

Supplemental Figure 1

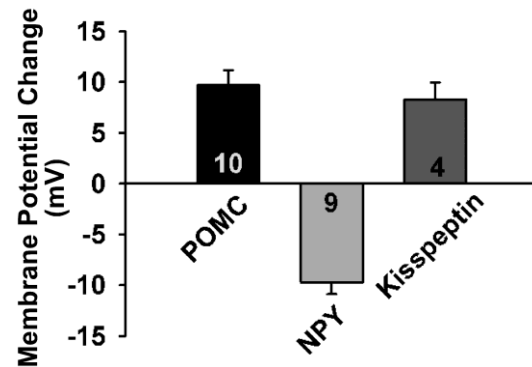


Figure S1, related to Figure 2. Summary of the effects of GP insulin (20 nM) on membrane potentials of mouse POMC, NPY/AgRP and kisspeptin neurons

The driving force was essentially equal in all of the cells for testing insulin. Insulin depolarized mouse POMC and kisspeptin neurons (9.7 ± 1.5 mV, $n=10$ and 8.3 ± 1.7 mV, $n=4$, respectively). In contrast, Insulin hyperpolarized NPY/AgRP neurons (-9.7 ± 1.1 mV, $n=9$).

Supplemental Figure 2

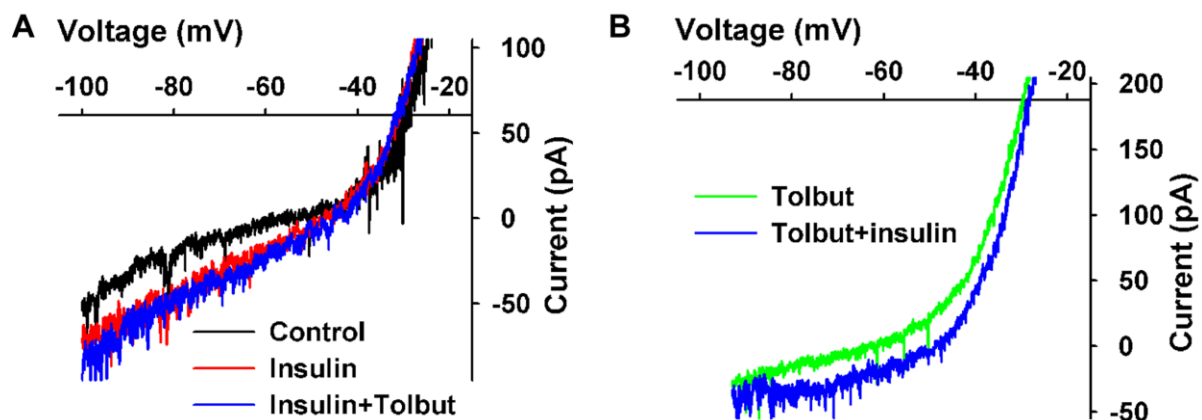


Figure S2, related to Figure 2. I/V curves generated in POMC-EGFP neurons in the presence of insulin (20 nM) and insulin + tolbutamide (200 μ M) (A), or in the presence of tolbutamide (200 μ M, 15 min exposure) and tolbutamide + insulin (B).

Supplemental Figure 3

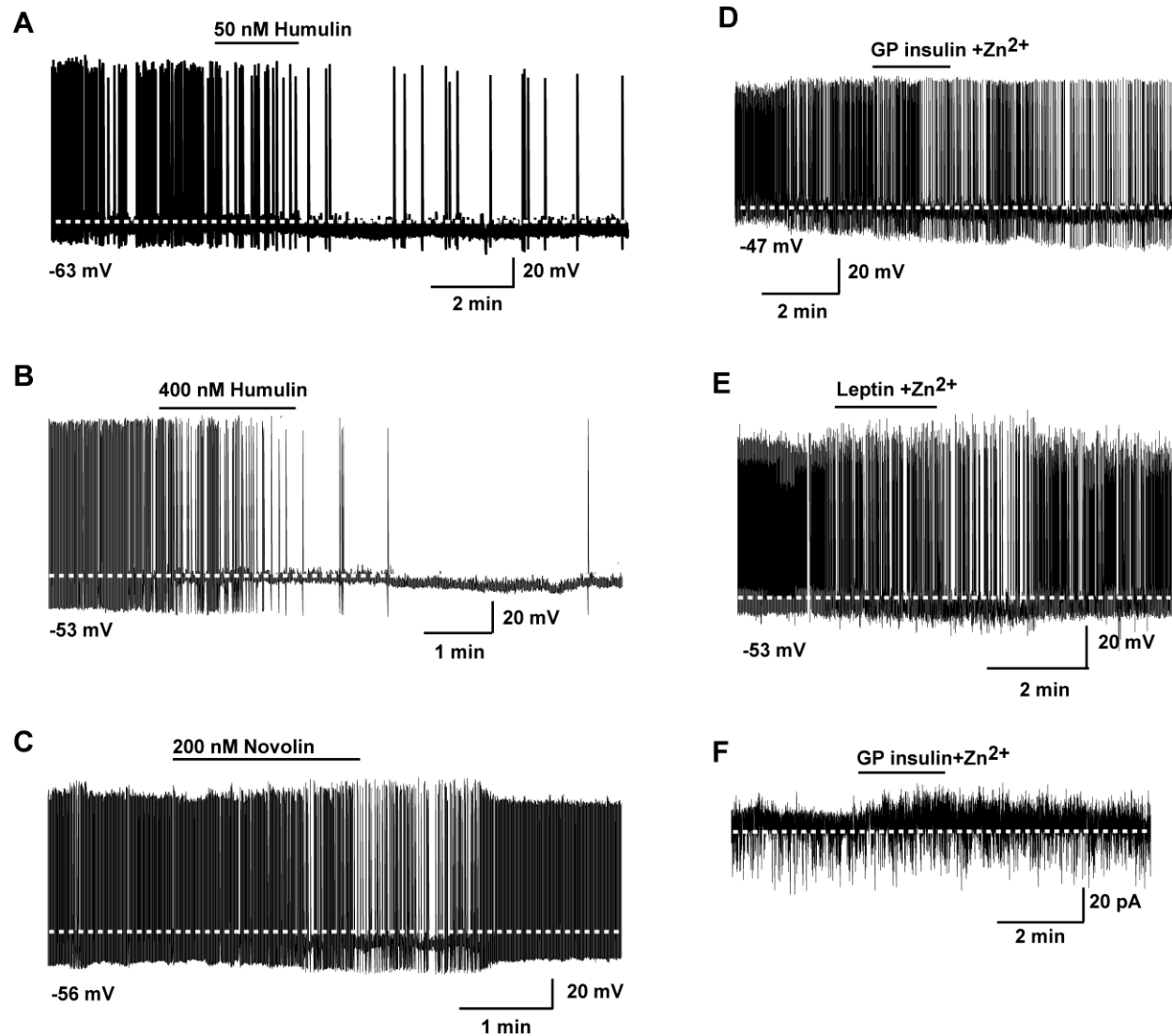


Figure S3, related to Figure 4. Insulin formulations, as well as purified insulin (or leptin) perfused in combination with Zn²⁺, inhibit POMC neurons

(A-C) Humulin and Novolin hyperpolarized POMC neurons. (D-E) Guinea pig insulin (150 nM) (or mouse leptin, 100 nM) containing Zn²⁺ (75 nM) hyperpolarized POMC neurons. (F) in voltage clamp and in TTX (1 μ M), guinea pig insulin (150 nM) containing Zn²⁺ (75 nM) induced an outward current. $V_h = -60$ mV. Drugs were bath applied at the concentrations indicated via the superfusion system.

Supplemental Figure 4

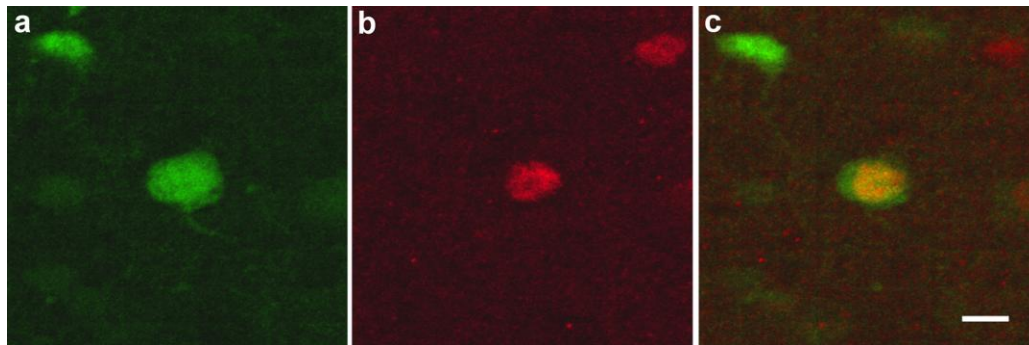


Figure S4, related to Figure 5. ICV insulin stimulates c-fos expression in POMC-EGFP neurons

a, POMC neurons (green). **b**, c-fos positive nuclei (red). **c**, overlay of **a** and **b** illustrating co-expression (yellow). Scale bar: 10 μ m.

Supplemental Figure 5

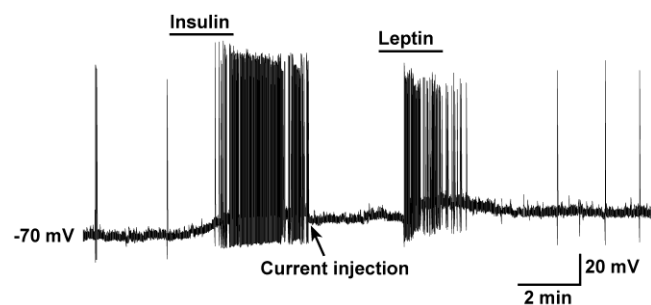


Figure S5, related to Figure 6. Insulin and leptin depolarized the same population of POMC neurons

GP-insulin (20 nM), added as a bolus directly to the bath, produced a 13 mV depolarization in this mouse POMC neuron. Then a constant current injection was used to hyperpolarize the cell and silence the firing, and leptin (100 nM) was added. Leptin induced a further depolarization (10 mV) and an increase in firing. Insulin and leptin depolarized the same population of mouse (n=3) and guinea pig (n=3) POMC neurons.