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Supplemental Material

Multi- and Transgenerational Outcomes of an Exposure to a Mixture of Endocrine-Disrupting Chemicals (EDCs) on Puberty and Maternal Behavior in the Female Rat

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Figure S1. Body weight and ovarian weight measured at P60 in F1 (n=14/group), F2 (n=16/group) and F3 (n=9-10/group) females. Measurements are displayed in grams. Bars represent mean ±s.e.m. (*P < 0.05, ***P < 0.001 vs. CTL, Student's t-test). Summary data are reported in Table S5.

Figure S2. Fold enrichment of gene ontology (GO) annotations using David pathway analysis across compared groups (CTL vs EDC) in the MBH-PoA of F3 generation females at P21. The gene enrichment analysis grouped the differentially expressed genes using GO annotations data. We selected enriched GO annotations using 2-fold enrichment criteria as a threshold and identifying annotations that were involved in brain and behavioral processes. Those annotations were then categorized in upregulated (orange) or downregulated (blue) annotations.

Figure S3. *Nr3c1*, *Crh*, *Grin2d*, *Grid2* and *Avp* mRNA expression in the female rat ancestrally (F3 generation) exposed to an EDC mixture or vehicle in the MBH-PoA of infant (P6), prepubertal (P1) and adult (P60) female rats as determined by qPCR (n=6/group). AU = arbitrary units. RNA expression data were normalized by dividing each individual value by the average of the control group at every time point. Bars represent mean ± s.e.m. (*P < 0.05, **P < 0.01, ***P < 0.001 vs. CTL, Student's t-test). Summary data are reported in Table S5.

Figure S4. Abundance of the TrxG-dependent activating marks H3K4me3 and H3K9ac and the PcG-dependent repressive mark H3K27me3 and H3K9me3 at the *Kiss1, Esr1, Oxt, Pomc, Cart, Nr3c1, Crh* and *Grin2d* promoter in the prepubertal MBH-PoA of females EDC and control from the F3 generation, as measured by ChIP (n=6/group). Dotted red lines represent repressive histone modifications while green lines represent activatory histone modifications. All data was normalized to control. Bars represent mean ± s.e.m. (*P < 0.05, **P < 0.01, ***P < 0.001 vs. CTL, Student's t-test). Summary data are reported in Table S5.

Figure S5. Maternal behavior displayed by F0 female rats directly exposed to a mixture of EDC or vehicle. Data shows time spent by dams displaying in-nest behavior (nest building, Arched-back, blancked and passive nursing; and total time spent in-nest) or off-nest behaviors (eating/drinking, self-grooming, being active and total time off-nest) from P2 to P8. Plotted lines represent average of time ± s.e.m. Summary data are reported in Table S5.

Figure S6. Maternal behavior displayed by F1 female rats exposed *in utero* to a mixture of EDC or vehicle. Data shows time spent by dams displaying in-nest behavior (nest building, Arched-back, blancked and passive nursing; and total time spent in-nest) or off-nest behaviors (eating/drinking, self-grooming, being active and total time off-nest) from P2 to P8. Plotted lines represent average of time ± s.e.m. Summary data are reported in Table S5.

Figure S7. Maternal behavior displayed by F2 female rats exposed as germ cells to a mixture of EDC or vehicle. Data shows time spent by dams displaying in-nest behavior (nest building, Arched-back, blancked and passive nursing; and total time spent in-nest) or off-nest behaviors (eating/drinking, self-grooming, being active and total time off-nest) from P2 to P8. Plotted lines represent average of time ± s.e.m. Summary data are reported in Table S5.

Figure S8. Maternal behavior displayed by F3 female rats directly ancestrally exposed to a mixture of EDC or vehicle. Data shows time spent by dams displaying in-nest behavior (nest building, Arched-back, blancked and passive nursing; and total time spent in-nest) or off-nest behaviors (eating/drinking, self-grooming, being active and total time off-nest) from P2 to P8. Plotted lines represent average of time ± s.e.m. Summary data are reported in Table S5.

Figure S9. Fold enrichment of gene ontology (GO) annotations using David pathway analysis across compared groups (CTL vs EDC) in the MBH-PoA of F1 generation females at P21. The gene enrichment analysis grouped the differentially expressed genes using GO annotations data. We selected enriched GO annotations using 2-fold enrichment criteria as a threshold and identifying annotations that were involved in brain and behavioral processes. Those annotations were then categorized in upregulated (orange) or downregulated (blue) annotations.

Figure S10. *Nr3c1* and *Crh* mRNA expression and *Darpp32* and *Dard1* promoter chromatin state in the female rat *in utero* and lactationally (F1 generation) exposed to an EDC mixture or vehicle. (**a**) Expression of *Nr3c1* and *Crh* mRNA in the MBH-PoA prepubertal (P21) female rats as determined by qPCR (n=6/group). AU = arbitrary units. RNA expression data were normalized by dividing each individual value by the average of the control group at every time point. (**b-c**) Abundance of the TrxG-dependent activating marks H3K4me3 and H3K9ac and the PcG-dependent repressive mark H3K27me3 and H3K9me3 at the *Darpp32* and *Drd1* promoter in the prepubertal MBH-PoA of females perinatally exposed to a mixture of EDC (F1 generation), as measured by ChIP (n=6/group). Dotted red lines represent repressive histone modifications while green lines represent activatory histone modifications. Bars represent mean ± s.e.m. Summary data are reported in Table S5.

Figure S11. Abundance of the TrxG-dependent activating marks H3K4me3 (a) and H3K9ac (b) and the PcG-dependent repressive mark H3K27me3 (c) at the *Kiss1, Esr1, Oxt* and *Th* promoter in the prepubertal (P21) MBH-PoA of the transgenerationally-exposed EDC pups or control raised by either *in utero* EDC exposed dams or control, as measured by ChIP (n=6/group). CC= control pup raised by control dam; EC: control pup raised by *in utero* EDC exposed dam; EE= germ-cell EDC exposed pup raised raised by *in utero* EDC exposed dam; CE= germ-cell EDC exposed pup raised by control dam. Bars represent mean ± s.e.m. (*$P < 0.05$, **$P < 0.01$ vs. CC, †$P < 0.05$ vs. CE, one-way ANOVA). Summary data are reported in Table S6.

Table S1. Primer Sequence.

Table S2. List of primary antibodies.

Table S3. Report of descriptive and statistical data.

Table S4. Report of descriptive data and statistical analysis of crossfostering data.

Table S5. Report of descriptive and statistical data from supplementary material.

Table S6. Report of descriptive data and statistical analysis of crossfostering data from the supplementary material.