

Review

Effects of xenoestrogens on the differentiation of behaviorally-relevant neural circuits

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Available online 6 August 2007

Abstract

It has become increasingly clear that environmental chemicals have the capability of impacting endocrine function. Moreover, these endocrine disrupting chemicals (EDCs) have long term consequences on adult reproductive function, especially if exposure occurs during embryonic development thereby affecting sexual differentiation. Of the EDCs, most of the research has been conducted on the effects of estrogen active compounds. Although androgen active compounds are also present in the environment, much less information is available about their action. However, in the case of xenoestrogens, there is mounting evidence for long-term consequences of early exposure at a range of doses.

In this review, we present data relative to two widely used animal models: the mouse and the Japanese quail. These two species long have been used to understand neural, neuroendocrine, and behavioral components of reproduction and are therefore optimal models to understand how these components are altered by precocious exposure to EDCs. In particular we discuss effects of bisphenol A and methoxychlor on the dopaminergic and noradrenergic systems in rodents and the impact of these alterations. In addition, the effects of embryonic exposure to diethylstilbestrol, genistein or ethylene,1,1-dichloro-2,2-bis(*p*-chlorophenyl) is reviewed relative to behavioral impairment and associated alterations in the sexually dimorphic parvocellular vasotocin system in quail. We point out how sexually dimorphic behaviors are particularly useful to verify adverse developmental consequences produced by chemicals with endocrine disrupting properties, by examining either reproductive or non-reproductive behaviors.

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Keywords: Bisphenol A; DES; DDE; Genistein; Phytoestrogens; Mouse; Japanese quail; Endocrine disrupting chemicals; Brain sexual differentiation; Vasotocin

1. Introduction

All living organisms depend upon a large and intricate array of chemical signaling systems to guide biological development and regulate cell and organ activity. The observation that early exposure to industrial pollutants could raise adverse effects on endocrine structures development, such as the reproductive organs, induced public concern about

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the existence of endocrine active compounds that may affect humans, farm animals and wildlife [67,146]. These environmental chemicals have been termed endocrine disrupting chemicals (EDCs)¹ and have been defined by the European Commission (1996) and by the United States Environmental Protection Agency (US-EPA) panel as: “Exogenous substances that change endocrine function and cause adverse effects at the level of the organism, its progeny, and of populations of organisms” [81].

There is evidence that EDCs may interact with several endocrine systems, including the thyroid, reproductive, and adrenal axes as well as other endocrine processes [12,42]. Because hormones play important regulatory roles in adults, EDCs can have transient effects that may be reversible [66,67]. Additionally, hormonal signals control normal development of many organs, including the brain, therefore, EDCs may alter developmental processes by interfering with these systems [180]. For these reasons, effects of EDCs on developing organisms are of greatest concern, since the disruptive effects of developmental exposure interfere in the organization of neural systems frequently exerting permanent and irreversible impacts (see for reviews [66,168,236]).

Among the hormonal signals with high impact on brain development, gonadal hormones, such as 17 β -estradiol (E₂) and androgens, play key roles in the development of primary and secondary sex characteristics in higher vertebrates, including several steroid dependent behaviors. Many of the EDCs are estrogens or estrogen-like molecules that have been classified as environmental estrogens or xenoestrogens (XEs) [151,211]. Due to their estrogenic effects, they could, even in very low concentrations, deeply influence the development and the function of estrogen-dependent neural circuits and related behaviors [165].

In recent years, the knowledge of how estrogen affects mammalian brain function and development has substantially broadened. After the demonstration that both nuclear estrogen receptors [α and β (ER α and ER β)] are expressed in many brain areas during ontogeny (for a summary of previous data see [191]), it was soon realized that estrogens may modulate neuronal differentiation, notably by influencing cell migration, survival and death, and synaptic plasticity of neurons (for reviews, see [51,68,174,209]). These effects were initially seen in the classical target area

for E₂, the hypothalamus and, later, also in other brain regions that revealed neurotrophic effects of estrogen [143,218].

Appropriate levels of steroid hormones are essential for normal development and sexual differentiation of the reproductive system, central nervous system, and reproductive behavior [174,207]. Therefore, disturbing this developmental milieu, via exogenous estrogen treatment or gonadectomy, during critical periods of the pre- and/or postnatal development, may induce irreversible changes in the organization of the central nervous system (for a reviews, see [19,74,143,144]) and determine behavioral alterations in many species [126]. Similarly, if an EDC is weakly estrogenic or androgenic, it still has the capability to impact developing systems, especially the central nervous system (CNS), resulting in functional alterations. Furthermore, because behavioral responses represent the culmination of several integrated systems, even small changes of neural or neuroendocrine components are likely to disrupt or modify behavior. Importantly, disturbances in normal behavior may influence the individual fitness and, therefore, assume a real biological significance in both animal and human ecosystems [165].

Our present understanding of the activity and metabolism of XEs has been based mainly on *in vitro* models [46], which do not provide detailed study of XEs low dose effects. In addition, the traditional testing paradigms consider measures that are not tailored for impact on endocrine systems, current studies of EDCs must consider end-points that are sensitive and reliable for assessing the effects of these compounds on the whole living organism as well as on the integrated systems (behaviors) that could be affected.

For example, the *in vivo* estrogenic action of a widespread environmental pollutant such as DDT has been demonstrated primarily at very high doses [58]. However, more recent studies suggest that subtle behavioral modifications may occur also in animals treated prenatally with low, environmentally relevant doses of DDT [170]. An interesting example is bisphenol A (BPA), which is widely used in the food industry and in dentistry. BPA is known to have a weak estrogenic action due to its low affinity for the ER α [214]. In spite of this weak binding activity, very low doses of BPA administered during the perinatal period have consequences on male mice, specifically increased prostate weight and decreased sperm production [155,227]. In addition, mice fetuses exposed to BPA and then raised by untreated foster mothers still showed significant increase of body weight at weaning, earlier vaginal opening signaling accelerated puberty onset, and altered maternal behavior as adult [107], as well as alterations of maternal behavior when adult [172].

EDCs acting at low levels can exert subtle effects by interfering with gene expression and other cellular activities which can cause transient activational responses, or permanent impairment [231,236,237]. Thus, the impact of EDCs will vary depending upon a variety of factors,

¹ *Abbreviations used:* AFP, α -fetoprotein; AMPH, amphetamine; ARO, aromatase; ARO-ir, aromatase immunoreactive; AVPV, anteroventral periventricular nucleus of the preoptic area; BPA, bisphenol A; BST, nucleus of the stria terminalis; BW, body weight; CNS, central nervous system; CPP, conditioned place preference; DA, dopamine; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl); DES, diethylstilbestrol; E₂, 17 β -estradiol; EB, estradiol benzoate; EDCs, endocrine disrupting chemicals; ER α -ER β , estrogen receptors [α and β]; GEN, genistein; GNX, gonadectomized; HPG axis, hypothalamic-pituitary-gonadal axis; LC, locus coeruleus; M, mount; MA, mount attempt; MXC, methoxychlor; NG, neck grab; POM, medial preoptic nucleus; SDN-POA, sexually dimorphic nucleus of the preoptic area; SL, lateral septum; T, testosterone; TH, tyrosine hydroxylase; VT, vasotocin; XE, xenoestrogens.

including when in the life-cycle of an organism exposure occurs, as well as the duration and amount of the exposure (Fig. 1). Until recently, the critical importance of life stage has not been fully appreciated. During the life-cycle of an organism, developmental stages are typically far more vulnerable to signal disruption than adult stages and the consequences of fetal exposure may be drastically different from those of adult exposure. This is thought to occur for several reasons, including the absence of fully developed protective enzyme systems and higher metabolic rates. Most importantly, however, the events underway in development involve a series of organizational alternatives that are (largely) irreversible once the “choice” in development is determined [176,180]. In sharp contrast, in adults, the processes can be reversed very often by removing the perturbing factor, thus returning gene expression levels and organ functioning to (almost) normal. These transient effects are termed “activational” effects [18,143].

One clear implication of this focus on low level exposure during fetal and neonatal development is that levels of exposure that have been considered as “background” and thus “safe” can have deleterious effects. Many laboratory studies on animal models now support the conclusion of high sensitivity of the embryo and neonate [180,184,212], as some epidemiological data from human studies do [121].

We have here outlined evidence from a diversity of sources indicating that a variety of EDCs can interfere with the neuroendocrine system, affecting sexual and brain development, and resulting in reduced fertility, decreased immune competence, altered brain function and behavior in wildlife, laboratory animals and humans. Four summary points emerge:

- EDCs at low levels can interfere with gene expression.
- Wildlife, laboratory animal and human effects are often concordant with potential differences in sensitivity to specific compounds.

- The available data on EDCs demonstrate that traditional toxicological assumptions of appropriate measurement end points are not sufficient for regulatory science and regulations.
- Due to the multiplicity of involved factors, traditional epidemiology has great difficulty establishing causation of specific EDCs’ effects in humans. All else being equal, the ability of an epidemiological study to identify the cause of an adverse outcome decreases as the prevalence of the outcome and the number of causal factors increases; thus multifactorial diseases of high incidence are only poorly handled by epidemiology.

There are a number of potential mechanisms for EDCs’ actions. Many EDCs have direct action on a specific steroid hormone receptor or on multiple receptors [72,92,117,122,127,188]. In addition, some compounds act on the enzyme systems (e.g., genistein [190]) and may impact hormone transport (for a review, see [55]). Finally, as for endogenous steroid hormones, they can act through signaling pathways including the activation of additional transcription factors as well as the action through estrogen receptors located outside the nucleus: in the plasma membrane, mitochondria and probably the cytosol (for a review, see [200]).

For the purpose of this review, we will concentrate on the effects of early exposure to selected EDCs on neural systems that may alter sexual differentiation of brain and behavior. There are a number of potential receptors for EDCs’ effects. In addition to humans, domestic animals and wildlife are likely to be impacted by EDCs’ exposure. We will review the data from two groups of the animal models in which EDCs’ effects have been studied, including the rodent and galliform birds.

2. Developmental exposure to EDCs alters sexual differentiation of brain and behavior in mice

Many studies on EDCs in rodent models reported effects on both reproductive system and performances

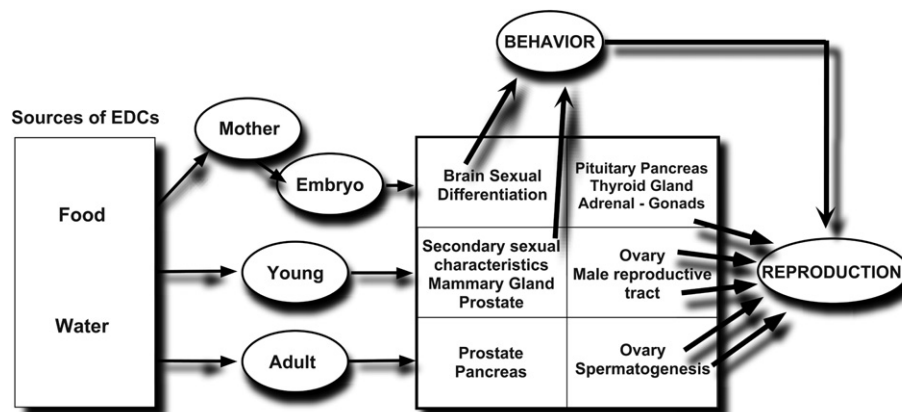


Fig. 1. Diagrammatic summary of effects of environmental disrupting chemicals (EDCs) on reproduction through the exposure during embryonic, postnatal, and adult life. During the postnatal and adult life, EDCs act primarily at the level of peripheral organs (gonads, reproductive tracts), interfering with circulating hormonal levels and therefore with the reproduction. During embryonic or early postnatal life EDCs interfere with the differentiation of steroid-sensitive neural circuits and in this way may heavily impact adult behavioral activities that, in turn, control reproduction.

[70,85,107,227,235]. However, more subtle and potentially insidious effects of chemicals can emerge due to interference with brain developmental processes, resulting in behavioral alterations [168–172]. Mothers can pass EDCs to their offspring transplacentally, and after birth by breastfeeding newborns. Through neurotrophic and differentiation promoting effects, estrogens are crucial for the sexual differentiation of CNS structures and functions.

According to the classic “hormonal” theory, during a critical period of brain development, which in mice and rats extends from late pregnancy until the first 1–2 weeks after birth, estrogens organize male-type specific circuitries permanently and irreversibly [18,143]; E_2 is actively synthesized in the brain from testosterone (T) by neurons expressing the enzyme aromatase [145]. In addition, the classical theory hypothesizes that, in mammals, the brain of the female fetus is protected from defeminization (decrease or disappearance of displaying female-typical behaviors, such as lordosis) or masculinization (displaying male-typical behaviors such as mounting a female) by α -fetoprotein (AFP, abundant during fetal and early neonatal life [45]) which binds circulating maternal E_2 . At the same time AFP is also protecting the brain of male fetus from the excess of maternal estrogens. This hypothesis has been recently demonstrated by elegant experiments in AFP knockout mice [28].

Contrary to estrogens, nonsteroidal estrogens, such as BPA, exhibit a lower affinity for plasma estrogen binding proteins [149], therefore they may avoid the protective action of AFP. Although recent evidence has pointed out that sex differences in non-reproduction related neural structure (i.e., the hippocampus) may be related also to different mechanisms, such as *de novo* synthesis of neural E_2 [143] or genetic sex [19], this does not seem to apply to sexual differentiation of hypothalamus and hypothalamus-dependent behaviors. XEs can thus interfere with the male- and female-typical development of brain areas that control the occurrence and pattern of a wide range of behaviors required for reproduction such as direct sexual behaviors, social and non-social behaviors in adult life.

2.1. Sexually differentiated neural circuits in rodents

Many brain structures show volumetric or neurochemical features that are sexually differentiated in rodents. In particular, hormone-dependent brain sexual dimorphism was at first demonstrated in the number of dendritic spines in the dorsal POA of the female compared with the male [197], and in the volume of a particular region of the rat preoptic area named sexually dimorphic nucleus (SDN-POA, [91]) that was four to six times larger in male than female. In addition, the whole accessory olfactory pathway is sexually dimorphic [98]. Other regions were demonstrated to have a reverse sex dimorphism (larger in female than in male), in particular the locus coeruleus (LC, [97]) and the anterior ventral periventricular nucleus (AVPV, [210]). It is now known that there is considerable variety

in the nature of structural sexual dimorphisms in the brain including not only the size of specific brain regions, but also the extent of dendritic arborization, the density and pattern of synaptic connections (e.g., spine and somatic synapses), size, number and phenotype of neurones in a particular region and astrocyte morphology (for an up to date review of these data see [240]).

In rodents, these differences are due to the effect of gonadal hormones' neonatal environment on programmed cell death, neurite growth, axon guidance and synaptogenesis (for reviews, see [87,209]). Functionally, the structural sexual dimorphisms in individual brain regions give rise to sex differences in neuronal circuitry and thus differences in circuitry-dependent functions, such as various behaviors related or not to reproduction. Among these circuitries the dopaminergic system has been particularly considered, due to its involvement in the control of rodent sexual behavior [108–110], as well as for its involvement in many other behaviors such as locomotion [44,61].

2.2. Effects of exposure to low dose XEs on reproduction

Prenatal exposure to doses of the estrogenic endocrine disruptors, BPA and methoxychlor (MXC), within an environmentally relevant range for human exposure, have produced abnormalities in daily sperm production per gram of testis, and epididymis, prostate, and seminal vesicles in male mice [155,227,235]. Exposure of rodents to low doses of BPA during fetal development has been shown to induce early vaginal opening [106], advance the onset of puberty [107], disrupt estrous cyclicity [137,202], and decrease serum levels of LH after ovariectomy [202] in females. Perinatal exposure to BPA alters postnatal mammary gland development [138,152] and increases the sensitivity to estradiol at puberty [152]. These changes are a consequence of altered development during the period of BPA exposure [220]. Exposure during prenatal and postnatal development to BPA (40 μ g/kg body weight (BW)) altered sexual activity in rats. BPA-exposed females showed an increase in receptive behaviors and in sexual motivation when in proestrus, while BPA-exposed males showed a general impairment of sexual behavior when compared to controls [85].

The mouse (*Mus domesticus*) is a good experimental model to investigate the effects of developmental exposure to EDCs on certain types of behavioral systems that are differently expressed in male and female, such as the occurrence and pattern of social (e.g., aggression, parental behavior) and non-social behaviors (e.g., exploration, emotionality, activity patterns, learning, and memory) in adult life. In mice and rats, as in other mammals, non reproductive behaviors have been described to show sex differences in quantity of performance expressed rather than being present in one sex and absent in the other [17,93]. Although some of these sex differences reflect activation effects of E_2 and T in the blood of adult males and females [167,192,243], differential actions of gonadal steroids during the perinatal period play a crucial role in organizing

the sexual dimorphism in behavior and its underlying neural substrates [18,43]. For instance, ER α deficient male mice have been shown to exhibit more female-like behavior (higher locomotion and rearing, more center crossing, and lower defecation) in the open field relative to wild-type males [158], unfortunately no data are published for ERKO female explorative behavior. However, this finding suggests that estrogen action during development is important for defeminization and/or masculinization of exploration and emotional behaviors. A number of studies have revealed higher levels of activity, sometimes associated by lower anxiety, in female relative to male mice, though many factors (such as strain, housing procedures, age, and experience) can affect these behaviors [113,141,167, 201,203].

Results of a number of recent studies in our laboratory and others [201,203] indicate that developmental exposure to low doses of EDCs affect the sexual differentiation of non reproductive behavioral systems in mice, such as explorative, emotional, and cognitive behaviors. We have previously shown that developmental exposure to sub-toxic low doses of estrogenic compounds, relevant with the concentrations wildlife and humans could be exposed to, show different effects than those observed at higher doses; specifically, prenatal exposure to the estrogenic compounds o,p'-DDT, MXC or BPA can affect different behavioral systems in mice [170–172].

We report here our more recent research examining the effects of maternal exposure to two estrogenic EDCs, BPA, and MXC, at doses within the range of human exposure and not patently teratogenic, on emotional, explorative, and sexual behaviors of male and female CD-1 house mice (*Mus domesticus*) [90,129]. MXC is widely used as insecticide on pets, in home gardens, and on crops and livestock. It acts as an agonist of the estrogenic receptor after being metabolized by the liver as shown by *in vitro* and *in vivo* studies (see for review [71]), it has also a weak anti-androgenic activity [94]. MXC was administered orally to pregnant/lactating mice at a dose of 20 $\mu\text{g}/\text{kg}/\text{day}$, which has been shown to affect behavioral development in previous experiments [171,172]. This dose is well below the lowest observed adverse effect level (LOAEL 50 mg/kg/day, according to Environmental Protection Agency, USA) and also below the 5 mg/kg maternal predicted no observed adverse effect level (World Health Organization, 1996). BPA is a widespread estrogenic chemical, with human exposure, due to release by polycarbonate plastics, lining of food cans, and dental sealants [56,159]. BPA has a weak estrogenic activity *in vitro* and *in vivo* [123,130,214], and interacts with ER α in a unique manner, somewhat different from E $_2$ [92]. We fed pregnant/lactating mice 10 $\mu\text{g}/\text{kg}/\text{day}$ BPA, a dose reported as the tolerable daily intake by the European Commission's Scientific Committee on Food [80]. This dosage is far below the LOAEL of 50 mg/kg/day that was used to calculate a reference dose of 50 $\mu\text{g}/\text{kg}/\text{day}$ (IRIS 1988). This xenoestrogen has been measured in maternal and fetal plasma and placental tissue

at birth in humans [111,205]. In a recent study, BPA was found in 95% of urine samples of a 394 Americans [59]. From these data, the mean exposure was estimated to be 40 ng/kg BW per day and the 95th percentile was 230 ng/kg BW per day, assuming that 70% of the daily dose was excreted into the urine. A smaller study estimated a maximum daily intake of BPA to be 230 ng/kg BW [16].

2.3. Maternal treatment procedure

We developed a procedure that allowed oral administration of the chemical to the pregnant female, without disturbing or stressing the animal. This is a critical issue, since handling procedures can be stressful to animals, and stressful events during pregnancy can change the hormonal milieu of the mother and affect neuroendocrine development of the offspring. Before and after time-mating, female mice were trained to spontaneously drink a small volume (50 μl) of corn oil from a modified syringe (without the needle and with a larger hole) introduced through the cage top every day. All females easily learned to drink the oil as soon as the syringe was introduced; this procedure allows accurate administration of chemicals without the stress associated with gavage or injection. Pregnant female mice spontaneously drank daily doses of corn oil with or without the estrogenic plastic derivative, BPA (10 $\mu\text{g}/\text{kg}$) or the insecticide MXC (20 $\mu\text{g}/\text{kg}$) from gestation day 11 to postpartum day 8 (perinatal exposure) or only for gestation day 11–18 (prenatal exposure). Their offspring were examined for exploratory behaviors before and after puberty, for sexual behaviors and conditioned place preference (CPP induced by amphetamine). Three different tests examined different components of explorative and emotional behaviors (see Fig. 2): (1) novelty test before puberty, which measures levels of impulsivity and novelty-seeking; (2) exploration and activity in a free-exploratory open field as adults to measure propensity to explore a novel environment and activity and anxiety levels; (3) exploration in the elevated plus maze, which is the traditional test for measuring anxiety responses in rodents. Sexual behavior was examined by pairing experimental male mice with unexposed estrous female and experimental estrous females with unexposed males. The CPP measures potential changes in the reinforcing effects of amphetamine. This paradigm provides a measure of incentive memory of rewarding drug effects, which do impinge on drug action within mesolimbic dopamine (DA) systems [129].

2.4. Effects of EDCs on exploration and emotional behavior

We assessed explorative and emotional behaviors of the maternally exposed offspring at different ages (pre-puberty and adulthood) and in three different experimental settings. As a general result, we found that while control mice showed sex differences on a number of behavioral

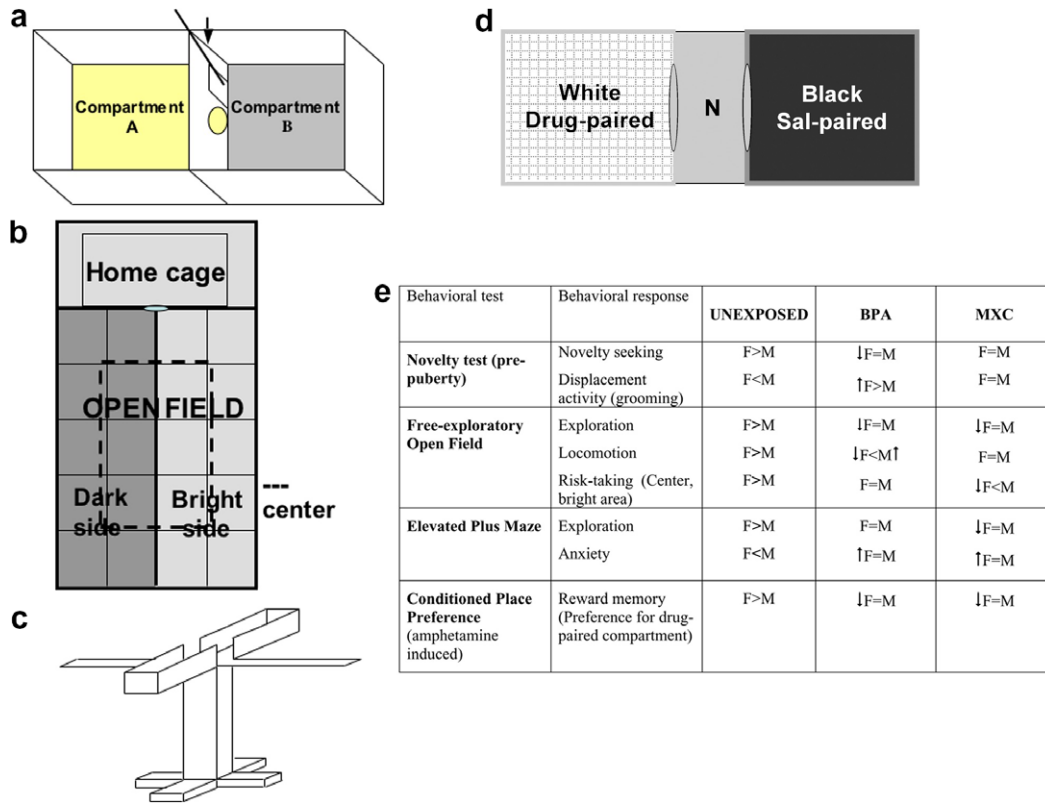


Fig. 2. Different test paradigms (left column) to examine behavioral responses of male and female house mice perinatally exposed to BPA (10 µg/kg BW) or MXC (20 µg/kg). (a) Novelty test before puberty; male or female sibling groups were housed in compartment A of the apparatus. After 24 h, all mice but one were randomly removed from the cage, so that only one mouse was tested and the door dividing the two compartments was opened thus allowing the mouse to enter the novel compartment (b). (b) A free-exploratory open field, consisting of a home-cage and an unfamiliar area (an open-field, OF) of 73 × 110 cm bordered along by a 50 cm high wall and in which a bright and a dark zone were created. A male and a female per litter were individually housed in the home cage section and after 24 h the removable barrier was removed allowing entrance in the OF. A cut-off of 10 min was used for those animals that did not enter the OF and starting from the first entrance in the OF, behavioral observation lasted 5 min. (c) The Elevated Plus Maze consists of two open arms and two closed arms that extended from a common central platform. A mouse was placed in the center and tests lasted 5 min. (d) The conditioned place preference (CPP) paradigm consists of a three-compartments opaque rectangular box; two cues, one visual (white or black walls) and one tactile (wide or narrow mesh floor), were associated with each of the two end-compartments. The white compartment of the apparatus was repeatedly paired with the administration amphetamine (1 or 2 mg/kg i.p.), whereas the black one was paired with an injection of saline. According to a split-litter design, one male and one female from each litter were randomly assigned to be conditioned with saline or one of the two amphetamine doses. On test day, mice were allowed free-access to both compartments, and a preference for the drug-paired side is taken as an index of the drug’s positive reinforcing properties. (e) Summary of the effects of perinatal exposure to BPA or MXC on sex differences in the different test paradigms. F = M, level of behavior do not significantly differ in males and females; F > M, levels of behavior significantly higher in females than males; F < M levels of behavior significantly lower in females than males. ↓ statistically significant decrease of behavior following perinatal exposure; ↑ statistically significant increase of behavior following perinatal exposure.

responses at both ages and in all the test paradigms, mice perinatally exposed to BPA or MXC showed decreased or no sex differences (see Fig. 2). Unexposed female mice, either pre- or post-puberty, when “stimulated” to explore a novel environment, were more reactive and explorative and less anxious as compared to unexposed males. Developmental exposure to the estrogenic pollutants BPA and MXC resulted in behavioral alterations mainly in females, which showed levels of exploratory behavior more similar to the typical behaviors observed in control males than to those recorded in the control females. Altogether these findings may well be seen as indexes of reduced reactivity of exposed females to novel stimuli and are consistent with an estrogenic action of BPA and MXC, and possible “defeminization” or “masculinization” effects of the perinatal exposure to these compounds. However, we also

reported that BPA-exposed males showed female-type behavior on a few measures. The overall result was a reduction or a reversal of sexual differences in EDCs exposed mice, relative to those displayed by controls. Sexual behavioral differences, as well as effects of estrogenic compounds altering such differences, occur before puberty (i.e., before the increase of sexual hormones production is activated by gonads). This can be interpreted as an index of an interference of EDCs in the processes of development and organization of CNS and of receptor systems of both sexes. Fig. 2 summarizes the main results of developmental exposure to BPA and MXC on mice behavioral responses. Interestingly, no effects of prenatal exposure to EDCs were recorded on male or female sexual behaviors or reproductive success (Palanza, unpublished observation).

2.5. Effects of EDCs on brain dopaminergic function

The specificity of the developmental changes affecting a central neurochemical system can be evaluated by assessing the effects of a psychoactive agent targeting to that system upon the behavioral responses modulated by that system. For this reason we assessed in adult animals the possibility that prenatal exposure to BPA or to MXC may influence the development of brain dopaminergic systems by investigating potential changes in the reinforcing effects of amphetamine (AMPH), using a widely validated paradigm, the CPP [129]. As a general result, AMPH treatment produced increased locomotor activity in mice regardless prenatal exposure. With respect to AMPH-induced place conditioning, females as a whole were more responsive than males, thus confirming previous results [128]. When compared to unexposed female mice, BPA- as well as MXC-exposed females failed to show AMPH-induced conditioning. Males showed no changes due to the prenatal treatment. Thus, prenatal exposure to BPA or MXC was apparently responsible in female mice for impairment of brain reward pathways targeted by the drug. Reduced novelty seeking and increased neophobia were also found in female rats perinatally exposed to BPA [9]. This behavioral profile could be related to gender-specific alterations in the function of brain neurochemical systems involved in the response to AMPH. As release of DA within the dorsal and ventral striatum is known to be involved in the behavioral effects that follow amphetamine administration [118,213], it is reasonable to assume that a potential alteration in the behavioral effects of amphetamine administration could be an index of BPA- and/or MXC-induced long-term effects on the dopaminergic function of the brain. Our preliminary neurobehavioral study has shown a decrease in D1-like receptors density in nucleus accumbens and olfactory tubercle of mice prenatally exposed to MXC, at the same doses that have caused alteration in exploration and novelty-induced locomotor activity (Morellini F., Palanza P., Fuchs E., unpublished data).

2.6. Mechanisms of EDCs action

As for possible action mechanisms, it should be noted that both MXC and BPA exhibit weak estrogenic activity in adult rats of both sexes [11,119,217]. In these studies, motor activity and motivation to explore were depressed at adulthood following maternal exposure to BPA [9,86]. Kubo et al. [125] reported that in rats, exposure to BPA abolished sex differences in behavioral patterns in an open field and reversed the normal sex differences in the locus coeruleus. Locus coeruleus (LC) and dopaminergic system are known to be involved in the regulation of animal reactivity to a novel environment and in the CNS, the dopaminergic system having been reported to be affected by early developmental exposure to EDCs. Sexual dimorphism in LC has been detected in rats, with females showing larger volume, higher number of neurons and more dopamine-

β -hydroxylase immunoreactive cells than males [193]. Perinatal exposure to low doses of BPA in rats reversed the sexual difference in volume and number of cells of the locus coeruleus [125]. The mesolimbic and nigrostriatal dopaminergic systems represent major structures of the CNS essential for locomotor activity, novelty induced behavior, reward learning, attention deficit [13,50,61]. In utero and lactational exposure to PCB77 resulted in elevations in concentrations of DA in the frontal cortex, and of DA and its metabolites in the substantia nigra in young (before puberty) and in adult rats [208].

We reported that prenatal exposure to the estrogenic pollutants BPA and MXC resulted in marked alterations in the psychopharmacological profile of female mice suggesting an impairment of brain reward pathways targeted by amphetamine, possibly involving brain monoaminergic (particularly dopaminergic) circuits [129]. On this basis, it may be supposed that prenatal exposure to BPA or MXC might interact with some steps in the development and organization of the monoaminergic system during the perinatal period. A convincing body of evidence indicates that estrogen can modulate basal and amphetamine-stimulated levels of DA release in rodent striatum as measured by in vivo microdialysis [44] and intrauterine exposure to estradiol has been reported to have a significant effect on the organization of monoamine systems within the foetal hypothalamus [116]. Developmental exposure to BPA has been shown to alter D₁ receptor expression and density in male mice [215]. The exposure to BPA during either organogenesis or lactation, but not implantation and parturition, significantly enhanced the morphine-induced hyperlocomotion and rewarding effects. Furthermore, exposure to BPA during either organogenesis or lactation also produced an up-regulation of DA receptor function to activate G-protein in the mouse limbic forebrain [156]. These results indicate that both organogenesis and lactation are more sensitive to the BPA-induced developmental neuronal toxicology than any other periods.

Recent studies demonstrated that mice or rats perinatally exposed to low doses of BPA showed alterations in sexually dimorphic population of tyrosine hydroxylase (TH) neurons in the AVPV [186,201] and in the open field behavior [201], an effect consistent with an estrogenic activity of BPA on the developing brain. This is consistent with our preliminary data on the effect of prenatal exposure to BPA or the synthetic estrogen diethylstilbestrol (DES) on the number of neurons producing TH in the locus coeruleus of pre-puberal mice. We found that, while control animals showed sex difference in the number of TH-stained neurons in the LC, the exposure to BPA eliminates this difference, as did DES [194].

It is recognized that DA is regulating male sexual behavior in rodents through its cooperation with the NO-producing system [108,204]. Our preliminary data indicate that pre- and postnatal exposure to BPA can alter selectively this system at the level of medial preoptic nucleus and of the bed nucleus of the stria terminalis [140]. These data

indicate in the NO system another potential and important target for the action of EDCs in mammals. Although we did not find significant alteration of sexual behaviors in male mice following perinatal exposure to EDCs, other studies [85,154] reported that male rats perinatally exposed to BPA showed less efficient sexual behaviors than controls.

However, none of these studies can delineate the mechanisms involved in BPA's or MXC's actions on the developing brain; further study is needed to clarify possible mechanisms underlying EDCs actions on brain development and their effects on behavior. At present, it must be recognized that in addition to well-documented estrogenicity, BPA and MXC may exert other effects on the developing brain. A number of studies have suggested that some of the neurobehavioral effects of XEs, such as BPA, cannot be explained by an estrogenic action of this compound, related to its binding to ER α and ER β [76,77,85]. MacLusky and co-workers have recently reported anti-estrogenic effects of BPA on hippocampal synaptogenesis of mice brain [136]. In another study, BPA has been shown to exert estrogenic or anti-estrogenic effects in the rat cerebellum, according to the concentration of the compound [244]. In addition, recent studies have shown that not all effects of BPA are mediated by the classical nuclear ERs. Non-genomic cell-signaling systems involve serial activation of kinases via ligand binding to cell-membrane receptors at very low concentrations [236].

3. Circuits controlling sexual behavior in birds

Birds, and in particular galliforms are characterized by extreme forms of sex dimorphism [i.e., the male copulatory behavior and neural circuits associated to it, as the vasotocin (VT) system]. In quail, contrary to what is observed in rodents, the male-type copulatory behavior is highly differentiated between males and females, while the female-type receptive behavior can be activated in both sexes by an appropriate treatment with estrogens [4] (for a review, see [31]). The sexual dimorphism affecting male copulatory behavior is quite extreme in this species: under laboratory test conditions, sexually mature males almost never fail to exhibit the complete copulatory sequence, including grabbing the female's neck feathers, mount attempts, mounts, and cloacal contact movements. These behaviors are never seen in females and are T-dependent [4,39,163]. The dimorphism is an all or none phenomenon; it is qualitative in nature (Fig. 3). Because these behaviors are T-dependent, they disappear after castration in males. However, gonadectomized or intact females still do not exhibit these behaviors due to a relative absence of circulating T. It has indeed been shown that treatment with high doses of T is not sufficient to activate cloacal contact movements in gonadectomized females, while such a treatment restores the full spectrum of sexual behaviors in males [4,39,162,206]. This suggests that the neuronal circuits

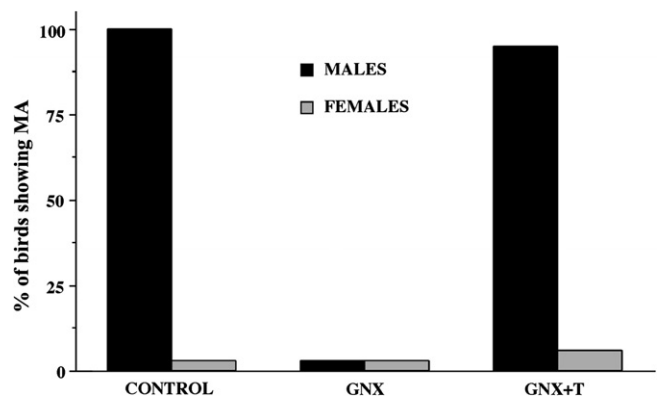


Fig. 3. Sex dimorphism in the control of copulatory behavior of Japanese quail (illustrated here by mount attempt, MA) by testosterone. (A) Adult male Japanese quail readily show copulatory behavior when presented to a sexually mature female. This response is never shown by intact females (Control). It disappears after gonadectomy (GNX). Treatment of gonadectomized birds with testosterone (T) activates MA in males but not in females (GNX + T). Females are insensitive to the activating effects of T. Redrawn from data in [39].

supporting male reproductive behavior are also sexually differentiated in this species.

A large number of investigations have elucidated the neural basis of this dimorphism and the results were particularly interesting for two nuclei of the limbic-hypothalamic region, the medial preoptic nucleus (POM, [183]) and the nucleus of the stria terminalis (BST, [20]). These two nuclei were described in detail for both their cytoarchitecture and for their neurochemical features (for a review, see [182]). Several studies have also demonstrated that, in particular the POM, they are profoundly implicated in the control of male sexual behavior (for detailed reviews, see [181,182]). Among the different neural circuits that have been characterized in POM and BST, two are particularly involved in the control of reproductive behavior, the neurons producing aromatase (the enzyme converting T into E_2 , [32]) and the parvocellular neuronal system producing VT located in the BST and POM [173]. The quail (and chicken) VT system shows however a stronger dimorphism than the aromatase system [182], and its sexual differentiation is influenced in the same way by estrogens as the copulatory behavior do [95,115,176].

Male copulatory behavior and VT parvocellular system are therefore major targets of the action of estrogens during development of galliforms, in this respect they are also privileged targets for the action of EDCs, in particular XEs and xenoandrogens, during the embryonic life [175].

In field birds, it is critical to consider endocrine, neuroendocrine, and behavioral components of reproduction, as all are critical to overall fitness. Therefore, the study of neural circuits as impacted by EDCs in specific avian models provides a fundamental understanding of the mechanisms involved in the behavioral impairment and other effects of exposure. This is especially important in light of the apparent negligible effects on testis weight, plasma steroids, and other more general measures of reproductive

function. Understanding specific neural effects associated with EDC effects provide an important measure for field birds in which more subtle effects may occur due to spotty or variable exposure. Furthermore, some of these endocrine active compounds may translate into epigenetic or impaired reproductive fitness and variable responses between individuals [14,15]. In this way, there may be long-term deleterious effects on individuals and then eventually on the population as a whole.

4. The Japanese quail as an avian model for testing EDCs

As briefly described in the previous chapter, the Japanese quail provides an advantageous model for understanding the impact of EDCs, in particular those provided by the diet, because there are well characterized embryonic effects of gonadal steroids. In the female embryo, plasma E_2 peaked at E10, E12, and declined post hatch, with levels always higher than in males [164], in males, plasma androgen peaked at E10–E12 and P1, with a decrease post hatch being always higher than in females [161] (Fig. 4). Administration of exogenous gonadal steroids alters sexual differentiation of reproductive behavior

in both sexes [6,7]. In male quail, the embryonic exposure to T or E_2 by E12 altered later expression of copulatory sexual behavior (demasculinized males [5,22,176]). On the contrary, early embryonic administration of specific inhibitors of the synthesis of E_2 (fadrozole, R76713) induces defeminization of sexual behavior in females [34,176]. These results indicate the important organizational role of the conversion of T into E_2 (aromatization) [29] in addition to its role in the induction of adult sexual behavior [33]. Increased 5β reductase enzyme activity was found in the brain of male quail embryos between E7 and E15, which may protect males from being demasculinized by inactivating T [37]. Finally, early steroid exposure also influences gonadal development, with fadrozole and tamoxifen exposure producing defeminization of the ovary and accessory structures [82]. These data were confirmed in several independent studies, including those that compare the effects of gonadal steroids and EDCs.

Neural systems that are responsive to steroids and key regulators of endocrine and behavioral components of reproduction are found in the preoptic, septal, limbic, and selected hypothalamic regions. In Japanese quail, neural systems that control male copulatory behavior include the POM and BST [181,182], these nuclei are characterized by a large sexually dimorphic population of aromatase-immunoreactive (ARO-ir) neurons [23,40] and a sexually dimorphic VT-immunoreactive parvocellular system [20,173]. ARO-ir cells are also controlled by several neurotransmitter/neuropeptide afferents: catecholamines [36], nitric oxide [38], VT [30] (for review, see [1]). Other neuropeptides and neurotransmitters are also present in the same regions: galanin [27], neuropeptide Y [25], vasointestinal polypeptide, substance P [26], and serotonin [69]. In addition, ARO, VT and vasointestinal polypeptide immunoreactive elements are modulated in adulthood by the circulating levels of T. ARO and VT show a marked decrease in the number of immunoreactive cell bodies in POM, BST and lateral septum of castrated or aged males [21,23,177,179,222], whereas, in the same endocrine situation, vasointestinal polypeptide fiber density in the caudal lateral septum increases [24]. Conversely, the expression of both VT-immunoreactive and ARO-ir elements are stimulated in castrated adults when E_2 is administered [21,223]. ARO-producing neurons (primarily in the POM) and neural input to these neurons are critical modulators of male copulatory behavior [1,181]. In addition, the parvocellular sexually differentiated VT system is particularly sensitive to estrogens during the embryonic period. Administration of estradiol benzoate (EB) at day 9 of incubation suppresses male copulatory behavior in adults and induces a female-type VT phenotype in adult males [176].

4.1. The effects of estrogenic EDCs in Japanese quail

Earlier studies showed that some pesticides have an ability to interfere with the hypothalamic–pituitary–gonadal axis (HPG axis; [198]). Insecticides such as *o,p'*-DDT

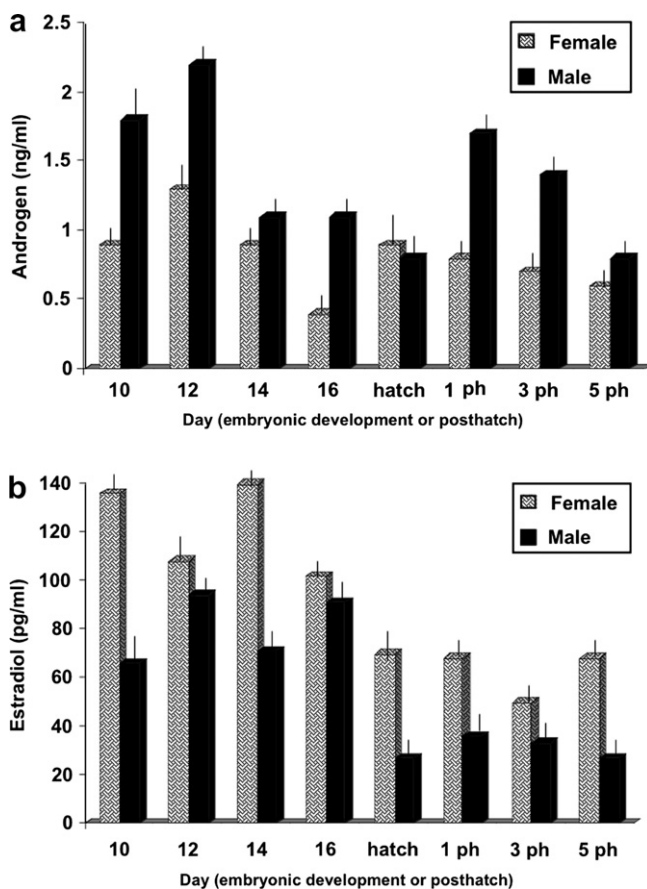


Fig. 4. (a) Plasma androgen concentrations in embryonic and post-hatch male and female Japanese quail at various ages. (b) Plasma 17β -estradiol concentrations in embryonic and post-hatch male and female Japanese quail at various ages. Redrawn from [164].

[103], DDE [178] or MXC [100,101], as well as other compounds such as ethinylestradiol [102], diethylstilbestrol [224], and genistein [225] significantly decrease male sexual performance when administered during embryonic development. Besides the effects on behavioral differentiation, these chemicals affect differentiation of gonads, accessory sexual organs, and brain circuits.

In birds, the female deposits steroids and steroid-like compounds into the yolk, thereby providing a primary route of exposure during embryonic development (for a review, see [60]). Female quail given E₂ implants were found to transfer estradiol to offspring via the yolk [7]. Therefore, a primary route of exposure is via maternal deposition of the EDC into the yolk. This is also the case for other endocrine active compounds, including MXC, which is an estrogenic pesticide [166]. Although EDCs are generally weaker in action than endogenous steroids, including exogenous E₂, the estrogenic pesticide MXC slowed sexual maturation in both males and females. Treated males also had impaired sexual behavior, similar to the effects of embryonic estradiol [160]. Similarly, soy phytoestrogens and especially genistein readily transfer into the yolk when ingested by the hen (Fig. 5) [135].

4.2. The Japanese quail sexually dimorphic parvocellular vasotocin system

Among the different neural circuits that have been previously illustrated, the sexually dimorphic parvocellular vasotocin system has been thoroughly investigated (for reviews, see [115,173]). This system is sensitive to gonadal steroids both in embryonic and in adult life [176,179]. In detail, POM and BST contain a dense population of vasotocinergic cells strongly sexually differentiated [182], in addition VT-ir fibers are present in much higher density in these nuclei and in the lateral septum in males than in females [20,221]. VT-ir cell bodies have been observed in POM

and BST of males but not in females, and accordingly, a sex difference in VT expression has been confirmed by *in situ* hybridization of VT mRNA in the BST and POM [20] (Fig. 6).

In adult birds, the VT innervation of the POM is steroid sensitive in males: the density of VT-ir fibers in this nucleus decreases in conditions where males experience low levels of circulating T (castration, photoregression, and aging) and are restored to levels typical of sexually mature males by exogenous treatments with T [177,222]. Similar changes have been demonstrated also for the expression of VT

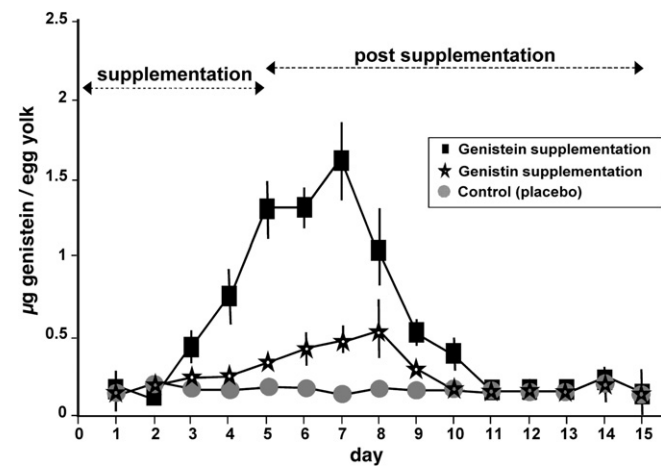


Fig. 5. Concentration of genistein in Japanese quail egg yolks from hens supplemented with genistein, genistein or placebo capsules. Data points represents average of 4 replies for treatment groups and 2 replicates for the control groups. Modified from [135].

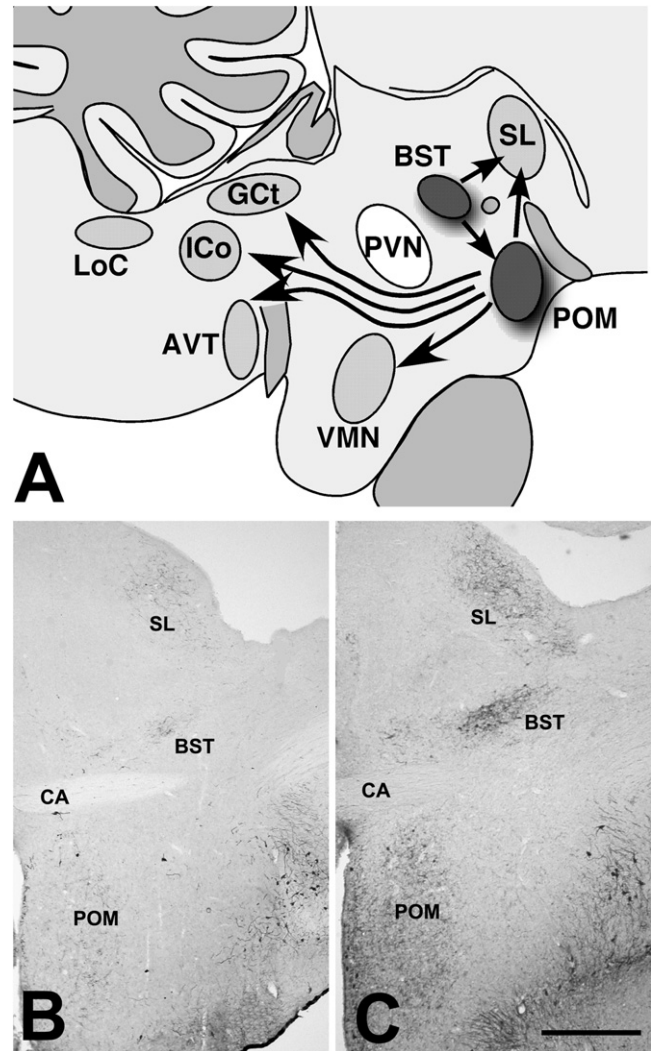


Fig. 6. (A) Schematic representation of connectivity of the parvocellular sexually dimorphic VT system. BST and POM are the two nuclei in which are concentrated the VT-ir cell bodies. BST is projecting to SL and POM [2]. POM is connected to several hypothalamic and brainstem nuclei [35]. (B and C) Comparison of the VT immunoreactivity in adult female (B) and male (C) Japanese quail. Black nuclei show VT-ir cell bodies, grey nuclei show a sexually dimorphic VT innervation [173,221]. Bar = 300 µm. AVT, ventral tegmental area; BST, bed nucleus of the stria terminalis; CA, anterior commissure; Gct, central grey; ICo, intercollicular nucleus; LoC, Locus coeruleus; POM, medial preoptic nucleus; PVN, paraventricular nucleus; VMN, ventromedial nucleus.

mRNA in the parvocellular elements of the BST and POM [179].

As described before, T activates male sexual behavior through its aromatization in estrogens (17β -estradiol, E_2), in parallel, E_2 administration to castrated adults induced VT innervation of POM, BSTm, and lateral septum comparable to that observed in the intact adult or in castrated treated with T [223]. There are therefore many experimental situations in which the VT parvocellular system within POM, BST and lateral septum vary with the expression of male copulatory behavior.

The existence of causal links between the peptide VT and this behavior has been investigated in experiments that demonstrated either peripheral or intracerebroventricular injection of VT or a specific V1-receptor antagonist markedly affected the appetitive and consummatory aspects of male sexual behavior in castrated T-treated male quail [63]. The VT structures (cells and fibers) of POM and BST are indeed sexually differentiated in the organizational sense [176]. This was demonstrated by injecting fertilized quail eggs of both sexes on day 9 of incubation with either EB (25 $\mu\text{g}/\text{egg}$, a treatment that suppresses the capacity to show copulatory behavior in adulthood) or the aromatase inhibitor R76713 (10 $\mu\text{g}/\text{egg}$, a treatment that makes adult females behaviorally responsive to T), or with the solvents as a control (C). At 3 weeks posthatch, all subjects were gonadectomized and later implanted with Silastic capsules filled with T. At the age of six weeks, when quail reach puberty, birds were perfused and brains were sectioned. Despite the similarity of the adult endocrine conditions of the subjects (all were gonadectomized and treated with T implants providing the same plasma level of steroid to all subjects), major qualitative differences were observed in the density of VT-ir structures in the POM of the different groups. Dense immunoreactive structures (fibers and a few cells) were observed in the POM of C males but not females; EB males had completely lost this immunoreactivity (and lost the capacity to display copulatory behavior) and, conversely, R76713 females displayed a male-typical VT-ir system in the nucleus (and also high levels of copulatory behavior). Similar changes in immunoreactivity were seen in the BST and in the lateral septum (VT-ir fibers only in this case) but not in the magnocellular vasotocinergic system. These neurochemical changes closely parallel the effects of the embryonic treatments on male copulatory behavior. These results clearly demonstrate that, in quail, the vasotocinergic innervation of the POM, lateral septum, and BSTm, and its sensitivity to T and E_2 in adulthood are organized during the embryonic life. Exposure to high levels of estrogens results in a female phenotype as far as these vasotocinergic inputs are concerned; the (relative) absence of estrogens in the embryos leads to the male phenotype. The sexual dimorphism observed in the adults is truly organizational in its nature, even if the presence of T is required for this dimorphism to be fully expressed during adult life. Similar findings have also been recently reported in the domestic fowl [96,114].

Therefore, both male copulatory behavior and the sexually dimorphic parvocellular VT system are particularly sensitive to the organizing effects of E_2 during embryonic development. Hence, these variables provide useful endpoints to detect the estrogenic capacity of different XEs [175]. To test this hypothesis, we administered BPA [229], diethylstilbestrol (DES, a powerful synthetic estrogen [79]), genistein (GEN, a phytoestrogen, [78,132]) and ethylene, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) (DDE, a common metabolite of DDT, with anti-androgenic activity [117]) to fertile quail eggs. Our hypothesis was that XEs may alter the animal physiology through their binding to estrogen receptor. In our model this means that a XE administered during embryonic life, should reduce or abolish male copulatory behavior and interfere with the differentiation of the sexually dimorphic parvocellular VT-ir system. We have always introduced two other experimental groups, one injected with the solvent (OIL) and a second one injected with estradiol benzoate (EB). The hatched birds were raised in heterosexual cages up to the age of 4 weeks when they were put in individual cages. At the age of 7 weeks we tested the male copulatory behavior, and the following week the animals were perfused to dissect the brain and perform immunocytochemical analyses (for the full description of methods see [224,225]).

4.3. Effects of BPA administration

As previously reported the xenoestrogen BPA [226] is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles, therefore it is largely diffused in the environment and in the food. Investigations were performed in both quail and chicken eggs using similar doses of BPA (from 67 to 200 $\mu\text{g}/\text{egg}$ [47]; embryos were sacrificed at the age of 15 or 19 days of incubation. BPA induced Mullerian duct (embryonic oviduct) malformation in female quail embryos and feminization of the left testis (ovotestis) in male chicken embryos. BPA caused mortality only in chicken embryos at 67 and 200 $\mu\text{g}/\text{egg}$. In this study no investigations were performed on brain circuits. In two different experiments we administered 50, 100, or 200 μg of BPA per egg. The result of these embryonic treatments was a dramatic decrease in the number of hatched animals: the percentage of living chicks was in fact ranging from 8% to 11% of injected eggs (controls and EB-injected were ranging from 55% to 60% in these experiments). The BPA-injected young quail did not survive after one week of life. The dissection of non-hatched embryos revealed that the large majority of the embryos was blocked immediately after the BPA administration (from 36% to 63%), whereas for the embryos that died later, we observed a high incidence of malformations of the gut, abdominal wall, and legs. With these experiments we were, therefore, unable to study any alteration in the brain (unpublished results). Contrary to what happens in mammals, BPA, even at low doses, has robust adverse effects in birds, inducing several malformations also of the reproductive tract, and

determining a strong reduction in their surviving after the exposure.

4.4. Effects of DES administration

Among various chemical XEs, diethylstilbestrol (DES) was initially synthesized as an orally effective estrogen for use in human medicine and as anti-abortive substance and then used as anabolic growth promoter in cattle, steer, and sheep [89]. In the beginning, the environmental impact of this compound was not considered. However, DES and its metabolites were also excreted into the ecosystem with unknown consequences. Detection of radiolabeled DES in a model ecosystem demonstrated that it was persistent and bioaccumulated (for a review, see [146]). In quail, previous studies demonstrated a potent effect of DES on the development of sex organs and the differentiation of male sexual behavior [49], however, no attention was dedicated to alterations of brain nervous circuits that should take place related to or inducing behavioral changes.

In a recent study [224], we have confirmed that embryonic treatment with 700 ng/egg of DES demasculinize sexual behavior and cloacal gland size of adult intact male quail. These effects are particularly strong and fully comparable to those obtained after administration of higher doses of EB (10 or 25 μ g) with the same procedure. These effects confirm that sexual behavior of adult male quail is an excellent *bioassay* for embryonic exposure to EDCs with estrogenic activity. It is interesting to emphasize that a similar dose of DES may induce in humans alterations of male genital system (for a review, see [146]). In addition, as illustrated in Fig. 7, this *in ovo* treatment significantly decreased the fractional area covered by VT-ir structures within BSTm, POM, and lateral septum [224].

The effects of DES on VT-ir structures appear to be anatomically specific in that no changes were observed in VT magnocellular neurons of the supraoptic and paraventricular nuclei. This further supports our previous findings showing that changes in VT-immunoreactivity related to endocrine status affect only VT parvocellular circuits of the preoptic and limbic regions [173,223].

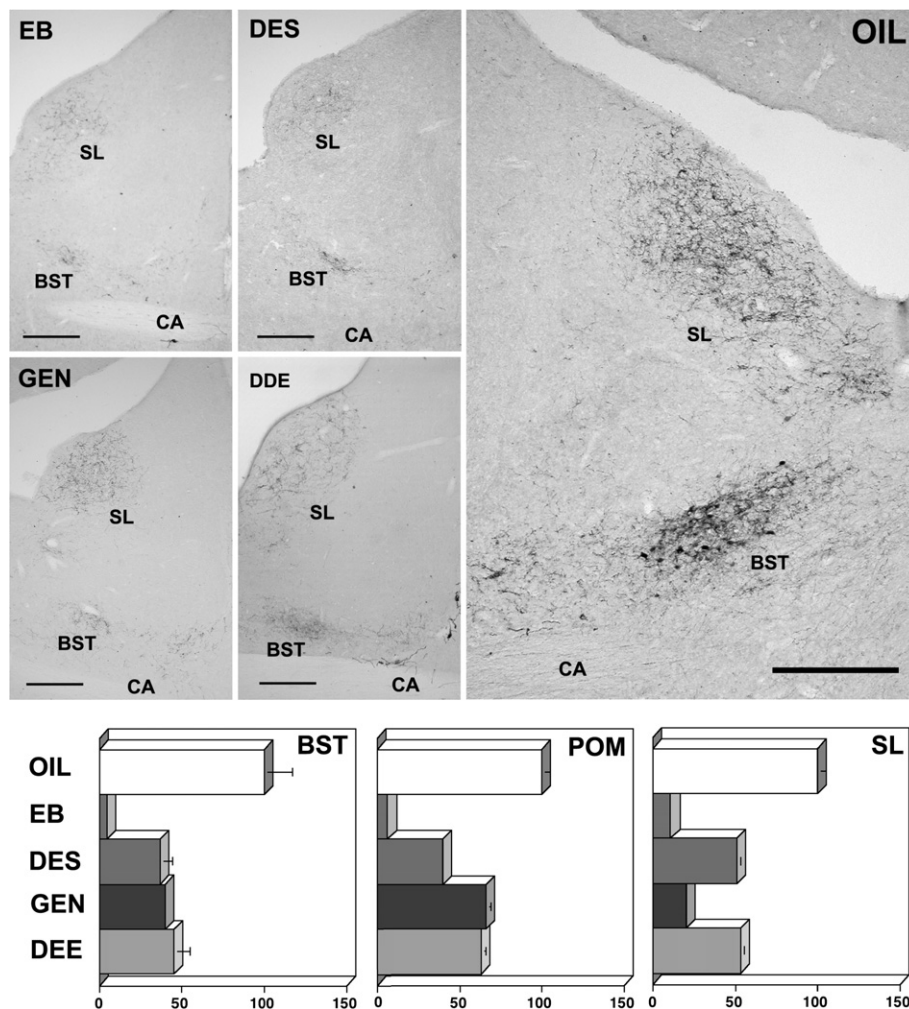


Fig. 7. (Upper) Changes in male VT immunoreactivity in different experimental conditions. OIL, control; EB, estradiol benzoate; DES, diethylstilbestrol; GEN, genistein; DDE, ethylene; 1,1-dichloro-2,2-bis(*p*-chlorophenyl). Bars = 300 μ m. (Lower) Fractional area covered by VT-immunoreactivity in BST, POM, and SL of male quail from different experimental groups. Data redrawn from [178,224,225].

4.5. Effects of genistein administration

Genistein is the simplest isoflavonoid compound produced by Leguminosae, particularly abundant in soybeans. In plants, together with the other isoflavonoids collectively called phytoestrogens, it has antimicrobial activity [78], as well as a specific activity to protect plants from insects [52,233]. This action is mediated by its activity as a ligand of ecdysone (the molt steroid hormone of invertebrates) receptor [157]. This molecule shares structural features with the 17 β -estradiol and therefore can bind ERs and sex hormone binding proteins [127,150]. Thus genistein, as other similar isoflavones collectively called phytoestrogens, can exert both estrogenic and antiestrogenic activity, the latter by competing with estradiol for receptor binding [53].

A number of studies have been performed to investigate possible alterations of brain circuitries or of behavioral activities after administration of genistein (or of a mixture of phytoestrogens) in mammals during adult life or during the critical period (for recent reviews, see [132,184]). The administration of genistein to neonatal female rats: (a) significantly increased SDN-POA volumes in adult gonadectomized female [8,84], or b) resulted in a non-significant volumetric decrease of SDN-POA in intact adult female [133,134]. The administration of genistein to neonatal male rats induced an increase in the number of calbindin positive cells within the SDN [187]. Neonatal administration of genistein is also influencing the sexual differentiation of TH system of the hypothalamic AVPV in rodents. In intact adult rat and mouse, female AVPV is larger than the male AVPV and contains a higher number of cells expressing TH. Peri- or postnatal exposure to genistein demasculinized TH-ir system in the male AVPV [186,201]. In addition, the number of TH-ir neurons colocalized with estrogen receptor α is strongly decreased in females [186]. Overall, these studies suggest that acute exposure to genistein and other phytoestrogens during a critical developmental period alters the development of some encephalic sexually dimorphic structures. Finally, other studies performed during adulthood demonstrated that phytoestrogens may have also activational effects. In fact, in adult rat, variation of the diet from a phytoestrogen-rich to a phytoestrogen-free diet determined the decrease of the SDN-POA volume in males and of the AVPV volume in females [131].

In our study we have treated quail eggs with 100 or 1000 μ g of genistein [225]. The lowest dose of GEN had significant effect only on one aspect of male copulatory behavior (mount attempt, MA), whereas the GEN-1000 treated males showed a significant reduction for most aspects of the copulatory behavior (neck grab, NG, MA, and mount, M). No effects were detected on the cloacal gland size.

The parvocellular VT-ir system (POM, BST, and lateral septum) was affected by GEN treatment, showing a significant decrease of immunoreactivity with both 100 and 1000 μ g of GEN. However, the reduction was not as strong as that observed after treatment with EB (Fig. 7). As for

the experiment with DES no significant effect was detected in the magnocellular VT system.

4.6. Effects of DDE administration

It took about 50 years after the initial use of DDT as a pesticide to understand some of the mechanisms of action of its main metabolite DDE. This is due in part to much of the early research concentrating on the estrogenic effects of *o,p'*-DDT, which only made up about 15% of technical DDT. *o,p'*-DDT was shown to cause estrogenic effects in female rats [65]. It was not until studies began to show phenotypic effects of this chemical on male rats that were very similar to those observed with exposure to known androgen receptor blocking chemicals that scientists first began to speculate about *p,p'*-DDE's anti-androgenic nature [117]. It is now known that DDE is a potent androgen receptor antagonist and a very potent testosterone hydroxylase modulator. Its androgen receptor blocking ability is almost equal to that of the anti-androgen hydroxyflutamide [117]. In birds, *in ovo* DDE administration has impact on brain structure (reduction in volume of the forebrain, and of the song controlling nuclei in a wild oscine species [112]) and on the immune system [195,196].

In a preliminary experiment, we have tested if the administration of 20 or 40 μ g of DDE per egg has an impact on male quail sexual behavior and parvocellular VT system [178]. At both doses DDE significantly decreases the number of MA, as well as the VT immunoreactivity in POM, BST, and lateral septum (Fig. 7). Therefore, this antiandrogen compound, when administered during the embryonic life, has also a powerful action on the differentiation of estrogen-dependent function (male copulatory behavior) and nervous circuitries (the parvocellular VT system).

5. General comments

In the conceptual frame of the evolutionary theory, sex-differences in behavior are thought to reflect adaptive differences of behavioral strategies in coping as resulting from sexual selection [73]. Longitudinal studies on effects of EDCs should be carried out in order to evaluate in which contexts, and with what intensity, reversing or leveling of sexual differences could have relevance, in particular whether behavioral alterations occur in systems influencing individual fitness/reproductive success.

EDCs are globally distributed through our atmosphere, our seas and wildlife. Many are persistent; others, while not persistent, should be treated as persistent because of their chronic and ubiquitous use. They act at a population level and many have the potential to (individually or cumulatively) affect future generations, for example by decreasing fertility, feminizing males, masculinizing females or altering cognitive abilities. All these endpoints have been studied in our or others' laboratories and many have been observed in wildlife. Recent data, which must be confirmed by further

studies, suggest that comparable changes can be produced in human populations as well [105,228,241]. These include increases in the frequency of preterm birth, obesity, cognitive/behavioral dysfunctions (such as autism and attention deficit hyperactivity disorder, ADHD, Parkinson's disease), and decreases in reproductive function (such as a decline in sperm count) and immune function [121,216].

In birds, the diversity of reproductive strategies, habitat and the migration of many species means that their exposure to EDCs may be extremely variable, also depending by the amount of body fat (many of the EDCs are lipophilic), so, for example, migratory birds might receive additional exposure during migration when lipid stores are utilized for energy. Exposure of the avian embryo to maternal hormones or to EDCs is increased by the use of fat stores for the production of vitellogenin that becomes a primary component of the yolk, which is then used by the embryo during development [7,48,60,99,104,135]. As we have demonstrated in our studies, it is clear that low, field relevant concentrations of EDCs do exert irreversible effects on endocrine, neuroendocrine, and behavioral systems that are often due to permanent changes in neural systems. Therefore, the embryonic period, during which irreversible alterations in the organization of neural systems occurs, appears to be the most vulnerable stage in the life. The use of laboratory species, as the Japanese quail, is valuable to fully characterize the impact and risk of EDCs to avian species, as well as to understand the mode and mechanisms of action of classes of EDCs, but further studies are needed to assess risk in field birds.

In rodents the reported experimental studies indicate that exposure to environmentally relevant doses of EDCs during developmental critical periods interacts with some steps in the sexual differentiation of the neural systems controlling explorative and emotional behavior. Therefore, from this point of view, rodents and galliforms are similarly sensitive to early exposure to EDCs, mainly in those "critical" periods that are important for brain sexual differentiation. However, experimental data suggest that the behavioral responses as well as the neural circuits sensitive to the action of EDCs differ between these vertebrate classes. In rodents, the majority of data suggest alterations mainly in females and in non-reproductive behaviors that could be related to altered development of central monoaminergic pathways, but further work is needed to clarify the neural basis of long-term consequences of developmental exposure to EDCs such as BPA and MXC at the level of neurobehavioral alterations. Some compounds target neuroendocrine systems, thereby affecting reproductive endocrine systems as well as other endocrine systems. Therefore, exposure to the estrogenic chemicals during fetal/early prenatal development has consequences beyond impaired function of the reproductive axis.

Most of the data that we discussed in the present review are summarized in Table 1. They are only considering studies in which behavioral effects following pre- and/or early post-natal exposure to low, environmentally relevant doses

of EDCs were recorded. Many other studies have analyzed only alteration of neural circuits, with no description of related behaviors [10,83,112,186,187], or the effects of feeding EDCs in adult animals [55,57,88,132,185,199,217,238,242]. A direct comparison of the EDCs effects in rodents and galliform is impossible, due to the variety of behaviors that were considered for rats and mice and the almost exclusive consideration of male copulatory behavior in quail. However, they strongly indicate that altered behavior is one of the most conspicuous endpoint produced by EDCs. Behavioral alteration, although a relatively insensitive indicator of the degree of exposure, has the advantage of revealing both direct or indirect effects of contamination and in some cases represents the only clue of functional deficits at different physiological levels [75] and can be more sensitive than other endpoints as biomarkers of exposure, either in terms of chemical concentration, response time or both. In particular, sexually dimorphic behaviors (either reproductive or non-reproductive) are useful to verify adverse developmental consequences produced by chemicals with endocrine disrupting properties: they interfere with sexual differentiation of the brain, consequently they can diminish, eliminate, reverse or widen sex differences in behaviors. In contrast, explicit recognition of sex differences in performance is not a prominent feature of toxicological studies, except for reproductive capacity studies and neurotoxicity testing does not typically recognizes sex differences in behavioral responses as an experimental criterion [234]. It should be noted that in a number of the reported studies no significant effects of perinatal exposure to EDCs on brain and/or behavior were found, but a consistent result was the elimination of the sex differences shown by unexposed animals [90,124,125,129,172,201]. If these studies had tested only males, as the majority of classic toxicological studies do, no effects of EDCs on behavioral/brain development would have been detected.

Collectively, the new behavioral data from studies of EDCs are strongly suggesting that prior methods of testing chemicals have been inadequate to detect adverse effects of the type now known to be caused by chemicals that are classified as endocrine disruptors. Foremost, there is a challenge to the operating assumption concerning appropriate dose. Because many EDC have effects at very low concentrations in water (for example, nanogram per litre levels for estradiol), it is obvious there is a need to develop analytical methods applicable at such trace levels. It is also important to integrate and correlate chemical analytical data with endocrine-disrupting effects [41].

The recent evidences for multiple roles of steroid hormones in the brain (neurogenesis, neuroprotection, regulatory, short-term and long-term activators) indicate that the nervous system is a target for two different pools of steroids [147]), one coming from the peripheral glands (i.e., gonadal steroids) and the second one originating in the nervous system partly by *de novo in situ* synthesis and partly for enzymatic activation of peripheral steroids (neuroactive steroids

Table 1

Sex-specific effects of developmental exposure (prenatal, or pre- and post-natal) to low, environmentally relevant concentrations of xenoestrogens or xenoandrogens on brain and behavior of rodent and quail animal models

EDC	Species	Sex specific behavioral effect	Neural circuits	References
Bisphenol A	Rat	Decreased explorative activity in females	—	[89]
Diethylstilbestrol			LC volume	[123,124]
			LC volume	[124]
Bisphenol A	Mouse	Decreased explorative activity in females	TH neurons in AVPV	[203]
Bisphenol A, Methoxychlor			Noradrenergic receptors in	[93]
Methoxychlor			LC	[170]
Bisphenol A, Ethynil estradiol	Mouse	Decreased anxiety in females	—	[205]
Bisphenol A	Rat	Increased exploratory activity in males	—	[90]
			Dopaminergic system	[157]
Bisphenol A, methoxychlor	Mouse	Decreased response to reward in females	LC, Dopaminergic system	[128]
Bisphenol A	Rat	Decreased drug-induced locomotion in males	Monoaminergic system	[9]
Ethynil Estradiol	Mouse	Increased spatial memory in females	—	[205]
Ethynil Estradiol	Rat	Increased spatial memory in males	—	[70]
Methoxychlor	Mouse	Decreased onset of aggression in males	—	[170]
Vinclozolin, Methoxychlor	Quail	Decreased male mating behavior	—	[85,101,161]
Diethylstilbestrol			Vasotocin system in BST	[225]
Genistein			Vasotocin system in BST	[226]
DDE			Vasotocin system in BST	[179]
Ethynilestradiol			No effect on Vasotocin system	[142]
Bisphenol A			(embryonic death)	[47]
Bisphenol A	Rat	Decreased male mating behavior	—	[89,155]
Methoxychlor			—	[85]
Bisphenol A	Rat	Increased female sexual interest	—	[88]
Bisphenol A	Mouse	Decreased maternal behavior	—	[173]
Methoxychlor	Rat	Estrous cyclicity	—	[141]
Genistein			TH neurons in AVPV	[190]
Genistein	Mouse	Estrous cyclicity	—	[64]
Bisphenol A, Ethynil estradiol	Mouse	Onset of puberty	—	[108,205]
Bisphenol A	Mouse	Memory impairment in male	ChAT neurons in hippocampus	[150]

[189]). Probably, the gonadal steroids are most important to determine irreversible changes in brain circuits as well as in sexually dimorphic behaviors, whereas the neuroactive steroids are more important for short-term regulations.

As reviewed before, the EDCs, chiefly the xenoestrogens, may have heavy biological effects that will vary over the life cycle of the animal as well as across species and phyla [180], moreover they accumulate not only because of environmental pollution, but also due to their wide presence in the food. The evolutionary implications of having some of these compounds in the normal food supply for certain human populations (i.e., phytoestrogen derivatives from soy, [54,62,153,219]), as well as for wild and farm animals have not yet been discussed, even if animals that, due to their specific diet, are largely exposed to these compounds, as the domestic ruminants, seem to show unfavorable effects of phytoestrogens at least on reproduction [3]. Research in reproductive endocrinology has been almost exclusively focused on a small group of domesticated species, but if ecological variables, such as dietary burden of phytoestrogens, have altered susceptibility to anthropogenic contaminants, then a more diverse research base is urgently needed. Furthermore, phytoestrogens in the diet can lead to a net decrease in estrogenic activity in the serum

in rodents and interfere with the action of other EDCs [8,139,230].

The mechanisms underlying the effects of these compounds needs to be further investigated; in fact, in many cases the effects of XEs cannot be easily superimposed to those of “natural” estrogens, suggesting that non-estrogenic or/and metabolic effects are involved [92,239]. For example, phytoestrogens may play different roles in the cells. On one side genistein and other isoflavones are direct regulators of the aromatase activity [190], while it has high affinity for some transcription factors as PPAR- γ [120,148] that have been recently localized also in the brain [64] where it, potentially, could have a role in nervous tissue differentiation [232]. Therefore, at least part of the effects of prenatal exposure to EDCs may involve non-steroidal mechanisms activating signaling cascades that in many cases finalize with the activation of transcription factors, turning a non-genomic response into a genomic one (rapid endocrine disruption [200]).

In parallel we should note that in rodents, in some cases, XEs may masculinize female brain morphology and feminize the male brain [125], as indicated also by the expression of sexually differentiated socio-sexual and non-social

behaviors [86]. A smaller number of reports support a similar masculinization of the female brain and feminization of the male brain in response to anti-androgenic EDCs [142]. It is possible that these contrasting effects depend by the alteration of androgen:estrogen balance during development (for a review, see [240]).

In conclusion, in addition to gonadal steroids and neuroactive steroids, xenoestrogens derived from food and environment should be considered as a third player within the nervous system that can regulate or alter its functions through multiple pathways.

Acknowledgments

Studies described in the present review have been supported by MURST-PRIN (C.V.P., G.C.P., P.P.), Regione Piemonte (C.V.P., G.C.P.), Fondazione CRT (G.C.P.), University of Torino (C.V.P., G.C.P.), EPA (M.A.O.), NSF (M.A.O.). E.M. is a fellow of Regione Piemonte.

References

- [1] P. Absil, M. Baillien, G.F. Ball, G.C. Panzica, J. Balthazart, The control of preoptic aromatase activity by afferent inputs in Japanese quail, *Brain Res. Rev.* 37 (2001) 38–58.
- [2] P. Absil, M. Papello, C. Viglietti Panzica, J. Balthazart, G.C. Panzica, The medial preoptic nucleus receives vasotocinergic inputs in male quail: a tract-tracing and immunocytochemical study, *J. Chem. Neuroanat.* 24 (2002) 27–39.
- [3] N.R. Adams, Detection of the effects of phytoestrogens on sheep and cattle, *J. Anim. Sci.* 73 (1995) 1509–1515.
- [4] E.K. Adkins, Hormonal basis of sexual differentiation in the Japanese quail, *J. Comp. Physiol. Psych.* 89 (1975) 61–71.
- [5] E.K. Adkins, Effect of embryonic treatment with estradiol or testosterone on sexual differentiation of the quail brain. Critical period and dose–response relationship, *Neuroendocrinology* 29 (1979) 178–185.
- [6] E.K. Adkins Regan, Hormonal bases of sexual differentiation in birds, in: *Hormones, Brain and Behaviour in Vertebrates*, in: J. Balthazart (Ed.), *Sexual Differentiation, Neuroanatomical aspects, Neuropeptides and Neurotransmitters*, vol. 1, Karger, Basel, New York, 1990, pp. 1–14.
- [7] E. Adkins-Regan, M.A. Ottinger, J. Park, Maternal transfer of estradiol to egg yolks alters sexual differentiation of avian offspring, *J. Exp. Zool.* 271 (1995) 466–470.
- [8] H. Adlercreutz, C. Bannwart, K. Wahala, T. Makela, G. Brunow, T. Hase, P.J. Arosemena, J.T. Kellis Jr., L.E. Vickery, Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens, *J. Steroid Biochem. Mol. Biol.* 44 (1993) 147–153.
- [9] W. Adriani, D. della Seta, F. Dessi-Fulgheri, F. Farabollini, G. Laviola, Altered profiles of spontaneous novelty seeking, impulsive behavior, and response to D-amphetamine in rats perinatally exposed to bisphenol A, *Environ. Health Perspect.* 111 (2003) 395–401.
- [10] R. Alò, R.M. Facciolo, M. Madeo, G. Giusi, A. Carelli, M. Canonaco, Effects of the xenoestrogen bisphenol A in diencephalic regions of the teleost fish *Coris julis* occur preferentially via distinct somatostatin receptor subtypes, *Brain Res. Bull.* 65 (2005) 267–273.
- [11] A.M. Aloisi, D. Della Seta, I. Ceccarelli, F. Farabollini, Bisphenol-A differently affects estrogen receptors- α in estrous-cycling and lactating female rats, *Neurosci. Lett.* 310 (2001) 49–52.
- [12] J.J. Amaral Mendes, The endocrine disruptors: a major medical challenge, *Food Chem. Toxicol.* 40 (2002) 781–788.
- [13] S.L. Andersen, M.H. Teicher, Sex differences in dopamine receptors and their relevance to ADHD, *Neurosci. Biobehav. Rev.* 24 (2000) 137–141.
- [14] G. Ankley, E. Mihaich, R. Stahl, D. Tillitt, T. Colborn, S. McMaster, R. Miller, J. Bantle, P. Campbell, N. Denslow, R. Dickerson, L. Folmar, M. Fry, J. Giesy, E. Gray, P. Guiney, T. Hutchinson, S. Kennedy, V. Kramer, G. LeBlanc, M. Mayes, A. Nimrod, R. Patino, R. Peterson, R. Purdy, R. Ringer, P. Thomas, L. Touart, G. Van der Kraak, T. Zacharewski, Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife, *Environ. Toxicol. Chem.* 17 (1997) 68–87.
- [15] M.D. Anway, M.K. Skinner, Epigenetic transgenerational actions of endocrine disruptors, *Endocrinology* 147 (2006) S43–S49.
- [16] C. Arakawa, K. Fujimaki, J. Yoshinaga, H. Imai, S. Serizawa, H. Shiraishi, Daily urinary excretion of bisphenol A, *Environ.. Health. Prev. Med.* 9 (2004) 22–26.
- [17] J. Archer, Rodent sex differences in emotional and related behavior, *Behav. Biol.* 14 (1975) 451–479.
- [18] A.P. Arnold, S.M. Breedlove, Organizational and activational effects of sex steroids on brain and behavior: a reanalysis, *Horm. Behav.* 19 (1985) 469–498.
- [19] A.P. Arnold, E.F. Rissman, G.J. De Vries, Two perspectives on the origin of sex differences in the brain, *Ann. N. Y. Acad. Sci.* 1007 (2003) 176–188.
- [20] N. Aste, J. Balthazart, P. Absil, R. Grossmann, E. Mühlbauer, C. Viglietti-Panzica, G.C. Panzica, Anatomical and neurochemical definition of the nucleus of the stria terminalis in Japanese quail (*Coturnix japonica*), *J. Comp. Neurol.* 396 (1998) 141–157.
- [21] N. Aste, G.C. Panzica, P. Aimar, C. Viglietti-Panzica, N. Harada, A. Foidart, J. Balthazart, Morphometric studies demonstrate that aromatase-immunoreactive cells are the main target of androgens and estrogens in the quail medial preoptic nucleus, *Exp. Brain Res.* 101 (1994) 241–252.
- [22] N. Aste, G.C. Panzica, C. Viglietti-Panzica, J. Balthazart, Effects of *in ovo* estradiol benzoate treatments on sexual behavior and size of neurons in the sexually dimorphic medial preoptic nucleus of Japanese quail, *Brain Res. Bull.* 27 (1991) 713–720.
- [23] N. Aste, G.C. Panzica, C. Viglietti-Panzica, N. Harada, J. Balthazart, Distribution and effects of testosterone on aromatase mRNA in the quail forebrain: a non-radioactive *in situ* hybridization study, *J. Chem. Neuroanat.* 14 (1998) 103–115.
- [24] N. Aste, C. Viglietti-Panzica, J. Balthazart, G.C. Panzica, Testosterone modulation of peptidergic pathways in the septo-preoptic region of male Japanese quail, *Poultry Avian Biol. Rev.* 8 (1997) 9–20.
- [25] N. Aste, C. Viglietti-Panzica, A. Fasolo, C. Andreone, H. Vaudry, G. Pelletier, G.C. Panzica, Localization of neuropeptide Y (NPY) immunoreactive cells and fibres in the brain of the Japanese quail, *Cell Tissue Res.* 265 (1991) 219–230.
- [26] N. Aste, C. Viglietti-Panzica, A. Fasolo, G.C. Panzica, Mapping of neurochemical markers in quail central nervous system: VIP- and SP-like immunoreactivity, *J. Chem. Neuroanat.* 8 (1995) 87–102.
- [27] Y. Azumaya, K. Tsutsui, Localization of galanin and its binding sites in the quail brain, *Brain Res.* 727 (1996) 187–195.
- [28] J. Bakker, C. De Mees, Q. Douhard, J. Balthazart, P. Gabant, J. Szpirer, C. Szpirer, Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens, *Nat. Neurosci.* 9 (2006) 220–226.
- [29] J. Balthazart, Steroid control and sexual differentiation of brain aromatase, *J. Steroid Biochem. Mol. Biol.* 61 (1997) 323–339.
- [30] J. Balthazart, P. Absil, C. Viglietti-Panzica, G.C. Panzica, Vasotocinergic innervation of areas containing aromatase-immunoreactive cells in the quail forebrain, *J. Neurobiol.* 33 (1997) 45–60.
- [31] J. Balthazart, E. Adkins-Regan, Sexual differentiation of brain and behavior in birds, in: D. Pfaff (Ed.), *Hormones, Brain and Behavior*, Elsevier Science, 2002, pp. 223–301.

- [32] J. Balthazart, M. Baillien, T.D. Charlier, C.A. Cornil, G.F. Ball, The neuroendocrinology of reproductive behavior in Japanese quail, *Domest. Anim. Endocrinol.* 25 (2003) 69–82.
- [33] J. Balthazart, G.F. Ball, New insights into the regulation and function of brain estrogen synthase (aromatase), *Trend. Neurosci.* 21 (1998) 243–249.
- [34] J. Balthazart, A. De Clerck, A. Foidart, Behavioral demasculinization of female quail is induced by estrogens: Studies with the new aromatase inhibitor, R76713, *Horm. Behav.* 26 (1992) 179–203.
- [35] J. Balthazart, V. Dupiereux, N. Aste, C. Viglietti-Panzica, M. Barrese, G.C. Panzica, Afferent and efferent connections of the sexually dimorphic medial preoptic nucleus of the male quail revealed by in vitro transport of DiI, *Cell Tissue Res.* 276 (1994) 455–475.
- [36] J. Balthazart, A. Foidart, M. Baillen, N. Harada, G.F. Ball, Anatomical relationships between aromatase and tyrosine hydroxylase in the quail brain: double-label immunocytochemical studies, *J. Comp. Neurol.* 391 (1998) 214–226.
- [37] J. Balthazart, M.A. Ottinger, 5beta-reductase activity in the brain and cloacal gland of male and female embryos in the Japanese quail (*Coturnix coturnix japonica*), *J. Endocrinol.* 102 (1984) 77–81.
- [38] J. Balthazart, G.C. Panzica, R.W. Krohmer, Anatomical relationships between aromatase-immunoreactive neurons and nitric oxide synthase as evidenced by NOS immunohistochemistry or NADPH diaphorase histochemistry in the quail forebrain, *J. Chem. Neuroanat.* 25 (2003) 39–51.
- [39] J. Balthazart, M. Schumacher, M.A. Ottinger, Sexual differences in the Japanese quail: behavior, morphology and intracellular metabolism of testosterone, *Gen. Comp. Endocr.* 51 (1983) 191–207.
- [40] J. Balthazart, O. Tlemçani, N. Harada, Localization of testosterone-sensitive and sexually dimorphic aromatase-immunoreactive cells in the quail preoptic area, *J. Chem. Neuroanat.* 11 (1996) 147–171.
- [41] D. Barcelo, A. Kettrup, Endocrine disruptors, *Anal Bioanal Chem* 378 (2004) 547–548.
- [42] E.R. Bauer, N. Bitsch, H. Brunn, H. Sauerwein, H.H. Meyer, Development of an immuno-immobilized androgen receptor assay (IRA) and its application for the characterization of the receptor binding affinity of different pesticides, *Chemosphere* 46 (2002) 1107–1115.
- [43] W.W. Beatty, Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences, *Horm. Behav.* 12 (1979) 112–163.
- [44] J.B. Becker, Gender differences in dopaminergic function in striatum and nucleus accumbens, *Pharmacol. Biochem. Behav.* 64 (1999) 803–812.
- [45] A. Belayew, S.M. Tilghman, Genetic analysis of alpha-fetoprotein synthesis in mice, *Mol. Cell. Biol.* 2 (1982) 1427–1435.
- [46] S.M. Belcher, A. Zarnovszky, Estrogenic actions in the brain: estrogen, phytoestrogens, and rapid intracellular signaling mechanisms, *J. Pharm. Exp. Therap.* 299 (2001) 408–414.
- [47] C. Berg, K. Halldin, B. Brunstrom, Effects of bisphenol A and tetrabromobisphenol A on sex organ development in quail and chicken embryos, *Environ. Toxicol. Chem.* 20 (2001) 2836–2840.
- [48] C. Berg, K. Halldin, B. Brunstrom, I. Brandt, Methods for studying xenoestrogenic effects in birds, *Toxicol. Lett.* 102–103 (1998) 671–676.
- [49] C. Berg, K. Halldin, A.K. Fridolfsson, I. Brandt, B. Brunstrom, The avian egg as a test system for endocrine disruptors: effects of diethylstilbestrol and ethynylestradiol on sex organ development, *Sci. Tot. Environ.* 233 (1999) 57–66.
- [50] K.C. Berridge, T.E. Robinson, What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* 28 (1998) 309–369.
- [51] C. Beyer, Estrogen and the developing mammalian brain, *An. Embryol.* 199 (1999) 379–390.
- [52] S.M. Boué, A.K. Raina, Effects of plant flavonoids on fecundity, survival, and feeding of the formosan subterranean termite, *J. Chem. Ecol.* 29 (2003) 2575–2584.
- [53] K.S. Bramlett, Y. Wu, T.P. Burris, Ligands specify coactivator nuclear receptor (NR) box affinity for estrogen receptor subtypes, *Mol. Endocrinol.* 15 (2001) 909–922.
- [54] F. Branca, S. Lorenzetti, Health effects of phytoestrogens, *Forum Nutr.* (2005) 100–111.
- [55] R.W. Bretveld, C.M. Thomas, P.T. Scheepers, G.A. Zielhuis, N. Roeleveld, Pesticide exposure: the hormonal function of the female reproductive system disrupted? *Reprod. Biol. Endocrinol.* 4 (2006) 30.
- [56] J.A. Brotons, F.F. Olea-Serrano, M. Villalobos, V. Pedraza, N. Olea, Xenoestrogens released from lacquer coatings in food cans, *Environm. Health Persp.* 103 (1995) 608–612.
- [57] L.H. Bu, E.D. Lephart, Effects of dietary phytoestrogens on core body temperature during the estrous cycle and pregnancy, *Brain Res. Bull.* 65 (2005) 219–223.
- [58] W.H. Bulger, D. Kupfer, Estrogenic action of DDT analogs, *Am. J. Industr. Med.* 4 (1983) 163–173.
- [59] A.M. Calafat, Z. Kuklenyik, J.A. Reidy, S.P. Caudill, J. Ekong, J.L. Needham, Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population, *Environm. Health Persp.* 113 (2005) 391–395.
- [60] C. Carere, J. Balthazart, Sexual versus individual differentiation: the controversial role of avian maternal hormones, *Trends Endocrinol. Metab.* 18 (2007) 73–80.
- [61] A. Carlsson, On the neuronal circuitries and neurotransmitters involved in the control of locomotor activity, *J. Neural. Transm. Suppl.* 40 (1993) 1–12.
- [62] A. Cassidy, Potential risks and benefits of phytoestrogen-rich diets, *Int. J. Vitam. Nutr. Res.* 73 (2003) 120–126.
- [63] C. Castagna, P. Absil, A. Foidart, J. Balthazart, Systemic and intracerebroventricular injections of vasotocin inhibit appetitive and consummatory components of male sexual behavior in Japanese quail, *Behav. Neurosci.* 112 (1998) 233–250.
- [64] A. Cimini, E. Benedetti, L. Cristiano, P. Sebastiani, M.A. D'Amico, B. D'Angelo, S. Di Loreto, Expression of peroxisome proliferator-activated receptors (PPARs) and retinoic acid receptors (RXRs) in rat cortical neurons, *Neuroscience* 130 (2005) 325–337.
- [65] J.G. Clement, A.B. Okey, Estrogenic and anti-estrogenic effects of DDT administered in the diet to immature female rats, *Can. J. Physiol. Pharmacol.* 50 (1972) 971–975.
- [66] T. Colborn, M.J. Smolen, R. Rolland, Environmental neurotoxic effects: the search for new protocols in functional teratology, *Environm. Health Persp.* 14 (1998) 9–23.
- [67] T. Colborn, F.S. vom Saal, A.M. Soto, Developmental effects of endocrine-disrupting chemicals in wildlife and humans, *Environm. Health Persp.* 101 (1993) 378–384.
- [68] B. Cooke, C.D. Hegstrom, L.S. Villeneuve, S.M. Breedlove, Sexual differentiation of the vertebrate brain: principles and mechanisms, *Front. Neuroendocrinol.* 19 (1998) 323–362.
- [69] B. Cozzi, C. Viglietti-Panzica, N. Aste, G.C. Panzica, The serotonergic system of the Japanese quail brain. An immunohistochemical study, *Cell Tissue Res.* 263 (1991) 271–284.
- [70] D. Crews, E. Willingham, J.K. Skipper, Endocrine disruptors: present issues, future directions, *Quart. Rev. Biol.* 75 (2000) 243–260.
- [71] A.M. Cummings, Methoxychlor as a model for environmental estrogens, *Crit. Rev. Toxicol.* 27 (1997) 367–379.
- [72] P. D'Ursi, E. Salvi, P. Fossa, L. Milanese, E. Rovida, Modelling the interaction of steroid receptors with endocrine disrupting chemicals, *BMC Bioinform.* 6 (Suppl 4) (2005) S10.
- [73] C. Darwin, *The Descent of Man*, Murray, J., London, 1871.
- [74] G.J. De Vries, Minireview: sex differences in adult and developing brains: compensation, compensation, compensation, *Endocrinology* 145 (2004) 1063–1068.
- [75] G. Dell'omo (Ed.), *Behavioural Ecotoxicology*, J. Wiley, New York, 2002.
- [76] D. Della Seta, I. Minder, V. Belloni, A.M. Aloisi, F. Dessi-Fulgheri, F. Farabolini, Pubertal exposure to estrogenic chemicals affects

- behavior in juvenile and adult male rats, *Horm. Behav.* 50 (2006) 301–307.
- [77] F. Dessi-Fulgheri, S. Porrini, F. Farabollini, Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats, *Environm. Health Persp.* 110 (Suppl 3) (2002) 403–407.
- [78] R.A. Dixon, D. Ferreira, Genistein, *Phytochemistry* 60 (2002) 205–211.
- [79] E.C. Dodds, L. Goldberg, W. Lawson, R. Robinson, Oestrogenic activity of certain synthetic compounds, *Nature* 141 (1938) 247–248.
- [80] ECSCF, European Commission – Scientific Committee on Food. Opinion on Bisphenol A, http://ec.europa.eu/food/fs/sc/scf/out128_en.pdf, 2002, pp. 1–22.
- [81] EDSTAC, Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC). Final Report, Environmental Protection Agency, Washington, DC, USA, 1998.
- [82] A. Elbrecht, R.G. Smith, Aromatase enzyme activity and sex determination in chickens, *Science* 255 (1992) 467–470.
- [83] K.A. Faber, C.L. Hughes, The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat, *Biol. Reprod.* 45 (1991) 649–653.
- [84] K.A. Faber, C.L. Hughes, Dose–response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats, *Reprod. Toxicol.* 7 (1993) 35–39.
- [85] F. Farabollini, S. Porrini, D. Della Seta, F. Bianchi, F. Dessi-Fulgheri, Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats, *Environm. Health Persp.* 110 Suppl. 3 (2002) 409–414.
- [86] F. Farabollini, S. Porrini, F. Dessi-Fulgheri, Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats, *Pharm. Biochem. Behav.* 64 (1999) 687–694.
- [87] N.G. Forger, Cell death and sexual differentiation of the nervous system, *Neuroscience* 138 (2006) 929–938.
- [88] M. Furuya, F. Sasaki, A.M.A. Hassanin, S. Kuwahara, Y. Tsukamoto, Effects of bisphenol-A on the growth of comb and testes of male chicken, *Can. J. Vet. Res.* 67 (2002) 68–71.
- [89] H. Galbraith, Response of cattle and sheep to hormonal anabolic compounds, *Vet. Res. Commun.* 7 (1983) 27–34.
- [90] L. Gioiosa, E. Fissore, G. Ghirardelli, S. Parmigiani, P. Palanza, Developmental exposure to estrogenic endocrine disruptors alters sex differences in exploration and emotionality in mice, *Horm. Behav.* 52 (2007) 307–316.
- [91] R.A. Gorski, J.E. Gordon, J.E. Shryne, A.M. Southam, Evidence for a morphological sex difference within the medial preoptic area of the rat brain, *Brain Res.* 148 (1978) 333–346.
- [92] J.C. Gould, L.S. Leonard, S.C. Maness, B.L. Wagner, K. Conner, T. Zacharewski, S. Safe, D.P. McDonnell, K.W. Gaido, Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol, *Mol. Cell. Endocrinol.* 142 (1998) 203–214.
- [93] R.W. Goy, B.S. McEwen, *Sexual Differentiation of the Brain*, MIT Press, Cambridge, Ma, 1980.
- [94] L.E. Gray, J. Ostby, R.L. Cooper, W.R. Kelce, The estrogenic and androgenic pesticide methoxychlor alters the reproductive tract and behavior without affecting pituitary size or LH and prolactin secretion in male rats, *Toxicol. Industr. Health* 15 (1999) 37–47.
- [95] R. Grossmann, A. Jurkevich, Estradiol treatment during embryogenesis affects masculine behavior and sex dimorphic vasotocin system in adult male chickens, *Soc. Neurosci. Abstr.* 26 (2000) 306.
- [96] R. Grossmann, A. Jurkevich, A. Kohler, Sex dimorphism in the avian arginine vasotocin system with special emphasis to the bed nucleus of the stria terminalis, *Comp. Biochem. Physiol. A Comp. Physiol.* 131 (2002) 833–837.
- [97] A. Guillamon, M.R. De Blas, S. Segovia, Effects of sex steroids on the development of the locus coeruleus in the rat, *Dev. Brain Res.* 40 (1988) 306–310.
- [98] A. Guillamon, S. Segovia, Sex differences in the vomeronasal system, *Brain Res. Bull.* 44 (1997) 377–382.
- [99] R. Hackl, V. Bromundt, J. Daisley, K. Kotschal, E. Mostl, Distribution and origin of steroid hormones in the yolk of Japanese quail eggs (*Coturnix coturnix japonica*), *J. Comp. Physiol. B* 173 (2003) 327–331.
- [100] K. Halldin, Impact of endocrine disrupting chemicals on reproduction in Japanese quail, *Domest. Anim. Endocrinol.* 29 (2005) 420–429.
- [101] K. Halldin, J. Axelsson, B. Brunstrom, Effects of endocrine modulators on sexual differentiation and reproductive function in male Japanese quail, *Brain Res. Bull.* 65 (2005) 211–218.
- [102] K. Halldin, C. Berg, I. Brandt, B. Brunström, Sexual behavior in Japanese quail as a test end point for endocrine disruption: effects of in ovo exposure to ethinylestradiol and diethylstilbestrol, *Environ. Health Perspect.* 107 (1999) 861–866.
- [103] K. Halldin, L. Holm, Y. Ridderstråle, B. Brunström, Reproductive impairment in Japanese quail (*Coturnix japonica*) after in ovo exposure to *o,p'*-DDT, *Archiv. Toxicol.* 77 (2003) 116–122.
- [104] A.M. Hanafy, T. Sasanami, M. Mori, Sensitivity of expression of perivitelline membrane glycoprotein ZP1 mRNA in the liver of Japanese quail (*Coturnix japonica*) to estrogenic compounds, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 144 (2007) 356–362.
- [105] J.J. Heindel, Role of exposure to environmental chemicals in the developmental basis of disease and dysfunction, *Reprod. Toxicol.* 23 (2007) 257–259.
- [106] S. Honma, A. Suzuki, D.L. Buchanan, Y. Katsu, H. Watanabe, T. Iguchi, Low dose effects of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction, *Reprod. Toxicol.* 16 (2002) 117–122.
- [107] K.L. Howdeshell, A.K. Hotchkiss, K.A. Thayer, J.G. Vandenberg, F.S. vom Saal, Exposure to bisphenol A advances puberty, *Nature* 401 (1999) 763–764.
- [108] E.M. Hull, J.M. Dominguez, Getting his act together: roles of glutamate, nitric oxide, and dopamine in the medial preoptic area, *Brain Res.* 1125 (2006) 66–75.
- [109] E.M. Hull, J. Du, D.S. Lorrain, L. Matuszewich, Testosterone, preoptic dopamine and copulation in male rats, *Brain Res. Bull.* 44 (1997) 327–333.
- [110] E.M. Hull, J.W. Muschamp, S. Sato, Dopamine and serotonin: influences on male sexual behavior, *Physiol. Behav.* 83 (2004) 291–307.
- [111] Y. Ikezuki, O. Tsutsumi, Y. Takai, Y. Kamei, Y. Taketani, Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure, *Hum. Reprod.* 17 (2002) 2839–2841.
- [112] A.N. Iwaniuk, D.T. Koperski, K.M. Cheng, J.E. Elliott, L.K. Smith, L.K. Wilson, D.R. Wylie, The effects of environmental exposure to DDT on the brain of a songbird: changes in structures associated with mating and song, *Behav. Brain Res.* 173 (2006) 1–10.
- [113] A.L. Johnson, S.E. File, Sex differences in animal tests of anxiety, *Phys. Behav.* 49 (1991) 245–250.
- [114] A. Jurkevich, R. Grossmann, Vasotocin and reproductive functions of the domestic chicken, *Domest. Anim. Endocrinol.* 25 (2003) 93–99.
- [115] A. Jurkevich, R. Grossmann, J. Balthazart, C. Viglietti-Panzica, Gender-related changes in the avian vasotocin system during ontogeny, *Microsc. Res. Techniq.* 55 (2001) 27–36.
- [116] W.M.J. Kaylor, C.H. Song, S.J. Copeland, F.P. -Zuspan, M.H. Kim, The effect of estrogen on monoamine systems in the fetal rat brain, *J. Reprod. Med.* 29 (1984) 489–492.
- [117] W.R. Kelce, C.R. Stone, S.C. Laws, L.E. Gray, J.A. Kemppainen, E.M. Wilson, Persistent DDT metabolite *p,p'*-DDE is a potent androgen receptor antagonist, *Nature* 375 (1995) 581–585.
- [118] P.H. Kelly, P.W. Seviour, S.D. Iversen, Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, *Brain Res.* 94 (1975) 507–522.

- [119] H.S. Kim, S.Y. Han, S.D. Yoo, B.M. Lee, K.L. Park, Potential estrogenic effects of bisphenol-A estimated by in vitro and in vivo combination assays, *J. Toxicol. Sci.* 26 (2001) 111–118.
- [120] S. Kim, H.J. Shin, S.Y. Kim, J.H. Kim, Y.S. Lee, D.H. Kim, M.O. Lee, Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPARalpha, *Mol. Cell. Endocrinol.* 220 (2004) 51–58.
- [121] C. Koopman-Esseboom, N. Weisglas-Kuperus, M.A. de Ridder, C.G. Van der Paauw, L.G. Tuinstra, P.J. Sauer, Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development, *Pediatrics* 97 (1996) 700–706.
- [122] D. Kostelac, G. Rechkemmer, K. Briviba, Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element, *J. Agricult. Food Chem.* 51 (2003) 7632–7635.
- [123] A.V. Krishnan, P. Stathis, S.F. Permeth, L. Tokes, D. Feldman, Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving, *Endocrinology* 132 (1993) 2279–2286.
- [124] K. Kubo, O. Arai, R. Ogata, M. Omura, T. Hori, S. Aou, Exposure to bisphenol A during the fetal and suckling periods disrupts sexual differentiation of the locus coeruleus and of behavior in the rat, *Neurosci. Lett.* 304 (2001) 73–76.
- [125] K. Kubo, O. Arai, M. Omura, R. Watanabe, R. Ogata, S. Aou, Low dose effects of bisphenol A on sexual differentiation of the brain and behavior in rats, *Neurosci. Res.* 45 (2003) 345–356.
- [126] A.E. Kudwa, V. Michopoulos, J.D. Gatewood, E.F. Rissman, Roles of estrogen receptors alpha and beta in differentiation of mouse sexual behavior, *Neuroscience* 138 (2006) 921–928.
- [127] G.G. Kuiper, B. Carlsson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson, J.A. Gustafsson, Comparison of ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta, *Endocrinology* 138 (1997) 863–870.
- [128] G. Laviola, G. Dell'Omo, F. Chiarotti, G. Bignami, D-Amphetamine conditioned place preference in developing mice: Relation with changes in activity and stereotypies, *Behav. Neurosci.* 108 (1994) 514–524.
- [129] G. Laviola, L. Gioiosa, W. Adriani, P. Palanza, d-Amphetamine-related reinforcing effects are reduced in mice exposed prenatally to estrogenic endocrine disruptors, *Brain Res. Bull.* 65 (2005) 235–240.
- [130] S.C. Laws, S.A. Carey, J.M. Ferrell, G.J. Bodman, R.L. Cooper, Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats, *Toxicol. Sci.* 54 (2000) 154–167.
- [131] E.D. Lephart, R.W. Rhees, K.D. Setchell, L.H. Bu, T.D. Lund, Estrogens and phytoestrogens: brain plasticity of sexually dimorphic brain volumes, *J. Steroid Biochem. Mol. Biol.* 85 (2003) 299–309.
- [132] E.D. Lephart, K.D.R. Setchell, T.D. Lund, Phytoestrogens: hormonal action and brain plasticity, *Brain Res. Bull.* 65 (2005) 193–198.
- [133] J.R. Levy, K.A. Faber, L. Ayyash, C.L. Hughes, The effect of prenatal exposure to the phytoestrogen genistein on sexual-differentiation in rats, *Proc. Soc. Exp. Biol. Med.* S208 (1995) 60–66.
- [134] R.W. Lewis, N. Brooks, G.M. Milburn, A. Soames, S. Stone, M. Hall, J. Ashby, The effects of the phytoestrogen genistein on the postnatal development of the rat, *Toxicol. Sci.* 71 (2003) 74–83.
- [135] F. Lin, J. Wu, M.A. Abdelnabi, M.A. Ottinger, M.M. Giusti, Effects of dose and glycosylation on the transfer of genistein into the eggs of the Japanese quail (*Coturnix japonica*), *J. Agricult. Food Chem.* 52 (2004) 2397–2403.
- [136] N.J. MacLusky, T. Hajszán, C. Leranth, The environmental estrogen bisphenol-A inhibits estradiol-induced hippocampal synaptogenesis, *Environ. Health Perspect.* 113 (2005) 675–679.
- [137] C.M. Markey, M.A. Coombs, C. Sonnenschein, A.M. Soto, Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid-hormone target organs, *Evol. Dev.* 5 (2003) 1–9.
- [138] C.M. Markey, E.H. Luque, M.M. Munoz de Toro, C. Sonnenschein, A.M. Soto, In utero exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland, *Biol. Reprod.* 65 (2001) 1215–1223.
- [139] M.E. Martin, M. Haourigui, C. Pelissero, C. Benassayag, E.A. Nunez, Interactions between phytoestrogens and human sex steroid binding protein, *Life Sci.* 58 (1996) 429–436.
- [140] M. Martini, D. Miceli, P. Palanza, C. Viglietti-Panzica, G.C. Panzica, Effects of bisphenol A on the hypothalamic nitricergic system of CD1 mouse, *Trabaj. Inst. Cajal* 81 (2007) 185–186.
- [141] J. Masur, M.T. Schutz, R. Boerngen, Gender differences in open-field behavior as a function of age, *Dev. Psychobiol.* 13 (1980) 107–110.
- [142] N. Masutomi, M. Shibutani, H. Takagi, C. Uneyama, N. Takahashi, M. Hirose, Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life, *Toxicology* 192 (2003) 149–170.
- [143] M.M. McCarthy, A.T.M. Konkle, When is a sex difference not a sex difference? *Front. Neuroendocrinol.* 26 (2005) 85–102.
- [144] B.S. McEwen, Estrogens effects on the brain: multiple sites and molecular mechanisms, *J. Appl. Physiol.* 91 (2001) 2785–2801.
- [145] B.S. McEwen, S.E. Alves, Estrogen actions in the central nervous system, *Endocr. Rev.* 20 (1999) 279–307.
- [146] J.A. McLachlan, Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals, *Endocr. Rev.* 22 (2001) 319–341.
- [147] R.C. Melcangi, G.C. Panzica, Neuroactive steroids: old players in a new game, *Neuroscience* 138 (2006) 733–739.
- [148] O. Mezei, W.J. Banz, R.W. Steger, M.R. Peluso, T.A. Winters, N. Shay, Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells, *J. Nutr.* 133 (2003) 1238–1243.
- [149] S.R. Milligan, O. Khan, M. Nash, Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (*Oncorhynchus mykiss*) plasma, *Gen. Comp. Endocrinol.* 112 (1998) 89–95.
- [150] K. Morito, T. Hirose, J. Kinjo, T. Hirakawa, M. Okawa, T. Nohara, S. Ogawa, S. Inoue, M. Muramatsu, Y. Masamune, Interaction of phytoestrogens with estrogen receptors alpha and beta, *Biol. Pharm. Bull.* 24 (2001) 351–356.
- [151] S.O. Mueller, Xenoestrogens: mechanisms of action and detection methods, *Anal. Bioanal. Chem.* 378 (2004) 582–587.
- [152] M.M. Munoz de Toro, C.M. Markey, P.R. Wadia, E.H. Luque, B.S. Rubin, C. Sonnenschein, A.M. Soto, Perinatal exposure to bisphenol A alters peripubertal mammary gland development in mice, *Endocrinology* 146 (2005) 4138–4147.
- [153] F. Naftolin, M.G. Stanbury, Phytoestrogens: are they really estrogen mimics? *Fertil. Steril.* 77 (2002) 15–17.
- [154] T. Nagao, Y. Saito, K. Usumi, M. Kuwagata, K. Imai, Reproductive function in rats exposed neonatally to bisphenol A and estradiol benzoate, *Reprod. Toxicol.* 13 (1999) 303–311.
- [155] S.C. Nagel, F.S. vom Saal, K.A. Thayer, M.G. Dhar, M. Boechler, W.V. Welshons, Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol, *Environ. Health Perspect.* 105 (1997) 70–76.
- [156] M. Narita, K. Miyagawa, K. Mizuo, T. Yoshida, T. Suzuki, Changes in central dopaminergic systems and morphine reward by prenatal and neonatal exposure to bisphenol-A in mice: evidence for the importance of exposure period, *Addict. Biol.* 12 (2007) 167–172.
- [157] E. Oberdorster, M.A. Clay, D.M. Cottam, F.A. Wilmot, J.A. McLachlan, M.J. Milner, Common phytochemicals are ecdysteroid agonists and antagonists: a possible evolutionary link between vertebrate and invertebrate steroid hormones, *J. Steroid Biochem. Mol. Biol.* 77 (2001) 229–238.
- [158] S. Ogawa, D.B. Lubahn, K.S. Korach, D.W. Pfaff, Behavioral effects of estrogen receptor gene disruption in male mice, *Proc. Natl. Acad. Sci. USA* 94 (1997) 1476–1481.

- [159] N. Olea, R. Pulsar, P. Perez, F.F. Olea-Serrano, A. Riva, A. Novillo-Fertrell, V. Pedraza, A.M. Soto, C. Sonnenschein, Estrogenicity of resin-based composites and sealants used in dentistry, *Environ. Health Perspect.* 104 (1996) 298–305.
- [160] M.A. Ottinger, M.A. Abdelnabi, P. Henry, S. McGary, N. Thompson, J.M. Wu, Neuroendocrine and behavioral implications of endocrine disrupting chemicals in quail, *Horm. Behav.* 40 (2001) 234–247.
- [161] M.A. Ottinger, M.R. Bakst, Peripheral androgen concentrations and testicular morphology in embryonic and young male Japanese quail, *Gen. Comp. Endocrinol.* 43 (1981) 170–177.
- [162] M.A. Ottinger, J. Balthazart, Altered endocrine and behavioral responses with reproductive aging in the male Japanese quail, *Horm. Behav.* 20 (1986) 83–94.
- [163] M.A. Ottinger, H.J. Brinkley, The relationship of testosterone and sexual behavior during maturation of the male Japanese quail, *Horm. Behav.* 11 (1978) 174–182.
- [164] M.A. Ottinger, S. Pitts, M.A. Abdelnabi, Steroid hormones during embryonic development in Japanese quail: plasma, gonadal, and adrenal levels, *Poultry Sci.* 80 (2001) 795–799.
- [165] M.A. Ottinger, F.S. vom Saal, Impact of environmental endocrine disruptors on sexual differentiation in birds and mammals, in: D. Pfaff, A. Arnold, A.M. Etgen, R. Rubin (Eds.), *Hormones and Behavior in Higher Vertebrates*, Academic Press, New York, 2002, pp. 325–383.
- [166] M.A. Ottinger, J.M. Wu, J.L. Hazelton, M.A. Abdelnabi, N. Thompson, M.L. Quinn, D. Donoghue, S.F.M. Ruscio, J. Beavers, M. Jaber, Assessing the consequences of the pesticide methoxychlor: neuroendocrine and behavioral measures as indicators of biological impact of an estrogenic environmental chemical, *Brain Res. Bull.* 65 (2005) 199–210.
- [167] P. Palanza, L. Gioiosa, S. Parmigiani, Social stress in mice: gender differences and effects of estrous cycle and social dominance, *Physiol. Behav.* 73 (2001) 411–420.
- [168] P. Palanza, F. Morellini, S. Parmigiani, F.S. vom Saal, Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development, *Neurosci. Biobehav. Rev.* 23 (1999) 1011–1027.
- [169] P. Palanza, F. Morellini, S. Parmigiani, F.S. vom Saal, Ethological methods to study the effects of maternal exposure to estrogenic endocrine disrupters. A study with methoxychlor, *Neurotoxicol. Teratol.* 24 (2002) 55–69.
- [170] P. Palanza, S. Parmigiani, H. Liu, F.S. vom Saal, Prenatal exposure to low doses of the estrogenic chemicals diethylstilbestrol and *o,p'*-DDT alters aggressive behavior of male and female house mice, *Pharmacol. Biochem. Behav.* 64 (1999) 665–672.
- [171] P. Palanza, S. Parmigiani, F.S. Vom Saal, Effects of prenatal exposure to low doses of diethylstilbestrol, *o,p'*-DDT and methoxychlor on postnatal growth and neurobehavioral development in male and female mice, *Horm. Behav.* 40 (2001) 252–265.
- [172] P. Palanza, K.L. Howdeshell, S. Parmigiani, F.S. vom Saal, Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice, *Environ. Health Perspect.* 110 Suppl 3 (2002) 415–422.
- [173] G.C. Panzica, N. Aste, C. Castagna, C. Viglietti-Panzica, J. Balthazart, Steroid-induced plasticity in the sexually dimorphic vasotocinergic innervation of the avian brain: behavioral implications, *Brain Res. Rev.* 37 (2001) 178–200.
- [174] G.C. Panzica, N. Aste, C. Viglietti-Panzica, M.A. Ottinger, Structural sex differences in the brain: influence of gonadal steroids and behavioral correlates, *J. Endocrinol. Invest.* 18 (1995) 232–252.
- [175] G.C. Panzica, J. Balthazart, M. Pessatti, C. Viglietti-Panzica, The parvocellular vasotocin system of Japanese quail: a developmental and adult model for the study of influences of gonadal hormones on sexually differentiated and behaviorally relevant neural circuits, *Environ. Health Perspect.* 110 (Suppl.3) (2002) 423–428.
- [176] G.C. Panzica, C. Castagna, C. Viglietti-Panzica, C. Russo, O. Tlemçani, J. Balthazart, Organizational effects of estrogens on brain vasotocin and sexual behavior in quail, *J. Neurobiol.* 37 (1998) 684–699.
- [177] G.C. Panzica, E. Garcia-Ojeda, C. Viglietti-Panzica, N.E. Thompson, M.A. Ottinger, Testosterone effects on vasotocinergic innervation of sexually dimorphic medial preoptic nucleus and lateral septum during aging in male quail, *Brain Res.* 712 (1996) 190–198.
- [178] G.C. Panzica, E. Mura, C. Barale, M.J. Quinn, N. Thompson, C. Viglietti-Panzica, M.A. Ottinger, Embryonic exposure to estrogenic and androgenic endocrine disrupting chemicals alters male quail sexual behavior and vasotocin system, *Soc. Neurosci. Abstr.* 320 (2005).
- [179] G.C. Panzica, M. Pessatti, C. Viglietti-Panzica, R. Grossmann, J. Balthazart, Effects of testosterone on sexually dimorphic parvocellular neurons expressing vasotocin mRNA in the male quail brain, *Brain Res.* 850 (1999) 55–62.
- [180] G.C. Panzica, C. Viglietti Panzica, M.A. Ottinger, Introduction: neurobiological impact of environmental estrogens, *Brain Res. Bull.* 65 (2005) 187–191.
- [181] G.C. Panzica, C. Viglietti-Panzica, J. Balthazart, The sexually dimorphic medial preoptic nucleus of quail: a key brain area mediating steroid action on male sexual behavior, *Front. Neuroendocrinol.* 17 (1996) 51–125.
- [182] G.C. Panzica, C. Viglietti-Panzica, J. Balthazart, Sexual dimorphism in the neuronal circuits of the quail preoptic and limbic regions, *Microsc. Res. Techniq.* 54 (2001) 364–374.
- [183] G.C. Panzica, C. Viglietti-Panzica, F. Sanchez, P. Sante, J. Balthazart, Effects of testosterone on a selected neuronal population within the preoptic sexually dimorphic nucleus of the Japanese quail, *J. Comp. Neurol.* 303 (1991) 443–456.
- [184] H.B. Patisaul, Phytoestrogen action in the adult and developing brain, *J. Neuroendocrinol.* 17 (2005) 57–64.
- [185] H.B. Patisaul, M. Dindo, P.L. Whitten, L.J. Young, Soy isoflavone supplements antagonize reproductive behavior and estrogen receptor alpha- and beta-dependent gene expression in the brain, *Endocrinology* 142 (2001) 2946–2952.
- [186] H.B. Patisaul, A.E. Fortino, E.K. Polston, Neonatal genistein or bisphenol-A expose alters sexual differentiation of the AVPV, *Neurotoxicol. Teratol.* 28 (2006) 111–118.
- [187] H.B. Patisaul, A.E. Fortino, E.K. Polston, Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A, *Neurotoxicology* 28 (2007) 1–12.
- [188] H.B. Patisaul, M. Melby, P.L. Whitten, L.J. Young, Genistein affects ER β - but not ER α -dependent gene expression in the hypothalamus, *Endocrinology* 143 (2002) 2189–2197.
- [189] S.M. Paul, R.H. Purdy, Neuroactive steroids, *FASEB J.* 6 (1992) 2311–2322.
- [190] C. Pelissero, M.J. Lenczowski, D. Chinzi, B. Davail-Cuisset, J.P. Sumpter, A. Fostier, Effects of flavonoids on aromatase activity, an in vitro study, *J. Steroid Biochem. Mol. Biol.* 57 (1996) 215–223.
- [191] S.E. Perez, E.Y. Chen, E.J. Mufson, Distribution of estrogen receptor alpha and beta immunoreactive profiles in the postnatal rat brain, *Dev. Brain Res.* 145 (2003) 117–139.
- [192] T. Perrot-Sinal, K.P. Ossenkopp, M. Kavaliers, Influence of a natural stressor (predator odor) on locomotor activity in the meadow vole (*Microtus pennsylvanicus*): modulation by sex, reproductive condition and gonadal hormones, *Psychoneuroendocrinology* 25 (2000) 259–276.
- [193] H. Pinos, P. Collado, M. Rodríguez-Zafra, C. Rodríguez, S. Segovia, A. Guillamón, The development of sex differences in the locus coeruleus of the rat, *Brain Res. Bull.* 56 (2001) 73–78.
- [194] D. Ponzi, P. Palanza, J.A. Maruniak, S. Parmigiani, F.S. Vom Saal, Sexual dimorphism in the number of TH-immunostained neurons in the Locus Coeruleus of young mice is eliminated by prenatal exposure to Bisphenol A, in: 5th Forum of European Neuroscience Abstract (2006) A008.21.

- [195] M.J. Quinn Jr., M.A. Ottinger, Embryonic effects of androgen active endocrine disrupting chemicals on avian immune and reproductive systems, *J. Poultry Sci.* 43 (2006) 1–11.
- [196] M.J. Quinn Jr., C.L. Summitt, M.A. Ottinger, Effects of androgen disruption by DDE on the development and functioning of the immune system in Japanese quail, *Immunopharmacol. Immunotoxicol.* 28 (2006) 535–544.
- [197] G. Raisman, P.M. Field, Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen, *Brain Res.* 54 (1973) 1–29.
- [198] B.A. Rattner, R.N. Clarke, M.A. Ottinger, Depression of plasma luteinizing hormone concentration in quail by the anticholinesterase insecticide parathion, *Comp. Biochem. Physiol.* 83C (1986) 451–453.
- [199] M. Razzoli, P. Valsecchi, P. Palanza, Chronic exposure to low doses bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils, *Brain Res. Bull.* 65 (2005) 249–254.
- [200] A.B. Ropero, P. Alonso-Magdalena, C. Ripoll, E. Fuentes, A. Nadal, Rapid endocrine disruption: environmental estrogen actions triggered outside the nucleus, *J. Steroid Biochem. Mol. Biol.* 102 (2006) 163–169.
- [201] B.S. Rubin, J.R. Lenkowski, C.M. Schaeberle, L.N. Vandenberg, P.M. Ronsheim, A.M. Soto, Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol, *Endocrinology* 147 (2006) 3681–3691.
- [202] B.S. Rubin, M.K. Murray, D.A. Damassa, J.C. King, A.M. Soto, Perinatal exposure to low doses of bisphenol-A affects body weight, patterns of estrous cyclicity and plasma LH levels, *Environ. Health Perspect.* 109 (2001) 675–680.
- [203] B.C. Ryan, J.G. Vanderbergh, Developmental exposure to environmental estrogens alters anxiety and spatial memory in female mice, *Horm. Behav.* 50 (2006) 85–93.
- [204] S.M. Sato, E.M. Hull, The nitric oxide-guanosine 3', 5'-cyclic monophosphate pathway regulates dopamine efflux in the medial preoptic area and copulation in male rats, *Neuroscience* 139 (2006) 417–428.
- [205] G. Schonfelder, W. Wittfoht, H. Hopp, C.E. Talsness, M. Paul, I. Chahoud, Parent bisphenol A accumulation in the human maternal-fetal-placental unit, *Environ. Health Perspect.* 110 (2002) A703–A707.
- [206] M. Schumacher, J. Balthazart, The effects of testosterone and its metabolites on sexual behavior and morphology in male and female Japanese quail, *Physiol. Behav.* 30 (1983) 335–339.
- [207] M. Schumacher, J.J. Legros, J. Balthazart, Steroid hormones, behavior and sexual dimorphism in animals and men: the nature-nurture controversy, *Exp. Clin. Endocrinol.* 90 (1987) 129–156.
- [208] R.F. Seegal, K.O. Brosch, R.J. Okoniewski, Effects of in utero and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function, *Toxicol. Appl. Pharmacol.* 146 (1997) 95–103.
- [209] R.B. Simerly, Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain, *Ann. Rev. Neurosci.* 25 (2002) 507–536.
- [210] R.B. Simerly, L.W. Swanson, R.A. Gorski, The distribution of monoaminergic cells and fibers in a periventricular preoptic nucleus involved in the control of gonadotropin release: immunohistochemical evidence for a dopaminergic sexual dimorphism, *Brain Res.* 330 (1985) 55–64.
- [211] D.W. Singleton, S.A. Khan, Xenoestrogen exposure and mechanisms of endocrine disruption, *Front. Biosci.* 8 (2003) s110–s118.
- [212] A.E. Smits-van Prooije, D.H. Waalkens-Berendsen, D.C. Morse, C. Koopman-Esseboom, M. Huisman, P.J. Sauer, E.R. Boersma, J.H. Lammers, K.J. van den Berg, G.C. van der Paauw, B.M. Kulig, N.J. Snoeij, The effects on mammals of pre- and postnatal environmental exposure to PCBs. the Dutch collaborative PCB/dioxin study, *Arch. Toxicol. Suppl.* 18 (1996) 97–102.
- [213] D.M. Staton, P.R. Solomon, Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differentially affects stereotypy and locomotion in the rat, *Physiol. Psychol.* 12 (1984) 159–162.
- [214] R. Steinmetz, N.G. Brown, D.L. Allen, R.M. Bigsby, N. Ben-Jonathan, The environmental estrogen bisphenol A stimulates prolactin release in vitro and in vivo, *Endocrinology* 138 (1997) 1780–1786.
- [215] T. Suzuki, K. Mizuo, H. Nakazawa, Y. Funae, S. Fushiki, S. Fukushima, T. Shirai, M. Narita, Prenatal and neonatal exposure to bisphenol-A enhances the central dopamine D1 receptor-mediated action in mice: Enhancement of the metamphetamine-induced abuse state, *Neuroscience* 117 (2003) 639–644.
- [216] S.H. Swan, R.L. Kruse, L. Fan, D.B. Barr, E.Z. Drobnis, J.B. Redmon, C. Wang, C. Brazil, J.W. Overstreet, Semen quality in relation to biomarkers of pesticide exposure, *Environ. Health Perspect.* 111 (2003) 1478–1484.
- [217] A. Tohei, S. Suda, K. Taya, T. Hashimoto, H. Kogo, Bisphenol A inhibits testicular functions and increases luteinizing hormone secretion in adult male rats, *Exp. Biol. Med.* 226 (2001) 216–221.
- [218] K. Tsutsui, H. Sakamoto, H. Shikimi, K. Ukena, Organizing actions of neurosteroids in the Purkinje neuron, *Neurosci. Res.* 49 (2004) 273–279.
- [219] T. Usui, Pharmaceutical prospects of phytoestrogens, *Endocr. J.* 53 (2006) 7–20.
- [220] L.N. Vandenberg, M.V. Maffini, P.R. Wadia, C. Sonnenschein, B.S. Rubin, A.M. Soto, Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters development of the fetal mouse mammary gland, *Endocrinology* 148 (2007) 116–127.
- [221] C. Viglietti-Panzica, G.C. Anselmetti, J. Balthazart, N. Aste, G.C. Panzica, Vasotocinergic innervation of the septal region in the Japanese quail: sexual differences and the influence of testosterone, *Cell Tissue Res.* 267 (1992) 261–265.
- [222] C. Viglietti-Panzica, N. Aste, J. Balthazart, G.C. Panzica, Vasotocinergic innervation of sexually dimorphic medial preoptic nucleus of the male Japanese quail: influence of testosterone, *Brain Res.* 657 (1994) 171–184.
- [223] C. Viglietti-Panzica, J. Balthazart, S. Fratesi, L. Plumari, P. Absil, G.C. Panzica, Estradiol mediates effects of testosterone on vasotocin-immunoreactivity in the adult quail brain, *Horm. Behav.* 40 (2001) 445–461.
- [224] C. Viglietti-Panzica, B. Montoncello, E. Mura, M. Pessatti, G.C. Panzica, Organizational effects of diethylstilbestrol on brain vasotocin and sexual behavior in male quail, *Brain Res. Bull.* 65 (2005) 225–233.
- [225] C. Viglietti-Panzica, E. Mura, G.C. Panzica, Effects of early embryonic exposure to genistein on male copulatory behavior and vasotocin system of Japanese quail, *Horm. Behav.* 51 (2007) 355–363.
- [226] F.S. vom Saal, Bisphenol A eliminates brain and behavior sex dimorphisms in mice: how low can you go? *Endocrinology* 147 (2006) 3679–3680.
- [227] F.S. vom Saal, P.S. Cooke, D.L. Buchanan, P. Palanza, K.A. Thayer, S.C. Nagel, S. Parmigiani, W.V. Welshons, A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior, *Toxicol. Industr. Health* 14 (1998) 239–260.
- [228] F.S. vom Saal, C. Hughes, An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment, *Environ. Health Perspect.* 113 (2005) 926–933.
- [229] F.S. vom Saal, S.C. Nagel, B.G. Timms, W.V. Welshons, Implications for human health of the extensive bisphenol A literature showing adverse effects at low doses: a response to attempts to mislead the public, *Toxicology* 212 (2005) 244–252.
- [230] F.S. vom Saal, C.A. Richter, J. Mao, W.V. Welshons, Commercial animal feed: variability in estrogenic activity and effects on body weight in mice, *Birth Defect Res. A Clin. Mol. Teratol.* 73 (2005) 474–475.

- [231] F.S. vom Saal, W.V. Welshons, Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A, *Environ. Res.* 100 (2006) 50–76.
- [232] K. Wada, A. Nakajima, K. Katayama, C. Kudo, A. Shibuya, N. Kubota, Y. Terauchi, M. Tachibana, H. Miyoshi, Y. Kamisaki, T. Mayumi, T. Kadowaki, R.S. Blumberg, Peroxisome proliferator-activated receptor gamma-mediated regulation of neural stem cell proliferation and differentiation, *J. Biol. Chem.* 281 (2006) 12673–12681.
- [233] S.F. Wang, T.J. Ridsdill-Smith, E.L. Ghisalberti, Levels of isoflavonoids as indicators of resistance of subterranean clover trifoliates to red legged earth mite, *J. Chem. Ecol.* 25 (1999) 795–803.
- [234] B. Weiss, Sexually dimorphic nonreproductive behaviors as indicators of endocrine disruption, *Environ. Health Perspect.* 110 (Suppl. 3) (2002) 387–391.
- [235] W.V. Welshons, S.C. Nagel, K.A. Thayer, B.M. Judy, F.S. vom Saal, Low dose bioactivity of xenoestrogens in animals: fetal exposure to methoxychlor and other xenoestrogens increases adult prostate size in mice, *Toxicol. Industr. Health* 15 (1999) 12–25.
- [236] W.V. Welshons, S.C. Nagel, F.S. vom Saal, Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure, *Endocrinology* 147 (2006) S56–S69.
- [237] W.V. Welshons, K.A. Thayer, B.M. Judy, J.A. Taylor, E.M. Curran, F.S. vom Saal, Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity, *Environ. Health Perspect.* 111 (2003) 994–1006.
- [238] K.W. Wilhelms, S.A. Cutler, J.A. Proudman, L.L. Anderson, C.G. Scanes, Atrazine and the hypothalamo–pituitary–gonadal axis in sexually maturing precocial birds: studies in male Japanese quail, *Toxicol. Sci.* 86 (2005) 152–160.
- [239] K.W. Wilhelms, C.G. Scanes, L.L. Anderson, Lack of estrogenic or antiestrogenic actions of soy isoflavones in an avian model: the Japanese quail, *Poultry Sci.* 85 (2006) 1885–1889.
- [240] C.A. Wilson, D.C. Davies, The control of sexual differentiation of the reproductive system and brain, *Reproduction* 133 (2007) 331–359.
- [241] M. Yang, M.S. Park, H.S. Lee, Endocrine disrupting chemicals: human exposure and health risks, *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* 24 (2006) 183–224.
- [242] R. Zhao, Y. Wang, Y. Zhou, Y. Ni, L. Lu, R. Grossmann, J. Chen, Dietary daidzein influences laying performance of ducks (*Anas platyrhynchos*) and early post-hatch growth of their hatchlings by modulating gene expression, *Comp. Biochem. Physiol. A Comp. Physiol.* 138 (2004) 459–466.
- [243] B. Zimmerberg, M.J. Farley, Sex differences in anxiety behavior in rats: role of gonadal hormones, *Physiol. Behav.* 54 (1993) 1119–1124.
- [244] A. Zsarnovszky, H.H. Le, H.S. Wang, S.M. Belcher, Ontogeny of rapid estrogen-mediated ERK1/2 signaling in the rat cerebellar cortex in vivo: potent non-genomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A, *Endocrinology* 146 (2006) 5388–5396.