Male Puberty: Neuroendocrine Disruption of Reproduction

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Abstract

The hypothalamus and the pituitary play crucial roles in the finely tuned integration of endogenous and environnemental factors influencing puberty and reproduction. Recent studies have shown that environnemental factors can influence the neuroendocrine control of puberty. Involved mechanisms differ depending on the period of exposure : fetal or neonatal life, prepubertal or adult life. Interpretation of the neuroendocrine effects of endocrine disrupting chemicals (EDCs) is made more complex by the possible coexistence of peripheral mechanisms. This chapter will discuss the central pathways by which EDCs affect the hypothalamic control of puberty.

Key Points

- Recent data indicate variations in male pubertal timing with potential involvement of environnmental factors, amongst which is exposure to endocrine disrupting chemicals.
- Endocrine disrupting chemicals affect pubertal timing differently depending on the sex and whether they come into action during the fetal/early postnatal period or the peripubertal period.
- The hypothalamic-pituitary control of puberty is a potential target of exposure to endocrine disrupting chemicals, especially the GnRH network.

Introduction

Male puberty involves the coordinated activation of the hypothalamus, pituitary gland and gonads and leads to the acquisition of reproductive function. Epidemiological and laboratory studies have shown that exposure to endocrine disrupting chemicals (EDCs) is associated with reproductive disorders such as alteration of sexual development, abnormal puberty timing, infertility and increased risk of testicular cancer in males (Brauner *et al.*, 2021; Johansson *et al.*, 2017; Lopez-Rodriguez *et al.*, 2021a; Skakkebæk *et al.*, 2022; Uldbjerg *et al.*, 2025). Some of those disorders have been shown to involve direct effects of EDCs on the gonads. For instance, the testis is directly targeted by EDCs during early human life, as indicated by intratesticular anomalies described in the testicular dysgenesis syndrome in human (Bay *et al.*, 2006; Sharpe and Skakkebaek, 2008; Wohlfahrt-Veje *et al.*, 2009) which present some similarities with testicular anomalies described in rodents after early exposure to phthalates (Sharpe *et al.*, 1995). In the last two decades, several studies have shown that phthalates alter morphology, survival, and differentiation of testicular germ cells and disrupt testis steroidogenesis (reviewed in Li and Spade (2021)). Central endocrine disruption initially received relatively less attention but increasing evidence during the past decade has shown that the neuroendocrine system is also targeted by EDCs (Bourguignon *et al.*, 2010; Frye *et al.*, 2012; Gore, 2001; Lopez-Rodriguez *et al.*, 2021b). This chapter will summarize the data illustrating the effects of EDCs on sexual maturation in males.

Neuroendocrine Disruption of Pubertal Timing

Secular Trend in Age at Puberty and Environment

Puberty is a life period characterized by the maturation of the hypothalamic-pituitary-gonadal axis. It involves both physiological and behavioral changes that ultimately lead to the achievement of reproductive capacity. Puberty results from the activation of a

complex neuroendocrine machinery which leads to an increase in frequency and amplitude of gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus (Herbison, 2016).

Changes in pubertal timing has been mostly studied in females. A reduction in menarcheal age has been reported between 1850 and 1960 in Scandinavian countries (Tanner, 1962) and further in many European countries and USA (reviewed in Lopez-Rodriguez *et al.* (2021a) and Parent *et al.* (2003, 2015)). These observations were thought to be the result of the improvement in health and nutritional status as well as socio-economical conditions. After a constant decrease between 1850 and 1960, it appears that, in several countries with relatively stable and uniform condition of life, menarcheal age has shown only minor progression during the past decades while breast development seems to still be advancing worldwide (Eckert-Lind *et al.*, 2020; Parent *et al.*, 2015). Some studies have identified a secular trend towards early onset of puberty, irrespective of the level of BMI (Aksglaede *et al.*, 2009), suggesting the involvement of other environmental influences, amongst which are endocrine disruptors chemicals. Similar to the observed pattern in girls, male puberty seems to occur earlier based on data collection from United States (Herman-Giddens *et al.*, 2012) Europe (Brix *et al.*, 2019) and Asia (Hu *et al.*, 2025). Similarly, a recent Swedish study has shown that age at peak height velocity was 1.5 months earlier for every decade increase in birth year in boys born between 1951 and 1996 (Ohlsson *et al.*, 2019). In parallel, some studies have reported an increased incidence of male central precocious puberty (CPP) over the last years (Huttunen *et al.*, 2024) and this increase was driven by growing number of idiopathic CPP. Interestingly, as reported in boys with Down syndrome, puberty timing can be differentially affected depending on the pubertal marker used: boys with Down syndrome entered puberty at the same age compared to healthy boys while they attained Tanner 5 stage later, suggesting that the duration of pubertal process is longer in those patients (Erdoğan and Güven, 2022).

Detailed analysis reveals that the pattern of age distribution is affected in boys and girls. This justifies a revision of the belief according to which current changes correspond to an advancement of pubertal timing in females only. Current variations in pubertal timing involve a trend towards earliness for initial pubertal stages and towards lateness for final pubertal stages (Parent *et al.*, 2015). Such rapid evolution suggests a role for environmental influences including nutrition, stress and endocrine disruptors. This hypothesis is supported by some observations of association between early exposure to endocrine disrupting chemicals or other environmental factors and the age at onset of puberty as well as rodent data which will be described below.

Challenges in Demonstrating the Endocrine Disruption of Puberty Timing

Before discussing the available data linking exposure to EDCs and neuroendocrine disruption of pubertal timing, it is important to be aware of some challenges that we face in this area.

The effects of environmental stressors on pubertal timing depend on timing of exposure but also vary by sex. In animal models, nutritional restriction during gestation is associated with early puberty in females (Sloboda *et al.*, 2009), while pubertal timing is not affected in males exposed to maternal undernutrition in utero (Sánchez-Garrido *et al.*, 2013). In human studies, classically, girls born small for gestational age (used as a marker of intra-uterine growth restriction) present with advanced age at menarche (Eplein *et al.*, 2010; Ibáñez *et al.*, 2006, 2011; Maisonet *et al.*, 2010; Verkauskiene *et al.*, 2013). Data are very scarce for boys. Some studies suggest an early pubertal age, but with differences less consistent and pronounced than for girls (Hvidt *et al.*, 2019). In contrast, low prepubertal BMI leads to delayed puberty in boys (Busch *et al.*, 2019; Oehme *et al.*, 2021) as in girls (Campisi *et al.*, 2021). Likewise, psychosocial stress shortly before or during puberty may cause delayed onset of different pubertal signs in both girls and boys (Liu *et al.*, 2025), whereas advancement of puberty has been described in girls who had experienced such stress in early postnatal life or infancy (Moffitt *et al.*, 1992; Wierson *et al.*, 1993). Only few human studies have investigated the association between early psychosocial stress and pubertal timing in boys, with contrasting results ranging from no effect to accelerated or delayed puberty timing (reviewed in Sear *et al.* (2019)). Using an animal model, Cowan and Richardson have highlighted sex specific response to early life maternal separation stress regarding the timing of puberty: stressed females entered puberty earlier than controls, while stressed males matured later (Cowan and Richardson, 2019). Those data are consistent with the concept that environmental clues affect pubertal timing differently depending on sex and on the life period when they come into action. In the early phase of organization, adverse conditions can be interpreted centrally as a risk for species survival and are translated into the need for early reproductive fitness. Reversely, in a period closer to puberty, similar adverse conditions can be interpreted as a risk for quality and outcome of pregnancy and are translated into a need for delayed reproductive fitness.

Another challenge comes from observations that are inconsistent with the classical toxicology perspective according to which there will be no effect of a chemical below a threshold of exposure. This principle implies that the dose-response relationship is linear. However, hormones have complex concentration-response patterns which lay the foundation for dose-response characteristics exhibited by EDCs (Kahn *et al.*, 2020). We have shown in female rats that neonatal exposure to BPA for 2 weeks leads to a delayed maturation of GnRH secretion after a low environmentally relevant dose and advanced maturation after exposure to a higher dose (Franssen *et al.*, 2016). Opposing effects on male rat puberty have also been observed after exposure to lower or higher doses of phthalates (Ge *et al.*, 2007; Saillenfait *et al.*, 2008). In addition, low-dose mixtures, consistent with human exposure, can have effects not conforming to simple additive models (Christiansen *et al.*, 2012; Christiansen *et al.*, 2020; Conley *et al.*, 2018; Hass *et al.*, 2017). However, most animal and human studies so far have focused on one or a single group of EDCs.

EDC exposure, by modifying the hormonal environment, can affect pubertal timing either through central mechanisms, acting at different levels of the hypothalamic-pituitary system or through interaction with the peripheral target tissues such as the breast, uterus, testis or ovaries. Peripheral mechanisms can coexist with central mechanisms or secondarily facilitate them. Such a concept is supported by the dissociation between advancement in age at onset of breast development in Denmark, the Netherlands and Belgium without parallel change in menarcheal age (Lopez-Rodriguez *et al.*, 2021b). A single pubertal event can be influenced by different endocrine pathways. For instance, breast development can be due to ovarian estrogen secretion under the stimulation by pituitary gonadotropins and/or estrogenic effects of EDCs independently of hypothalamic-pituitary maturation. Moreover, EDCs can interfere with the physiological inhibitory feedback mechanisms of sex steroids at the hypothalamic-pituitary level while they can also stimulate neuroendocrine maturation of GnRH secretion (Rasier *et al.*, 2006).

Disruption of Male Puberty : Epidemiological Studies and Animal Models

EDC exposure during the perinatal or prepubertal period has been associated with early or late pubertal onset in boys. A longitudinal follow up of Russian Boys studied the association between childhood exposure to dioxin-like compounds and PCBs and pubertal development. Higher exposure to dioxin-like compounds delayed pubertal onset and the age at attaining Tanner stage 5, while non-dioxin-like PCBs advanced pubertal onset and the age at attaining sexual maturity defined by Tanner stage 5 (Burns *et al.*, 2016). In the CHAMACOS longitudinal cohort, boys with higher prenatal exposure to BPA and phthalates presented earlier onset of puberty, especially boys with overweight or obesity (Berger *et al.*, 2018). In a recent study based on three European cohorts, higher maternal exposure to two phthalate metabolites increased the probability of having started puberty in boys while higher exposure to BPA or some parabens had an opposite effect with less probability to present gonadarche (Freire *et al.*, 2024). Those results differ from previous data from Ferguson *et al.* (2014) that did not report association between prenatal exposure to BPA and pubertal onset in boys. Another study evaluated pubertal timing in boys after prenatal or peripubertal exposure to phthalates, parabens, and phenols and did not find any association except for exposure to propyl paraben at peripubertal stage and earlier gonadarche (Harley *et al.*, 2019). In the study from Kasper-Sonnenberg *et al.* (2017) peri-pubertal exposure to BPA and phthalates was not associated with pubertal development. In a longitudinal cohort study of predominantly Mexican origin families in Northern California, prenatal and peripubertal exposure to 4 PBDEs did not affect boy's pubertal development (Harley *et al.*, 2017). In contrast, in Spanish adolescent boys, higher urinary concentration of non persistent pesticide metabolites was associated with delayed gonadal development (Castiello *et al.*, 2023). Interestingly, in addition to the timing of exposure that can impact the effects of EDCs on pubertal timing, specific pubertal endpoints can be differentially affected by EDCs, as suggested by association between peripubertal urinary BPA levels and earlier pubertal onset accompanied by delayed pubertal progression with delayed attainment of genital stage 5 in a cohort of Chinese adolescent boys (Wang *et al.*, 2017). Although human studies are very limited to explore the neuroendocrine effects of EDCs, animal models indicate that changes in pubertal timing involve disruption of neuroendocrine mechanisms.

In male rodents, balano-preputial separation is commonly used as a marker of puberty. With the exception of some studies reporting early balano-preputial separation after exposure to phthalates (Curi *et al.*, 2023; Ge *et al.*, 2007; Saillenfait *et al.*, 2008), EDCs appear to cause either no effect or a delay of sexual maturation in the male rodent. For instance, a recent study has shown that gestational and lactational exposure to phthalates delayed pubertal onset in males (Guerra *et al.*, 2023). Similarly, late pubertal onset was observed after peripubertal exposure to phthalates (Liu *et al.*, 2023). Several studies with BPA did not show any effect whatever the dose and the window of exposure (Ashby and Lefevre, 2000; Kato *et al.*, 2006; Nagao *et al.*, 1999; Tan *et al.*, 2003; Tinwell *et al.*, 2002). Similarly, exposure during pregnancy and lactation to BPA analogs (BPB, BPS and BPF) did not show any effect on pubertal timing in males (Ullah *et al.*, 2019). However, we have shown that neonatal exposure to a very low dose of BPA for 5 days reduced GnRH pulse frequency without affecting the age at balano-preputial separation, suggesting a delayed maturation of GnRH secretion caused by BPA neonatally. This indicates that GnRH secretion could be extremely sensitive to disruption without being sufficient or lasting sufficiently to account for phenotypic changes. When similar doses of different EDCs are used at different periods of exposure in the male rodent, they are found to be effective in delaying puberty after postnatal (after weaning) exposure as opposed to no effects after prenatal exposure. For instance, it has been shown that exposure to BPA during late pregnancy and postnatally (from GD18 to PN5) delayed puberty in male offspring (Oliveira *et al.*, 2017). Such findings are obtained using dichlorodiphenylichloroethylene (DDE) (Yamasaki *et al.*, 2009), vinclozolin (Eustache *et al.*, 2020) or diethylstilbestrol (DES) (Shin *et al.*, 2009) suggesting that the fetus is less sensitive than the juvenile animal. However, pubertal onset appears to be also delayed when exposure occurs at the end of gestation, as observed after PCBs exposure at GD16 and GD18 in male rat (Dickerson *et al.*, 2011).

Importantly, both periods of exposure fall into the so-called "programming window" indicating that, even within this particular period, there may be differences in sensitivity to endocrine disruption. The dose of EDC however plays a critical role since, when investigated at a given period of life, higher doses appear to be more effective such as shown after prenatal exposure to DES (Shin *et al.*, 2009) or after pubertal exposure to phthalates (Liu *et al.*, 2023; Noriega *et al.*, 2009). It is noteworthy that in two studies using phthalates, opposing effects are observed since lower doses are associated with early puberty and higher doses with delay (Curi *et al.*, 2023; Deng *et al.*, 2012). In addition to the doses of EDCs and the period of exposure, pubertal onset can be also differentially affected by exposure to mixtures of EDCs. For instance, *in utero* exposure to a mixture of pesticides and phthalates delayed male pubertal development, using a mixture of chemicals each present at the no observed adverse effect level dose (Conley *et al.*, 2018). Moreover, the effects of EDCs exposure on pubertal timing can be transmitted to the next generations as illustrated by delayed puberty observed in male F3 generation after gestational xposure to Vinclozolin (Nilsson *et al.*, 2018).

Mechanisms of EDCs Effects on the Overall Neuroendocrine Control of puberty

The hypothalamic-pituitary-gonadal axis is responsible for the development of puberty and acquisition of the reproductive function. A subset of neurons in the anterior hypothalamus secrete gonadotropin-releasing hormone (GnRH) which, through its pattern of release, controls all aspects of reproductive function throughout life. The secretory activity of GnRH neurons depends on trans-synaptic and glial inputs mediated by neurotransmitters and cell–cell signaling molecules. New methods have provided more information regarding the dynamic coordination of networks contributing to the central control of the pubertal process and reproduction (Herbison, 2016; Kaprara and Huhtaniemi, 2018; Lopez-Rodriguez *et al.*, 2021b). When studying the central mechanisms potentially involved in the neuroendocrine disruption of puberty, the GnRH network appears to be a potential target of exposure to endocrine disruptors (Lopez-Rodriguez *et al.*, 2021b). As neuroendocrine disruption of puberty has been mostly studied in females, we will describe effects of EDC on the activation of the GnRH system both in male and female animals.

Using a murine model of neonatal exposure to dichlorodiphenyltrichloroethane (DDT), our team has confirmed the involvement of neuroendocrine disruption in the alteration of pubertal timing. Pulsatile GnRH secretion was accelerated after early exposure to DDT and this effect was followed by early female sexual maturation (Rasier *et al.*, 2007). Further studies have indicated that such effects involve the estrogen receptor, the orphan dioxin (AhR, aryhydrocarbon) receptor and a subtype of glutamate receptor (Rasier *et al.*, 2008). Another study of our group showed that exposure to DES (10 µg/kg/day) during the first 5 days of life was associated with early vaginal opening while a dose of 1 µg/kg/day was associated with delayed vaginal opening and delayed developmental increase in GnRH pulse frequency (Franssen *et al.*, 2014). DES is a potent synthetic estrogen which was prescribed to prevent miscarriages during the 1950s and 1960s. Its prescription is now prohibited because of the increased risk of vaginal cancer after in utero exposure. DES is still used as a paradigmatic EDC in animal models. Our observations counteract the classical hypothesis that EDCs cause sexual precocity. Although precocity is indeed observed in several studies and possibly reflects both peripheral and central mechanisms, delayed female puberty after neonatal exposure to a potent estrogenic EDC is a new finding that is likely to be of neuroendocrine origin. In addition, it appears that prenatal food restriction could have additive effects to neonatal DES exposure on the neuroendocrine effects of leptin. When females were exposed in utero to prenatal food restriction and postnatally to DES, the stimulatory effect of leptin on GnRH secretion in vitro was completely blunted (Franssen *et al.*, 2014). This reinforces the demonstration of a neuroendocrine disruption of GnRH secretion and illustrates the potential additive effects of different environmental stressors.

Other studies have shown effects of EDCs on GnRH secretion. Fernández *et al.* (2009) demonstrated an increase of GnRH pulsatility in infantile female rats after neonatal exposure to BPA. Interestingly, GnRH pulsatility remained disrupted in adult female rats neonatally exposed to BPA (Fernández *et al.*, 2009). Losa-Ward *et al.* (2012) have reported morphological alteration of RFamide-related peptide-3 (RFRP3) neurons known to inhibit GnRH neuron activity after neonatal exposure to BPA. They showed reduced RFRP3 perikaria, fiber density and contacts on GnRH neurons, suggesting that BPA-induced premature puberty could result from decreased inhibition of GnRH neurons. GnRH neurons response to steroid-positive feedback, however, was not affected by neonatal exposure to BPA in the study from Adewale *et al.* (2009). Our group has reported that the developmental increase in frequency of GnRH release at the time of puberty is delayed in female rats exposed neonatally to a low dose of BPA (25 ng/kg/day). Interestingly, the effects were opposite with a high dose of BPA (5 mg/kg/day). The mechanism involved respectively increased or reduced the tone of γ-aminobutiric acidergic (GABAergic) neurotransmission which is known to exert inhibitory control on GnRH secretion (Franssen *et al.*, 2016). In males, early exposure to BPA (from GD18 to PND5) delayed the onset of puberty (Oliveira *et al.*, 2017) and the same exposure affected the hypothalamic expression rate of GnRH in adult offspring, supporting the involvement of GnRH network in mediating the effects of early BPA exposure on puberty control.

The GnRH network is sensitive to EDCs other than BPA as illustrated by the delayed maturation of GnRH secretion observed in female rats after perinatal exposure to ketoconazole, a steroidogenesis inhibitor, whereas pubertal or adult exposure had no effect on GnRH pulsatility, highlighting as for BPA exposure the high sensitivity of perinatal period (Franssen *et al.*, 2023).

Neuroendocrine disruption of reproduction can act through interference with the production or the action of brain neuropeptides which regulate GnRH secretion. Kisspeptin, encoded by the KISS1 gene, is known to stimulate GnRH secretion and plays a crucial role in the onset of puberty (Sobrino *et al.*, 2022). Navarro *et al.* (2009) have shown a reduction in hypothalamic expression of *KiSS1* mRNA in female rats at puberty after neonatal exposure to a high dose of BPA, as was observed after exposure to estradiol benzoate. Similarly, Patisaul *et al.* (2009) found a reduction in kisspeptin immunoreactivity (estimated in term of fibers density) in the arcuate nucleus in adult female rats after neonatal exposure. In contrast, kisspeptin immunoreactivity was not affected in the adult male hypothalamus (Patisaul *et al.*, 2009), suggesting that female hypothalamus could be more sensitive to early endocrine disruption than male. However, Bai *et al.* (2011) reported an increased number of kisspeptin-immunoreactive cells in the anteroventral periventricular nucleus of adult male offspring exposed perinatally to BPA, highlighting that kisspeptin system can be differentially affected depending on the dose and the period of exposure to BPA.

The kisspeptin system can be targeted by endocrine disruptors other than BPA. For instance, gestational exposure to PCBs leads to reduced kisspeptin immunofluorescent density in the AVPV of female adult rat, while no changes were observed in male adult after the same exposure (Dickerson *et al.*, 2011). Similarly, neonatal exposure to Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) decreased Kisspeptin expression in the AVPV and the ARC nucleus in adult female rats (Du *et al.*, 2019). Interestingly, similar alterations were observed after prepubertal exposure, suggesting that the prepub rtal period could be also a potential critical period regarding some EDCs effects of some EDCs on kisspeptin network.

Regarding the earlier vaginal opening that occurs after exposure to a high dose of BPA (Franssen *et al.*, 2016), those observations were unexpected since Kiss1 is considered as a major gatekeeper of puberty onset. The reduction in kisspeptin expression could then be part of a reactive mechanism to BPA exposure. Similarly, a study performed in sheep showed a decrease of *Kiss1* mRNA levels at the rostral, mid and caudal regions of the hypothalamus in foetus exposed in utero to a complex mixture of EDCs (Bellingham *et al.*, 2009). Such effects were not observed after adult exposure, supporting the hypothesis that the period of exposure is critical for such regulatory processes. Xi *et al.* (2011) showed that perinatal exposure to a high dose of BPA resulted in the up-regulation of the expression levels of KiSS-1 and GnRH in both male and female pups. This observation was not replicated after postnatal exposure to BPA, highlighting once again the critical perinatal period for BPA affecting reproductive neural circuits in hypothalamus (Xi *et al.*, 2011). Recently, Jiang *et al.* (2024) showed that gestational exposure and postnatal exposure (GD1 to PND21) to BPA increased expression of KiSS-1 and GnRH in females during the prepubertal period (Jiang *et al.*, 2024). Interestingly, recent data suggests that specific populations of kisspeptin neurons could have divergent sensitivity to BPA. Mice exposed to BPA at low doses (GD11-PN8) exhibited a persistent, but divergent, impairment of Kiss1 neuronal maturation, with more kisspeptin cells in the AVPV but consistently fewer kisspeptin neurons and lower KISS1 and TAC2 expression in the ARC, 2 genes playing a crucial role in puberty onset (Ruiz-Pino *et al.*, 2019). BPA exposure in immortalized hypothalamic cell lines has been shown to affect the expression of phoenixin, a neuropeptide involved in the positive regulation of gonadotropin-releasing hormone (GnRH) and kisspeptin (McIlwraith *et al.*, 2018). Acute infusion of BPA in the stalk-median eminence of mid to late pubertal female rhesus monkeys led to a suppression of GnRH and kisspeptin secretion for the highest doses while lower doses did not have any apparent effects (Kurian *et al.*, 2015).

Beyond kisspeptin, the hypothalamic transcriptome in the mediobasal hypothalamus was found to be very sensitive to perinatal exposure to ketoconazole or DES in female rats (Franssen *et al.*, 2023). A high number of genes regulating the activity of the extrinsic GnRH pulse generator were affected by all doses of DES and ketoconazole before puberty. GnRH neurons themselves express ERβ but also G protein coupled receptor 30 (GPR30) and estrogen-related receptor γ, all potential receptors for endocrine disrupting chemicals. Thus GnRH neurons could be directly targeted by EDCs. BPA significantly decreases calcium activity in GnRH neurons in explants. Blockade of GABAergic and glutamatergic input did not abrogate the inhibitory effect of BPA, suggesting a direct effect of BPA on GnRH neurons (Klenke *et al.*, 2016). Other studies in fish suggest that early exposure to BPA or bisphenol S could increase the number of GnRH3 neurons during development which suggests a broader effect of BPA and BPS on neurodevelopment (Qiu *et al.*, 2016).

Evidence suggests that epigenetic regulation of the transcriptional activity of neurons involved in stimulating GnRH release plays a fundamental role in the timing of puberty (Ojeda and Lomniczi, 2014; Lomniczi *et al.*, 2015). In addition, a multilayered microRNA-operated switch appears to be involved in the increase in GnRH expression during the prepubertal period (Messina *et al.*, 2016). Recent studies have shown that exposure to one single EDC or mixtures of EDCs leads to transgenerational effects on the hypothalamic control of puberty in rats and mice (Lopez Rodriguez *et al.* 2021; Rattan *et al.*, 2018; Rogers *et al.*, 2023; Shi *et al.*, 2019). For example, ancestral exposure to a mixture of 13 EDCs delayed puberty and GnRH maturation in the third generation of rats (F3), affecting hypothalamic Kiss1 expression. In F3 females, the Kiss1 promoter exhibited a disrupted histone configuration. Another study has reported that F3 juvenile males exposed to BPA had increased levels of Maternally expressed gene 3 (Meg3) in the mPOA (Drobna *et al.*, 2018), an imprinted lncRNA associated with precocious puberty (Geoffron *et al.*, 2018) and neurobehavioral problems (Fuemmeler *et al.*, 2016). However, DNA CpG methylation of this gene was not affected by BPA (Drobna *et al.*, 2018), suggesting that other epigenetic mechanisms, such as histone modifications, could explain the altered expression of Meg3 across generations. In a recent study from Dong *et al.* (2022), neonatal exposure to BPA increased Kiss1 expression in the ARC nucleus of female rat during the juvenile period, through changes in histone methylation at the Kiss1 promoter.

Conclusion and Perspectives

The hypothalamus provides a unique site for finely tuned integration of endogenous and environmental factors influencing the control of puberty and reproduction. While initial studies focused on the effects of EDCs on peripheral organs, recent data have shown that the hypothalamic-pituitary control of reproduction can be targeted by environmental pollutants. While such effects are extremely difficult to study in humans, animal models play a crucial role in deciphering the neuroendocrine mechanisms of action of such compounds. Neuroendocrine effects of EDCs can be contradictory and involve different mechanisms depending on the dose or whether exposure takes place early, during fetal and neonatal life or late, during prepubertal life.

During these last few years, the knowledge of molecular and genetic mechanisms controlling the pubertal process has accelerated considerably. Puberty results from coordinated changes in a multiplicity of genes organized into functional networks. Recent advances in high throughput approaches have helped identify new molecular pathways targeted by EDCs in the hypothalamus. Proteins, mRNAs, and metabolites are sensitive to EDCs and exhibit rapid changes after exposure while epigenetic modifications may help explain some of the long-term effects. Moving forward, single cell and spatial transcriptomics, or proteomics, approaches will serve to characterize cell type-specific responses to EDCs at the lev 1 of the hypothalamus.

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