS. Animal models to study environmental epigenetics. *Biol Reprod*. 2010; 82:473–488. [PubMed: 19828779]
70. Palanza PL, Howdeshell KL, Parmigiani S, vom Saal FS. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. *Environ Health Perspect*. 2002; 110 (Suppl 3):415–422. [PubMed: 12060838]
71. Chung YW, Nunez AA, Clemens LG. Effects of neonatal polychlorinated biphenyl exposure on female sexual behavior. *Physiol Behav*. 2001; 74:363–370. [PubMed: 11714501]
72. Salama J, Chakraborty TR, Ng L, Gore AC. Effects of polychlorinated biphenyls on estrogen receptor-beta expression in the anteroventral periventricular nucleus. *Environ Health Perspect*. 2003; 111:1278–1282. [PubMed: 12896846]
73. Steinberg RM, Juenger TE, Gore AC. The effects of prenatal PCBs on adult female paced mating reproductive behaviors in rats. *Horm Behav*. 2007; 51:364–372. [PubMed: 17274994]
74. Steinberg RM, Walker DM, Juenger TE, Woller MJ, Gore AC. Effects of perinatal polychlorinated biphenyls on adult female rat reproduction: development, reproductive physiology, and second generational effects. *Biol Reprod*. 2008; 78:1091–1101. [PubMed: 18305224]
75. Desaulniers D, et al. Effects of postnatal exposure to a mixture of polychlorinated biphenyls, p,p'-dichlorodiphenyltrichloroethane, and p,p'-dichlorodiphenyldichloroethene in prepubertal and adult female Sprague-Dawley rats. *Int J Toxicol*. 2005; 24:111–127. [PubMed: 16036770]
76. Desaulniers D, et al. Effects of mixtures of polychlorinated biphenyls, methylmercury, and organochlorine pesticides on hepatic DNA methylation in prepubertal female Sprague-Dawley rats. *Int J Toxicol*. 2009; 28:294–307. [PubMed: 19636072]
77. Shimada M, et al. Gene expression profiles in the brain of the neonate mouse perinatally exposed to methylmercury and/or polychlorinated biphenyls. *Arch Toxicol*. 2010; 84:271–286. [PubMed: 20020106]
78. Walker, DM.; Gore, AC. Endocrine-Disrupting Chemicals: From Basic Research to Clinical Practice. Gore, AC., editor. Vol. Ch 4. Humana Press; New Jersey: 2007. p. 63-109.
79. Qiu J. Epigenetics: unfinished symphony. *Nature*. 2006; 441:143–145. [PubMed: 16688142]
80. Kavlock RJ, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect*. 1996; 104 (Suppl 4):715–740. [PubMed: 8880000]

81. Bowler PJ. The changing meaning of “evolution”. *J Hist Ideas*. 1975; 36:95–114. [PubMed: 11610247]
82. Gorski RA, Harlan RE, Jacobson CD, Shryne JE, Southam AM. Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *J Comp Neurol*. 1980; 193:529–539. [PubMed: 7440781]
83. Simerly RB, Swanson LW, Gorski RA. The distribution of monoaminergic cells and fibers in a periventricular preoptic nucleus involved in the control of gonadotropin release: immunohistochemical evidence for a dopaminergic sexual dimorphism. *Brain Res*. 1985; 330:55–64. [PubMed: 2859086]
84. Ryan BC, Hotchkiss AK, Crofton KM, Gray LE Jr. *In utero* and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. *Toxicol Sci*. 2010; 114:133–148. [PubMed: 19864446]
85. Myers JP, et al. Why public health agencies cannot depend on good laboratory practices as a criterion for selecting data: the case of bisphenol A. *Environ Health Perspect*. 2009; 117:309–315. [PubMed: 19337501]

Review criteria

We selected the articles for this Review by focusing on the four main endocrine disruptors (vinclozolin, diethylstilbestrol, bisphenol A and polychlorinated biphenyls) for which there is substantial evidence of both epigenetic and transgenerational effects on the reproductive system. Because the number of primary literature publications on this topic is limited, we did not have any other parameters for exclusion. We found most of the literature cited by searching PubMed and using the search terms “epigenetics” and “transgenerational.” Additionally, we used a few review articles to help us cross-reference our citations and confirm that we had uncovered the complete body of literature on this topic. Although we initially identified some articles through their abstracts, we read all of the primary literature and review articles in order to compile as complete an understanding as possible on this topic.

Key points

- The hypothalamic neuroendocrine systems develop in a sexually dimorphic manner, largely because of differences in levels of gonadal steroids
- Environmental endocrine disrupting chemicals (EDCs) impair the function of the neuroendocrine systems that control reproduction
- Developmental exposure to EDCs, particularly during embryonic and early postnatal periods, permanently impairs functions and predisposes individuals to disease later in life owing to altered epigenetic programming
- The mechanisms of EDC action include effects on the epigenetic molecular machinery that controls gene expression in hypothalamic and reproductive tissues
- Effects of EDCs may be transmitted transgenerationally through molecular changes to the germline or through context-dependent modifications to somatic cells by continued exposures to EDCs or the individual's social or environmental context

Figure 1.

The hypothalamic–pituitary–gonadal (HPG) axis. The HPG axis of mammals is shown with hypothalamic-releasing factor neurons of the hypothalamus terminating in the median eminence above the anterior pituitary gland, where the portal capillary system communicates signals from the hypothalamus to the pituitary. The release of gonadotropin-releasing hormone from hypothalamic neurons specifically targets the anterior pituitary gonadotropes to synthesize and secrete luteinizing hormone and follicle-stimulating hormone. From there, these circulating gonadotropins act upon receptors in the gonad, ovary or testis, to activate gonadal steroidogenesis and gametogenesis. Steroid hormones in the circulation act upon peripheral targets and also feed back upon steroid-sensitive neurons in the brain and hypothalamus. Endocrine disrupting chemicals can disrupt HPG systems through interference with any or all of these pathways, particularly during developmental exposures. Shown in the figure is how endocrine disrupting chemicals may disrupt the hypothalamic level of the HPG axis through actions on neurons containing steroid hormone receptors.

Figure 2. DNA methylation and histone modifications. Two major epigenetic mechanisms, DNA methylation and histone modifications, act in concert to regulate gene transcription. The DNA double helix backbone is shown in blue. In DNA methylation, methyl groups are added to a cytosine that is immediately 5' to a guanine. In general, an increase in DNA methylation leads to a decrease in mRNA transcription. Histone modifications occur when functional groups are added to the tails of histones (histones shown in pink, with blue tails), leading to a 'relaxed' or 'condensed' chromatin state. Depending on the modification, the DNA will be more or less tightly wound around the histone thereby increasing or decreasing the likelihood of gene transcription. Adapted with permission from Macmillan Publishers Ltd © Qiu, J. *Nature* **441**, 143–145 (2006).⁷⁹

Figure 3.

Context-dependent epigenetic transmission. EDC effects can be passed from one generation to another through actions on genes and proteins that control hormone levels, neurobiological physiological functions, and behaviors, particularly maternal behaviors towards the offspring. As depicted here, prenatal exposure to an EDC in a dam can alter the hypothalamic control of her hormone levels and her behaviors towards her F1 pups. The F2 generation may exhibit differences in their own hormones and behavior due to the context of what they were exposed to during postnatal development from their F1 mother. Abbreviation: EDC, endocrine disrupting chemical.

Figure 4.

Programming of methylation in the germline. **a** | Methylation reprogramming in the germ line. Primordial germ cells (PGCs) in the mouse become demethylated early in development. Remethylation begins in prospermatogonia on embryonic day 16 in male germ cells and after birth in growing oocytes. Some stages of germ cell development are shown. **b** | Methylation reprogramming in preimplantation embryos. The paternal genome (blue) is demethylated by an active mechanism immediately after fertilization. The maternal genome (red) is demethylated by a passive mechanism that depends on DNA replication. Both are remethylated around the time of implantation to different extents in the embryonic and extra-embryonic lineages. Methylated imprinted genes and some repeat sequences (dashed line) do not become demethylated. Unmethylated imprinted genes (dashed line) do not become methylated. Abbreviations: EM, embryonic lineages; EX, extra-embryonic lineages; PGC, primordial germ cells. Permission obtained from the American Association of the Advancement of Science © Reik, W. *et al. Science* **293**, 1089–1093 (2001).³⁰

Figure 5.

Transgenerational inheritance of epigenetic traits across three generations. Depiction of how exposure of a pregnant dam (F0) exposes the fetal F1 offspring, as well as germ cells within the F1 that will develop into the F2 generation. The first generation devoid of any personal exposure is F3.

Figure 6. Effects of endocrine disrupting chemicals on mate preference in the F3 generation. A partner preference test is depicted. The testing chamber is partitioned on either end with acrylic glass, with an opening covered with mesh wire. In this figure, a male from each lineage (F3-vehicle, F3-vinclozolin) is housed in each opposite end chamber. In the center, a female from one of the lineages is videotaped for a 10-minute trial, during which time the nature and location of her behaviors relative to each male is recorded. A preference is indicated by a significantly greater amount of time spent towards one male over the other.

Table 1

Summary of epigenetic and transgenerational effects of four endocrine disrupting chemicals

EDC	Epigenetic effects	Transgenerational effects
Vinclozolin		
Mice	DNA methylation males (sperm): ³⁴ ↓ H19 and Gtl2 and ↑ Peg1, Snrpn and Peg3 (F1) ↓ Maternally and paternally imprinted genes (F3) DNA methylation in females (various tissues): ³⁴ ↑ H19 in tail; ↓ Peg1, Snrpn and Peg3 (F1–F3)	Motile sperm concentrations: ³⁴ 56% of controls in F1; 90% of controls in F2; 100% of controls in F3
Rats	↔ DNA methylation of LPLase gene 1 in sperm (F0s) or testes (F1 and F2) ⁴⁰ ↓ Dnmt3A (F1 and F2) and Dnmt1, Dnmt3L and Ehmt1 (F1–F3) gene expression in testes (E 16) ³⁷ DNA methylation altered in the testes (PND 6); ³³ F2 and F3 males have altered methylation in sperm ³³ Transcriptome altered in hippocampus and amygdala of F3 males (12 months) and females (15 months), ⁴³ and testes (E 16) ³⁶	↔ Spermatogenesis, fertility (F0–F2) or organ weights (F1 and F2 at 13 weeks) ⁴⁰ ↔ Sexual development or sperm evaluations in F1 or F3 ⁴¹ ↓ Testes and caudal epididymidis weight; ↔ sperm evaluations in F2 ⁴¹ ↓ Apoptosis of germ cells F1 and F2 (low dose), F1 and F3 (high dose) ⁴¹ ↑ Apoptosis in all control animals relative to vinclozolin ⁴¹ ↑ Spermatogenic apoptosis ^{33,35,37} ↓ Sperm motility and sperm concentrations in F1–F3 (PND 60–150) ^{33,35,37} ↑ Disease states in F1–F4 ³⁵ males and F1–F3 females ³⁹ Behavior: ↓ Anxiety-like behavior in young F3 males and ↑ in old and young females ⁴³ F3 females (treated or untreated) show a preference for untreated males over the treated males; no preference of F3 males for either female lineage ⁷
Diethylstilbesterol (DES)		
Mice	Uterus: ↑ Methylation of homeobox 10A promoter region and protein expression (PND 14) ⁵⁷ ↑ cFos mRNA expression (PND 5–60) and methylation on exon 4 (PND 5) ⁵⁶ ↓ Methylation of cFos on exon 4 (PND 8–60) ⁵⁶ ↓ Methylation of lactotransferrin gene (PND 21 and 30) ⁵⁴ ↑ Expression of Dnmt1 (PND 14 and 30), ^{57,59} 3b (PND 5 and 14) ^{57,59} and Dnmt3a (PND 30) ⁵⁹ Epididymis: ↑ Expression of Dnmt 1, 3a and 3b ⁵⁸	↑ Tumors, proliferative lesions of the testis (F2 males) ⁵¹ ↔ Fertility unaltered (F2 males and females) ^{51,52} ↓ Serum E ₂ (F2 males) ⁵¹ ↑ Uterine adenocarcinomas (F2 females) ⁵² ↑ Tumors of reproductive system (F2 females) ⁵³
Rats	Uterus: ↓ Total histone trimethylation of K27 (PND 12) ⁶⁰ ↑ Phosphorylation of histone methyltransferase EZH2 (PND 12) ⁶⁰ ↑ Expression of CaBP-9k and Dio2; ↓ Gdf10, Car8, Gria2 and Mmp3 (5 months and PND 12) ⁶¹	–
Humans	–	↑ Birth defects (grandsons and daughters) ⁴⁷ ↑ Risk for heart conditions (granddaughters) ⁴⁷ ↓ Number of live births and altered menstrual cycles (daughters) ⁴⁸ Small cell carcinoma of the ovary (granddaughter); ⁵⁰ ↑ hypospadias (grandsons) ⁴⁹
Bisphenol A (BPA)		
Mice	Altered CpG methylation in the forebrain (embryo) ⁶⁷ Shifts agouti coat color by decreasing DNA methylation ⁶⁸	Maternal behavior altered with treatment in both F0 and F1 mothers ⁷⁰
Rats	Prostate: ↑ Methylation of several genes (PND 10 and 90); ⁶⁶ ↓ methylation promoter region and ↓ mRNA expression of PDE4D3 ⁶⁶ Protein expression in testes: ↓ AR, ERβ, SRC-1 and NCor in F1–F3 (adult); ^{64,65} ↑ ERα in F1, p/CIP in F2, GRIP-1 in F2 and F3 (adult) ^{64,65}	↑ Prostate intraepithelia neoplasia and cell proliferation (PND 200) ⁶⁶ ↓ Fertility F1–F3 males (PND 75) ⁶⁴ ↑ Body weight in F2 and F3 males (PDN 125) ⁶⁴

EDC	Epigenetic effects	Transgenerational effects
<i>Polychlorinated biphenyls (PCBs)</i>		
Rats	↓ Dnmt1 expression in hypothalamus and liver (PND 21) ^{75,76} ↓ Dnmt1, 3a and 3b and methylation of 16 genes in the liver (PND 21) ⁷⁶	Skews sex ratio towards females F1 and F2 (slightly) ⁷⁴ ↓ LH and P ₄ on proestrus and uterine weight in F2 (adult females) ⁷⁴

↑ = increase, ↓ = decrease and ↔ = no changes or no effect. Abbreviations: AR, androgen receptor; CaBP-9k, calbindin D9k; Car8, carbonic anhydrase related protein 8; Dio2, diiodinase type 2; Dnmt, DNA methyltransferase; E, embryonic day; E₂, estradiol; Ehmt1, histone methyltransferase; ER, estrogen receptor; EZH2, enhancer of zeste homolog 2; Gdf10, growth differentiation factor 10; Gria2, glutamate receptor, ionotropic, AMPA 2; GRIP-1, glutamate receptor interacting protein 1; K27, lysine 27; LH, luteinizing hormone; LPLase, lysophospholipase; Mmp3, matrix metalloproteinase 3; NCor, nuclear receptor co-repressor; P₄, progesterone; p/CIP, cointegrator-associated protein; PDE4D3, phosphodiesterase type 4 variant 4; Peg, paternally expressed gene; PND, postnatal day; Snrpn, small nuclear ribonucleoprotein polypeptide N; SRC-1, steroid receptor coactivator 1.