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Supplemental Information

Blunted Vagal Cocaine- and Amphetamine-Regulated Transcript Promotes Hyperphagia and Weight Gain

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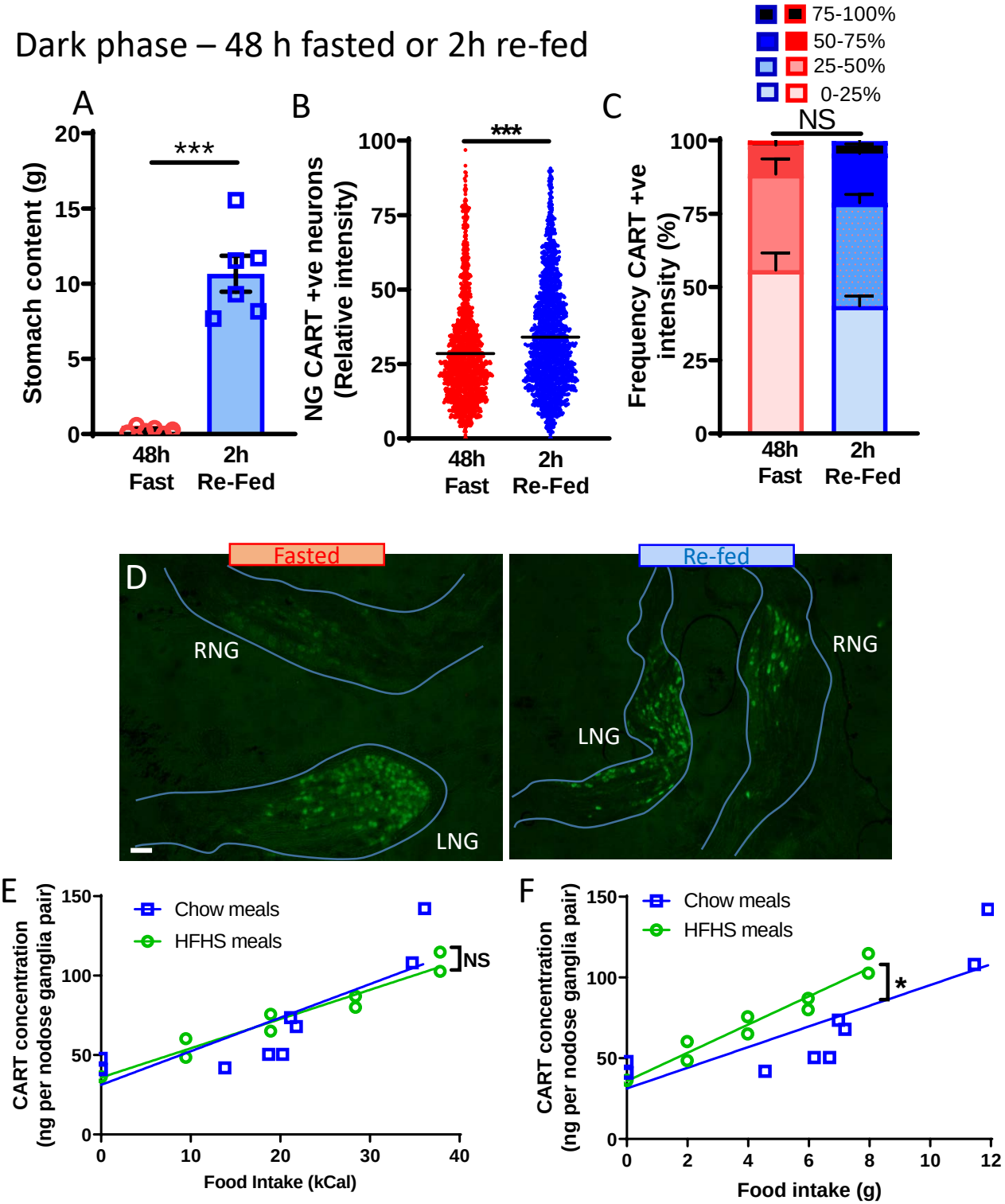


Fig S1. Vagal afferent neuron CART expression increases proportionally to caloric intake in the dark phase. Related to figure 1. A-D) Rats fasted 48h or refed for 2 h after 46 h fasting (n=6). A) Stomach content at termination 2.5 h into the dark phase (unpaired two-tailed t-test, p<0.0001). B) The relative intensity of CART labeling in combined left and right NG neuron increases with re-feeding compared with fasting (unpaired two-tailed Mann Whitney test p<0.0001). C) The frequency of low or high CART antibody expression in neurons was not different between groups (two-way ANOVA, F(3,32)=2.7, p=0.06). D) Images of left and right NG from lean rats 48 h fasted or 2 h re-fed, sectioned and stained on the same slide and imaged at x4 magnification. Scale bar 300µm. E-F) EIA quantification of CART in NG in lean animals fed either chow or HFHS diet. E) ANCOVA indicates a similar association between CART concentration and the food consumed irrespective of meal type (Chow or HFHS) when energy intake is used as covariate (no difference in slopes p=0.59 or intercepts p=0.97). F) This association is significantly different between meal types when food intake is expressed as grams (no difference in slopes p=0.25 but significantly different intercepts p=0.039).

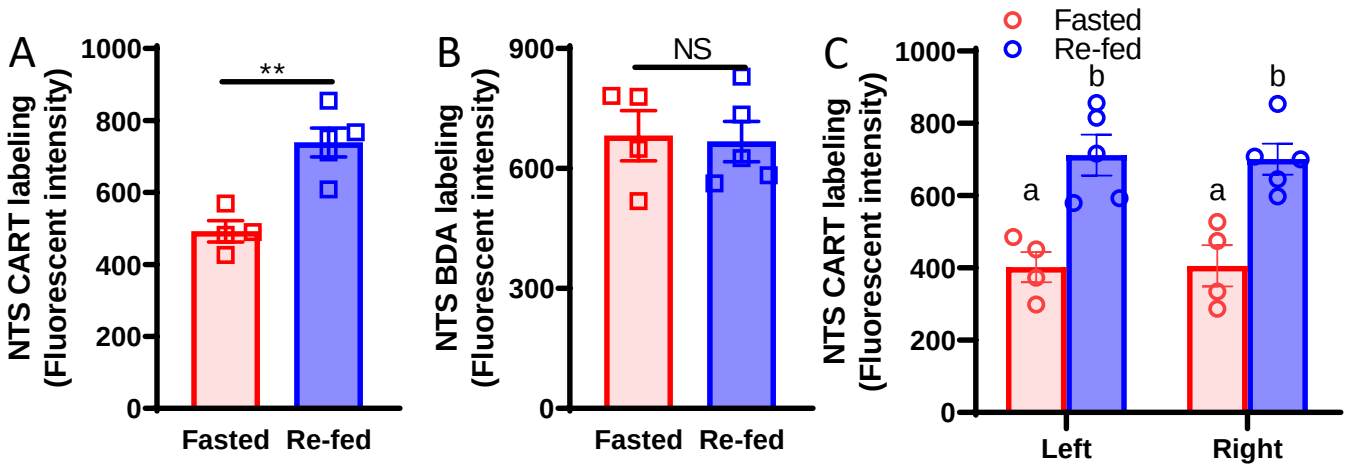
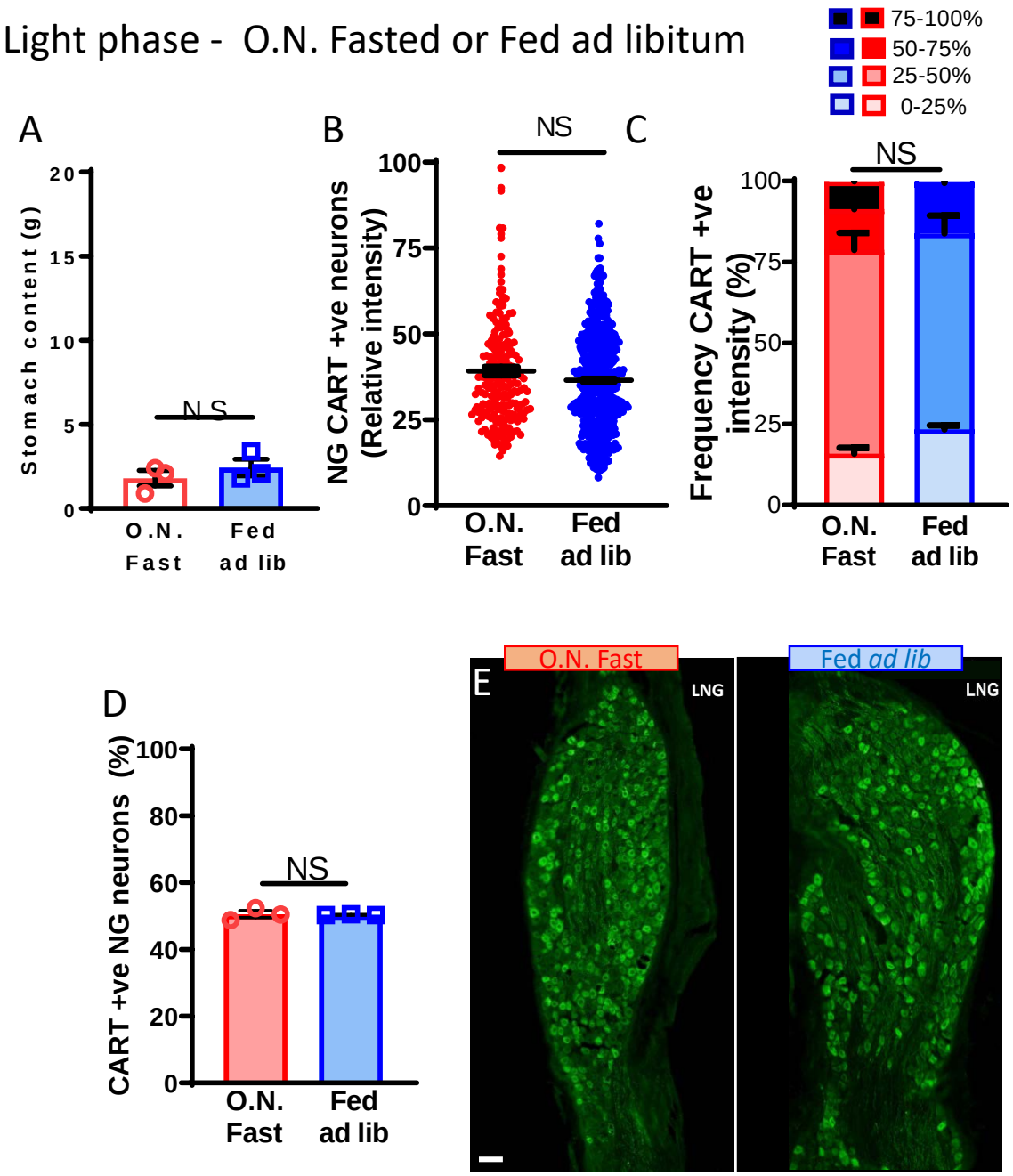
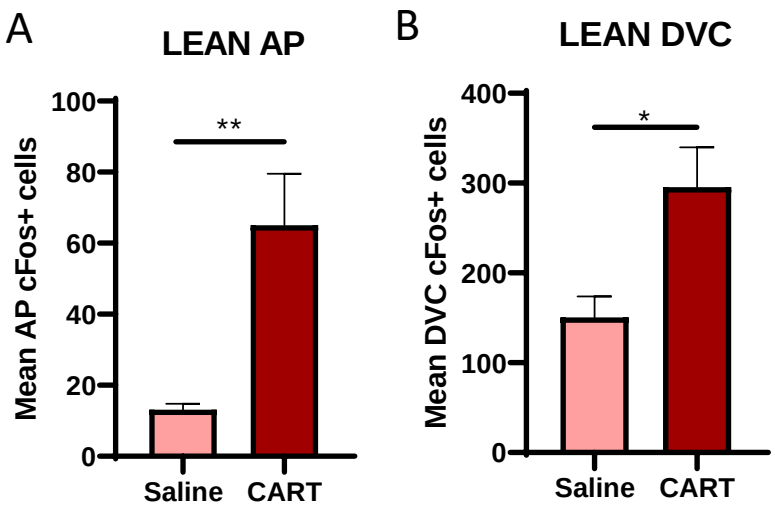


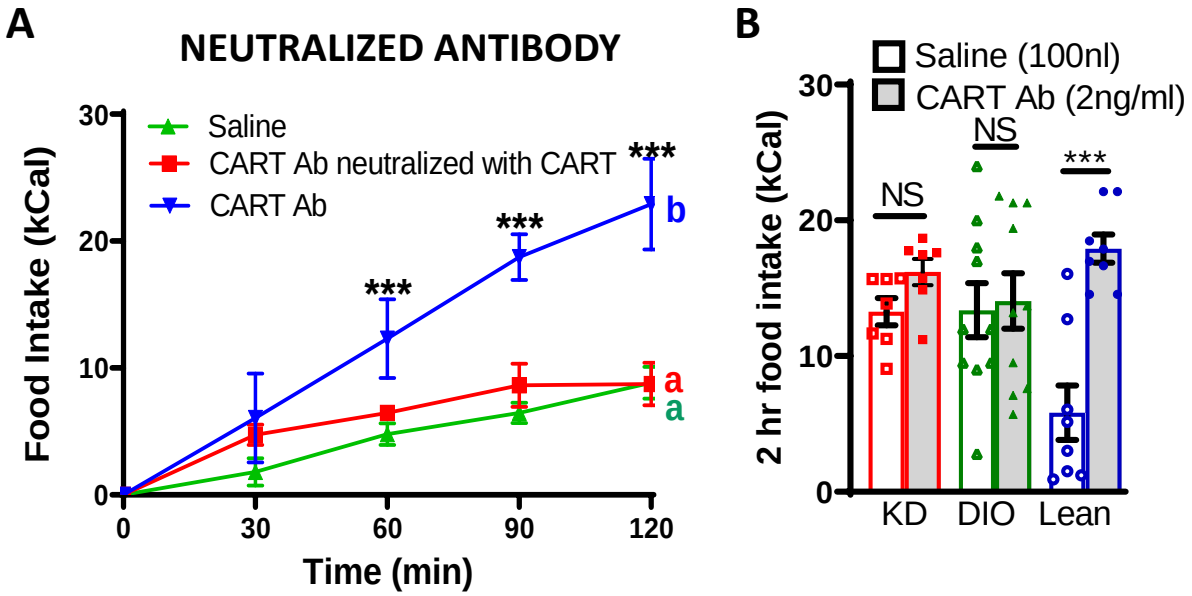
Fig S2. Feeding increases CART expression in the NTS. Related to figure 1. A-C) Quantification of images in Fig 1D. A) NTS CART increases in refed compared to fasted conditions (n=4-5. unpaired two-tailed t-test, $p<0.0022$). B) Vagal fibers labeled with BDA are unchanged between refed and fasted conditions (n=4-5, unpaired two-tailed t-test, $p<0.86$). C) CART labeling in both the left and right NTS are identical in fasted and refed conditions (n=4-5; Two way ANOVA, interaction $F(1,7)=0.135$, $p=0.72$; Sidak's multiple comparisons **< $p<0.003$).



Sup Fig3. Vagal afferent neuron CART does not differ between fasted and re-fed rats in the light phase. Related to figure 1. A-E) Rats were either fasted overnight (O.N.) or fed ad libitum (n=6). A) Stomach content at termination 4 h into light phase (unpaired two-tailed t-test, p=0.399). B) No difference in relative intensity of CART labeling in the left NG neurons (unpaired two-tailed Mann Whitney test; p=0.284). C) No change in frequency of relative intensity (two-way ANOVA, $F(3,16)=1.197$, p=0.343). D) No difference in the percent of CART+ neurons (unpaired two-tailed t-test, p=0.842). E) Stitched image of CART expression in NG fed or O.N. fasted in the light phase. Scale bar represents 100µm.



Sup Fig4. CART increases activity in cells of the dorsal vagal complex. . Related to figure 2 .Fos expression was quantified in cells from Fig2E and F in the (A) area postrema and (B) whole dorsal vagal complex in fasted rats following bilateral injection of saline (100nL) or CART (200pM). n=6; t-test, * $p<0.05$, ** $p<0.01$.



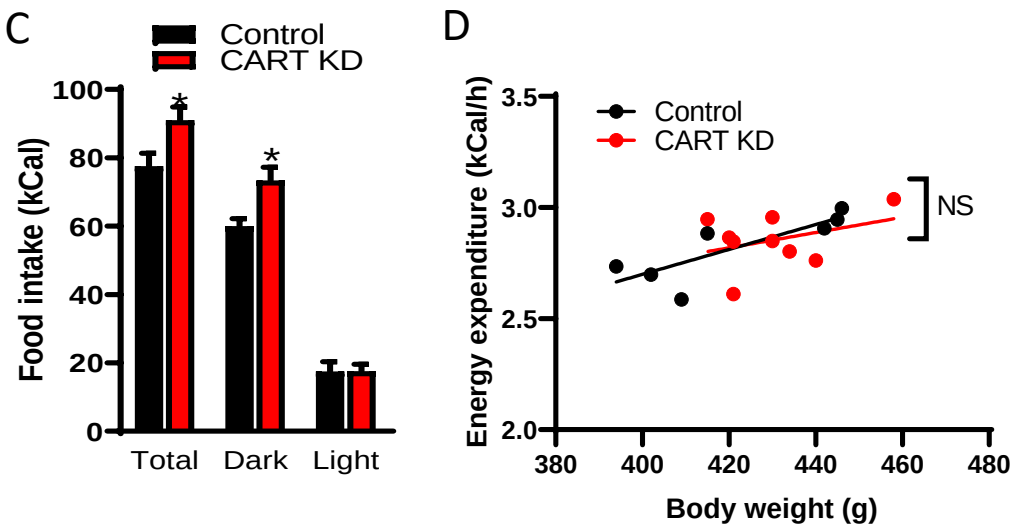
Sup Fig5. Validation of CART antibody injected into the NTS. Related to figure 3. In fed lean animals, intraNTS CART antibody injection (2ng/ml) increases food intake compared to saline treatment. Neutralized CART antibody does not cause hyperphagia. (n=5/6/6; two-way ANOVA: interaction $F(8,32)=3.739$, $p=0.003$, Tukey multiple-comparisons). B) 2 h food intake in lean, KD and DIO rats following intraNTS CART antibody administration. CART antibody fails to increase food intake in rats with CART knockdown in NG, suggesting that in the NTS CART antibody blocks endogenous CART released from NG (n=7-10; one-way ANOVA, Tukey's multiple-comparison letters represent $p<0.05$).

A **Dark phase**

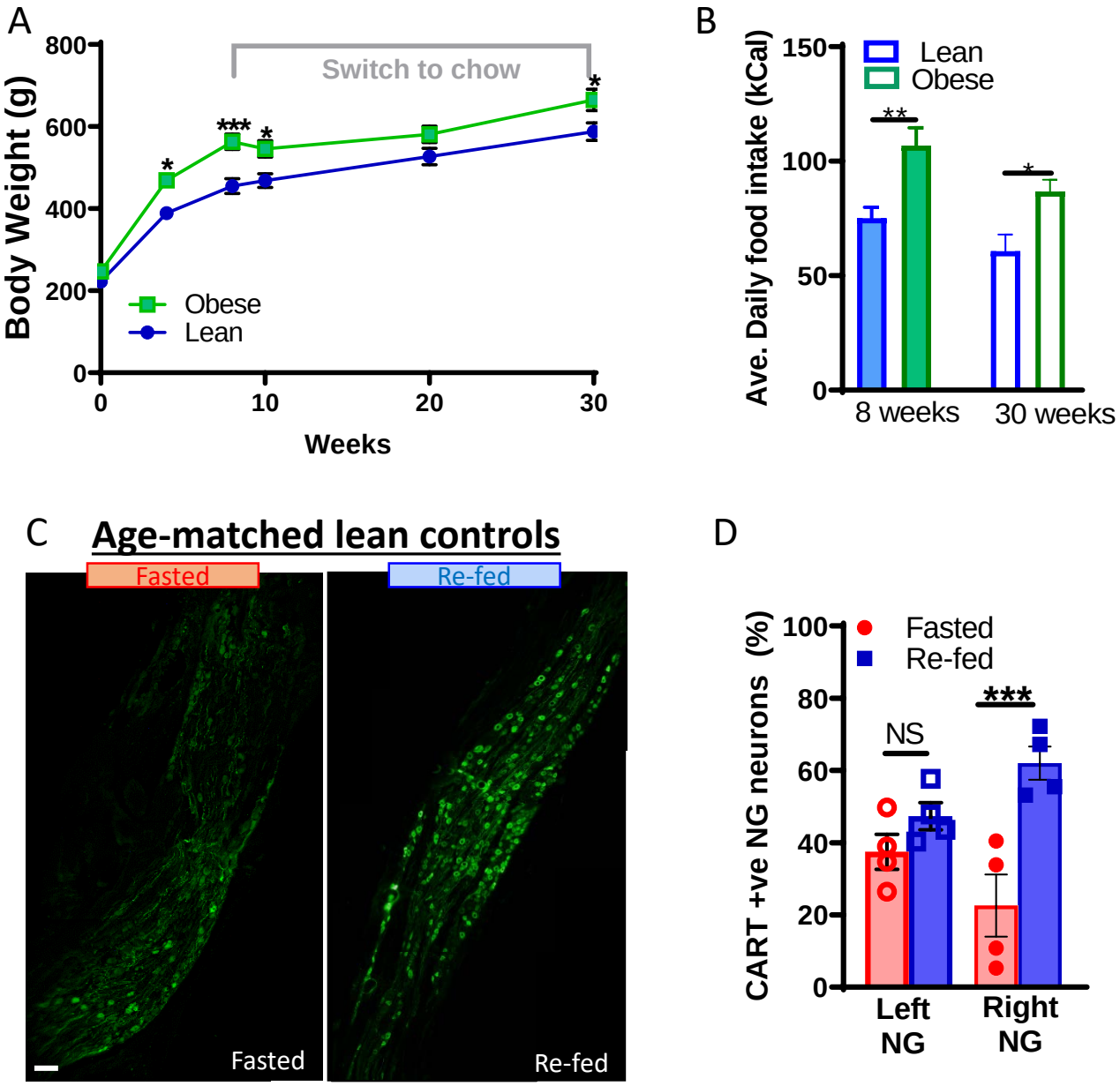
	Control (N=8)	CART KD (N=7)	p value
Meal Number	7.07±0.21	7.04±0.46	0.475
Meal Size (g)	2.84±0.13	3.20±0.13	**0.028**
Meal Duration (min)	14.93±2.24	9.29±0.58	0.071
Inter-Meal Interval (min)	89.10±3.34	100.070±5.69	**0.044**
Satiety ratio	45.81±2.51	46.88±2.14	0.369
Ingestion Rate (g/min)	0.35±0.02	0.45±0.02	**<0.001**

B **Light phase**

	Control (N=8)	CART KD (N=7)	p value
Meal Number	2.29±0.24	2.09±0.13	0.227
Meal Size (g)	2.79±0.31	3.42±0.29	0.073
Meal Duration (min)	11.84±2.13	9.85±1.27	0.245
Inter-Meal Interval (min)	257.95±43.4	335.41±27.11	0.071
Satiety ratio	85.48±5.22	81.30±5.60	0.284
Ingestion Rate (g/min)	0.32±0.02	0.44±0.02	**<0.001**



Sup Fig6. NG CART KD meal patterns are altered in dark but not light phase with no change on energy expenditure. Related to figure 4. A+B) Table comparing average meal patterns from control and CART KD of in the A) dark phase and B) light phase. C) Average daily energy intake spanning the full duration of the 5 week study broken down into 24 h, light or dark phase (n=8/7; Two way ANOVA: interaction F(2,28)=5,487, p=0.0098; Sidak's multiple comparisons * p<0,05). F) Energy expenditure over 24h in CART KD and control rats in function of their body weight (ANCOVA, no difference in slopes F(1,12)=0.3543, p=0.56 or intercepts F(1,13)=0.02202, p=0.8843).



Sup Fig7. Blunted vagal CART expression in DIO is not restored by a dietary intervention
Related to Fig 6. A) Body weight of rats (Fig 6F and GC fed a HFHS diet 8 weeks and switched back to chow compared to rats maintained on chow (n=7; two-way ANOVA interaction $F(5, 60)=3.927$, $p=0.003$; Sidak's multiple-comparison *$p<0.05$, ***$p=0.0002$). B) Daily food intake of DIO rats before and after dietary switch compared to lean rats fed chow diet (n=7; one-way ANOVA, Tukey's multiple-comparison letters represent $p<0.05$). C) Representative stitched image of age-matched control rats never fed HFHS diet retain postprandial CART up-regulation compared obese rats switched back to chow for 5 months (Fig6 F and G). Scale bar 100µm. D) Percent CART$^+$ neurons increase with refeeding in right, but not left, NG (n=4; two-way ANOVA, interaction $F(1,12)=6.597$, $p=0.025$, Sidak's multiple comparisons *** $p=0.0008$).