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Supplementary Materials for

Hedonic eating is controlled by dopamine neurons that oppose GLP-1R satiety

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The PDF file includes:

Figs. S1 to S20
Table S1

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist

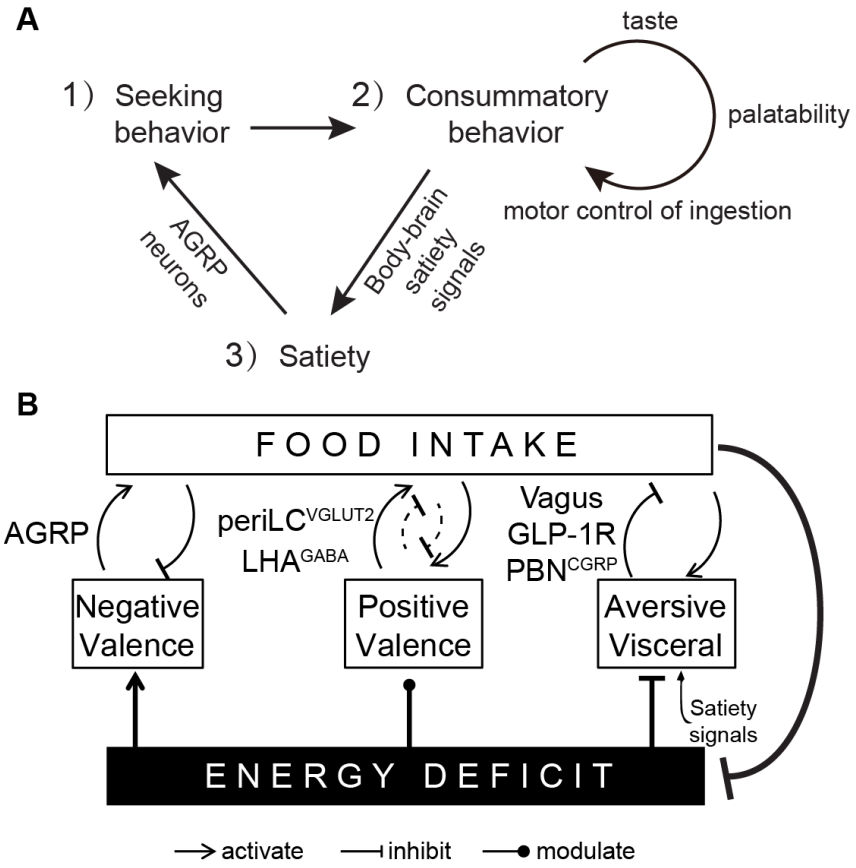

Fig. S1. Three phases of feeding behavior. (A) Feeding behavior starts with food-seeking. Next, food consumption is initiated and is sustained by feedback from palatable taste. Satiety pathways from the body respond to ingested nutrients, signal to the brain, and terminate consumption. (B) The phases of feeding behavior are mediated by different pathways with distinct neural dynamics and motivational characteristics. First, food-seeking systems have been identified that are activated by energy deficit and promote food-seeking but are inhibited by cues that predict food and have low activity during food ingestion, e.g. Agouti-related protein (AGRP) neurons. Second, neurons engaged during consummatory behavior can also promote consumption, have positive valence, and are modulated by energy deficit. However, energy deficit is not required for these consummatory pathways because they are also engaged by palatable food intake. Examples of consummatory control neurons include lateral hypothalamic area (LHA^{GABA}) neurons that show positive feedback between food ingestion and their activity or $periLC^{VGLUT2}$ neurons that show a double negative feedback relationship where food ingestion inhibits these neurons, which correspondingly promotes further food intake and is rewarding. Third, neurons responsive to bodily signals mediated by the vagus nerve, circulating hormones, GLP-1R agonists, and parabrachial nucleus Calcitonin gene-related peptide (PBN^{CGRP}) neurons induce satiety to terminate a meal. These pathways often show a progressive rise in activity during food intake, and some have negative valence when activated. Interactions between these systems exist but are not shown here.

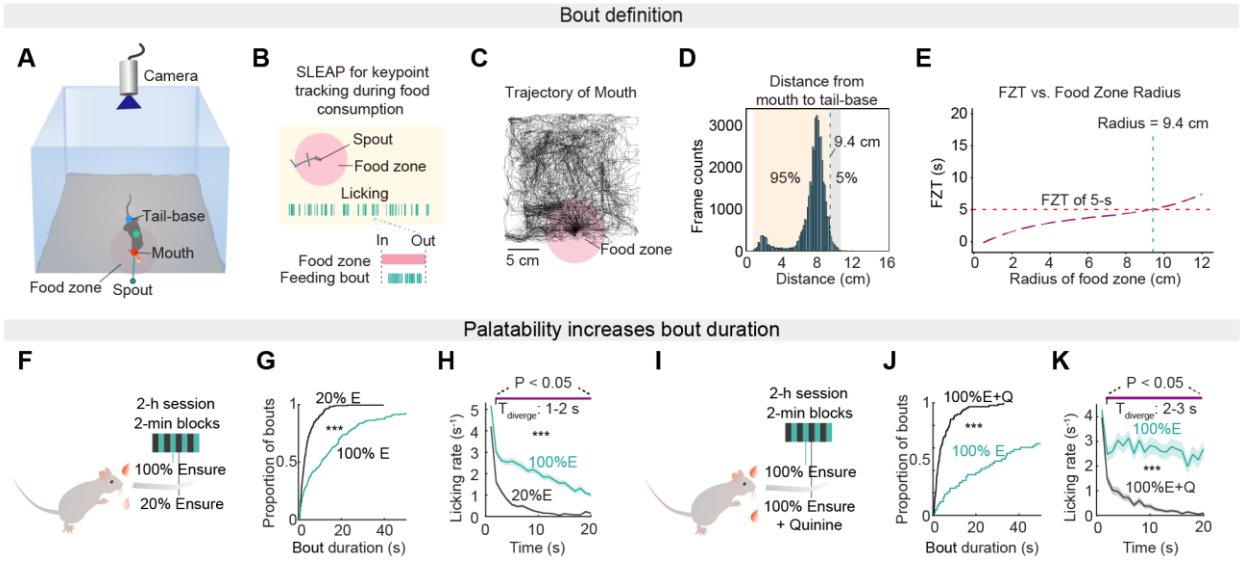

Fig. S2. Bout duration is based on sequential licking events in the food zone and is increased by higher palatability food. (A, B) A feeding bout is a sequence of licks that is terminated by an interlick interval (ILI) greater than a threshold value. We defined a bout ILI greater than the shortest 10% of times in which the mouse was in the food zone (FZ), which was based on the notion that a feeding bout ended when the mouse leaves the FZ. We defined the FZ as one body length (mouth to tail-base) from the lick spout. To empirically determine the FZ size and the shortest 10% of FZ time (FZT), we used an experimental setup with a camera capturing video for tracking key points, including the mouth, tail-base, and body position relative to the lick spout at the center of the FZ (A). (B) Raster of lick events when a mouse enters the FZ. Key points indicate mouse position and the pink area represents the FZ boundary. (C) Representative data showing the position of the mouth, which was tracked during one session of palatable food consumption. Scale bar: 5 cm. (D) The mean body length was calculated from video tracking as the 95% probability distance between the mouth and tail-base during a feeding session, which was 9.4 cm (n = 4 mice). (E) From the tracking data, we calculated the shortest 10% of FZT values based on different radii defining the FZ. For the FZ radius defined by body length (9.4 cm), the shortest 10% of FZT values was 5-s, which was used as the inter-lick interval to define the bout threshold. (F, I) Feeding bout analysis comparing the consumption of higher and lower palatability food (100% Ensure *vs* 20% Ensure (F-H, n = 11 mice) or 100% Ensure with Quinine (I-K, n = 7 mice) available in alternating 2-minute blocks (teal and black blocks, respectively). (G-K) Higher palatability food leads to a greater proportion of long bouts (G, J), with a higher licking rate compared to lower palatability food after 1-s (H) or after 2-s of licking onset (K). ***p<0.001. Statistical details are in Table S1.

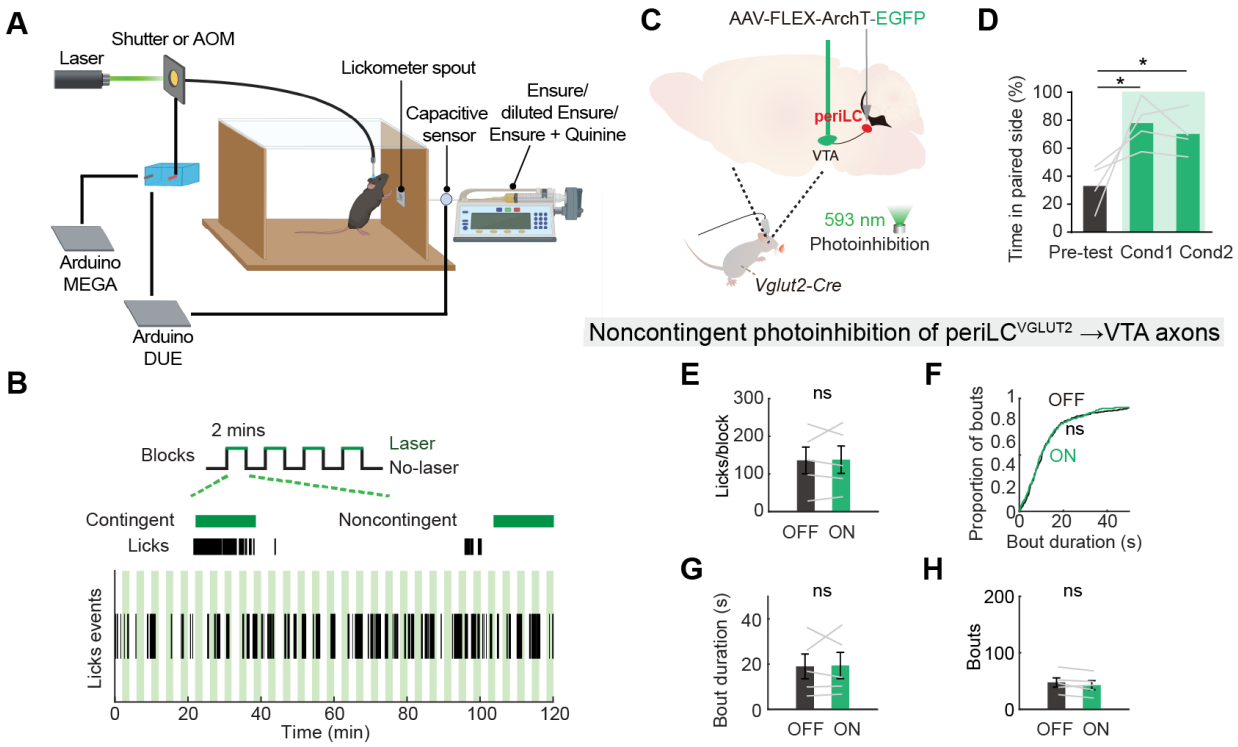

Fig. S3. Photoinhibition of periLC^{VGLUT2} axon projections to the VTA conditions place preference but noncontingent photoinhibition does not affect food intake. (A) Schematic of lick-triggered optogenetics apparatus. A capacitive lick detector on the liquid food spout registers licks that activate a syringe pump to deliver food as well as trigger a pulse generator program to deliver laser light in optogenetic experiments by controlling a shutter or an acoustic-optic modulator (AOM). (B) Interleaved block structure for closed-loop lick-contingent and open-loop lick-noncontingent perturbation and example lick events (black ticks) in one session. For lick-contingent sessions, licking the food spout delivers food and concurrently triggers the laser in laser-ON blocks (green, 2-min), and for alternating laser-OFF blocks (white, 2-min) licking delivers food but no laser. The same blockwise session structure is used for lick-noncontingent sessions but the optogenetic stimulation pattern from the prior lick-contingent session is used independently of the mouse licking behavior. (C) Schematic for photoinhibition of periLC^{VGLUT2} axon projections to downstream VTA during place preference test and feeding in *Vglut2-IRES-Cre* mice. (D) Photoinhibition of periLC^{VGLUT2} →VTA axon projections conditioned place preference (rmANOVA, n = 4 mice). (E-H) Noncontingent photoinhibition of periLC^{VGLUT2} →VTA axon projections did not significantly affect food consumption (E), bout duration (F-G), or bout number (H) (KS-test and paired t-test, n = 5 mice). Data are represented as mean ± SEM. ns $p>0.05$, *$p<0.05$. Statistical details are in Table S1.

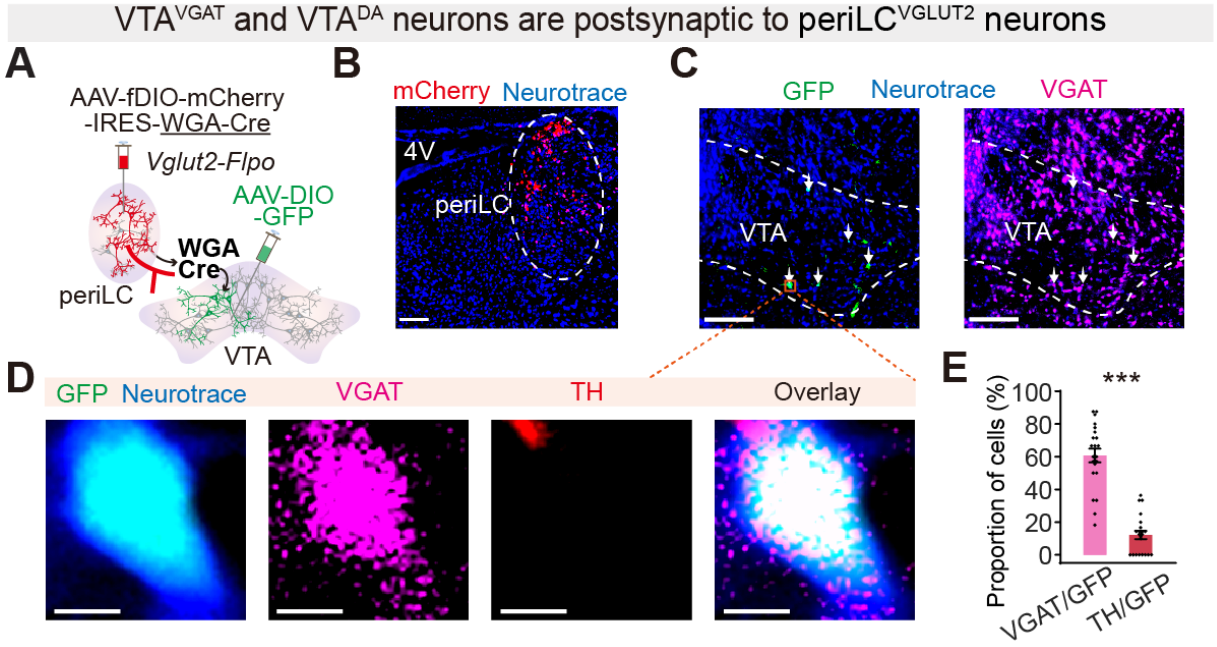

Fig. S4. Intersectional anterograde tracing of periLC^{VGLUT2} neurons to VTA^{VGAT} and VTA^{DA} neurons. (A) Schematic of the experimental strategy for intersectional anterograde transsynaptic labeling of VTA neurons downstream of periLC^{VGLUT2} neurons. AAV-fDIO-mCherry-IRES-WGA-Cre was injected into the periLC region of *Vglut2-IRES-Flpo* mice, along with AAV-DIO-GFP injected into the VTA. (B) Representative image showing mCherry expression (co-expressed fluorescent protein marker for transduction with WGA-Cre) in the periLC region. Dashed circle indicates periLC, and 4V marks the fourth ventricle. Scale bar: 100 µm. (C) Coronal sections of the VTA show GFP (green, left), Neurotrace (blue) staining, and VGAT (magenta, right) labeling. Arrows indicate GFP+ VGAT+ neurons, demonstrating periLC^{VGLUT2} input to GABAergic neurons. Scale bar: 200 µm. (D) Insets in (C) show a GFP+/VGAT+ neuron that is TH-negative. Scale bars: 5 µm. (E) Quantification of GFP labeling in VGAT+ (GABAergic) and TH+ (dopaminergic) neurons in the VTA (t-test, n = 22 VTA sections from 2 mice). Data are represented as mean ± SEM. ***p<0.001. Statistical details are in Table S1.

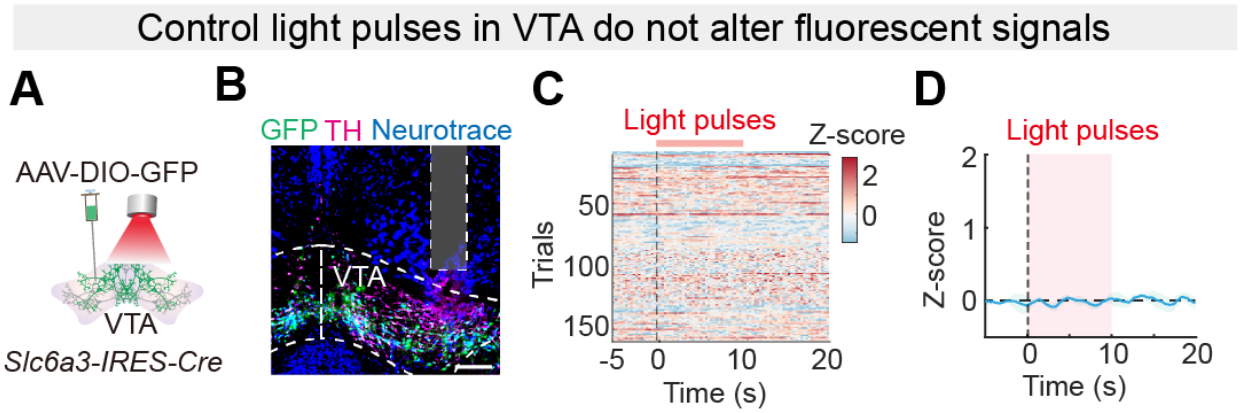

Fig. S5. Negative control experiments for photostimulation during photometry recordings in the VTA. (A) Photometry setup for monitoring GFP fluorescence in VTA^{DA} neurons during 635 nm light pulses in the VTA (*Slc6a3-IRES-Cre* mice). (B) Expression of GFP (green) and TH (magenta) within VTA. Scale bar, 200 μm. (C) Photometry recordings show the absence of responses in GFP-expressing neurons during 10-s light pulses. (D) GFP negative control mean responses (blue) during 10-s light pulses (n = 2 mice). Data are represented as mean ± SEM.

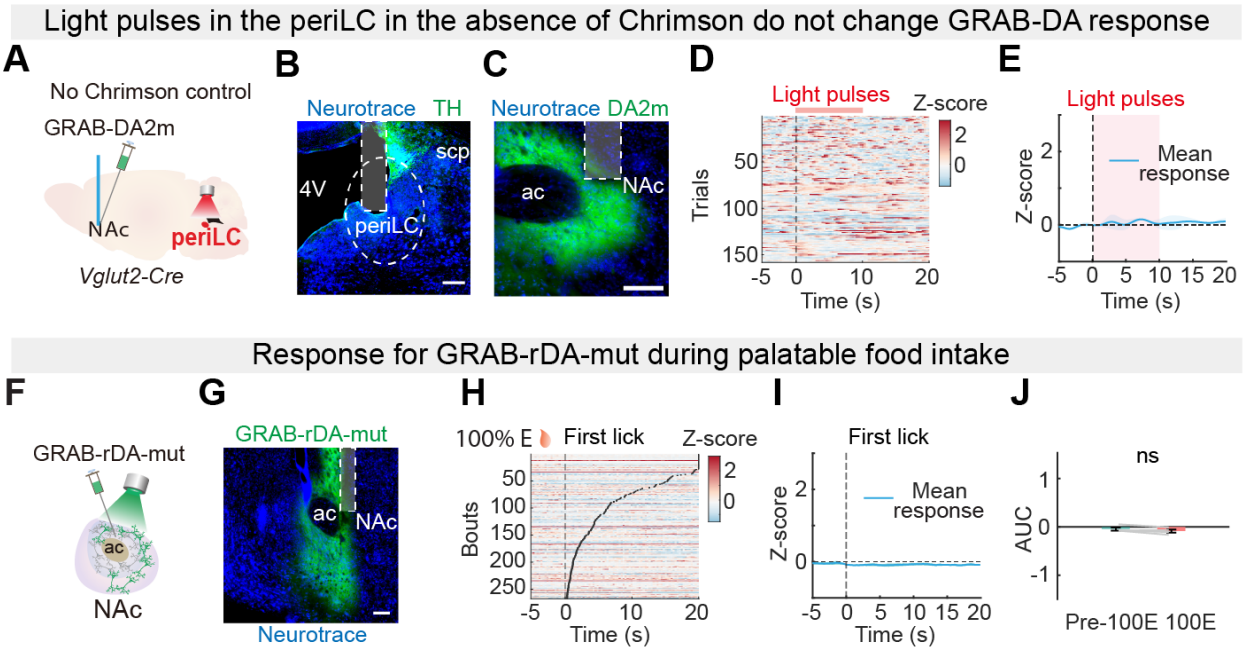

Fig. S6. Negative controls for GRAB-DA experiments in NAc during palatable food consumption. (A) Photometry setup for monitoring NAc dopamine during light pulses in the periLC without Chrimson in periLC^{VGLUT2} neurons (n = 2, *Vglut2-IRES-Cre* mice). (B, C) Fiber implantation (grey shading) over periLC and expression of TH (green) in the LC (B) and expression of GRAB-DA2m in NAc (C). Scale bars, 200 µm. (D) NAc GRAB-DA responses during delivery of 10-s light pulses. (E) GRAB-DA response was not significantly different during 10-s light pulses in the periLC. (F) Photometry setup recording the GRAB-rDA-mut control during consumption of 100% Ensure. (G) Expression of GRAB-rDA-mut (green) within NAc. Scale bar, 200 µm. (H) GRAB-rDA-mut control responses during consumption of 100% Ensure. (I, J) GRAB-rDA-mut control mean responses (blue) (I), showed no significant change of AUC during consumption of 100% Ensure (J, paired t-test, n = 4 C57BL6/J mice). Data are represented as mean ± SEM. ns p>0.05. Statistical details are provided in Table S1.

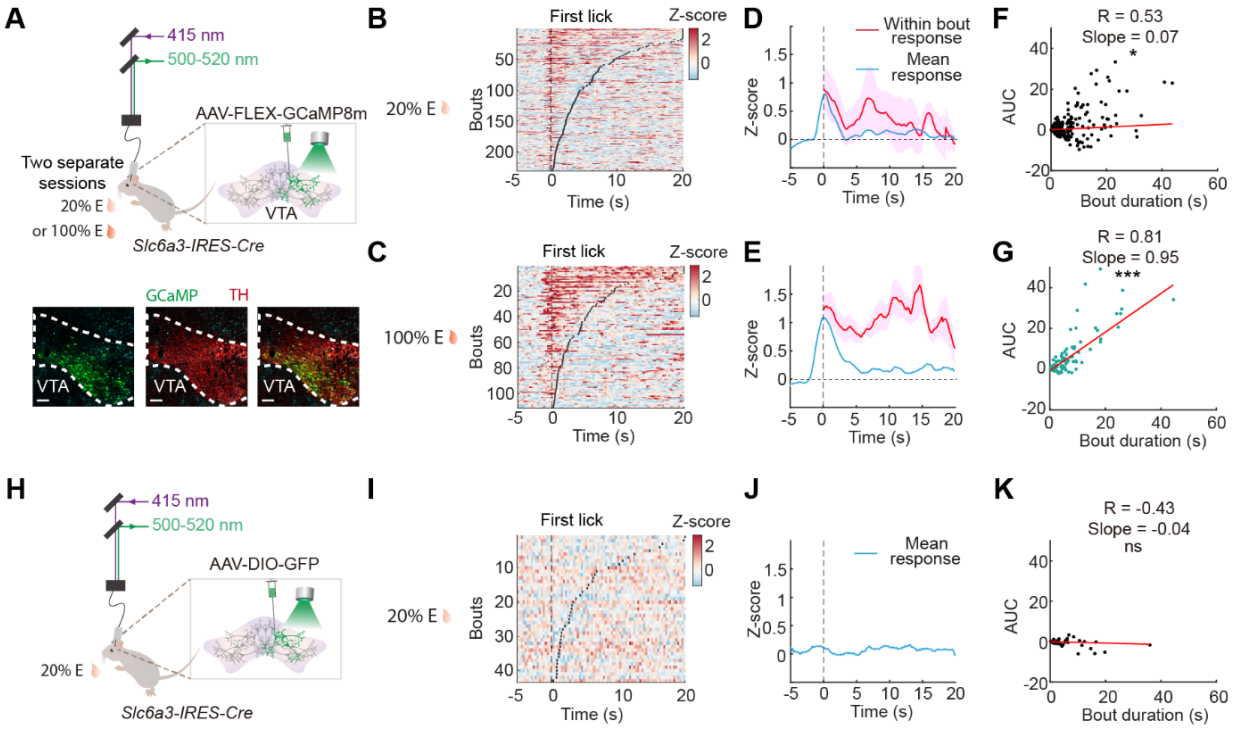

Fig. S7. Reproducibility of VTA^{DA} neuron calcium dynamics relationship with food consumption duration, effects of palatability, and GFP control experiment. (A) Upper panel, photometry setup showing VTA^{DA} neuron dynamics during consumption of 20% or 100% Ensure during separate sessions (*Slc6a3-IRES-Cre* mice). Bottom panels, viral expression of GCaMP8m in VTA (green, left), anti-TH (red, center), and overlaid images (right). E: Ensure. Scale bars, 100 µm. (B-E) GCaMP8m responses during consumption of 20% (B, D) and 100% Ensure (C, E). (D, E) GCaMP8m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 20% Ensure and 100% Ensure. (F-G) Regression of GCaMP8m AUC with bout duration during consumption of 20% Ensure (F) and 100% of Ensure (G) (n = 4 mice). (H) Photometry setup recording the GFP control of VTA^{DA} neuron dynamics during consumption of 20% or 100% Ensure (n = 3 *Slc6a3-IRES-Cre* mice). (I) GFP control responses during consumption of 20% Ensure. (J) GFP control mean responses (blue) during consumption of 20% Ensure. (K) Regression of GFP control AUC with bout duration during consumption of 20% Ensure (n = 3 mice). Data are represented as mean ± SEM. ns $p>0.05$, *$p<0.05$, ***$p<0.001$. Statistical details are provided in Table S1.

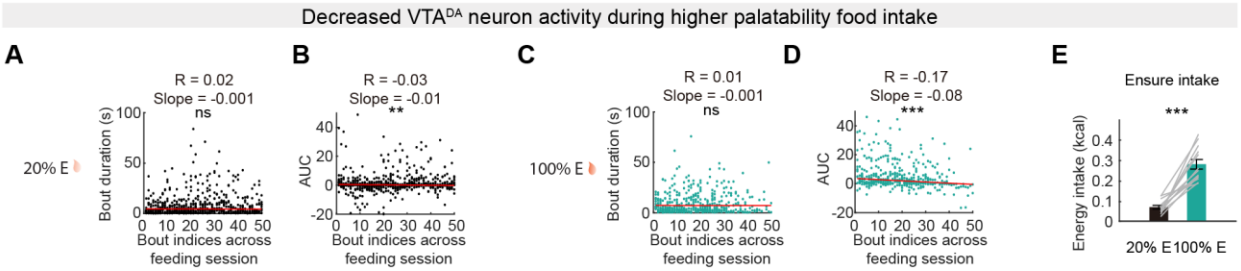

Fig. S8. Across-session bout duration and VTA^{DA} neuron dynamics. (A-D) Linear regression of bout duration (A, C) or AUC of GCaMP8s responses (B, D) with the bout indices across each separate feeding session during consumption of 20% Ensure (A, B) or 100% Ensure (C, D) (n = 13 mice). E: Ensure. (E) Energy intake for 20% and 100% Ensure sessions (n = 13 mice). ns p>0.05, **p<0.01, ***p<0.001. Statistical details are provided in Table S1.

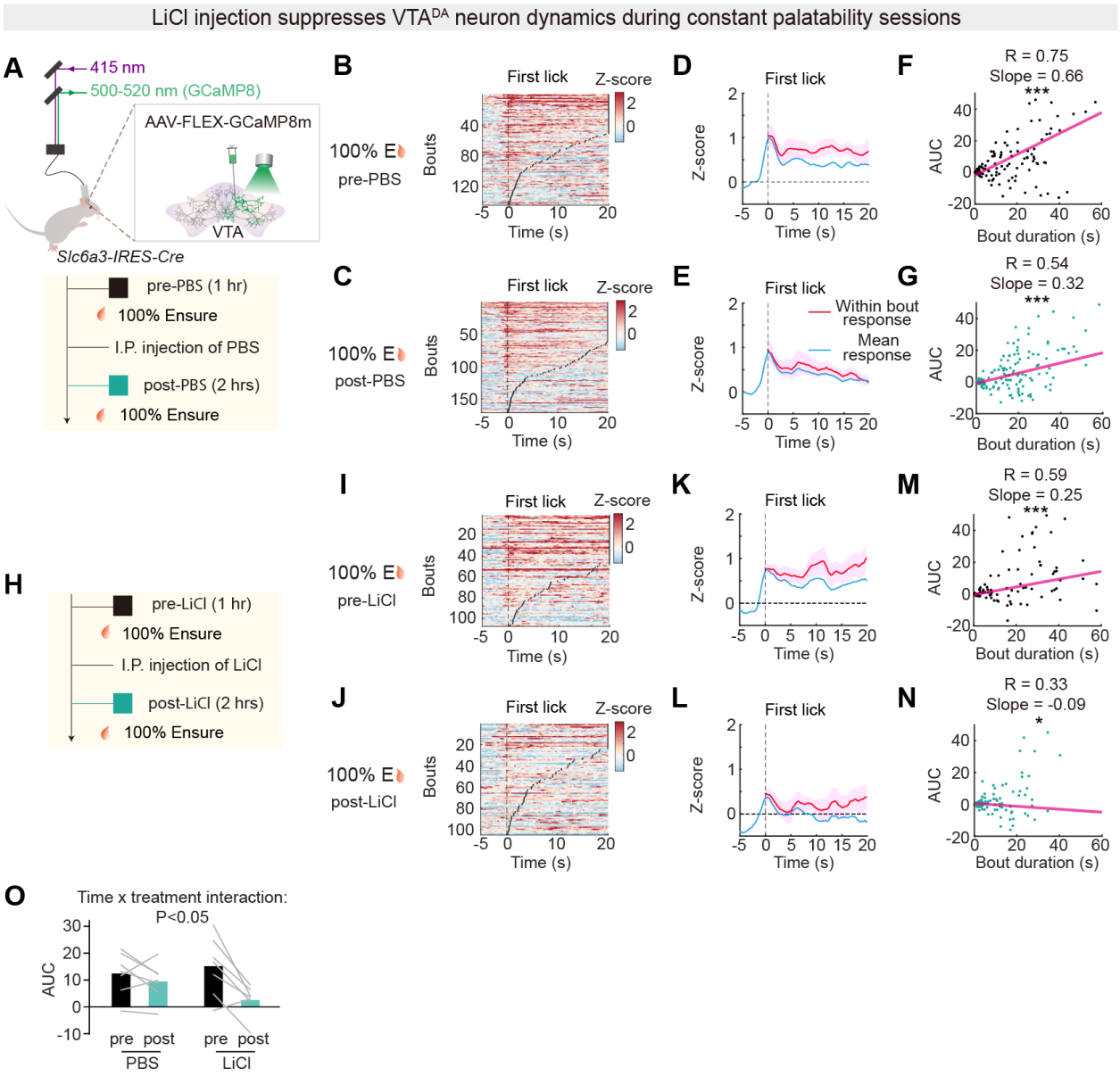

Fig. S9. LiCl suppresses VTA^{DA} neuron dynamics during food consumption. (A) Photometry setup for monitoring VTA^{DA} neuron dynamics during consumption of 100% Ensure before or after PBS injection (*Slc6a3-IRES-Cre* mice). (B-E) GCaMP8m responses during consumption of 100% Ensure before (B, D) or after PBS injection (C, E). (D, E) GCaMP8m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 100% Ensure before or after PBS injection. (F-G) Regression of GCaMP8m AUC with bout duration during consumption of 100% Ensure before (F) or after (G) PBS injection (n = 7 mice). (H) Schematic of recording VTA^{DA} neuron dynamics during consumption of 100% Ensure before or after LiCl injection during separate sessions (n = 7 *Slc6a3-IRES-Cre* mice). (I-L) GCaMP8m responses during consumption of 100% Ensure before (I, K) or after LiCl injection (J, L). (K, L) GCaMP8m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 100% Ensure before (K) or after (L) LiCl injection. (M, N) Regression of GCaMP8m AUC with bout duration during consumption of 100% Ensure before (M) or after (N) LiCl injection (n = 7 mice). (O) VTA^{DA} neuron calcium dynamics show a lower

AUC for palatable food after LiCl injection (rmANOVA, n = 7 mice). Data are represented as mean ± SEM. ns $p>0.05$, *$p<0.05$, ***$p<0.001$. Statistical details are in Table S1.

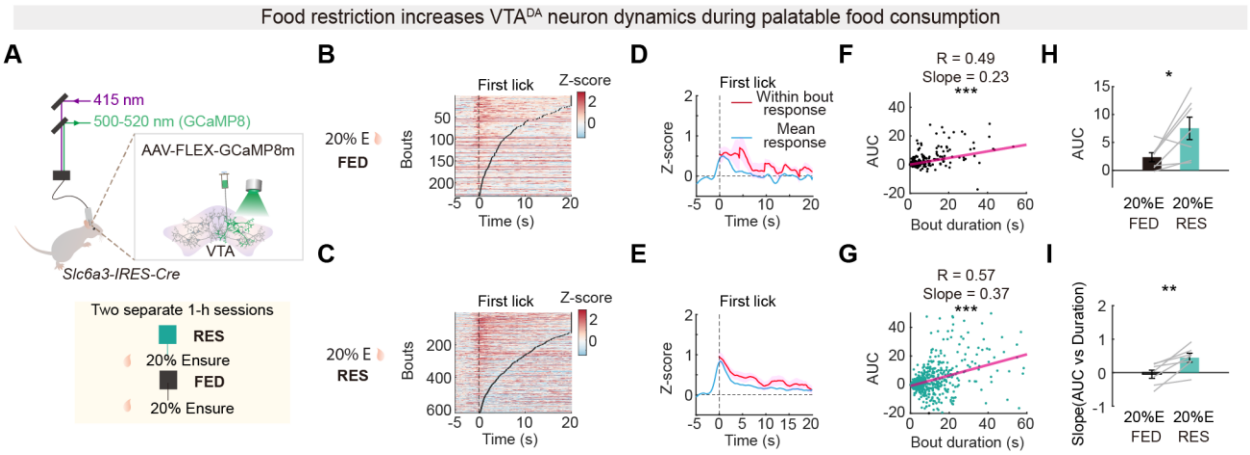

Fig. S10. Food restriction increases VTA^{DA} neuron response during palatable food consumption. (A) Photometry setup showing VTA^{DA} neuron dynamics during consumption of 20% Ensure in *ad-libitum* fed (FED) or food-restricted (RES) mice during separate sessions (*Slc6a3-IRES-Cre* mice). (B-E) GCaMP8m responses during consumption of 20% Ensure in FED (B, D) and RES mice (C, E). (D, E) GCaMP8m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 20% Ensure in FED (D) or RES mice (E). (F-G) Regression of GCaMP8m AUC with bout duration during consumption of 20% Ensure in FED (F) and RES mice (G) (n = 7 mice). (H-I) VTA^{DA} neuron dynamics show a significantly larger AUC (H) and a steeper slope of GCaMP8s AUC/Bout Duration (I) for palatable food in RES mice (paired t-test, n = 7 mice). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. Statistical details are provided in Table S1.

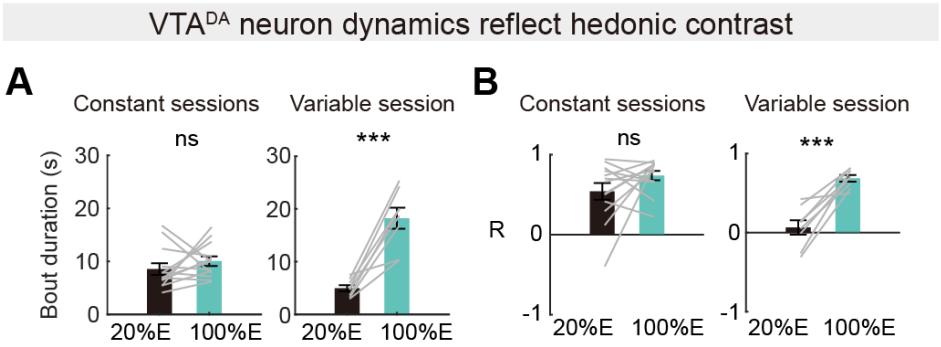

Fig. S11. Across-session bout duration and VTA^{DA} neuron dynamics. (A) Bout durations for 20% and 100% Ensure in constant palatability sessions (n = 13 mice) and variable palatability sessions (n = 8 mice). (B) Pearson correlation coefficients (R) for regression of photometry AUC and bout duration in constant palatability sessions (n = 13 mice) and variable palatability sessions (n = 8 mice). Data are represented as mean ± SEM. ns $p>0.05$, ***$p<0.001$. Statistical details are in Table S1.

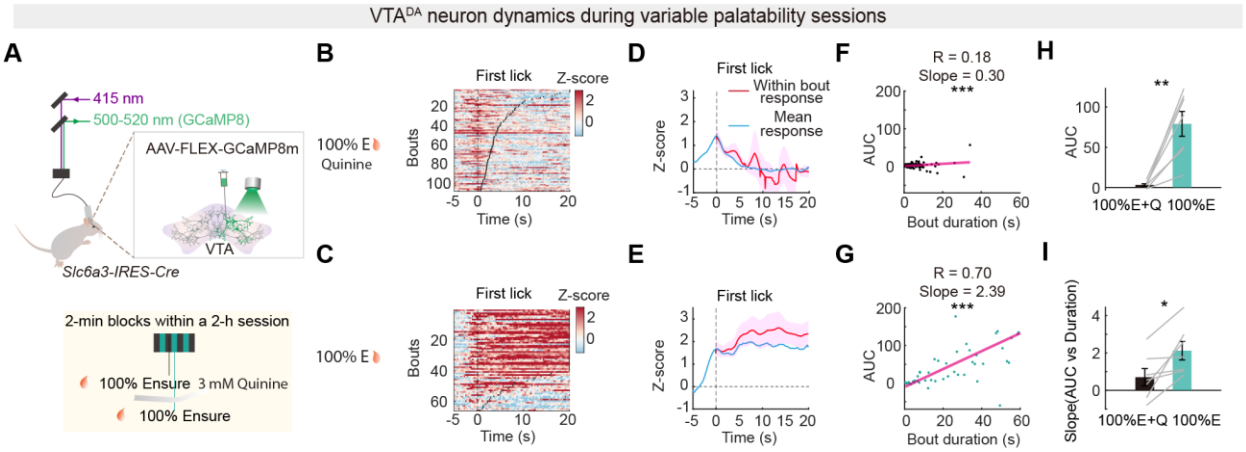

Fig. S12. Hedonic contrast with quinine adulteration increases VTA^{DA} neuron responses during consumption of higher palatability food with constant nutrient content. (A) Photometry setup showing VTA^{DA} neuron dynamics during consumption of 100% Ensure or 100% Ensure adulterated with Quinine during the same session (*Slc6a3-IRES-Cre* mice). (B-E) GCaMP8m responses during consumption of 100% Ensure with Quinine (B, D) and 100% Ensure (C, E). (D, E) GCaMP8m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 100% Ensure with Quinine and 100% Ensure. (F-G) Regression of GCaMP8m AUC with bout duration during consumption of 100% Ensure with Quinine (F) and 100% of Ensure (G) (n = 7 mice). (H-I) VTA^{DA} neuron dynamics show a larger AUC (H) and a steeper slope of GCaMP8m AUC/Bout Duration (I) for the higher palatability food lacking quinine (paired t-test, n = 7 mice). Data are represented as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001. Statistical details are in Table S1.

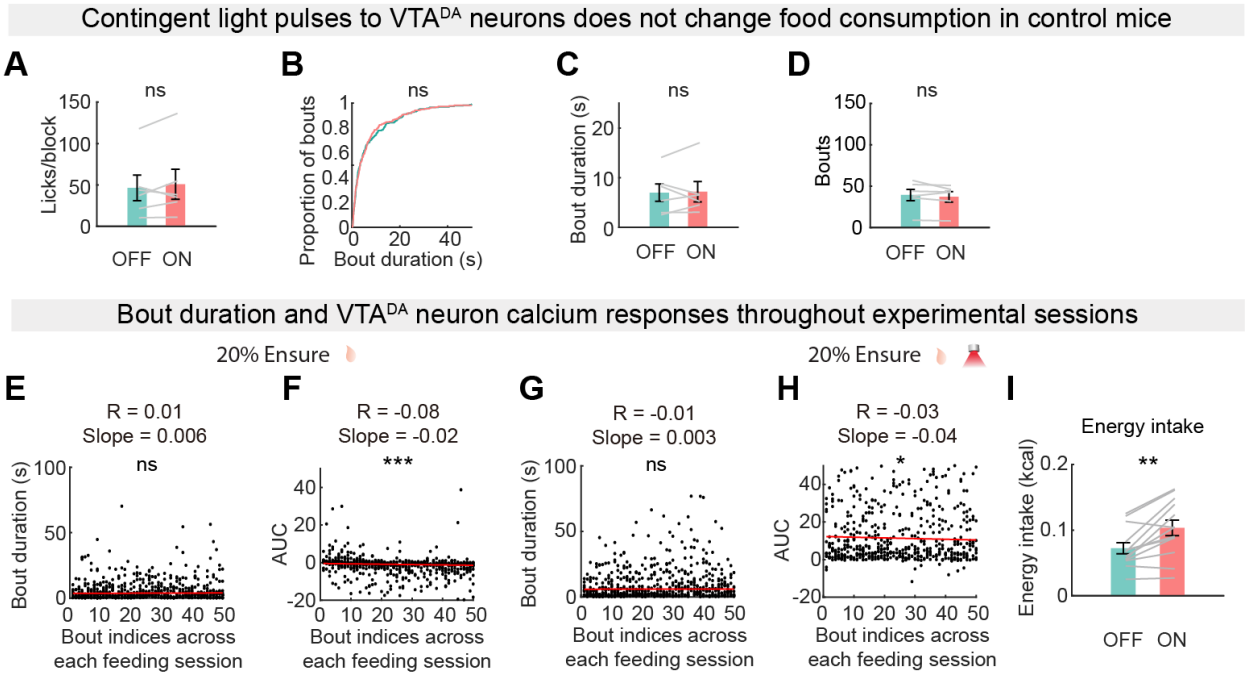

Fig. S13. VTA^{DA} neuron photostimulation control experiments and comparison of bout duration and neuron activity of each feeding session. (A-D) Contingent light pulses to VTA^{DA} neurons do not change consumption (A), bout duration, and bout number during laser-on blocks in GCaMP8m or GFP control mice (both lacking Chrimson in VTA^{DA} neurons) (B-D, KS-test and paired t-test, n = 6). (E-H) Linear regression of bout duration (E, G) or GCaMP8s AUC (F, H) with the bout indices across each separate feeding session during consumption of 20% Ensure (E, F) or 20% Ensure with photometry-calibrated VTA^{DA} neuron photostimulation (G, H) (n = 13 mice). (I) Energy intake for 20% and 20% Ensure with photometry-calibrated VTA^{DA} neuron photostimulation sessions (n = 13 mice). Data are represented as mean ± SEM. ns p>0.05, *p<0.05, **p<0.01, ***p<0.001. Statistical details are provided in Table S1.

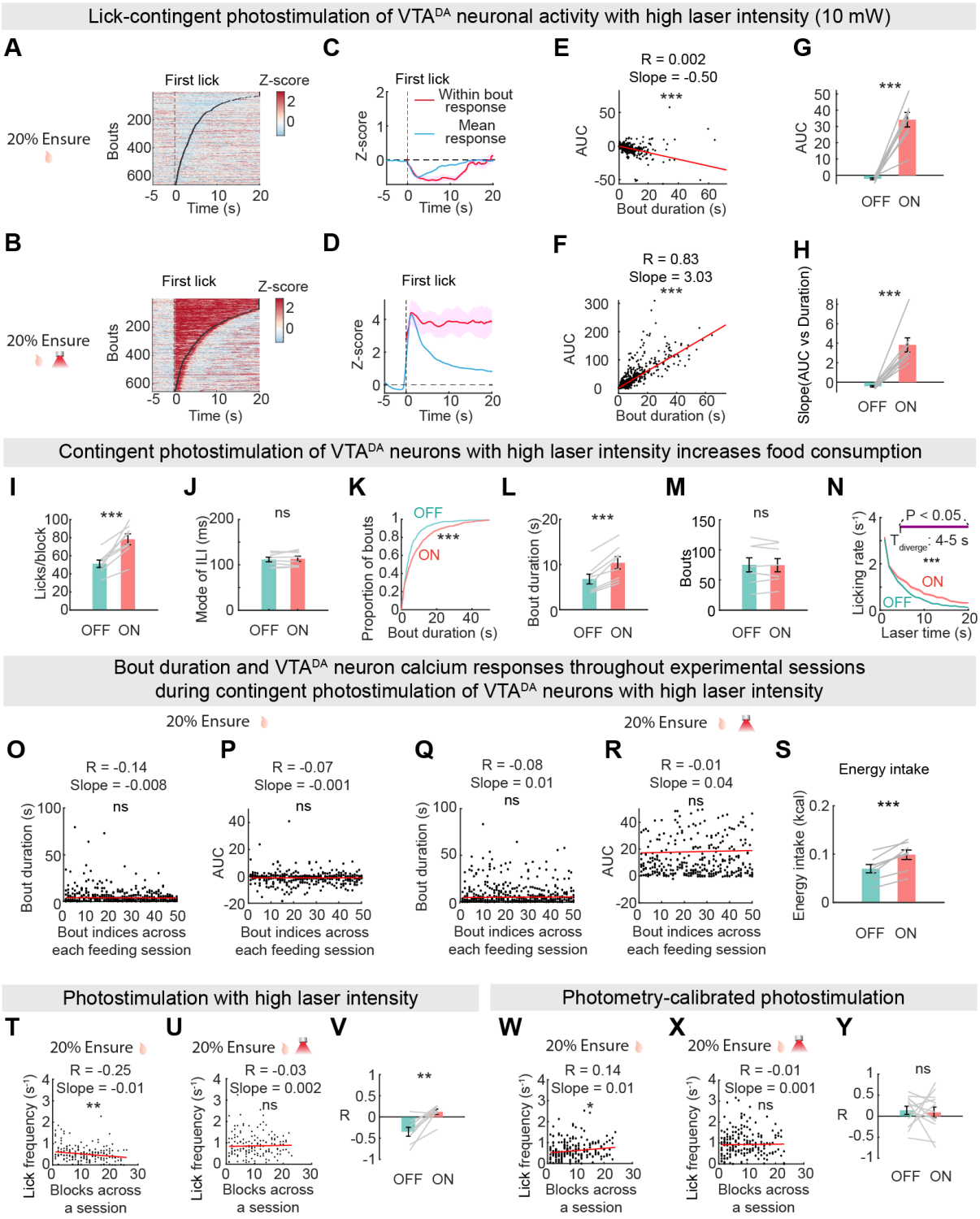

Fig. S14. Photostimulation of VTA^{DA} neuron dynamics with a higher laser intensity reinforces food consumption. (A-D) GCaMP8s responses during consumption of 20% (A, C) and 20% Ensure with lick-contingent VTA^{DA} neuron photostimulation with a higher laser intensity (10 mW) (B, D). (C, D) GCaMP8s mean responses (blue) and mean response within a bout (magenta) during consumption of 20% Ensure and 20% Ensure with contingent photostimulation with a higher laser intensity. (E-F) Regression of GCaMP8s AUC with bout duration during

consumption of 20% Ensure (E) and 20% Ensure with contingent photostimulation with a higher laser intensity (F) (n = 8 mice). (G-H) VTA^{DA} neuron dynamics show a larger area under the curve (AUC) (G) and a steeper slope of GCaMP8s AUC/Bout Duration (H) for contingent photostimulation with a higher laser intensity (paired t-test, n = 8 mice). (I-N) Contingent photostimulation of VTA^{DA} neurons with a higher laser intensity increases consumption (I-J), and bout duration but not bout number during laser-ON blocks (K-N) (negative binomial generalized linear mixed model, KS-test and paired t-test, n = 8). (O-R) Linear regression of bout duration (O, Q) or AUC of GCaMP8s responses (P, R) with the bout indices across each separate feeding session during consumption of 20% Ensure (O, P) or 20% Ensure with contingent photostimulation with a higher laser intensity (Q, R) (n = 8 mice). (S) Energy intake for 20% Ensure during ON and OFF blocks of VTA^{DA} contingent photostimulation with a higher laser intensity (n = 8 mice). (T-V) Linear regression of mean lick frequency within the 2-minute blocks index across a session on laser-OFF (T) and laser-ON (U) periods during the photostimulation with high laser power and comparison of correlation coefficient in ON and OFF blocks (V, paired t-test, n = 8 mice). (W-Y) As for T-V with photometry-calibrated photostimulation (n = 13 mice). Data are represented as mean ± SEM. ns $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$. Statistical details are provided in Table S1.

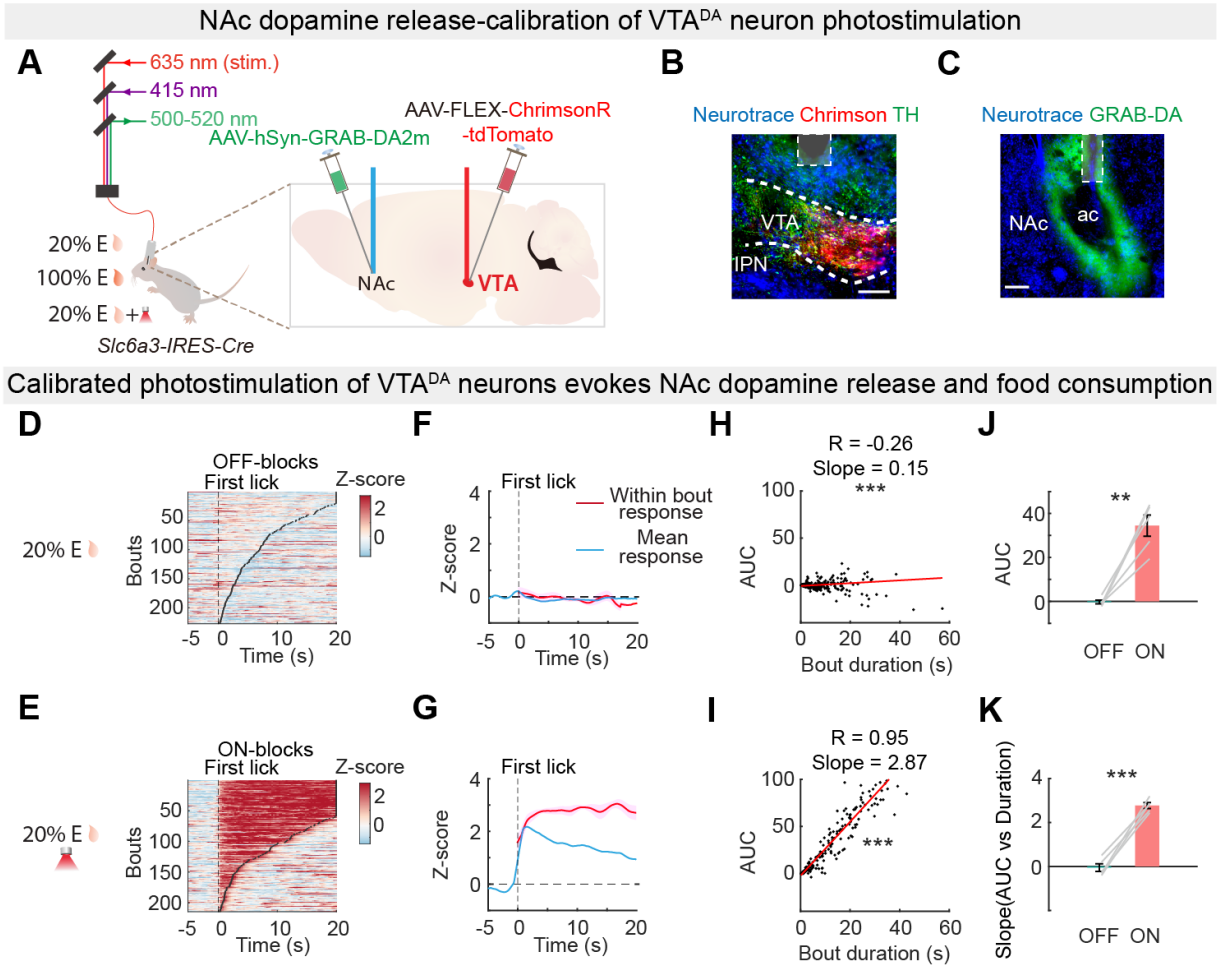

Fig. S15. Dopamine-calibrated photostimulation of VTA^{DA} neurons promotes food consumption duration and palatability. (A) Photometry-calibrated photostimulation experimental setup (*Slc6a3-IRES-Cre* mice). (B) Expression of Chrimson (red) and TH (green) within VTA. Scale bar, 200 µm. (C) Expression of GRAB-DA2m (green) within NAc. Scale bar, 200 µm. (D-G) NAc dopamine responses during consumption of 20% Ensure with photometry-calibrated VTA^{DA} neuron photostimulation in 20% Ensure in OFF-blocks (D, F) and ON-blocks (E, G). (F, G) NAc GRAB-DA2m mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 20% Ensure (F) and 20% Ensure with photometry-calibrated VTA^{DA} neuron photostimulation (G). (H-I) Regression of NAc dopamine AUC with bout duration during consumption of 20% Ensure (H) and 20% Ensure with photometry-calibrated VTA^{DA} neuron photostimulation (I) (n = 5 mice). (J-K) NAc dopamine dynamics show a larger area under the curve (AUC) (J) and a steeper slope of NAc dopamine AUC/Bout Duration (K) for photometry-calibrated VTA^{DA} neuron photostimulation (paired t-test, n = 5 mice). Data are represented as mean ± SEM. **p<0.01, ***p<0.001. Statistical details are provided in Table S1.

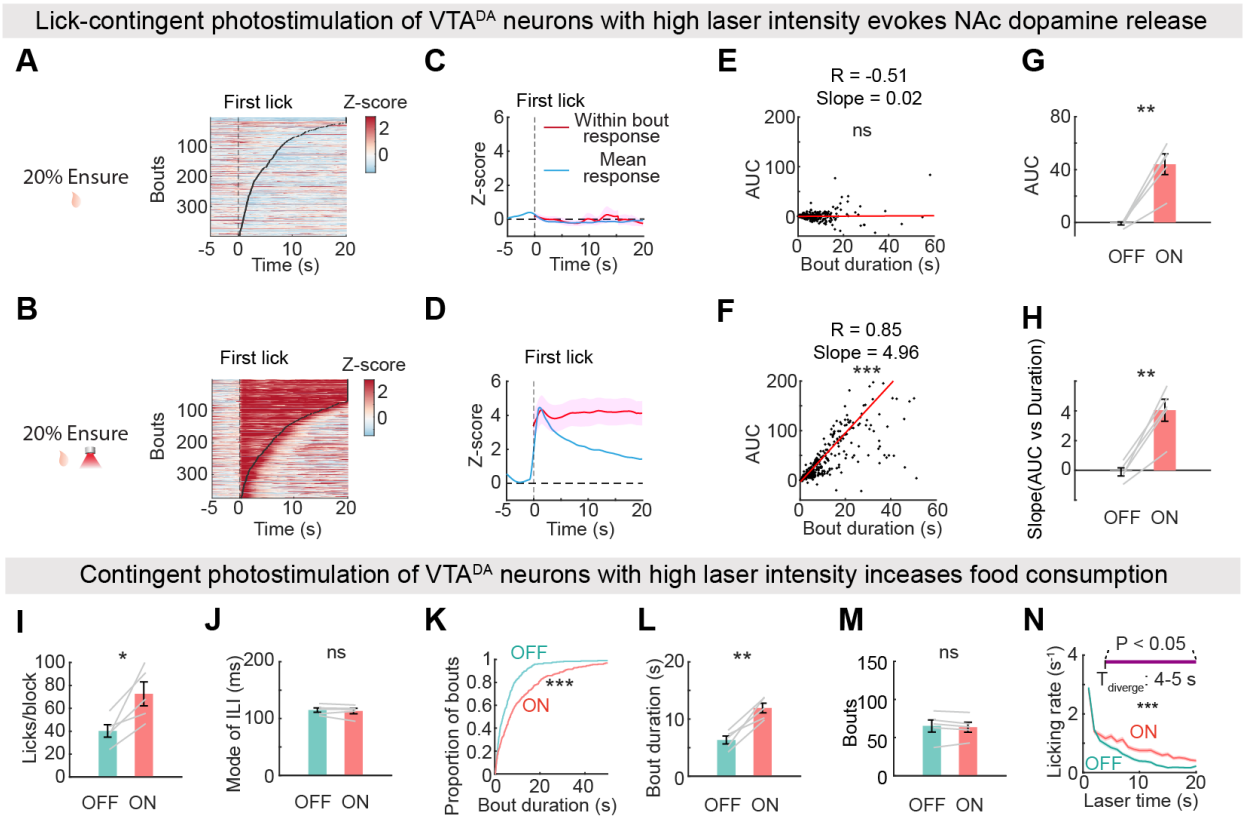

Fig. S16. Photostimulation of VTA^{DA} neuron dynamics with a higher laser intensity increases food consumption. (A-D) NAc dopamine responses during consumption of 20% (A, C) and 20% Ensure with lick-contingent VTA^{DA} neuron photostimulation with a higher laser intensity (10 mW) (B, D). (C, D) NAc dopamine mean responses (blue) and variable-length time mean response across all bouts (magenta) during consumption of 20% Ensure and 20% Ensure with contingent photostimulation with higher laser intensity. (E-F) Regression of NAc dopamine AUC with bout duration during consumption of 20% Ensure (E) and 20% Ensure with contingent photostimulation with a higher laser intensity (F) (n = 5 mice). (G-H) NAc dopamine dynamics show a larger AUC (G) and a steeper slope of GCaMP8s AUC/Bout Duration (H) for contingent photostimulation with a higher laser intensity (paired t-test, n = 5 mice). (I-N) Contingent photostimulation of VTA dopamine neurons with a higher laser intensity increases consumption (I-J), and bout duration but not bout number during laser-ON blocks (K-N) (negative binomial generalized linear mixed model, KS-test and paired t-test, n = 5 mice). Data are represented as mean ± SEM. ns p>0.05, *p<0.05, **p<0.01, ***p<0.001. Statistical details are provided in Table S1.

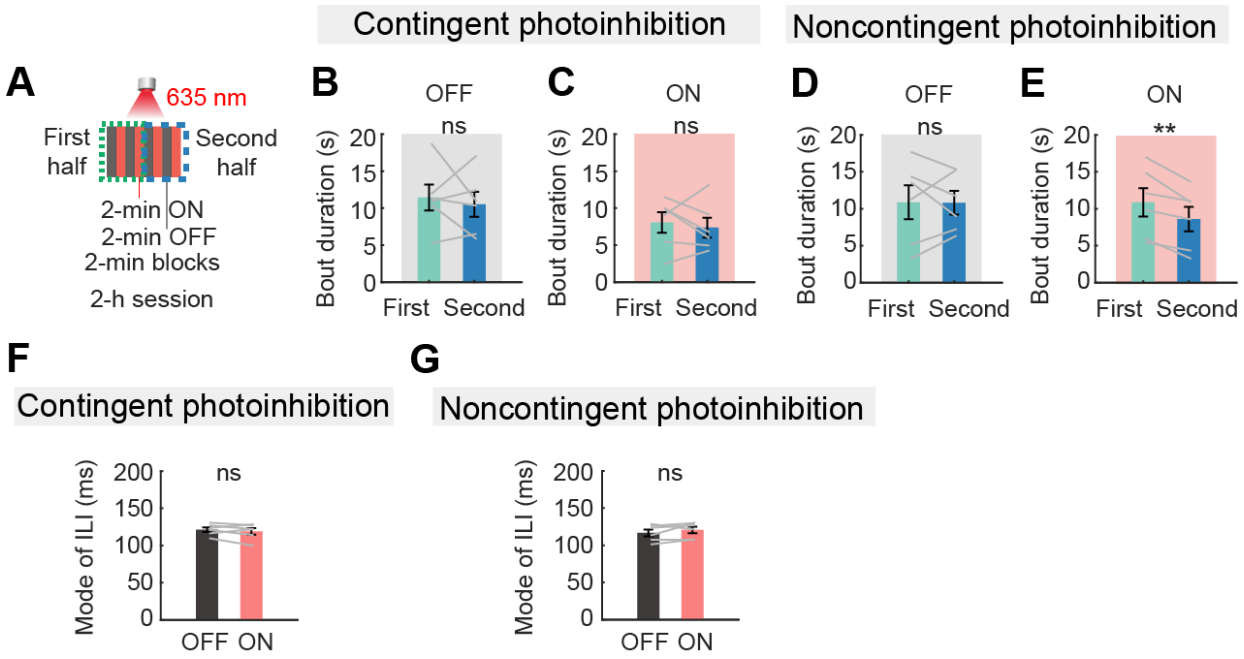

Fig. S17. Comparison of lick-triggered photoinhibition of VTA^{DA} neurons in the first and second halves of lick-contingent and noncontingent photoinhibition sessions. (A) Schematic for comparison of bout duration from the first and second half of the session during lick-triggered photoinhibition of VTA^{DA} neurons. (B-C) Bout duration was similar in the first and second halves of laser-OFF blocks (B) and laser-ON blocks (C) with lick-contingent photoinhibition of VTA^{DA} neurons (paired t-test, n = 6 mice). (D-E) The bout duration in the first and second half during laser-OFF blocks was similar (D) but the bout duration was shorter in the second half of laser-ON blocks (E) of noncontingent photoinhibition of VTA^{DA} neurons (paired t-test, n = 6 mice). (F-G) Contingent (F) or noncontingent (G) photoinhibition of VTA^{DA} neurons does not change the fundamental lick oscillator interval (paired t-test, n = 6 mice). Data are represented as mean ± SEM. ns $p>0.05$, **$p<0.01$. Statistical details are provided in Table S1.

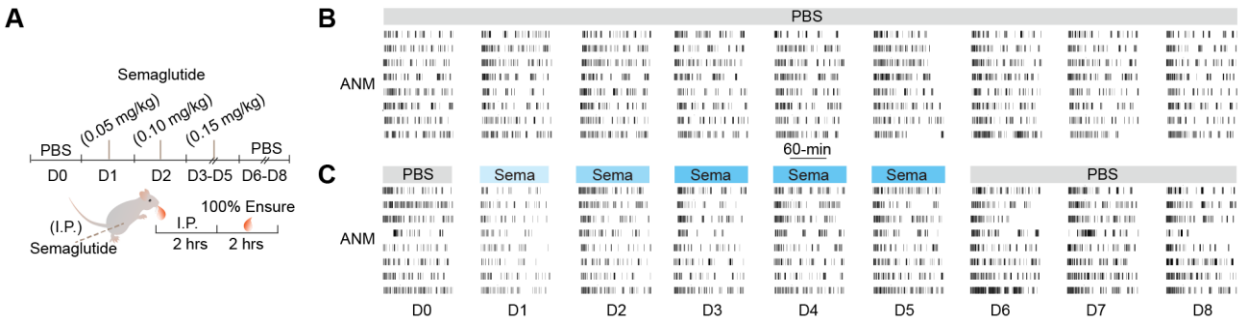

Fig. S18. Palatable food intake during semaglutide treatment. (A) Experimental design to test the effects of semaglutide on food consumption. (B-C) For each animal (ANM), lick raster plots of all licks for a 2-h session following injection of PBS (B) or semaglutide (C) (n = 8 mice).

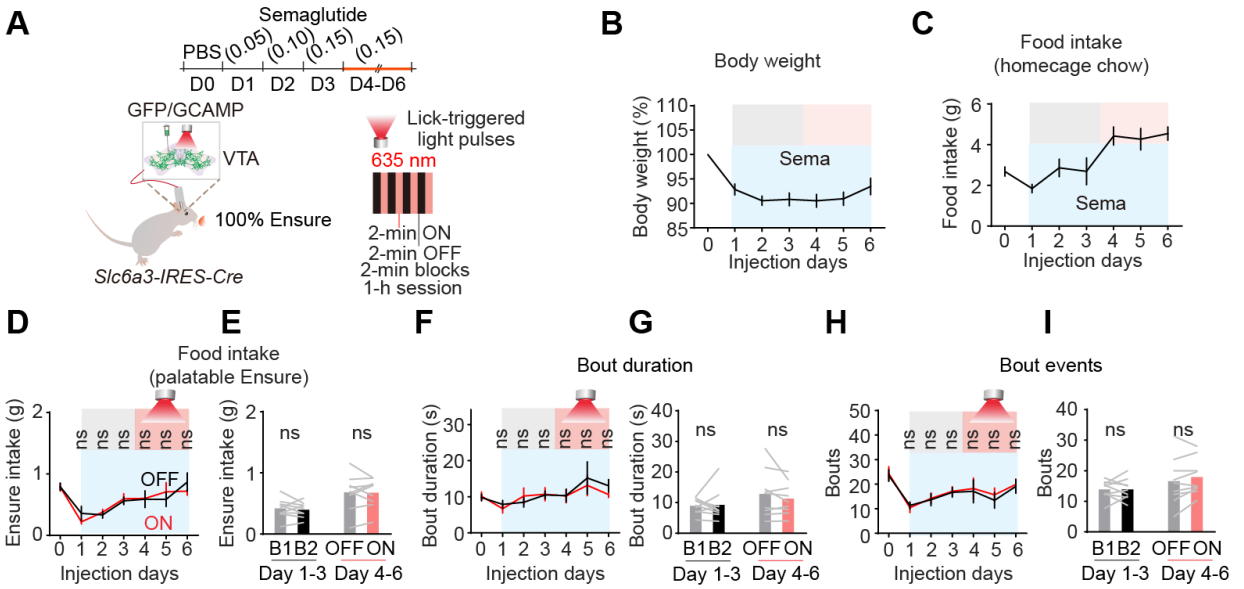

Fig. S19. Control experiment for photoinhibition of VTA^{DA} neurons during semaglutide treatment. (A) Design of control experiment with light pulses to VTA^{DA} neurons expressing GFP or GCaMP (no JAWS) during Ensure food intake from day 4 to day 6 with highest dose semaglutide treatment. (B-C) Body weight (B) and home cage chow food intake (C) during semaglutide treatment. (D-I) Similar Ensure intake (D, E), bout duration (F, G), and bout numbers (H, I) during semaglutide treatment (n = 9 mice). Days 1-3 are analyzed to show Ensure intake, bout duration, and bout number across the same alternating 2-min blocks (B1, B2) in the absence of photoinhibition. Data are represented as mean ± SEM. ns $p>0.05$. Statistical details are provided in Table S1.

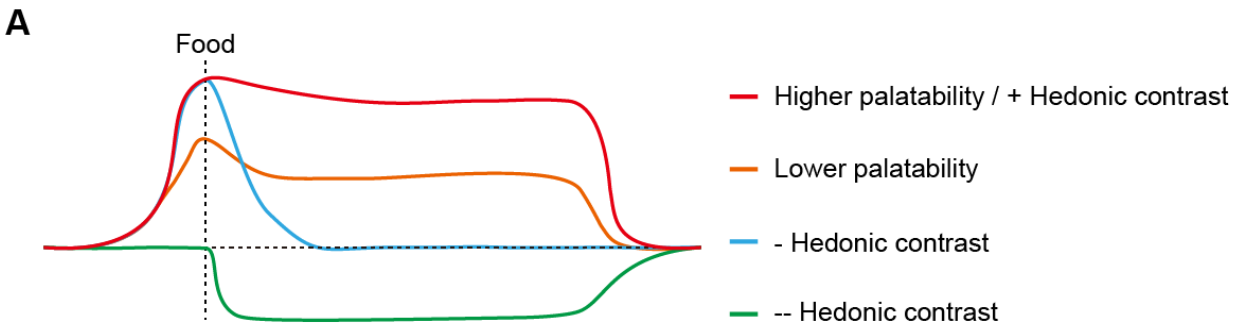

Fig. S20. Summary of VTA^{DA} neuron activity during hedonic feeding. VTA^{DA} neuron activity during consumption is scaled by palatability differences or positive and negative hedonic contrast.

Table S1. Statistical data for all Figures.

Figure	Comparison	Analysis	Statistic value	N
1B	Licks/block	Paired t-test	20%E vs 100%E: $p < 0.001$	n = 11 mice
1C	Bout duration	Paired t-test	20%E vs 100%E: $p < 0.001$	n = 11 mice
1D	Bouts	Paired t-test	20%E vs 100%E: $p = 0.794$	n = 11 mice
1E	Licks/block	Paired t-test	100%E + Q vs 100%E: $p < 0.001$	n = 7 mice
1F	Bout duration	Paired t-test	100%E + Q vs 100%E: $p < 0.001$	n = 7 mice
1G	Bouts	Paired t-test	100%E + Q vs 100%E: $p = 0.055$	n = 7 mice
1L	Licks/block	Paired t-test	BNST/LHA/VTA/PCRt (OFF vs ON): $p = 0.706$/0.910/0.012/0.377	n = 3,6,9,4 mice
1M	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p = 0.410$	n = 3 mice
1M	Bouts	Paired t-test	OFF vs ON: $p = 0.836$	n = 3 mice
1N	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p = 0.129$	n = 6 mice
1N	Bouts	Paired t-test	OFF vs ON: $p = 0.872$	n = 6 mice
1O	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p < 0.001$	n = 9 mice
1O	Bouts	Paired t-test	OFF vs ON: $p = 0.693$	n = 9 mice
1P	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p = 0.830$	n = 4 mice
1P	Bouts	Paired t-test	OFF vs ON: $p = 0.803$	n = 4 mice
1S	Licks/block	Paired t-test	OFF vs ON: $p = 0.007$	n = 4 mice
1T	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p < 0.001$	n = 4 mice
1U	Bout duration	Paired t-test	OFF vs ON: $p = 0.024$	n = 4 mice
1V	Bouts	Paired t-test	OFF vs ON: $p = 0.006$	n = 4 mice
2F	AUC before vs during photostimulation	Paired t-test	PRE vs PS: $p = 0.049$	n = 4 mice
2I	AUC/Duration of laser-OFF vs laser-ON blocks	Paired t-test	OFF vs ON: $p = 0.021$	n = 4 mice
2J	Licks/block	Paired t-test	OFF vs ON: $p = 0.025$	n = 4 mice
2K	Licking rate	Negative binomial	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 722.07$, $p < 0.001$	n = 4 mice

		generalized linear mixed models	Likelihood ratio test: Factor B (Time): $Chi^2(38) = 296.63$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 96.16$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.009 1–2 s: p < 0.001 2–3 s: p < 0.001 3–4 s: p < 0.001 4–5 s: p < 0.001 5–6 s: p < 0.001 6–7 s: p < 0.001 7–8 s: p < 0.001 8–9 s: p < 0.001 9–10 s: p < 0.001 10–11 s: p < 0.001 11–12 s: p < 0.001 12–13 s: p < 0.001 13–14 s: p < 0.001 14–15 s: p < 0.001 15–16 s: p < 0.001 16–17 s: p < 0.001 17–18 s: p < 0.001 18–19 s: p < 0.001 19–20 s: p < 0.001	
2L	Proportions of bouts vs bout duration	KS test	OFF vs ON: *p < 0.001*	n = 4 mice
2M	Bout duration	Paired t-test	OFF vs ON: *p = 0.005*	n = 4 mice
2N	Bouts	Paired t-test	OFF vs ON: *p = 0.391*	n = 4 mice
2S	AUC before vs during photostimulation	Paired t-test	PRE vs PS: *p = 0.018*	n = 5 mice
2V	AUC/Duration of laser-OFF vs laser-ON blocks	Paired t-test	OFF vs ON: *p = 0.036*	n = 5 mice
3F	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.483; Slope = 0.168; *p < 0.001*	n = 13 mice
3G	Regression of GCaMP8s AUC	Linear regression	R = 0.779; Slope = 0.549; *p < 0.001*	n = 13 mice

	with bout duration of 100% Ensure consumption			
3H	AUC of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p = 0.003$	n = 13 mice
3I	Slope of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p = 0.001$	n = 13 mice
3O	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.11; Slope = 0.015; $p = 0.659$	n = 8 mice
3P	Regression of GCaMP8s AUC with bout duration of 100% Ensure consumption	Linear regression	R = 0.617; Slope = 0.705; $p < 0.001$	n = 8 mice
3Q	AUC of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p < 0.001$	n = 8 mice
3R	Slope of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p = 0.002$	n = 8 mice
3X	Regression of GRAB-DA AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.713; Slope = 0.364; $p < 0.001$	n = 9 mice
3Y	Regression of GRAB-DA AUC with bout duration of 100% Ensure consumption	Linear regression	R = 0.781; Slope = 1.169; $p < 0.001$	n = 9 mice
3Z	AUC of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p = 0.015$	n = 9 mice
3AA	Slope of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p = 0.003$	n = 9 mice
3AG	Regression of GRAB-DA AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.614; Slope = 1.215; $p < 0.001$	n = 13 mice

3AH	Regression of GRAB-DA AUC with bout duration of 100% Ensure consumption	Linear regression	R = 0.838; Slope = 2.364; $p < 0.001$	n = 13 mice
3AI	AUC of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p < 0.001$	n = 13 mice
3AJ	Slope of 20% vs 100% Ensure consumption	Paired t-test	20%E vs 100%E: $p < 0.001$	n = 13 mice
4J	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption with calibrated photostimulation	Linear regression	R = 0.76; Slope = 2.31; $p < 0.001$	n = 13 mice
4K	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.21; Slope = -0.48; $p < 0.001$	n = 13 mice
4L	AUC of OFF and ON period	Paired t-test	OFF vs ON: $p < 0.001$	n = 13 mice
4M	Slope of OFF and ON period	Paired t-test	OFF vs ON: $p < 0.001$	n = 13 mice
4N	Licks/block	Paired t-test	OFF vs ON: $p < 0.001$	n = 13 mice
4O	Mode of ILI	Paired t-test	OFF vs ON: $p = 0.186$	n = 13 mice
4P	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p < 0.001$	n = 13 mice
4Q	Bout duration	Paired t-test	OFF vs ON: $p < 0.001$	n = 13 mice
4R	Bouts	Paired t-test	OFF vs ON: $p = 0.386$	n = 13 mice
4S	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 597.05$, $p < 0.001$	n = 13 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 9944.7$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 207.37$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.345	

			1–2 s: p = 0.893 2–3 s: p = 0.659 3–4 s: p = 0.044 4–5 s: p < 0.001 5–6 s: p < 0.001 6–7 s: p < 0.001 7–8 s: p < 0.001 8–9 s: p < 0.001 9–10 s: p < 0.001 10–11 s: p < 0.001 11–12 s: p < 0.001 12–13 s: p < 0.001 13–14 s: p < 0.001 14–15 s: p < 0.001 15–16 s: p < 0.001 16–17 s: p < 0.001 17–18 s: p < 0.001 18–19 s: p < 0.001 19–20 s: p < 0.001	
4T	Licks/block	Paired t-test	OFF vs ON: *p = 0.986*	n = 8 mice
4U	Mode of ILI	Paired t-test	OFF vs ON: *p = 0.050*	n = 8 mice
4V	Proportions of bouts vs bout duration	KS test	OFF vs ON: *p = 0.772*	n = 8 mice
4W	Bout duration	Paired t-test	OFF vs ON: *p = 0.194*	n = 8 mice
4X	Bouts	Paired t-test	OFF vs ON: *p = 0.062*	n = 8 mice
4Y	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 26.301$, $p = 0.156$	n = 8 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 6395.9$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 19.371$, $p = 0.433$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.855 1–2 s: p = 0.855 2–3 s: p = 0.971 3–4 s: p = 0.971 4–5 s: p = 0.855 5–6 s: p = 0.617 6–7 s: p = 0.971 7–8 s: p = 0.896	

			8–9 s: p = 0.971 9–10 s: p = 0.896 10–11 s: p = 0.715 11–12 s: p = 0.324 12–13 s: p = 0.855 13–14 s: p = 0.117 14–15 s: p = 0.715 15–16 s: p = 0.896 16–17 s: p = 0.971 17–18 s: p = 0.263 18–19 s: p = 0.263 19–20 s: p = 0.263	
4AC	Bout duration	Paired t-test	OFF vs ON: *p = 0.008*	n = 5 mice
4AD	Bouts	Paired t-test	OFF vs ON: *p = 0.495*	n = 5 mice
5C	Licks/block	Paired t-test	OFF vs ON: *p = 0.038*	n = 6 mice
5D	Bout duration	Paired t-test	OFF vs ON: *p = 0.023*	n = 6 mice
5E	Bouts	Paired t-test	OFF vs ON: *p = 0.926*	n = 6 mice
5F	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 102.04$, $p < 0.001$	n = 6 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 2187.1$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 46.584$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.772 1–2 s: p = 0.748 2–3 s: p = 0.650 3–4 s: p = 0.858 4–5 s: p = 0.240 5–6 s: p = 0.007 6–7 s: p = 0.007 7–8 s: p = 0.050 8–9 s: p = 0.013 9–10 s: p = 0.007 10–11 s: p = 0.002 11–12 s: p = 0.002 12–13 s: p = 0.003 13–14 s: p = 0.061 14–15 s: p = 0.033 15–16 s: p = 0.015	

			16–17 s: p = 0.007 17–18 s: p = 0.013 18–19 s: p = 0.013 19–20 s: p = 0.007	
5G	Licks/block	Paired t-test	OFF vs ON: *p = 0.985*	n = 6 mice
5H	Bout duration	Paired t-test	OFF vs ON: *p = 0.153*	n = 6 mice
5I	Bouts	Paired t-test	OFF vs ON: *p = 0.133*	n = 6 mice
5J	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 29.036$, $p = 0.087$	n = 6 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 3196.6$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 26.825$, $p = 0.109$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.586 1–2 s: p = 0.646 2–3 s: p = 0.583 3–4 s: p = 0.440 4–5 s: p = 0.727 5–6 s: p = 0.440 6–7 s: p = 0.821 7–8 s: p = 0.583 8–9 s: p = 0.583 9–10 s: p = 0.583 10–11 s: p = 0.433 11–12 s: p = 0.433 12–13 s: p = 0.287 13–14 s: p = 0.433 14–15 s: p = 0.732 15–16 s: p = 0.433 16–17 s: p = 0.568 17–18 s: p = 0.599 18–19 s: p = 0.821 19–20 s: p = 0.238	
6B	Normalized body weight during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction;	Factor A (Treatment): F (1, 14) = 85.52, *p < 0.001*;	n = 8 mice
			Factor B (Time): F (1.986, 27.80) = 7.881, *p = 0.002*;	
			A * B (Interaction): F (4, 56) = 5.607, *p < 0.001*;	

		Holm-Šídák's multiple comparisons test	Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 5.289, $p < 0.001$; Day 2: t = 7.153, $p < 0.001$; Day 3: t = 8.674, $p < 0.001$; Day 4: t = 8.491, $p < 0.001$; Day 5: t = 8.616, $p < 0.001$;	
6C	Homecage chow intake during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 30.50, $p < 0.001$; Factor B (Time): F (2.098, 29.37) = 2.285, $p = 0.117$; A * B (Interaction): F (4, 56) = 3.983, $p = 0.007$; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 8.895, $p < 0.001$; Day 2: t = 6.863, $p < 0.001$; Day 3: t = 1.396, $p = 0.253$; Day 4: t = 5.137, $p = 0.001$; Day 5: t = 1.591, $p = 0.253$;	n = 8 mice
6D	Ensure intake during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 21.69, $p < 0.001$; Factor B (Time): F (2.665, 37.31) = 18.01, $p < 0.001$; A * B (Interaction): F (4, 56) = 14.93, $p < 0.001$; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 8.677, $p < 0.001$; Day 2: t = 7.545, $p < 0.001$; Day 3: t = 2.685, $p = 0.049$; Day 4: t = 2.764, $p = 0.049$; Day 5: t = 0.737, $p = 0.475$;	n = 8 mice
6E	Bout duration during days 1-5 with PBS treatment versus semaglutide treatment	Two-way ANOVA; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 70) = 7.783, $p = 0.007$; Factor B (Time): F (2.883, 40.36) = 2.523, $p = 0.073$; A * B (Interaction): F (4, 70) = 0.366, $p = 0.832$; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 1.881, $p = 0.282$ Day 2: t = 1.881, $p = 0.282$; Day 3: t = 0.913, $p = 0.672$; Day 4: t = 1.021, $p = 0.672$; Day 5: t = 0.541, $p = 0.672$;	n = 8 mice

6F	Bout numbers during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 0.121, *p = 0.734* Factor B (Time): F (3.165, 44.31) = 5.514, *p = 0.002*; A * B (Interaction): F (4, 56) = 3.086, *p = 0.023*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 3.267, *p = 0.035*; Day 2: t = 0.294, *p = 0.987*; Day 3: t = 0.251, *p = 0.987*; Day 4: t = 0.452, *p = 0.987*; Day 5: t = 0.308, *p = 0.987*;	n = 8 mice
6H	Normalized body weight during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 57.55, *p < 0.001*; Factor B (Time): F (3.034, 42.48) = 0.867, *p = 0.467*; A * B (Interaction): F (4, 56) = 2.163, *p = 0.085*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 4.800, *p < 0.001*; Day 2: t = 6.316, *p < 0.001*; Day 3: t = 6.855, *p < 0.001*; Day 4: t = 5.192, *p < 0.001*; Day 5: t = 6.362, *p < 0.001*;	n = 8 mice
6I	Homecage chow intake during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 6.077, *p = 0.027*; Factor B (Time): F (2.429, 34.01) = 3.702, *p = 0.028*; A * B (Interaction): F (4, 56) = 7.763, *p < 0.001*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 6.368, *p < 0.001*; Day 2: t = 2.474, *p = 0.104*; Day 3: t = 2.569, *p = 0.104*; Day 4: t = 0.145, *p = 0.887*; Day 5: t = 2.119, *p = 0.105*;	n = 8 mice
6J	Ensure intake during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction;	Factor A (Treatment): F (1, 14) = 9.816, *p = 0.007*; Factor B (Time): F (2.927, 40.97) = 6.726, *p < 0.001*; A * B (Interaction): F (4, 56) = 8.298, *p < 0.001*;	n = 8 mice

		Holm-Šídák's multiple comparisons test	Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 6.904, *p < 0.001*; Day 2: t = 4.670, *p = 0.004*; Day 3: t = 1.035, *p = 0.686*; Day 4: t = 0.887, *p = 0.686*; Day 5: t = 0.028, *p = 0.978*;	
6K	Bout duration during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 1.294, *p = 0.274*; Factor B (Time): F (3.063, 42.88) = 1.250, *p = 0.304*; A * B (Interaction): F (4, 56) = 7.053, *p<0.001*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 3.922, *p = 0.021*; Day 2: t = 3.417, *p = 0.027*; Day 3: t = 0.078, *p = 0.940* Day 4: t = 1.614, *p = 0.339*; Day 5: t = 1.494, *p = 0.339*;	n = 8 mice
6L	Bout numbers during days 1-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 4.039, *p = 0.064*; Factor B (Time): F (3.530, 49.42) = 6.384, *p < 0.001*; A * B (Interaction): F (4, 56) = 0.803, *p = 0.529*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 3.223, *p = 0.052*; Day 2: t = 0.889, *p = 0.549*; Day 3: t = 1.023, *p = 0.549*; Day 4: t = 1.649, *p = 0.405*; Day 5: t = 1.337, *p = 0.497*;	n = 8 mice
6N	AUC during days 0-5 with PBS treatment versus semaglutide treatment	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 14) = 0.050, *p = 0.826*; Factor B (Time): F (3.207, 44.89) = 0.359, *p = 0.796*; A * B (Interaction): F (5, 70) = 2.430, *p = 0.043*; Post hoc Holm-Šídák's multiple comparisons test Day 0: t = 0.150, *P =0.883*; Day 1: t = 2.441, *p = 0.033*; Day 2: t = 1.215, *p = 0.244*; Day 3: t = 0.378, *p = 0.715*; Day 4: t = 1.101, *p = 0.291*;	n = 8 mice

			Day 5: t = 1.865, *p = 0.091*;	
7C	Proportions of lick vs licking rate	KS test	OFF vs ON: *p = 0.003*	n = 10 mice
7D	Bout duration	Paired t-test	OFF vs ON: *p = 0.020*	n = 10 mice
7E	Bouts	Paired t-test	OFF vs ON: *p = 0.654*	n = 10 mice
7I	Daily Ensure intake of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 0.9492, *p = 0.355*; Factor B (Time): F (1.702, 15.32) = 45.53, *p < 0.001*; A * B (Interaction): F (1.287, 11.58) = 0.7985, *p = 0.421;* Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 0.580, *p = 0.741*; Day 2: t = 1.333, *p = 0.517*; Day 3: t = 0.718, *p = 0.741*;	n = 10 mice
7I	Daily Ensure intake of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 18.89, *p = 0.002*; Factor B (Time): F (1.747, 15.72) = 3.100, *p = 0.079*; A * B (Interaction): F (1.989, 17.90) = 2.570, *p = 0.105;* Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 3.263, *p = 0.024*; Day 5: t = 1.039, *p = 0.326*; Day 6: t = 3.381, *p = 0.024*;	n = 10 mice
7J	Averaged Ensure intake of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.355*; OFF vs ON (day 4-6): *p = 0.002*	n = 10 mice
7K	Daily bout duration of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 0.029, *p = 0.869*; Factor B (Time): F (1.715, 15.44) = 8.059, *p = 0.005*; A * B (Interaction): F (1.719, 15.47) = 0.297, *p = 0.715*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 0.486, *p = 0.925*; Day 2: t = 0.576, *p = 0.925*; Day 3: t = 0.351, *p = 0.925*;	n = 10 mice

7K	Daily bout duration of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 18.31, *p = 0.002*; Factor B (Time): F (1.644, 14.80) = 3.370, *p = 0.070*; A * B (Interaction): F (1.311, 11.80) = 1.881, *p = 0.198*; Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 3.794, *p = 0.013*; Day 5: t = 3.632, *p = 0.013*; Day 6: t = 2.775, *p = 0.021*;	n = 10 mice
7L	Averaged bout duration of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.869;* OFF vs ON (day 4-6): *p = 0.002*	n = 10 mice
7M	Daily bout numbers of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 0.787, *p = 0.398*; Factor B (Time): F (1.415, 12.73) = 2.714, *p = 0.116*; A * B (Interaction): F (1.781, 16.03) = 1.858, *p = 0.190*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 1.076, *p = 0.524*; Day 2: t = 1.964, *p = 0.224*; Day 3: t = 0.179, *p = 0.862*;	n = 10 mice
7M	Daily bout numbers of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 9.000) = 4.520, *p = 0.062*; Factor B (Time): F (1.334, 12.01) = 1.451, *p = 0.263*; A * B (Interaction): F (1.898, 17.09) = 0.655, *p = 0.524*; Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 2.187, *p = 0.160*; Day 5: t = 0.825, *p = 0.676*; Day 6: t = 0.220, *p = 0.831*;	n = 10 mice
7N	Averaged bout numbers of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.398;* OFF vs ON (day 4-6): *p = 0.969*	n = 10 mice

S2G	Proportions of bouts vs bout duration	KS test	20%E vs 100%E: $p < 0.001$	n = 11 mice
S2H	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 1919.9$, $p < 0.001$	n = 11 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 4018.7$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 520.89$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.101 1–2 s: p < 0.001 2–3 s: p < 0.001 3–4 s: p < 0.001 4–5 s: p < 0.001 5–6 s: p < 0.001 6–7 s: p < 0.001 7–8 s: p < 0.001 8–9 s: p < 0.001 9–10 s: p < 0.001 10–11 s: p < 0.001 11–12 s: p < 0.001 12–13 s: p < 0.001 13–14 s: p < 0.001 14–15 s: p < 0.001 15–16 s: p < 0.001 16–17 s: p < 0.001 17–18 s: p < 0.001 18–19 s: p < 0.001 19–20 s: p < 0.001	
S2J	Proportions of bouts vs bout duration	KS test	100%E+Q vs 100%E: $p < 0.001$	n = 7 mice
S2K	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 1042.3$, $p < 0.001$	n = 7 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 1305.5$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 350.15$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment:	

			0–1 s: p = 0.172 1–2 s: p = 0.051 2–3 s: p < 0.001 3–4 s: p < 0.001 4–5 s: p < 0.001 5–6 s: p < 0.001 6–7 s: p < 0.001 7–8 s: p < 0.001 8–9 s: p < 0.001 9–10 s: p < 0.001 10–11 s: p < 0.001 11–12 s: p < 0.001 12–13 s: p < 0.001 13–14 s: p < 0.001 14–15 s: p < 0.001 15–16 s: p < 0.001 16–17 s: p < 0.001 17–18 s: p < 0.001 18–19 s: p < 0.001 19–20 s: p < 0.001	
S3D	Place preference	Repeated measures ANOVA; Holm-Šídák's multiple comparisons test	Factor A (Photostimulation): F (2, 6) = 7.032, $p = 0.027$ Pre-test vs Cond1: t = 3.508, $p = 0.025$; Pre-test vs Cond2: t = 2.902, $p = 0.027$	n = 4 mice
S3E	Licks/block	Paired t-test	OFF vs ON: $p = 0.904$	n = 5 mice
S3F	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p = 0.893$	n = 5 mice
S3G	Bout duration	Paired t-test	OFF vs ON: $p = 0.904$	n = 5 mice
S3H	Bouts	Paired t-test	OFF vs ON: $p = 0.143$	n = 5 mice
S4E	Proportions of cells	Unpaired t-test	VGAT/GFP vs TH/GFP: $p < 0.001$	n = 22 VTA sections from 2 mice
S6J	AUC of before vs during food consumption	Paired t-test	Pre-100E vs 100E: $p = 0.128$	n = 4 mice
S7F	Regression of GCaMP8m AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.531; Slope = 0.065; $p = 0.022$	n = 4 mice
S7G	Regression of GCaMP8m AUC with bout duration of	Linear regression	R = 0.812; Slope = 0.948; $p < 0.001$	n = 4 mice

	100% Ensure consumption			
S7K	Regression of GFP AUC with bout duration of 20% Ensure consumption	Linear regression	R = -0.434; Slope = -0.037; $p = 0.131$	n = 3 mice
S8A	Regression of bout duration of 20% Ensure consumption with bout indices across feeding session	Linear regression	R = 0.02; Slope = -0.001; $p = 0.904$	n = 13 mice
S8B	Regression of GCaMP8m AUC with bout indices across feeding session	Linear regression	R = -0.031; Slope = -0.013; $p = 0.003$	n = 13 mice
S8C	Regression of bout duration of 100% Ensure consumption with bout indices across feeding session	Linear regression	R = 0.014; Slope = -0.001; $p = 0.987$	n = 13 mice
S8D	Regression of GCaMP8m AUC with bout indices across feeding session	Linear regression	R = -0.167; Slope = -0.082; $p < 0.001$	n = 13 mice
S8E	Calories of 20% vs 100% Ensure consumption	Paired t-test	$p < 0.001$	n = 13 mice
S9F	Regression of GCaMP8m AUC with bout duration of 100% Ensure consumption before PBS injection	Linear regression	R = 0.754; Slope = 0.659; $p < 0.001$	n = 7 mice
S9G	Regression of GCaMP8m AUC with bout duration of 100% Ensure consumption after PBS injection	Linear regression	R = 0.537; Slope = 0.321; $p < 0.001$	n = 7 mice
S9M	Regression of GCaMP8m AUC with bout duration of	Linear regression	R = 0.588; Slope = 0.245; $p < 0.001$	n = 7 mice

	100% Ensure consumption before LiCl injection			
S9N	Regression of GCaMP8m AUC with bout duration of 100% Ensure consumption after LiCl injection	Linear regression	R = 0.328; Slope = -0.092; *p = 0.038*	n = 7 mice
S9O	AUC of 100% Ensure consumption before and after PBS treatment versus before and after LiCl treatment	1-Factor (Time) Repeated measures ANOVA	Factor A (Treatment): F (1, 12) = 0.295, *p = 0.597*; Factor B (Time): F (1, 12) = 12.66, *p = 0.004*; A * B (Interaction): F (1, 12) = 4.822, *p = 0.049*;	n = 7 mice
S10F	Regression of GCaMP8m AUC with bout duration of 20% Ensure consumption for FED mice	Linear regression	R = 0.489; Slope = 0.231; *p < 0.001*	n = 7 mice
S10G	Regression of GCaMP8m AUC with bout duration of 20% Ensure consumption for RES mice	Linear regression	R = 0.573; Slope = 0.367; *p < 0.001*	n = 7 mice
S10H	AUC of 20% Ensure consumption FED vs RES	Paired t-test	FED *vs* RES: *p = 0.035*	n = 7 mice
S10I	Slope of 20% Ensure consumption FED vs RES	Paired t-test	FED *vs* RES: *p = 0.006*	n = 7 mice
S11A	Bout duration of 20% vs 100% Ensure consumption in separate sessions	Paired t-test	*p = 0.335*	n = 13 mice
S11A	Bout duration of 20% vs 100% Ensure consumption within a session	Paired t-test	*p < 0.001*	n = 8 mice
S11B	Pearson correlation coefficient of 20% vs 100% Ensure consumption in separate sessions	Paired t-test	*p = 0.153*	n = 13 mice

S11B	Pearson correlation coefficient of 20% vs 100% Ensure consumption within a session	Paired t-test	$p < 0.001$	n = 8 mice
S12F	Regression of GCaMP8m AUC with bout duration of 100% Ensure with quinine consumption	Linear regression	R = 0.180; Slope = 0.297; $p < 0.001$	n = 7 mice
S12G	Regression of GCaMP8m AUC with bout duration of 100% Ensure consumption	Linear regression	R = 0.704; Slope = 2.390; $p < 0.001$	n = 7 mice
S12H	AUC of 100% Ensure with quinine vs 100% Ensure consumption	Paired t-test	100% Ensure with quinine vs 100% Ensure: $p = 0.002$	n = 7 mice
S12I	Slope of 100% Ensure with quinine vs 100% Ensure consumption	Paired t-test	100% Ensure with quinine vs 100% Ensure: $p = 0.021$	n = 7 mice
S13A	Licks/block	Paired t-test	OFF vs ON: $p = 0.433$	n = 6 mice
S13B	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p = 0.912$	n = 6 mice
S13C	Bout duration	Paired t-test	OFF vs ON: $p = 0.863$	n = 6 mice
S13D	Bouts	Paired t-test	OFF vs ON: $p = 0.357$	n = 6 mice
S13E	Regression of bout duration of 20% Ensure consumption with bout indices across feeding session	Linear regression	R = 0.011; Slope = 0.006; $p = 0.188$	n = 13 mice
S13F	Regression of GCaMP8s AUC with bout indices across feeding session	Linear regression	R = -0.08; Slope = -0.02; $p < 0.001$	n = 13 mice
S13G	Regression of bout duration of 20% Ensure consumption and calibrated photostimulation with bout indices	Linear regression	R = -0.01; Slope = 0.003; $p = 0.680$	n = 13 mice

	across feeding session			
S13H	Regression of GCaMP8s AUC with bout indices across feeding session	Linear regression	R = -0.03; Slope = -0.04; *p = 0.029*	n = 13 mice
S13I	Calories of OFF and ON periods	Paired t-test	*p = 0.002*	n = 13 mice
S14E	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption	Linear regression	R = 0.002; Slope = -0.501; *p < 0.001*	n = 8 mice
S14F	Regression of GCaMP8s AUC with bout duration of 20% Ensure consumption with contingent photostimulation of high laser intensity	Linear regression	R = 0.833; Slope = 3.029; *p < 0.001*	n = 8 mice
S14G	AUC of OFF and ON period	Paired t-test	OFF vs ON: *p < 0.001*	n = 8 mice
S14H	Slope of OFF and ON period	Paired t-test	OFF vs ON: *p < 0.001*	n = 8 mice
S14I	Licks/block	Paired t-test	OFF vs ON: *p < 0.001*	n = 8 mice
S14J	ILI	Paired t-test	OFF vs ON: *p = 0.478*	n = 8 mice
S14K	Proportions of bouts vs bout duration	KS test	OFF vs ON: *p < 0.001*	n = 8 mice
S14L	Bout duration	Paired t-test	OFF vs ON: *p < 0.001*	n = 8 mice
S14M	Bouts	Paired t-test	OFF vs ON: *p = 0.782*	n = 8 mice
S14N	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 491.1$, $p < 0.001$	n = 8 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 7933.2$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 219.05$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.835	

			1–2 s: p = 0.340 2–3 s: p = 0.688 3–4 s: p = 0.112 4–5 s: p = 0.005 5–6 s: p < 0.001 6–7 s: p < 0.001 7–8 s: p < 0.001 8–9 s: p < 0.001 9–10 s: p < 0.001 10–11 s: p < 0.001 11–12 s: p < 0.001 12–13 s: p < 0.001 13–14 s: p < 0.001 14–15 s: p < 0.001 15–16 s: p < 0.001 16–17 s: p < 0.001 17–18 s: p < 0.001 18–19 s: p < 0.001 19–20 s: p < 0.001	
S14O	Regression of bout duration of 20% Ensure consumption with bout indices across each feeding session	Linear regression	R = -0.14; Slope = -0.008; *p = 0.065*	n = 8 mice
S14P	Regression of GCaMP8s AUC with bout indices across each feeding session	Linear regression	R = -0.07; Slope = -0.001; *p = 0.701*	n = 8 mice
S14Q	Regression of bout duration of 20% Ensure consumption and calibrated photostimulation with bout indices across each feeding session	Linear regression	R = -0.08; Slope = 0.01; *p = 0.180*	n = 8 mice
S14R	Regression of GCaMP8s AUC with bout indices across each feeding session	Linear regression	R = -0.01; Slope = 0.04; *p = 0.240*	n = 8 mice
S14S	Calories of Laser-OFF and Laser-ON periods	Paired t-test	*p < 0.001*	n = 8 mice

S14T	Regression of lick frequency with blocks across feeding sessions (20% Ensure)	Linear regression	R = -0.25; Slope = - 0.01; $p = 0.005$	n = 1 mouse
S14U	Regression of lick frequency with blocks across of feeding session (20% Ensure and high laser photostimulation)	Linear regression	R = -0.03; Slope = 0.002; $p = 0.763$	n = 1 mouse
S14V	Pearson correlation coefficient of 20% Ensure consumption vs 20% Ensure consumption and high laser photostimulation	Paired t-test	$p = 0.003$	n = 8 mice
S14W	Regression of lick frequency with blocks across feeding sessions (20% Ensure)	Linear regression	R = 0.14; Slope = 0.01; $p = 0.020$	n = 1 mouse
S14X	Regression of lick frequency with blocks across of feeding session (20% Ensure and calibrated photostimulation)	Linear regression	R = -0.01; Slope = 0.001; $p = 0.853$	n = 1 mouse
S14Y	Pearson correlation coefficient of 20% Ensure consumption vs 20% Ensure consumption and calibrated photostimulation	Paired t-test	$p = 0.776$	n = 13 mice
S15H	Regression of GRAB-DA2m AUC with bout duration of 20% Ensure consumption	Linear regression	R = -0.262; Slope = 0.149; $p < 0.001$	n = 5 mice
S15I	Regression of GRAB-DA2m AUC with bout duration of	Linear regression	R = 0.947; Slope = 2.865; $p < 0.001$	n = 5 mice

	20% Ensure consumption with calibrated photostimulation			
S15J	AUC of OFF and ON period	Paired t-test	OFF vs ON: $p = 0.002$	n = 5 mice
S15K	Slope of OFF and ON period	Paired t-test	OFF vs ON: $p < 0.001$	n = 5 mice
S16E	Regression of GRAB-DA2m AUC with bout duration of 20% Ensure consumption	Linear regression	R = -0.514; Slope = 0.022; $p = 0.187$	n = 5 mice
S16F	Regression of GRAB-DA2m AUC with bout duration of 20% Ensure consumption with high-intensity laser photostimulation	Linear regression	R = 0.849; Slope = 4.957; $p < 0.001$	n = 5 mice
S16G	AUC of OFF and ON period	Paired t-test	OFF vs ON: $p = 0.003$	n = 5 mice
S16H	Slope of OFF and ON period	Paired t-test	OFF vs ON: $p = 0.002$	n = 5 mice
S16I	Licks/block	Paired t-test	OFF vs ON: $p = 0.022$	n = 5 mice
S16J	Mode of ILI	Paired t-test	OFF vs ON: $p = 0.457$	n = 5 mice
S16K	Proportions of bouts vs bout duration	KS test	OFF vs ON: $p < 0.001$	n = 5 mice
S16L	Bout duration	Paired t-test	OFF vs ON: $p = 0.004$	n = 5 mice
S16M	Bouts	Paired t-test	OFF vs ON: $p = 0.461$	n = 5 mice
S16N	Licking rate	Negative binomial generalized linear mixed models	Likelihood ratio test: Factor A (Treatment): $Chi^2(20) = 437.6$, $p < 0.001$	n = 5 mice
			Likelihood ratio test: Factor B (Time): $Chi^2(38) = 3677.2$, $p < 0.001$	
			Likelihood ratio test: A * B (Interaction): $Chi^2(19) = 186.36$, $p < 0.001$	
			Wald tests with Benjamini-Hochberg P-value adjustment: 0–1 s: p = 0.363 1–2 s: p = 0.168 2–3 s: p = 0.147 3–4 s: p = 0.136	

			4–5 s: $p < 0.001$ 5–6 s: $p = 0.017$ 6–7 s: $p < 0.001$ 7–8 s: $p < 0.001$ 8–9 s: $p < 0.001$ 9–10 s: $p < 0.001$ 10–11 s: $p < 0.001$ 11–12 s: $p < 0.001$ 12–13 s: $p < 0.001$ 13–14 s: $p < 0.001$ 14–15 s: $p < 0.001$ 15–16 s: $p < 0.001$ 16–17 s: $p < 0.001$ 17–18 s: $p < 0.001$ 18–19 s: $p < 0.001$ 19–20 s: $p < 0.001$	
S17B	Bout duration at first and second half sessions during the laser-OFF period	Paired t-test	First vs Second: $p = 0.653$	n = 6 mice
S17C	Bout duration of first and second half sessions during the laser-ON period	Paired t-test	First vs Second: $p = 0.609$	n = 6 mice
S17D	Bout duration at first and second half sessions during the laser-OFF period	Paired t-test	First vs Second: $p = 0.970$	n = 6 mice
S17E	Bout duration of first and second half sessions during the laser-ON period	Paired t-test	First vs Second: $p = 0.007$	n = 6 mice
S17F	Mode of ILI	Paired t-test	OFF vs ON: $p = 0.384$	n = 6 mice
S17G	Mode of ILI	Paired t-test	OFF vs ON: $p = 0.289$	n = 6 mice
S19D	Daily Ensure intake of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 8.000) = 0.280, $p = 0.735$; Factor B (Time): F (1.804, 14.43) = 7.288, $p = 0.008$; A * B (Interaction): F (1.319, 10.55) = 1.893, $p = 0.200$ Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 1.418, $p = 0.418$; Day 2: t = 1.527, $p = 0.418$;	n = 9 mice

			Day 3: t = 0.479, *p = 0.645*;	
S19D	Daily Ensure intake of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 8.000) = 0.007, *p = 0.934*; Factor B (Time): F (2, 16) = 1.044, *p = 0.375*; A * B (Interaction): F (2, 16) = 1.948, *p = 0.175*; Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 0.135, *p = 0.895*; Day 5: t = 1.256, *p = 0.403*; Day 6: t = 1.519, *p = 0.382*;	n = 9 mice
S19E	Averaged Ensure intake of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.611;* OFF vs ON (day 4-6): *p = 0.934*	n = 9 mice
S19F	Daily bout duration of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1, 48) = 0.042, *p = 0.839*; Factor B (Time): F (2, 48) = 2.328, *p = 0.108*; A * B (Interaction): F (2, 48) = 0.444, *p = 0.644*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 0.761, *p = 0.718*; Day 2: t = 1.111, *p = 0.655*; Day 3: t = 0.140, *p = 0.892*;	n = 9 mice
S19F	Daily bout duration of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 8.000) = 1.578, *p = 0.244*; Factor B (Time): F (1.123, 8.981) = 1.773, *p = 0.219*; A * B (Interaction): F (1.777, 14.22) = 1.018, *p = 0.377*; Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 0.100, *p = 0.923*; Day 5: t = 0.939, *p = 0.610*; Day 6: t = 1.909, *p = 0.253*;	n = 9 mice
S19G	Averaged bout duration of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.860;* OFF vs ON (day 4-6): *p = 0.244*	n = 9 mice

S19H	Daily bout numbers of day 1-3 semaglutide treatment during Block 1 compared to Block 2	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 8.000) = 0.001, *p = 0.972*; Factor B (Time): F (1.852, 14.81) = 2.205, *p = 0.148*; A * B (Interaction): F (1.591, 12.73) = 0.331, *p = 0.676*; Post hoc Holm-Šídák's multiple comparisons test Day 1: t = 0.517, *p* = 0.945; Day 2: t = 0.370, *p* = 0.945; Day 3: t = 0.326, *p* = 0.945;	n = 9 mice
S19H	Daily bout numbers of day 4-6 semaglutide treatment during the laser-OFF blocks compared to laser-ON blocks	Repeated measures ANOVA with the Geisser-Greenhouse correction; Holm-Šídák's multiple comparisons test	Factor A (Treatment): F (1.000, 8.000) = 2.459, *p = 0.156*; Factor B (Time): F (1.258, 10.07) = 0.649, *p = 0.474*; A * B (Interaction): F (1.686, 13.49) = 0.285, *p = 0.720*; Post hoc Holm-Šídák's multiple comparisons test Day 4: t = 0.640, *p = 0.788*; Day 5: t = 1.993, *p = 0.225*; Day 6: t = 0.543, *p = 0.788*;	n = 9 mice
S19I	Averaged bout numbers of day 1-3 or day 4-6 semaglutide treatment during different blocks	Paired t-test	B1 vs B2 (day 1-3): *p = 0.972;* OFF vs ON (day 4-6): *p = 0.156*	n = 9 mice