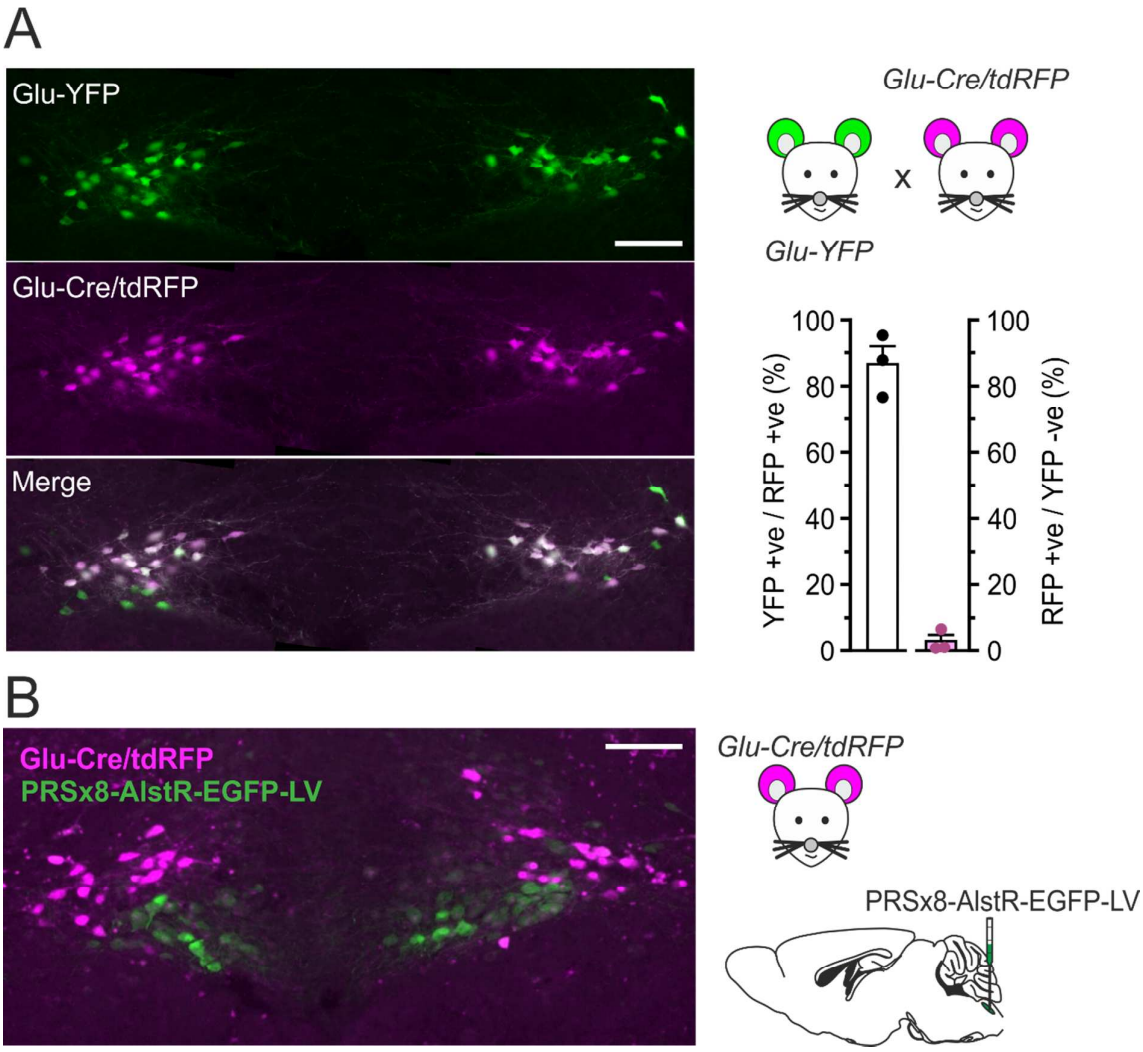
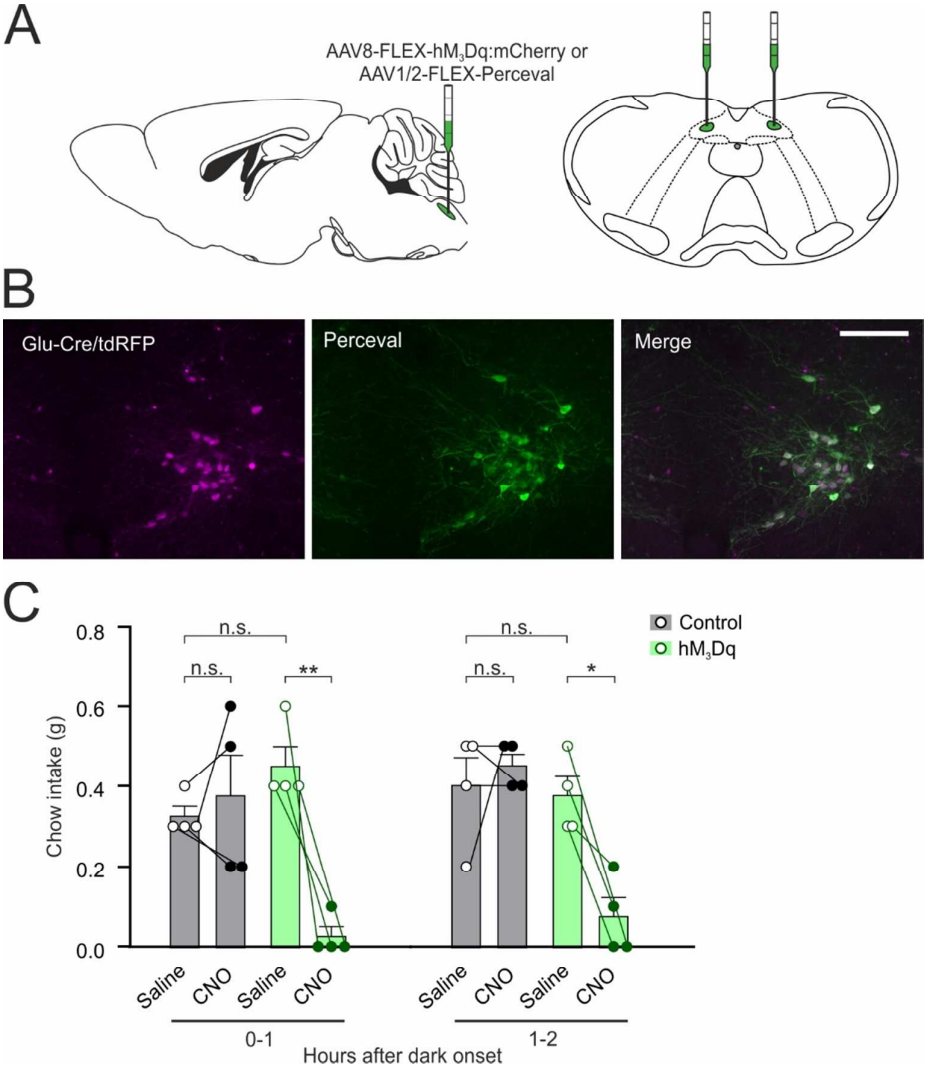
Supplemental Material

Supplemental figures



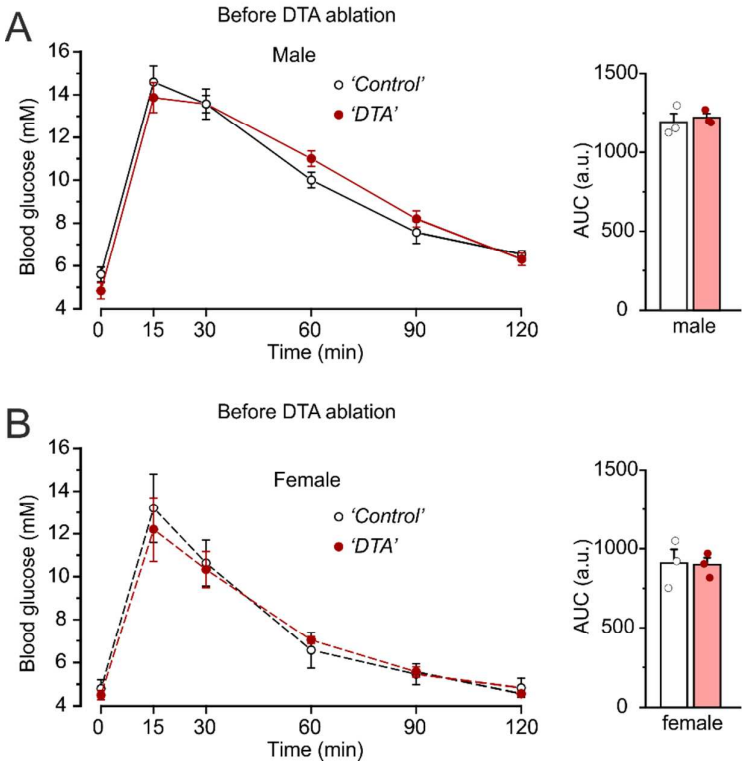
Supplemental figure 1 – related to Fig 1. RFP-positive Glu-Cre cells overlap with Glu-YFP cells, but not Phox2b-expressing cells.

(A) GFP- and RFP-immunoreactivity in the NTS of the result of crossbreeding Glu-YFP and Glu-Cre/tdRFP mice, demonstrating clear overlap between these neuronal populations. Scale: 100 µm. Right panel: Percentage of RFP-positive cells also expressing YFP (left) as well as RFP-positive cells *not* expressing YFP (right) throughout the NTS. Data given as mean±SEM, n=3 mice. (B) RFP-immunoreactivity and Phox2b-dependent expression of EGFP in the NTS of Glu-Cre/tdRFP mice injected with PRSx8-AlstR-EGFP-LV. Scale: 100 µm. N=3 mice.

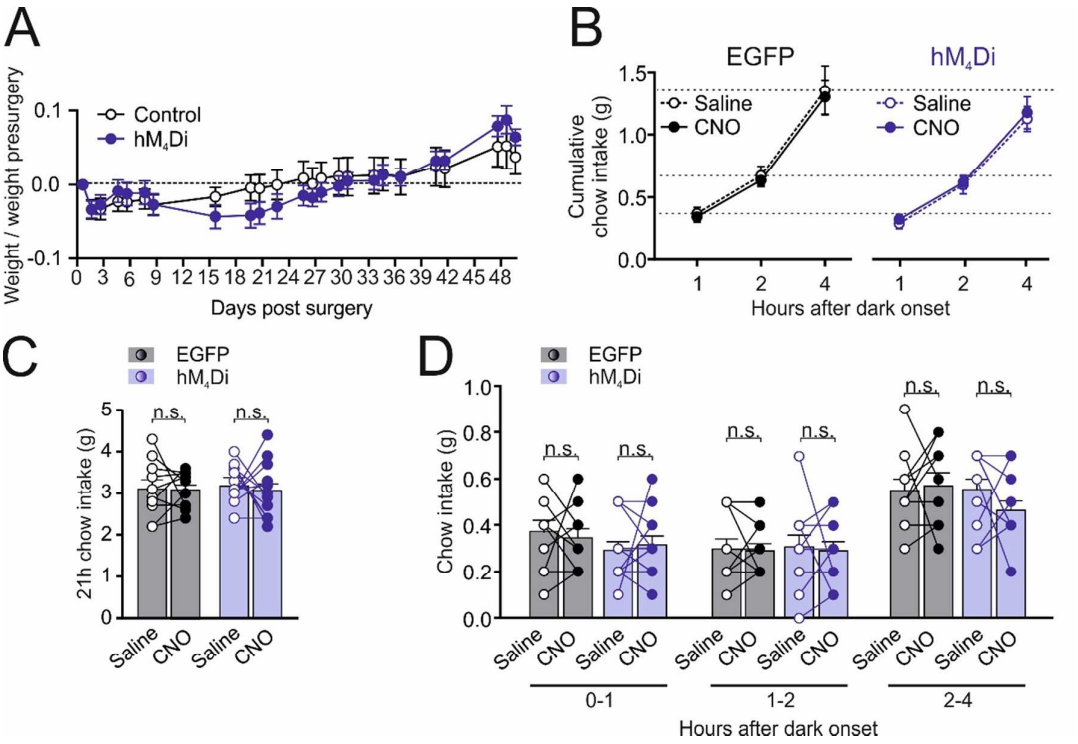


Supplemental figure 2 – related to Fig 1. hM$_3$Dq lacks intrinsic activity on food intake.

(A) Schematic showing injection of AAV2-FLEX-hM$_3$Dq:mCherry or AAV1/2-FLEX-Perceval into the NTS of Glu-Cre mice. (B) Expression of tdRFP and Perceval (detected with an anti-GFP antibody). Scale: 100 µm. (C) Non-cumulative chow intake of Glu-Cre mice expressing Perceval or hM$_3$Dq in the first two hours after dark onset following injection of 2 mg/kg CNO i.p. 30 mins prior to dark onset. Data given as mean±SEM, n=4 (Control), n=4 (hM$_3$Dq). There was a significant virus × drug interaction at both hour 0-1 ($F(1, 6)=17.19$, $p=0.006$) and hour 1-2 ($F(1, 6)=8.65$, $p=0.026$) and a significant effect of CNO in the hM$_3$Dq group in hour 0-1 ($p=0.0038$) and in hour 1-2 ($p=0.024$), but no effect of CNO in the control group in hour 0-1 ($p=0.81$) or hour 1-2 ($p=0.82$) (Sidak's multiple comparisons test). *: $p<0.05$; **: $p<0.01$; n.s.: not significant.



Supplemental figure 3 – related to Fig 3. Glucose tolerance before PPG ablation.
(A-B) Blood glucose in response to an i.p. injection of glucose (1 g/kg) at t=0 prior to stereotaxic injection of -DTA or control virus in six male (A) and six female (B) Glu-Cre mice. Area under the curve (AUC) of the i.p. glucose tolerance test for each group is given on the right of each graph. Data given as mean±SEM.



Supplemental figure 4 – related to Fig 4. hM$_4$Di lacks intrinsic activity on body weight and food intake.

(A) Change in bodyweight following stereotaxic injection of AAV2-FLEX-hM$_4$Di (n=13) or AAV2-FLEX-EGFP (n=11, control). There was a significant virus × time interaction ($F(23, 506)=2.93$, $p=0.0001$), but no significant difference between groups at any timepoint (Sidak's multiple comparisons test). (B-D) Cumulative (B-C) and non-cumulative (D) chow intake in the first four hours (B,D) and 21 hours (C) after dark onset. Mice were expressing either hM$_4$Di or EGFP in NTS PPG neurons and were injected with 2 mg/kg CNO i.p. 30 mins prior to dark onset. Data given as mean±SEM, n=11 (control), n=12 (hM$_4$Di). B: There was no significant drug × time interaction for either the control ($F(2, 20)=0.086$, $p=0.92$) or hM$_4$Di group ($F(2, 22)=0.036$, $p=0.96$), and there was no significant main effect of CNO in the control ($F(1, 10)=0.22$, $p=0.65$) or in the hM$_4$Di group ($F(1, 11)=0.11$, $p=0.74$). C: There was no significant drug × virus interaction ($F(1, 20)=0.22$, $p=0.65$) and no main effect of CNO ($F(1, 20)=0.47$, $p=0.5$) or virus ($F(1, 20)=0.11$, $p=0.75$). D: No significant drug × virus interactions (Hour 0-1: ($F(1, 21)=0.18$, $p=0.67$), hour 1-2: ($F(1, 21)=0.011$, $p=0.92$), and hours 2-4 ($F(1, 19)=1.30$, $p=0.27$)). n.s.: not significant.