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Supplemental information

$G\alpha_{i/o}$-coupled *Htr2c* in the paraventricular nucleus of the hypothalamus antagonizes the anorectic effect of serotonin agents

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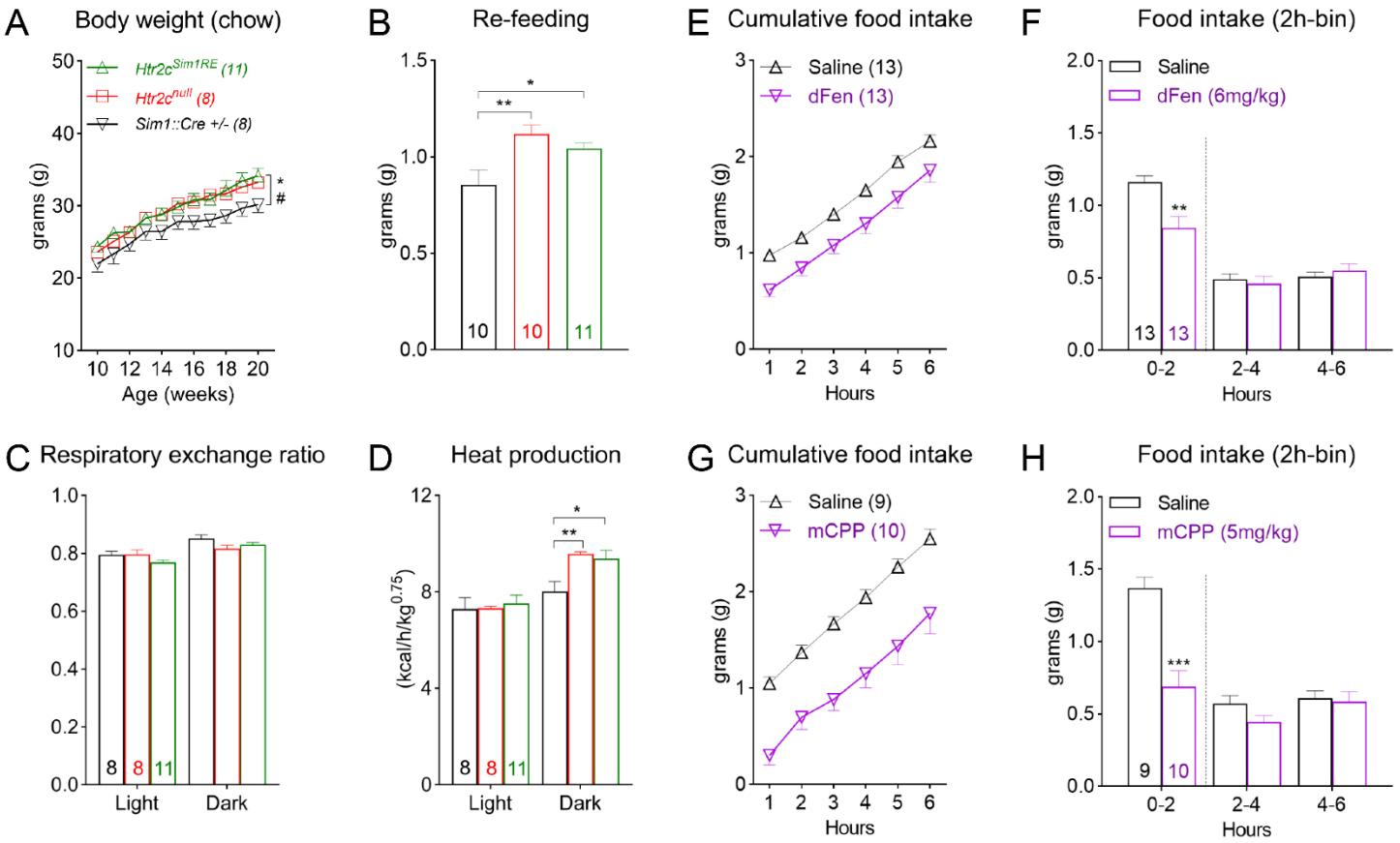


Figure S1. Selective reactivation of *Htr2c* in *Sim1* neurons does not normalize defects in refeeding or energy expenditure of chow-fed *Htr2c* null mice. Related to Figure 1**.** (A) Body weight curves in chow-fed male mice. * p<0.05 between *Htr2c*^{Sim1RE} and *Sim1::Cre* +/- mice; # p<0.05 between *Htr2c*^{null} and *Sim1::Cre* +/- mice. (B) Food intake during the first two hours of re-feeding after an overnight fast. (C) Respiratory exchange ratio during light (6:00-18:00) and dark (18:00-6:00) phases of a day. (D) Heat production. (E) Cumulative food intake (six hours) after an i.p. dose of saline or dFen (6 mg/kg) in wild-type male mice. (F). Food intake in (E) is binned into two-hour time frames for the six hours. (G) Cumulative food intake (six hours) after an i.p. dose of saline or mCPP (5 mg/kg) in wild-type male mice. (H) Food intake in (G) is binned into two-hour time frames for the six hours. Experiments in E-F were conducted in chow-fed mice. Values represent mean ± SEM; n=8-13, Two-way ANOVA with Tukey's *post hoc* tests in A, C, D, E-H. One-way ANOVA with Tukey's *post hoc* test in B. *p<0.05, **p<0.01, and ***p<0.001.

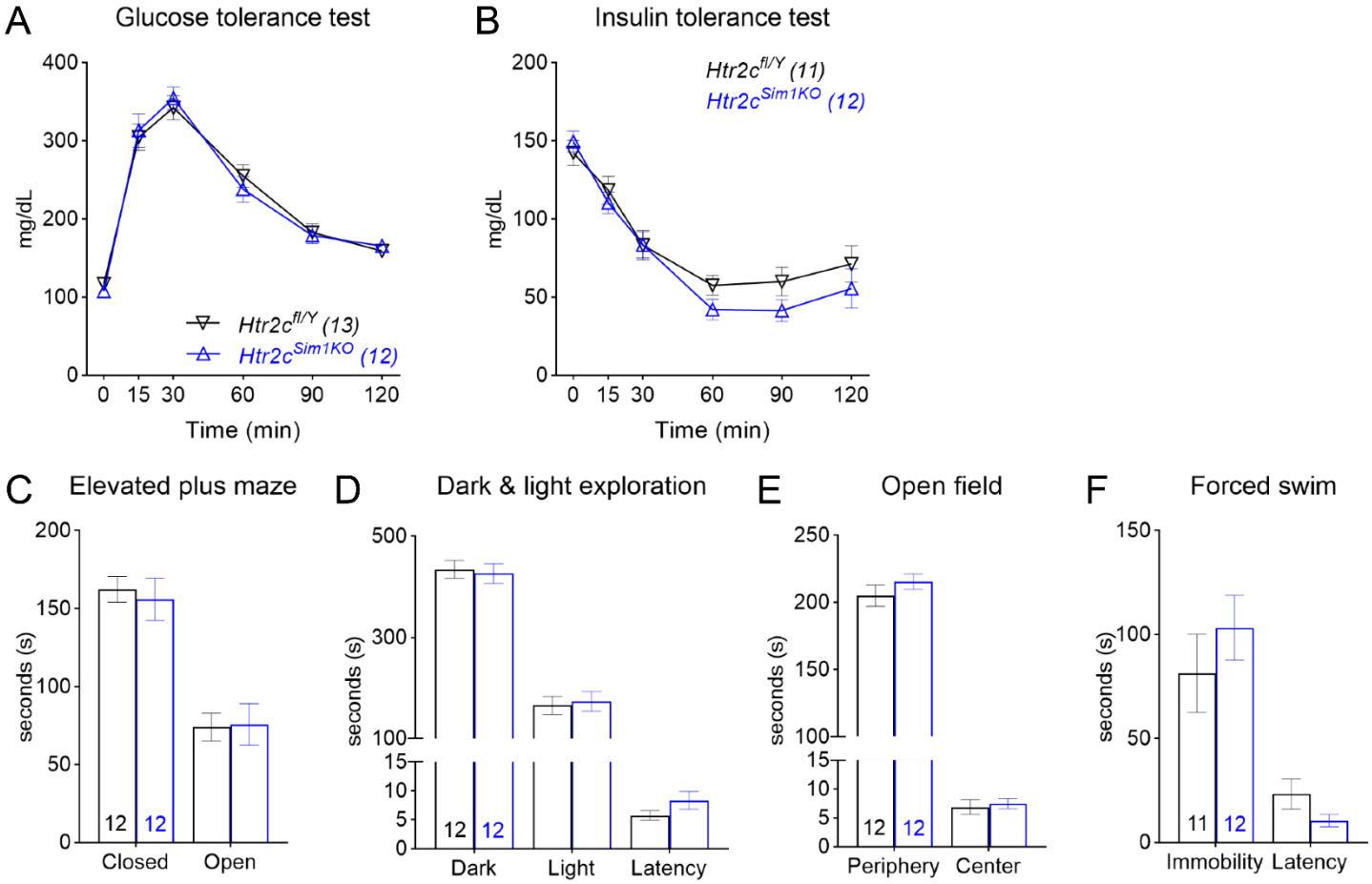


Figure S2. Deletion of *Htr2c* in *Sim1* neurons does not affect glucose homeostasis, anxiety, or depressive-like behaviors in male mice in male mice. Related to Figure 2. (A) Glucose tolerance test. (B). Insulin tolerance test. (C) Elevated plus-maze, F(1, 22)=0.07, p=0.79. (D) Dark light exploration, F(1, 22)=2.21, p=0.15. (E) Open field, F(1, 22)=0.81, p=0.38. (F) Forced swim test, F(1, 21)=1.31, p=0.26. Values represent mean ± SEM; n=11-13, two-way ANOVA with Tukey's *post hoc* tests.

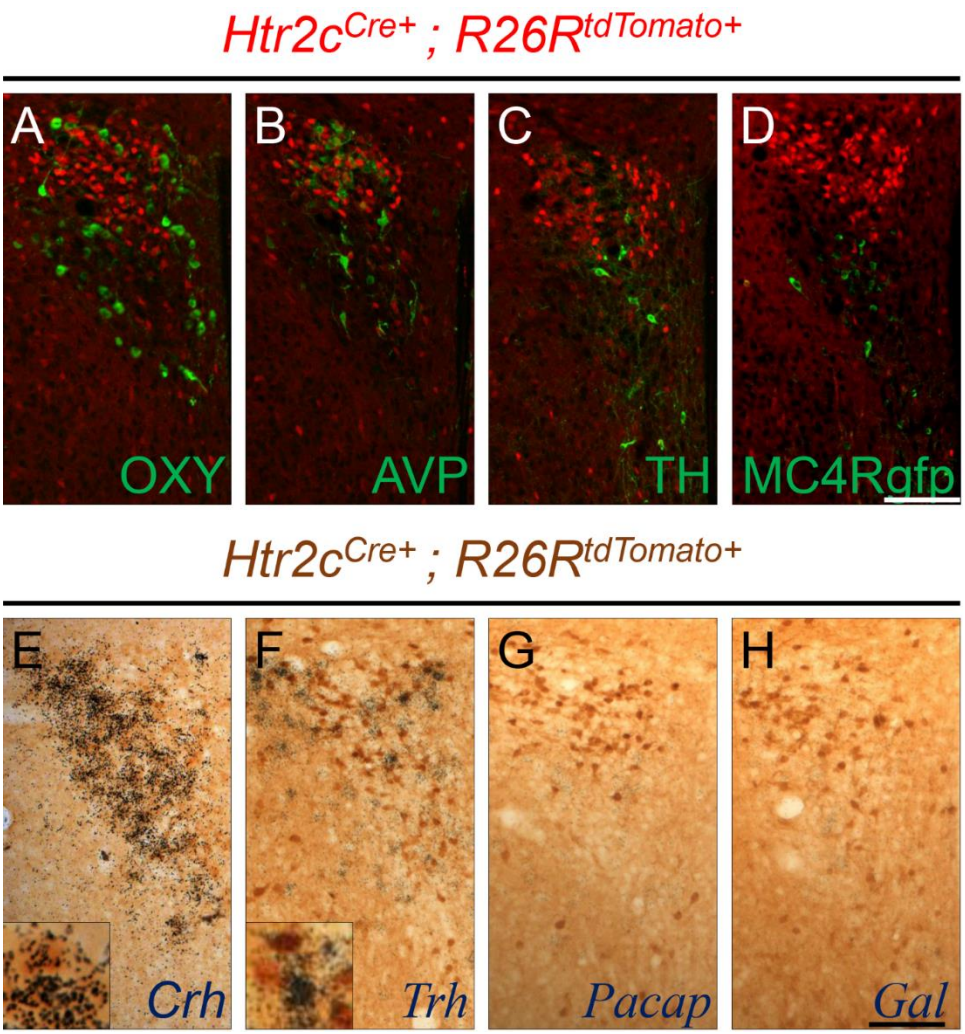


Figure S3. Fate mapping of *Htr2c* expressing neurons in the PVH. Related to Figure 3 (A-D) Double immunofluorescence between tdTomato reporter (red) and proteins (green) for OXY, AVP, TH, and MC4R-GFP in *Htr2c*$^{Cre+;\ tdTomato+}$ mice. (E-H) Dual-label of S^{35}-labelled RNA *in situ* hybridization and immunohistochemistry between tdTomato protein (brown) and mRNAs (blue) for *Crh, Trh, Pacap*, and *Gal* in *Htr2c*$^{Cre+;\ tdTomato+}$ mice. Scale bars are 50 µm.

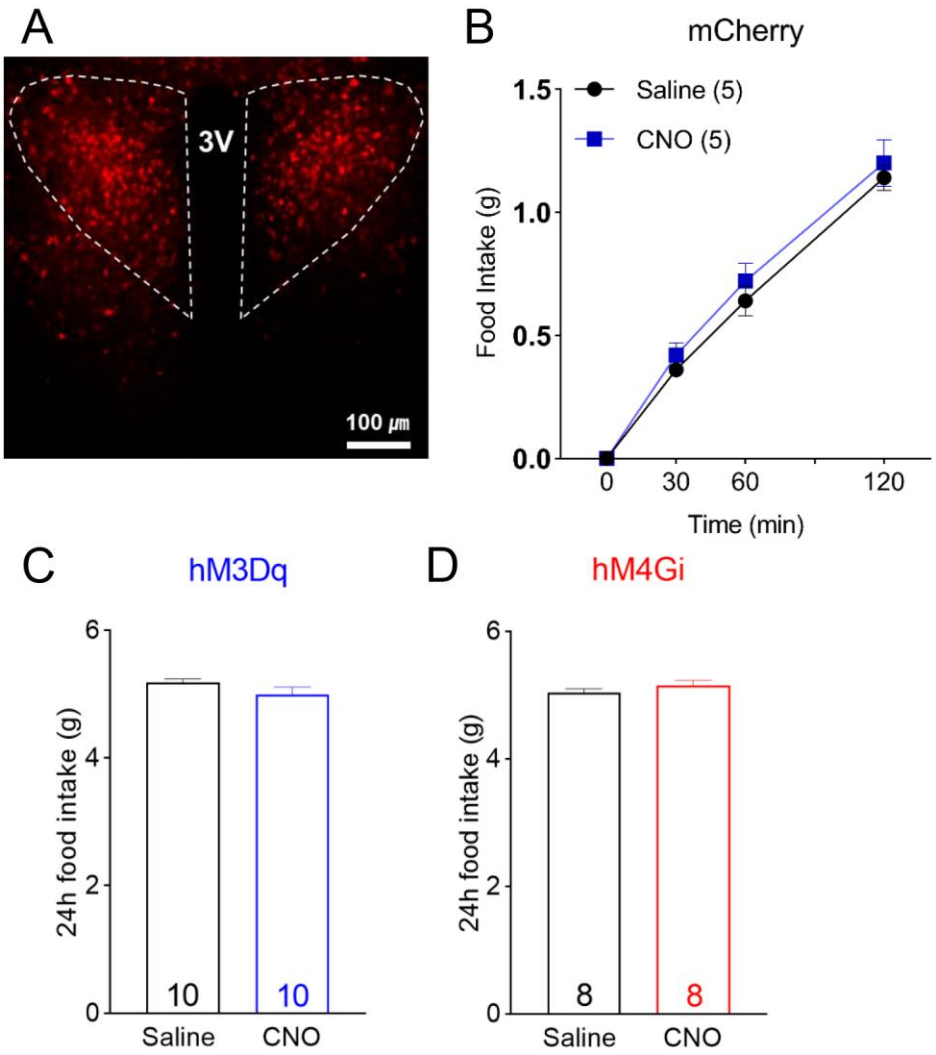
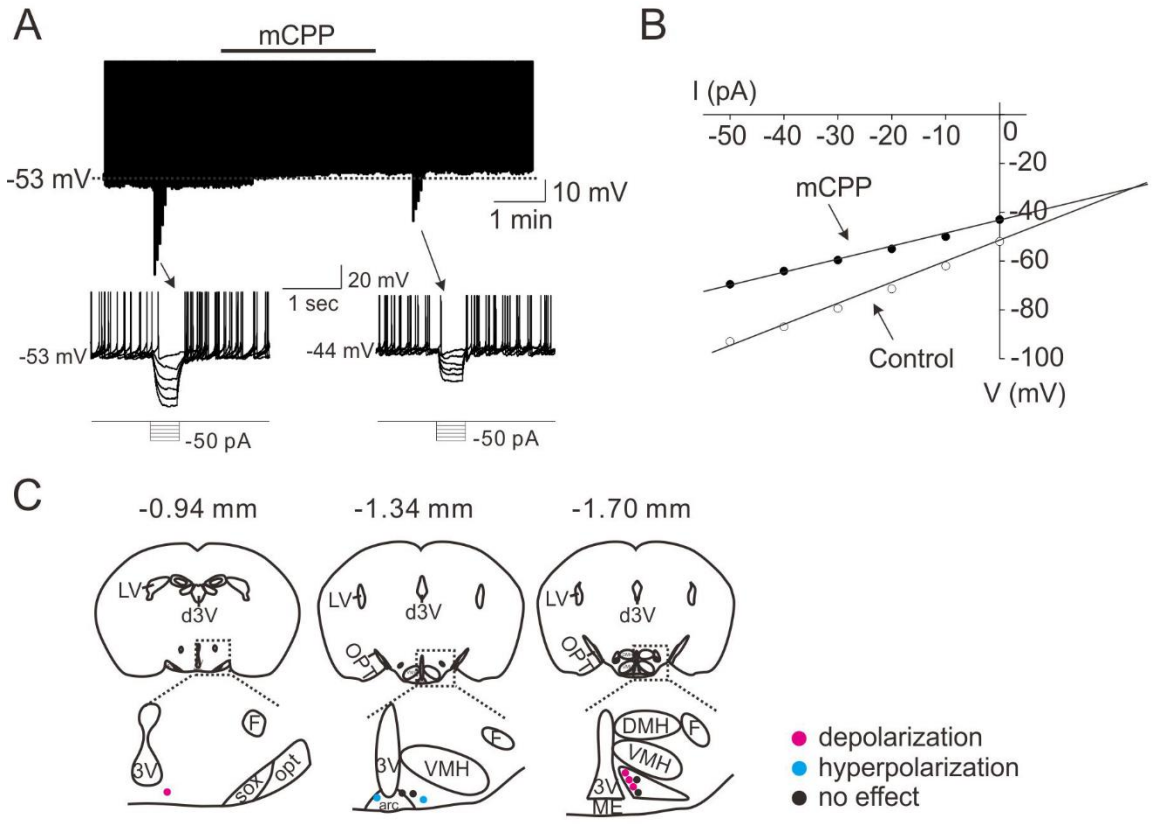


Figure S4. CNO alone did not alter food intake in *Htr2c*$^{Cre+}$ male mice. Related to Figure 3 (A) Immunofluorescence for mCherry. (B) Refeeding in *Htr2c*$^{Cre+}$ mice that received the control virus (AAV-DIO-mCherry) injections in the PVH. CNO treatment (1 mg/kg) did not change food intake in these mice. (C and D). Refeeding during twenty-four hours after saline or CNO treatment in *Htr2c*$^{Cre+}$ mice that received either hM3Dq (C) or hM4Gi DREADD constructs. Two-way ANOVA with Sidak's *post hoc* tests are in B, paired t-test in C and D. Scale bar in A is 100 μm.



D

Acute effects of mCPP on ARH *Htr2c* neurons (n=11)	Membrane Potential	Decrease in Input Resistance	Reversal Potential
Depolarized (n=4, 36.4 %)	6.3 ± 1.1 mV	-27.3 ± 5.6 %	-28.1 ± 4.7 mV
Hyperpolarized (n=2, 18.2 %)	-8 & -7 mV	-25.1 & -27.6 %	-79.5 & -85.7 mV
No response (n=5, 45.4 %)	0.2 ± 0.4 mV	N.A.	N.A.

Figure S5. mCPP activates a subpopulation of ARH *Htr2c* neurons. Related to Figure 4. (A) Bath application of mCPP caused a prompt depolarization of membrane potential. Downward deflections shown in the trace represent voltage responses to current steps, which are shown as insets below. (B) Current-voltage (IV) relationship before (control) and after (mCPP) application. (C) A schematic that summarizes the location of the recorded *Htr2c* neurons within the ARH. (D) A table that summarizes the number, percentage, and electrophysiological characteristics, of responses to mCPP treatment.

Oligonucleotides		
Htr2c probe primer set, F: GAATTAACCCTCACTAAAGGGTGAAACAATACTGTTAACTTCCCAAT, R: GTAATACGACTCACTATAGGGCATAAAGAATTGCAAGCAGAGACAG;	Integrated DNA Technologies	N/A
Galanin probe primer set, F: GAATTAACCCTCACTAAAGGGACCGAGAGAGCCTTGATCCT, R: GTAATACGACTCACTATAGGGCGCTTGAGGAGTTGGCAGAAG;	Integrated DNA Technologies	N/A
Trh probe primer set, F: GAATTAACCCTCACTAAAGGGGAGGGGAGATTTGGGAGAAG, R: GTAATACGACTCACTATAGGGCCCAGTGAAGGGACTGGGATA;	Integrated DNA Technologies	N/A
Crh probe primer set, F: GAATTAACCCTCACTAAAGGGCCAAGGGAGGAGAAGAGAGC, R: GTAATACGACTCACTATAGGGCGGTGGAAGGTGAGATCCAGA;	Integrated DNA Technologies	N/A
Pacap probe primer set, F: GAATTAACCCTCACTAAAGGGAATGACTTGGGGAATTGCTG, R: GTAATACGACTCACTATAGGGCGCATGAACAGCACTGGAGAA	Integrated DNA Technologies	N/A
Htr2c::Cre genotyping primer set, CL-9631: TTTGTGGGAAGGCCTGTAAC; CL-10057: GGAGTGGGGGACTTTCCTAC; CL-R309: TCCCTCACATCCTCAGGTTC	Integrated DNA Technologies	N/A
Htr2c flox genotyping primer set, Htr2c-flox1: TGTCATCTCTCAATGCACAAAA; Htr2c-flox2: GCCACTAGAGGGCAACAATAA; Htr2c-flox3: GCATCAGATCTCCTGGGACT;	Integrated DNA Technologies	N/A
Htr2c RN genotyping primer set, Htr2c-RN 1: AAGAGCTACAGGAAGGCAGGTCA; Htr2c-RN 2: AGGCTTTAGTGACCTCCCATGA; Htr2c-RN 3: CGGAAGAGAAGGACCTGGATAG;	Integrated DNA Technologies	N/A

Table S1. Oligonucleotide information, related to STAR Methods.