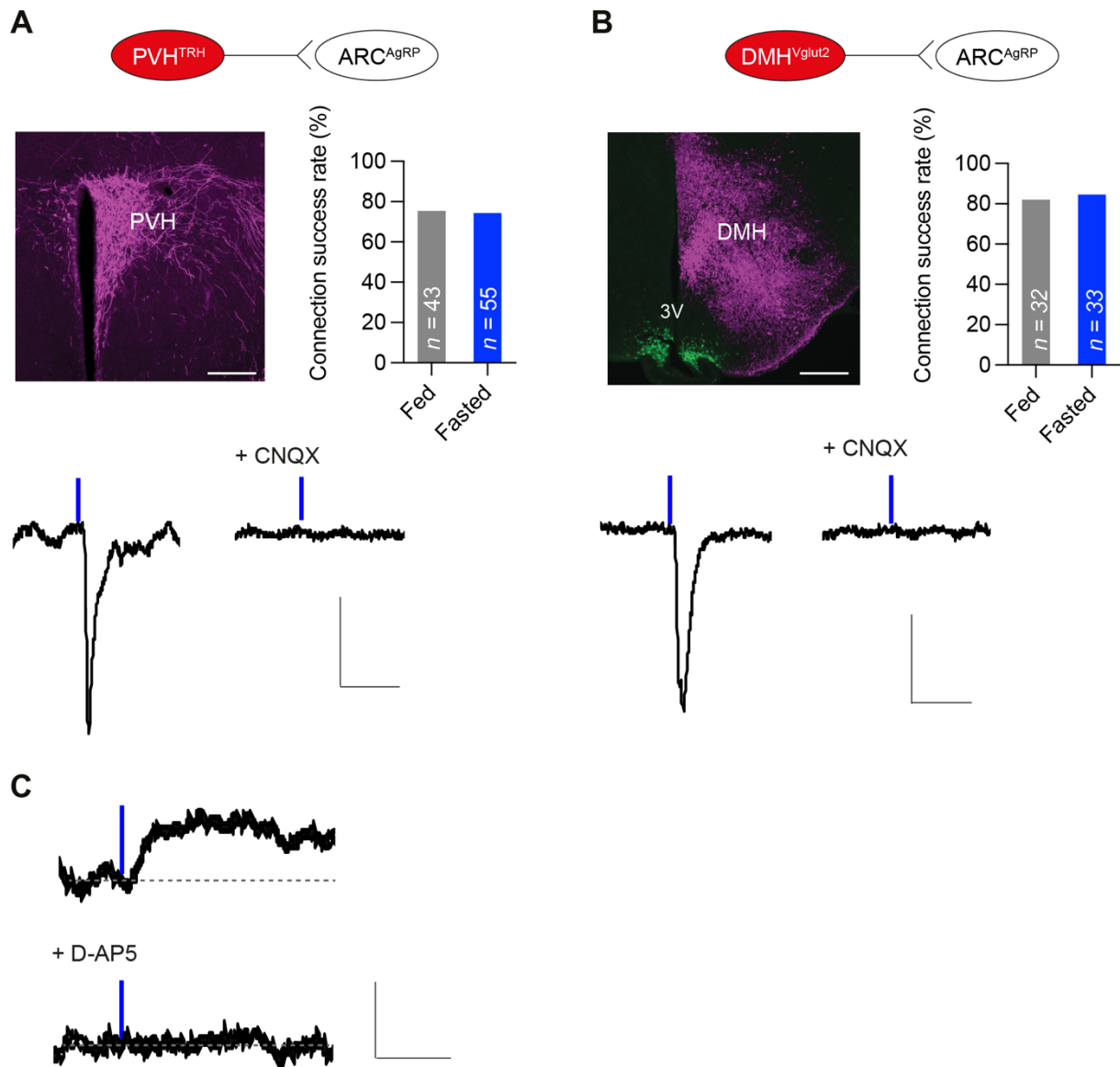


Supplemental Data Figures

Figure S1



**Figure S1 (refers to Figure 1): AgRP neurons receive robust glutamatergic input from PVH<sup>TRH</sup> and DMH<sup>Vglut2</sup> neurons**

**A, B)** Representative fluorescence images showing expression of ChR2-mCherry (magenta) in PVH<sup>TRH</sup> neurons (**A**) and DMH<sup>Vglut2</sup> neurons (**B**), and AgRP neurons positive for NPY-hrGFP (green, **B**). Scale bars represent 100  $\mu$ m.

Summary of connections show that light-evoked glutamatergic currents were detected in the vast majority of AgRP neurons in fed and fasted mice expressing ChR2 in PVH<sup>TRH</sup> (**A**) or DMH<sup>Vglut2</sup> neurons (**B**; right).

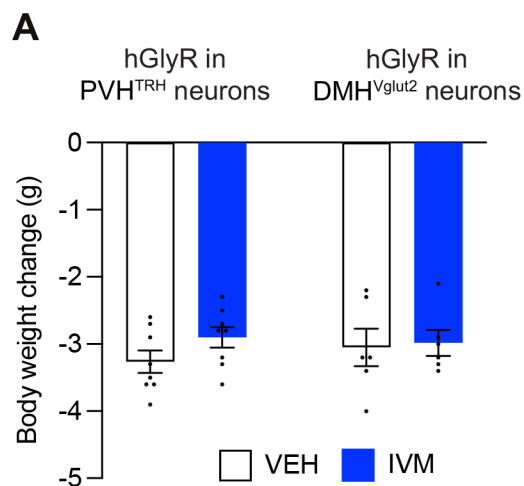
Representative traces showing postsynaptic currents recorded at  $V_h = -70$ mV from AgRP neurons in mice expressing ChR2 in PVH<sup>TRH</sup> (**A**) or DMH<sup>Vglut2</sup> neurons (**B**). Bath administration

of the AMPAR antagonist CNQX completely blocked le-EPSCs, confirming the glutamatergic nature of connections.

**C)** Representative trace of postsynaptic currents from AgRP neurons in presence of CNQX. Bath administration of the NMDAR antagonist D-AP5 completely blocked le-EPSCs recorded at  $V_h = +40\text{mV}$ .

Scale bars represent 30 pA, 30 ms.

## Figure S2

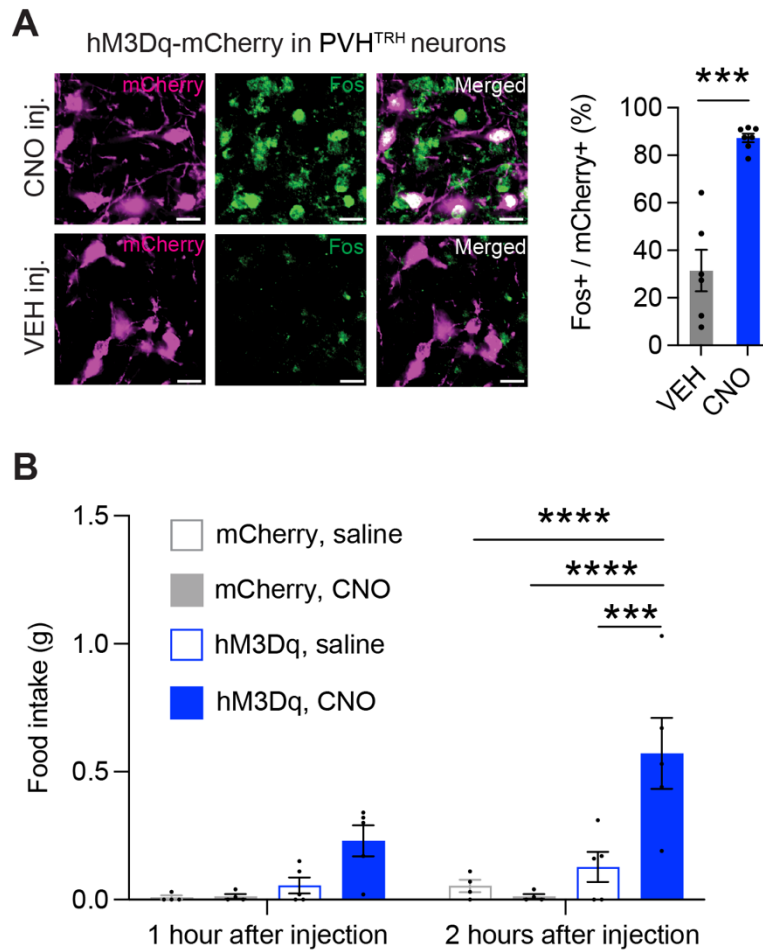


**Figure S2 (refers to Figure 2): Selective silencing of PVH<sup>TRH</sup> and DMH<sup>Vglut2</sup> neurons using the IVM-sensitive glycine receptor hGlyR**

**A)** Summary of body weight changes show that IVM injection in mice expressing hGlyR in PVH<sup>TRH</sup> or DMH<sup>Vglut2</sup> neurons did not affect body weight loss during fasting, compared to control experiments when the same mice received the vehicle.

All data are presented as mean  $\pm$  s.e.m.; two-tailed unpaired Student's *t*-test.

**Figure S3**



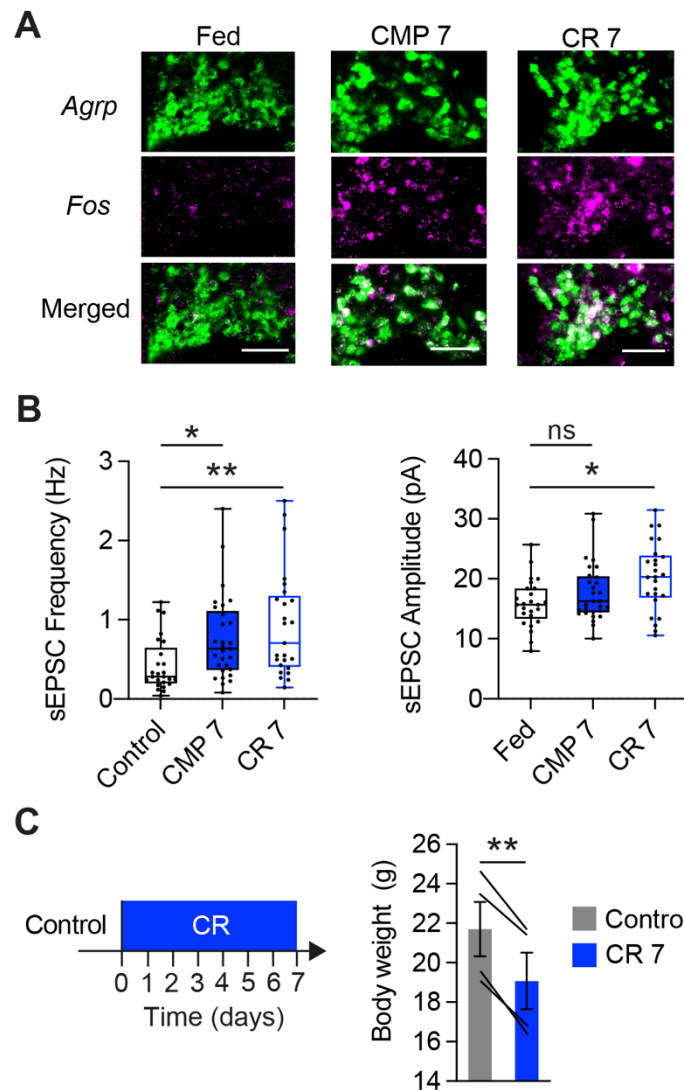
**Figure S3 (refers to Figure 3): Selective chemogenetic stimulation of PVH<sup>TRH</sup> neurons acutely increases food intake**

**A)** Representative fluorescence images showing expression of hM3Dq-mCherry (magenta) and *Fos* (green) in PVH<sup>TRH</sup> neurons. Mice were treated with CNO or vehicle one hour before transcardial perfusion. Summary of *Fos* expressing and mCherry-positive cells show that CNO treatment effectively activates PVH<sup>TRH</sup> neurons. Scale bars represent 20  $\mu$ m.

**B)** Mice expressing hM3Dq in PVH<sup>TRH</sup> neurons significantly increase food intake after CNO injection at the onset of the light cycle, compared to control mice expressing mCherry in PVH<sup>TRH</sup> neurons. Saline injection in the same mice did not alter food intake.

All data are presented as mean  $\pm$  s.e.m.; \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ ; two-tailed unpaired Student's *t*-test (**A**), and ordinary two-way ANOVA followed by Tukey's multiple comparisons test (**B**).

**Figure S4**



**Figure S4 (refers to Figure 4): Activation of AgRP neurons in circumstances in which weight gain is promoted**

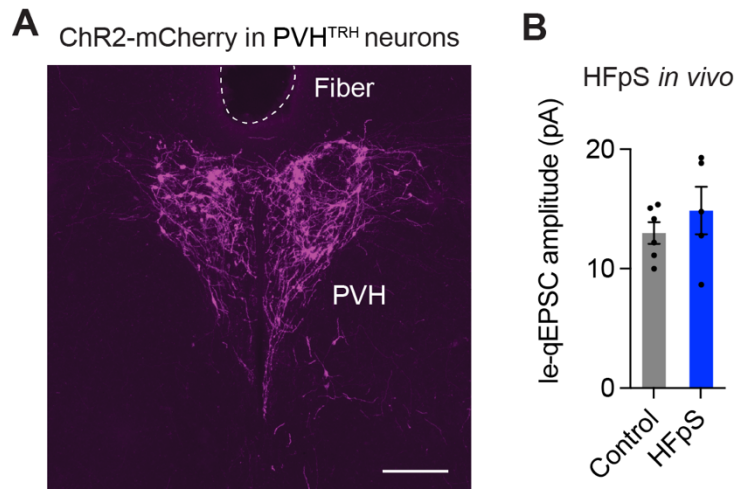
**A)** Representative histological images showing *Fos* expression in AgRP neurons in fed mice, in mice that were kept on the caloric maintenance paradigm (CMP) after fasting, or in mice that were subjected to the caloric restriction paradigm (CR). Scale bars represent 50  $\mu$ m.

**B)** Quantification of sEPSCs recorded from AgRP neurons in fed mice, from mice kept on the CMP, or from mice that were subjected to the CR. ( $N = 2/2/2$  mice)

**C)** Timeline showing the experimental scheme for the CR (left). Mice were provided 75% of the amount of food that they consumed on average in *ad libitum* control conditions for 7 days. Body weight was decreased at day 7 when mice were kept on the CR (right).

Data are presented as boxplots with median and min/max-whiskers (**B**) and mean  $\pm$  s.e.m. (**C**); \*  $p < 0.05$ , \*\*  $p < 0.01$ ; Kruskal-Wallis test (**B**) and two-tailed paired Student's *t*-test (**C**).

## Figure S5



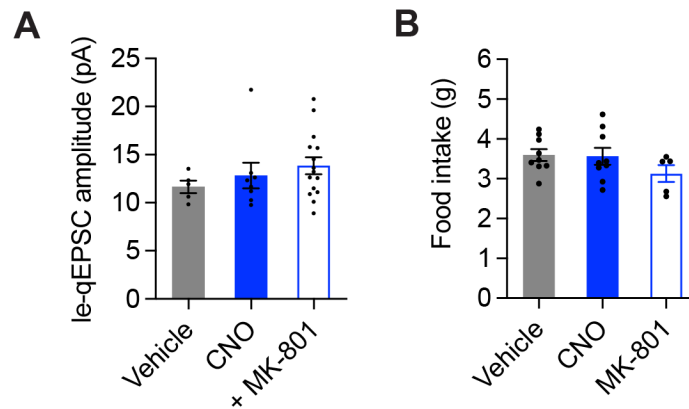
**Figure S5 (refers to Figure 5): High-frequency optogenetic stimulation of PVH<sup>TRH</sup> neurons**

**A)** Representative image showing expression of ChR2-mCherry (magenta) in PVH<sup>TRH</sup> neurons and placement of optical fiber above the PVH. Scale bar, 100  $\mu$ m.

**B)** Brief, high-frequency photostimulation (HFpS; 50 Hz for 10 minutes) of PVH<sup>TRH</sup> neurons *in vivo* does not alter the amplitude of le-qEPSCs recorded from AgRP neurons in presence of strontium.

All data are presented as mean  $\pm$  s.e.m.; two-tailed unpaired Student's *t*-test.

**Figure S6**



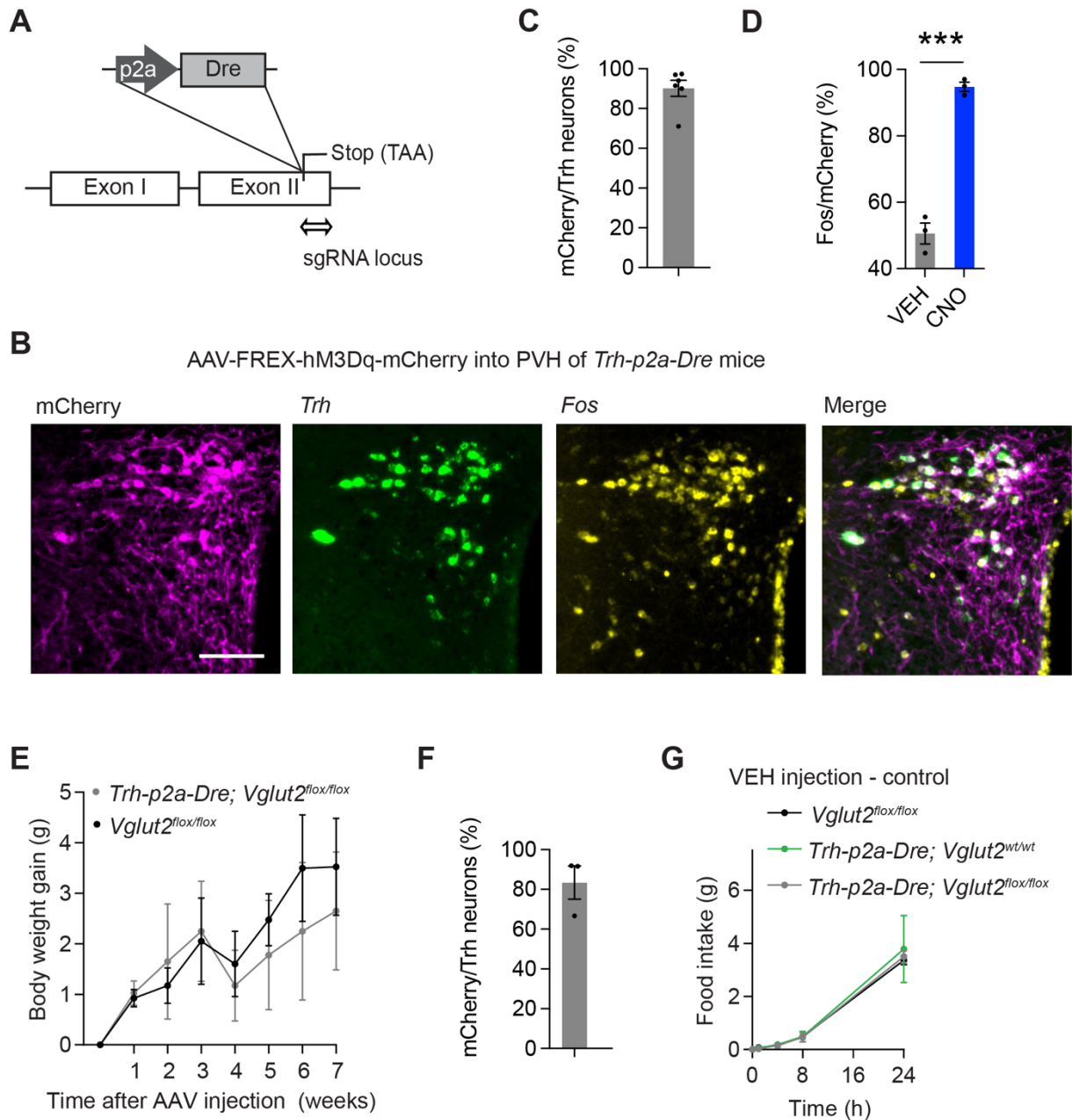
**Figure S6 (refers to Figure 6): Additional analysis for le-qEPSCs recordings after NMDAR blockade, and food intake in mCherry-expressing control mice.**

**A)** Summary of le-qEPSCs amplitude recorded from AgRP neurons in mice co-expressing hM3Dq and ChR2 in PVH<sup>TRH</sup> neurons following injection of CNO or CNO + MK-801 ( $N = 3/4$  mice).

**B)** Summary of 24-hour food intake in mice virally expressing mCherry in PVH<sup>TRH</sup> neurons show that injection of CNO or CNO + MK-801 does not significantly alter food intake, as compared to control conditions (vehicle injected).

All data are presented as mean  $\pm$  s.e.m.; ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

**Figure S7**



**Figure S7 (refers to Figure 6 and 7): Dre-recombinase-dependent viral vectors allow for specific targeting of PVH<sup>TRH</sup> neurons in *Trh-p2a-Dre* mice**

**A)** Schematic of the *Trh-p2a-Dre* knock-in allele. A *p2a-Dre-recombinase* cassette was inserted in front of the *Trh* stop codon in the 2nd exon.

**B)** Representative fluorescence images showing expression of *Trh* (green), mCherry (magenta), and *Fos* (yellow) in the PVH from *Trh-p2a-Dre* mice that were injected with an AAV for the Dre-dependent expression of hM3Dq (AAV-FREX-hM3Dq-mCherry). Assessment of *Trh* and *Fos* was performed by FISH, and mCherry expression was determined by immunohistochemistry. Scale bar represents 100 $\mu$ m and applies to all images.

**C)** Summary of *Trh* and mCherry expression in the PVH show that AAV-FREX-hM3Dq-mCherry is selectively expressed in PVH<sup>TRH</sup> neurons.

**D)** Summary of mCherry and *Fos* show that CNO administration effectively activates PVH<sup>TRH</sup> neurons in *Trh-p2a-Dre* mice expressing AAV-FREX-hM3Dq in the PVH (\*\*\*p < 0.001; two-tailed unpaired Student's *t*-test).

**E)** Body weight development of *Slc17a6*<sup>flox/flox</sup> mice and *Trh-p2a-Dre; Slc17a6*<sup>flox/flox</sup> mice (*N* = 4/4 mice) mice following co-injection of AAV-FREX-Cre (Dre-dependent) and AAV-FLEX-hM3Dq-mCherry (Cre-dependent) into the PVH.

**F)** Summary of *Trh* and mCherry expression in the PVH of *Trh-p2a-Dre; Slc17a6*<sup>flox/flox</sup> mice demonstrate selective targeting of PVH<sup>TRH</sup> neurons.

**G)** Cumulative food intake following injection of VEH in *Slc17a6*<sup>flox/flox</sup>, *Trh-p2a-Dre; Slc17a6*<sup>wt/wt</sup>, and *Trh-p2a-Dre; Slc17a6*<sup>flox/flox</sup> (*N* = 5/3/4 mice) mice. All mice were injected with an AAV expressing Dre-dependent Cre (AAV-FREX-Cre) together with an AAV expressing Cre-dependent hM3Dq (AAV-FLEX-hM3Dq-mCherry) into the PVH.

All data are presented as mean ± s.e.m.