Supporting Information

Ma *et al.* 10.1073/pnas.0800952105

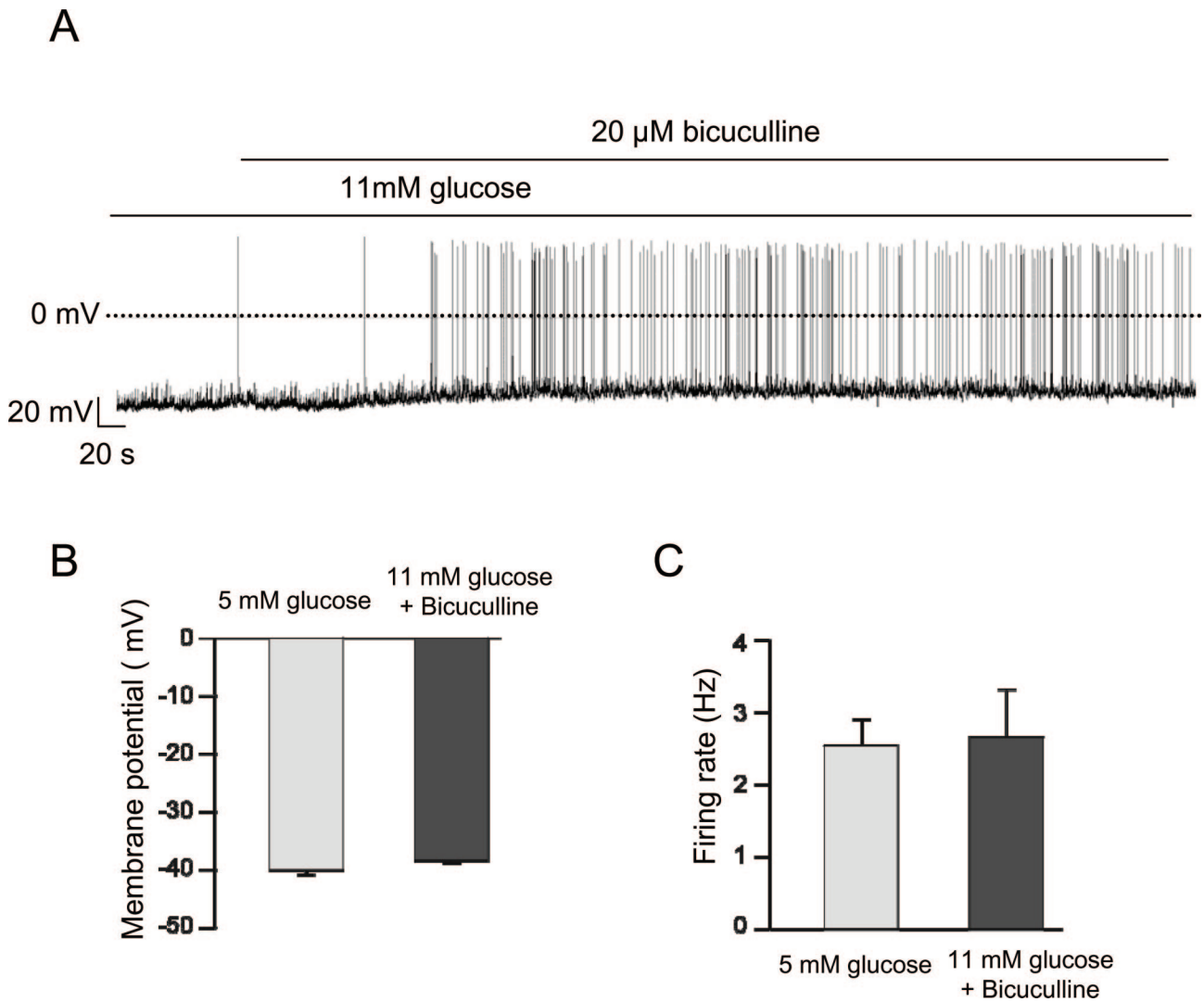

Fig. S1. Effects of bicuculline on the electrical activity of POMC neurons. (*A*) Representative recordings of electrical activity from an identified POMC neuron. Experiments were performed at 11 mM glucose and bicuculline (20 μM) was applied as indicated on the bars. (*B* and *C*) Mean ($\pm$ SEM) membrane potential (*B*) or action potential frequency (*C*) in the presence of 5 mM glucose (*Left*; $n = 31$ cells) or 11 mM glucose + 20 μM bicuculline (*Right*; $n = 10$ cells).

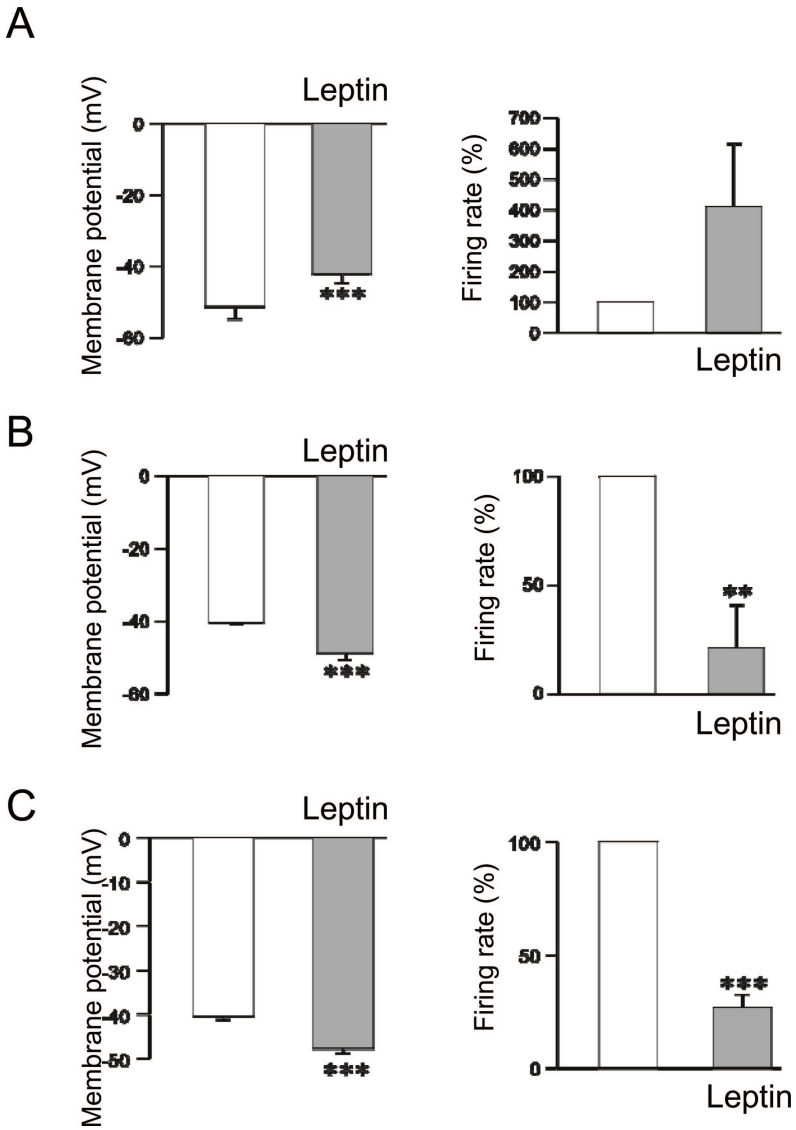

Fig. S2. Effects of leptin on the electrical activity of POMC neurons at different glucose concentrations. Mean (± SEM) membrane potential (*Left*) or changes in action potential frequency (*Right*) before and 10 min after addition of 100 nM leptin. (*A*) 11 mM glucose, 32°C, $n = 9$ cells. (*B*) 5 mM glucose, 32°C, $n = 5$ cells. (*C*) 5 mM glucose, 22°C, $n = 26$ cells. **, $P < 0.01$; ***, $P < 0.001$, versus before the addition of leptin.

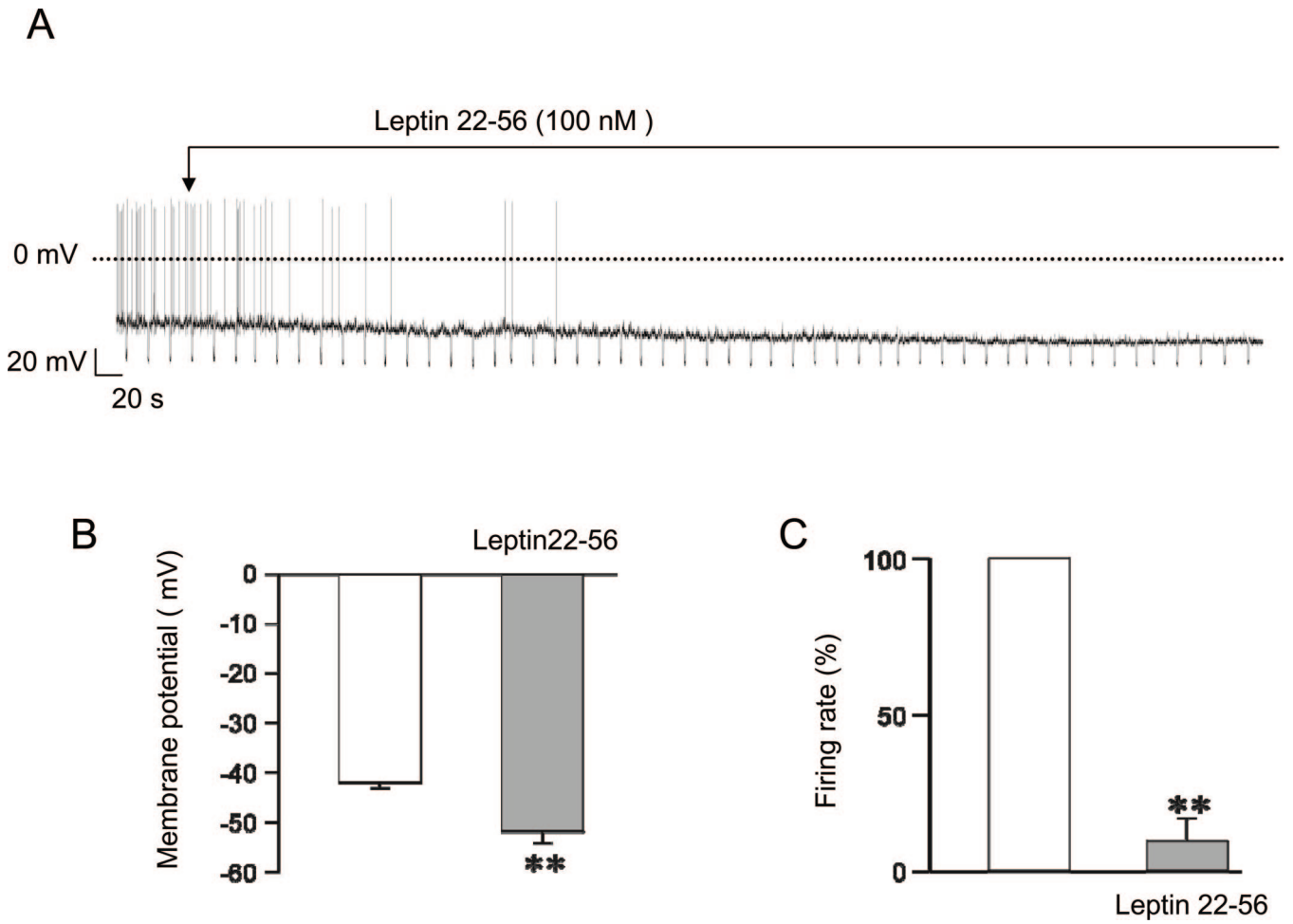

Fig. S3. Effect of the leptin fragment 22-56 on POMC neurons. (*A*) Representative recordings of electrical activity from an identified POMC neuron. Downward voltage deflections are caused by periodic injections of hyperpolarizing current, which were applied to monitor membrane resistance. Leptin 22-56 (100 nM) was applied as indicated by the bar. (*B* and *C*) Mean (± SEM) membrane potential (*B*) or action potential frequency (*C*) before and 10 min after addition of 100 nM leptin 22–56 ($n = 7$ cells). **, $P < 0.01$ versus before addition of leptin 22-56.

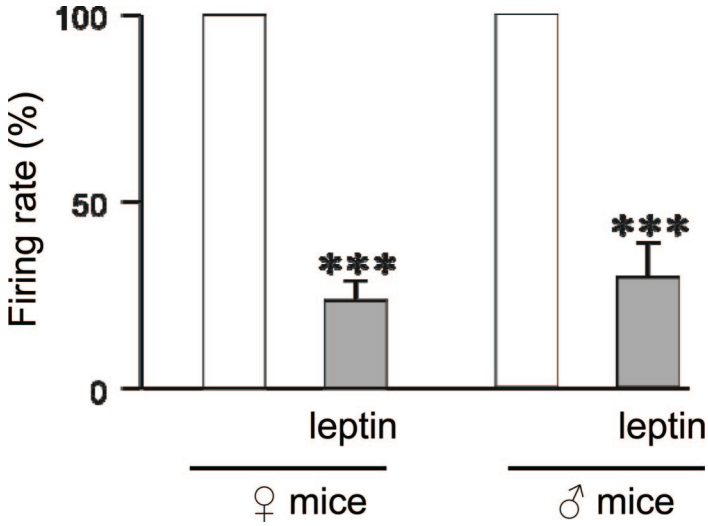

Fig. S4. Effect of leptin on firing rate of POMC neurons in male and female mice. Mean firing rate was measured from the same identified POMC neuron first in control solution (white bar) and 10 min after addition of 100 nM leptin (gray bar) for male ($n = 15$ cells) and female ($n = 11$ cells) 2- to 4-week-old mice. Data are expressed relative to the firing rate in control solution. ***, $P < 0.01$ versus control.

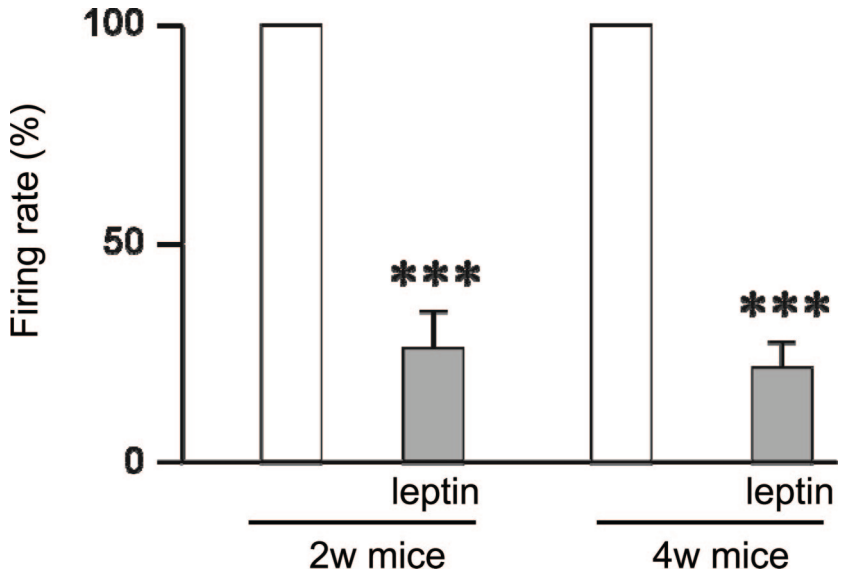

Fig. S5. Effect of leptin on the firing rate of POMC neurons in 2-and 4-week mice. Mean firing rate measured from the same identified POMC neuron first in control solution (white bar) and then 10 min after addition of 100 nM leptin (gray bar) for 2-week-old ($n = 9$ cells) and 4-week-old ($n = 17$ cells) mice. Data are expressed relative to the firing rate in control solution. ***, $P < 0.01$ versus control.

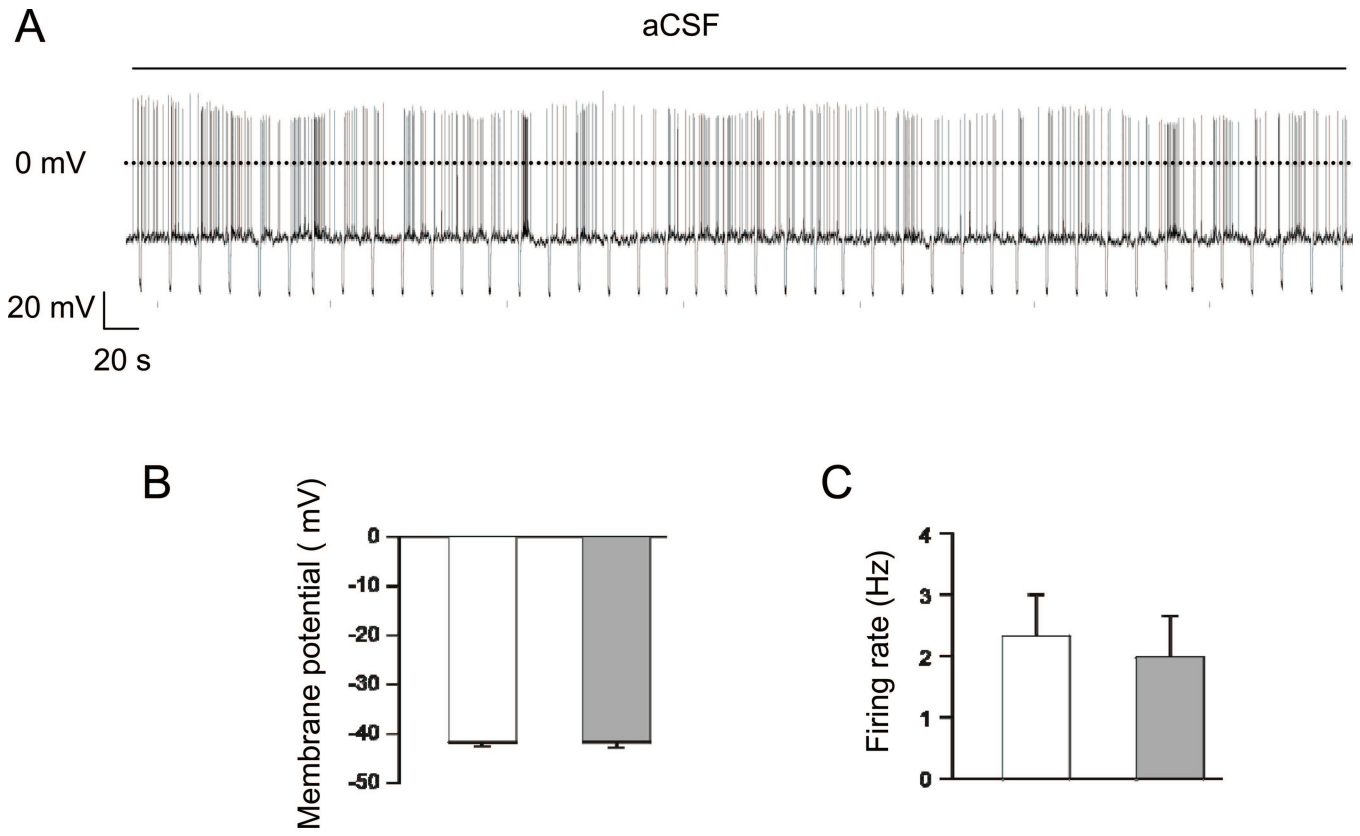

Fig. S6. Electrical activity of POMC neurons recorded in aCSF solution. (*A*) Representative recordings of electrical activity from identified POMC neurons. Downward voltage deflections are caused by periodic injections of hyperpolarizing current, which were applied to monitor membrane resistance. Recordings were made in the continued presence of aCSF. (*B* and *C*) Mean (± SEM) membrane potential (*B*) or action potential frequency (*C*) before and 10 min after continued perfusion of aCSF ($n = 11$ cells).

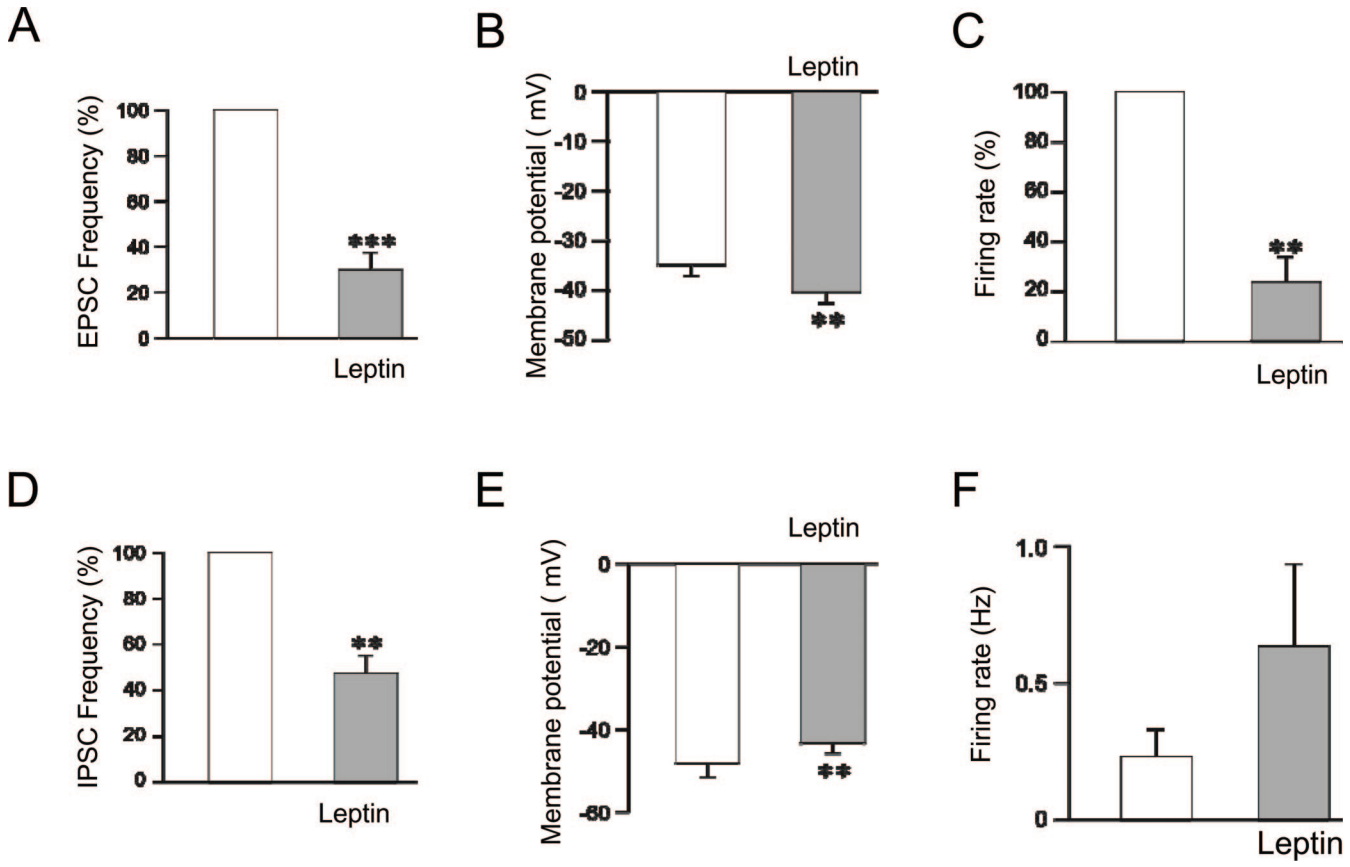

Fig. S7. Leptin modulates POMC electrical activity by changing presynaptic inputs. (*A* and *D*) Mean glutamatergic EPSC frequency (*A*) and GABAergic IPSC frequency (*D*) before and after exposure to 100 nM leptin. **, $P < 0.01$ ($n = 6$ cells; *D*); ***, $P < 0.001$ ($n = 5$ cells; *A*) versus before the addition of leptin. Glutamatergic EPSCs were identified by their sensitivity to DAP5/CNQX. IPSCs were identified by their sensitivity to picrotoxin. (*B*, *C*, *E*, and *F*) Mean membrane potential (*B* and *E*) and firing rate (*C* and *F*) in the presence and absence of leptin. **, $P < 0.01$ ($n = 5$ cells; *B* and *C*) and ($n = 9$ cells; *E* and *F*) versus before the addition of leptin.

Table S1. PSCs at 11 and 5 mM glucose

Parameters	5 mM glucose	11 mM glucose
EPSCs, Hz	3.58 ± 0.39 (2.50∼4.75; $n = 6$)*	2.33 ± 0.33 (0.69∼3.63; $n = 10$)
IPSCs, Hz	2.53 ± 0.52 (0.85∼3.98; $n = 6$)	2.94 ± 0.56 (1.76∼5.51; $n = 10$)
Size, pF	10.35 ± 0.73 ($n = 6$)	11.71 ± 0.87 ($n = 10$)

*, $P = 0.014$ versus IPSCs (paried t test).

Table S2. Leptin transiently stimulates 9/26 POMC neurons over 2 min at 5 mM glucose

Parameters	Control	Leptin
Resting potential, mV	-39 ± 1 ($n = 9$)	-37 ± 1 ($n = 9$)*
Firing frequency, Hz	2.13 ± 0.67 ($n = 9$)	2.31 ± 0.76 ($n = 9$)
Membrane resistance, GΩ	1.44 ± 0.16 ($n = 9$)	1.36 ± 0.11 ($n = 9$)
Capacitance, pF	12 ± 1 ($n = 9$)	12 ± 1 ($n = 9$)

Parameters were measured from the same neuron first in control solution (ACSF), then 2 min after addition of 100 nM leptin.

*, $P < 0.05$ versus before leptin (paried *t* test)

Table S3. Leptin stimulates 4/31 POMC neurons at 5 mM glucose

Parameters	Control	Leptin
Resting potential, mV	−38 ± 3 ($n = 4$)	−35 ± 2 ($n = 4$)*
Firing frequency, Hz	2.9 ± 1.2 ($n = 4$)	3.9 ± 1.5 ($n = 4$)
Membrane resistance, GΩ	1.68 ± 0.24 ($n = 4$)	1.83 ± 0.29 ($n = 4$)
Capacitance, pF	13 ± 2 ($n = 4$)	13 ± 2 ($n = 4$)

Parameters were measured from the same neurone first in control solution (ACSF), then 10 min after addition of 100 nM leptin.

*, $P < 0.05$ versus before leptin (paried t test).

Table S4. Whole-cell K_{ATP} conductance in the absence and presence of leptin

Parameters	Control	Leptin
Membrane conductance, pS/pF (before tolbutamide)	75 ± 24 (n = 4)	73 ± 13 (n = 9)
Membrane conductance, pS/pF (after tolbutamide)	55 ± 25 (n = 4)	48 ± 10 (n = 9)
K_{ATP} conductance, pS/pF	20 ± 5 (n = 4)	25 ± 6 (n = 9)
Non-K_{ATP} condutance, pS/pF	55 ± 25 (n = 4)	48 ± 10 (n = 9)

No significant differences in all the parameters between leptin and control.