



Published in final edited form as:

*J Toxicol Environ Health B Crit Rev.* 2011 ; 14(5-7): 328–345. doi:10.1080/10937404.2011.578556.

## Early developmental actions of endocrine disruptors on the hypothalamus, hippocampus and cerebral cortex

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### Abstract

Sex steroids and thyroid hormones play a key role in the development of the central nervous system. The critical role of these hormonal systems may explain the sensitivity of the hypothalamus, the cerebral cortex and the hippocampus to endocrine disrupting chemicals (EDCs). This review examines the evidence for endocrine disruption of glial-neuronal functions in the hypothalamus, the hippocampus and the cerebral cortex. We focus on two well-studied EDCs, the insecticide dichlorodiphenyltrichloroethane (DDT) and the polychlorinated biphenyls (PCBs). DDT is involved in neuroendocrine disruption of the reproductive axis whereas PCBs interact with both the thyroid hormone- and sex steroid-dependent systems and disturb the neuroendocrine control of reproduction and the development of the hippocampus and cortex. These results highlight the impact of EDCs on the developing nervous system and the need for more research in this area.

### Keywords

diethyl-dichloroethane (DDT); Polychlorinated biphenyls (PCBs); puberty; cerebral cortex; hypothalamus

### Introduction: early regulation and disruption of hypothalamic, hippocampal and cortical function

As illustrated in Figure 1, the hypothalamus, hippocampus and cerebral cortex mediate many of the essential functions of the central nervous system. These brain regions are complex networks of neurons and surrounding glial cells, which are modulated by paracrine or autocrine neurotransmitters as well as peripheral hormones and chemicals produced in the body or in the environment. Sex steroids and thyroid hormones play a crucial role in the development of the hypothalamus, the hippocampus and the cerebral cortex. They have lifelong effects on central functions by influencing cellular proliferation, dendritic outgrowth or synaptogenesis. Structural changes in the brain following hormonal alterations during fetal and perinatal life result in functional consequences in adolescence and adulthood. Typical examples are anovulation and infertility after perinatal exposure to sex steroids (Sawaki M et al., 2003) and cognitive dysfunction after fetal hypothyroidism (DeLange F, 2000).

According to the World Health Organization, an endocrine disrupting chemical (EDC) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or

(sub)population. Knowing the critical role of sex steroids and thyroid hormones in the development of the central nervous system, one can speculate that the young brain will be particularly sensitive to endocrine disruption. Therefore, this paper will first review the role of sex steroids and thyroid hormones in the developing hypothalamus, hippocampus and cortex in mammals. Secondly, we will discuss the effects of perinatal exposure to EDCs on structural development of the cortex, hypothalamus and hippocampus during early life as well as long-term consequences on structure and function in adulthood. We will review the available evidence of endocrine disruption of neuro-glial function in those regions. Emphasis will be put on two EDCs for which the most data is available: the insecticide dichlorodiphenyltrichloroethane (DDT) which causes neuroendocrine disruption (Rasier et al., 2007 & 2008) and the polychlorinated biphenyls which interact with thyroid hormones and sex steroids and disturb neuroendocrine control of reproduction and the development of the cortex and hippocampus (Bansal, 2008; Steinberg 2008).

The insecticide DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] has been banned from the United States and Western Europe since the late 1960s but is still used in developing countries. DDT behaves as an estrogen agonist and/or androgen antagonist. Due to their long half-life and its lipophilic nature, DDT and its metabolite DDE (dichlorodiphenyl dichloroethylene) are still detected in the serum of western pregnant and lactating mothers (Llop, 2010; Glynn, 2007).

PCBs are a group of 209 different congeners used in lubricating oils and plasticizers. Because of their long half-life (Ogura, 2009), they are still ubiquitous environmental contaminants, found in high concentrations in human and animals, even though they have been banned in Europe and the USA in the seventies. Although PCBs effects on brain development have been well documented (Schantz et al, 1995 and 2003), their mode of action is still not completely understood. Based on their chemical structure, PCBs can act through different pathways (Mc Kinney & Waller, 1994). Coplanar congeners have carcinogenic, immunogenic and teratogenic effects mostly through binding to cytosolic aryl hydrocarbon receptors (AhR), a ligand-dependent transcription factor involved in cell proliferation and differentiation (Dietrich, C. & Kaina, B., 2010). However, the neurotoxic effects on development might not be entirely explained by AhR. Three types of mechanisms have been described: alteration of thyroid hormones, neurotransmission, or intracellular signaling (Kodavanti, 2006).

## **Developmental brain processes regulated by thyroid hormones and sex steroids and potentially targeted by endocrine disruption**

### **Hypothalamus and neuroendocrine system**

The neuroendocrine control of female reproduction through the preovulatory gonadotrophin surge and its alteration following exposure to sex steroids during fetal or perinatal life has been known for several decades (Gorski, 1968). Although this finding provided a rationale for studies on neuroendocrine effects of EDCs, these studies received relatively little attention compared to the direct gonadal effects on the testis and ovary (Bay et al., 2006; Sharpe et al., 2006; Skakkebaek et al., 2001; Mc Lachlan et al., 2006) as well as effects on sex steroid-sensitive peripheral structures such as the prostate or breast (Darbre et al., 2006; Fenton, 2006). This historical emphasis resulted from several issues. First, the direct gonadal and peripheral effects of EDCs complicate efforts to delineate neuroendocrine effects *in vivo* because of changes in gonadal function. In addition, the gonads and target tissues are relatively more accessible to study, and better known in terms of structure – function relationships compared to the neuroendocrine system.

Sex steroids, and more specifically estradiol, are important regulators of the neuronal control of reproduction in the hypothalamus. The gonadotropin-releasing hormone (GnRH) system controlling puberty and reproduction involves numerous types of secretory, inhibitory and excitatory neurons and glial cells. Those cells are regulated by estrogens. The effects of estrogens on different components of the GnRH system will be reviewed in this paragraph. GnRH neurons themselves express estrogen receptor beta (ER $\beta$ ) (Maffucci JA et al., 2009) but estrogen effects on GnRH neurons are mostly mediated by a very complex network of neurons such as glutamate and gamma-aminobutyric acid (GABA) neurons and glial cells expressing estrogen receptors (Maffucci JA et al., 2009). Estrogens also modulate Kisspeptin and its receptor expression, both of them being key players in the regulation of GnRH secretion. Kisspeptin mediates a negative feedback regulation of gonadotropin secretion by gonadal steroids in the arcuate nucleus.

Estrogen receptor alpha (ER $\alpha$ ) and beta are also expressed in astrocytes membrane and cytoplasmic fractions. ER $\alpha$  is able to transactivate metabotropic glutamate receptor mglur1a in astrocytes, which seems to be necessary to initiate some sexual behaviour and to induce the preovulatory luteinizing hormone (LH) surge (Micevych et al., 2010). However, astrocytes sensitivity to chemicals disrupting estrogen action is still unknown and deserves further study.

The neuroendocrine functions potentially affected by early events *in vivo* (table 1) include centrally-mediated (gonadotropin-dependent) onset of puberty (Rasier et al., 2007; Gore, 2008); ovulation that is dependent on stimulation by the gonadotropin surge (Savabieasfahani et al., 2006; Steinberg et al., 2008); and sexual behaviour (Patisaul et al., 2001; Funabashi et al., 2003; Viglietti-Panzica et al., 2005; Rubin et al., 2006; Steinberg et al., 2007). The regulation by sex steroids, and possibly disruption by EDCs, of these three sexually dimorphic processes are different in males and females in several species such as rodents or birds.

Other common endpoints in experimental studies on neuroendocrine effects include expression or transcripts of sex steroid receptors (Patisaul et al., 2001) as well as enzymes that are involved in sex steroid metabolism or dependent on sex steroids (Khan et al., 2001; Kuhl et al., 2005; Rubin et al., 2006). Indeed, it has appeared recently that alpha-fetoprotein and aromatase play a fundamental role in sexual differentiation of the hypothalamus. It appears that in fetal female mice, circulating alpha-fetoprotein binds estradiol in order to protect the brain, including the hypothalamus, from the defeminising action of this hormone that would normally occur in males in response to testosterone locally transformed in estradiol by aromatase (Bakker & Brok, 2010).

### Cerebral cortex

The role of sex steroids, particularly estradiol, in the central nervous system extends far beyond the hypothalamic/neuroendocrine control of reproduction (figure 2). Estradiol is a possible factor promoting development, function and survival of neurons (Mc Ewen and Alves, 1999) through classical genomic interactions with the nuclear ER and also non-genomic interactions with membrane receptors. Neurons, astrocytes and neuronal progenitors express ERs. In particular, astrocytes influence neural development in part by synthesizing estrogens (Garcia-Segura et al., 2006). Aromatase, expressed by radial glial cells in rodents, is activated by E2 and stimulates local E2 production (Pellegrini et al., 2007). This positive feedback loop suggests that E2 and maybe some estrogen-like EDCs may alter E2 production early in development. Interestingly, alpha-fetoprotein (AFP) is expressed at high levels in radial glial cells but at lower levels by intermediate progenitors. Thus high levels of AFP in the ventricular zone could inhibit E2-promoted proliferation in this region while low levels of AFP in the subventricular zone could allow a stronger effect

of E2 on intermediate progenitors (Martinez-Cerdeno et al., 2006). Estrogens also stimulate neurogenesis in adult rodents and increase proliferation in cortical progenitor cells by shortening the G1 phase (Martinez-Cerdeno et al., 2006). Because EDCs can affect the ER directly or indirectly through estrogen biosynthesis or metabolism, it is important that studies of the action EDCs examine those different structures and functions in the cortex.

During foetal and neonatal life, neuronal and glial proliferation, migration, and differentiation depend on thyroid hormones (figure 2). Thyroid hormone action is mediated by 2 classes of nuclear receptors (Forrest, D. & Vennstrom, B., 2000) that exhibit differential spatial and temporal expression in the brain, suggesting that thyroid hormones have multiple functions during brain development (Horn, S. & Heuer, H., 2010). Thyroid hormone receptors are expressed in neurons, astrocytes, and oligodendrocytes and precursors before the foetal thyroid is functional, suggesting a role for hormones of maternal origin. Triiodothyronine (T3) regulates the expression of genes coding for growth factors, cell surface receptors and transcription factors involved in cell cycle regulation and proliferation (reviewed in Puzianowska et al., 2006). The action of T3 is not homogenous and depends on the cell type and its developmental state. T3 blocks proliferation and induces differentiation of oligodendrocyte progenitor cells (Baas et al., 1997). This effect results from a rapid decrease of the transcription factor E2F1 in oligodendrocyte precursors, which induces a decrease of proliferation by arresting the cells in G1 and S phases (Nygard et al., 2003). Tokumoto et al. (2001) also showed that thyroid hormones promote oligodendrocyte differentiation through another pathway involving p53 proteins.

In addition to these few studies suggesting a role for thyroid hormones on cell proliferation in the cortex, several studies have reported an effect on cell migration and differentiation. For example, T4 promotes actin polymerization through non-genomic action in developing neurons (reviewed in Cheng et al., 2010). Actin polymerization is necessary to recognize the laminin guidance molecule during migration (Farwell et al., 2005). Thyroid hormones also regulate the organization of the actin cytoskeleton in astrocytes during development, thus affecting the production and deposition of laminin at the surface of astrocytes that is necessary for neuronal migration (Farwell et al., 1999). In *ex vivo* studies, maternal hypothyroxinemia alters radial and tangential neuronal migration (Lavado-Autric et al., 2003; Auso et al., 2004). In these experiments, green fluorescent protein- medial ganglionic eminence (GFP-MGE) - derived neurons from hypothyroxinemic mothers showed a normal migratory behaviour whereas GFP-MGE-neurons from normal or hypothyroxinemic mothers showed disrupted migration when explanted into the neocortex of embryos from hypothyroxinemic dams. These studies suggest a non-cell autonomous effect caused not by the migratory neurons themselves, but by elements guiding the migration (Cuevas et al., 2005). Thyroid hormones also regulate the expression and distribution of molecules such as actin or tenascin (Farwell et al., 2005; Alvarez-Dolado et al., 1998) that interact with the extracellular matrix and facilitate neurite outgrowth. Overall, these examples illustrate that thyroid hormones are involved in multiple aspects of early brain development including proliferation, differentiation and migration of progenitors. Disruption of thyroid function by EDCs such as PCBs could thus cause neurological deficits that are very similar to hypothyroidism.

## Hippocampus

Sex steroids also influence hippocampus development. Androgen receptors, ERalpha and ERbeta are expressed by neurons and glial cells throughout the hippocampus, and mediate both genomic and non-genomic effects. The primary source of estrogens is peripheral, even though hippocampal neurons seem to be able to synthesize estradiol (Moult and Harvey, 2008) from pregnenolone by cytochrome P45017alpha and P450 aromatase (Hojo et al., 2003). Androgens and estrogens sculpt the gender-specific differences in hippocampal size.

Both steroids increase the number of newborn neurons, but androgens preferentially support neurogenesis whereas estrogens promote gliogenesis (Zhang et al., 2008). Estradiol also increases the depolarizing GABA response in developing hippocampal neurons, and delays the maturational change from GABA-mediated excitation to inhibition, which is influential in brain development (Nunez et al., 2005).

Some hippocampal functions such as spatial cognition are sexually dimorphic. This dimorphism as well as sex differences in CA3 pyramidal cell layer and neuronal soma size and dendritic length in adults can be reversed by neonatal castration in males or prenatal testosterone treatment of females (Isgor & Sengelaub, 2003). This result underscores the importance of androgens in the development of the hippocampus during critical periods in late prenatal life and early postnatal life. Androgen receptors are expressed in the hippocampus at high levels during development (ref Sar in Zhang). This high expression of androgen receptors correlates with high levels of testosterone and dihydrotestosterone during postnatal period. Zhang et al (2008) showed that a neonatal treatment with testosterone or dihydrotestosterone increased the number of newly born cells in the immature female, an effect blocked by an androgen receptor antagonist.

Even though thyroid hormones receptors are widely distributed in the brain, the hippocampus appears to be more sensitive than cortex to thyroid hormone depletion during the perinatal period (Zhang et al., 2009). Pre- or perinatal hypothyroidism illustrates the potential consequences of a lack of thyroid hormones on hippocampal development. Even more than the degree of thyroid insufficiency, the timing and duration of that insufficiency seem to be important. This underlines the notion of a window of sensitivity. Thus exposure to EDCs that disrupt thyroid function would be expected to have different effects depending on the timing of exposure. Very few studies have concentrated on the effects of thyroid hormones on progenitor proliferation in the neonatal hippocampus. However, some authors have reported a decrease in cell survival in the dentate gyrus, CA1 and CA3 after developmental hypothyroidism (Gong et al., 2010). Others have reported decreased progenitor proliferation in the dentate gyrus after early postnatal hypothyroidism (Zhang et al., 2009; Uchida et al., 2005). However, the adult brain seems to be able to compensate to this early insult (Zhang et al., 2009).

Perinatal hypothyroidism decreases synaptogenesis in the dentate gyrus of the developing rat (Rami et al., 1990), and decreases neurite outgrowth and dendrite elaboration (Thompson and Potter, 2000). These alterations seem to be irreversible as they persist in adulthood even after correction of hypothyroidism (Gilbert et al., 2004). Expression of markers of synaptic formation such as RC3/neurogranin or srg1 also depend on thyroid hormones (Martinez de Arrieta et al., 1999; Thompson and Potter, 2000). Even mild prenatal hypothyroidism alters expression of hippocampal genes involved in neurite outgrowth, synaptogenesis and plasticity (Royland et al., 2008). Thus even moderate alterations in thyroid hormone levels following exposure to EDCs could be functionally significant.

## **Early endocrine disruptor effects in the central nervous system: structural and functional aspects**

It is now well accepted that most endocrine disruptors cross the blood brain barrier and can have a direct action on brain cells. However, it remains difficult to correlate serum concentration of such chemicals and the doses at the hormone receptors. Knowing the long half life of some endocrine disruptors such as PCBs or DDT and their lipophilic nature, they accumulate in the brain. For example, DDT and PCBs concentrations in human brain were in the range of mg/kg of lipids (Dewailly et al., 1999).



## Neuroendocrine

*In vitro* models enable the direct evaluation of EDCs on neuro-glial function. Using PCBs, Gore et al. reported both facilitatory and inhibitory ER-mediated effects on GnRH peptide and mRNA levels in immortalized GnRH neurons, depending on the PCB compound and its dose (Gore et al., 2002; Dickerson et al., 2009). Moreover, in the same model, PCBs appeared to affect GnRH neuron viability as a result of increased expression of cleaved caspase-9 (Dickerson et al., 2009). We used hypothalamic explants to study the effects of DDT, an estrogenic EDC. Our paradigm included not only axons and terminals of the final effector, i.e. the GnRH neuron (Purnelle et al., 1997), but also afferent neuro-glial components (neurotransmitters, neuropeptides). Hypothalamic explants retain important functional and developmental characteristics in that they release GnRH in a pulsatile manner with a frequency increasing from birth to onset of puberty (Bourguignon JP et al., 1992). Using hypothalamic explants of immature female rats aged 15 days, o,p'-DDT increased GnRH pulse frequency through a mechanism involving ER, AhR, and intracellular kinases (Rasier et al., 2007 & 2008). The effects of DDT were dose- and time- dependent with both rapid and slow effects mediated respectively by membrane and nuclear receptors. The glutamate-evoked release of GnRH progressively increased after 4 h of incubation with DDT which suggests slow effects (Rasier et al., 2007 & 2008). Though such *in vitro* investigations provide evidence of the neuroendocrine effects of EDCs and allow mechanistic studies, the conditions are far different from environmental exposure to EDCs. Specifically, the *in vitro* models were deafferented, required concentrations higher than those toxicologically relevant in humans likely because of low diffusion in the explant, and involved gene manipulation in immortalized neurons.

Do *in vivo* experiments provide an insight into neuroendocrine effects of EDCs? Suggestive evidence can be obtained by studying physiological processes that involve neuroendocrine regulation. Such processes include gonadotropin-dependent onset of puberty; ovulation that is dependent on stimulation by the gonadotropin surge in many species; and sexual behaviour that is also known to involve hypothalamic mechanisms in several species. Not unexpectedly, these three processes are sexually dimorphic, and thus are likely to be differentially regulated by sex steroids (and disrupted by EDCs) in males and females. However, *in vivo* studies are further complicated by variables that can influence the response to EDCs including dose, age at exposure, duration of exposure, route of administration, gender, and the components of PCB mixture.

In ovines, prenatal exposure to testosterone can alter pubertal timing and estrous cyclicity as a result of neuroendocrine alteration of estradiol positive feedback (Unsworth et al., 2005). Fetal exposure to EDCs delayed (Methoxychlor, MXC) or severely reduced (BPA) the LH surge without affecting pubertal timing in the ewe (Savabieasfahani et al., 2006). As emphasized by these authors, the exposed animal also had ovarian anomalies, growth retardation and metabolic disorders that could have contributed to disrupted reproductive function. In other studies, a single prenatal administration of a PCB mixture on gestational day 16 in rats caused postnatal growth retardation (Gore, 2008) while 2 injections on gestational day 16 and 18 disturbed sexual behaviour, most prominently in females (Steinberg et al., 2007). Increased GnRH mRNA levels in the hypothalamus of female adult rats previously exposed to aroclor 1221, a mixture of PCBs (Gore, 2008) provides direct evidence of the neuroendocrine effects of EDC exposure. This EDC also caused, after prenatal exposure on gestational days 16 and 18, a marked reduction in the proestrous LH surge (Steinberg et al., 2008). The behavioral consequences of EDC exposure can be transgenerational as shown by female preference for male rats with no history of exposure, three generations after the progenitors were exposed to vinclozolin (Crews et al., 2007). Such findings suggest epigenetic changes in the neuroendocrine components of sexual behaviour regulation. The reduction in the normal female mouse predominance of tyrosine

hydroxylase-expressing neurons in the anterior hypothalamus after fetal and early postnatal exposure to BPA illustrate the sexually dimorphic characteristics of EDCs on the neuroendocrine system (Rubin et al., 2006).

We studied the effect of DDT in the female rat with pubertal timing and estrous cyclicity as endpoints. Our rationale was to use early and transient exposure to DDT to model of neuroendocrine effects of the pesticide that might account for sexual precocity reported in girls migrating for international adoption (Krstevska-Konstantinova et al., 2001; Parent et al., 2003). Because fetal or early postnatal exposure to testosterone or estradiol masculinizes the central nervous system and alters the mechanism of estrous cycling, we exposed the animals to DDT on postnatal day 6–10. This age window allows for a sexually differentiated response to estradiol both *in vitro* and *in vivo*, and subsequent female sexual precocity (Matagne et al., 2004). In such conditions, *in vivo* exposure to DDT followed by *ex vivo* measurement of GnRH release in hypothalamic explants showed an acceleration of pulsatile secretion, consistent with subsequent sexual precocity (Rasier et al., 2007). Though both vaginal opening and first estrus occurred earlier after exposure to estradiol or DDT, the time interval between these two events increased. To obtain further evidence of a neuroendocrine action of DDT, we studied the response of pituitary LH to a bolus administration of synthetic GnRH. The LH response reflects previous stimulation by the endogenous neuropeptide and, in human, increased LH response is regarded as evidence of central neuroendocrine maturation that leads to puberty (Lebrethon & Bourguignon, 2001). The rat pattern differs from human because neuroendocrine maturation is associated with an ontogenetic reduction of LH response in rat, a confounding situation with LH reduction resulting from negative feedback such as seen in peripheral precocity. Nevertheless, DDT exposure led to premature developmental reduction in the LH response (Rasier et al., 2007). This result may reflect effects on negative feedback, as it resembles the reduced LH responses following 30-day exposure of the Atlantic croaker to the PCB mixture Aroclor 1254 (Khan & Thomas, 2001).

### Cerebral cortex

Impaired memory as well as altered learning abilities and motor deficits have been associated with prenatal exposure to some EDCs such as PCBs in human and rodents (Schantz, 2003). Although those observations suggest that EDCs may alter cortex or hippocampus development, little is known about the underlying cellular and molecular mechanisms. It is known that EDCs such as PCBs disturb thyroid and sex steroid actions on brain development or act directly as a neurotoxicant.

*In vitro* models have helped unravel some direct effects of PCBs on neurons, identify potential alteration of neurotransmission and intracellular signaling, and differentiate effects caused by coplanar and noncoplanar congeners. Coplanar and non-coplanar PCBs induce apoptosis and a reduction of cell viability in cerebellar and cortex neurons cultures (Mariussen et al., 2002; Sanchez-Alonzo et al., 2003), but the mechanism of action may differ. Coplanar PCBs seem to act through AhR to induce cell death, whereas it has been suggested that non-coplanar PCBs act through alteration of intracellular secondary messengers (Mundy et al., 1999), alteration of the cell membrane (Tan et al., 2004) or inhibition of dopamine synthesis (Seegal et al., 1997). Consistent with *in vitro* data, early studies showing neurobehavioral changes in mice developmentally exposed to PCBs (Chou et al., 1979; Agrawal et al., 1981) have implicated alterations of the dopamine system. All these *in vitro* data suggest a direct neurotoxicity of PCBs in addition to their action as endocrine disruptors.

In rodents, developmental exposure to PCBs induces alterations of long-term potentiation (Gilbert et al., 2000) and impairs memory and learning (Schantz et al., 1995). Because

intracellular secondary messengers modulate long-term potentiation, it has been hypothesized that PCBs disrupt secondary messenger signaling. *In vitro* studies with cerebellar granule cells and cortical neurons show slow increases in intracellular calcium after exposure to non-coplanar PCBs (Kodavanti et al., 1993; Seegal, 2000) as a result of inhibition of calcium uptake by mitochondria and microsomes. This increase in intracellular calcium level might lead to the translocation of protein kinase C to the membrane where it is activated, and thus alteration of second messenger pathways. This effect was observed at low PCBs concentrations in the absence of cytotoxicity.

PCBs may also induce developmental neurotoxicity by interfering with neuronal connectivity. Prenatal exposure *in vivo* has been used to test this possibility. Developmental exposure to PCBs accelerates dendritic growth cerebellum (Lein et al., 2007); and also interferes with experience-dependent dendritic plasticity, which is necessary to learning and memory (Yang et al., 2009). This effect might involve altered expression and activity of the ryanodine receptors (RyRs). Indeed, PCBs appear to enhance the activity of RyRs, a group of intracellular Ca<sup>2+</sup> channels regulating cellular signalling in neurons (Wong, 1997). This activation of RyRs could also explain the neurotoxic effects of PCBs on auditory cortex development by disrupting the balance of neuronal inhibition to excitation (Kenet et al., 2007).

Prenatal exposure to PCBs mimicks alterations of brain development that occur with hypothyroidism. In addition to potential direct effects of PCBs on the developing brain, *in vivo* models have been used to study possible interactions of these EDCs with thyroid function. Rodent studies consistently report a decrease of foetal and maternal thyroid hormones following prenatal exposure to PCBs (Bastomsky, 1974; Brouwer et al., 1998). This decrease might result from a direct action of PCBs on thyroid hormone synthesis (Collins & Capen, 1980), an increased biliary excretion (Van Birgelen, 1995) or a displacement of T4 from transthyretin (Lans et al., 1993). However, PCBs exposure does not mimick the increased TSH levels or reduced brain weight associated with hypothyroidism, suggesting that PCBs may have a direct action on the foetal brain. Gauger et al. (2004) showed that PCBs caused a decrease in thyroid hormones levels as well as an increased expression of thyroid hormones responsive genes in the prenatal and neonatal cerebral cortex. This result suggests a direct action of PCBs on foetal thyroid receptors (Gauger et al., 2004) and is consistent with other studies in which PCBs mimicked the ability of T3 to increase oligodendrocytes differentiation (Fritsche, 2005). Some congeners also exhibit weak thyroid hormone activity in yeast two-hybrid assays (Arulmozhiraja & Morita, 2004). Interactions of PCBs with thyroid hormones receptors are very complex involving both agonist and antagonist actions. Likewise, PCBs include more than 200 congeners that may exert different effects.

## Hippocampus

Hippocampus development seems to be targeted by EDCs, in particular PCBs, as well. While some of the cellular and molecular effects of EDCs on the hippocampus might be similar to those observed in cortex, one can hypothesize that the resulting phenotype should be different.

Royland et al (2008b) have studied the effect of a neonatal exposure to Aroclor 1254 on gene expression in the hippocampus and the cerebellum in animals at postnatal day 7 and 14. Expression of developmental genes related to cell cycle, synaptic function and neurogenesis were altered by exposure to Aroclor. The effect appeared to be more important in the cerebellum, which could be explained by the different developmental timing of the cerebellum and the hippocampus. Functional analysis of those results seemed to indicate that Aroclor delayed cerebellum and hippocampus development.



Hippocampus represents a very nice model to study the effects of EDC on dendritogenesis and synaptogenesis. Lein et al (2007) showed that perinatal exposure to Aroclor 1254 caused a decrease of dendritic length in young rats at postnatal day 22 but the rate of growth was accelerated at later ages leading to similar dendritic length at postnatal day 60. Using a similar mode of exposure, Pruitt et al (1999) showed a decreased growth of hippocampal intra- and infra-pyramidal mossy fibers. Knowing the role of dendritic plasticity in learning and memory (Hering and Sheng, 2001), alteration of dendritic growth by PCBs could explain some of the impairment in learning and memory after perinatal exposure.

Developmental exposure to Aroclor 1254 causes a significant decrease of serum T4 but similarly to what happens in the cortex, some PCB congeners could have a thyroid hormone agonist action on some markers such as neurogranin (Bansal and Zoeller 2008).

Perinatal exposure to PCBs is associated to alteration of some neurotransmitters in the hippocampus similarly to what is observed in the cortex. PCBs such as PCB153 causes alterations of the levels of dopamine, a neurotransmitter involved in the control of memory, in the hippocampus (Honma et al, 2009).

## **Translational aspects: bridging human and experimental observations**

### **EDCs and neuroendocrine control of sexual maturation, reproduction and energy balance**

Because EDCs likely involve both neuroendocrine and peripheral pathogenetic mechanisms, the evidence for neuroendocrine effects in humans is only partial and indirect. Data in a cohort of internationally adopted children in Denmark indicate increase in gonadotropin secretion before onset of puberty, providing evidence of early hypothalamic-pituitary maturation (Teilmann et al., 2007). In Belgium, central precocious puberty is much more frequent in internationally adopted girls, and commonly presents with detectable plasma levels of the DDT subproduct, DDE (Krstevska-Konstantinova et al., 2001). Although these data suggest a neuroendocrine mechanism, it does not exclude other mechanisms that may coexist or even precede EDC exposure. Indeed, besides endocrine disruption, other stressors such as psychological stress and undernutrition are likely involved in those children and should be taken into account. Early timing of menarche as well as reduced rate of pregnancy have been reported in relation to exposure to DDT and or DDE, possibly involving peripheral and neuroendocrine mechanisms (Vasilu et al., 2004; Ouyang et al., 2005; Cohn et al., 2003).

A role for EDCs in the epidemic of obesity in industrialized countries has been postulated recently. The hypothesis is that changes in energy balance by EDCs may contribute to obesity (Heindel, 2003). Peripheral mechanisms could occur. For example in mature abdominal adipocytes *in vitro*, BPA reduces the secretion of adiponectin that protects against obesity and insulin resistance (Hugo et al., 2008). Fetal malnourishment as well as fetal exposure to endocrine disruptors such as DES and BPA can lead to low birth weight, early puberty, ovulatory disorders, obesity in adulthood and metabolic syndrome (Gluckman & Hanson, 2004; Newbold et al., 2008; Heindel & vom Saal, 2009).

### **EDCs and cognitive functions**

The first observation pointing to the neurotoxic effects of PCBs followed an accidental exposure in Taiwan in which children exposed *in utero* showed impaired cognitive function at 5 years of age (Mc Kinney JD et al., 1994). The major difficulty in such studies is the long delay between the exposure and its measurable effect. Several other follow-up studies have shown a negative correlation between *in utero* exposure to PCBs and cognitive performance and memory in infants and children (reviewed in Schantz, 2003). Those results are consistent with observations made in rodents. It is interesting to note that the levels of

exposure in recent studies are lower than in earlier studies, but still negatively correlate with cognitive function. More recent studies are developing analytic methods to correlate neurodevelopmental toxicity with specific congeners.

## Conclusion and further research needs

Thyroid hormones and sex steroids play a key role in the development of the central nervous system. In addition to those hormones and their receptors, other receptors such as peroxisome proliferator-activated receptors and AhRs are expressed in the brain and appear to be targeted by EDCs (Kodavanti, 2006; Devergne, 2009). These EDC targets underlie the potential sensitivity of developing neuroendocrine systems in the hypothalamus, cortex and hippocampus (Table 1). Those systems appear to be especially sensitive to endocrine disruption during their development; this period has been named the critical window of exposure. The more porous blood brain barrier around the hypothalamic-pituitary junction might explain why most of the central effects of EDCs occur in that system. *In vitro* and *in vivo* models have shown that EDCs have several mechanisms of action. Although the first described mechanisms involved alterations of the transport and metabolism of hormones, other mechanisms including non-genomic effects have emerged recently. It now appears that EDCs could have direct effects on several aspects of developing cells in the brain. Most cellular and molecular studies have focused on the effects of EDCs on neurons. Given the role of astrocytes in the regulation of GnRH secretion as well and their involvement in neuronal plasticity in the hypothalamus, the cortex and the hippocampus, further studies are warranted to characterize effects of EDCs on glial cells. By interfering with peripheral hormones or acting directly at the glial-neuronal level, EDCs can disrupt the neuroendocrine regulation of reproduction and metabolism (Bourguignon et al., 2010), as well as cortical and hippocampal function. Those systems all receive neuroendocrine regulation as well as feedback from peripheral tissues. Diethylstilbestrol (DES) is a potent estrogenic agent that was used forty years ago to prevent miscarriages. This molecule illustrates the potential multisystemic action of EDCs. Beside the increase rate of cervix cancer and decreased fertility, an early exposure to DES causes metabolic disorders (Newbold et al., 2008) with increased weight and body fat as well as alteration of the hippocampus development (Ramos et al., 2007). This crosstalk underlines the need for studies integrating the multi-system aspects of endocrine disruption.

Programming of the hypothalamus, the cortex and the hippocampus can be affected by many different stressors such as nutritional, chemical or emotional stresses or anoxia. Further studies are needed to explore the effects of combined stressors on the development of these structures because the organism is usually exposed to concomitant stresses. Disorders of puberty associated with early exposure to EDCs could contribute to a spectrum of diseases throughout life involving intrauterine growth retardation, precocious pubarche, precocious puberty, disorders of ovulation, metabolic syndrome, and sensitivity to cancer (Parent et al., 2003). A spectrum of abnormalities associated with EDC exposure of the developing cerebral cortex and hippocampus to EDCs is still to be described, but recent data suggest a link between exposure to EDCs and oxidative damage or neurodegenerative disease later in life (Obata & Kubota, 2000; Jones & Miller 2008). As it is the case for other systems targeted by EDCs, the effects of a prenatal exposure can only be seen when symptoms arise, which usually means years after exposure. This delay underlines the necessity for further research to identify early endpoints indicating an exposure.

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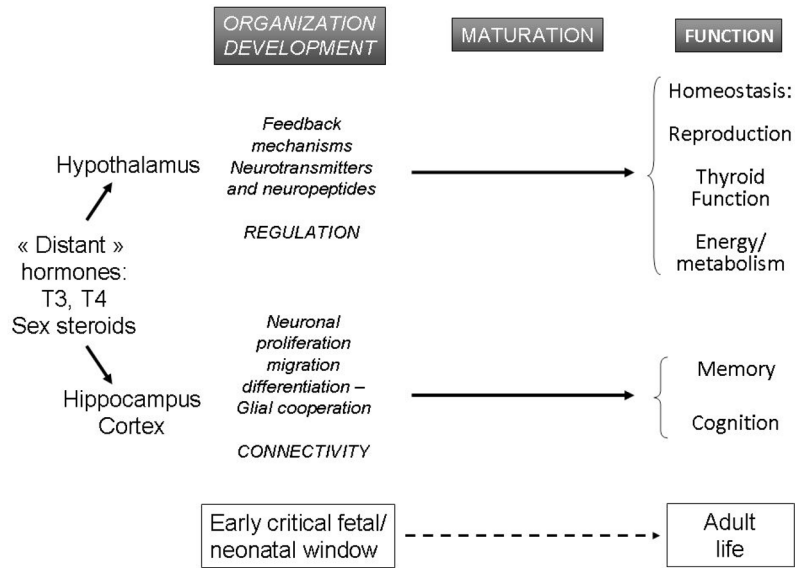
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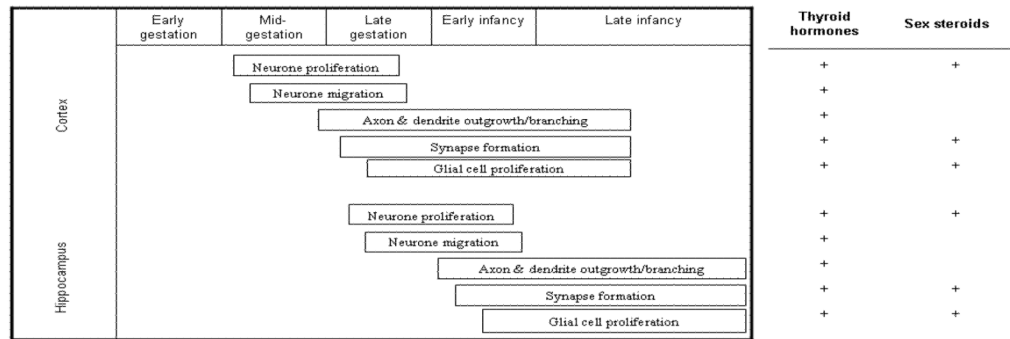
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**Figure 1.**

The hypothalamus, the hippocampus and cerebral cortex show dependency on thyroid hormones and sex steroids starting with early organizational and developmental events during fetal and neonatal life and leading to late functional consequences in adolescence and adulthood. Such a lifelong hormonal dependency provides a basis for endocrine disruption. GnRH: gonadotropin-releasing hormone; TRH: thyrotropin-releasing hormone; NPY: neuropeptide Y, AgRP: agouti-related protein



**Figure 2.** The left panel illustrates the relative sequence of cellular changes during the stages of the cortex and hippocampus development in rodents. The right panel indicates the reported effects of sex steroids and thyroid hormones, or thyroid hormones alone, on the different aspects of cerebral cortex and hippocampus during early development. Based on Williams, 2008



**Table 1**

Common and distinct features in effects of EDCs on the hypothalamic-pituitary axis and the cortex and hippocampus. IUGR: intrauterine growth retardation; BBB: blood brain barrier; AFP: alpha-fetoprotein

	<b>Neuroendocrine effects Hypothalamic-pituitary axis</b>	<b>Effects on cortex</b>	<b>Effects on hippocampus</b>
<b>Hormones involved</b>	T4, T3, sex steroids, ...		
<b>Critical window of exposure</b>	Prenatal		
<b>Age at clinical effects</b>	Delayed from exposure		
<b>Target cells</b>	GnRH, TRH neurons, Other neurons Astrocytes? Less protected by BBB	Neurons Astrocytes, oligodendrocytes Protected by BBB	
<b>Cellular endpoints</b>	Neurosecretion	Proliferation, migration, differentiation (neurite/dendrite outgrowth), synaptogenesis, cell survival	
<b>Possible EDC mechanisms of action</b>	Genomic/non genomic effects Alteration of synthesis (aromatase), binding (AFP) of sex steroids		
	Alteration of metabolism and transport of sex steroids	T3 responsive genes controlling cell proliferation; Actin (cell migration), Alteration of binding, metabolism and transport of thyroid hormones Neurotoxicity	Genes involved in neurite outgrowth, synaptogenesis and plasticity
<b>Interaction with peripheral effects</b>	Disturbed feedback mechanisms	?	
<b>Possible consequences of EDC exposure</b>	IUGR, early puberty, dysovulation, disturbed sexual behaviour, metabolic syndrome	Impaired cognitive function, alteration of response to oxidative stress, Alzheimer, Parkinson	Impaired cognitive function, altered learning and memory