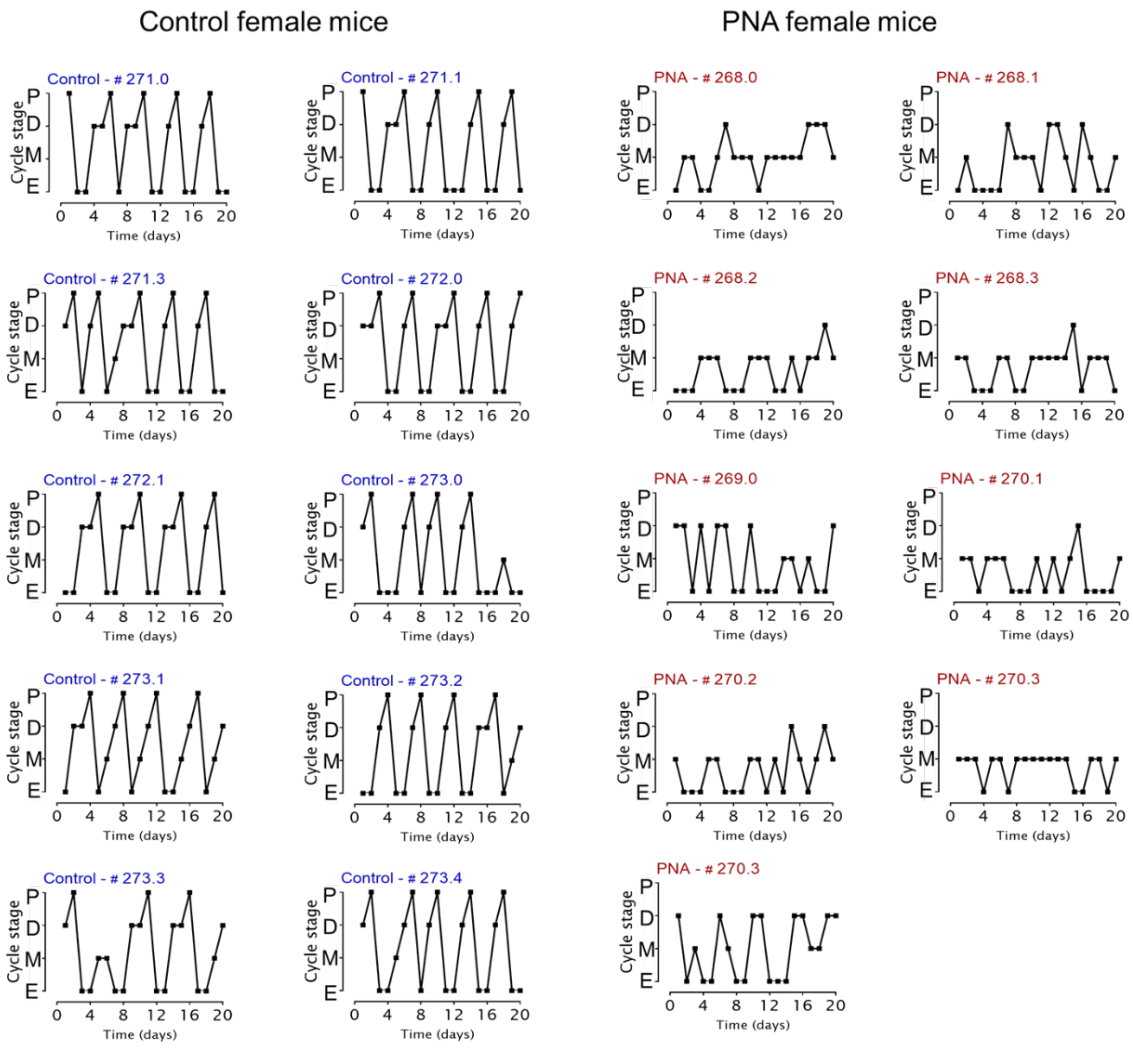
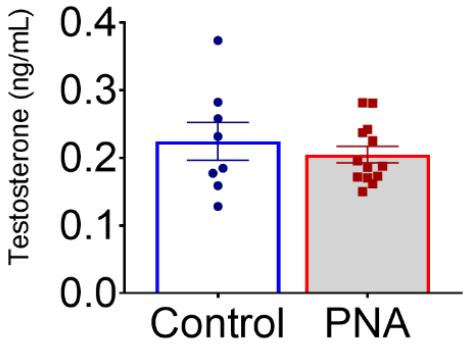
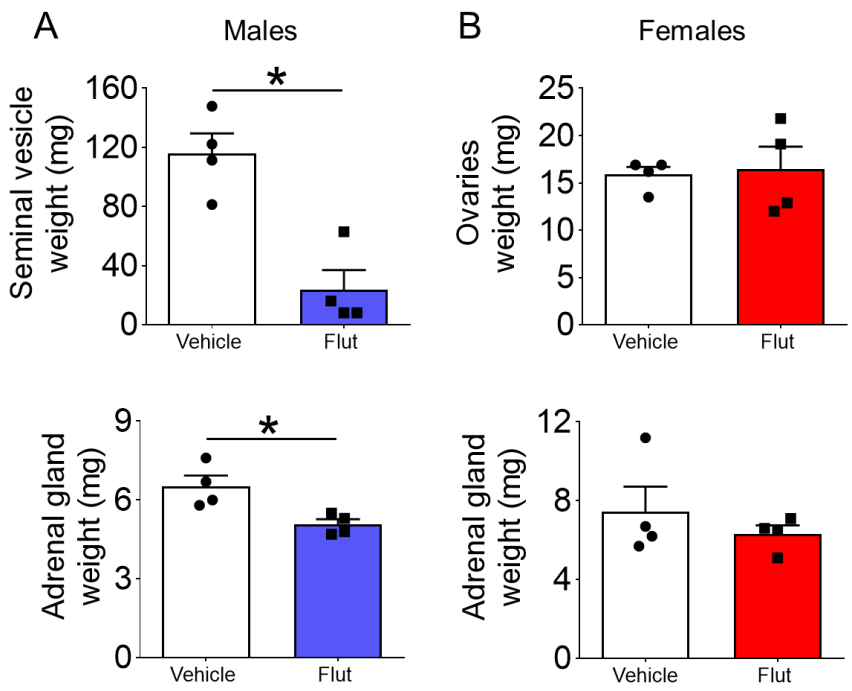
Supplemental data



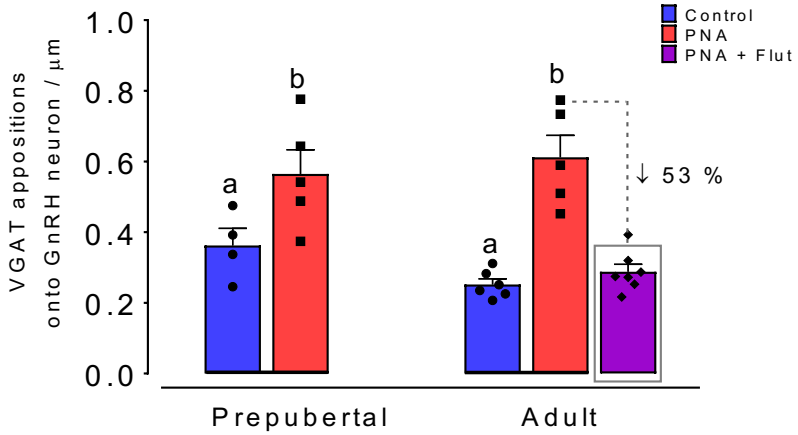
Supplemental Figure 1. PNA treatment significantly disrupts reproductive cycles. Estrous cycles from control (N = 10) and PNA (N = 9) from PND 60 to PND 80. Vaginal smears and cytological evaluation were used to identify the estrous stages proestrus (P), diestrus (D), metestrus (M) and estrus (E). All mice here presented are the same animals used to measure testosterone blood levels from PND 30 to PND 60 as shown in *Figure 1* of this manuscript.



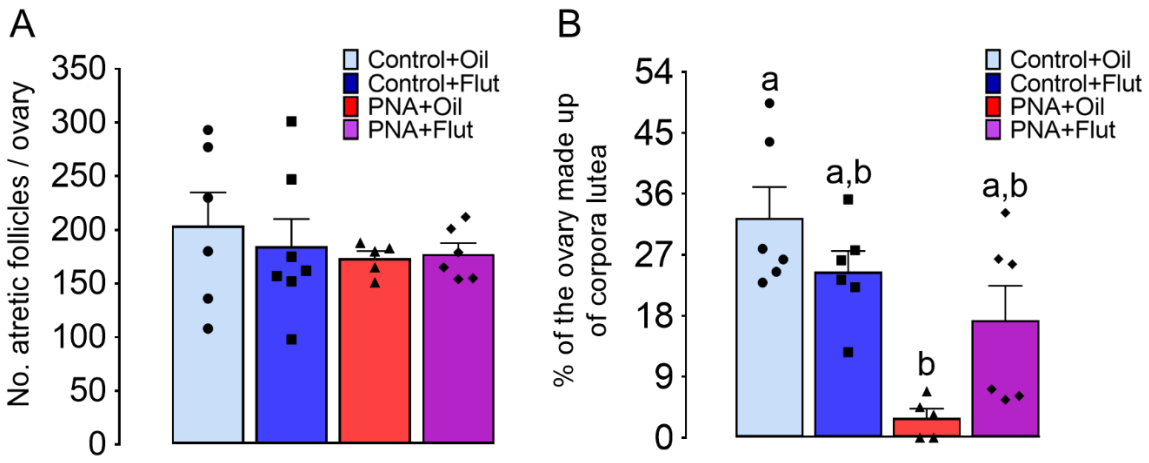
Supplemental Figure 2. Prepubertal PNA mice have similar testosterone blood levels. Testosterone blood levels from control (N = 8) and PNA (N = 13) mice at PND 25. Two-tailed Student's *t*-test.



Supplemental Figure 3. Anti-androgenic effect of flutamide (Flut; 25mg/kg/day) administration in control male and female mice. Flut was administered with subcutaneous (s.c.) injections daily for 15 days. Antiandrogenic effect at the dose of 25mg/kg/day was determined by measuring the weight of seminal vesicles and adrenal glands from male mice (N = 4), as a positive biological control, and the weight of ovaries and adrenal glands from female mice (N = 4). Data are expressed as means ± SEM. *$P < 0.05$; Mann-Whitney *U* test.



Supplemental Figure 4. PNA mice show similar amount of GABA inputs onto GnRH neurons across pubertal development. Total VGAT appositions onto GnRH neuron were compared among prepubertal control (N = 4) and PNA (N = 5) mice, and adult oil-vehicle control (N = 6) and oil-vehicle PNA (N = 5) mice. Histogram values are represented as mean ± SEM. Different letters indicate significant statistical differences with $P < 0.05$; two-way ANOVA with Bonferroni`s post hoc test. Graphical representation illustrates the effect of androgen receptor (AR) blockade attenuating GABA inputs onto GnRH neurons (percentage of decrease) in PNA+Flut mice when compared to PNA+oil group.



Supplemental Figure 5. Androgen receptor (AR) blockade effect on the number of atretic follicles and corpora lutea area within the ovary. (A) Total number of atretic follicles in adult control and PNA mice receiving daily s.c. injections of an oil-vehicle or flutamide (Flut) 25 mg/kg/day from PND 40 to PND 60. **(B)** Percentage of the ovarian follicle area made up of corpora lutea across groups. Different letters indicate significant statistical differences with $P < 0.05$; two-way ANOVA followed by Tukey's post hoc test. Histograms show mean ± SEM data from control+oil (N = 6), control+Flut (N = 7), PNA+oil (N = 5) and PNA+Flut (N = 6) groups.