Leptin’s hunger-suppressing effects are mediated by the hypothalamic–pituitary–adrenocortical axis in rodents

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Abstract

Leptin informs the brain about sufficiency of fuel stores. When insufficient, leptin levels fall, triggering compensatory increases in appetite. Falling leptin is first sensed by hypothalamic neurons, which then initiate adaptive responses. With regard to hunger, it is thought that leptin-sensing neurons work entirely via circuits within the central nervous system (CNS). Very unexpectedly, however, we now show this is not the case. Instead, stimulation of hunger requires an intervening endocrine step, namely activation of the hypothalamic–pituitary–adrenocortical (HPA) axis. Increased corticosterone then activates AgRP neurons to fully increase hunger. Importantly, this is true for 2 forms of low leptin-induced hunger, fasting and poorly controlled type 1 diabetes. Hypoglycemia, which also stimulates hunger by activating CNS neurons, albeit independently of leptin, similarly recruits and requires this pathway by which HPA axis activity stimulates AgRP neurons. Thus, HPA axis regulation of AgRP neurons is a previously underappreciated step in homeostatic regulation of hunger.

Significance

Low levels of leptin, a hormone secreted by adipocytes that signals the body as to the availability of fuel stores, are known to increase food intake. Here, we demonstrate a mechanism by which low leptin stimulates food intake in rodents: Under conditions of hyperleptinemia, stress hormone (glucocorticoid) production is increased, and in turn stimulates AgRP neurons to promote appetite.

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receptor antagonist, mifepristone, suppressed caloric intake to that measured in recently fed animals without altering plasma leptin concentrations. Plasma adrenocorticotropic hormone (ACTH) concentrations mirrored plasma corticosterone, with the exception of mifepristone-treated rats, which exhibited the expected increase in ACTH resulting from glucocorticoid receptor antagonism. Taken together, these data suggest that hypercorticosteronemia mediates the majority of fasting-induced hyperphagia (Fig. 1A–C and SI Appendix, Fig. S1A–F). Next, we performed similar studies in insulin-deficient, poorly controlled type 1 diabetic rats, as we and others have shown that poorly controlled diabetes is a state of severe hypoleptinemia (25–33). In this model, we again found that hypoleptinemia caused hypercorticosteronemia in T1D rats as replacement leptin normalized plasma corticosterone concentrations and reversed hyperglycemia without affecting plasma insulin concentrations. Hypoleptinemia caused hyperphagia in T1D rats, an effect mediated through hypercorticosteronemia: Animals with poorly controlled T1D consumed twice as many calories within a 2-h span compared with nondiabetic rats, an observation corrected by replacement leptin infusion and restored by coinfusion of corticosterone.
in rats treated with leptin. Mifepristone treatment abrogated the effect of poorly controlled diabetes to cause hyperphagia, suppressing food intake after an overnight fast to rates measured in nondiabetic controls (Fig. 2 A–C and SI Appendix, Fig. S2 A–F).

HPA Axis Activation Causes Hyperphagia in Hypoglycemia. We next hypothesized that HPA axis activation would drive hyperphagia during acute, insulin-induced hypoglycemia (34). Consistent with this, following a 4-h hyperinsulinemic–hypoglycemic clamp, rats consumed 4 times as many calories as euglycemic rats infused with the same dose of insulin, despite unchanged plasma leptin concentrations. The majority of this hyperphagia was driven by hypercorticosteronemia, as evidenced by the fact that infusing euglycemic rats with corticosterone to increase plasma corticosterone concentrations to levels measured in hypoglycemic rats recapitulated the majority of the effect of hypoglycemia to cause hyperphagia. In contrast, mifepristone treatment reduced food intake in hypoglycemic rats to rates measured under euglycemic conditions (Fig. 3 A–C and SI Appendix, Fig. S3 A–F), thereby demonstrating that hypercorticosteronemia, and not central hypoglycemia, is ultimately responsible for the majority of hypoglycemia-induced hyperphagia.

Elevated Leptin Abrogates HPA Axis-Mediated Hyperphagia. To directly test the role of corticosterone in modulating food intake, we studied adrenalectomized (ADX) mice infused with low (0.75 mg/d) or high (2 mg/d) doses of corticosterone s.c. Corticosterone drove food intake: Both total caloric intake and caloric intake following a 24-h fast were reduced in the ADX-low corticosterone group, and increased to control rates in ADX-high corticosterone-treated mice (SI Appendix, Fig. S4 A–D). We then placed the ADX mice on a high-fat diet (HFD) for 2 wk to test whether increased adiposity may prevent hyperphagia after a fast. High-fat feeding more than doubled fat mass and reduced caloric intake upon refeeding by 40% only in sham-operated mice (SI Appendix, Fig. S4 E–H). Given the decreased food intake after a fast in sham-operated mice fed an HFD, these data suggest that obesity generates a negative-feedback signal dependent on glucocorticoid activity to suppress fasting-induced hyperphagia. We tested this hypothesis in ADX rats, which, unlike mice, afford the ability to measure plasma glucocorticoid concentrations in the unrestrained, awake state. In ADX rats, fasting lowered leptin and refeeding increased it independent of adrenal function. However, corticosterone drove fasting-induced hyperphagia: Upon refeeding, 24-h–fasted ADX-low corticosterone-treated rats exhibited a 50% reduction in caloric intake compared with both sham-operated and high-corticosterone treated ADX rats. After 10 d of high-fat feeding, plasma leptin concentrations increased: After a 24-h fast, plasma leptin concentrations dropped only to ~2 ng/mL, compared with 0.5 ng/mL in chow-fed rats. This increase in plasma leptin concentrations prevented the fasting-induced increase in plasma corticosterone in sham-operated rats, and consequently reduced food intake after a 24-h fast to levels measured in ADX-low corticosterone-treated rats (Fig. 3 A–F and SI Appendix, Fig. S5 A–D).

Correcting Body Weight in Obese Rodents Restores Fasting-Induced Hyperphagia. Because these data demonstrate that elevated leptin reduced fasting-induced hypoleptinemia and consequently abrogated hypercorticosteronemia-mediated hyperphagia in the fasted–refed state, we next sought to determine whether these alterations could be reversed by normalizing body weight in rats with diet-induced obesity. To that end, we placed 4-wk HFD rats on a very-low–calorie diet (VLCD) (10.4 kcal/d, ~20% of their typical daily caloric intake) to reduce their body weight to that of healthy rats. This intervention lowered plasma leptin and increased plasma corticosterone during a 48-h fast to those of lean rats, resulting in a 3.5-fold increase in food intake upon refeeding (Fig. 3 G–I and SI Appendix, Fig. S6 A–C), suggesting a threshold for plasma leptin at which the HPA axis and, consequently, hyperphagia are activated.
Corticosterone Increases the Activity of AgRP Neurons. Because genetic tools in mice more readily allow for a mechanistic examination of neural processes, we next set out to confirm a similar role for corticosterone in modulating feeding behavior in mice. We therefore assessed the effect of elevated corticosterone on food intake in mice. To do so, slow-release corticosterone or placebo pellets were implanted subcutaneously 2 h prior to dark cycle onset. As a consequence, mice that received corticosterone pellets should have elevated corticosterone in the following light period, compared with placebo-implanted animals, in which corticosterone is at its circadian nadir (35–38) (Fig. 4A). Indeed, corticosterone implants significantly increased plasma corticosterone to levels similar to previous reports in fasted mice (36) and our starvation, T1D, hypoglycemia, and corticosterone infusion studies in rats (Fig. 4B; see Figs. 1–3). Food intake in the early light period was also significantly increased (Fig. 4C), while food intake in the preceding dark cycle, when corticosterone is naturally high, was comparable to placebo-implanted animals (Fig. 4D). Thus, corticosterone increases AgRP neuron activity compared with vehicle injection (Fig. 4K and L and SI Appendix, Fig. S7B). Thus, corticosterone not only causes persistent increases in AgRP neuron firing in ex vivo cell-attached recordings from AgRP neurons under 2 conditions in which corticosterone levels are high, concomitant with an increase in appetite. AgRP neurons were identified in ex vivo brain slices for electrophysiology studies by using Npy-hrGFP mice (44) (Fig. 4D). First, we confirmed previous findings that AgRP neurons fire with higher frequency in the fasted state (41–43) (Fig. 4E and F). This effect persisted in the presence of blockers of AMPA, kainate, NMDA, and GABA-A receptor-mediated synaptic transmission, suggesting a cell-autonomous component to their firing under caloric deficit. Second, we assessed whether administering exogenous corticosterone to mice would increase the firing rate of AgRP neurons. We therefore implanted placebo or corticosterone pellets into Npy-hrGFP mice (as in Fig. 4J), and mice were killed for electrophysiology recordings 2 h into the following light period when corticosterone levels are naturally low. AgRP neuron firing rates were significantly higher in mice that received corticosterone pellets, which persisted in the presence of synaptic blockers (Fig. 4G and H). Exogenous corticosterone administration did not have an effect on the firing of Npy-hrGFP-negative neurons in the arcuate (Fig. 4I).

To further examine the effect of corticosterone on AgRP neuron firing, we assessed whether corticosterone increases the activity of AgRP neurons in vivo. To do so, we virally expressed the Ca$^{2+}$ sensor, GCaMP6s, in AgRP neurons using Agrp-IRES-Cre mice (45) and performed fiber photometry recordings in the early light cycle in awake, behaving mice (46) (Fig. 4J). Subcutaneous injection of corticosterone (2 mg/kg) significantly increased AgRP neuron activity compared with vehicle injection (Fig. 4K and L and SI Appendix, Fig. S7B). Thus, corticosterone not only causes persistent increases in AgRP neuron firing in ex vivo cell-attached recordings from AgRP neurons under 2 conditions in which corticosterone levels are high, concomitant with an increase in appetite. AgRP neurons were identified in ex vivo brain slices for electrophysiology studies by using Npy-hrGFP mice (44) (Fig. 4D). First, we confirmed previous findings that AgRP neurons fire with higher frequency in the fasted state (41–43) (Fig. 4E and F). This effect persisted in the presence of blockers of AMPA, kainate, NMDA, and GABA-A receptor-mediated synaptic transmission, suggesting a cell-autonomous component to their firing under caloric deficit. Second, we assessed whether administering exogenous corticosterone to mice would increase the firing rate of AgRP neurons. We therefore implanted placebo or corticosterone pellets into Npy-hrGFP mice (as in Fig. 4J), and mice were killed for electrophysiology recordings 2 h into the following light period when corticosterone levels are naturally low. AgRP neuron firing rates were significantly higher in mice that received corticosterone pellets, which persisted in the presence of synaptic blockers (Fig. 4G and H). Exogenous corticosterone administration did not have an effect on the firing of Npy-hrGFP-negative neurons in the arcuate (Fig. 4I).

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brain and may activate AgRP neurons through modulation of their neural afferents, we sought to determine whether direct action of corticosterone on AgRP neurons was necessary to augment their activity. To do so, we overexpressed the glucocorticoid-
inactivating enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) in AgRP neurons. 11β-HSD2 is a key regulator of active glucocorticoid levels as it converts corticosterone to its biologically inert isomer, dehydrocorticosterone, thus preventing GR-mediated effects on gene expression (47–49). To visualize AgRP neurons in ex vivo brain slices, Agrp-IRES-Cre mice were crossed to L10-GFP Cre-reporter mice (50) (Agrp-IRES-Cre::L10-GFP mice). Agrp-IRES-Cre::L10-GFP mice were injected unilaterally with a Cre-dependent adenovirus-associated virus (AAV) driving the expression of mCherry-tagged 11β-HSD2 into the arcuate nucleus (Fig. 5A). Expression of 11β-HSD2 was confirmed to be specific to AgRP neurons (Fig. 5B). Subsequently, we recorded from AgRP neurons with (mCherry+, GFP+) or without (mCherry-, GFP+) 11β-HSD2 expression after fasting or corticosterone pellet treatment. Indeed, 11β-HSD2 overexpression in AgRP neurons blunted both the fasting-induced (Fig. 5 C and D) and corticosterone-mediated increase in AgRP neuron firing rate (Fig. 5 E and F). Together, these data suggest that corticosterone promotes appetite by directly increasing the firing of orexigenic AgRP neurons.

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Corticosterone Promotes Fasting- and Hypoglycemia-Induced Hyperphagia through AgRP Neurons. To examine whether corticosterone signaling in AgRP neurons is necessary for the hyperphagia caused by elevated corticosterone, we bilaterally injected Cre-dependent AAV-DIO-GCaMP6s-2a-mCherry into the arcuate of Agrp-IRES-Cre mice. 11β-HSD2 overexpression in AgRP neurons completely abolished the hyperphagic effect of corticosterone pellets during the early light cycle (Fig. 6 A and B). Given that our work in rats demonstrated that corticosterone drives fasting and hypoglycemia-induced hyperphagia, we assessed whether food intake under these conditions is mediated by action of corticosterone on AgRP neurons. Indeed, expression of 11β-HSD2 in AgRP neurons significantly reduced food consumption following a fast (Fig. 6 C and D), while hypoglycemia-induced feeding was entirely abolished by 11β-HSD2 expression in AgRP neurons (Fig. 6 E and F). Furthermore,
11β-HSD2–expressing mice ate less during the early dark cycle when corticosterone is at its daily peak (SI Appendix, Fig. S7 C and D), and 24-h food intake was also reduced (SI Appendix, Fig. S7E). These data demonstrate that corticosterone’s effects on food intake under multiple conditions is mediated by its action on AgRP neurons and that this mechanism is entirely responsible for hypoglycemia-induced food intake.

Discussion

Despite their differing etiologies, hypoglycemia (51–59), poorly controlled diabetes (17, 55, 60–69), and starvation (70–74) are all well-known triggers of hyperphagia. Our current study demonstrates that hyperphagia driven by these states is dependent upon hypercorticosteronemia and that the effect of leptin to reduce food intake in starvation and poorly controlled diabetes is mediated through suppression of the HPA axis. Furthermore, we show that activation of appetite-promoting AgRP neurons in the hypothalamic arcuate nucleus by corticosterone underlies these effects. While surprising, these results are in line with prior work linking elevated corticosterone to hyperphagia, weight gain, and increased expression of the orexigenic neuropeptides NPY and AgRP in the arcuate (22–24, 75). Furthermore, most prior studies (76–81), but not all (82), have found that adrenalectomy moderates the development of obesity in leptin-deficient rodents; however, these studies attributed the finding that adrenalectomy reduced food intake in leptin-deficient rodents to multiple alternative mechanisms and did not implicate stimulation of AgRP neuron firing by glucocorticoids.

It is generally believed that leptin does not regulate the HPA axis in humans because some leptin-deficient humans, unlike leptin-deficient rodents, do not have elevated glucocorticoids (83, 84). However, evidence to the contrary exists: Patients with congenital leptin deficiency (85, 86) or endocrine dysfunction concomitant with hypothalamic insufficiency (87) present with elevated cortisol, which can be rescued with leptin treatment (87). Furthermore, humans with low glucocorticoids have reduced hunger and body weight (88, 89), and increased cortisol stimulates appetite in humans (90, 91). Our current findings and the discrepancies regarding HPA axis regulation of hyperphagia in humans with leptin deficiency strongly suggest that further examination of this system is warranted.

The hyperphagia that occurs in response to glucose deprivation is also dependent upon hypercorticosteronemia, but independent of leptin deficiency, in contrast to fasting and T1D. This suggests a different mechanism for HPA axis activation during hypoglycemia. Indeed, C1 catecholaminergic neurons in the
ventrolateral medulla, which are activated by hypoglycemia and are viewed as playing a key role in mediating the vital counterregulatory responses (92), stimulate both glucocorticoids and feeding (34). This is likely mediated through their projections to the paraventricular nucleus of the hypothalamus, suggesting that these neurons directly regulate the HPA axis (34, 92). Previously, insulin or 2-deoxyglucose–induced glucoprivation was shown to increase \( \text{Agrp} \) and \( \text{Npy} \) transcripts in the arcuate (93, 94), suggesting that AgRP neurons are likely involved in driving the hypoglycemia-induced hyperphagia. Strikingly, 11\( \beta \)-HSD2 expression in AgRP neurons blocked hypoglycemia-induced hyperphagia (93, 94), indicating that hypoglycemic activation of the HPA axis and subsequent corticosterone-mediated stimulation of AgRP neurons is necessary for the induction of hypoglycemia-driven feeding.

Inhibition of glucocorticoid signaling in AgRP neurons blocked hyperphagia induced by hypercorticosteronemia and hypoglycemia, while prominently reducing food intake after fasting. However, the molecular mechanism underlying corticosterone activation of AgRP neurons is unknown. Glucocorticoids primarily regulate gene expression, but the genomic effects of corticosterone signaling are dependent upon many factors, including cell type, and are not fully characterized (95). AgRP neurons may have potential firing caused by fasting or hypercorticosteronemia persists ex vivo in the presence of synaptic blockers, corticosterone signaling may drive expression of genes that promote cell-autonomous activity. We have previously proposed similar actions by aldosterone on neurons that drive sodium appetite in the hindbrain (96). In addition, non-genomic effects of corticosterone signaling, which are often mediated through modulation of synaptic inputs (97), may contribute to driving AgRP neuron activity. Indeed, excitatory inputs to AgRP neurons are strengthened during fasting (15, 42), and corticosterone was previously reported to regulate synaptic input organization and excitability of AgRP neurons (98). Our findings that corticosterone signaling in AgRP neurons is necessary for hyperphagia contrast with a previous report that mice lacking glucocorticoid receptors specifically in AgRP neurons show little to no reduction in food intake or body weight (99). However, compensatory actions of mineralocorticoid receptors, which bind corticosterone with high affinity, may account for these findings. In addition, compensation for AgRP neuron loss of function has been reported in other studies including neonatal ablation (100, 101) and developmental deletion of leptin receptors (17, 102). Future studies leveraging RNA-sequencing technology to examine gene targets and recordings of AgRP neuron activity in the presence and absence of corticosterone signaling are needed to decipher which genomic and nongenomic mechanisms are involved in hypercorticosteronemia-mediated stimulation of AgRP neurons.

In summary, these findings reveal that hunger driven by hypoleptinemia or hypoglycemia is not entirely mediated via CNS circuits. Unexpectedly, both hypoleptinemia and hypoglycemia recruit and require an HPA axis–AgRP neuron pathway to fully induce hunger. We further demonstrate that the majority of leptin-mediated suppression of hyperphagia occurs through its ability to suppress hypercorticosteronemia in the transition from low to physiologic leptin concentrations (25, 26, 103). Finally, this study provides evidence for stimulation of a glucocorticoid–AgRP neuron axis that promotes food intake and suggests that AgRP neurons may be an attractive therapeutic target to suppress hyperphagia under conditions of leptin deficiency or glucocorticoid excess including Cushing’s disease.

**Methods**

**Animals.** All protocols were approved by the Institutional Animal Care and Use Committees of Yale University or Beth Israel Deaconess Medical Center. In the rat studies, healthy male rats weighing 250–300 g were ordered from Charles River Laboratories and maintained on regular chow (Harlan Teklad; 2018). Where indicated, adrenalectomy was performed by Charles River, and ADX rats were maintained on drinking water containing 0.9% NaCl and 2%
sucre. Upon arrival, they were housed in a 12-h light/dark cycle at −25 °C, and underwent surgery under isoflurane anesthesia to place catheters in the jugular vein and left carotid artery as well as an Alzet osmotic pump to deliver low-dose (7.5 μg/d) or high-dose (20 μg/d) corticosterone. Arterial catheters were used for all rat infusions, while venous catheters were used for blood sampling. To avoid any effects of diurnal variation on food intake, all acute food intake measurements were obtained between 2:00 and 4:00 PM following the fasting times listed in the figure legends. At 10:00 AM, treatment with leptin, corticosterone, and/or mifepristone, as described below, was begun following 42 h of food withdrawal or at the time of food withdrawal as designated in the figure legends (final fasting time, 6 or 48 h). ADX rats were given access to NaCl/sucrose-containing water throughout the fast, but food was removed 48 h before the study. After a fast–refeeding study, ADX rats were plained on HFD (Research Diets; 12492) for 2 wk, after which the fast–refeeding study was repeated. In all rat studies, food intake was measured by a blinded investigator, who weighed food before and after refeeding.

To induce poorly controlled T1D, rats were injected with 65 mg/kg streptozotocin after an overnight fast at 9:00 AM the morning before the study, and then refed. At 7:00 PM, food was removed. Plasma insulin concentrations were measured after a 15-h fast, and those with plasma glucose concentrations <160 μg/dL were later removed from analysis. Beginning at 10:00 AM, they were treated with leptin, corticosterone, and/or mifepristone as described below. Food was provided at 2:00 PM, and rats were allowed to eat ad libitum until they were killed at 4:00 PM.

Four-hour euglycemic and hypoglycemic clamp studies were performed beginning at 10:00 AM after an overnight fast. In both cases, insulin was infused intraperitoneally (prime 40 μU/kg, continuous infusion 4 μU/[kg.min]); and a variable infusion of 20% dextrose was administered to maintain euglycemia (∼0.16 mmol/L) (Fig. 1, A–D). The Jackson Laboratory and singly housed before food was removed ad libitum and the insulin and glucose infusions were continued, but the infusion rates were not adjusted for the remaining 2 h of study. Before the VLCD study, rats were fed HFD for 4 wk, and plasma hormones and food intake were measured following a 48-h fast. They were then placed on a VLCD (2 g diet per day, ~25% of their usual intake) for 4 wk, thereby returning their body weights to the typical healthy range. The fasting study was repeated in mice with hypoglycemic measurements as described below. Food was provided at 2:00 PM, and rats were allowed to eat ad libitum until they were killed at 4:00 PM.

For behavior experiments AAV8-H-syn-DIO-mCherry (UNC Vector Core) or AAV8-H-syn-DIO-Hsd11b2-t2a-mCherry was injected into Agrp-ires-Cre mice at 6 sites to cover the anterior-posterior extent of the arcuate (50 μL per site; bregma: anteroposterior [AP], −1.30 and −1.45 mm; dorsoventral [DV], −5.85 and −6.08 mm; lateral [ML], −0.3 mm). For electrophysiology studies, AAV-DIO-Hsd11b2-t2a-mCherry was injected unilaterally into Agrp-ire-Cre:L10-GFP mice (50 μL; bregma: AP, −1.45 mm; DV, −5.85; ML, −0.35 mm). For fiber photometry experiments, AAV1-H-syn-DIO-GCaMP6s (University of Pennsylvania Vector Core) was injected into the arcuate of Agrp-Ire-Cre mice. Animals were allowed to recover from stereotaxic surgery a minimum of 14 d before experiments. Following each experimental paradigm, animals were deeply anesthetized by an i.p. injection of the drug (10 mg/kg) solubilized in 10% ethanol/90% normal saline at 12:00 PM, 2 h before refeeding.

Stereotactic AAV Injections. pAAV-H-syn-DIO-Hsd11b2-t2a-mCherry was constructed by inserting the Hsd11b2-t2a-mCherry in reverse orientation in Agrp-ires-Cre mice at 6 sites to cover the anterior-posterior extent of the arcuate nucleus, mice were anesthetized with xylazine (5 mg per kg) and ketamine (75 mg per kg) diluted in saline (350 μg/dL), and placed into a stereotactic apparatus (KOPF; model 963). For postoperative care, mice were injected s.c. with sustained release meloxicam (4 mg/kg). After exposing the skull via a small incision, a small hole was drilled for injection. A pulled-glass pipette with a 20- to 40-μm tip diameter was inserted into the brain and virus was injected by an air pressure system. A micromanipulator (Grass Technologies; model S4B stimulator) was used to deliver the injection at 25 nl/min1 and the pipette was withdrawn 5 min after injection. For behavior experiments AAV8-H-syn-DIO-mCherry (UNC Vector Core) or AAV8-H-syn-DIO-Hsd11b2-t2a-mCherry was injected into Agrp-Ires-Cre mice at 6 sites to cover the anterior-posterior extent of the arcuate (50 μL per site; bregma: anteroposterior [AP], −1.30 and −1.45 mm; dorsoventral [DV], −5.85 and −6.08 mm; lateral [ML], −0.3 mm). For fiber photometry experiments, AAV1-H-syn-DIO-GCaMP6s (University of Pennsylvania Vector Core) was injected into the arcuate of Agrp-Ires-Cre mice. Animals were allowed to recover from stereotaxic surgery a minimum of 14 d before experiments. Following each experimental paradigm, animals were deeply anesthetized by an i.p. injection of the drug (10 mg/kg) solubilized in 10% ethanol/90% normal saline at 12:00 PM, 2 h before refeeding.

Optic Fiber Implantation. For fiber photometry recordings, an optic fiber was implanted in the same slice as virus injection. A metal ferrule optic fiber (480 μm external diameter; BFH37 coil; NA 0.48; Doric Lenses) was attached to the implanted optic fiber with a ceramic mating sleeve (Thor Labs). Light intensity was measured as 0.2 mW/mm² at the ferrule. For experiments targeting AgRP neurons, mice aged 8–12 wk were used. The mice were singly housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle before experiments. Mice had ad libitum access to NaCl/sucrose-containing water throughout the fast, but food was removed 48 h before the study. After a fast–refeeding study, ADX rats were fed HFD for 4 wk, and plasma hormones and food intake were measured following a 48-h fast. They were then placed on a VLCD (2 g diet per day, ~25% of their usual intake) for 4 wk, thereby returning their body weights to the typical healthy range. The fasting study was repeated in mice with hypoglycemic measurements as described below. Food was provided at 2:00 PM, and rats were allowed to eat ad libitum until they were killed at 4:00 PM.

In Vivo Fiber Photometry Recordings. Fiber photometry was performed on a rig constructed as follows: A 465-nm LED (PlexBright LED Module and LD-1 Driver; Plexon) was used as the excitation source, which was passed through a fluorescence microscope (excitation, 460–490 nm; detection, 500–550 nm; Doric Lenses) and transmitted onto the sample via a fiber optic cable (1 m long; 400 μm diameter; N.A. 0.48; Doric Lenses). The optic fiber was coupled to the implanted optic fiber with a ceramic mating sleeve (Thor Labs). Light intensity was measured as 0.2–0.3 mW at the end of the patch cord. A glass micropipette kept constant current through the NA = 0.48, 2 mW/mm² with 300 μm core (AAD Labs). Emitted light was collected by a photodetector (2151; Newport). The signal was digitized at 1 kHz with a National Instruments data acquisition card and collecting with a custom MATLAB (MATLAB 2016a; MathWorks) script. To reduce photobleaching during 1-h-long recordings, the LED was pulsed for 1 s every 10 s by a pulse generator (Arduino) as in ref. 46.

Data were analyzed using a custom Python (Python 3.6) script. The median fluorescence value of each LED pulse was taken to condense each pulse into a single data point. The average fluorescence change was calculated as F/F0 = (F – F0)/F0, where F0 was the mean of all data points from the 15-min baseline before injection, for each recording session.

Biochemical Analysis. Plasma glucose was measured using the YSI Glucose Analyzer. Plasma insulin, leptin, corticosterone, and ACTH concentrations were measured by ELISA (Bethyl Laboratories Inc., Alpco, and MyBioSource, respectively), with the exception of the hypoglycemic clamps in which insulin concentrations were measured by radioimmunoassay by the Yale Diabetes Research Core. To assess corticosterone levels in mice implanted with
slow-release pellets, trunk blood was taken following sacrifice and centrifuged for plasma collection. Plasma was run in duplicate in a 96-well plate ELISA kit for corticosterone (Enzo Life Sciences) according to the manufacturer's protocol.

Electrophysiology Studies. Loose-seal, cell-attached recordings were performed as described previously with minor modifications (96). Briefly, brains were quickly removed and placed into ice-cold cutting solution consisting of 310 mM NaCl, 21.4 mM NaHCO3, 2.5 mM KCl, 1.2 mM Na2HPO4, 1.2 mM MgCl2, 2.4 mM CaCl2, 10 mM glucose) at 34 °C for 15 min and then stored in the same solution at room temperature (20 °C) for at least 60 min before recording. Loose-seal, cell-attached recordings (seal resistance, 20–50 MΩ) were made in voltage-clamp mode with a configuration as internal solution and holding current maintained at Vh = 0 mV. Where indicated, synaptic blocks (including kynurenate (1 mM) and picrotoxin (100 µM)) were included in the bath solution to synthetically isolate AgRP neurons. All recordings were made using a Multiclamp 700B amplifier, and data were filtered at 2 kHz and digitized at 10 or 20 kHz before analysis off-line using Clampfit 10.

Histology. Immunofluorescence was performed as described previously (96). Briefly, mice were terminally anesthetized with 7% choral hydrate and digitized at 10 or 20 kHz before analysis off-line using Clampfit 10.
42. T. Liu et al., Fastening activation of AgRP neurons requires NMDA receptors and in-
termittent refeeding defines excitatory tone. Br. J. Nutr. 113, 260–266 (2015).
43. Y. Yang, D. Atasoy, J. E. Jones, J. K. Elmquist, B. B. Lowry, Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. Nat. Neurosci. 11, 998–1000 (2008).
44. L. L. Bellush, S. G. Reid, Metabolic and neurochemical profiles in insulin-treated di-
abetic NPY-deficient mice. Diabetes 68, 381–387 (2019).
45. M. A. Votteler, M. A. Rosas Fernández, 11β-Hydroxysteroid dehydrogenase: Fact or fancy? Steroids 44, 383–417 (1984).
46. C. S. Wynwoll, M. C. Holmes, J. R. Seck, 11β-Hydroxysteroid dehydrogenases and the brain: From zero to hero, a decade of progress. Front. Neuroendocrinol. 32, 265–286 (2011).
47. T. P. Nedungadi, K. P. Briski, Effects of intracerebroventricular administration of the neuropeptide Y receptor antagonist, 1229U91, on hypoglycemic and hyperglycic responses to acute and chronic intramydial-induced insulinhypoglycemia in female rats. Regul. Pept. 159, 14–18 (2010).
48. L. L. Bellush, S. G. Reid, Metabolic and neurochemical profiles in insulin-treated di-
abetic rats. Am. J. Physiol. 266, R87–R94 (1994).
49. S. E. Corrin, H. D. McCarthy, P. E. McElhinney, G. Williams, W. Langhans. Effect of CCB-
O on insulin-induced hyperglycemia and hypothalamic orexin neuropeptide expres-
sion in the rat. Peptides 26, 437–445 (2005).
50. K. J. Cai et al., Hypoglycemia activates orexin neurons and selectively increases hy-
pothalamic orexin-B levels: Responses inhibited by feeding and possibly mediated by thalamic melanocortin tone in leptin-deficient diabetic rats. Diabetes 60, 2265–2273 (2011).
51. J. W. Funder, P. T. Pearce, R. Smith, A. I. Smith, Mineralocorticoid action: Target tissue specificity is enzyme, not receptor, mediated. Science 242, 583–585 (1988).
52. T. P. Nedungadi, K. P. Briski, Effects of intracerebroventricular administration of the NPY-1 receptor antagonist, 1229U91, on hypothalamic and glycic responses to acute and chronic intramydial-induced insulinhypoglycemia in female rats. Regul. Pept. 159, 14–18 (2010).
53. E. Goulandris, A. Antoniou, M. Spengler, G. Williams, W. Langhans. Effect of CCB-
O on insulin-induced hyperglycemia and hypothalamic orexin neuropeptide expres-
sion in the rat. Peptides 26, 437–445 (2005).
54. J. Solomon, J. Mayer, The effect of adrenalectomy on the development of the obese-
hyperglycemic syndrome in ob-ob mice. Endocrinology 93, 510–512 (1973).
55. J. M. Resch et al., Elevated hypothalamic ghrelin levels are associated with obesity and hyperphagia in male mice. Endocrinology 157, 4257–4265 (2016).
56. J. Solomon, J. Mayer, The effect of adrenalectomy on the development of the obes-
eye syndrome in ob-ob mice. Endocrinology 93, 510–512 (1973).
57. K. Clément et al., A mutation in the human lepin receptor gene causes obesity and pyloric dysfunction. Nature 392, 398–401 (1998).
58. P. Mnatzagian, A. J. Li, Q. Wang, S. Ritter, Selective pharmacogenetic activation of catecholamine-
receptors in hypothalamus account male and female hyperphagia in Akita diabetes. Diabetes 62, 431–437 (2000).
59. I. S. Farrow et al., Effects of lepin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital lepin deficiency. J. Clin. Invest. 110, 1093–1103 (2002).
60. M. Ozata, I. C. Ozdemir, J. Licinio, Human lepin deficiency caused by a missense mutation: Multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for lepin action, greater central than per-
ipheral resistance to the effects of lepin, and spontaneous correction of lepin-
meditated defects. J. Clin. Endocrinol. Metab. 84, 3686–3695 (1999).
61. S. H. Chou et al., Leptin is an effective treatment for hypothalamic amenorrhea. Proc. Natl. Acad. Sci. U.S.A. 108, 6558–6590 (2011).
62. E. Charmandari, N. C. Nicolaides, G. P. Chrousos, Adren al insufficiency. Lancet 383, 2152–2167 (2014).
63. R. Salanti, Adrenal insufficiency. JAMA 294, 2481–2488 (2005).
64. B. S. Berthon, L. K. MacDonald-Wicks, L. G. Wood, A systematic review of the ef-
effect of oral glucocorticoids on energy intake, appetite, and body weight in humans. Nutr. Res. 34, 179–190 (2014).
65. P. A. Tataranni et al., Effects of glucocorticoids on energy metabolism and food intake in humans. Am. J. Physiol. 271, E317–E325 (1996).
66. A. J. Li, Q. Wang, S. Ritter, Selective pharmacogenetic activation of catecholamine-
subgroups in the ventrolateral medulla elks key glucoregulatory responses. En-
docrinology 159, 341–350 (2018).
67. V. Sergeyev, C. Broberger, O. Gorbatyuk, T. Höffken, Effect of 2-mercaptoacetate and 2-deoxy-o-glucose administration on the expression of NPY, AGRP, POMC, MCH and hypocretinorexin in the rat hypothalamus. Neuropeport 11, 117–121 (2000).
68. D. K. Sindelar et al., Neuropeptide Y is required for hyperphagic feeding in response to neuroglucopenia. Endocrinology 145, 3368–3368 (2004).
69. M. A. Sacta, Y. Chinenov, I. Rogatsky, Glucocorticoid signaling: An update from a genomic perspective. Annu. Rev. Physiol. 78, 155–180 (2016).
70. J. M. Resch et al., Aldosterone-sensing neurons in the NTIS exhibit state-dependent pacemaker activity and drive sodium appetite via synergy with angiotensin II sig-
naling. Neuron 96, 190–206.e7 (2017).
71. F. L. Groeneveld, H. Karrt, E. R. de Kloet, M. Joels, Rapid non-genomic effects of corticosteroids and their role in the central stress response. J. Endocrinol. 209, 153– 167 (2011).
72. E. Gyeneti et al., Corticosterone regulates synaptic input organization of POMC and NPY/AgRP neurons in adult mice. Endocrinology 151, 5395–5402 (2010).
73. M. Shibata et al., Neuropeptide Y-specific deletion of glucocorticoid receptor leads to increased energy expenditure and decreased body weight in female mice on a high-
fat diet. Endocrinology 157, 1457–1466 (2016).
74. S. Luquet, F. A. Perez, T. S. Hinako, R. D. Palmeter, NP/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science 310, 683–685 (2005).
75. E. Gropp et al., Agouti-related peptide-expressing neurons are mandatory for feeding. Nat. Neurosci. 8, 1289–1291 (2005).
76. E. van de Wall et al., Collective and individual functions of leptin receptor modu-
lated neurons controlling metabolism and ingestion. Endocrinology 149, 1773–1785 (2008).
77. R. J. Perry et al., Leptin mediates a glucose-fatty acid cycle to maintain glucose homeostasis in starvation. Cell 172, 234–248.e7 (2018).
78. H. Holeckova, P. Fabry, Hypothalamic and gastric hyperplasty in rats adapted to in-
termittent refeeding. Br. J. Nutr. 53, 260–266 (1985).