Supplemental Material

Rat hypothalamic cDNA was used as template to generate gpr54 specific PCR fragment. The PCR reaction was carried out using gpr54 forward and T7 gpr54 antisense primers. The amplicon was visualized by gel electrophoresis (1.8 % agarose gel, 0,5xTBE buffer). The gpr54 specific PCR product (473 bp) was isolated from the gel (Gel/PCR DNA Fragment Extraction Kit, Genaid). This fragment (10 ng) was served as a template to second PCR reaction. This step increases the specificity of the gpr54 amplicon. The second PCR reaction was carried out using T7 and gpr54 forward primers. The PCR product visualized by gel electrophoresis (1.8 % agarose gel, 0,5xTBE buffer) and isolated from the gel (Gel/PCR DNA Fragment Extraction Kit, Genaid). This amplicon (50 ng) was served as a template for in vitro radionuclide (35S-uridine triphosphate, DuPont NEN, USA) labeled cRNA probe synthesis using T7 RNA polymerase. The specificity of the designed primers was verified by Primer Blast software.

Experimental conditions of the PCR reaction using gpr54 forward and T7 gpr54 antisense primers:

95 °C, 3 min
95 °C, 30 sec
65 °C, 30 sec 35 cycles
72 °C, 1 min
72 °C, 3 min

The experimental conditions for the PCR reaction using gpr54 forward and T7 primers:

95 °C, 3 min
95 °C, 30 sec
55 °C, 30 sec 35 cycles
72 °C, 1 min
72 °C, 3 min
Supplemental Figure 1. EE2 dose-response relationship

Mean ± SEM values of normalized uterus weight (A) and timing of vaginal opening (B) in vehicle-treated controls and ethinyl estradiol (EE2-1, 10, 50 µg/kg/day)-treated immature rats. Animals (n=16) were treated via oral gavage between PN days 18-28. A group of naïve rats (n=5) was left undisturbed until the “natural” vaginal opening (NVO).
Supplemental Figure 2. Effect of xenoestrogen treatment on the ovaries

Representative hematoxylin-eosin stained ovarian sections from rats treated with vehicle, ZEA or EE2 in the prepubertal period (PND 18-28).
Supplemental Figure 3. Food and water intake in xenoestrogen exposed prepubertal female rats.

Daily food and water intake of female rats exposed to vehicle (control)-, ZEA- and EE2 as measured between postnatal days 18-28. On the right, cumulative food consumption of rats measured during the whole treatment period (mean ± SEM values).
Supplemental Figure 4. Effect of xenosestrogens ZEA and EE2 on the puberty-controlling transcription factors.

Mean ± SEM values of relative quantities of makorin 3 (MKR3) and EED in the AVPV and ARC.
Supplemental Figure 5. Expression of various factors implicated in development of puberty in the anterior hypothalamic region

Mean ± SEM values of relative quantities of cbx7, eap1, cux1, ttf1 transcription factors, estrogen receptor alpha (erα) and tyrosine hydroxylase (th) in the anterior hypothalamic block (AVPV/POA) of vehicle-treated control, ZEA and EE2-treated prepubertal female rats.
Supplemental Figure 6. Expression of various factors implicated in development of puberty in the arcuate region

Mean ± SEM values of relative quantities of cbx7, eap1, cux1, ttf1 transcription factors and estrogen receptor alpha (ERα) in the arcuate region (ARC) of vehicle-treated control, ZEA and EE2-treated prepubertal female rats.