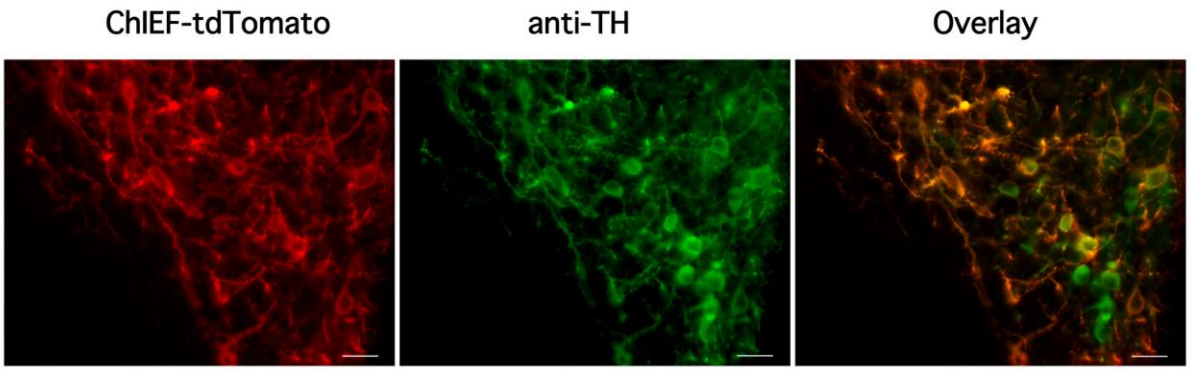

Supplementary Figure 1

Arcuate ChIEF-tdTomato neurons expressed TH

These micrographs show that TH-Cre-ChIEF-tdTomato (magenta), expressed by AAV in a TH-Cre mouse, were immunostained with TH (green) in the arcuate nucleus. Scale: 20 μm.

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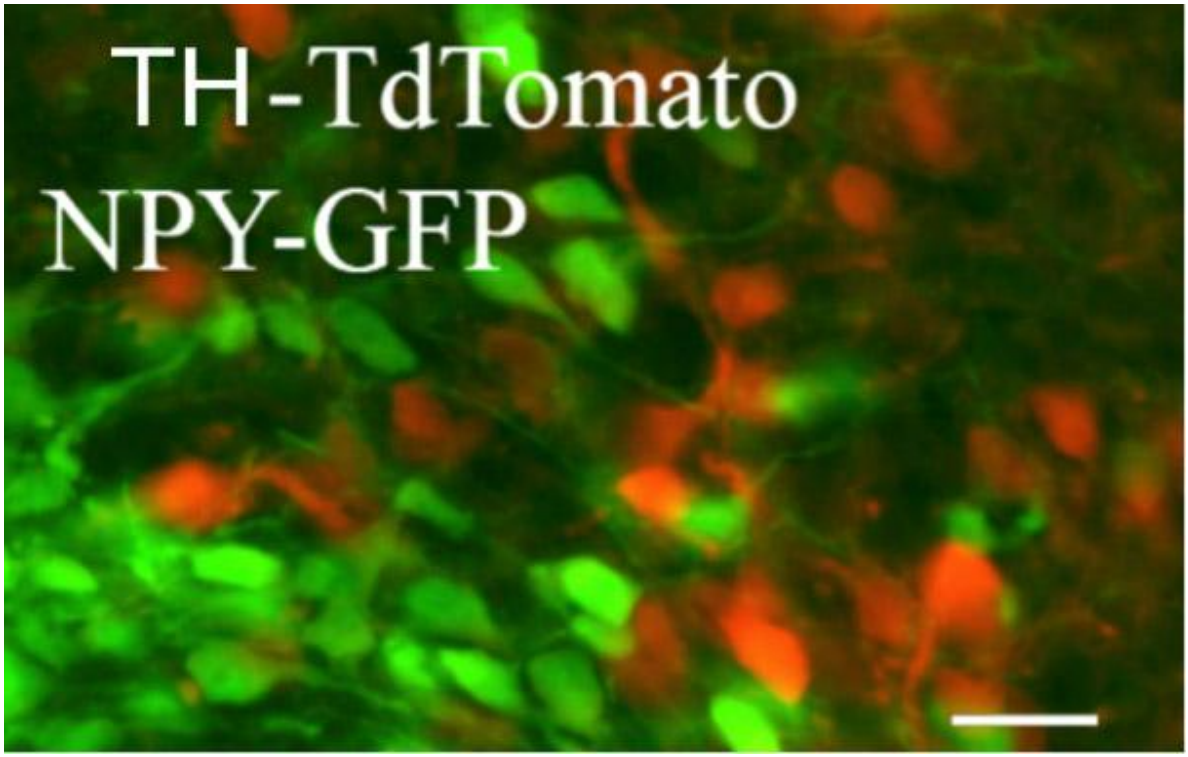

Supplementary Figure 2

Micrographs show sections from a transgenic mouse with red TH neurons and green NPY/AgRP neurons

No immunostaining was used. TH-tdTomato transgenic mice were crossed with NPY-GFP transgenic mice. Arcuate TH neurons are red, NPY/AgRP neurons are green. No cells showed both green and red fluorescence, indicating completely different populations of neurons. Bar, 30 μm. In corroborative immunocytochemical tests, we found that almost all red tdTomato reporter expression under control of the TH promoter was colocalized with immunostaining using TH antisera. In our experiments we focused on those areas of the ARC where TH neurons also contain dopamine; we recently showed that 94% of the TH neurons in the dorsomedial ARC expressed dopamine immunoreactivity, whereas in the ventrolateral ARC the number of TH neurons that contained dopamine were only a minority, as previously shown in rats.

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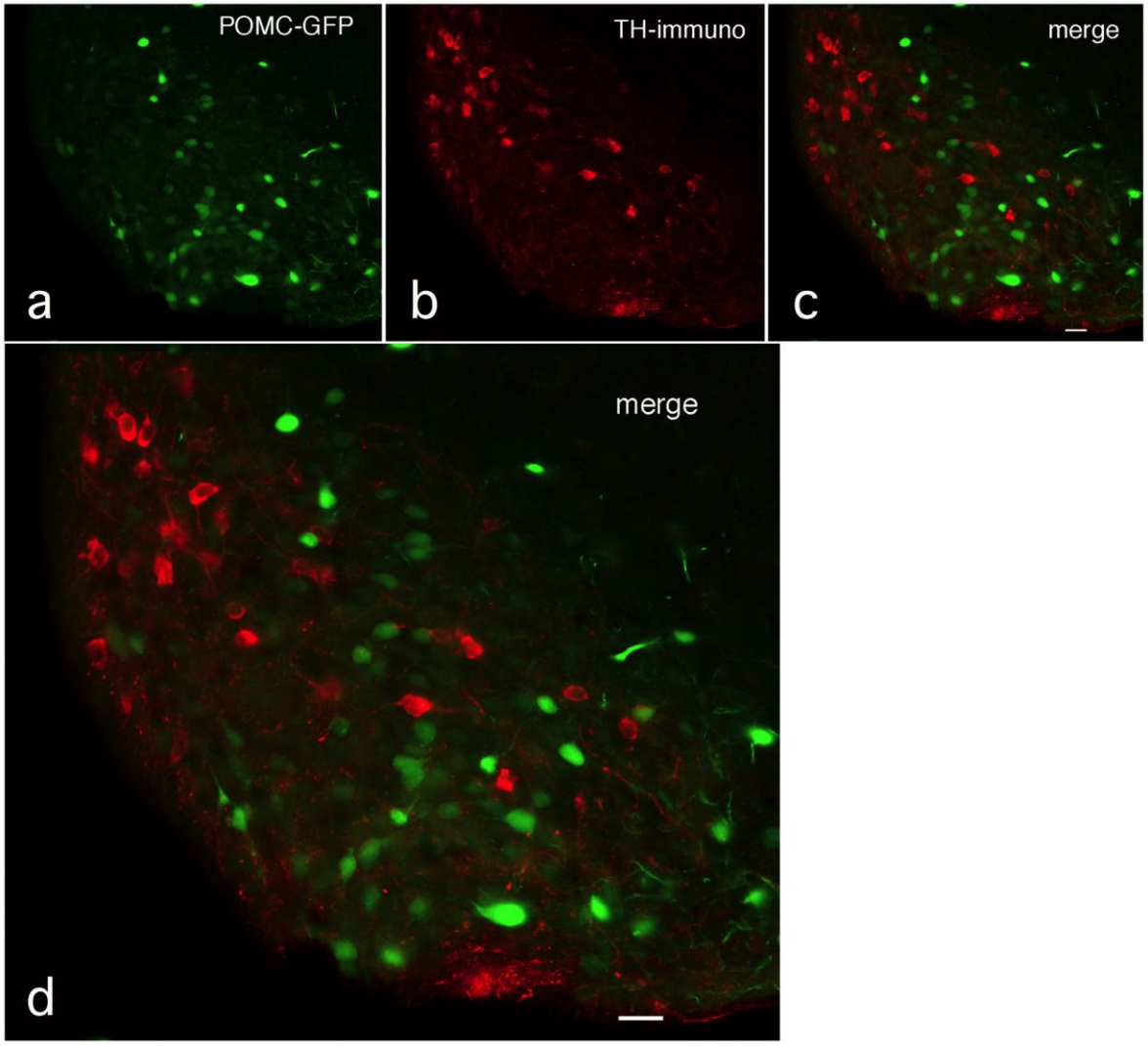
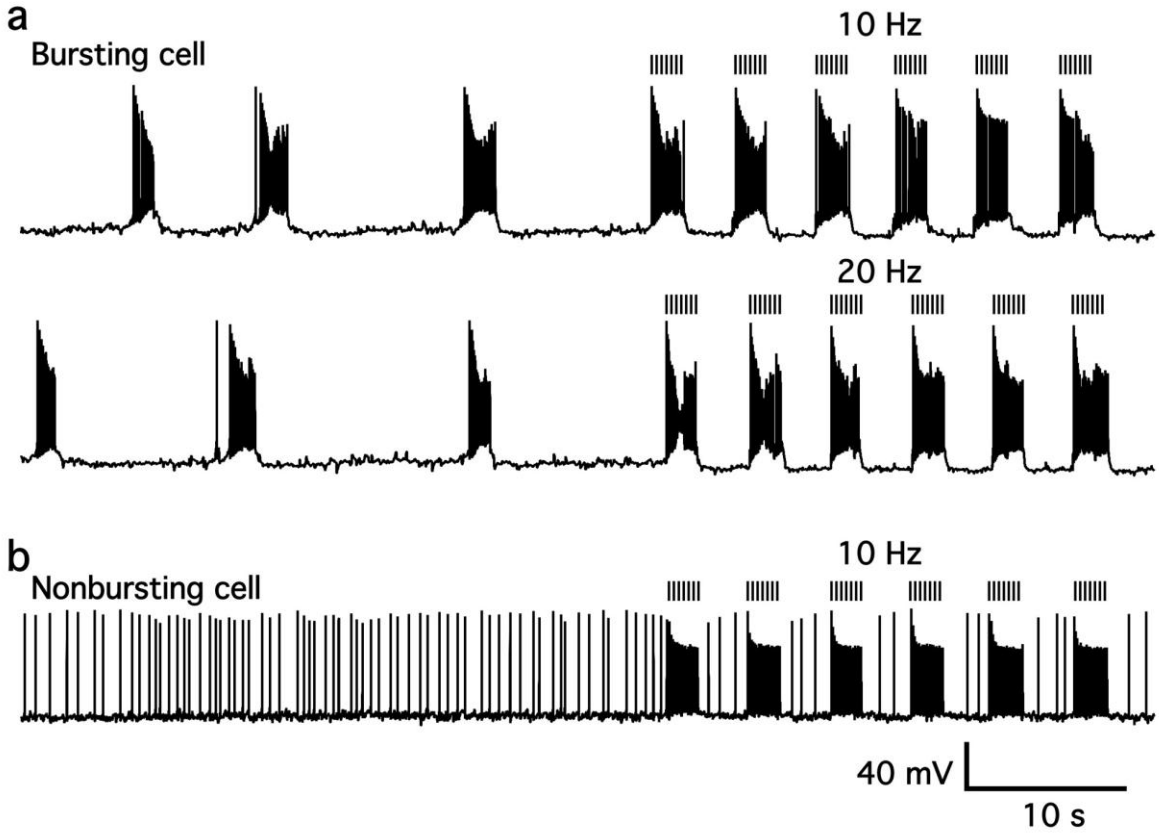


Supplementary Figure 3

No colocalization of TH and POMC neurons

a. Neurons from a POMC-GFP mouse are green. **b.** TH immunostaining with a red fluorophore shows TH immunopositive neurons with red color. **c.** Merge: No coexpression of POMC and TH is found. Scale: 20 μm. **d,** Enlarged image *c* to show the absence of co-expression. Scale: 20 μm.



Supplementary Figure 4

Optogenetic activation of an arcuate TH neuron

a, Representative traces show that repeated optogenetic stimulation (6 sets of stimuli) using 10 Hz (above) and 20 Hz (bottom) light pulses of 2 s followed by 3 s interval activates a bursting TH neuron in the dorsomedial ARC. **b,** Representative trace showing optogenetic activation of a nonbursting TH neuron in the same area by repeated stimulation using 10 Hz light pulses of 2 s followed by a 3 s non-stimulation interval.

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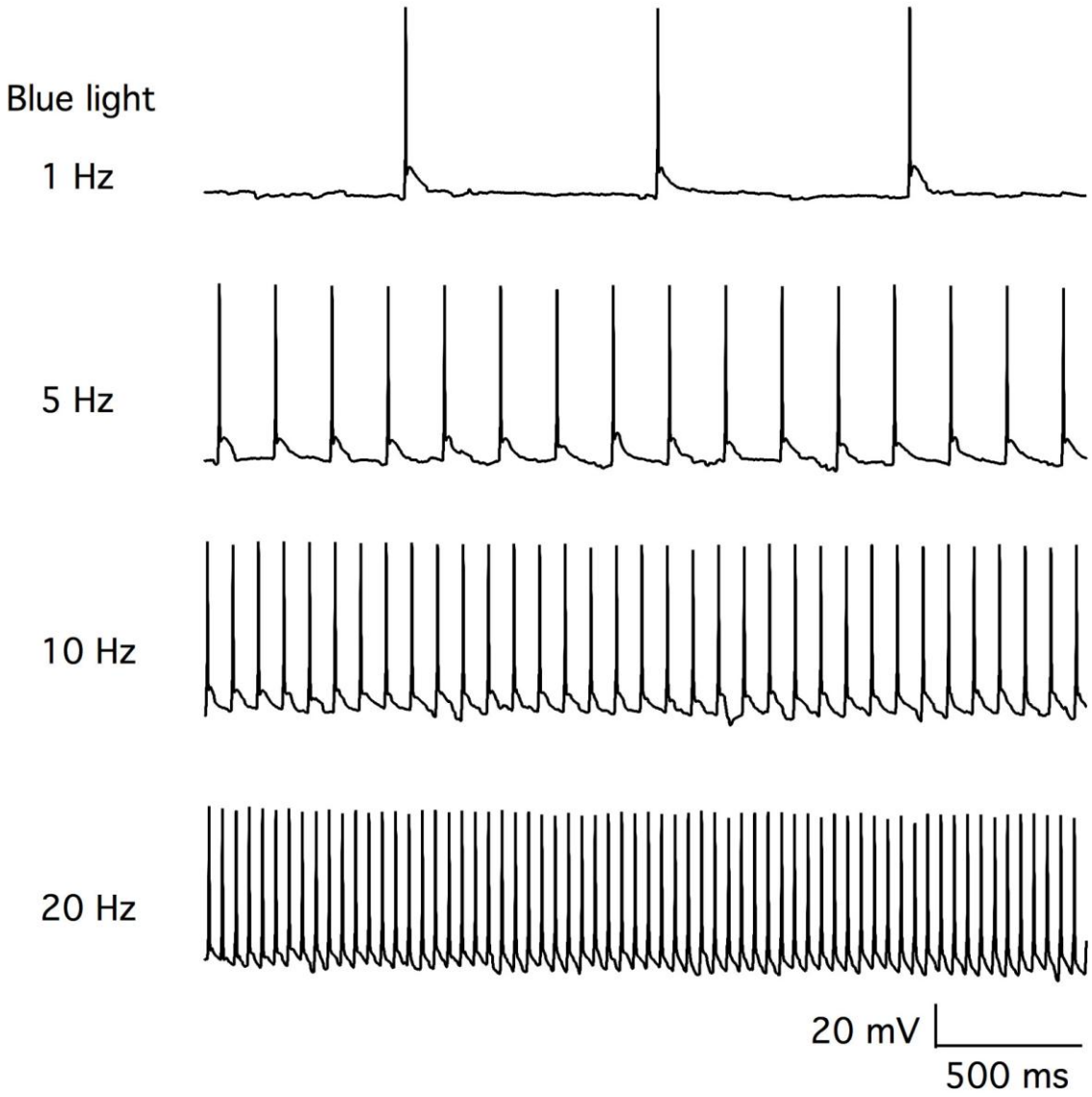
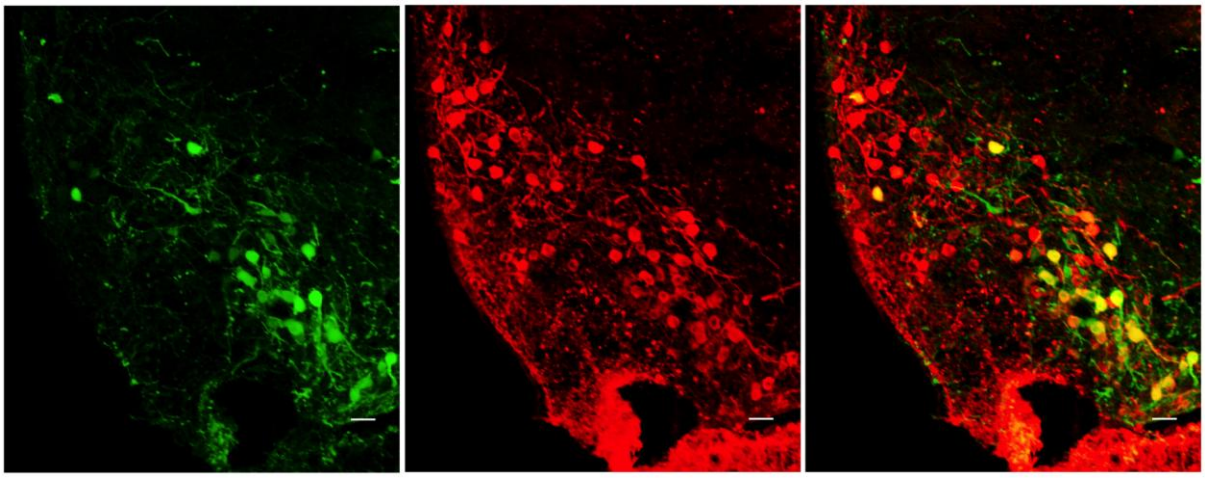


Supplementary Figure 5

Blue light stimulation at different frequencies activates arcuate TH neurons with high fidelity



Supplementary Figure 6

Arcuate TeNT-GFP neurons express TH

left, Green image shows TH-Cre-TeNT-GFP neurons expressed by AAV in a TH-Cre mouse, **middle**, Orange image shows neurons immunostained for TH in the arcuate nucleus. **right,** Merged photo shows colocalization of TeNT-GFP and TH immunostaining. Scale: 20 μm.

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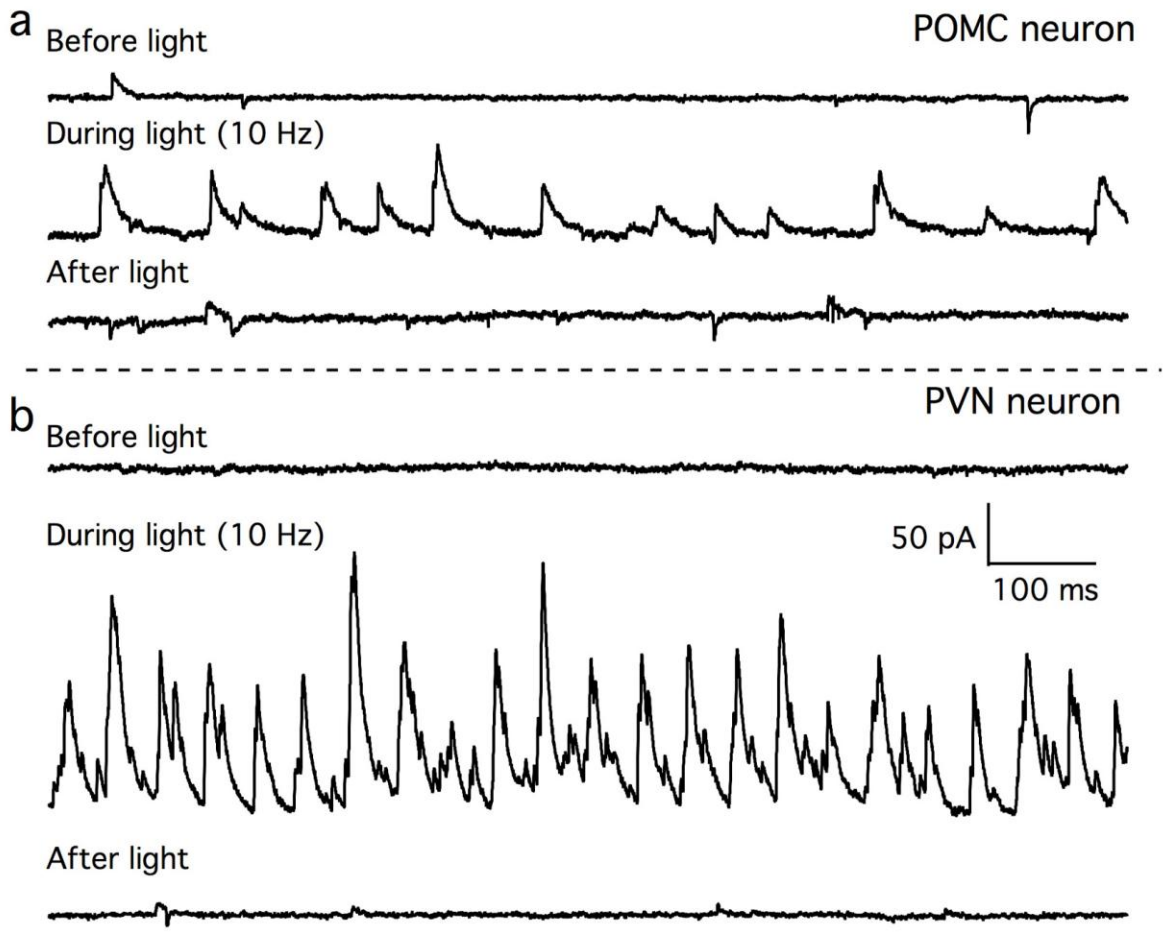

Supplementary Figure 7

Photostimulation-evoked inhibitory postsynaptic current in TH neurons

a, Representative traces showing inhibitory postsynaptic currents (IPSCs) before, during and after TH neuron photostimulation (10 Hz) as recorded in a POMC neuron. IPSCs are evoked by each photostimulation. **b,** representative traces showing IPSCs before, during and after photostimulation (10 Hz) in PVN neuron.

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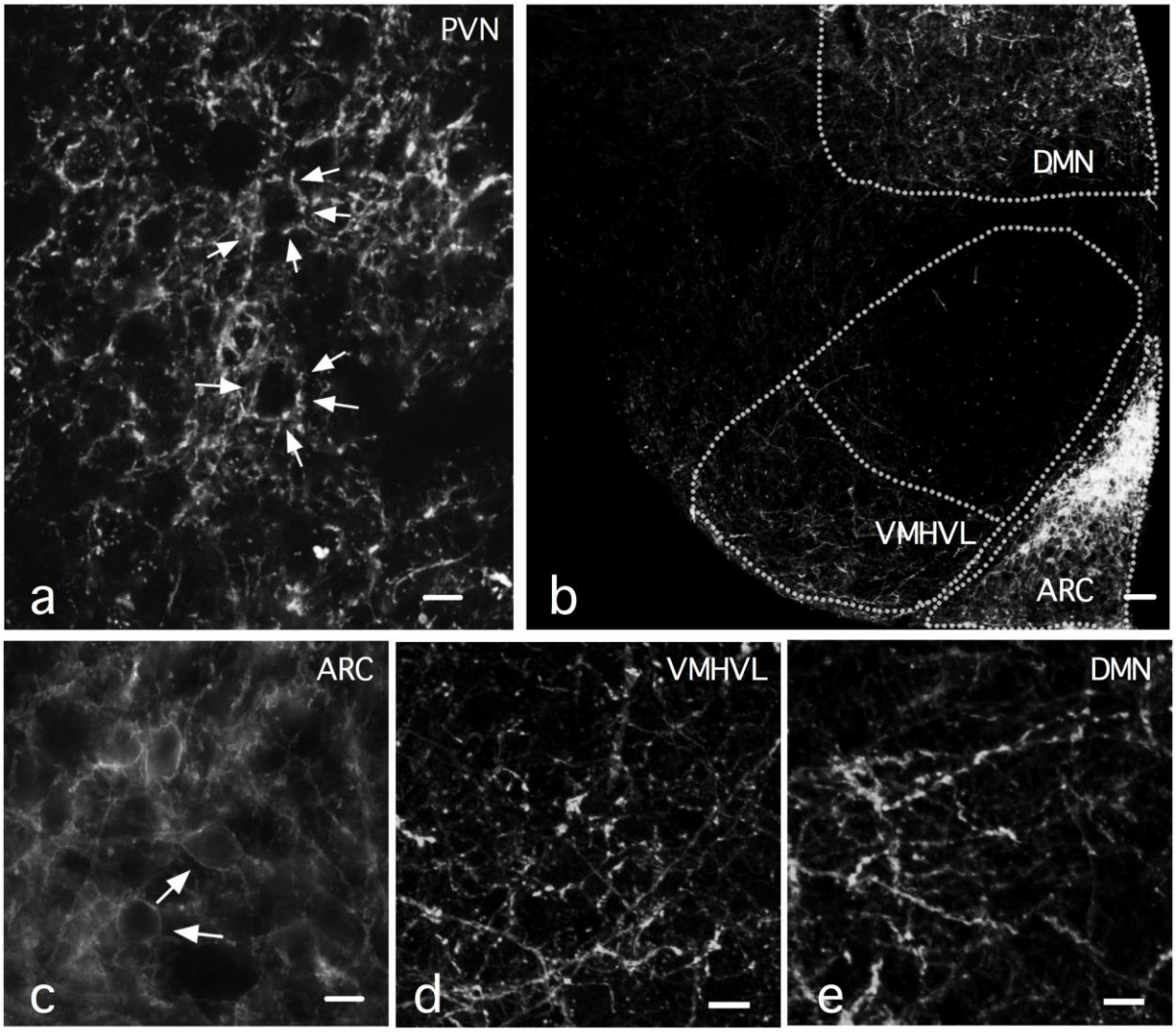

Supplementary Figure 8

(AAV)dj-ChIEF-tdTomato-mediated ChIEF-tdTomato expression in ARC and PVN

a. High density of axons was found in PVN after the AAV was microinjected into the ARC. Arrows indicate some examples where PVN neurons are surrounded by positive axons arising from ARC TH neurons. PVN cell bodies did not express ChIEF-tdTomato. Bar, 10 μm. **b.** Low-resolution photo shows ChIEF-tdTomato fluorescence in ARC, the ventral part of the hypothalamic ventromedial nucleus (VMH-VL) and the dorsomedial nucleus (DMN) of same slice. Bar: 50 μm. **c,** High-resolution image showing ChIEF-tdTomato expression in cell body and axons in ARC 21 days after AAVdj-CAG-DIO-ChIEF-tdTomato was injected into the ARC of the TH-cre mouse. Arrows point to neurons that express tdTomato-Chief with typical smooth plasma membrane expression. Bar, 9 μm. **d-e,** Labeled axons from ARC TH cells were found in VMH-VL and DMN with relatively lower density. Bar, 10 μm. Outside the hypothalamus, AgRP neurons project to the parabrachial nucleus (PBN). We found no positive axons in the PBN after ARC labeling.

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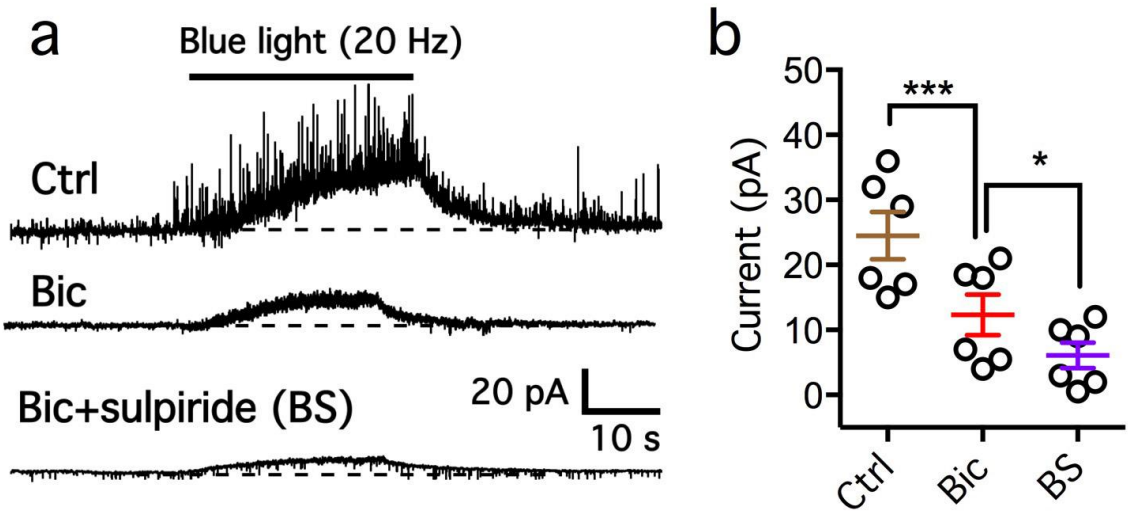
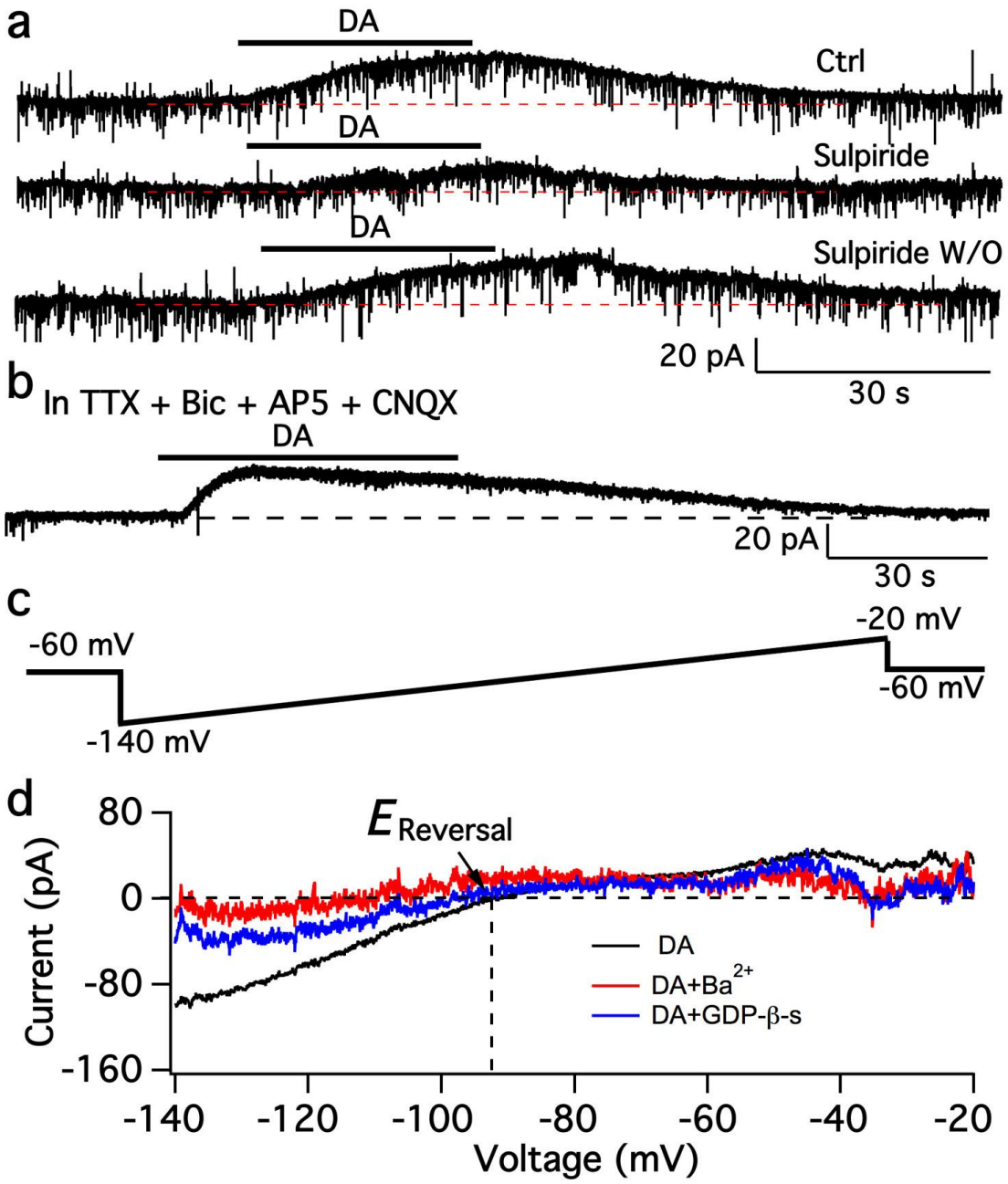


Supplementary Figure 9

Arcuate TH neuron inhibition in the PVN

a, Tonic outward current evoked by 20 Hz photostimulation for 30 s, showing control, bicuculline (Bic, 30 μM) and bicuculline (30 μM) + sulpiride (10 μM) in the PVN. **b,** Graph shows tonic outward current (n=6) in Ctrl and in the presence of Bic and Bic+sulpiride (BS), one-way repeated measure ANOVA, $F(5,10)=21.6$, $P<0.0001$; Bic versus Ctrl: ***, $P=0.0003$, BS versus Bic: *$P=0.026$,. Data are consistent with release of GABA and dopamine from arcuate TH axons in the PVN.



Supplementary Figure 10

Dopamine evokes GIRK current through direct activation of D2 receptors in POMC neurons

a, Representative traces show dopamine (30 μM) evoked outward current in control and in the presence of the D2 antagonist sulpiride (10 μM). Holding potential, -60 mV. **b,** Dopamine activates outward current in the presence of TTX (0.5 μM), AP5 (50 μM), CNQX (10 μM) and Bic (30 μM). Holding potential, -60 mV. **c,** A voltage ramp protocol from -140 to -20 mV was used to test GIRK current. **d,** Inward rectifying current with $E_{Reversal}$ around -

95 mV showing K^+ current in control and in the presence of dopamine (black line)(30 μM). The red line shows the response of dopamine in the presence of barium (Ba^{2+}). The blue line shows the response to dopamine (30 μM) in the presence of GDP-β-s. The recordings were done in the presence of TTX (0.5 μM), AP5 (50 μM), CNQX (10 μM) and Bic (30 μM).

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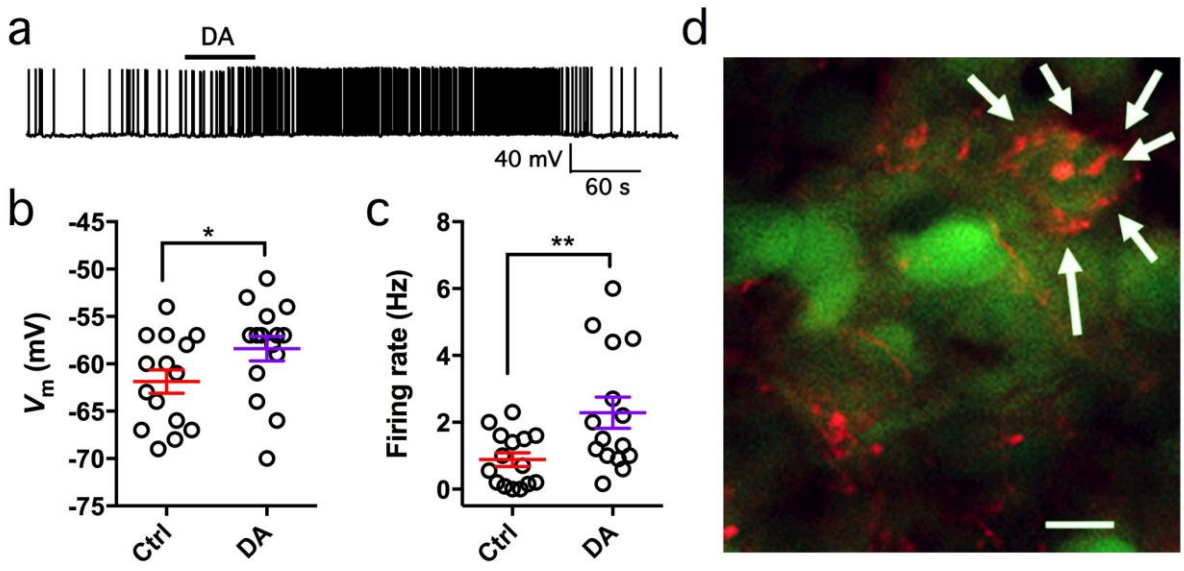

Supplementary Figure 11

Excitatory postsynaptic effect of dopamine on NPY/AgRP neurons

a, Representative trace shows dopamine (30 μM) excites an NPY/AgRP neuron in the presence of AP5 (50 μM), CNQX (10 μM) and Bic (30 μM). **b,** Graph shows the mean resting membrane potential (n=15) before and in the presence of dopamine after blocking synaptic transmission. Paired t test, t(14)=2.313, **P*=0.036. **c,** Graph shows firing rate (n=15) in control and in the presence of dopamine after blocking synaptic transmission. Paired t test, t(14)=3.149, **P*=0.007. **d,** DA-antibody immunostained orange axons surround GFP-expressing NPY/AgRP cell. Arrows denote multiple contacts between DA-immunoreactive axons and NPY/AgRP neuron. Scale bar, 12 μm.

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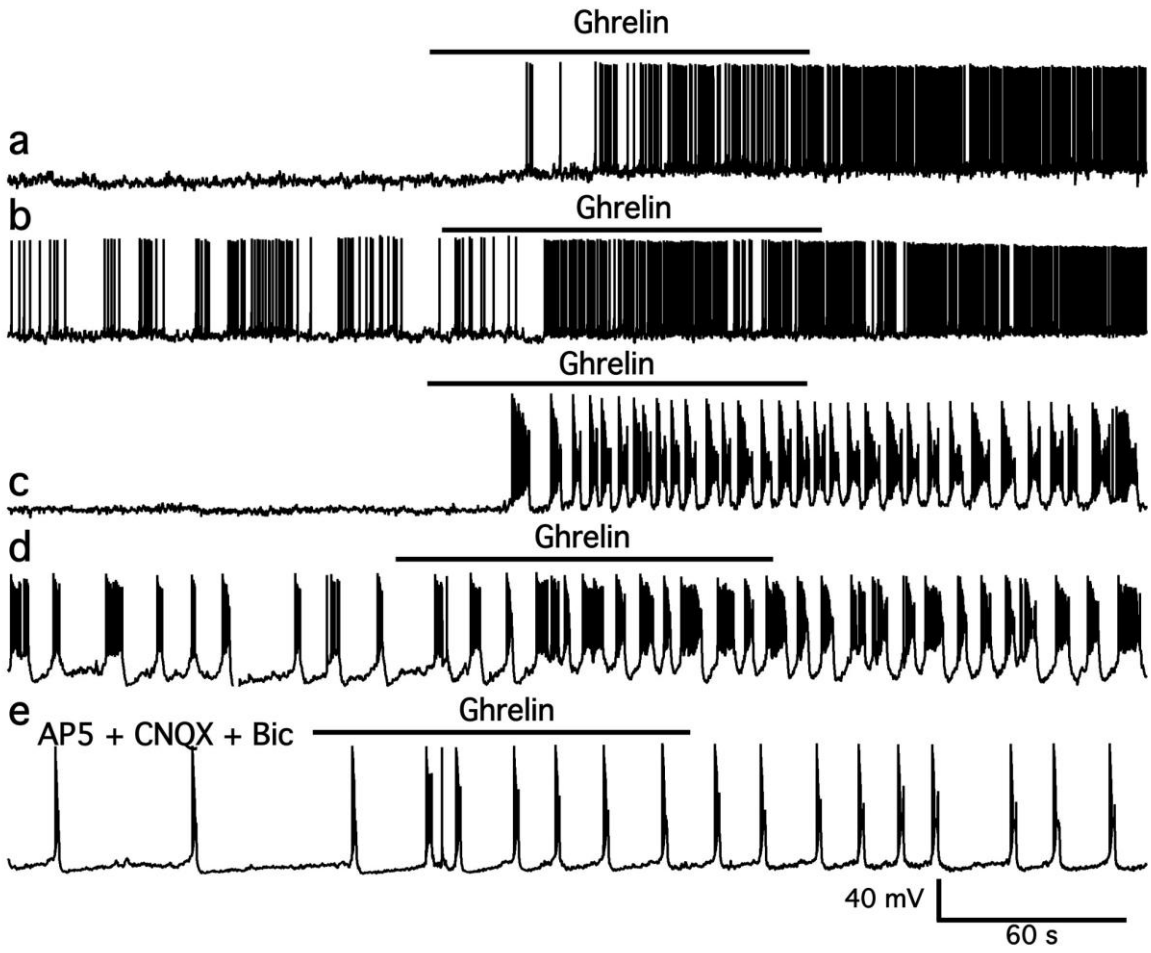

Supplementary Figure 12

Ghrelin excites arcuate TH neurons

a. Ghrelin (100 nM) depolarizes and excites a previously silent TH neuron to tonic firing in a hypothalamic brain slice from transgenic mice with the tdTomato reporter expressed selectively under control of the tyrosine hydroxylase promoter. **b**. Ghrelin increases the firing rate of an irregularly firing cell. **c**. Ghrelin induces a previously silent TH neuron to a burst firing state with a high frequency of spike bursts. **d**. Ghrelin increases burst rate of a bursting TH neuron, and reduces inter-burst intervals. All recorded cells were from the dorsomedial arcuate nucleus where dopamine has been found in almost all tyrosine hydroxylase positive neurons. **e,** Ghrelin increases burst rate of a bursting dopamine neuron in the presence of AP5 (50 μM), CNQX (10 μM) and Bic (30 μM).

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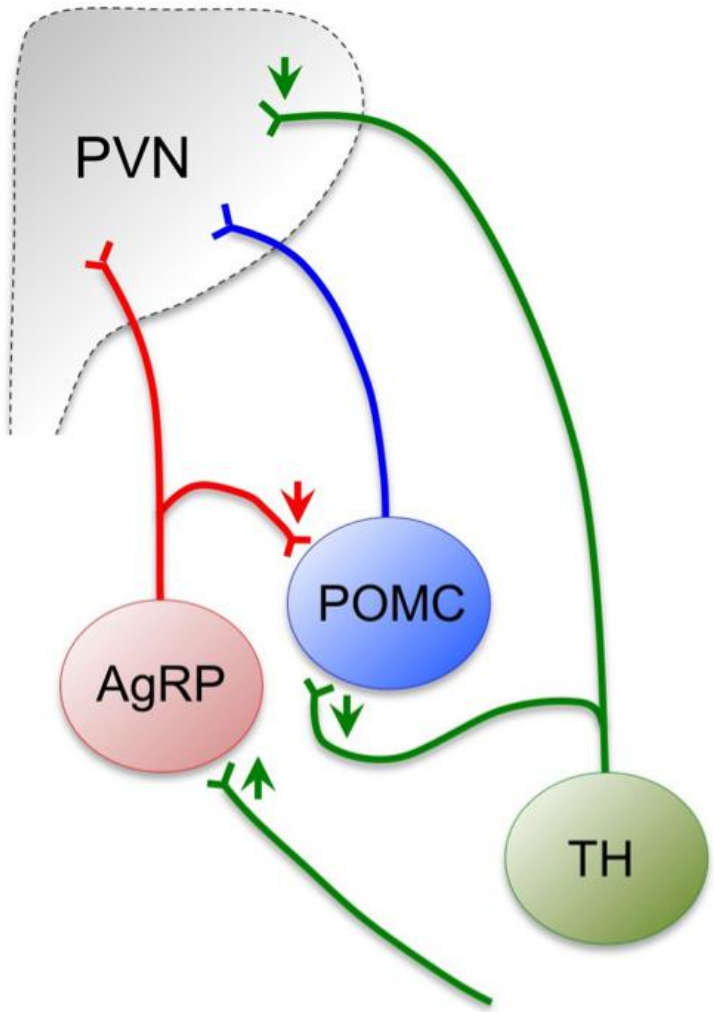

Supplementary Figure 13

Schematic showing the hypothalamic projections of ARC TH neurons

Dopamine excites NPY/AgRP neuron; dopamine inhibits POMC neurons and also inhibits PVN neurons. Arcuate TH neurons inhibit PVN neurons by release of both GABA and dopamine; TH neurons inhibit POMC neurons by GABA release. Arrows indicate axonal excitation (up arrow) or inhibition (down arrow). Dopamine is released in enhanced amounts during burst firing, and dopamine can be released non-synaptically. Therefore hypothetically, TH axons on POMC neurons or near NPY/AgRP neurons may release dopamine to act by bulk flow mechanisms to increase the excitability of NPY/AgRP neurons. NPY/AgRP cells may receive a dopamine input from an unidentified source. This diagram only shows local projections of these cells, and does not include extrahypothalamic projections of TH, NPY/AgRP or POMC neurons or projections of PVN neurons.

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