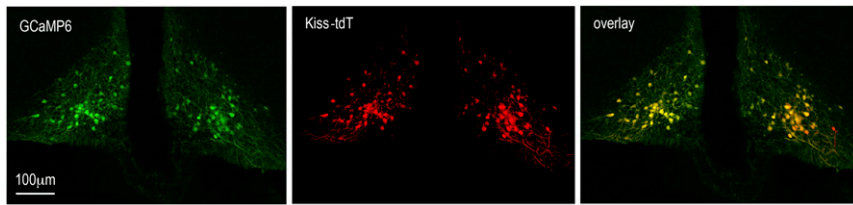


Supporting Information

Clarkson et al. 10.1073/pnas.1713897114



	No. KP neurons	No. GCaMP neurons	No. dual labelled	% KP with GCaMP	% GCaMP with KP
rARN	31.4±4.4	19.8±9.6	16.7±5.9	51.3±13.1	95.5±5.5
mARN	40.8±5.4	31.7±5.9	28.5±6.7	68.6±6.9	89.2±6.3
cARN	56.0±12.3	32.0±16.3	32.7±12.4	56.4±12.8	91.5±1.9

Fig. S1. Expression of GCaMP6 in ARN^{KISS} neurons. Photomicrographs show confocal images of GCaMP6 and kisspeptin (tdTomato reporter) and an overlay of the two at the middle coronal level of the ARN in a gonadectomized male mouse. The table below provides mean (\pm SEM) numbers of single- and dual-labeled kisspeptin- (KP) and GCaMP-expressing neurons and respective percentages at the rostral (rARN), middle (mARN), and caudal (cARN) levels of the ARN ($n = 3$).

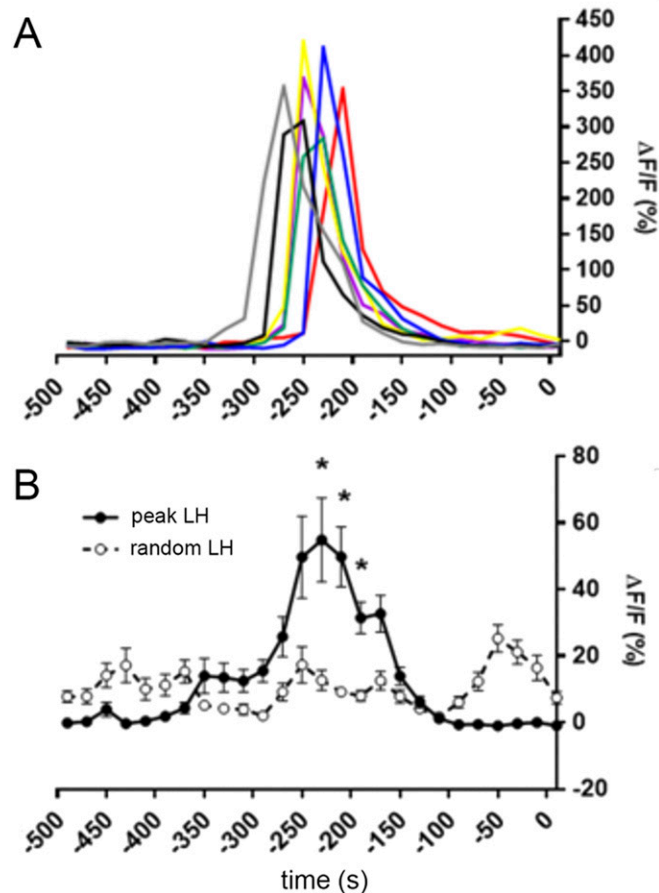
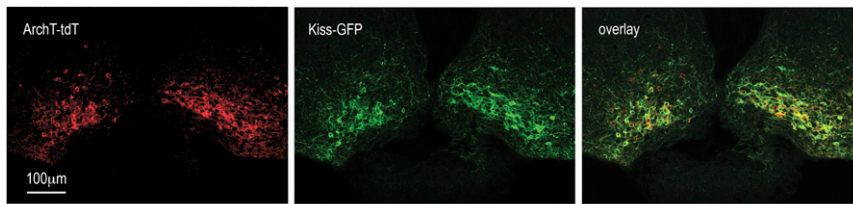
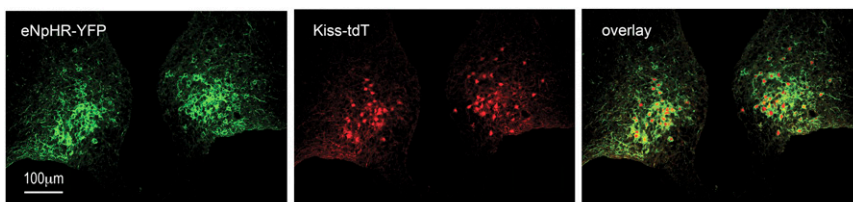


Fig. S2. Temporal relationship between LH and calcium peaks. (A) Example of one individual mouse where the six calcium peaks are aligned over the 500 s preceding the time of the LH pulse peaks set at $t = 0$. (B) Mean \pm SEM calcium levels across all animals in relation to either the peak pulse LH level (black circles; 43 pulses, $n = 7$) or an equal number of random LH time points (open circles; 43 samples, $n = 7$). A significant increase in calcium is observed in relation to LH pulses ($*P < 0.05$, repeated-measures ANOVA) but not with random LH time points.



	No. KP neurons	No. ArchT neurons	No. dual labelled	% KP with ArchT	% ArchT with KP
rARN	26.6±3.2	23.6±2.6	19.6±3.6	64.6±13.1	82.9±6.0
mARN	46.1±8.9	38.9±8.8	32.0±12.1	69.0±11.5	80.9±7.4
cARN	46.5±6.9	34.5±3.7	26.4±4.9	60.9±15.5	77.1±10.4

Fig. S3. Expression of ArchT in ARN^{KISS} neurons. Photomicrographs show confocal images of ArchT (tdTomato reporter) and kisspeptin (GFP reporter) and an overlay of the two at the caudal coronal level of the ARN in a gonadectomized female mouse. The table below provides mean (\pm SEM) numbers of single- and dual-labeled kisspeptin- and ArchT-expressing neurons and respective percentages at the rostral, middle, and caudal levels of the ARN ($n = 5$).



	No. KP neurons	No. Halo neurons	No. dual labelled	% KP with Halo	% Halo with KP
rARN	33.1±4.2	26.6±1.6	22.3±0.33	70.2±10.8	94.2±0.3
mARN	62.4±5.4	64.5±3.2	50.3±5.4	62.7±6.6	85.4±5.6
cARN	68.7±9.6	51.1±10.4	44.8±9.1	65.7±12.5	91.2±1.0

Fig. S4. Expression of eNpHR3.0 (halorhodopsin) in ARN^{KISS} neurons. Photomicrographs show confocal images of eNpHR-YFP and kisspeptin (tdTomato reporter) and an overlay of the two at the caudal coronal level of the ARN in a gonadectomized female mouse. The table below provides mean (\pm SEM) numbers of single- and dual-labeled kisspeptin- and eNpHR (Halo)-expressing neurons and respective percentages at the rostral, middle, and caudal levels of the ARN ($n = 4$).

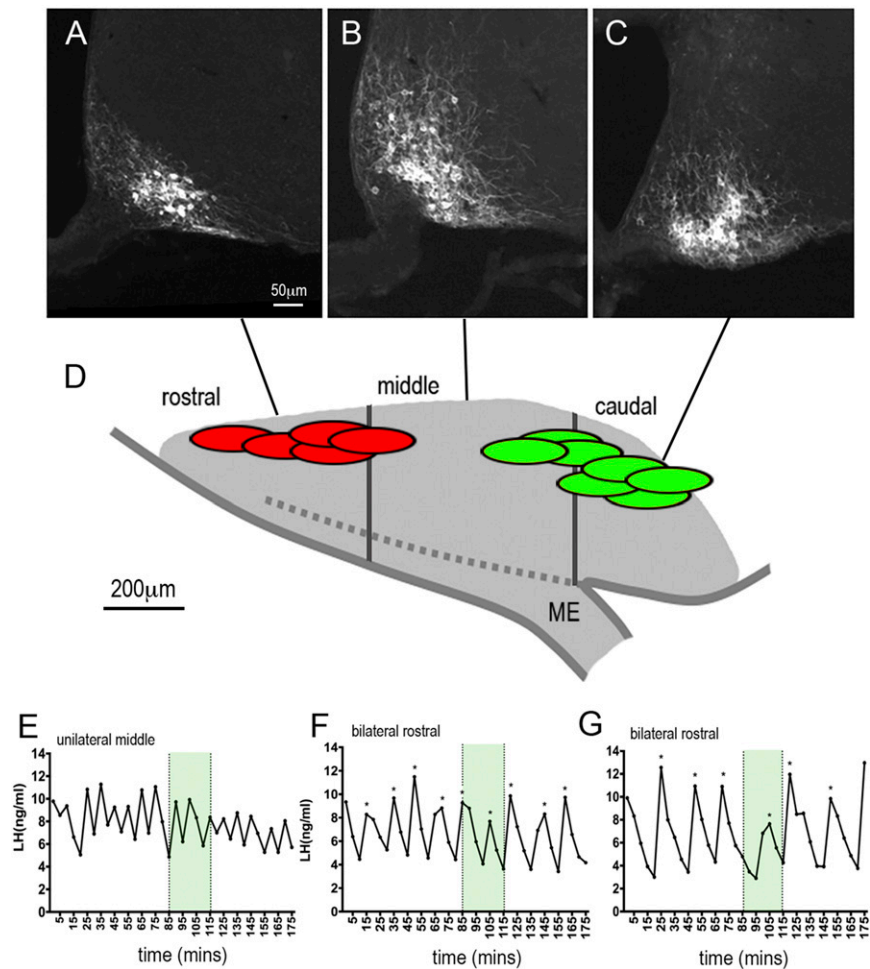


Fig. 55. Rostrocaudal relationship of effective eNpHR3.0 inhibition of pulsatile LH secretion. (A–C) eNpHR3.0 (eYFP) expression at the rostral, middle, and caudal levels of the ARN. ME, median eminence. (D) Schematic sagittal view of the ARN showing fiber optic placements that were effective (green) or ineffective (red) at suppressing pulsatile LH secretion. (E–G) Examples of pulsatile LH secretion in gonadectomized female mice with unilateral eNpHR3.0 activation (E) and two examples of bilateral rostral placements of the optic fibers (F and G).