Supporting Information

Moore et al. 10.1073/pnas.1415038112

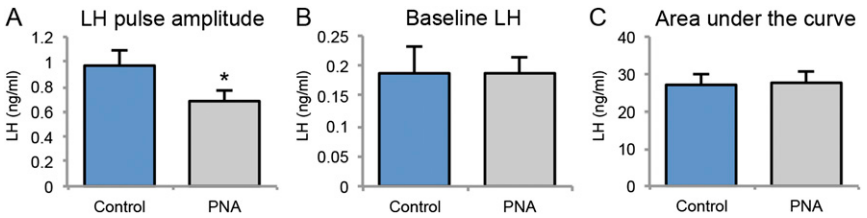

Fig. S1. Serial blood sampling of whole blood LH concentrations from control ($n = 14$) and PNA ($n = 19$) mice in diestrus at 6- to 10-min intervals for 2 h reveals that LH pulse amplitude (*A*) is significantly reduced by PNA treatment. No differences in mean baseline LH (*B*) and the area under the curve (*C*) were detected between control and PNA mice.

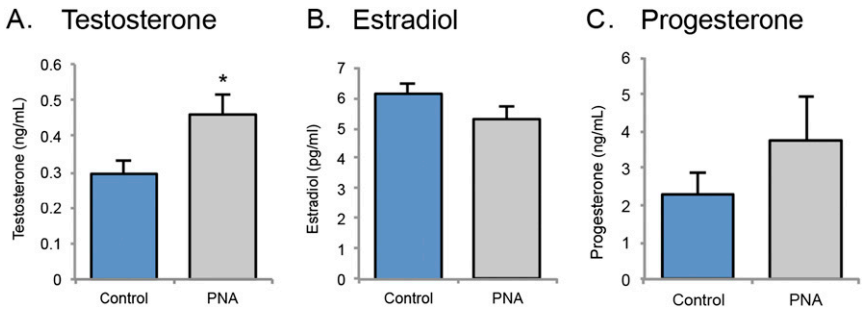

Fig. S2. (*A*) Plasma testosterone concentration is significantly increased in PNA mice ($n = 17$) compared with controls ($n = 17$). Reproduced with permission from ref. 1. (*B*) Serum estradiol concentration is not significantly different between control ($n = 18$) and PNA mice ($n = 18$) in diestrus. (*C*) Plasma progesterone concentration is not significantly different between control ($n = 6$) and PNA ($n = 5$) mice in diestrus.

1. Moore AM, Prescott M, Campbell RE (2013) Estradiol negative and positive feedback in a prenatal androgen-induced mouse model of polycystic ovarian syndrome. *Endocrinology* 154(2):796–806.

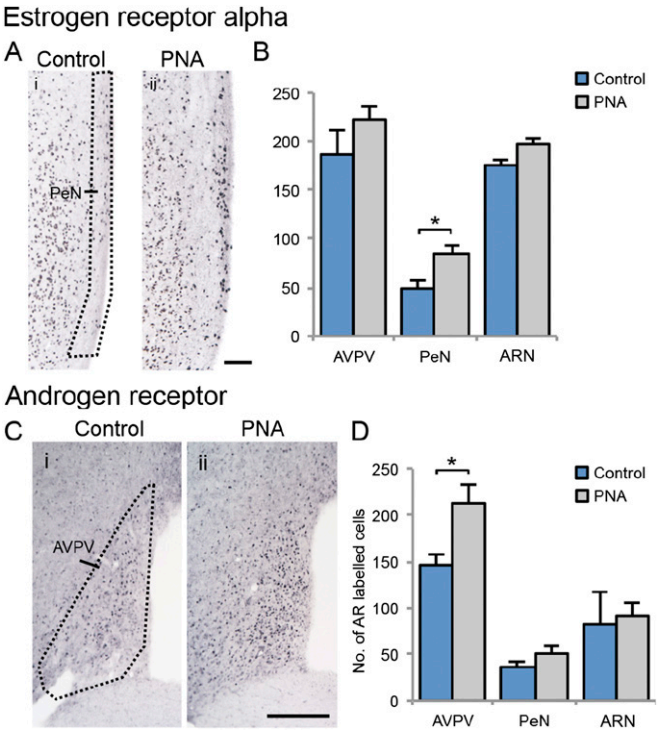

Fig. S3. (*A*) ERα immunoreactivity in representative unilateral sections containing the PeN (outlined) in control (*i*, $n = 8$) and PNA (*ii*, $n = 7$) mice. (*B*) The number of ERα-labeled cells is significantly increased in the PeN of PNA mice compared with controls but unchanged in the AVPV and ARN. (*C*) AR immunoreactivity in representative unilateral sections containing the AVPV (outlined) in control (*i*, $n = 6$) and PNA (*ii*, $n = 5$) mice. (*D*) The number of AR-labeled cells is significantly increased in the AVPV of PNA mice compared with controls but unchanged in the PeN and ARN. (Scale bar, 0.2 mm.)

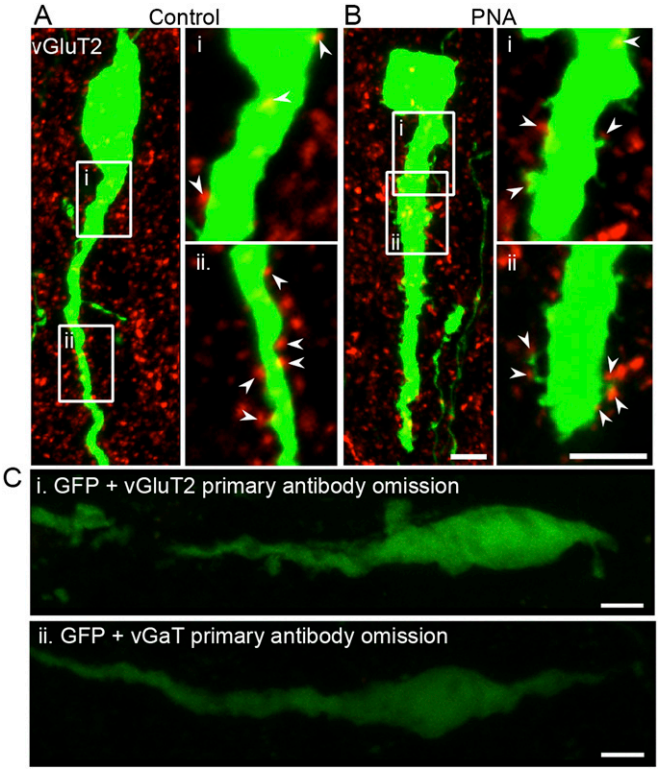

Fig. S4. Projected confocal images (8-μm optical thickness) of GnRH neurons (green) closely apposed by vGluT2-ir puncta (red) from control (*A*, $n = 9$) and PNA (*B*, $n = 8$) mice. (*i* and *ii*) Projected confocal images (1.35-μm optical thickness) of the GnRH neuron dendrite from corresponding white boxes. Arrowheads indicate points where vGluT-ir puncta are considered to be contacting the GnRH neuron. (*C*) Projected confocal images (8-μm optical thickness) from negative control sections, in which primary antibodies have been omitted, with endogenous GFP in GnRH neurons and the absence of vGluT2 (*i*) and vGaT (*ii*) immunoreactivity. (Scale bars, 5 μm.)

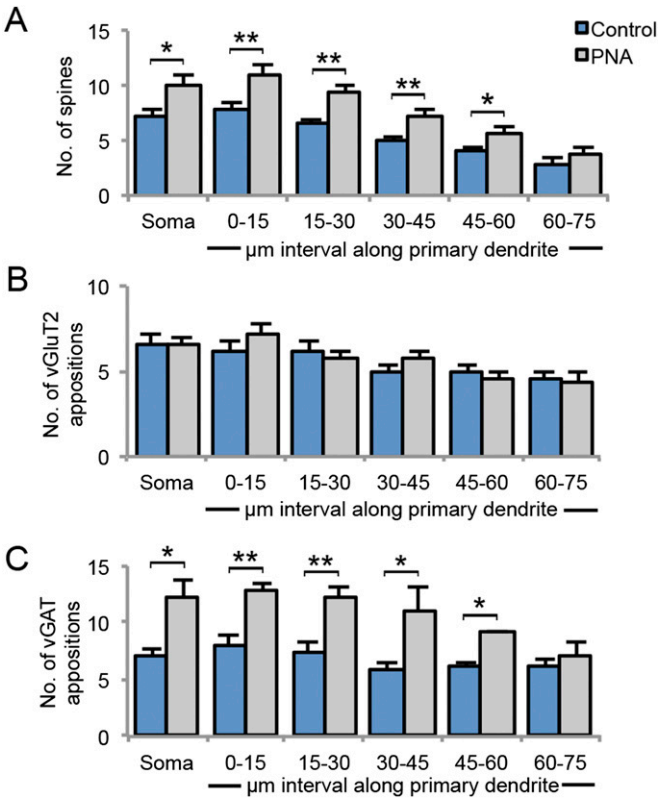

Fig. S5. (*A*) The number of GnRH neuron spines at the soma and proximal 60 μm of the dendrite is significantly increased in PNA mice ($n = 9$) compared with controls ($n = 8$). (*B*) The number of vGluT2-ir puncta closely apposed to the GnRH neuron soma and dendrite is not significantly different between control ($n = 9$) and PNA ($n = 8$) mice. (*C*) The number of vGaT-ir puncta closely apposed to the GnRH neuron soma and proximal 60 μm of the dendrite is significantly increased in PNA mice ($n = 5$) compared with controls ($n = 5$).

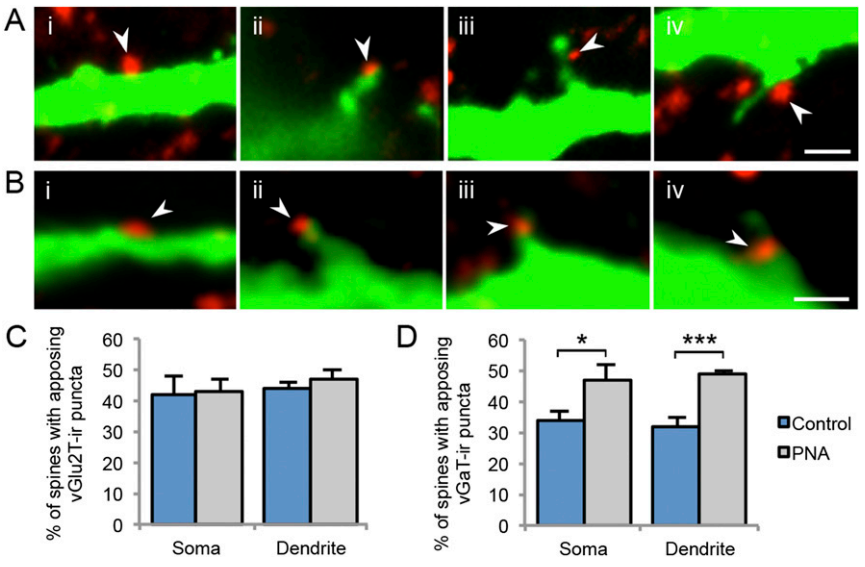

Fig. S6. Representative confocal images from projected slices (total 1.35-μm thickness) of vGluT2-ir puncta (*A*) and vGaT-ir puncta (*B*) apposed (white arrows) to the GnRH neuron dendritic shaft (*i*), spine head (*ii*), spine neck (*iii*), and spine base (*iv*). The percentage of spines apposed by vGluT2-ir puncta (*C*) is not altered by PNA treatment, however the percentage of spines apposed by vGaT-ir puncta (*D*) is significantly increased in PNA mice compared with controls. [Scale bar, (*A*) 2.5 μm and (B) 1.25 μm.]

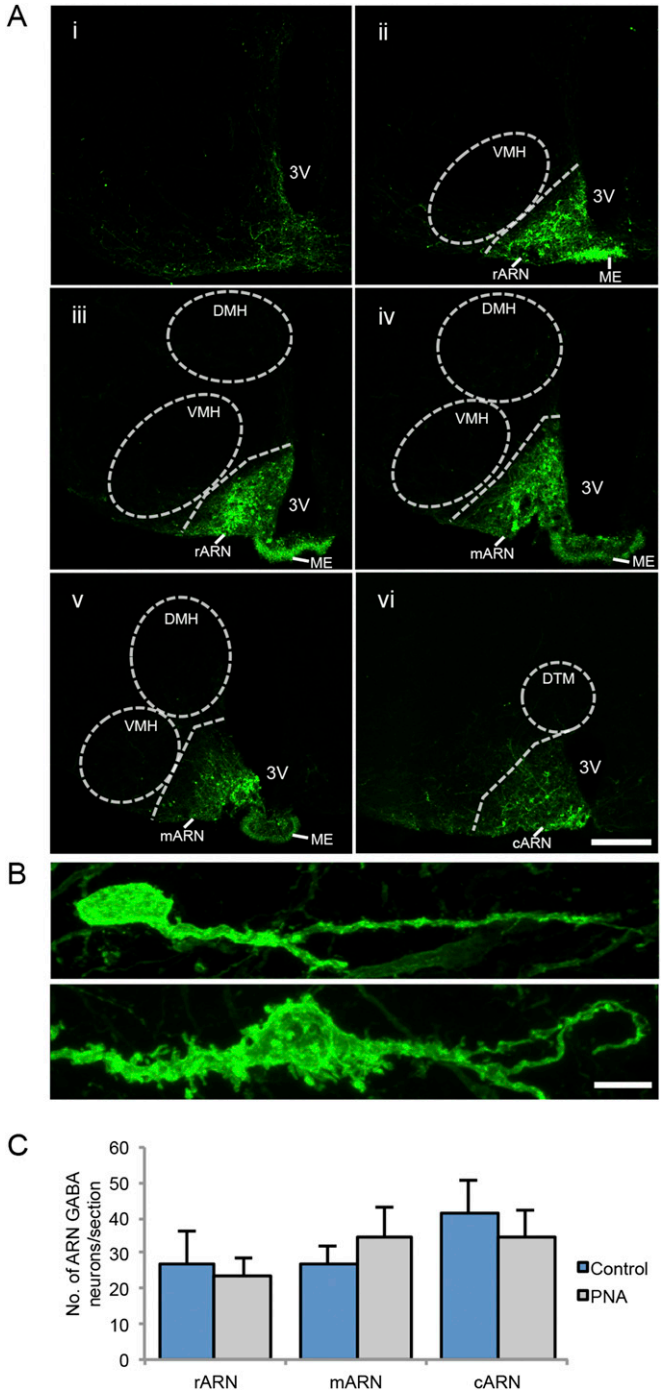

Fig. S7. (*A*) A single injection of Ad-iZ/EGFPf induces EGFPf expression in GABAergic neurons of the rostral (*ii* and *iii*), middle (*iv* and *v*), and caudal (*vi*) regions of the ARN and avoids EGFPf expression in surrounding GABAergic populations. (Scale bar, 0.5 mm.) (*B*) High-magnification images of ARN GABAergic neurons expressing EGFPf. (Scale bar, 5 μm.) (*C*) The number of EGFPf-positive GABA neurons in the rostral, middle, and caudal ARN is not significantly different between regions in control and PNA mice. 3V, third ventricle; DMH, dorsomedial hypothalamus; DTM, dorsal tuberomammillary nucleus; ME, median eminence; VMH, ventral medial hypothalamus.

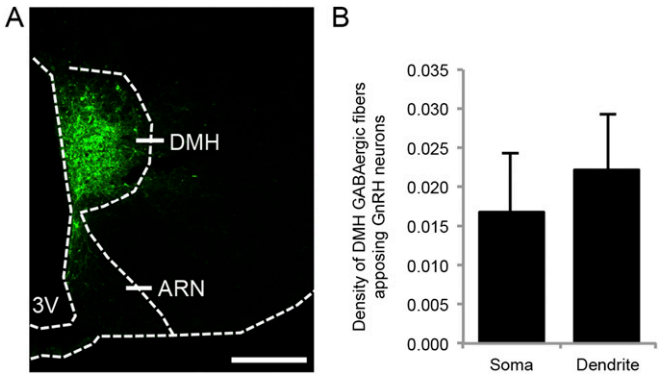

Fig. S8. (*A*) Injection of Ad-iZ/EGFPf 1 mm posterior to bregma, 0.3 mm lateral to bregma, and 5.2 mm ventral to dura induces EGFPf expression in GABAergic neurons of the DMH ($n = 4$). (Scale bar, 0.5 mm.) (*B*) The density of closely apposed fibers from DMH GABAergic neurons to GnRH neurons in control injections.