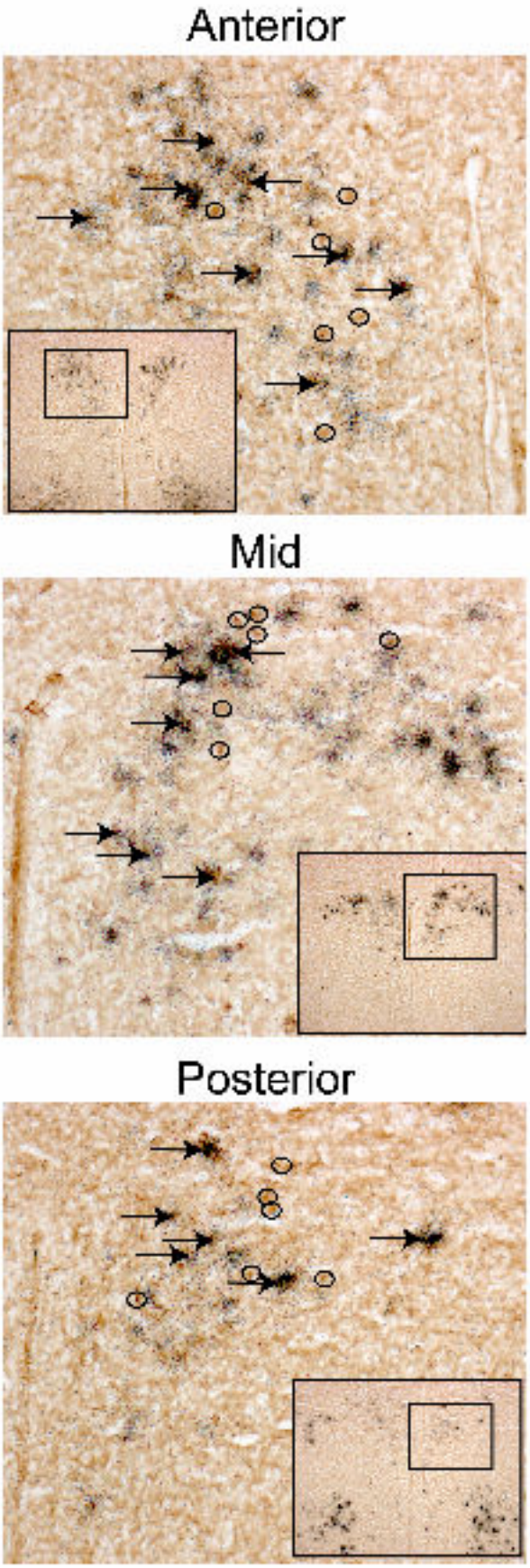

Figure 1: Colocalization of GFP immunostaining and TRH mRNA in the anterior, mid and posterior regions of the PVN containing hypophysiotropic TRH neurons. Dual-label immunohistochemistry/*in situ* hybridization was performed on TRH-Cre x R26-GFP mice. Images detail GFP-immunostaining and TRH mRNA in regions defined in Table 1, anterior, mid and posterior regions of the PVH containing hypophysiotropic TRH neurons. Circles indicate GFP-only staining (brown). Arrows indicate colocalization of GFP (brown) and TRH mRNA (black grains). Magnification: 20X (highlighted area of 10X inset).

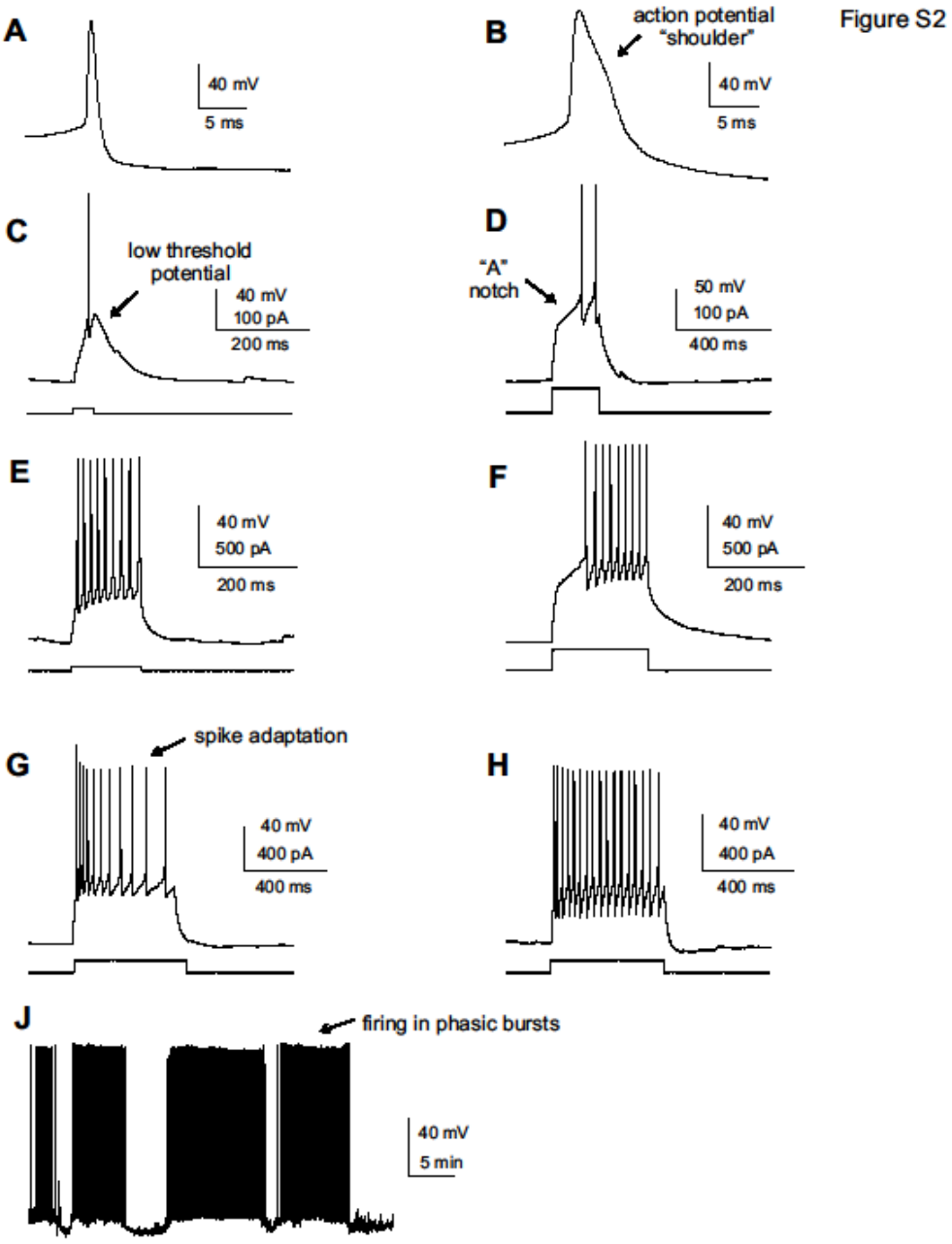

Figure 2: Electrogenic membrane properties of TRH -expressing neurons in PVN. A) The width of action potential recorded from a parvocellular neuroendocrine (type II) cell is narrower than that from a magnocellular neurosecretory (type I) neuron that is associated with a Ca^{+2} -dependent action potential "shoulder" (B). C) a brief (~40 ms duration) positive current (~20-50 pA) pulse generates a low threshold (~ -40 mV) transient depolarizing potential lasting tens of milliseconds. Its expression, observed mainly in type III pre-autonomic neurons, can be associated with superimposed firing of action potentials. However, in type I mangnocellular neurons such a positive current pulse applied from low (<-70 mV) membrane potentials was associated with delayed in onset of action potential firing, the "A" notch, D. E & F panels demonstrate trains of action potentials respectively without and with expression of the "A" notch. G, indicates frequency adaptation of firing in a train of action potentials, mostly observed in type II parvocellualar neuroendocrine compared with type I magnocellular neurosecretory neurons that did not express any frequency adaptation to firing, H. J) Spontaneously firing action potentials in phasic bursts, a property observed in Type I neuroendocrine magnocellular neurons.

Table 1: Electrogenic membrane properties of TRH -expressing neurons in mouse PVN.

Characteristics	n value	Percentage of TRH neurons exhibiting property	Indication
"A" notch	34	35%	I
Action potential "shoulder", >1.1 ms at half-peak	34	35%	I
Firing phasic bursts	17	35%	I
Low threshold potentials (bursting and non-bursting)	34	13%	III & II
Action potential frequency adaptation	34	53%	II & III

Table 2: Comparison of responses of type I and II PVN neurons to the peptides.

	Type I		p value	n=	Type II		p value	n=
	Control	peptide			control	peptide		
α-MSH-induced firing activity (Hz)	1.8±0.3	3.2±0.5	<0.01	19	1.6±0.4	2.9±0.6	P<0.001	16
α-MSH-induced depolarization (mV)	-45.9±1.6	-39.6±1.1	<0.0001	19	-47.9±1.6	-41.4±1.3	<0.0001	16
NPY-induced inhibition (Hz)	4.3±1.1	1.3±0.9	<0.05	5	3.9±1.5	0.5±0.2	<0.05	8
NPY-induced hyperpolarization (mV)	-45.0±2.1	-52.6±2.3	P<0.05	5	-46.6±2.1	-56.0±2.1	<0.001	8
leptin–induced firing activity (Hz)	0.9±0.2	3.5±0.6	< 0.0001	21	1.1±0.2	2.6±0.3	< 0.0001	12
leptin –induced depolarization (mV)	-51.0±1.7	-40.0±3.6	<0.005	21	-52.1±1.8	-44.7±1.5	< 0.0001	12