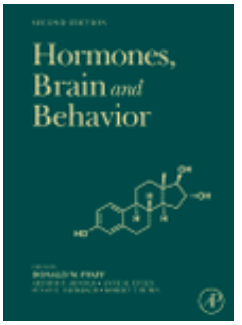

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56 Environmental Endocrine Disruption of Brain and Behavior

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Chapter Outline

Glossary

endocrine disruption The process by which exogenous or endogenous chemicals cause perturbations in endocrine and/or reproductive systems.

epigenetics The interactions between the environment and the genome by mechanisms, independent of genetic mutation, resulting in modifications of gene expression.

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fetal basis of adult disease The scientific discipline studying the interactions of an individual with its fetal environment (e.g., the uterus or the egg), its external environment (e.g., exposures to environmental endocrine-disrupting chemicals), and its genome and epigenome, resulting in an individual predisposition (or lack thereof) for the development of a disease later in life. An important component of this hypothesis is that early developmental organisms are particularly vulnerable to external perturbations.

gonadotropin-releasing hormone (GnRH) The hypothalamic ten-amino-acid peptide that controls reproductive function in vertebrates.

hypothalamic–pituitary–gonadal axis The reproductive system of vertebrates, comprising the hypothalamus at the base of the brain, the anterior pituitary gland, and the gonads (ovary or testis).

no observed adverse effect level (NOAEL) It is a commonly used, but flawed, approach in risk assessment.

sexual differentiation of the brain The process by which hormones, chromosomes, and the environment interact in the developing nervous system to permanently affect sexual behavior and physiology in adulthood.

transgenerational effects Effects that are observed not only in an exposed organism but also in that organism's offspring and future generations.

xenobiotic Literally, a foreign biological agent; an exogenous substance that exerts biological effects.

56.1 Introduction to Endocrine Disruption

Endocrine-disrupting chemicals (EDCs) comprise a wide variety of natural and synthetic compounds that exert actions upon hormonally sensitive pathways, resulting in endocrine and/or reproductive dysfunctions. There are numerous industrial chemicals, pesticides, fungicides, plasticizers, pharmaceuticals, phytoestrogens, and other compounds that may cause endocrine disruption. Although the mechanisms of their actions vary, they may include any or all of the following: nuclear hormone receptors, membrane hormone receptors, enzymes involved in the biosynthesis/metabolism/degradation of hormones, coregulatory factors, and neurotransmitter systems in the brain that control neuroendocrine functions, among others (reviewed in Gore (2007) and Gore et al. (2006)). An example of a prototypical EDC is provided by polychlorinated biphenyls (PCBs), a family of highly stable compounds that were used in industry for decades until they were banned in 1977 in the United States. Ironically, the very structural properties of PCBs and similar compounds that made them useful in industry were what caused them to be EDCs. Their lipophilic, biphenolic structures enable PCBs to interact with nuclear hormone receptors, including the estrogen receptor (ER), thyroid receptor, androgen receptor, and the aryl hydrocarbon receptor (e.g., Kuiper et al., 1998). PCBs also exert actions on other steroid-regulatory or -sensitive pathways (Connor et al., 1997; Kester et al., 2000; Ohtake et al., 2003). With specific regard to neuroendocrine systems, PCBs can disrupt specific aspects of reproductive physiological and behavioral functions controlled by the hypothalamus–preoptic area (Chung and Clemens, 1999; Chung et al., 2001; Steinberg et al., 2007). This example provided by PCBs demonstrates effects of EDCs on reproductive and endocrine functions.

This chapter provides a summary of the evidence of effects of EDCs on reproductive neuroendocrine systems, focusing on the consequences for the development of sexually dimorphic behaviors and physiological functions. A key point from the neuroendocrine perspective is that timing is everything. As discussed in more detail below, the ability of organisms to exhibit sex-appropriate reproductive physiological and behavioral functions in adulthood is, in most species, contingent upon exposures (or the lack thereof) to specific hormones during critical developmental windows (reviewed in Gore (2008); see **Chapter 23, Hormones, Brain, and Behavior in Reptiles**; **Chapter 25, Neuroendocrine Regulation of Reproductive Behavior in Birds**; **Chapter 54, Sexual Differentiation of the Brain: Mode, Mechanisms, and Meaning**; **Chapter 55, Sexual Differentiation of Brain and Behavior in Birds**; and **Chapter 58, Sexual Differentiation of Behavior in Nonhuman Primates**). This concept of timing is particularly relevant to the field of neuroendocrine disruption, as exposure to exogenous substances during these critical periods, or even to endogenous hormones (e.g., from the maternal environment), may have a permanent imprinting effect. We present a comparative approach to the field of neuroendocrine disruption, as the different vertebrate

classes may be differentially affected by EDCs, and research across the evolutionary spectrum has been particularly enlightening about the mechanisms by which EDCs exert their actions. For information on sex differences in EDC actions in invertebrates, the reader is referred to McClellan-Green et al. (2007). In this chapter, we focus on the key questions for consideration in effects of EDCs on hormones, the brain, and behavior in vertebrates.

56.1.1 Critical Issues about Endocrine Disruption

Several key topics have emerged in an effort to reconcile the disparate effects of EDCs and to understand their mechanisms. In general, consequences of EDC exposure are dependent upon both the properties of the chemical(s) to which an organism is exposed, together with the individual's genetic susceptibility and developmental or life stage. More specifically, the following points determine the effects of EDCs (for further information, see Crews et al. (2000), Dickerson and Gore (2007), Gore (2008), McLachlan, (2001), Walker and Gore (2007), Willingham and Crews (2000), and Witorsch (2002)).

Before describing these topics, it is important, primarily, to distinguish between the various categories of effects of EDCs. The first, and most obvious, are those gross effects on development, whether on specific structures or on systems. Examples would include the development of supernumerary, split, and duplicated limbs in frogs, stunted growth resulting in smaller individuals, abnormal or erratic escape behaviors from predators or hostile conditions, etc. Such individuals are more likely to die early in life than those not affected. A second category is to do with the effects on those animals that appear otherwise normal in appearance, but are grossly abnormal internally, either structurally or in physiology. Examples of this are hermaphroditic fish with ovotestes and/or abnormal accessory sex structure development, delayed or absent puberty, high vitellogenin levels, etc. Such individuals usually are sterile. The third category includes effects in which exposed individuals might be capable of breeding but do not engage in normal courtship and copulatory behavior with opposite-sex individuals. Examples are individuals that do not respond to species-typical courtship signals, are unable to produce the appropriate signals, or cannot integrate properly the necessary sensory and motor information necessary for successful copulation. Birds that form pair bonds, but with a member of the same sex, fall into this category. Taken together, these individuals could be said to be exhibiting effects of EDC exposure, and might live as long as normal, unexposed individuals. However, they represent a form of evolutionary death in that they do not reproduce.

56.1.1.1 *Life stage and timing*

There are critical windows of developmental sensitivity to natural or exogenous hormones or hormone-like molecules. In nonmammalian species, the embryo may be exposed to EDCs through the yolk. Bern (1990) first noted the significance of maternal steroids accumulation in yolking follicles and that these hormones can influence offspring phenotype in a number of species, a finding now supported in studies with fish, amphibians, reptiles, and birds (Bowden et al., 2000; Cariello et al., 2006; Eising et al., 2001; Elf, 2004; Elf et al., 2002; Gil et al., 1999; Groothuis and Schwabl, 2008; Lipar and Ketterson, 2000; Martin and Schwabl, 2007; Ottinger et al., 2005a; Pilz et al., 2005; Radder et al., 2007; Rhen et al., 2006; Schwabl, 1993, 1996; Sockman et al., 2006; Verboven et al., 2003). Studies have established that, both for aquatic and groundwater environments, contamination penetrates into the egg resulting in EDC profiles in the yolk that match those of the surrounding environment (cf. Bishop et al., 1998; Gale et al., 2002; Halldin, 2005; Heinz et al., 1991; Peterman et al., 1996; Podreka et al., 1998).

In mammals, the late embryonic/early postnatal period is considered a critical period for brain sexual differentiation, during which exposure to a hormone, or the lack thereof, causes permanent molecular changes in the brain (Gore, 2008; Phoenix et al., 1959). Later in life, in response to increased gonadal steroid hormones during puberty, these organizational effects of hormones on the brain are manifested as appropriate masculine or feminine reproductive physiology and behavior (Dohler, 1991; Rhees et al., 1990). Endocrine disruption, during the critical periods of brain sexual differentiation, is therefore particularly detrimental. Animals may retain responsiveness to EDCs throughout life (Brezner et al., 1984), although, in general, the adult is less adversely affected by EDCs than is a developing organism. Thus, life stage is a key consideration in interpretation of EDC data.

56.1.1.2 *Latency of effects*

The fetal/developmental basis of adult disease hypothesis postulates that early exposures to subtoxic levels of EDCs may not have any immediate apparent effects, but can predispose an organism to the latent development of a disease or disorder (Barker, 2003;

Gore et al., 2006). Animal models consistently demonstrate that low-dose exposures of fetuses to EDCs often have no discernible effects at birth, but result in infertility, abnormalities, and cancers much later in life (Welshons et al., 2006). A compelling example comes from humans, as millions of women who took the estrogenic pharmaceutical diethylstilbestrol (DES), under physicians' advice to avert miscarriage, inadvertently exposed their fetuses to a potent estrogen. At birth, the infant girls appeared externally normal, but later in life they were found to have a disproportionately high level of reproductive-tract abnormality and increased incidence of development of rare vaginocervical cancers (Newbold, 2004). Laboratory rodent models of DES are quite consistent with the human data, as fetal DES is associated with the latent development of uterine cancer (Newbold et al., 2006). This fetal basis of adult disease also applies to the context of hormones and reproductive behaviors (Gore, 2008; Gore et al., 2006; Steinberg et al., 2007, 2008).

56.1.1.3 Sensitivity to EDCs

Species and even strains differ in their sensitivity to EDCs (Crews et al., 2000; Spearow et al., 1999; Thigpen et al., 2007). This should not be surprising considering the substantial differences among species in the nature and amount of circulating concentrations of steroid hormones, with differences of similar magnitude existing between inbred strains (Shire, 1976). In some species, testosterone may be the predominant steroid in males, but in other species it may be dihydrotestosterone or 11-ketotestosterone. In females, estrone (E1), 17β-estradiol (E2), and estriol (E3) are commonly found, and research in mammals has led to the supposition that E2 is the most potent of these steroidal estrogens. While this may be true for human beings, it does not follow for all other mammals and other vertebrate classes. For example, in the red-eared slider turtle sex-determination assay, E3 is far more potent than E2 in overcoming the effects of a male-producing incubation temperature (Bergeron et al., 1999). Similarly, *o,p'*-DDE (DDE) and its metabolites have been implicated in a variety of reproductive anomalies, but not in all species. In the red-eared slider turtle, DDE causes female development when applied in concentrations found in contaminated environments (Willingham and Crews, 1999), but it has no detectable effect in the green sea turtle, *Chelonia mydas* (Podreka et al., 1998), and is antiestrogenic in the tiger salamander, *Ambystoma tigrinum* (Clark et al., 1998). In the rat, methoxychlor causes delayed anovulatory syndrome (Gray et al., 1989), but in hamsters it is without apparent effect on estrous cycling (Gray et al., 1985). On the other hand, the pesticide Kepone (chlordecone) masculinizes the behavior of female hamsters (Gray, 1982) and, in rats, seems to inhibit female sexual behavior when administered at proestrus (Brown et al., 1991), fails to induce lordosis in ovariectomized females (Uphouse et al., 1986), but accelerates puberty (Gellert, 1978).

Species and individual differences in behavioral sensitivity to a steroid hormone are also due to the abundance of the associated receptor in the brain, which is inversely proportional to the typical levels in the circulation (Young and Crews, 1995). This has implications for EDCs in that species with relatively low levels of endogenous estrogen(s) in their circulation will be more sensitive to chemicals that mimic the action of estrogen (Crews et al., 2000). Thus, rather than concentrating on a few species to serve as standards for defining a chemical as an EDC, a more instructive approach is to document the effects and then understand why different species vary in their sensitivity to specific compounds.

Finally, genetics play a role in sensitivity to EDCs. The genetics of endocrine systems has been demonstrated repeatedly in selection studies and has been extended to sensitivity to EDCs. For example, Ozburn and Morrison (1962) produced *o,p'*-DDT (DDT)-tolerant mice after eight generations of selection, and Poonacha et al. (1973) fed selected lines of quail a diet containing 200 ppm of DDT during the quails' first 30 days of life. The development of resistance was evident after the third generation of selection, as indicated by lower mortality among the selected lines when compared to a control line that was fed DDT. Interestingly, there was a sex difference in resistance, with females being less resistant than males.

56.1.1.4 Degradation and metabolism, mixtures, and synergism

The stability of EDCs in the environment is well documented, as is the degradation into more (or less) active compounds. For example, *o,p'*-DDT is biotransformed in the environment to form *o,p'*-DDE, a compound equally persistent and, in some instances, more biologically active than its parent.

Living organisms are exposed constantly to mixtures of chemicals, generated by the body, that serve to regulate critical developmental, homeostatic, and

reproductive events, as well as communicate vital information to conspecifics (e.g., pheromones). When this communication crosses species boundaries, these endogenously produced chemicals can stimulate or inhibit these life-history events in the recipient. Contamination occurs when the source(s) and nature of the chemicals are man-made. It is important to keep in mind that as in natural systems described above, such contamination never consists of single chemicals but of mixtures of chemicals with the compounded result that over time, degradation and/or metabolism occurs such that the metabolized/degraded product may be even more biologically active than the parent chemical.

It is now recognized that low doses/concentrations are biologically relevant when EDCs are considered (Welshons et al., 2003). Similarly, the issue of ecologically relevant mixtures continues to be underappreciated. Many chemicals are already mixtures, as is the case of PCBs that consist of different chlorinated biphenyls depending upon the industrial application, for example, Aroclors. Relatively few studies have identified the active chemicals in such compound(s), nor have they demonstrated how they may synergize to produce their effects in a complex biological system. When it is considered that, in nature, virtually all contamination is the form of mixtures, the importance of this aspect of endocrine disruption cannot be overestimated. While synergism is a commonly demonstrated phenomenon in endocrinology, the work in this avenue on EDCs has been limited (e.g., Christiansen et al., 2008).

One problem facing researchers in the field of endocrine disruption (and relevant to the concept of mixtures, as will become apparent in the following) is identifying a suitable animal model for *in vivo* studies of EDC effects. The red-eared slider turtle (*Trachemys scripta elegans*) serves as a biological-marker system for endocrine disruption at organismal (sex determination), physiological (circulating steroid hormone levels), and now genetic levels (regulation of gene networks) for studying EDCs singly, in mixtures, and in low doses. Indeed, work with the slider turtle established two of three foundational principles in EDC actions, namely the synergistic actions of ecologically relevant levels of mixtures of individual PCB compounds (Bergeron et al., 1994) and the absence of a threshold for EDC compounds (Sheehan et al., 1999). In the slider turtle, gonadal sex is determined by the temperature of the incubating egg (see Crews (1996) for review). For example, incubating slider turtle eggs at a temperature of 26 °C produces all males, and 31 °C produces all females; there is a very narrow range of temperatures (less than 1 °C) in which different ratios of male and female offspring are produced, with 29.2 °C or the threshold temperature giving a 1:1 sex ratio. Even at these intermediate incubation temperatures there are no hermaphroditic individuals but varying and predictable ratios of males or females.

Application of exogenous steroid hormones or steroidogenic enzyme inhibitors redirects the effects of incubation temperature (see Crews (1996) for review). Thus, a dose of 1 μg of E2 applied to eggs incubating at 26 °C, normally an all-male temperature, results in all of the hatchlings being gonadal females, whereas at the threshold incubation temperature (29.2 °C), the effective dose is 0.01 μg. An incubation temperature of 29.2 °C normally produces a female-biased sex ratio, but administration of an aromatase inhibitor results in 100% males. Considering that only 0.2% of the E2 applied to the eggshell ends up in the embryo (Crews et al., 1991), it becomes apparent that even very low doses of steroid hormones or their mimics can have profound biological effects (see Section 56.1.1.5).

The lability of sex determination in the slider turtle offers the opportunity to use gonadal sex as a marker for EDC effects both as mixtures as well as the effects of extremely low doses because it is possible to have both negative (ethanol-treated) and positive (E2-treated) controls in which either all males or all females were produced normally. In one study of mixtures, the effects of 11-hydroxylated and nonhydroxyated derivatives of heavily chlorinated PCBs, all ingredients in common-use mixtures such as Aroclors (Bergeron et al., 1994), were examined. These particular chemicals were selected because of their known affinity for the ER as demonstrated in mouse uterotropic assays and were likely to be less neurotoxic or teratogenic but more estrogenic as a result of their conformational structure. The mixture of all compounds produced sex reversal, but this activity was due to only two of the PCB compounds tested, 2′,4′,6′-trichloro-4-biphenylol (F) and 2′,3′,4′,5′-tetrachloro-4-biphenylol (G). This was established in studies in which mixtures containing the other nine compounds were tested (with no effect), and in studies where these compounds alone, or in combination, were tested. In the latter instance, both compounds showed significant sex reversal at this temperature and when combined, their effect was not additive but synergistic, resulting in a significant increase in ovarian development at a dose of 10 μg or

less than 1 ppm, whereas F alone and G alone required at least a tenfold higher dose to show that sex reversal was found to have estrogenic activity, as indicated by the production of female hatchlings from eggs incubated at a male-producing incubation temperature.

Environmentally relevant doses of commonly used compounds and their mixtures have been examined using the slider turtle. In one study, eight compounds identified in the yolk of alligator eggs from Lake Apopka, Fla (Heinz et al., 1991) were tested (Willingham and Crews, 1999). When applied as single compounds, the PCB mixture Aroclor 1242, *trans*-Nonachlor, *cis*-Nonachlor, DDE, and chlordane altered sex-ratio outcomes; Aroclor 1242 produced the most powerful effects, shifting the ratio of females almost twofold, while chlordane had the greatest effect when combined with E2. The combined effect of all eight compounds also significantly altered the sex ratio in the female direction. Further work indicated that when administered at even lower doses, some of these compounds (0.25 ng *trans*-Nonachlor, 7 ng DDE, and 0.125 ng chlordane) were still effective in overriding a male-producing incubation temperature (Willingham, 2004).

56.1.1.5 Low doses and thresholds (or the lack thereof)

Traditionally, toxicological studies have focused on dosage ("the dosage makes the poison" – Paracelsus) of substances administered that results in mortality and/or disease. Indeed, risk assessments for virtually all chemicals, except genotoxic chemicals, assume that, for any substance, there exists a threshold dose below which exposure is safe. This assumption underlies the concept of no observed adverse effect level (NOAEL), a commonly used approach in risk assessment. As in other risk-assessment approaches, the NOAEL relies on standard toxicology dose–response studies to designate the lethal dose low (LD_{Lo}) (concentration for inhalants) as the dose at which mortality or disease is still detectable (as compared to the LD_{50}, or the dose at which 50% of a group of test animals that die or manifest disease when administered as a single exposure at a specific time period). It is the dose tested below that of the LD_{Lo}, or the dose that shows no significant difference between it and the control, or no-dose, group, that becomes the basis for setting the acceptable exposure risk. This value is typically then divided by 100, or a factor of 10 for variability in test responses and another factor of 10 for species extrapolation (animals to humans; Bokkers and Slob, 2007). However, acceptable exposure levels that are considered safe are rarely tested empirically. The reason is simple. The number of doses needed to be tested as well as the number of individuals required to detect significant and valid deviations is prohibitively large. As a consequence, safety standards for most chemicals depend on the validity of the threshold assumption.

The threshold assumption was first challenged mathematically by Hoel (1980) who demonstrated that if an endogenous chemical normally produces a quantifiable response, then exogenous administration of that same chemical cannot produce a threshold because it automatically exceeds that which is present. For example, in any endocrine-mediated process, such as sexual development, the individual, during any stage of its normal development, is already producing the levels of the chemical(s) necessary for that process. In other words, the individual is already at its own threshold and any additional added ligand from the internal or the external environment is necessarily above threshold and adverse. Subsequently, Daston (1993), Gaylord et al. (1988), Sheehan (2006), and Welshons et al. (2003) questioned the underlying assumptions of the NOAEL, specifically the threshold hypothesis, and suggested that it may not apply to chemicals that share a common mechanism with endogenous chemicals important to normal development. Specifically, they proposed that if the threshold for the exogenous chemical is already exceeded by the endogenous chemical, then administration of the same chemical should lead to a curve showing no threshold dose, no matter how low the background incidence caused by the endogenous chemical. The slider turtle enabled a strong test of this hypothesis because previous work demonstrated that estrogen is involved in normal ovarian sex determination and that exogenous estrogen can override incubation temperatures that, otherwise, would produce males. Using over 2400 individuals, Sheehan et al. (1999) established even a dose of 40 ng kg^{-1} of E2 surpassed the endogenous threshold of 1.7 ng of E2 in an egg. This can be interpreted as indicating that, in the developing embryo, endogenous compounds such as E2, necessary for normal development, already exist at threshold levels and any additional ligand exceeds that threshold. Such biologically based dose–response models better describe the relationships between different components of the continuum between exposure to, and the adverse effects of, a chemical (Andersen and Dennison, 2001; Sheehan, 2006;

Welshons et al., 2003) and have been extended to other EDC-associated phenomena (Sheehan, 2006; Welshons et al., 2006).

56.1.1.6 Transgenerational, epigenetic effects of EDCs

EDCs may exert actions not only on the exposed individual but on subsequent generations. This has been best illustrated for an endocrine-disrupting fungicide, vinclozolin. Exposure of pregnant rats to vinclozolin resulted in latent development of reproductive dysfunctions, infertility, and cancers in their male F1 offspring (Anway et al., 2005; Anway and Skinner, 2006). Moreover, if the F1 males mated prior to the development of disease, their F2 male offspring developed a similar phenotype. To date, Skinner's lab has demonstrated that this effect carries at least as far as in the F5 offspring, and that the mechanism involves (at least in part) an epigenetic modification caused by a change in methylation patterns to the male germline (Anway et al., 2005). Recently, and as expanded upon below (see Section 56.5), we showed that male F3 descendants of vinclozolin-treated dams, compared to the F3 descendants of vehicle-treated dams, were significantly less attractive to a female rat in a mate preference test, even though the experimental F3 males did not yet express any obvious phenotypic dysfunction (Crews et al., 2007). This result suggests evolutionary consequences of EDCs on mate preference and the ability of an animal to successfully reproduce. Further discussion of this subject is provided in Section 56.5.

56.1.1.7 Toxicological death versus evolutionary death

The majority of studies on the impact of EDCs continue to focus on the individual in its own lifetime. This is valuable information, but says little about the impact of the chemical on the population through time (proximate or ultimate). One measure is whether an individual will breed. There are many studies documenting altered sexual development as a consequence of embryonic exposure to EDCs, with the consequence that the individual is sterile (usually as an intersexual, and not that the individual attempts, but fails, to breed). Still, if an affected individual succeeds in breeding, and its young do not develop properly and themselves do not breed, then the overall negative result in terms of the population is the same. This might be termed evolutionary death, which in turn may lead to extinction. It is here that we must turn our attention in evaluating the impact on EDCs for wildlife and human health. A valuable example was recently provided by Kidd et al. who showed that low exposure to ethinyl estradiol of a population of fathead minnows (*Pimephales promelas*) in an experimental lake led to its near extinction (Kidd et al., 2007).

56.1.1.8 Sex differences in sensitivity

It is remarkable that although the issue of sex differences has been a dominant topic in behavioral neuroendocrinology for the past 50 years, and within the larger biomedical community since 1993 (Food and Drug Administration, 1993), it is only recently that sex differences are coming to the fore as an important factor in EDC research. Sex matters, and readers are recommended to peruse the following recent papers to review this literature (Burger et al., 2007; Cummings et al., 2007; Dickerson and Gore, 2007; Gochfeld, 2007; Orlando and Guillette, 2007; Walker and Gore, 2007).

This above list, while not exhaustive, provides examples of the enormous complexity of the field of endocrine disruption. It highlights the necessity of considering sex, mixtures, timing of exposure, assaying a variety of potential mechanisms, testing low-dose, ecologically relevant levels of exposure, and considering effects beyond the exposed generation, in the evaluation of EDC effects.

56.2 Neuroendocrine Effects of EDCs on the Hypothalamic–Pituitary Control of Reproduction

A growing body of evidence strongly supports effects of EDCs on reproductive and endocrine function *in vivo*, and in *in vitro* models (Gore, 2002b; Kholkute et al., 1994; Kilic et al., 2005; Shekhar et al., 1997; Woodhouse and Cooke, 2004; reviewed in Dickerson and Gore (2007) and Walker and Gore (2007)). Whereas much of the original endocrine disruption work showed effects on vitellogenesis (fish), reproductive tract, genitalia, serum hormone levels, and reproductive developmental landmarks (e.g., timing of puberty), little of this work focused on the effects of EDCs on the neuroendocrine control of reproduction. This is surprising for several reasons. First, neuroendocrine circuits in the brain, specifically the hypothalamus and preoptic area (POA), control much of reproductive function. The hypothalamic

neurons that synthesize and secrete the neuropeptide, gonadotropin-releasing hormone (GnRH), provide the primary driving force of reproductive development and puberty, and maintain reproductive function in adult males and females (Gore, 2002a; Yin and Gore, 2006). Second, hypothalamic–preoptic brain regions are highly sensitive to steroid hormones throughout life, and have robust expression of ERα and ERβ, androgen receptors (ARs), and progesterone receptors (PRs; Chakraborty et al., 2003a,b; McAbee and DonCarlos, 1999; Quadros et al., 2002), any of which are targeted by EDCs. Third, effects of hormones on the hypothalamic GnRH neural system are mostly mediated by afferent neural and glial inputs, rather than directly upon GnRH neurons, which do not express ERα, PR, or AR (Herbison and Theodosis, 1992; Huang and Harlan, 1993; Shivers et al., 1983; Skinner et al., 2001). Although GnRH cells co-express ERβ, this is not enough to explain the effects of estrogen actions on GnRH function (Wintermantel et al., 2006). This point is important because other neurotransmitter factors that control GnRH release and synthesis do express steroid hormone receptors, and therefore EDCs that act via nuclear hormone receptors may exert these actions through the neurotransmitter systems that regulate GnRH neuronal functions. Indeed, EDCs can act upon cells in the nervous system that synthesize/release dopamine, serotonin, glutamate, norepinephrine, and others (e.g., Chakraborty et al., 2003b,c; Sar, 1984; Sar et al., 1990; Sar and Stumpf, 1981), and all of these systems are part of the central nervous circuitry controlling GnRH neuroendocrine functions. Thus, the neuroendocrine hypothalamus is a largely understudied key node for the integration of the neurological and endocrine effects of EDCs.

56.2.1 Endocrine Disruption of GnRH Neurons

56.2.1.1 Mammals

Studies published on effects of EDCs on the HPG axis suggest hypothalamic and/or pituitary actions of these compounds. In some cases it is not possible to differentiate between these two regulatory levels in cases when the measured outcome is a pituitary gonadotropin, luteinizing hormone (LH), and/or follicle-stimulating hormone (FSH), as a proxy for GnRH, the latter which is not easily measured *in vivo*. However, in all likelihood, EDCs can exert actions on both of these HPG targets. A study on effects of dioxin (TCDD) on serum gonadotropin levels in immature (25-day-old) female rats showed profoundly elevated LH and FSH in response to a challenge by chorionic gonadotropin (Petroff et al., 2003). A preliminary study from one of our laboratories (Andrea C. Gore) showed that female rats treated with PCB congeners on embryonic day 16 had upregulated GnRH mRNA levels in the POA when assayed at P40, and similar exposure to organochlorine pesticides was also associated with elevated GnRH mRNA levels at P50 (Gore, 2001). Thus, even very short-term exposures during critical developmental periods, particularly the late embryonic or early postnatal stages, have long-lasting consequences on GnRH mRNA expression.

A strong example of disruption of the HPG axis is provided by a study on two phytoestrogens: coumestrol (a coumestan) and genistein (a soy isoflavone). In this report by McGarvey et al. (2001), adult female rats were treated intravenously with coumestrol, genistein, E2, or a vehicle for an 8-h period, during which blood samples were collected frequently for subsequent radioimmunoassay of LH. Coumestrol and E2, but not genistein or the vehicle, caused significant suppressions of parameters of pulsatile LH release (McGarvey et al., 2001). That this effect is at least in part due to a hypothalamic action was elegantly proven through multiunit activity recording in the hypothalamus during the experiment. Multiunit activity volley frequency was significantly suppressed by coumestrol in concert with the suppression of LH pulses. Another part of the effect of coumestrol is also integrated at the pituitary gland because the pituitary response to GnRH challenge was attenuated in these animals (McGarvey et al., 2001). Thus, a specific class of phytoestrogen has suppressive actions on both the hypothalamus and pituitary in the suppression of HPG-axis function.

Other *in vivo* studies have reported effects of EDCs on serum LH concentrations. Lyche et al. treated goat kids with PCBs during gestation, and exposure presumably continued postnatally through lactational transfer (Lyche et al., 2004). Blood samples were collected regularly through the pubertal period for assay of serum LH. Results showed that PCB153 exposure was associated with significantly lower prepubertal LH concentrations, along with a delay in the timing of puberty. Again, these findings are consistent with effects of EDCs on HPG-axis function, and this

latter study is particularly interesting since exposure occurred early in life during a critical developmental window, but effects were not manifested until much later in postnatal life.

56.2.1.2 Birds

As reviewed in this book series previously by Ottinger and Vom Saal (2002), the GnRH neurosecretory system is highly conserved across the vertebrate classes. Nevertheless, specific evidence for endocrine disruption of GnRH neurons in birds is largely lacking. It is likely that this is the case, as EDCs affect neurotransmitter systems in birds that are known to affect GnRH cells (see Ottinger et al. (2005b) for a review). Future research will be very important in determining specific effects of EDCs on avian GnRH cells.

56.2.1.3 Amphibians

To our knowledge there is only one published study documenting the endocrine disruption of the GnRH system in amphibians, and none in reptiles. Sower et al. (2000) showed that two species of frogs in New Hampshire with developmental limb malformations had significantly lower hypothalamic GnRH peptide concentrations and serum androgens than normal frogs. Although the cause of the malformations was unknown, the authors speculated that it may be due to environmental pollutants, as the nature of the malformations was consistent with animals exposed to DDT, pesticides, or PCBs. Further research on a causal association between EDCs, limb malformation, and neuroendocrine outcomes is warranted.

56.2.1.4 Fish

The laboratories of I.A. Khan and P. Thomas have used the fish model of the Atlantic croaker (*Micropogonias undulates*) very effectively to ascertain effects of EDCs on HPG-axis function. They have shown that treatment of adult males for 30 days with the PCB mixtures Aroclor 1254 diminished pituitary gonadotropin responsiveness to treatment with a GnRH analog (LHRHa) *in vitro*, and reduced serum concentrations of the major fish androgens, 11-ketotestosterone and testosterone (Khan and Thomas, 1997). Moreover, concentrations of the monoaminergic neurotransmitters dopamine and serotonin in the POA were significantly reduced, whereas their metabolites were concomitantly increased. This latter finding suggests enhanced degration of these monoamines through potential actions on their degradatory enzymes. Although this can only be indirectly inferred from that study (Khan and Thomas, 1997), hypothalamic GnRH neurons in the Atlantic croaker are regulated by dopaminergic and serotonergic inputs, and changes in levels of these neurotransmitters suggest diminished drive to GnRH neurons, which in turn may alter GnRH release and subsequent pituitary responsiveness to GnRH. This conclusion was substantiated by a further study from this group (Khan and Thomas, 2001), which demonstrated that PCBs inhibited hypothalamic tryptophan hydroxylase activity, the rate-limiting enzyme for serotonin synthesis. Furthermore, that effects of PCBs on the HPG axis were due to actions on hypothalamic GnRH neurons was further tested by quantifying GnRH content in POA, and demonstrating a significant reduction in the PCB-treated fish (Khan and Thomas, 2001). As a whole, this story from the Atlantic croaker demonstrates alterations at the three levels of the HPG axis, and implicates the hypothalamic GnRH neuronal circuitry, including serotonergic and dopaminergic inputs, in mediating some of these effects.

56.2.1.5 In vitro studies

In vitro studies using the hypothalamic GnRH GT1–7 cell lines (Mellon et al., 1990) have also supported direct effects of EDCs on GnRH neurons. Low doses of PCBs significantly increased GnRH mRNA levels through a post-transcriptional mechanism that was partially, but not entirely, mediated by nuclear ERs (Gore et al., 2002). GnRH-peptide release from GT1–7 cells was also stimulated by Aroclor 1221, a PCB cocktail (Gore et al., 2002). In a similar study on organochlorine pesticides, treatment with methoxychlor or chlorpyrifos affected GnRH mRNA levels with an inverted U-shaped dose–response curve: low doses stimulated GnRH mRNA while higher doses suppressed this endpoint (Gore, 2002b). The methoxychlor metabolite 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) suppressed both GnRH transcription and mRNA levels (Roy et al., 1999). Finally, a study of the phytoestrogen, coumestrol, showed a suppression of GnRH mRNA levels, an effect that was prevented in the presence of a selective ERβ-antagonist (Bowe et al., 2003). These *in vitro* studies on GT1–7 GnRH cells are consistent with these neurons being direct targets of EDCs through mechanisms that are at least partially mediated through nuclear ERs. By contrast, a study by Petroff et al. on effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

(TCDD) on HPG-axis function found *in vivo* effects (described above) but no effect on GT1-7 cell numbers, GnRH peptide in the medium, or GnRH promoter activity in a promoter-reporter assay (Petroff et al., 2003). Thus, effects of EDCs may be limited to certain categories of compounds. Consistent with this, Petroff et al. could not detect the aryl hydrocarbon receptor mRNA in the GT1-7 cells, explaining the lack of effect of TCDD in this model (Petroff et al., 2003).

A report on cultured pituitary cells of young, adult female Sprague-Dawley rats investigated effects of the PCB mixture Aroclor 1242 on gonadotropin release (Jansen et al., 1993). The results showed that Aroclor 1242, at high doses and high duration (48 h), stimulated basal LH and FSH release. Moreover, Aroclor 1242 altered GnRH-stimulated LH and FSH release with an inverted U-shaped dose–response curve. We interpret these data as indicating effects of PCBs on pituitary gonadotropin responsiveness to GnRH, possibly through actions of the PCBs on GnRH receptors, as well as potential effects on the synthesis and/or release of the gonadotropins themselves.

Together, these *in vivo* and *in vitro* studies strongly support hypothalamic GnRH neurons as both a direct and indirect target of endocrine disruption. These hypothalamic cells provide key links between central nervous actions of EDCs, including on steroid hormone receptors and neurotransmitter receptors and synthetic/degradatory enzymes in the brain, and endocrine outputs to the anterior pituitary gland. An understanding of EDC effects on neuroendocrine systems is, therefore, critical to discerning the mechanisms by which EDCs alter sexual differentiation, puberty, fertility, and adult reproductive functions.

56.3 Endocrine Disruption of the Sexual Differentiation of the Brain

As reviewed in numerous articles, including elsewhere in this work (see **Chapter 23, Hormones, Brain, and Behavior in Reptiles**), the brain undergoes sexually dimorphic developmental processes due to influences from hormones, the environment, and chromosomes. These processes are highly subject to perturbation during critical developmental windows through exposures to EDCs (Dickerson and Gore, 2007; Gore, 2008). Specifically, there is an extensive literature showing specific effects of EDCs on the development of sexually dimorphic limbic brain regions, and on the expression of molecules in the brain that are involved in the control of sexually dimorphic sexual and nonsexual behaviors. Although it is impossible to include all of the examples from the literature, in the following we provide some representative examples of these effects of EDCs, focusing on hypothalamic–preoptic brain regions and the expression of hormone receptors in these areas.

56.3.1 Mammals

Numerous studies in experimental laboratory animals, principally rats, show that low levels of EDC exposure during gestation and early postnatal life, the critical period for brain sexual differentiation, cause permanent morphological changes in sexually dimorphic, steroid-sensitive hypothalamic–limbic brain regions. As reviewed in Dickerson and Gore (2007), much research has focused on developmental effects of EDCs on subregions of the hypothalamus–preoptic systems that control reproductive physiology and behavior in adulthood. To follow is a brief presentation of evidence for endocrine disruption of hypothalamic morphology and cellular phenotype.

Anteroventral periventricular nucleus (AVPV). The AVPV is a preoptic region that is important for the neuroendocrine control of ovulation in female rodents (Simerly, 2002; Wiegand et al., 1978), and it is sexually dimorphic in size, being larger in female than male rats. Research on EDCs shows consistent demasculinizing and/or feminizing effects of EDCs in AVPVs of neonatally exposed males, and defeminizing and/or masculinizing effects in their female counterparts. For example, one of us (Andrea C. Gore; Salama et al., 2003) showed that fetal exposures of rats to low levels of PCBs in early life (late embryonic through early postnatal) resulted in a significant (50%) suppression, in adulthood, of numbers of cells expressing ERβ-immunoreactivity in the AVPV. Patisaul et al. (2006) reported that early postnatal treatment of rat pups with bisphenol A (BPA) or genistein demasculinized the sexually dimorphic expression of tyrosine hydroxylase in the AVPV of male rats. Such treatment also defeminized the co-expression of ERα and tyrosine hydroxylase in the AVPV of females (Patisaul et al., 2006). Similarly, a diminution of the normal sex difference in both AVPV size, and in tyrosine hydroxylase-positive cell numbers in AVPV, was reported by Rubin et al. (2006). In this same brain region, Petersen's group showed a sex difference in the expression of mRNA for glutamic acid decarboxylase (GAD) 67, the

enzyme that synthesizes the neurotransmitter GABA and the expression of which indicates that a cell is GABAergic. They further reported that this sex difference was abolished by the dioxin TCDD given during prenatal life (Hays et al., 2002), presumably through actions on the aryl hydrocarbon receptor that is co-expressed in GABAergic cells. Together, these studies show that both the morphology of the AVPV and the phenotypes of its specific cells are significantly perturbed by early-life exposures to EDCs.

Sexually dimorphic nucleus of the preoptic area (SDN-POA). The SDN-POA of male rats is substantially larger than that of females, and early developmental manipulations of sex steroid hormones diminish this sex difference (Gore, 2008; Gorski, 2002). Thus, it is another part of the hypothalamic–preoptic circuitry that is vulnerable to fetal/early postnatal exposures to EDCs. It is important to mention that not all studies have reported effects of fetal exposure to EDCs on SDN-POA volume (Masutomi et al., 2003; Takagi et al., 2004). However, such effects have been shown for DES, genistein, and zearalenone (the latter is an estrogenic fungal metabolite), whereby postnatal treatment of rat pups during days 1–10 of life with one of these EDCs abolished the sex difference in SDN-POA volume, primarily through increasing this parameter in the females (Faber and Hughes, 1991). The pharmaceutical oral contraceptive estrogen, ethinyl estradiol, decreased SDN-POA volumes in prenatally exposed male rats at adulthood (Shibutani et al., 2005). Although both masculinizing effects of EDCs in females, and their demasculinizing effects in males, have been reported, differences between these reports, as well as the lack of effects in others, are likely attributable to the timing of exposure and the nature of the EDC.

Ventromedial nucleus of the hypothalamus (VMN). The VMN is linked to sexual behavior in adult male animals. Both the size of the VMN and its cellular phenotypes are disrupted through fetal and early postnatal exposures to EDCs, as well as treatments during puberty (Ceccarelli et al., 2007) and in adulthood (Funabashi et al., 2005). A good example in laboratory rats is provided by 4-methylbenzylidene camphor (4-MBC), an ultraviolet (UV) filter with actions both on estrogen and thyroid receptors. Prenatal exposure to 4-MBC affected a suite of genes in the VMN at adulthood. Specifically, it decreased ERα mRNA in both males and females, decreased PR mRNA only in females (to male levels), and increased both insulin-like growth factor I (IGF-1) and steroid receptor coactivator-1 (SRC-1) in females only (Maerkel et al., 2007).

Medial preoptic area (mPOA). The counterpart of the VMN is the mPOA, which plays key roles in controlling sexual behavior in adult females. Exposures to EDCs at various life stages, including perinatal and adult (Funabashi et al., 2001), affect expression of genes and proteins in this region. For example, prenatal BPA had latent effects on ERβ, but not ERα, mRNA levels in the POA at adulthood (Ramos et al., 2003). As described above for the VMN, exposures to 4-MBC altered expression of several genes in the mPOA, including ERα (decreased in both sexes), PR (increased in males), and IGF-1 and SRC-1 (increased in females; Maerkel et al., 2007).

56.3.2 Birds, Reptiles, and Amphibians

The considerable information on how EDC exposures modify gonadal differentiation and, in turn, influence adult brain–behavior relationships in various mammalian model systems, and the more limited but growing literature on birds, is absent in reptiles and amphibians. There is a literature on the sexual differentiation of the brain in reptiles and the reader is referred to (**Chapter 23, Hormones, Brain, and Behavior in Reptiles**). However, beyond the basic hormone-replacement studies *per se*, there is at present no information on the effects of EDCs on sexual differentiation of the reptilian brain.

In amphibians, research continues to focus on the effects of EDCs on the development or activation of the reproductive system (intersex gonads, accessory sex structures, induction of vitellogenin production in males, etc.); a literature that has been reviewed by others (see recent such reviews by Crews et al. (2003), Hayes et al. (2006a), Kloas and Lutz (2006), McLachlan (2001), Milnes et al. (2006), Mosconi et al. (2002), Reeder et al. (2004), and Vos et al. (2000)). To date, there is no information on the effects of EDCs on sexual differentiation of the brain.

56.3.3 Fish

Reports abound for effects of EDCs on reproduction in fish, with virtually all of the studies focusing on the gonad, vitellogenesis in males, the manifestation of intersex gonads, alterations in secondary sexual characteristics, and/or reduced fertility (Bayley et al., 2003; Jobling et al., 2002; Koger et al., 2000; Toft et al., 2003; reviewed in Arukwe (2001)). Nevertheless, environmental EDCs are highly likely to exert

effects on the fish brain, similar to those described above for mammals. To our knowledge, sexually dimorphic effects of EDCs on brain morphology are not known, although there are effects of EDCs on gene expression in the nervous system (e.g., Greytak and Callard, 2007; Lyssimachou et al., 2006).

Although the above sections highlight the need for further research in nonmammalian systems, they provide strong evidence for neural substrates that underlie effects of EDCs on reproductive systems. The following section will take these observations to the next level, focusing on the implications of developmental endocrine disruption on the manifestation of sexual behaviors in adulthood.

56.4 Endocrine Disruption of the Sexual Differentiation of Behavior

Extensive and compelling evidence shows that exposure of vertebrates to EDCs during the critical period of brain sexual differentiation has permanent effects on the manifestation of these behaviors in adulthood. Evidence for a range of representative EDCs follows, demonstrating consistent effects on sex-typical behaviors. Although in most cases the outcomes of EDC exposures are small, they are biologically relevant because a perturbation of behavior, no matter how small, may eliminate an animal from the mating pool if it is not selected by a conspecific mate. This may not be relevant in the laboratory setting, but is highly relevant in a natural setting. Readers are also referred to an excellent review by Zala and Penn (2004) for additional details, references, and information.

56.4.1 Mammals

Laboratory rodents have been used extensively in tests of sexual behaviors, with most studies focusing on exposure to EDCs during the organizational period, followed by behavioral testing in adulthood. A subset of published research has utilized the model of exposure and testing in adulthood. Examples for several representative EDCs, first for mating behavior in adult females, then in adult males, are provided in the following. The results provide overwhelming support for small but significant effects of EDCs on behaviors in most studies.

A good example of the fetal basis of adult disease, as it relates to endocrine disruption of reproductive behaviors, has been provided by PCBs. Early-life exposures to PCBs alter paced mating behaviors in adult female rats, with effects varying slightly depending upon the age of exposure. The paced mating model enables females to pace the timing of mating and enhances reproductive success (Coopersmith and Erskine, 1994), so it better approximates a naturalistic mating experience. One experiment involved exposure of rats on embryonic day (E) 14, the day of parturition (postnatal day (P) 0), and P10 to either Aroclor 1221 or Aroclor 1254 (Chung and Clemens, 1999). In adulthood, rats were ovariectomized (OVX) and treated sequentially with EB plus progesterone (P_4) to induce receptivity. Aroclor 1221 decreased lordosis quotient and other aspects of paced mating, whereas Aroclor 1254 had fewer effects. A second report from this group tested effects of longer-term postnatal treatment with Aroclor 1221 or 1254 in a similar mating paradigm (Chung et al., 2001). In that case, rats were administered the toxicants daily from P0 to P6. In adulthood, significant effects of A1254, but not A1221, were found on mating behaviors in adult OVX + EB + P_4 rats, a result that suggests that A1254 is more effective when given postnatally (Chung et al., 2001), compared to A1221, effects of which are exerted prenatally (Chung and Clemens, 1999). In addition, neither A1221 nor A1254 given to adult OVX + EB + P_4 females had any effect on mating behavior (Chung et al., 2001), indicating that effects are limited to exposure earlier in life.

One of our laboratories (Andrea C. Gore) assessed paced mating behavior in the adult female offspring of pregnant rat dams dosed with Aroclor 1221 at days 16 and 18 of pregnancy (Steinberg et al., 2007). Unlike the studies from Clemens' group described above, mating tests were performed on ovary-intact adult females, used on the evening of proestrus, when females were anticipated to be receptive. The paced mating tests demonstrated several significant differences in mating behavior in the F1 adult females that had been prenatally treated with PCBs. They spent significantly more time away from the male, and they also took significantly more trials to successfully mate (Steinberg et al., 2007). Thus, we found significant effects of fetal PCBs on adult paced mating behavior in F1 female rats, and we interpret these data to mean that reproductive success is diminished by low-level fetal PCB exposure.

Numerous other classes of EDCs alter mating behaviors in adult female rats. In many of these studies, rats were exposed in early life (late gestation and early postnatal development) and then in adulthood they were OVX and given sequential treatment of an estrogen followed by progesterone to induce

receptivity, similar to some of the PCB studies described above. An example of an EDC which altered sexual behaviors in adulthood is fenvalerate, a pyrethroid insecticide (Moniz et al., 2005) which when given prenatally, decreased the lordosis quotient in adult OVX, hormone-primed rats. Similarly, gestational exposure to DES reduced lordosis quotient and increased rejection rate (Kubo et al., 2003).

Phytoestrogens have been the subject of considerable interest for their effects on sexual behaviors. Again, most studies involved perinatal treatments, with behavioral tests done in adulthood in OVX + E2 + progesterone-treated females. Neonatal exposure to prenatal resveratrol, a phytoestrogen found in grapes, decreased lordosis quotient and increased rejections, consistent with diminutions in sexual receptivity (Kubo et al., 2003). Another study by Henry and Witt (2006) showed that when resveratrol exposure occurred via lactation, the only effect on sexual behavior was on the latency of males to mount the females, which was shorter in the resveratrol group (Henry and Witt, 2006). This surprising result suggests a potentially increased attractiveness of the resveratrol females, although the mechanism remains unknown. Genistein and daidzein, two soy isoflavones, were similarly tested for effects of early postnatal treatment at P0–P4 (Kouki et al., 2003). As adults, genistein-exposed rats had lower lordosis quotients on the third test compared to control rats. Daidzein had no influence on these behaviors. This result is interesting because it shows an interaction of sexual experience with the developmental treatment. This same lab performed a separate study for another class of phytoestrogen, coumestrol, a member of the coumestan family that is produced from clover and alfalfa sprouts (Kouki et al., 2005). When pups were exposed to a single injection of coumestrol at either a low (1 mg) or high (3 mg) dose on P4, in adulthood, the low dose of coumestrol resulted in higher lordosis quotient compared to control rats. By contrast, the higher dose of coumestrol (3 mg) almost completely suppressed the lordosis quotient to a similar extent as neonatal E2 (Kouki et al., 2005). These latter results emphasize the importance of dose in endocrine disrupting effects of phytoestrogens.

Some experiments on sexual behavior have involved exposure of adults to the EDCs (cf. the studies involving perinatal exposures described above). For example, Henry and Witt (2002) administered resveratrol or EB to adult OVX females, and 2 days later rats were given P_4 to induce receptivity (Henry and Witt, 2002). In this model, female rats given resveratrol did not exhibit lordosis behavior, indicating that resveratrol could not substitute for endogenous estrogens. In a second experiment, rats were pretreated with resveratrol prior to OVX, then tested for sexual behaviors following EB + P_4 treatment 10 days post-OVX. Although lordosis behavior did not differ between groups, resveratrol pretreated rats showed a delay in the exhibition of rejection behaviors, possibly due to a decrease in interactions with the males. Overall these experiments suggest few robust effects of resveratrol on sexual behaviors in adult female rats. Similar studies were done using zearalonone, a dietary estrogen produced by the *Fusarium* mold on cereal and grains, and it acts upon both ERα and ERβ (Kuiper et al., 1998). While not technically a phytoestrogen, it is consumed in the diet. Adult female rats were OVX for 1 week and treated with zearalonone for three consecutive days, with P_4 administered on the fourth day (Turcotte et al., 2005). While low-dose zearalonone had no effect, higher-dose zearalonone treatment enabled P_4-facilitated sexual behavior to occur. This result suggests that zearalonone has enough estrogenic activity to substitute for endogenous E2 in the adult OVX rat.

Several types of EDCs have been tested for their effects on sexual behavior in adult males. However, males may not be as sensitive as females to some compounds. For example, Wang et al. (2002) reported no effect of either PCB77 or PCB47 in males, despite this same group's findings of significant effects in females (Chung and Clemens, 1999; Chung et al., 2001). By contrast, Sager (1983) reported that "males exposed to PCBs showed a reluctance to mate when compared to control animals," and the latter group also showed an effect of sexual experience. In a series of four mating trials, 20 out of 32 males that had been exposed via lactation to Aroclor 1254 mated on their first trial (compared to 100% of control males), another seven on a subsequent trial, and five did not mate at all (Sager, 1983). Thus, sexual experience may interact with prenatal exposure to determine a behavioral outcome. Another study evaluating effects of a single fetal exposure (on gestational day 15) to PCBs showed that PCB 126 caused an increase in numbers of intromissions compared to the vehicle group in sexual-behavior tests of the males in adulthood, but no effects on other measured parameters (Faqi et al., 1998). These disparate results on PCBs indicate that the timing of exposure, the type of PCB, and the sensitivity of the behavioral assay has an effect on the result. Importantly, many of these studies did not use very sensitive outcomes, and much more careful

and naturalistic behavioral tests are necessary to provide stronger conclusions.

Other EDC classes given in gestational and early postnatal periods can affect sexual behaviors in adult male rats. Fenvalerate, the pyrethroid insecticide, was administered to rat dams late in gestation and through postnatal life (Moniz et al., 1999). When male offspring matured, effects on a range of sexual behaviors were quantified. Of these, the only significant effects of fenvalerate were on the number of mounts prior to first ejaculation, which increased, and the number of ejaculations, which decreased (Moniz et al., 1999). These data suggest detrimental effects of perinatal fenvalerate on masculine mating behaviors in rats. Low-dose BPA treatment given during gestation caused a significant decrease in the intromission rate in male rats, although it did not alter other behaviors, including numbers of mounts and timing of mating (Kubo et al., 2003). A study evaluating effects of postnatal BPA (administered from P0 to P9) found no significant change in the percentage of males that copulated (Kato et al., 2006). However, DES, when given to male rats during gestation had no effect on any measured parameters of masculine sexual behavior (Kubo et al., 2003). The latter result again suggests differential effects of EDCs on the two sexes, a finding that is not surprising considering their likely differential vulnerability.

Phytoestrogens have been evaluated in males for their effects on sexual behaviors. Resveratrol treatment throughout gestation suppressed intromission rate without affecting other masculine behaviors in rats (Kubo et al., 2003). A more recent study by Henry and Witt (2006) evaluated lactational exposure to resveratrol in male rats, and reported decreased mount frequency without affecting other behaviors. These reports suggest specific and modest effects of resveratrol that may differ in nature depending upon the timing of exposure (prenatal *vs.* postnatal). A report by Whitten et al. (1995) determined effects of lactational coumestrol, given either during the first 10 days of life, on sexual behavior in the male rats in adulthood. Early postnatal-coumestrol-exposed male rats showed significantly fewer mounts and ejaculations, and the latency to the first mount and first ejaculation was significantly longer, compared to control males.

56.4.2 Birds, Reptiles, and Amphibians

Although not widely recognized, some of the first demonstrations of endocrine disruption in wildlife were on effects of pesticides (DDT and DDE) on reproductive measures in birds (Crews et al., 2003; McLachlan, 2001; Zala and Penn, 2004). Subsequent work documented EDC-induced alterations in nesting and/or courtship behavior in ring doves (*Streptopelia risoria*; Haegele and Hudson, 1977), Bengalese fishes (*Lonchura striata*; Jefferies, 1967), Japanese quail (*Coturnix c. japonica*; Adkins-Regan and Garcia, 1986; Bryan et al., 1989; Halldin et al., 1999), Western, California, and herring gulls (*Larus occidentalis*, *L. californicus*, and *L. argentatus*; Fry and Toone, 1981; Fry et al., 1987; Hunt, 1977), and tree swallows (*Tachycineta bicolor*; McCarty and Secord, 1999a,b).

The reports of the effects of EDCs on sexual differentiation on gross morphology in birds are extensive, and there is a limited but increasing literature on how exposure might influence behavior of the adult (see recent reviews of Halldin (2005), Halldin et al. (1999), Ottinger et al. (2001, 2005a), and Panzica et al. (2005a,b, 2007)). In male quail, administration *in ovo* with estrogenic EDCs, such as BPA, DES, DDT, ethinyl estradiol, genistein, methoxychlor, and PCBs, reduced sexual behavior in adulthood (Bryan et al., 1989; Halldin, 2005 Halldin et al., 1999, 2001, 2003; Hoogesteijn et al., 2005; Ottinger et al., 2005b; Panzica et al., 2005a). Similar results were obtained following administration of antiestrogens and aromatase inhibitors (cf. Adkins, 1976; Adkins and Nock, 1976; Balthazart et al., 1992). In field studies, correlations between contaminant burdens in food with reduced or abnormal reproductive behaviors have been documented in both fish-eating birds and seed-eating birds (cf. Bosveld and van den Berg, 2002; Fox et al., 1978; Fry et al., 1987; Haegele and Hudson, 1977; McArthur et al., 1983).

Birds are particularly interesting in terms of EDC effects on brain and behavior because of the extensive work on the avian song system, an interconnected series of brain nuclei involved in the sensory integration and motor output of species-typical vocalizations (see **Chapter 26, Neural and Hormonal Control of Birdsong**). Although the role of steroid hormones in masculinizing sexual behavior in birds began in the mid-1970s (Adkins, 1976), it was the demonstration of Gurney and Konishi (1980) that early estrogen can alter the sexually dimorphic morphology of the song control system that provided the first direct link between early sex hormone exposure, brain development, and adult sexual behavior. The then-surprising result that administration of E2 to chicks would masculinize the brain and behavior of female offspring has since been documented by a

number of laboratories (cf. Noorden et al., 1987). Recent work of Adkins-Regan and colleagues has demonstrated that early estrogen treatment also altered the sexual-partner preference of adult female zebra finches, but only if they were raised in all-female colonies (Adkins-Regan, 1988, 1999; Adkins-Regan and Ascenzi, 1987; Mansukhani et al., 1996). Early estrogen treatment also led to a female-biased secondary sex ratio in this species (Von Englehardt et al., 2004; Williams, 1999). Taken together, these studies show a strong parallel between this observation and that of Fry et al. (1987) and Hunt (1977) on female–female pairing in areas where DDT had skewed the sex ratio of adult gulls.

Several research groups have capitalized on the proven relationship between brain morphology and behavior in birds, and are beginning to investigate how EDCs may act on specific brain regions to influence reproductive behaviors. Millam and colleagues focused most of their efforts on the zebra finch, demonstrating that oral administration (to mimic parental feeding) of exogenous estradiol benzoate administered shortly after hatching not only reduced fertility but masculinized the song system in the female (but not in males; Millam et al., 2001; Quaglino et al., 2002). Interestingly, the administration of octylphenol, methoxychlor, and dicofol in the doses given had no effect on fertility. Male zebra finches, however, did show deficits in mating behavior if they received posthatch treatment with estrogen, as Adkins-Regan and colleagues have shown (Adkins-Regan and Ascenzi, 1987), a finding also supported by Millam et al. (2001). Administration of perchlorate by oral gavage affected various developmental behaviors, but did not alter song-control nuclei size (Rainwater et al., 2007), while methoxychlor diminished fertility and hatching success (Gee et al., 2004). This method of oral gavage has been extended to pigeons for diazinon (Millam et al., 2000).

Iwaniuk et al. (2006) found that, in the American robin, (*Turdus migratorius*) increasing levels of DDT and DDE (as determined by analysis of yolk content in eggs taken from the same nests shortly after laying) were correlated with a reduction in males in the size of two song nuclei, nucleus robustus arcopallialis (RA) and HVC, and in both males and females with reduced neuronal size and overall volume of nucleus intercollicularis (ICo; see Reiner et al. (2004) for revised nomenclature of song-control nuclei); there is no change, however, in area X in either sex of the species. In the European starling (*Sturnus vulgaris*) a somewhat different picture emerges. Markman et al. (2008) reported that starlings foraging in the winter on the worms in sewage effluent filter beds received significantly higher amounts of synthetic and natural estrogens and other EDCs than those foraging on worms found in garden soil. The hypothesis that these contaminants might influence both the behavior and brain morphology was tested by feeding captive starlings mealworms containing 200 ng of E2 or a mixture of 200 ng of E2, 520 ng dioctylphthalate, 80 ng BPA, and 120 ng of dibutl phthalate (EDCs that are also found in worms in contaminated sites). The following spring, both males and females were assessed for the amount and complexity of song by males and the size of song nuclei (HVC). Male song and HVC volume were increased in individuals receiving the mixture; males receiving E2 alone did not differ from control (peanut oil) in any of these trait measures (Markman et al., 2008). Treatment did not affect testosterone levels or body mass. In a separate experiment, female preference for male song was assessed by measuring the time females spent on the perch adjacent to song playback. Females preferred the more complex song of males that had received the EDC mixture. It should be noted that the females used in the preference tests were wild-caught and hence their ingestion of EDCs during the previous winter was not known. The authors also measured immunosuppression in the males using cell-mediated immune function (wing-web swelling after injection of phytohemagglutinin) and secondary humoral response (response to injection of sheep red blood cells), finding that both the E2-alone and mixture-group males showed significantly lower immune function. Thus, by selecting males with more complex song, the females were also selecting males who were immunocompromised.

The neurohypophysial hormone arginine vasopressin (arginine vasotocin or AVT in nonmammalian vertebrates) is involved in a range of male-typical behaviors in vertebrates, including aggression and courtship, and its distribution in the brain is sexually dimorphic in all vertebrates studied to date (cf. fish (Goodson and Bass, 2001); amphibians (Boyd, 1994; Boyd et al., 1992; Marler et al., 1999; Moore et al., 2000); reptiles (Hillsman et al., 2006; Propper et al., 1992; Smeets et al., 1990; Stoll and Voorn, 1985; Thepen et al., 1987); birds (Aste et al., 1998; Grossman et al., 2002; Jurkevich et al., 1997, 1999, 2001; Kimura et al., 1999; Panzica et al., 2001; Panzica and Viglietti-Panzica, 1999; Viglietti-Panzica et al., 1994; Voorhuis et al., 1988); rodents (Aragona and Wang, 2004)). In the Japanese quail, the absence of, or reduced, sexual behavior of adult males exposed to EDCs *in ovo* is also

reflected in disrupted AVT-ir in the in bed nucleus of the stria terminalis, medial preoptic nucleus, and lateral septum (Panzica et al., 2005b, 2007).

Although amphibians are becoming symbolic of EDC effects in both field and laboratory studies, to the best of our knowledge there have been virtually no published studies at this time demonstrating that such compromised individuals show differences in behavior. However, the many demonstrations that EDCs alter gonadal differentiation and influence genes coding for steroidogenic factor-1 (SF-1) and other steroidogenic enzymes make this very likely. Strong evidence for such effects might be found in the *Xenopus*, in which both atrazine and PCB exposure inhibited laryngeal (both cartilage and muscle) development (Hayes et al., 2002; Qin et al., 2007), a sexually dimorphic structure important in the male calling behavior (Kelley and Brenowitz, 2002). Treating female red-spotted newts (*Notophthalmus viridescens*) with the insecticide endosulfan disrupted mate choice and lowered mating success (Park et al., 2001; Park and Propper, 2002). Recently, Helbing et al. (2007) demonstrated that perchlorate exposure to developing tadpoles modified gene expression profiles (using both cDNA array analysis and qPCR), with the greatest effect on the levels of mRNAs encoding proteins important in neural development and function.

56.4.3 Fish

Despite the considerable evidence for disruption and even complete failure of reproductive functions caused by EDCs (Arukwe, 2001; Bayley et al., 2003; Jobling et al., 2002; Kidd et al., 2007; Koger et al., 2000; Toft et al., 2003), the literature on effects of exposures on adult sexual behaviors is relatively sparse. There are many reports showing effects of exogenous hormones on sexual behaviors in male and female fish of many species (reviewed in Milnes et al. (2006)), suggesting similar consequences of xenobiotics. One challenge in fish is studying behaviors of populations in the wild. The extent (dose, duration, nature, mixture, etc.) of contamination is almost impossible to assess in these studies and results have sometimes been inconclusive. For example, courtship behavior in males was compared between mosquitofish in Lake Apopka, FL, which was polluted with DDT and metabolites and other EDCs such as dicofol, toxaphene, *trans*-nonachlor, dieldrin, and aldrin (Toft et al., 2003) and those in a reference lake, Orange Lake. Courtship behavior in the males was assayed by pairing with an uncontaminated adult female, and in particular, two behaviors, referred to as following behavior and close-following behavior in reference to the female's genital opening, were studied. Although certain sexual characteristics of the fish were depressed in the Lake Apopka fish, there were no statistical differences in the following and close-following behaviors. A correlational study comparing sexual behaviors of mosquitofish (*Gambusia affinis holbrooki*) living upstream versus downstream to a paper mill showed aberrations in the behaviors of both the males and females (Howell et al., 1980). Other work on fish has assessed reproductive behaviors in a very generalized way, that is, whether animals mate or not, and the time it takes them to initiate mating. These reports show detrimental effects of EDC exposures on these behaviors (reviewed in Segner et al. (2003)), such as in the Japanese medaka (*Oryzias latipes*) in which developmental exposure to ethinyl estradiol completely obliterated mating behaviors in adult females, and virtually obliterated it in the males (Balch et al., 2004). A laboratory study was carried out in male guppies, testing effects of vinclozolin, a fungicide that acts at least in part as an anti-androgen, on secondary sex characteristics and on male courtship behavior (Bayley et al., 2003). Courtship was assayed through quantification of specific, stereotyped swimming patterns by the male toward the female. Vinclozolin-treated males had smaller first clutch sizes, reduced sperm count, and a significant decline in the number of sexual displays exhibited toward a nonreceptive adult female (Bayley et al., 2003). Interestingly, this effect was limited to the males, as exposure of the females to vinclozolin did not alter clutch size. Similarly, in male guppies, anti-androgenic pesticides decreased sigmoid displays toward females, with lower doses having more potent effects (Baatrup and Junge, 2001). In this same species, 4-*tert*-octylphenol, a xenoestrogen, decreased male sexual displays toward females (Bayley et al., 1999). Similar effects of octylphenol were found in Japanese medaka (Gray et al., 1999). Considering the extent of exposure of fish to EDCs through their lifelong contact with potentially contaminated waters, their value as a model for neuroendocrine and behavioral effects has not been sufficiently exploited.

56.5 Transgenerational Effects of EDCs

Clearly, there are effects of EDC exposures on brain and behavior. What are missing are causal links and

underlying mechanisms. Changes in gene and/or protein expression in sexually dimorphic regions cannot necessarily explain behavioral differences. The mechanisms are beginning to be investigated, and three examples are provided for the model of BPA on DNA methylation. Monje et al. (2007) showed that early postnatal (P1–P7) treatment of rat pups with BPA caused changes in expression of the ERα gene in the POA, and immunohistochemical expression of the ERα protein in the AVPV, measured either at 8 or 21 days of age. Further, the methylation status of the ERα promoter was perturbed by BPA (Monje et al., 2007). This is a nice link between promoter utilization and gene and protein expression. It remains to be determined what the functional consequence on behavior is, but this is an important first step. Another potential mechanism involves imprinting effects of EDCs on promoters of estrogen- or hormone-responsive genes (as opposed to the hormone receptors themselves), through actions on DNA-response elements, such as the estrogen-response element (ERE). Again, BPA illustrates this point, using a model of fetal exposure (days 9–16) in mice and consequent effects on uterine phenotype in the F1 adults, specifically, expression of the developmental-patterning gene, Hoxa10 (Smith and Taylor, 2007). Prenatal BPA-exposed uteri had substantially (up to tenfold) increased Hoxa10 gene expression compared to control mice. Using a cell-line model, the authors went on to provide evidence that these effects are mediated through actions of BPA on the ERE of the Hoxa10 promoter (Smith and Taylor, 2007). Finally, BPA has also been used to demonstrate not only hypomethylating effects of fetal exposure, but also the rescue of this effect by maternal nutrition. Dolinoy et al. (2007) used an agouti mouse model (A^{vy} genotype), the gene of which is differentially methylated to result in a range of coat colors as an external phenotype, as well as differing in other phenotypic manifestations (reviewed in Jirtle and Skinner (2007)). BPA exposure of a pregnant mouse alters the expression of coat color concomitant with a shift in the A^{vy} site-specific methylation (BPA-caused hypomethylation), and further, this was compensated by supplementing the maternal diet with a methyl donor, such as genistein (Dolinoy et al., 2007).

56.5.1 Evolution and Epigenetics

Evolution selects for outcomes, not mechanisms. The individual with its adapted morphological, physiological, and behavioral traits is both a result and a cause of evolutionary change. Only phenotypic change is subject to selection and novelty can arise via several processes. Evolutionary change can result from mutation, although outside of the laboratory, most mutations are maladaptive or lethal when in the coding or regulatory regions of the gene. However, such changes, if successful, are incorporated and carried forward in the genome. Most traits, however, are not due to variability in a single gene but result from the interaction of many genes and their products and are not transmitted according to Mendelian inheritance. This is common in the evolution of simple as well as complex traits.

This ability of the genotype to produce different phenotypes in response to different environments has been termed plasticity. However, plasticity genes are unlikely. Rather, selection of an individual's capacity to respond to environmental change or insult with heritable phenotypic variation is highly likely. This ability of particular features of systems to facilitate change has been termed evolvability (Kirschner and Gerhart, 1998; West-Eberhard, 1998, 2003) or adaptability (Bateson, 2005). For example, it is likely that there are suites of genes that underlie the fundamental plasticity of an organism, particularly during development or life-history transitions. This characteristic of trait variability, whether molecular, cellular, physiological, morphological, or behavioral, represents the leading edge of evolution.

Epigenomic regulation of networks of genes and their products more accurately reflects the evolutionary process, particularly as it relates to the heritability of complex traits (Lewontin, 2000; West-Eberhard, 2003). Here the construction of new traits is via more of a stepwise process; the constituent elements of a trait are accrued as genes and their products interact both positively and negatively in a temporal, spatial, and conditional (tissue or external environment) context (Kirschner and Gerhart, 1998; Nijhout, 2003). Adaptive responses emerge that, in turn, set the stage for future variation. Thus, evolution is a tandem process first involving development, with its built-in flexible responsiveness to both gene products and environment, followed by selection, which dictates which variants are spread and maintained (West-Eberhard, 1998). In this sense it might be said that the 'genome learns from its experience' (Jaenisch and Bird, 2003).

56.5.2 EDCs and Epigenetics

How likely is it that EDCs and other environmental signals could actually contribute to the process of

evolution? In this present context, it is the persistence of EDC challenge that yields the altered function over generations. A striking but underappreciated aspect of environmental contamination is that some individuals are less affected than others (Orlando and Guillette, 2001), a common observation in centers of populations where there is greater variability. Even if fitness is compromised in all individuals, those that are less affected will have greater reproductive success than those who are rendered sterile or develop diseases. On the other hand, they may be epigenetically compromised such that their offspring are themselves affected. Whether these molecular epigenetic effects and their transgenerational consequences are eventually incorporated in the genome so that selection might act is a question of great interest.

A plausible scenario for EDC influencing changes in methylation patterns leading to epigenetic inheritance has been proposed (Guerrero-Bosagna et al., 2005; Guerrero-Bosagna and Valladares, 2007). But evidence suggesting that EDCs might reprogram methylation patterns that are, in turn, incorporated into the germ line and hence transmitted to future generations is still sparse. The best evidence is probably provided in work from Skinner's lab using the fungicide vinclozolin. This group showed that fetal exposure to high concentrations of vinclozolin caused latent reproductive and other dysfunctions later in life, and further, that these effects were transmitted via the paternal germline for up to four generations (Anway et al., 2005, 2006; Anway and Skinner, 2006). Although there may be other mechanisms at work, at least one of these involved changes in DNA-methylation patterns in germ cells, such as within the lysophospholipase gene and the cytokine-inducible SH2 gene, both of which had altered methylation patterns across generations (Anway et al., 2005). Recent work from this same group has since identified additional differentially imprinted genes altered in this manner in the sperm of F2 and F3 male vinclozolin descendents (Chang et al., 2006).

56.5.3 EDCs, Epigenetics, and Behavior

The role of behavior in evolutionary change is perhaps the most accessible avenue to pursue as mating relies on the successful interaction of individuals. In nature, individuals (particularly females) actively discriminate and choose among potential mates, and this choice enables a reciprocal synchronicity in behavior and physiology among males and females for successful mating (Crews, 2002). Extrapolating from altered behaviors due to EDC exposure to evolutionary significance is straightforward. How might EDCs influence an individual's ability to discriminate and choose between possible mates? This fundamental study, to the best of our knowledge, has never been done. There are two possibilities to consider: (1) an unaffected individual discriminating and preferentially mating with an affected individual and (2) an affected individual discriminating and preferentially mating with an affected individual. In terms of evolutionary significance, the first has the least impact and the second the greatest impact. That is, in the former case the individual male or female loses a breeding opportunity (albeit a more serious consequence for a female than for a male) while in the latter instance both individuals are removed from the gene pool.

We have begun to address this question in laboratory rats, using F3 descendents of vinclozolin-treated pregnant dams. Animals were produced at Washington State University by M. Anway and M.K. Skinner, and shipped to our laboratories at The University of Texas at Austin. Our experiment sought to determine whether there were any phenotypic differences between F3 vinclozolin- and vehicle-lineage males, prior to their development of disease, which could be discerned by females. A 10-min mate-preference test was conducted in which a female was allowed to choose between a vehicle-lineage and a vinclozolin-lineage male (males were separated from the female by a wire mesh). Results showed that females spent significantly more time in proximity to the F3 vehicle-lineage males compared to the F3 vinclozolin-lineage males (Crews et al., 2007). These data indicate that F3 descendants of vinclozolin-treated pregnant great-grandmothers are phenotypically different from control F3 descendants in a way that can be discerned by female rats, prior to the males' development of any overt disease. Future work is necessary to determine the nature of the phenotype that enables females to make this distinction. Notably, as these male animals aged in our laboratories, they developed the same reproductive dysfunctions (small testes, tumors, etc.) as originally reported by Skinner's group (Anway et al., 2005).

56.5.4 Two-Generational Studies on Effects of EDCs

In order to apply the fetal basis of adult disease in a truly transgenerational manner, it is necessary to take experiments to the F3 generation. As reviewed

recently (Skinner, 2008), when the pregnant F0 dam is exposed to an EDC, her F1 pups were exposed directly *in utero*. At this developmental stage, the primordial germ cells of the fetuses (the future F2 generation) are developing and can have direct exposure to the EDC. Although they are unlikely to have an appreciable body burden as adults, nevertheless, the F2 generation may have been influenced by direct exposure to the EDC. Thus, only their F3 offspring are the first to be free of direct contact to the original insult.

This multigenerational approach has rarely been used in the laboratory setting, and although there are inevitable exposures of multiple generations in the wild, the lack of a controlled setting has made it virtually impossible to draw causal relationships. In the arena of reproductive neuroendocrinology, there is a wealth of evidence for effects of fetal and early postnatal exposures to both endogenous and exogenous hormones on the adult phenotype (reviewed in Gore (2008)). There is beginning to be interest in this field from the perspective of endocrine disruption, although most reports are limited to two-generational studies (up to the F2) and/or do not extend to neuroendocrine or behavioral endpoints. We refer readers to Fernie et al. (2001), Keneko et al. (1974), McCoy et al. (1999) and provide a brief discussion of a few for illustrative purposes. When Japanese and bobwhite female quail were exposed to dietary methoxycholor or vinclozolin, similar deficits were observed in the sexual behavior of the male offspring of the two generations when they reached adulthood (Ottinger et al., 2005b). In fish, lifelong exposures of zebra fish (*Danio rerio*) to ethinyl estradiol reduced reproductive success although affected males continued to try to mate (Nash et al., 2004). The latter result is important because it hearkens back to the concept of competition between affected and unaffected individuals and its detrimental effect on the population. DES has also been an informative model, as its transgenerational effects on the reproductive tract have been studied in models from mice to humans (Newbold, 2004; Newbold et al., 2006; Titus-Ernstoff et al., 2006; Walker and Haven, 1997). However, effects on the brain and behavior are still lacking in a transgenerational approach.

One of us (Andrea C. Gore) investigated effects of low-level fetal PCB (Aroclor 1221) exposure to F0 rat dams during the critical period of brain sexual differentiation (gestational days 16 and 18) on the reproductive development and behavior in the F1 generation of female offspring in adulthood (Steinberg et al., 2007, 2008). We also evaluated the female F2 generation of offspring in early adulthood for several reproductive physiological parameters. These F2 rats exhibited aberrant hormone profiles across the estrous cycle (Steinberg et al., 2008). In particular, LH and progesterone levels were significantly lower on proestrus in the F2-PCB females compared to the F2-vehicle group. These results show that exposure of the F2 rats' pregnant grandmother to PCBs had effects on the F2 descendants' reproductive physiology in adulthood (Steinberg et al., 2008). We are currently developing models to assess effects of F0 exposure to PCBs up through the F3 generation.

56.6 Summary and Recommendations for the Future

Research into the effects of EDCs on wildlife and humans continues to evolve. It might be useful to consider how this evolution reflects certain parallels in any newly emerging science, namely first the verification and validation of a phenomenon in a variety of organisms. This stage is fraught with controversy and denial from the both the parent field(s) and other interests as the inherent varieties of the phenomenon become evident (e.g., egg-shell thinning, gross morphological anomalies, hermaphroditism/intersex, and sex-atypical metabolism). Once deniability becomes specious, the guiding principles are developed, again with acrimony and contest (e.g., low dose, mixtures, synergy, and thresholds). There is the tendency, during this period, for research to become mainstream with most content to solidify major principles in its variety of forms, usually with one venue being the mechanistic, and ultimately the genetic, basis and the other capturing the complexity of the phenomenon. This arena might also be widened as proven techniques and test systems from other fields are implemented to deepen our understanding of the mechanisms that underlie the variety of EDC effects. It is curious that it always seems that behavioral modifications are the last to be discovered. Perhaps this is simply an ontogenetic process as disciplines mature, but for those of us who are principally interested in behavior, knowing as we do that behavior is at the leading edge of evolutionary change, it is perplexing that the ultimate consequences of global ecological change are not often studied from the behavioral standpoint.

There is no question that certain chemicals disrupt endocrine systems essential to normal

development. Although still in its early stages, the discipline has learned quite a bit about the what, why, where, and how such anomalies result following exposure. Our focus now should be on those individuals that are not morphologically compromised but do not breed due to EDC exposure. In these instances, we will be dealing with how such chemical affect the brain mechanisms that process, produce, or integrate social signals necessary for successful reproduction that warrant research attention. For example, it is very likely that such individuals are responsible for the widely reported precipitous declines in amphibian populations worldwide, and this may have similar effects in fish (cf. Nash et al., 2004). The logic behind this revolutionary statement is quite simple. While it is true that disease caused by exogenous agents can eliminate population within a single generation, this is not what happens typically in EDC-contaminated areas. Rather the incidence of disease and malformed amphibians increases and then stabilizes, yet the population is still in decline. The reason would appear obvious, namely that there are otherwise normal appearing individuals that simply are not breeding, leading to the precipitous declines observed.

Finally, it should be said that we will never know the true extent of the effects of EDCs simply by tallying grossly and obviously malformed individuals in a population any more than we will understand EDC effects on wildlife and human populations by detailing exactly the mechanism of their action. In the former instance, the poster-child approach, the emphasis is misplaced by focusing on such individuals, even if the broader interpretation is taken by noting that such individuals are a negative burden on the population because they consume what may be limited resources. Similarly, the focus on how a particular chemical reacts with chaperones to interfere with normal ligand-binding at the DNA will not lead to new therapeutic advances. Take, for example, atrazine, a widely used herbicide. We now know that atrazine causes morphological abnormalities in amphibians in both nature and the laboratory and does so by affecting aromatase by acting on the SF-1 gene, thereby changing the relative production of androgen and estrogen during the time period when the reproductive system is forming (Fan et al., 2007a,b; Hayes et al., 2006a,b). The solution may seem quite simple. Stop making atrazine. It is important to acknowledge that the problem for which atrazine was developed is serious and should not be minimized, but new green chemistry might result in a chemical that equals, or betters, the performance of atrazine in the field without the endocrine-disrupting consequences (Thornton, 2000, 2007). Finally, the recently discovered phenomenon of transgenerational imprints by EDCs on DNA-regulatory mechanisms carried forward for four or perhaps more generations is a particularly sobering one. What this means is that simply cleaning up an environment and no longer using particular chemicals on a global level may have no ultimate effect. That is, the damage is already done and the most we can do for remediation is to stop polluting, and thus maintain a *status quo* by employing green-manufacturing methods (Thornton, 2000, 2007).

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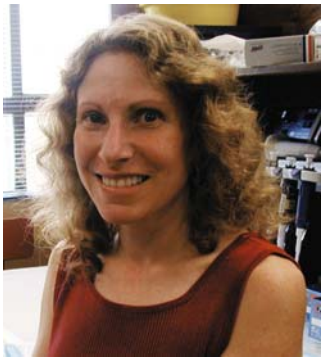
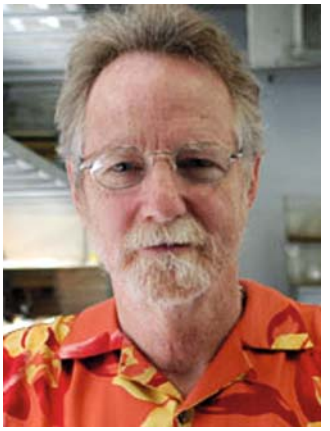
Biographical Sketch



Andrea C. Gore, PhD, is Gustavus and Louise Pfeiffer Professor of pharmocology and toxicology at the University of Texas. Dr. Gore's laboratory studies the mechanisms by which the brain controls reproductive neuroendocrine function in female and male mammals. Ongoing research projects focus on the regulation of major reproductive life transitions, namely, the developmental processes leading to the onset of puberty, and the transition from reproductive competence to failure during senescence. Dr. Gore studies the cellular and molecular processes in the hypothalamus that underlie these physiological changes, both in animal models of normal reproductive function, as well as in experiments in which these functions are perturbed by environmental endocrine-disrupting chemicals. Among her publications is the book, *GnRH: The Master Molecule of Reproduction* (Kluwer, ISBN 0-7923-7681-1), and the edited book, *Endocrine-Disrupting Chemicals: From Basic Research to Clinical Practice* (Humana, ISBN 1-58829-830-2). Outside of the laboratory, Dr. Gore is a 'serious amateur' violinist and pianist, and enjoys spending time with her rescue dogs and husband.



David Crews is Ashbel Smith Professor of zoology and psychology at the University of Texas at Austin. He received his PhD in psychobiology in 1973 under the supervision of the late Daniel S. Lehrman and Jay S. Rosenblatt from the Institute of Animal Behavior of Rutgers University. After a postdoctoral fellowship in biochemical endocrinology at the University of California at Berkeley he moved to Harvard University in 1975 first as an assistant, then associate professor of biology and psychology. He moved to the University of Texas at Austin in 1982. His research primarily concerns sex determination and sexual differentiation; specifically his research has included studies such as (1) the mechanisms and outcomes of sex determination in vertebrates lacking sex chromosomes; (2) the evolution of hormone–brain–behavior mechanisms; (3) understanding how the environment and behavior influence the structure and function of the brain; and (4) the role of epigenetics in behavioral neuroendocrinology. Dr. Crews has worked with a wide variety of organisms, from fruit flies to

mammals, but focuses on reptiles. He has received various honors, including a Sloan Fellowship in Neuroscience, the Distinguished Scientific Award from the American Psychological Association, and a MERIT Award and a 20-year Research Scientist Award from the NIMH. He has been elected fellow of the American Association for the Advancement of Science, American Psychological Society, and the American Academy of Arts and Sciences.