conference abstract *experimental study* *animals*

Environmental toxins can impair sexual development and fertility of future generations

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Exposure to environmental pollutants can cause alterations in brain development that affect sexual development and fertility for several generations, according to findings to be presented in Lyon, at the European Society of Endocrinology annual meeting, ECE 2019. The offspring of pregnant rats exposed to a mixture of common endocrine-disrupting chemicals (EDCs), at doses equivalent to those commonly experienced by people, showed impairments in sexual development and maternal behaviour that were passed on through several generations. These findings suggest that current levels of endocrine-disrupting chemicals in our environment may already be causing long-lasting harm and that people and agencies should take measures to minimise exposure.

Endocrine-disrupting chemicals can interfere with the normal function of our hormones and have previously been associated with infertility and altered sexual development in animals and people. We are exposed to hundreds of these pollutants in our daily lives, as they are used in the manufacture of plastics, pesticides and medicines. However, the extent of damage being done to our health and the consequences to future generations remains unclear. Rodent studies have suggested that exposure to EDCs can affect brain development through several generations but the generational effects on sexual development and reproduction have not previously been investigated.

In this study, David Lopez Rodriguez a graduate student in Anne-Simone Parent's lab at the University of Liege in Belgium monitored the sexual development of three generations of rats, whose parent generation only were exposed to a mixture of common EDCs during pregnancy and lactation. The female rats born in the first and second generation showed impairments in their care for their own pups. However, the female rats in the second and third generation exhibited a delayed onset of puberty and altered reproductive cycle and ovarian follicle development, indicating that their fertility was affected, even though they were never themselves exposed to the EDCs. These changes were associated with altered gene expression in their brains that are known to affect how reproductive hormones are regulated.

Prof Parent says, "Our results raise real concerns about the effects of these pollutants in our environment. We found effects of EDCs in generations of animals that had not been directly exposed to the chemicals. We exposed the parent generation only and found long-term effects on fertility. Of course, in everyday life this would not happen and exposure to these harmful chemicals would continue, which means even more damage could be done."

The team are now interested in how the changes are carried through generations, and are looking at whether impaired maternal care is the trigger for the altered development in the following generations.

Mr Rodriguez comments, "These findings raise questions about the legacy we are leaving future generations. Current European legislation on EDCs does not consider how mixtures of low dose pollutants in our environment could be causing harm and affecting our children and wildlife in future generations, our data suggest an urgent need to follow the precautionary principle."

Abstract

OC8.3

Endocrine Disruptors transgenerationally alters pubertal timing through epigenetic reprogramming of the hypothalamus.

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Endocrine disruptors are a rising concern for public health due to their ubiquitous presence affecting reproductive development throughout generations. We aim at studying the transgenerational effect of an EDC mixture on female sexual development and reproduction.

Female rats (F0 generation) were orally exposed to a mixture of 14 anti-androgenic and estrogenic EDCs or corn oil for 2 weeks before and throughout gestation and until weaning. The mixture was composed of plasticizers (BPA, DBP, DEHP), fungicides/pesticides (Vinclozolin, Procymidon, Prochloraz, Epoxynazole, Linurone, p-p'-DDT), UV filters (4-MBC, OMC), Butylparaben and the analgesic Acetaminophen. Doses were in the human exposure range (µg/kg). Sexual development and reproductive parameters (vaginal opening, GnRH secretion, estrous cyclicity and folliculogenesis) were studied from F1 to F3 generations. Maternal behavior was measured from F0 to F2 generations. At PND21, mediobasal hypothalamus of the F1 and F3 were removed for gene expression analysis (RNAseq, RT-PCR) as well as for Chromatin Immunoprecipitation of histone modifications at regulatory regions of target genes.

The results show multiple multi- and transgenerational effects after ancestral EDC exposure. While F2 and F3 females showed delayed vaginal opening, decreased percentage of regular estrous cycles, decreased GnRH interpulse interval and altered folliculogenesis, no such changes were detected in F1 animals. These alterations were accompanied with transcriptional and histone posttranslational modifications of key hypothalamic genes involved in puberty and reproduction. We observed a downregulation of estrogen signaling (Esr1), genes involved in the GnRH network (Kisspeptin, Grin2d, Tac3R), maternal behavior (Th, Oxt, Avp, Drd1, Drd2) and stress responsiveness (Nr3c1). Upregulated gens involved glucocorticoid activity (Crh) and metabolism (Pomc, Cart). Concomitantly with transcriptional levels, while downregulated genes present higher levels of repressive histone marks (H3K9me3, H3K27me3) and decreased levels of activational histone marks (H3K4me3, H3K9ac), upregulated genes present the opposite pattern. Such histone marks related to changes in the polycomb/thritorax group of protein balance, involved in the control of female puberty.

F1 and F2 females displayed decreased licking while spending more time resting alone. F1 RNAseq showed a reduction in *Th*, *Drd1* and *Drd2* mRNA expression. These alterations on maternal behavior are known to cause transgenerational alterations of the development of the corticotropic and gonadotropic axis.

In conclusion, exposure to an environmentally relevant EDC mixture transgenerationally affects sexual development and reproduction throughout epigenetic reprogramming of the hypothalamus. While not yet clear, such effects could be mediated by alterations of maternal behavior caused by exposure to the first generation.

Notes for Editors

1. The oral communication, “Endocrine Disruptors transgenerationally alters pubertal timing through epigenetic reprogramming of the hypothalamus” was presented on Monday 20 May 2019, at the European Congress of Endocrinology at the Lyon Convention Centre, Lyon, France.

2. For other press enquiries please contact the ECE 2019 press office:

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3. The European Congress of Endocrinology was held at Lyon Convention Centre, Lyon, France on the 18-21 May 2019.

4. The European Society of Endocrinology was created to promote research, education and clinical practice in endocrinology by the organisation of conferences, training courses and publications, by raising public awareness, liaison with national and international legislators, and by any other appropriate means.