Activation of a classic hunger circuit slows luteinizing hormone pulsatility

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Running Title: NPY/AgRP neurons slow LH pulsatility

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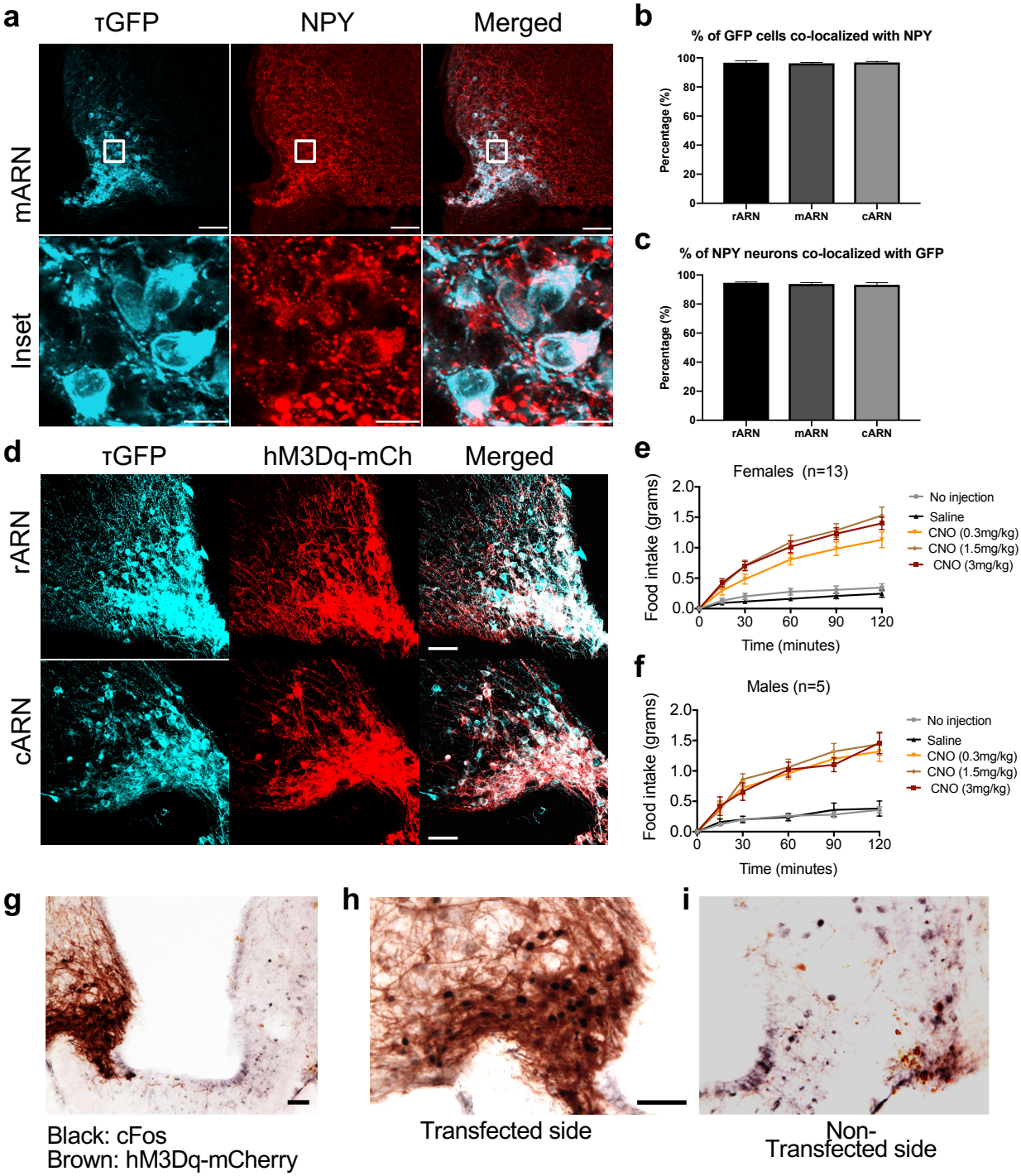
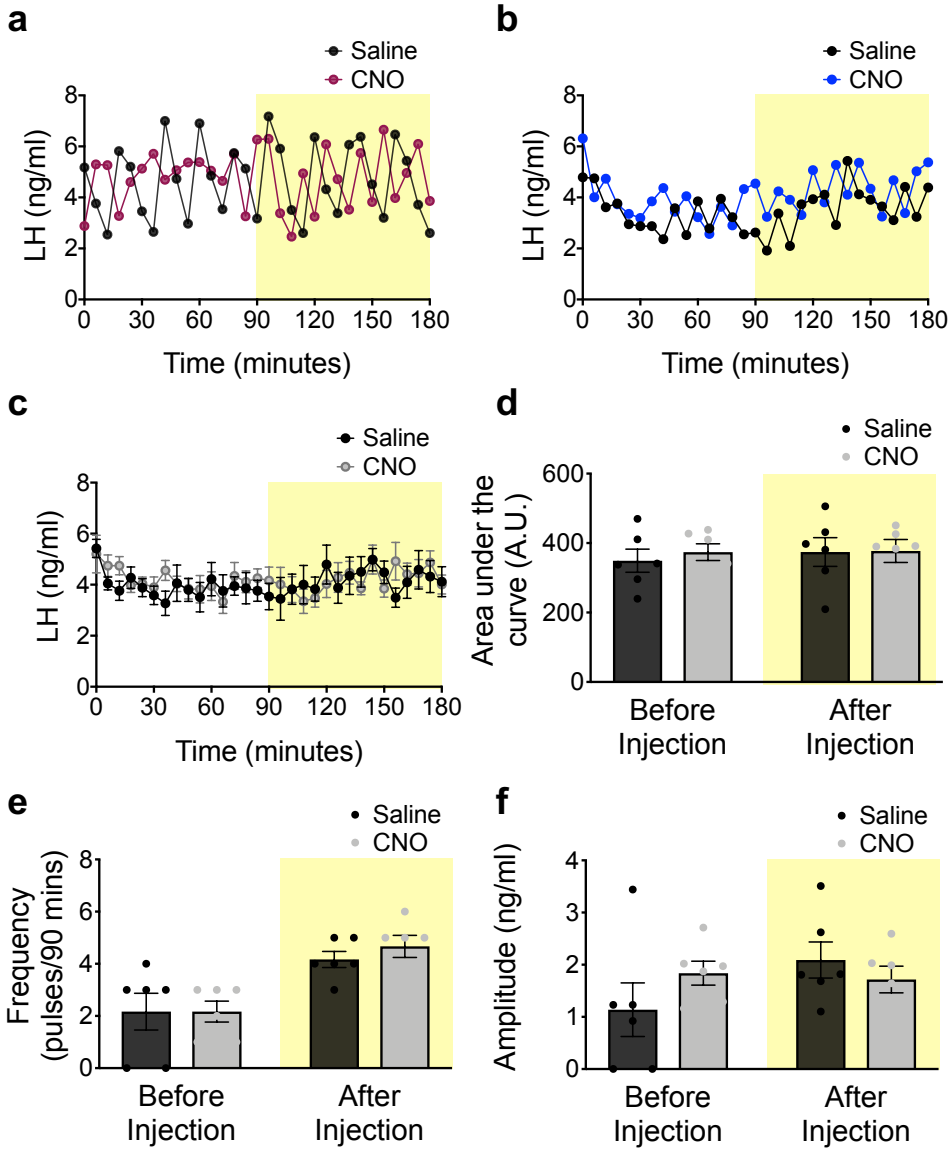
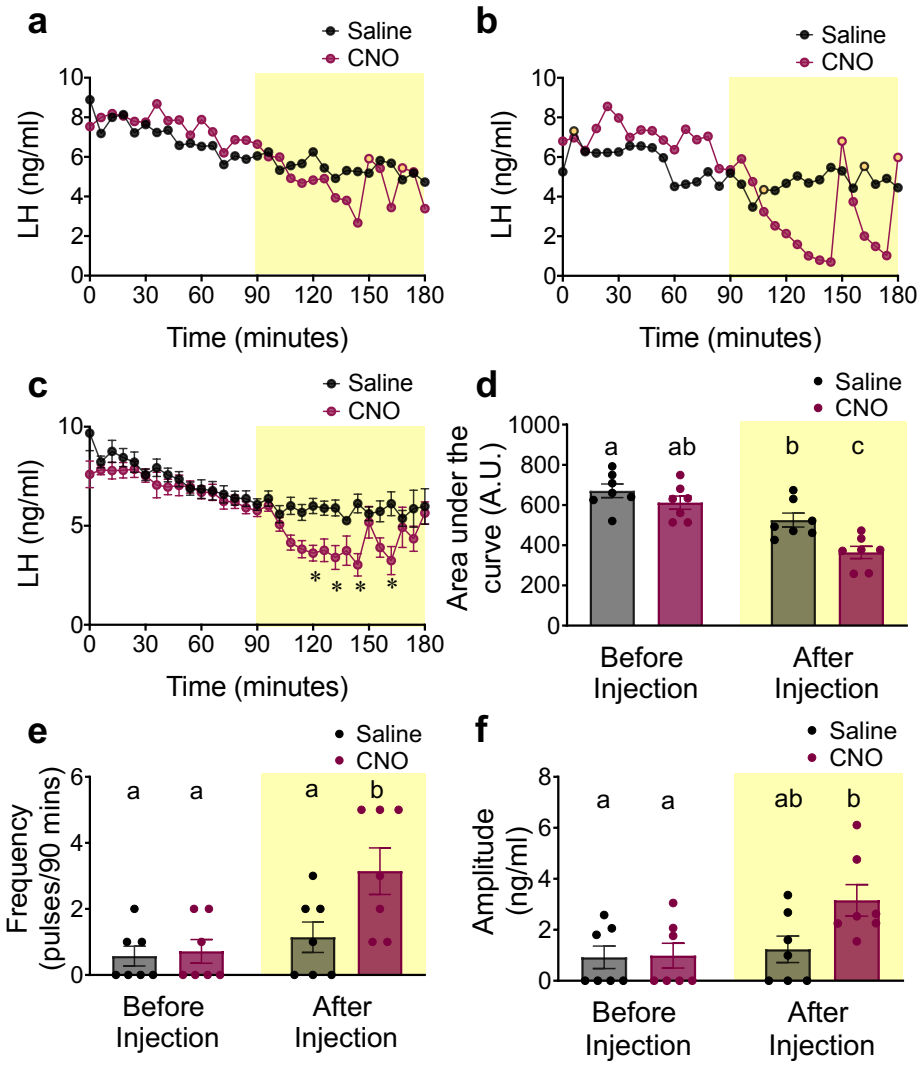


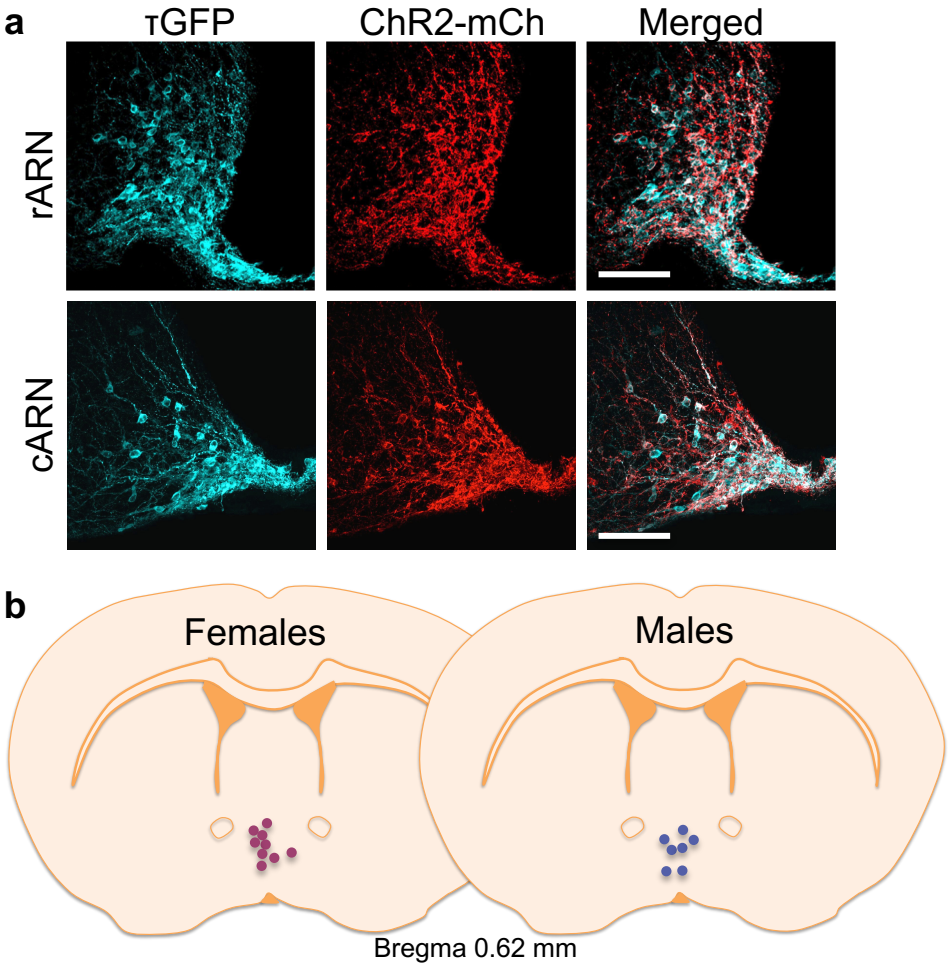
Supplementary Figure 1 Specific expression and activation of hM3Dq-mCherry in ARN NPY/AgRP neurons (a) Expression of τGFP (cyan), NPY (red) and merged images of the middle (mARN) arcuate nucleus. Scale = 50 μm (whole ARN), or 10 μm (inset). (b) Percentage of GFP cells expressing NPY (c) Percentage of NPY neurons co-localised with GFP (d) Specific expression of hM3D-mCherry (red) in only τGFP (cyan) expressing cells in the rostral (rARN) and caudal (cARN) arcuate nucleus. Scale = 100 μm. Food intake in (e) females (n = 13) and (f) males (n = 5) over 2 hours following injection of varying doses of CNO. (g) Image of a unilaterally hM3Dq transfected arcuate nucleus to illustrate cellular activation following 1.5mg/kg CNO delivery. Scale = 50 μm Staining for cFos (Black dots) and mCherry (brown) in the transfected (h) and non-transfected (i) sides of the arcuate nucleus. Scale = 50 μm



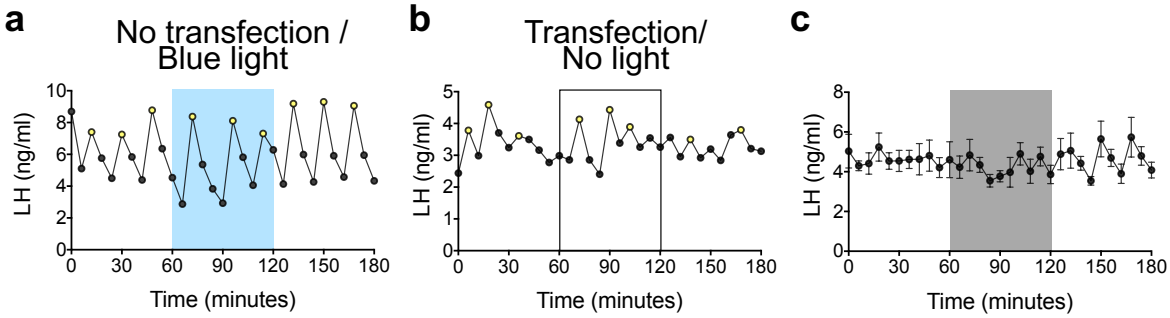
Supplementary Figure 2 Chemogenetic activation of NPY/AgRP neurons in control animals Example of LH secretion in non-transfected, gonadectomised (a) female and (b) male mice. (c) Mean LH secretion and (d) Area under the curve (e) pulse frequency and (f) amplitude for control animals (total n = 6) [Control animals were comprised of Cre-positive animals with no transfection (n = 3 males) and Cre-negative animals injected with hM3Dq-mCherry (n = 2 males, n = 1 female).]



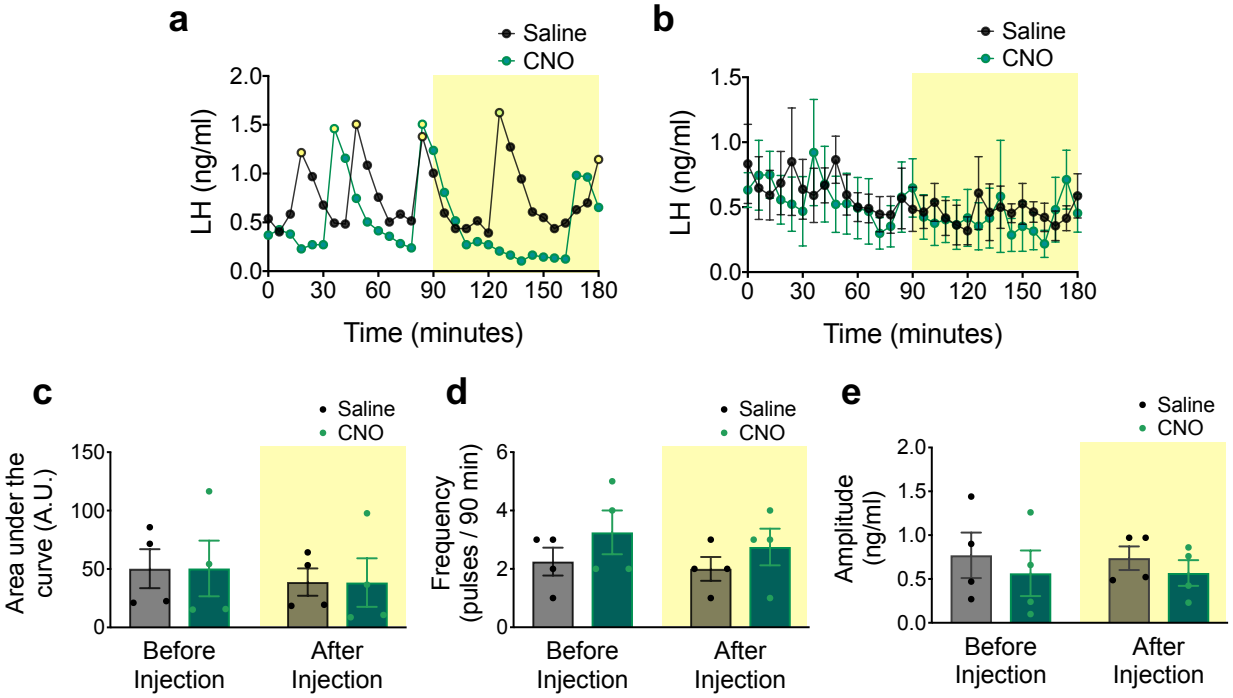
Supplementary Figure 3 Chemogenetic activation of NPY/AgRP neurons decreased LH secretion in long term ovariectomised females (a) & (b) Examples of LH secretion during saline (black) and CNO (magenta) trials in long term ovariectomised females. Yellow filled circles indicate peaks in LH secretion. Yellow shaded region indicates time after injection. (c) Mean LH secretion, (d) area under the curve (e) pulse frequency (f) amplitude from the saline and CNO trials for long term ovariectomised females (n = 6). Statistical analysis was carried out with two-way ANOVA and Sidak's *post-hoc* test. A *p*-value of less than 0.5 was considered significant. Significant groups are indicated by different letters.



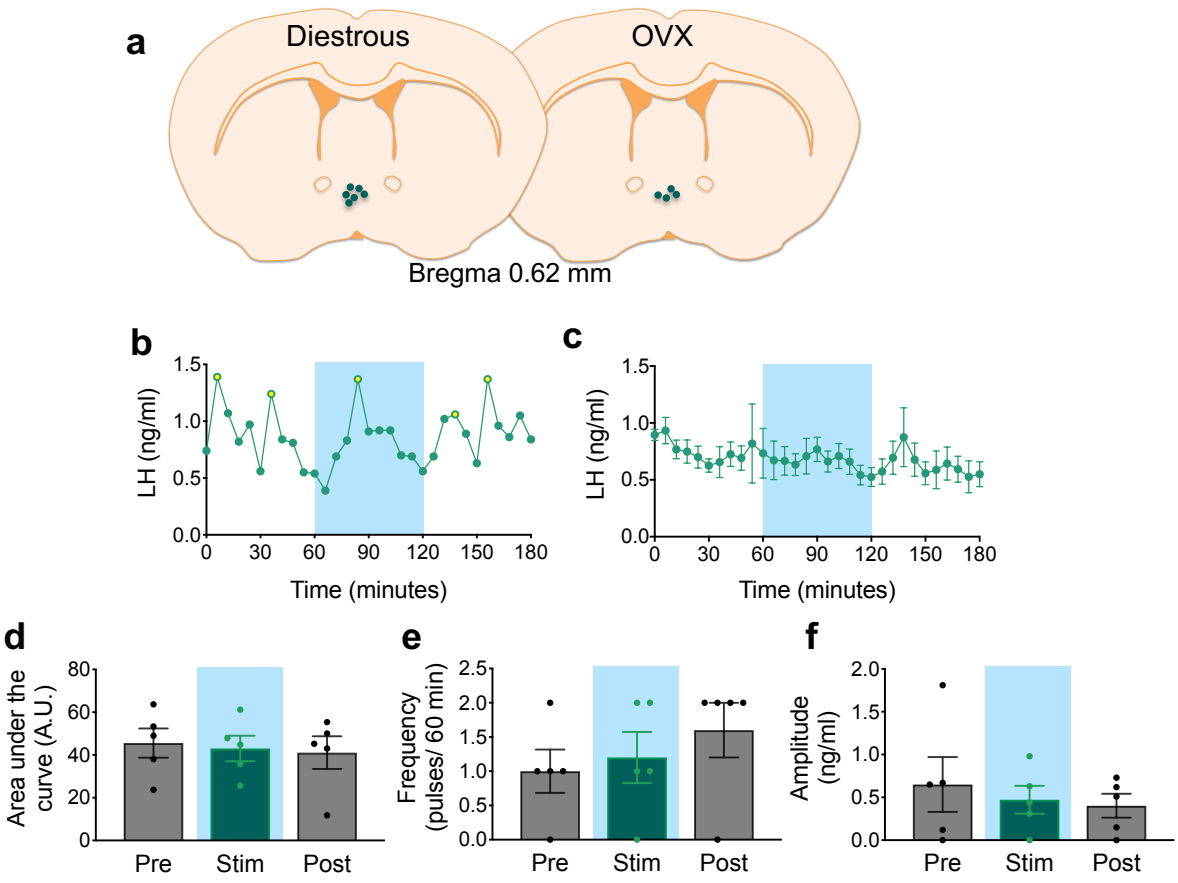
Supplementary Figure 4: (a) Specific expression of ChR2-mCherry (red) in only τGFP (cyan) expressing cells in the rostral (rARN) and caudal (cARN) arucate nucleus. Scale = 100 μm. (b) Position of the optic fibres in the rPOA of gonadectomised females (n=9) and males (n = 7).



Supplementary Figure 5: Optogenetic activation of NPY/AgRP terminals in the rPOA of control animals (a) Example of LH secretion in a non-tranfected animal with blue light stimulation. (b) Example of LH secretion in a transfected animal with no light stimulation. (d) Mean LH secretion for all control animals (n = 7).



Supplementary Figure 6 Chemogenetic activation of NPY/AgRP neurons in gonadally intact PNA mice. (a) Example of LH secretion during saline (black) and CNO (teal) trials in a diestrous PNA female. Yellow filled circles indicate LH pulses. Yellow shaded region indicates time after injection. (b) Mean LH secretion from the saline and CNO trials in diestrous PNA females (n = 4). (c) Area under the curve (d) pulse frequency and (e) amplitude before and after saline (black bars) and CNO (teal bars) injection. Statistical analysis was carried out with two-way ANOVA and Sidak's *post-hoc* test. A *p*-value of less than 0.5 was considered significant and significant groups are indicated by different letters. Absence of letters indicates no significant difference between any groups.



Supplementary Figure 7 (a) Optic fibre positions in diestrous (left) and ovariectomised (right) PNA females. (b) Example of LH secretion before, during and after blue light stimulation in a diestrous PNA female. Yellow filled circles indicate peaks in LH secretion. Blue shaded region indicates time of light stimulation (c) Mean LH secretion before, during and after blue light stimulation for diestrous PNA females (n = 6). (d) Area under the curve (e) pulse frequency and (f) amplitude before and after (black bars) and during (teal bar) light stimulation in diestrous PNA females (n = 6). Statistical analysis was carried out with repeated measures one-way ANOVA and Tukey's *post-hoc* test. Significant differences indicated as * for $p<0.05$, ** for $p<0.01$ and **** for $p<0.0001$.