Ciliary neurotrophic factor (CNTF) exerts anorectic effects by overcoming leptin resistance via activation of hypothalamic neurons. However, the exact site of CNTF action in the hypothalamus has not yet been identified. Using Cre-loxP-mediated recombination in vivo, we have selectively ablated the common cytokine signaling chain gp130, which is required for functional CNTF signaling, in proopiomelanocortin (POMC)-expressing neurons. POMC-specific gp130 knockout mice exhibit unaltered numbers of POMC cells and normal energy homeostasis under standard and high fat diet. Endotoxin (LPS) and stress-induced anorexia and adrenocorticotropic hormone regulation were unaffected in these animals. Strikingly, the anorectic effect of centrally administered CNTF was abolished in POMC-specific gp130 knockout mice. Correspondingly, in these animals, CNTF failed to activate STAT3 phosphorylation in POMC neurons and to induce c-fos expression in the paraventricular nucleus. These data reveal POMC neurons as a critical site of CNTF action in mediating its anorectic effect.

CNS | obesity

Obesity is commonly linked to leptin resistance (1). Physiologically, leptin activates the JAK/STAT signaling pathway through the long form of leptin receptors (ObRb), which belong to the family of cytokine receptors (2). Multiple mechanisms for the development of leptin resistance have been proposed such as reduced transport of leptin across the blood–brain barrier (3), as well as desensitization of the leptin target cells by increased expression of the suppressor of cytokine signaling (SOCS) (4) proteins. One therapeutic approach to overcoming leptin resistance could be to mimic intracellular leptin-evoked signaling through alternative receptors.

The ciliary neurotrophic factor receptor (CNTFR) also belongs to the family of cytokine receptors. In contrast to leptin, CNTF only fully activates this pathway as part of a tripartite complex consisting of CNTFR-α, leukemia inhibitory factor receptor (LIFR) β, and gp130. JAK2 associated with gp130 becomes activated upon homodimerization of gp130 resulting in the activation of the JAK/STAT pathway. Both CNTFR-α and gp130 are expressed in neurons of the arcuate nucleus (ARC) of the hypothalamus (5), and CNTF application is able to inhibit food intake and to reduce body weight both in control and leptin-resistant mice (6–9). Pharmacological experiments have indicated that CNTF, similar to leptin, is capable of suppressing neuropeptide Y mRNA levels, and neuropeptide Y application counteracts the weight-reducing effects of CNTF (9). Nevertheless, co-administration of an anorexigenic and an orexigenic factor does not prove that both are part of or targeting the same pathway(s). On the other hand, CNTF and leptin initiate differential patterns of gene expression in the ARC, indicating diverging mechanisms of action and potentially differential target sites of both hormones (10, 11). Currently, it is not known which neuronal subpopulation mediates the effects of CNTF. Because proopiomelanocortin (POMC) neurons are an important primary site of leptin action in the brain and critical for energy homeostasis (12), we have explored the role of these cells in mediating CNTF’s biological effect on feeding.

**Results**

**Abolished CNTF-Stimulated STAT3 Tyrosine Phosphorylation in POMC Cells of gp130<sup>a</sup>POMC Mice.** To determine the effect of POMC cell-restricted deficiency of gp130 on CNTF’s induction of STAT3 phosphorylation, control and gp130<sup>a</sup>POMC mice expressing β-gal in POMC neurons were injected with recombinant CNTF i.v. In control mice, CNTF treatment resulted in profound activation of STAT3 phosphorylation in both POMC- and non-POMC-expressing neurons of the ARC (Fig. 1a). Consistent with this, Western blot analysis revealed no alterations in overall brain and hypothalamic gp130 protein expression (Fig. 1b). Accordingly, gp130 expression in peripheral tissues remained unchanged in gp130<sup>a</sup>POMC mice (Fig. 1b).

**Conflict of interest statement:** No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office. Abbreviations: CNTF, ciliary neurotrophic factor; i.e., intracerebroventricularly; POMC, proopiomelanocortin; PVN, paraventricular nucleus.

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receptors such as the CNTF receptor, without abolishing gp130-independent cytokine receptor signaling such as that mediated by the leptin receptor.

Unaltered POMC Neuron Number and Stress Response in gp130KO POMC Mice. gp130 has been demonstrated to play a critical role in neurogenesis (16). Thus, we next determined whether lack of functional gp130 signaling alters the number or distribution of hypothalamic POMC neurons. This analysis revealed equal numbers and distribution of POMC neurons in both control and gp130KO POMC mice (Fig. 1e). These data indicate that disrupted gp130 signaling in our animals did not affect neurogenesis and cell survival of POMC neurons.

Consistent with the expression pattern of endogenously expressed POMC, the only non-hypothalamic site of recombination detectable in PomcCre-transgenic mice was the pituitary (Fig. 2a). Because cytokines that signal through gp130-dependent receptors such as leukemia inhibiting factor (LIF) and IL-6 have been demonstrated to regulate adrenocorticotropic expression and release in cultured pituitary cells (17), we next characterized the functional stress response of gp130KO POMC mice. Pomc mRNA expression in the pituitary of control and gp130KO POMC mice did not show a significant difference (Fig. 2b). To directly determine the stress response in control and gp130KO POMC mice, we used two paradigms activating the corticotropic response via distinct mechanisms, i.e., restraint stress and LPS injection. Both stimuli dramatically increased plasma corticosterone concentrations to a comparable extent in control and gp130KO POMC mice (Fig. 2c). As expected, LPS injection resulted in a profound increase of circulating IL-6 concentrations (18) (Fig. 2d). This increase was indistinguishable between control and gp130KO POMC mice (Fig. 2d). Taken together, these data indicate that inactivation of gp130 signaling in POMC cells of the hypothalamus and pituitary does not impair responses to restraint and LPS-induced stress in these animals.

Normal Energy and Glucose Homeostasis in gp130KO POMC Mice. To determine the importance of endogenous gp130-dependent signaling in POMC cells on the regulation of energy homeostasis, we first

**Fig. 1.** Generation of POMC-specific knockout (gp130KO POMC) mice. (a) PomcCre mice were mated with Z/EG reporter mice, and immunohistochemistry for enhanced GFP was performed in double-transgenic mice. (Scale bar: 100 μm.) (b) Western blot analysis of gp130 and pten (loading control) in brain, hypothalamus (HT), liver, and white adipose tissue (WAT) in control (CO) and gp130KO POMC (KO) mice. (c) Double immunohistochemistry for p-STAT3 (green) and β-gal (red) in POMC neurons of control (gp130+/−) and gp130KO POMC (gp130−/−) mice 30 min after i.v. stimulation with NaCl, leptin, or recombinant CNTF. (d) Quantification of p-STAT3-positive POMC cells after CNTF stimulation in control (CO) and gp130KO POMC (KO) mice (mean ± SEM of three animals in each group) (**P < 0.001**). (e) Quantitative and spatial analysis of POMC neurons in control (open bars) and gp130KO POMC (filled bars) mice (n = 3 of each genotype).

**Fig. 2.** Unaltered stress response in gp130KO POMC mice. (a) Immunohistochemistry for enhanced GFP performed in the pituitary of PomcCre, Z/EG mice. (b) Relative expression of Pomc mRNA in pituitaries of control (CO) and gp130KO POMC (KO) mice (n = 7–8). (c) Plasma corticosterone concentrations: basal and 1 h after NaCl injection, restraint stress, or LPS treatment in control (open bars; n = 6–10) and gp130KO POMC (filled bars; n = 7–12) mice. (d) IL-6 plasma concentrations: basal and 1 h after NaCl injection, restraint stress, or LPS treatment in control (open bars; n = 6) and gp130KO POMC (filled bars; n = 7) mice.
monitored the body weight of control and gp130ΔPOMC mice from weaning until 6 months of age. This analysis revealed no change in body weight in the presence of POMC-restricted gp130 deficiency both under standard and high-fat diets (Fig. 3a). White adipose tissue mass and circulating plasma leptin concentrations were indistinguishable between control and gp130ΔPOMC mice (Fig. 3b and c). In control and gp130ΔPOMC mice, there was an increase in adipose tissue mass and plasma leptin concentrations upon exposure to high-fat diet (Fig. 3b and c). Food intake and basal metabolic rate were also indistinguishable between control and gp130ΔPOMC mice (Fig. 3d and e). The expression of anorexigenic neuropeptides such as POMC and cocaine- and amphetamine-related transcript (CART) was unaltered in control and gp130ΔPOMC mice (Fig. 3f). Body length was indistinguishable between control and gp130ΔPOMC mice, a further indication of intact function of the melanocortin pathway in the absence of gp130 signaling in POMC neurons (Fig. 3g).

Consistent with unaltered energy homeostasis in gp130ΔPOMC mice, these animals exhibited normal glucose metabolism as assessed by glucose tolerance tests, insulin tolerance tests, and blood glucose and plasma insulin concentrations (Fig. 5a, c, e, and f, which is published as supporting information on the PNAS website). Exposure to high-fat diet resulted in impaired glucose tolerance, increased blood glucose concentrations, and elevated plasma insulin concentrations as an indirect measure of insulin resistance in a similar manner in control and gp130ΔPOMC mice (Fig. 5b and d–f). Taken together, these data indicate that gp130 signaling in POMC neurons is dispensable for normal control of body weight, food intake, energy expenditure, and glucose metabolism.

Blunted Anorectic Response to Intracerebroventricularly (i.c.v.) Injected CNTF in gp130ΔPOMC Mice. To analyze the effect of exogenous CNTF on food intake, we implanted cannulae into the lateral ventricle of female and male control and gp130ΔPOMC mice. In females, analysis of food intake 4 h after CNTF injection revealed a reduction in food intake in control mice by ~80% compared with 0.9% NaCl injection (Fig. 4a). Strikingly, however, this effect was severely blunted in gp130ΔPOMC mice, which exhibited only a 35% reduction in food intake (Fig. 4a). Similarly, in females, food intake over 24 h after a single injection of CNTF was significantly reduced by 40% in control animals but only resulted in a decrease of 18% in gp130ΔPOMC mice (Fig. 4a). In addition, CNTF reduced body weight significantly 24 h after injection in control females but exhibited only a minor effect in gp130ΔPOMC mice (Fig. 4b). Similar results were obtained when male control and gp130ΔPOMC mice were acutely treated with CNTF: 6-h food intake after single injection was significantly reduced by 60% in control animals, whereas gp130ΔPOMC mice failed to exhibit a significant reduction of food intake (Fig. 4c). Body weight significantly decreased by 4% in CNTF-treated controls, whereas it remained unchanged in gp130ΔPOMC mice (Fig. 4d). Taken together, CNTF very rapidly and severely impairs food intake over a 24-h period in control animals, an effect that is blunted in gp130ΔPOMC mice.

CNTF Fails to Induce c-Fos Immunoreactivity in the Paraventricular Nucleus (PVN) of gp130ΔPOMC Mice. The PVN of the hypothalamus is a major site of action of POMC efferents in the regulation of feeding (19). Therefore, we analyzed the activation of PVN neurons in response to acute CNTF treatment in control and gp130ΔPOMC mice. In control animals, CNTF evoked a strong induction of c-Fos immunoreactivity in the PVN (Fig. 4e and f). Strikingly, however, CNTF treatment completely failed to enhance c-Fos immunoreactivity in the PVN of male gp130ΔPOMC mice (Fig. 4f). Taken together, CNTF very rapidly and strongly activates neurons in the PVN that are characterized as important mediators of energy homeostasis regulation (19). The absence of PVN neuronal activation in response to CNTF application in gp130ΔPOMC mice clearly indicates that PVN activation critically depends on functional CNTF signaling in POMC neurons.

Discussion

CNTF has provided an attractive therapeutic tool for the treatment of obesity, although its clinical use has been limited due to the development of neutralizing antibodies in patients treated with a recombinant variant of CNTF (20). Despite intense research over the years, the exact site of CNTF action in mediating its anorectic effect has not been resolved.

Fig. 3. Unaltered energy homeostasis in gp130ΔPOMC mice. (a) Body-weight curve of control (open squares and open triangles) and gp130ΔPOMC (filled squares and filled triangles) mice on standard diet (SD) (squares; n = 12–18) and high-fat diet (HFD) (triangles; n = 12–31). (b) Epidiagnostic fat pads were dissected and weighed. Data represent the mean ± SEM of 14–31 mice in each group. (c) Serum leptin levels of control (n = 10–16) and gp130ΔPOMC (n = 10–16) mice on SD and HFD at the age of 24 weeks. (d) Basal metabolic rate (BMR) of control (CO) (n = 6) and gp130ΔPOMC (KO) (n = 9) mice at the age of 11 weeks. (e) Relative expression of POMC and CART in control (open bars; n = 6–7) mice. (f) Basal metabolic rate (BMR) of control (open squares and open triangles) and high-fat diet (HFD) (triangles; n = 12–18) and high-fat diet (HFD) (triangles; n = 12–31). (g) Body length of control (n = 13) and gp130ΔPOMC (n = 13) mice.
Here, we show that the POMC-specific deletion of gp130 (gp130<sup>POMC</sup>) has no impact on body weight, food intake, energy expenditure, and glucose metabolism, indicating that endogenous CNTF does not influence energy homeostasis via POMC neurons. In contrast, pharmacological doses of CNTF, injected i.c.v., resulted in a blunted anorectic response in gp130<sup>POMC</sup> mice, associated with abolished neuronal activation in the PVN. Thus, our current experiments define a functional neural circuit in which CNTF signaling in POMC neurons is essential for CNTF’s acute inhibitory effect on food intake and the activation of PVN neurons. These results are in line with the fact that CNTF engages similar intracellular signaling mechanisms to those activated by leptin stimulation (7, 11).

Nevertheless, the pattern of STAT3 phosphorylation within the hypothalamus was different after stimulation with CNTF vs. leptin. This is consistent with previous studies reporting a robust phospho-(p)-STAT3 staining in the ventromedial part of the ARC after CNTF stimulation, whereas leptin-stimulated STAT3 phosphorylation was more prominent in the lateral ARC and the ventromedial hypothalamus (6). However, even though this points to a differential expression of CNTF and leptin receptors within different neuronal populations of the ARC, our functional results reveal that CNTF signaling plays a critical role in POMC cells. Thus, CNTF and leptin seem to target at least this cell type similarly.

Our data reveal a blunted reduction of body weight and food intake in gp130<sup>POMC</sup> females upon acute CNTF treatment. However, in male gp130<sup>POMC</sup> mice, POMC-specific deletion of gp130 completely abolished the anorectic response to i.c.v. applied CNTF. Even though the underlying mechanism for this sexually dimorphic phenotype remains unsolved, our data are in line with other studies using genetic mouse models of obesity (21–23) or analyzing the effect of centrally administered agents such as insulin and leptin on energy homeostasis (24) also finding different phenotypes and pharmacological responses depending on gender.

In this study, we have addressed the acute pharmacological effect of centrally administered CNTF, but analyzing the effect of chronic CNTF treatment in gp130<sup>POMC</sup> mice will certainly provide additional important information. CNTF exhibits a unique feature, which is protection from weight rebound upon termination of treatment (7). Therefore, it will be crucial to determine whether this long-term protection also depends on functional gp130 signaling in POMC neurons.

More recently, additional intriguing mechanisms for CNTF action have been described. CNTF has been demonstrated to induce neurogenesis in the ARC of the adult brain. Importantly, this effect appeared to be necessary for protection against weight rebound (25). Kokoeva <i>et al.</i> also provided evidence that newly proliferated neurons exhibit both neuropeptide Y and POMC expression. Therefore, one could speculate that CNTF’s effect on neurogenesis requires gp130 signaling in adult neuronal stem cells before the acquisition of a POMC phenotype. According to this model, the protective effect against weight rebound would be retained in gp130<sup>POMC</sup> mice.

Furthermore, it has become evident that CNTF also exerts effects in peripheral tissues such as skeletal muscle and liver to improve metabolism via activation of AMP-dependent kinases (AMPK) (26, 27). Therefore, gp130<sup>POMC</sup> mice as well as mice with targeted disruption of gp130 signaling in peripheral organs will allow for the definition of the contribution of organ-specific gp130 signaling to the improvement of glucose metabolism and sustained weight loss upon chronic CNTF treatment.

Moreover, gp130<sup>POMC</sup> mice provide an excellent tool to dissect the primary site of other cytokines acting in the central nervous system to regulate energy homeostasis and signaling via gp130-dependent receptor complexes. These comprise IL-6 and the leukemia inhibitory factor (LIF) (28). Therefore, it will be interesting to analyze the effect of centrally administered IL-6 and LIF in gp130<sup>POMC</sup> mice and in mice with targeted disruption of gp130 in other neuronal populations such as AgRP/neuropeptide Y neurons. Ultimately, these experiments will allow for the dissection of neuronal networks targeted by different cytokines to regulate energy homeostasis and metabolism.
Methods

Animals. All animal procedures were conducted in compliance with protocols approved by local government authorities (Bezirksregierung Köln) and were in accordance with National Institutes of Health guidelines. Mice were housed in groups of three to five at 22–24°C in a 12-h light/12-h dark cycle with lights on at 6 a.m. Animals were fed either regular chow food (Global Rodent T.2018.R12 from Harlan Teklad, containing 12% of calories from fat) or a high-fat diet (C1057 from Altromin, containing 55.2% of calories from fat). Water was available ad libitum, and food was only withdrawn if required for an experiment. Body weight was measured once per week. At the end of the study period, the animals were killed under isoflurane anesthesia.

Generation of PomcCre-gp130lox/lox Mice. PomcCre mice (14) were mated with gp130lox/lox mice (13), and a breeding colony was maintained by mating gp130lox/lox with PomcCre-gp130lox/lox mice. gp130lox mice had been backcrossed for at least five generations on a C57BL/6 background, and PomcCre mice (initially established on an FVB background) had been backcrossed for two generations on a C57BL/6 background before intercrossing them with gp130lox mice. Only animals from the same mixed background strain generation were compared with each other. PomcCre mice were genotyped by PCR as described in ref. 14. gp130 mice were genotyped by PCR with the following primers: 5′-gpt1′, 5′-GGT GGC TGA TTC ACC TGC A-3′; and 3′gp130, 5′-TAC GGT GGG CAG CGT CCT-3′. For visualization of Cre-mediated recombination, mice were crossed with either the indicator strain Rosa26lacZ reporter (Fig. 6, which is published as supporting information on the PNAS web site) (29) or the Z/EG reporter strain (15).

Restraint Stress and LPS Treatment. Mice were adapted to gentle handling for ~8 weeks before the experiment. To determine basal serum corticosterone and IL-6 levels, blood was drawn from the tail vein during the first 3 h of the light phase. The next day, mice were subjected to 1 h of restraint stress at the same time of the light phase. Restraint stress was achieved by enclosing the animals in a plastic tube with a diameter of 3 cm and openings at both ends for tail and nose. At the end of the experiment, blood samples were again drawn from the tail vein.

Seven days later, the animals were injected i.p. with 0.9% NaCl during the first 3 h of the light phase, and blood was collected from the tail vein 1 h after injection. After 5 days of recovery, each mouse received an i.p. injection of 300 μL of LPS (L2630 from Sigma) 1 h after injection blood samples were taken. Two days after LPS injection, the animals were killed under isoflurane anesthesia.

Glucose and Insulin Tolerance Tests. Glucose and insulin tolerance tests were performed as described in ref. 30.

Indirect Calorimetry and Food Intake. All measurements were performed with the Comprehensive Laboratory Animal Monitoring System (CLAMS) (Oxyxam; Columbus Instruments, Columbus, OH). Mice were placed at room temperature (22–24°C) in 3.0-liter chambers of the CLAMS open circuit calorimetry. Setting time was set at 150 s, and measuring time was set at 60 s with room air as reference. Food and water were provided ad libitum in the appropriate devices. Mice were allowed to acclimate in the chambers for 24 h. Parameters of indirect calorimetry were measured for at least the following 72 h. Food intake was measured continuously in the CLAMS during the experiment. The data presented are average values obtained in these recordings.

Western Blot Analysis. Indicated tissues were dissected and homogenized in homogenization buffer with a Polytron homogenizer (IKA Werke, Staufen, Germany), and Western blot analyses were performed as described in ref. 31 with antibodies raised against gp130 (sc-656 from Santa Cruz Biotechnology) and Pten (MS-1250-P from NeoMarkers) as loading control.

Hypothalamic Neuropeptide Expression. Measurements of mRNA levels were carried out by quantitative RT-PCR on RNA extracted from dissected hypothalamic or pituitary tissue. Total RNA for each hypothalamus was quantified by spectrophotometry after purification with the Qiagen RNeasy kit. Two hundred nanograms of each total RNA sample was reverse-transcribed and PCR-amplified by using the TaqMan-principles ABI PRISM 7700 sequence detection system (Applied Biosystems). Efficiency for the primers was estimated from standard curves made with serial cDNA dilutions.

Analytical Procedures. Blood glucose levels and insulin and leptin serum levels were determined as described in ref. 30. IL-6 serum levels were measured by ELISA with mouse standards according to the manufacturer’s guidelines (BD OptEIA mouse IL-6 ELISA kit; BD Biosciences Pharmingen). Corticosterone serum levels were determined by RIA using mouse standards according to the manufacturer’s guidelines (ImmunoChem Double Antibody Corticosterone 125I RIA kit; ICN).

Statistical Methods. Data were analyzed for statistical significance by using a two-tailed unpaired Student t test.

i.c.v. Cannula Implantation. Twelve-week-old mice were anesthetized by i.p. injection of Avertin (240 mg/kg) (2,2,2-tribromoethanol; Sigma) and placed in a stereotactic device. A sterile osmotic pump connector cannula (Bilaney Consultants, Düsseldorf, Germany) was implanted into the lateral brain ventricle (0.2 mm posterior and 1.0 mm lateral relative to bregma and 2.5 mm below the surface of the skull). The support plate of the catheter was attached to the skull with superglue. The catheter was prefilled with 0.9% NaCl and connected to a sealed microporethane catheter (MRE-025 from Braintree Scientific, Braintree, MA).

i.c.v. Injection. After 7 days of recovery, 2 μL of recombinant rat CNTF (0.5 mg/mL; 557-NT/CF from R & D Systems) or 0.9% NaCl was injected, and 1 μL of 0.9% NaCl was postinjected to ensure a complete drug administration to the brain. To avoid a backflow, the catheter was sealed. Injections were performed under isoflurane anesthesia 1 h before the onset of the dark cycle.

Food intake and body weight were measured 4 and 24 h after the injection for females and 6 h after the injection for males. The correct position of the i.c.v. cannula was verified by injection of methyl blue after the mice were killed.

Tissue Preparation for Immunocytochemistry and Immunocytochemical Procedures. Control and gp130POMC mice were mated with Rosa26 reporter mice (29). At the age of 10 weeks, mice fed ad libitum received one i.v. injection of 5 μg of recombinant rat CNTF, 20 μg of leptin (L3772 from Sigma), or 0.9% NaCl into the tail vein. Thirty minutes after injection, the mice were perfused with 0.9% NaCl followed by 4% paraformaldehyde, and the brains were extracted, kept in 4% paraformaldehyde overnight at 4°C, and stored in 20% sucrose at 4°C.

For one series, we performed double-labeling of p-STAT3 and β-galactosidase (indicating POMC-expressing neurons) using free-floating immunohistochemistry as described in ref. 32. In brief, sections were washed, pretreated with 100% methanol/0.3% glycine/0.03% SDS, and blocked in 3% normal donkey serum followed by incubation of the primary antibodies [rabbit anti-p-STAT3 (1:3,000 dilution; Cell Signaling Technology, Beverly, MA) and goat anti-β-galactosidase (1:3,000 dilution; Biogenesis, Poole, U.K.)] for 48 h at 4°C. Secondary labeling was done with anti-rabbit Alexa 488 (Invitrogen/Molecular Probes) and biotinylated anti-
giant antibody (1:200; Jackson ImmunoResearch) followed by streptavidin-Alexa 568 (1:200) for detection of p-STAT3 and β-galactosidase antibodies. Sections were mounted onto gelatin-coated slides, dried, and coverslipped with using mounting media (ProLong Gold; Invitrogen/Molecular Probes). Fluorescence signals were detected under a fluorescent microscope (BX51; Olympus), and representative pictures of the ARC were taken with a digital color camera (DP70; Olympus).

Fet better visualization of GFP expression after Cre-recombination, brain sections (25 μm) of double-heterozygous POMC-Cre Z/EK mice were washed, pretreated with 0.3% H₂O₂, blocked with PBT-Azide containing 3% donkey serum, and incubated overnight with primary antibody (anti-GFP rabbit serum, 711-065-125 from Jackson ImmunoResearch) was followed by incubation with the VECTASTAIN Elite ABC kit (Vector Laboratories) for 1 h and 0.4% DAB/0.01% H₂O₂. Afterward the sections were mounted onto gelatin-coated slides and covered with glycerin.

Plutitary was stained with the same antibodies, using the Tyramide Signal Amplification kit (TSA Plus Fluorescence Systems; PerkinElmer).

**POMC Cell Counting.** Quantification of β-galactosidase-positive POMC cells was done as described in ref. 32. In brief, every fourth section throughout the ARC was taken and allocated rostral to caudal to visualize the distribution of POMC neurons throughout the ARC. By using Adobe PHOTOSHOP software, β-galactosidase-positive neurons were counted and marked digitally to prevent multiple counts. Cell counts were performed on three animals per group. The percentage of double-positive cells compared with the total amount of β-galactosidase-positive POMC cells after CNTF stimulation was also determined by cell counting.


**Immunoassay for c-Fos and Quantification.** As described above, catheter-implanted animals received an injection of either 0.9% NaCl or CNTF 1 h before onset of the dark phase. Six hours later, the mice were anesthetized by using Avertin and perfused with 0.9% NaCl followed by 10% Formalin. The brains were dissected, kept in glutaraldehyde-free fixative at 4°C for at least 2 h, and stored in 0.1 M phosphate buffer at 4°C until further preparation.

Free-floating brain sections (50 μm) containing the PVN were immunostained for c-Fos [rabbit a-c-Fos, Oncogene (EMD Bio-Science) used at 1:20,000] by our standard protocol using diaminobenzidine as the chromogen as described in ref. 33. Sections were examined under a Zeiss Axioplan 2 microscope outfitted with an AxioCam HRC camera and AXIOVISION 4.2 imaging software. Every section containing the PVN (*n* = 4) was examined for each animal (with the operator blinded to the experimental conditions). For each hemisphere of the PVN, at a magnification of ×40, a single plane of focus was chosen and a black and white image was captured; a 100-μm² box was placed in the center of the PVN. All of the c-Fos-labeled cell nuclei were counted within the box for each section. Only those labeled nuclei that were clearly in the plane of focus were selected. The results were expressed as the number of c-Fos-labeled cells per 100-μm² area of the PVN.

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a) gp130

b) Pomc-Cre

Rosa (lacZ reporter)

Control

gp130\(\Delta^{POMC}\)