SUPPLEMENTAL FIGURES AND LEGENDS

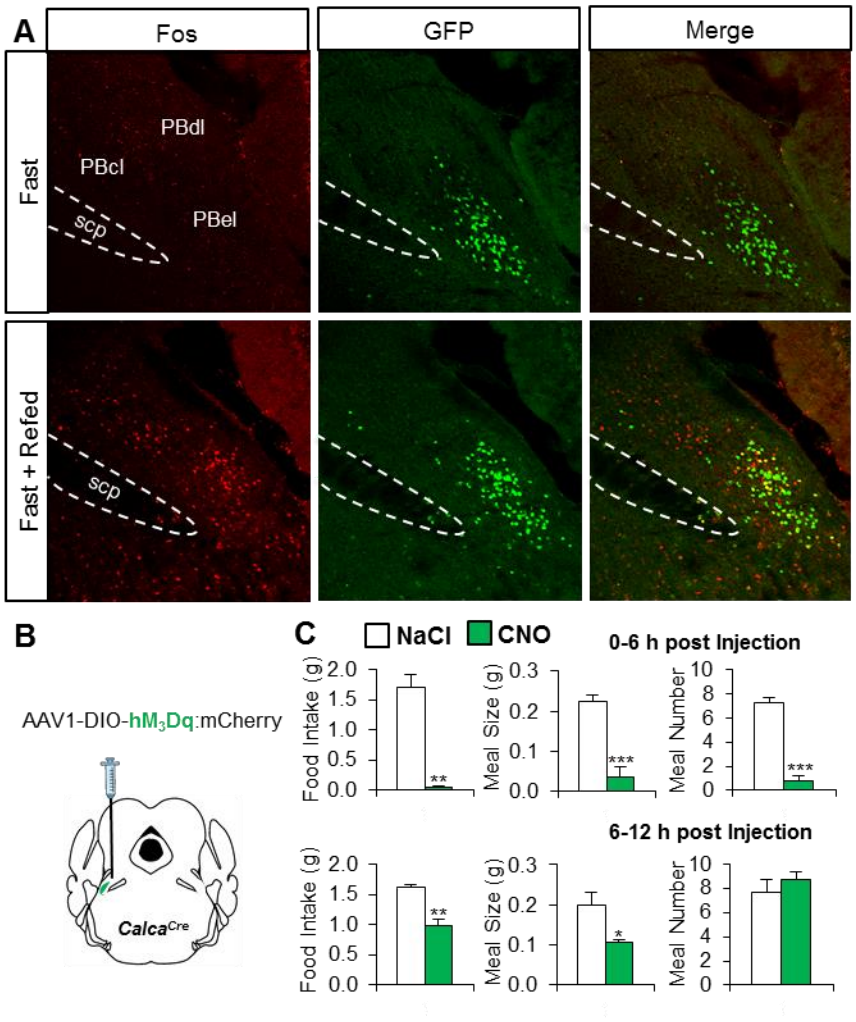

Figure S1. CGRP^{PBel} Neuron Activation Controls Meal Termination; Related to Figure 1

(A) Representative histological images showing Fos immunoreactivity in GFP-labeled CGRP^{PBel} neurons following a fast or fast-refeeding. Superior cerebellar peduncle, scp; central lateral parabrachial nucleus, PBcl; dorsal lateral parabrachial nucleus, PBdl; external lateral parabrachial nucleus, PBel.

(B) Unilateral delivery of AAV carrying Cre-dependent hM3Dq:mCherry into the PBel of *Calca*^{Cre} mice (4 x 10^9 viral genomes per microliter compared to 2 x 10^9 viral genomes per microliter in Figure 1).

(C) Cumulative food intake and meal pattern analysis following saline or CNO (1 mg/kg, i.p.) administration immediately prior to onset of dark cycle in non-food-deprived mice (n = 4) in. All data shown are means ± s.e.m. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Statistical analysis was performed with a two-way ANOVA followed by Bonferroni's post-hoc test.

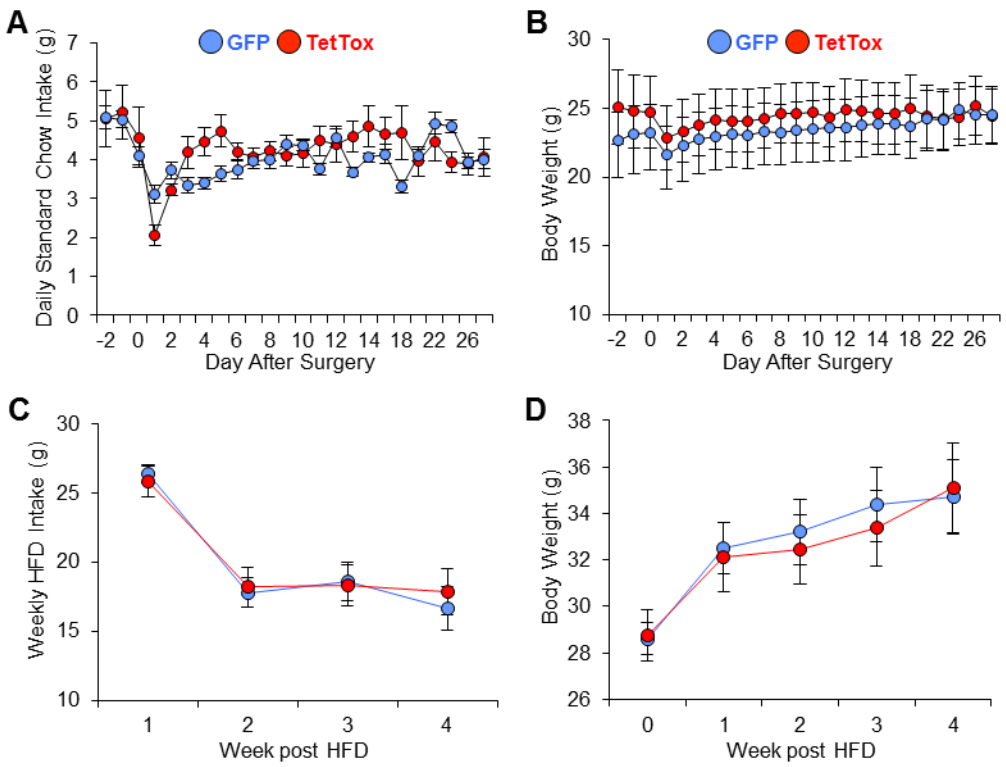

Figure S2. Functional Inactivation of CGRP^{PBel} Neurons Does Not Increase Cumulative Food Intake or Body Weight; Related to Figure 2

(A) Average daily standard chow intake in GFP (n = 6) and TetTox (n = 7) mice before and after virus injection.

(B) Body weights before and after virus injection in mice fed a standard chow diet.

(C) Average weekly food intake of GFP (n = 8) and TetTox (n = 6) mice fed a palatable high-fat diet (HFD).

(D) Body weights after given access to high-fat diet. All data shown are means ± s.e.m.

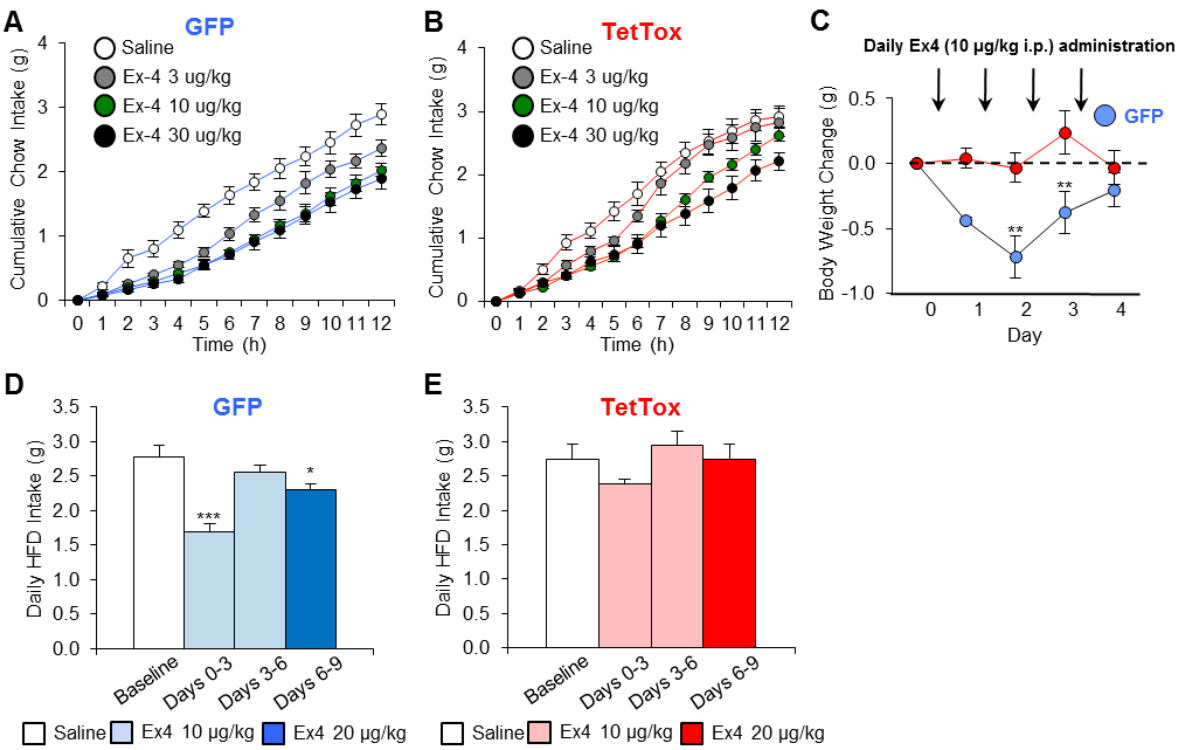

Figure S3. Functional Inactivation of CGRP^{PBel} Neurons Attenuates Anorexia and Body Weight Loss Induced by GLP-1 Receptor Agonist; Related to Figure 4

(A and B) 12-h cumulative standard chow intake following administration of several Ex4 doses in GFP **(A)** and TetTox **(B)** mice (n = 8 per group).

(C) In mice maintained on standard chow diet, daily Ex4 administration decreases body weight in GFP (n = 7) but not TetTox mice (n = 10).

(D and E) Average daily food intake in obese GFP **(D)** and TetTox **(E)** mice maintained on a high-fat diet and treated with twice-daily i.p. injections of saline (baseline), 10 µg/kg Ex4 (days 0-6), and 20 µg/kg Ex4 (days 6-9). All data shown are means ± s.e.m. (n = 8 GFP; n = 6, TetTox). * $P < 0.05$; ** $P < 0.01$. Statistical analysis was performed with a two-way ANOVA followed by Bonferroni's post-hoc test (C) and one-way ANOVA followed by Tukey's post-hoc test (D-E).

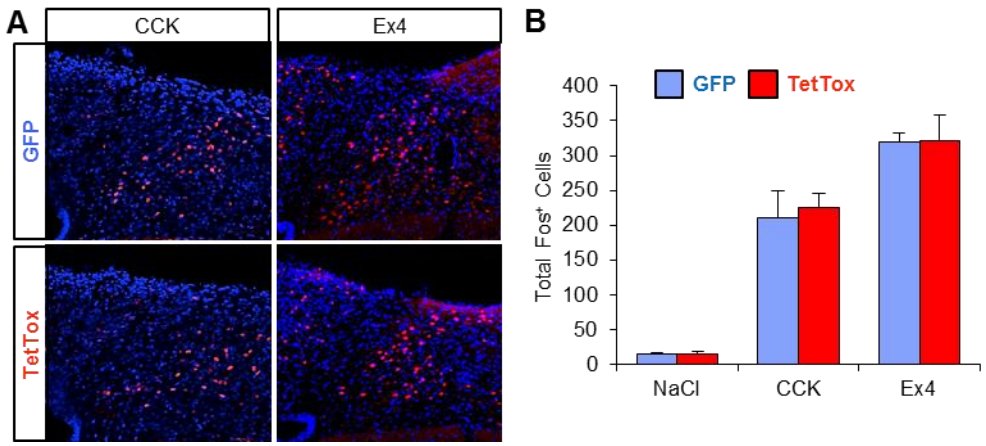

Figure S4. Fos Immunoreactivity in the NTS Following CCK and GLP-1 Receptor Agonist Administration; Related to Figure 5

(A and B) Representative histological images **(A)** and quantification **(B)** of Fos immunoreactivity in the NTS from GFP and TetTox mice treated with saline, CCK, and Ex4. NTS Fos induction was used as positive control for successful gut peptide administration. All data shown are means ± s.e.m (n = 4-6 per treatment).

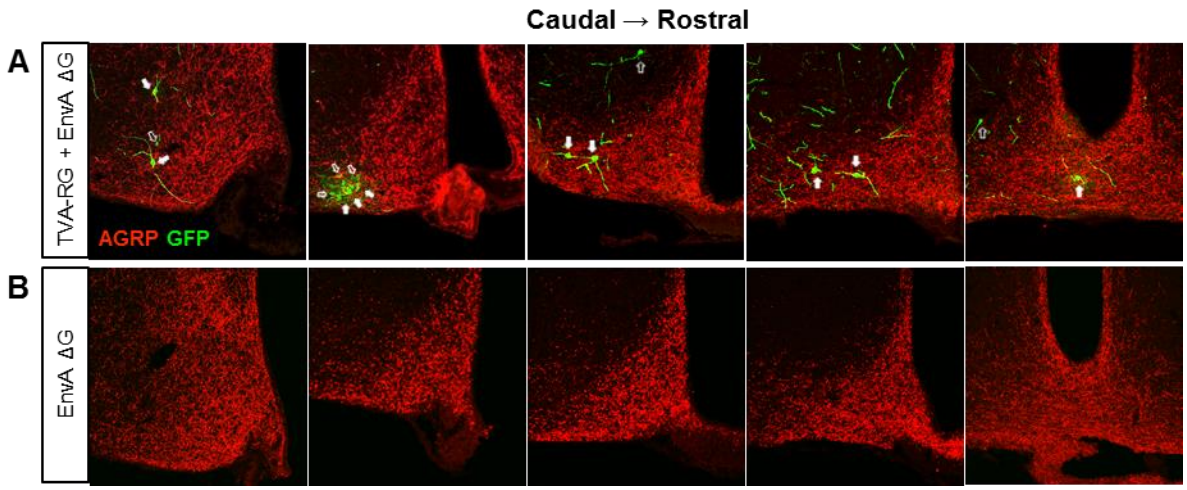

Figure S5. Retrograde Monosynaptic Rabies Tracing of CGRP^{PBel} Neurons; Related to Figure 6

(A) Representative confocal images of the arcuate hypothalamus showing overlap of EGFP-labeled neurons (green) and AgRP immunoreactivity (red) of *Calca*^{Cre} mice following PBel injection of AAV8-DIO-GFP-TVA-RG "helper virus" and EnvA-ΔG Rabies:EGFP virus (n = 4). Filled arrows indicate EGFP and AgRP double-labeling; hollow arrows indicate EGFP single-labeling.

(B) Representative images of the arcuate hypothalamus showing AgRP immunoreactivity (red) and lack of EGFP labeling (green) following PBel injection of EnvA-ΔG Rabies:EGFP without prior injection of "helper virus" (n = 2).

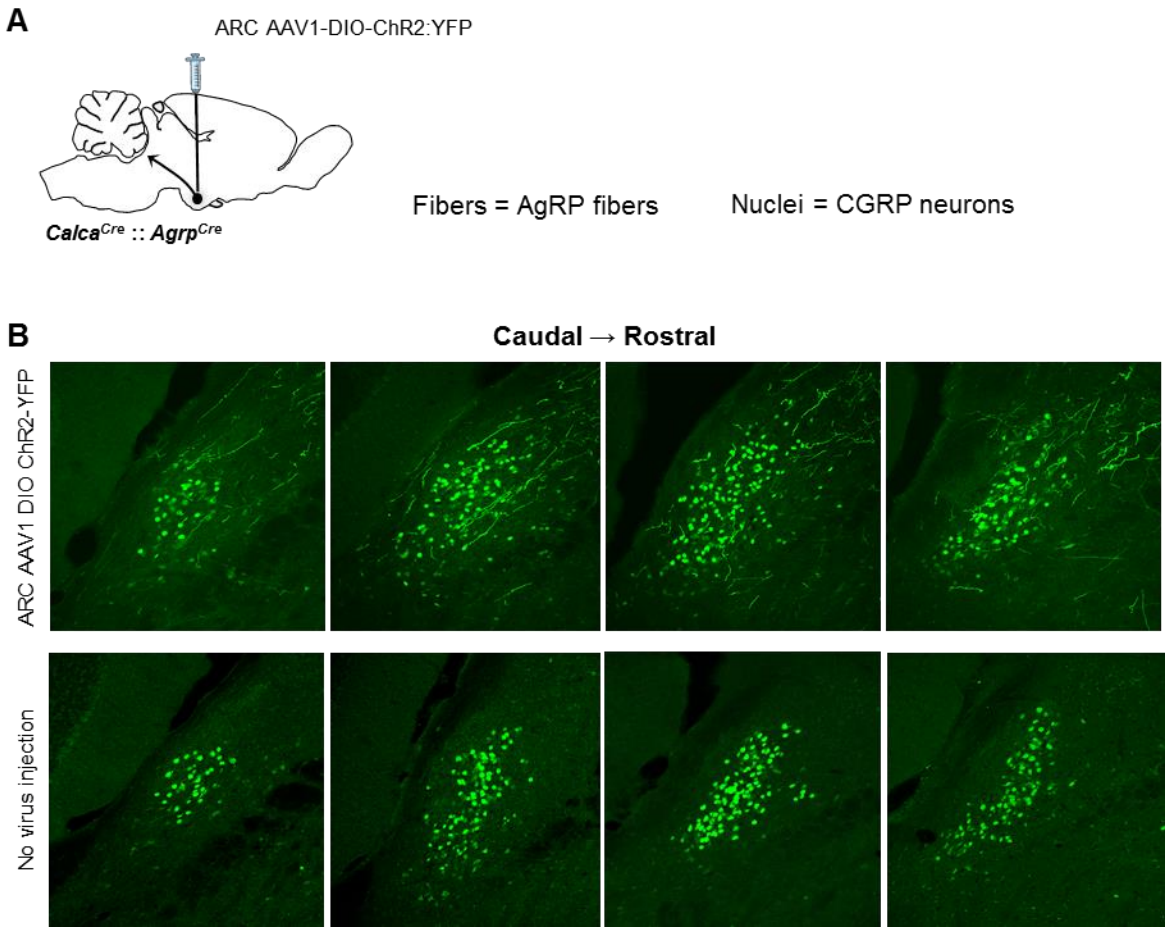

Figure S6. AgRP Axons Innervate the PBel; Related to Figure 6

(A) Bilateral delivery of AAV carrying Cre-dependent ChR2:YFP into the arcuate hypothalamus of *Calca*^{Cre}::*Agrp*^{Cre} mice.

(B) Representative histological images showing GFP-labeled CGRP^{PBel} neurons (nuclear labeling) and YFP-labeled AgRP fibers. Top row is following virus injection and bottom row is from a non-treated *Calca*^{Cre}::*Agrp*^{Cre} mouse (note absence of axon fibers).

Video S1. Concurrent Inactivation of CGRP^{PBel} Neurons and Somatic AgRP Photostimulation; Related to Figure 6

Video footage of *Calca*^{Cre}::*Agrp*^{Cre} mouse with bilateral inactivation of CGRP^{PBel} neurons (PBel delivery of AAV1-DIO-GFP:TetTox) and photostimulation of AgRP neurons (fiber-optic cannula placed above the arcuate following bilateral delivery of AAV1-DIO-ChR2:YFP into the arcuate nucleus). In non-food-deprived mice, inactivation of CGRP^{PBel} neurons enhances hyperphagic response induced by AgRP neuronal activation (2 h photostimulation). Video is 64X normal speed.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice

Calca$^{Cre:GFP}$ and *Agrp*$^{Cre:GFP}$ mice (C57Bl/6 background) were generated and maintained as previously described (Carter et al., 2013; Sanz et al., 2015). Following stereotaxic surgery, mice were singly housed with *ad libitum* access to standard chow diet (LabDiet 5053) in temperature- and humidity-controlled facilities with 12-h light/dark cycles. Heterozygous male *Calca*$^{Cre:GFP/+}$ and *Agrp*$^{Cre:GFP/+}$ mice were used for behavioral experiments whereas both male and female mice were used for immunohistochemical Fos studies. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Washington.

Virus Production and Stereotaxic Surgeries

Cre-dependent AAV1-DIO-hM$_3$Dq:mCherry, AAV1-DIO-GFP, AAV1-DIO-GFP:TetTox, and AAV1-DIO-ChR2:YFP viral vectors were produced by transfecting HEK cells and then purifying cell extracts by pelleting through sucrose and by CsCl-gradient ultracentrifugation. Final pellets were suspended in 0.1 M PBS at an approximate titer of 2 x 10^9 viral genomes per microliter (unless stated otherwise in the main text) measured with a fluorometer (Hoefer, DQ300). AAV8-DIO-GFP-TVA-RG and EnvA-ΔG Rabies:EGFP were generous gifts from Martin Myers lab. Stereotaxic injection of virus into the PBel of *Calca*^{Cre} mice was done as described (Carter et al., 2013). Virus (0.5 µl per side) was injected bilaterally (TetTox studies) or unilaterally (hM$_3$Dq-mCherry and retrograde rabies tracing studies) into the PBel using the following coordinates: antero-posterior (AP), -5.10 mm; medio-lateral (ML), ± 1.30; dorso-ventral (DV), 3.25 mm. The following coordinates were used to stereotaxically inject virus into the arcuate nucleus of *Agrp*^{Cre} mice (bilaterally, 0.5 µl per side): AP, -1.25 mm; ML, ± 0.35 mm; DV, -5.80 mm. For optogenetic control of AgRP neurons, a single fiber-optic cannula was implanted above the arcuate nucleus (AP, -1.25 mm; ML, 0.00 mm; DV, -5.50 mm) or bilaterally over the PBel (AP, -4.8 mm; ML, ± 1.40 mm; DV, -2.80 mm). Mice were given at least 3 weeks of post-surgery recovery.

Pharmacology

CNO (1 mg/kg, Sigma), CCK-8s (2-10 µg/kg, Bachem), recombinant murine leptin (2 µg/kg, PeproTech), and Ex4 (3-30 µg/kg, American Peptide) were dissolved in sterile 0.9% saline. All drugs were administered i.p. (10 µl per gram body weight).

Meal Pattern Analysis and Food Monitoring Experiments

Meal-patterning analysis. Mice were habituated to food monitoring home cages (BioDAQ, v. 2.2) for at least 10 days before experimental manipulation. Feeding records were analyzed using BioDAQ Viewer (software v. 2.2.01). A feeding bout was defined as a meal if ≥ 0.06 g of food was ingested and if it was separated from another meal by ≥ 5 min.

Mice had *ad libitum* access to water during all food-monitoring experiments. All pharmacological behavioral experiments were conducted in non-food-deprived mice injected with drug or control saline injection ~20 min before onset of the dark cycle. A cross-over, within-subjects experimental design was used for pharmacological experiments (except for during chronic Ex4 administration) with at least five days elapsing between experimental trials.

Ad libitum food monitoring. After stereotaxic injection of AAV1-DIO-GFP:TetTox into the PBel of *Calca*^{Cre} mice, food intake and body weights were measured on a daily basis. One month later, mice were housed in BioDAQ cages for meal-patterning analysis. Meal patterns from mice with *ad libitum* food access were analyzed from a 72-h continuous recording and meal parameters are presented as average daily values.

Fast and refeeding. Following a 24-h food deprivation, mice were given food access at the start of the dark cycle. Food intake was manually measured 1, 2, 3, and 12 h later.

Palatable diet intake. For acute studies, one week prior to the experiment, mice were given access to 1 g of palatable liquid diet (Ensure Original). On the day of the experiment, non-food-deprived mice were given *ad libitum* access to the palatable diet for 2 h during the light cycle. To examine the long-term effects of inactivating CGRP^{PBel}

neurons in mice with *ad libitum* access a palatable diet, a separate group of mice were fed a high-fat diet (45 kcal% fat; Research Diets, D12451) for 4 consecutive weeks.

Satiety peptides/hormones. Feeding records from the first 2 h post-injection were used to analyze CCK-induced anorexia and 12-h post injection for analyzing leptin- and Ex4-induced anorexia. For experiments involving chronically administered Ex4, mice with *ad libitum* access to standard chow diet received a single i.p. Ex4 injection immediately prior to dark cycle onset for four consecutive days. A separate group of mice were placed on a high-fat diet (45 kcal% fat; Research Diets, D12451) for two months prior to chronic Ex4 administration. After becoming obese (~35-40 g), these mice received twice-daily saline injections (every 12 h) for two consecutive days to gather baseline food intake and body weight followed by nine consecutive days of twice-daily Ex4 injections.

Photostimulation

A blue light laser (473 nm, LaserGlow) was used to deliver light pulses to the brain through fiber-optic cables (200-µm diameter, Doric Lenses) firmly attached to implanted fiber-optic cannula with zirconia sleeves (Doric Lenses). The frequency and pulse width of the laser light was programmed using a waveform generator (Agilent Technologies, catalog #33220A). The light power exiting the fiber tip (4.8 mW) was estimated to correspond to 3.52 mW mm^{-2} at the arcuate or PBel based on an online light transmission calculator for brain tissue (http://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php). Based on an established photostimulation protocol for activating AgRP neurons and their fiber projections (Aponte et al., 2011; Atasoy et al., 2012), all of our experiments used the following settings: 10-ms pulses, 20 pulses for 1 s on / 3 s off. During habituation sessions (2 h daily for 5 days), mice were attached to fiber-optic cables but did not receive photostimulation or access to food. All photostimulation experiments were conducted 3 h into the light cycle in non-food-deprived mice.

PBel AgRP fiber photostimulation. Mice with bilateral PBel fiber-optic cannulae implants were given access to 1 g of Ensure one week prior to experimental manipulation. On the day of experiment, mice were attached to fiber-optic cables 1 h prior to receiving *ad libitum* access to Ensure for 2 h. In one cohort of mice, light pulses were delivered 15 min before and during access to Ensure. In a separate cohort, light pulses were delivered for 1 h immediately prior to, but not during, access to Ensure. A total of three experimental trials were conducted (at least 5 days apart) in the following order: photostimulation, no photostimulation, and photostimulation.

Somatic AgRP photostimulation. In a separate group of mice with fiber-optic cannulas implanted over the arcuate nucleus, fiber-optic cables were attached 30 min prior to photostimulation. After the acclimation period, light pulses were delivered for 2 h during which mice were given access to pre-weighed food pellets. Food pellets were manually weighed every 30 min during photostimulation and a USB camera was used to record eating behavior. Based on published observations and criteria for successful ChR2 infection and photostimulation of AgRP neurons (Aponte et al., 2011; Atasoy et al., 2012), mice that consumed less than 0.5 g of food during the first 1 h of photostimulation were excluded from data analysis. For video analysis of time spent eating, eating behavior was defined as the time spent in direct contact with food and/or masticating. The period during which food was weighed was excluded from analysis.

Immunohistochemistry and Fos Studies

Fos, PKC-δ, and GFP immunolabeling were performed as described (Han et al., 2015). For all immunohistochemical experiments, mice were anesthetized (Beuthanasia, 320 mg/kg delivered i.p.) and intracardially perfused with 0.1 M PBS followed by 4% paraformaldehyde. Brains were then extracted, post-fixed in 4% paraformaldehyde overnight, and cryoprotected in 0.1 M PBS containing 20% sucrose until the brains sunk in the sucrose solution. Coronal cryostat sections 30-µm thick were collected and every third section of the PBel and every fourth section of the CeA were processed for immunolabeling and quantification. For co-labeling of EGFP and AgRP, sections were incubated for 16 h at room temperature in chicken anti-GFP (1:10000, Abcam) and rabbit anti-AgRP (1:300, Phoenix Pharmaceuticals). The sections were then washed and incubated for 2 h at room temperature in Alexa488-conjugated donkey anti-chicken (1:400, Jackson ImmunoResearch) and Alexa594-conjugated donkey anti-rabbit (1:400, Jackson ImmunoResearch). For co-labeling of GFP and PKC-δ in the CeA, sections were incubated for 16 h in chicken anti-GFP and mouse anti-PKC-δ (1:1000, BD Biosciences) with subsequent incubation for 2 h in Alexa488-conjugated donkey anti-chicken and Alexa594-conjugated donkey anti-mouse (1:400, Jackson ImmunoResearch). For co-labeling of GFP and Fos in the PBel and CeA, sections were incubated for 16 h at room temperature in chicken anti-GFP (1:10000, Abcam) and goat anti-Fos (1:400, Santa Cruz

Biotechnology). The sections were then washed and incubated for 2 h at room temperature in Alexa488-conjugated donkey anti-chicken (1:400, Jackson ImmunoResearch) and Alexa594-conjugated donkey anti-goat (1:400, Jackson ImmunoResearch). GFP antisera and corresponding secondary antisera were excluded during Fos immunolabeling of NTS sections. Unilateral images of the NTS, PBel and CeA were taken using a laser-scanning confocal microscope (Olympus, FV1200). Fos and GFP colocalization counts were performed using the Cell Counter feature on ImageJ (v. 1.48). Cell counts were obtained from two sections for each brain region corresponding to the following coordinates: -7.35 mm and -7.45 mm bregma, NTS; -5.15 mm and -5.25 mm bregma, PBel; -1.55 mm and -1.75 mm bregma, CeA. Detailed experimental procedures for Fos studies are provided below.

Fast and refeeding. 24-h fasted mice were given *ad libitum* food access during the light cycle and perfused 120 min later.

Satiety peptides. Non-food-deprived mice received an i.p. injection of saline, CCK (5 µg/kg), or Ex4 (3 µg/kg) and were perfused 90 min later.

AgRP→PBel photostimulation. Non-food-deprived mice received an i.p. injection of Ex4 (3 µg/kg) while receiving unilateral AgRP→PBel photostimulation (half the mice received photostimulation over the left PBel and half over the right PBel). Light pulses were delivered after Ex4 injection until mice were perfused 90 min later.

Statistics

All data were analyzed using Prism 5.0 (GraphPad Software). Statistical tests used are described in the figure legends.