Supplemental Data

Supplemental Table 1.
Membrane potential of EGFP-identified LHA LepRb neurons in the presence or absence of leptin and inhibitors of synaptic transmission.

	Inhib	Vm (mV)		n (%)
		Control	Leptin (100nM)	
Depolarization	**-**	**-60.5±3**	**-54.5±2***	**14/41 (34%)**
	+	**-69±3**	**-61.6±3***	**6/18 (33%)**
Hyperpolarization	**-**	**-64.3±4**	**-76±3***	**9/41 (22%)**
	+	**-64.7±6**	**-69.4±5***	**4/18 (22%)**

Supplemental Figures

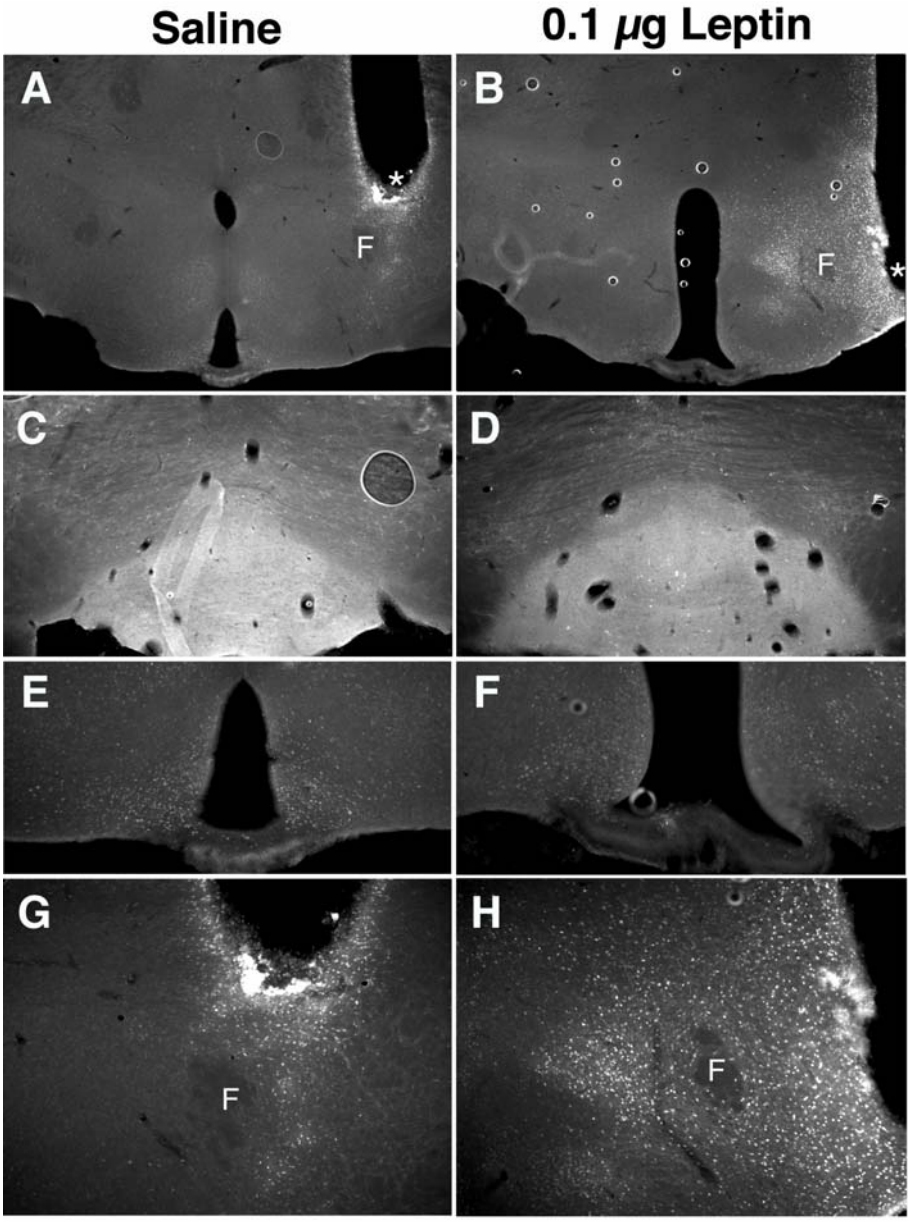
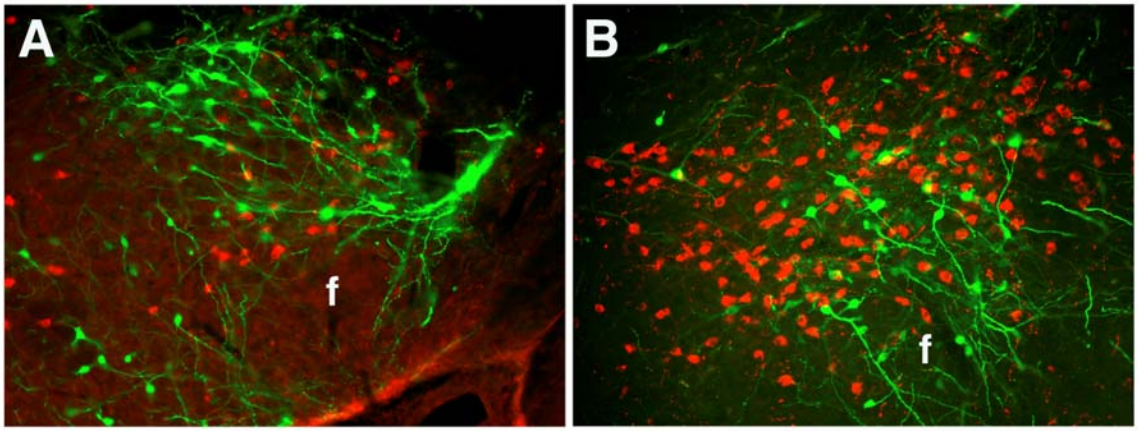
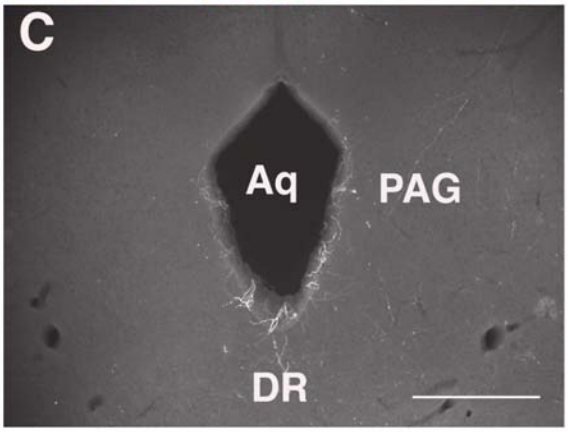


Figure S1. Leptin-induced pSTAT3 in LHA cannulated rats. To determine the spread of leptin via intra-LHA injection in rats, we examined leptin-induced pSTAT3-IR in response to saline (A,C,E,G) or 0.1 µg leptin (B,D,F,H). Cannulation sites in the LHA of saline- (A) and leptin-treated (B) rats showing that pSTAT3 is predominantly confined within the LHA. Iinjection sites are denoted by (*). No pSTAT3 was observed in the VTA (C, D), and neither intra-LHA leptin does not alter increase pSTAT3 in the ARC (F) relative to saline (E) (note that some baseline ARC pSTAT3-IR in both cases results from endogenous circulating leptin, as previously shown (Faouzi et al., 2007)). Intra-LHA LHA leptin dramatically increased pSTAT3-IR in the LHA (G, H). These data indicate that the 0.1 µg leptin dose is mainly confined within the LHA, and thus the effects of this dose are predominantly due to leptin action via LHA LepRb neurons.

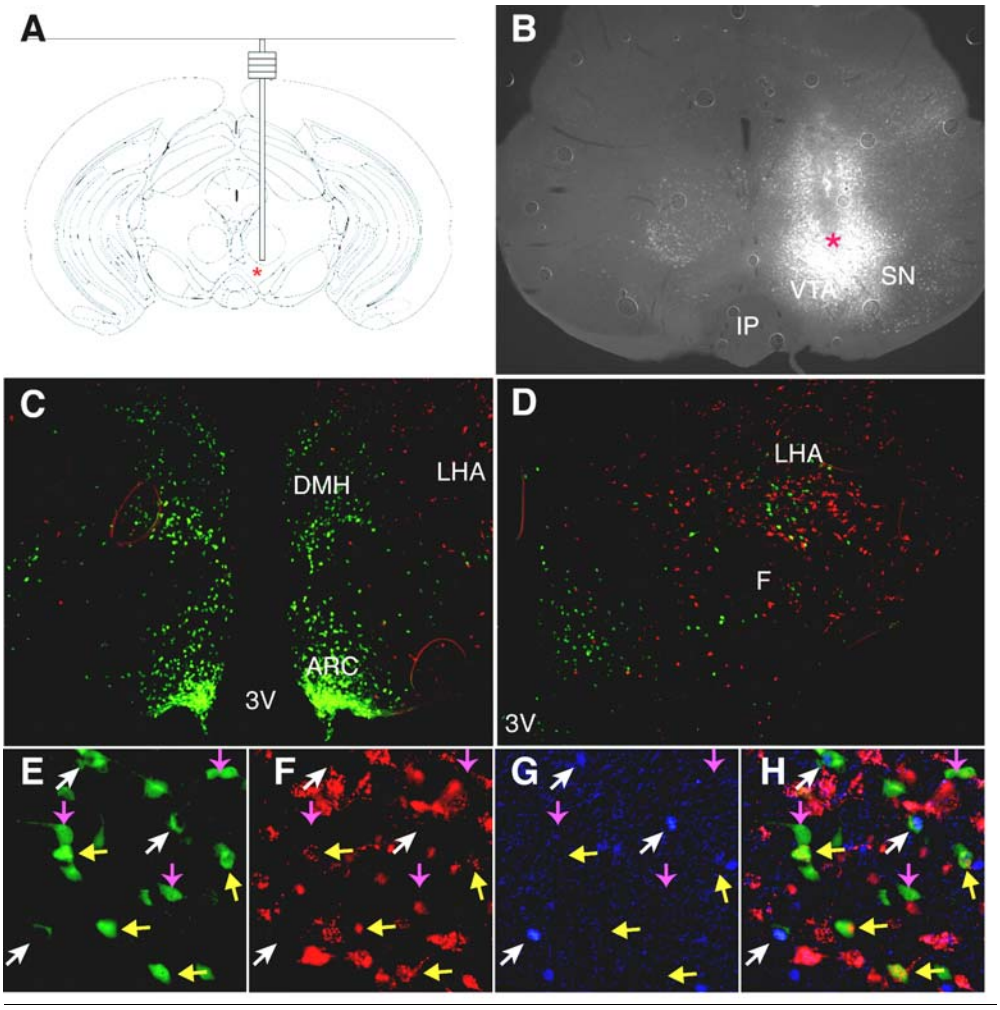




D

Region	Relative # of Fibers
LHA	++++
ARC	-
DMH	+
PAG	++
DR	++
VTA	+++
SN	++
NAc	-

Figure S2: Tracing of LHA LepRb neurons following stereotaxic injection of Ad-iZ/EGFPf into the LHA of *Lepr*^{cre} mice. Immunofluoresent analysis reveals no colocalization of EGFPf (green) in MCH (A, red) neurons or OX (B, red) in the LHA of *Lepr*^{cre} mice following injection of Ad-iZ/EGFPf. Note the appearance of some yellow due to superimposition of parts of EGFPf-expressing soma or projections upon occasional OX or MCH neurons; closer inspection reveals no colocalization. (C) Example of EGFPf-containing projections from LHA LepRb neurons to the periaqueductal grey matter (PAG) and dorsal raphe (DR). (D) Relative projection densities throughout the brain are tabulated. Areas with (-) or not listed contain few or no projections. F=fornix. Aq= cerebral aqueduct.



Supplemental Figure 3. Retrograde (fluorogold) tracing of VTA-projecting neurons in LepRb^{EGFP} mice. (a-d) To validate our Ad-iZ/EGFPf anterograde labeling from LHA LepRb neurons to the VTA, we stereotaxically injected 10 nl of the retrograde tracer flurogold (FG) into the VTA of LepRb^{EGFP} mice. 5 days later, the mice were treated with leptin for 4 hours to induce cFos in leptin-activated neurons and were perfused. Case 1 of 4 similar cases is shown. (A) Schematic showing FG injection site. (B) FG immunostaining in the injection site is mainly concentrated in the ipsilateral VTA, with minimal diffusion to the substantia nigra (SN) or aqueduct (Aq) and no labeling of the interpeduncular nucleus (IP). Contralateral FG staining is dimmer that ipsilateral staining, suggesting that these contralateral cells are retrogradely labeled and not primary labeled cells. (C) Staining for fluorogold (red) and EGFP (green) in the hypothalamus reveals few co-labeled cells in the ARC or DMH, but dense FG labeling in the LHA. (D) The ipsilateral LHA displays robust FG labeling distributed among and including numerous EGFP-expressing neurons. (E-H) Digitally enlarged views of the LHA depicting (E) EGFP, (F) fluorogold, (G) cFos (blue) and (H) the merged image. Yellow arrows: LepRb^{EGFP} neurons containing FG, indicating that these neurons project to the VTA. White arrows: LepRb^{EGFP} neurons containing cFos-IR, but not FG. Pink arrows: LepRb^{EGFP} neurons that do not contain cFos or FG. . F = fornix; 3V= third ventricle, ARC = arcuate nucleus, DMH = dorsomedial nucleus.

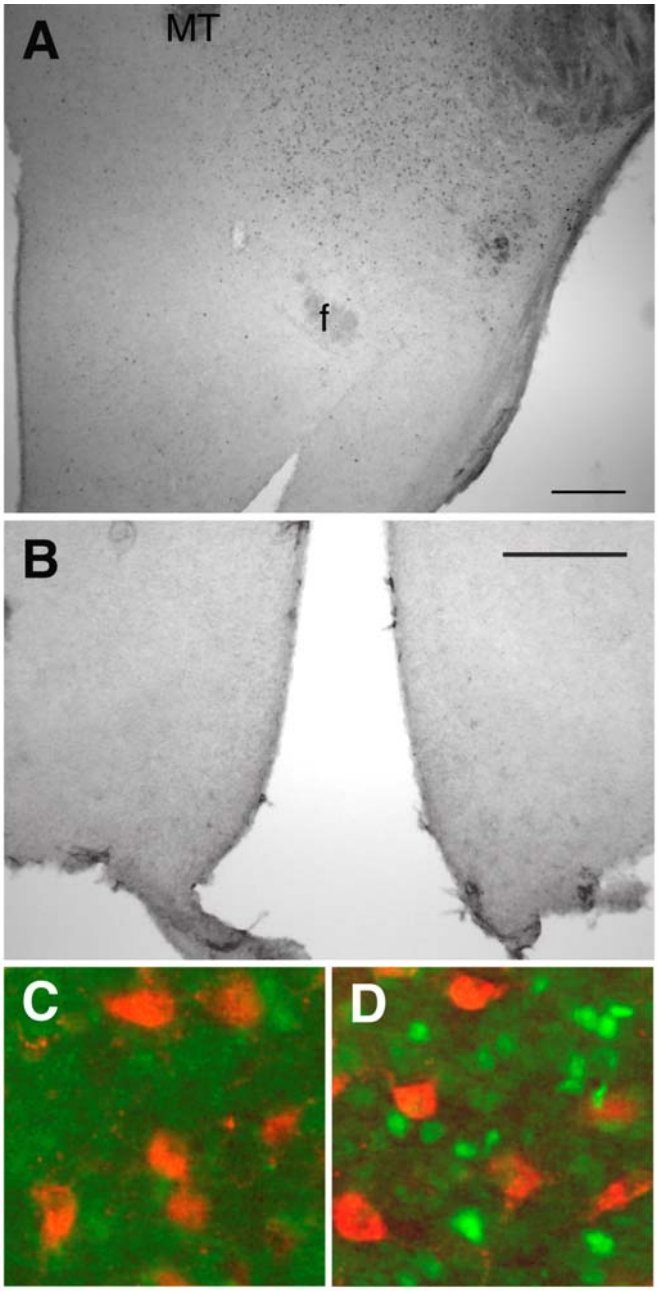


Figure S4. Cannula placement and LHA leptin action in LHA-cannulated *Lep*$^{ob/ob}$ mice. (A) Overview of pSTAT3-IR in the LHA an example of a *Lep*$^{ob/ob}$ animal receiving intra-LHA leptin treatment. Intra-LHA leptin treatment does not induce pSTAT3-IR in the ARC (B), nor other regions of the MBH. (C,D) immunohistochemical detection of pSTAT3-IR (red nuclei, psuedocolored green) and immunofluorescent OX (red) in the LHA of *Lep*$^{ob/ob}$ mice treated with intra-LHA vehicle (C) or leptin (D). Scale bar = 10 µm. f= fornix; MT= mammilothalamic tract.

Supplemental Experimental Methods

Fluorogold Methods: 10-12 wk old $LepRb^{EGFP}$ animals were anesthetized and prepared for stereotaxic injection as described in manuscript, but injectors were placed into the VTA (A/P: -3.2, M/L: -0.48, D/V: -4.6) and injected with 10 nl of 4% fluorogold (Biotium) in the VTA. 3 days later, the mice were treated with leptin (5 mg/kg IP, 4 hours), perfused and brains prepared for analysis, with DAB staining for cFos as described, and immunofluorescent detection of Fluorogold (Chemicon, 1:3000) and GFP (Abcam, 1:1000).

Supplemental Reference List

Faouzi,M., Leshan,R., Bjornholm,M., Hennessey,T., Jones,J., and Munzberg,H. (2007). Differential Accessibility of Circulating Leptin to Individual Hypothalamic Sites. Endocrinology 148(11):5414-23.