A hindbrain dopaminergic neural circuit prevents weight gain by reinforcing food satiation

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The neural circuitry mechanism that underlies dopaminergic (DA) control of innate feeding behavior is largely uncharacterized. Here, we identified a subpopulation of DA neurons situated in the caudal ventral tegmental area (cVTA) directly innervating DRD1-expressing neurons within the lateral parabrachial nucleus (LPBN). This neural circuit potently suppresses food intake via enhanced satiation response. Notably, this cohort of DA⁵VTA neurons is activated immediately before the cessation of each feeding bout. Acute inhibition of these DA neurons before bout termination substantially suppresses satiety and prolongs the consummatory feeding. Activation of postsynaptic DRD1⁵LPBN neurons inhibits feeding, whereas genetic deletion of Drd1 within the LPBN causes robust increase in food intake and subsequent weight gain. Furthermore, the DRD1⁵LPBN signaling manifests the central mechanism in methylphenidate-induced hypophagia. In conclusion, our study illuminates a hindbrain DAergic circuit that controls feeding through dynamic regulation in satiety response and meal structure.

INTRODUCTION

Feeding, an evolutionarily conserved process key to survival, is orchestrated by multiplexed neural circuits in response to diverse peripheral signals (1–3). Feeding behavior is composed of appetitive feeding as characterized by seeking and approaching rewarding stimuli such as food and consummatory feeding involving meal initiation, engagement, and satiation (4, 5). In particular, food satiation is a critical process that governs the size and termination of a meal (6, 7). Peripheral signals such as cholecystokinin and glucagon-like peptide 1 elicit impact on satiation through the afferent vagus nerve that, in turn, excites neurons within the nucleus of the solitary tract (NTS) and parabrachial nucleus (PBN) of the hindbrain (8–10). While emerging evidence suggests that the satiety-defeating overeating fundamentally contributes to weight gain and obesity (11), the key neural circuits and neurotransmitter systems underlying food satiation remain poorly understood.

The PBN, previously known for relaying taste, somatosensory, and visceral signals to the forebrain, has an established critical role in control of feeding, glucose metabolism, and body weight (12–17). Some recent studies have shown that GABAergic projections from the hypothalamic agouti-related peptide (AgRP) neurons to the PBN are critical for transmitting hunger signals from the periphery (15). Acute ablation of AgRP neurons or genetic disruption of their GABA (γ-aminobutyric acid) biosynthesis leads to anorexia and severe weight loss (18, 19). This lethal phenotype can be rescued by enhancing GABAₐ₅R signaling or by suppressing NMDAR (N-methyl-D-aspartate receptor) signaling within the PBN during a critical adaptation period (15, 16). The PBN is innervated densely by the excitatory inputs from the NTS, which, in turns, receives serotonergic afferents from the caudal raphe nuclei, forming a circuitry that inhibits food intake (20). However, the circuitry mechanism as to how the PBN neurons are involved in food satiation is not yet known.

Emerging evidence shows that dopaminergic (DA) neurons located in the ventral tegmental area (VTA) are highly heterogeneous in both their anatomical localization and functional significance (21–24). Previous studies showed that the forebrain DA signaling system plays a role in the control of food reward and food-conditioned stereotypic behavior (25–28). However, intra–nucleus accumbens (Acb) infusion of either DRD1 agonist, DRD2 agonist, or DRD1/DRD2 antagonists fails to affect food intake (29), and DA depletion in the forebrain or within the Acb does not affect appetitive taste reactivity for food (30). Genetic inactivation of DA transporter or injection of amphetamine into the Acb, both resulting into DA release, fails to enhance appetitive response to sucrose (31, 32). Chemosynaptic activation of the mesolimbic VTA→Acb circuit does not affect total food intake (33). These studies implicate that the potential role of the forebrain DA signaling on innate feeding behavior and body weight control might be inadvertently masked or secondary to those stereotypic responses (34). On the other hand, the DRD1 signaling is distributed abundantly within the PBN, yet with no physiological role established (35, 36). Systemic treatment of DRD1 agonist into mice has been shown to suppress the feeding of palatable diets (37, 38), whereas pretreatment with a DRD1 antagonist was sufficient to block amphetamine-induced anorexia (39). Although these studies implicated an inhibitory role of DA in control of feeding, the mechanistic significance of the DAergic circuit in food satiation remains poorly understood (4, 34, 40).

In this study, we revealed the functional role of a previously unidentified DAergic neural circuit comprising of DA⁵VTA neurons and DRD1⁵LPBN neurons on the control of consummatory feeding. We examined the neural activities of DA⁵VTA neurons during an integrated eating bout. We observed notably enhanced neural activities of DA⁵VTA neurons immediately before the bout termination. Genetic inhibition of these neurons prolonged the bout duration and led to an increase in food intake. We further showed that enhanced neural activities of DRD1⁵LPBN neurons are associated with meal termination. Last, we tested and validated the hypothesis that methylphenidate (MPH)–induced hypophagia and weight loss were mediated by the DRD1⁵LPBN signaling pathway. Our findings suggest that the neural circuit from DA⁵VTA neurons to DRD1⁵LPBN neurons plays a critical role in the suppression of consummatory feeding by governing food satiation.
RESULTS
We found that the DA VTA axonal terminals were not only distributed in several forebrain structures such as the Acb but were also shown with high density within the lateral PBN (LPBN) (fig. S1). Using a viral retrograde-tracing technique (Fig. 1A), our results showed that ~50% of LPBN-projecting VTA neurons or Acb-projecting VTA neurons are DA neurons (Fig. 1, B to E, and fig. S2). Most of the VTA neurons projecting to the LPBN were located within the caudal VTA (cVTA), which shows that the VTA neurons projecting to the LPBN and Acb were two cohorts of nonoverlapping neurons (Fig. 1, C to G, and fig. S2). These results reveal the existence of a distinctive cVTA→LPBN neural circuit.

To examine the function of this neural circuit on controlling feeding, we performed optogenetic stimulation of DA VTA axonal terminals within the LPBN and Acb by expressing channelrhodopsin (ChR2) in cVTA neurons and placing fiber-optic cannulae in the projection areas. Photostimulation of the cVTA→LPBN circuit significantly suppressed fasting-induced intake of chow diet while locomotive activity remained intact (Fig. 1, H and J). In contrast, activation of the VTA→Acb circuit significantly increased locomotive activity without affecting feeding (Fig. 1, I and J). To perform more precise manipulation of DA VTA→LPBN neurons, we used a combinatorial strategy (41) using retrograde CAV-Flp, which was injected into the LPBN coupled with AAV9-Con-Fon-ChR2-EYFP injected into the cVTA of Slc6a3Cre mice, permitting specific expression of ChR2 within DA VTA→LPBN neurons (Fig. 1K). In line with our retrograde-tracing study, we found that DA VTA→LPBN neurons expressing ChR2-EYFP were mainly located within the cVTA (Fig. 1, L and M). Optogenetic activation of these DA VTA→LPBN neurons significantly decreased food intake after a fast, without affecting locomotive activity (Fig. 1, N and O), showing that a cohort of LPBN-projecting DA VTA neurons inhibits feeding.

To functionally delineate the cVTA→LPBN neural circuit, we injected a combination of AAV2-DIO-Chr2-GFP and AAV-CBA-DIO-WGA-ZsGreen (transsynaptic tracer) into the cVTA of Slc6a3Cre mice (42, 43) to tag the LPBN neurons innervated by DA VTA neurons (Fig. 2, A and B). Patch-clamp recording revealed that ZsGreen neurons within the LPBN received monosynaptic input from DA VTA axonal terminals (Fig. 2, C to E). Most ZsGreen neurons (92.3%) were activated upon treatment of SKF81297, a selective DRD1 agonist (Fig. 2, F to H). Moreover, photostimulation of ChR2-expressing DA terminals depolarized ~67% of postsynaptic LPBN neurons in a reversible manner, which could be further blocked by the selective DRD1 antagonist SCH23390 (Fig. 2, I to K). There were ~37.5% of postsynaptic LPBN neurons recorded that did not respond to the DRD1 antagonist, suggesting the existence of other neurotransmitter signaling pathways (e.g., glutamatergic). These electrophysiological results demonstrate a monosynaptic connection between DA VTA neurons and DRD1-expressing LPBN (DRD1LPBN) neurons.

![Fig. 1. A subset of DA neurons projecting from the cVTA to the LPBN neurons control food intake.](image-url)
We examined how DA<sup>cVTA</sup> neurons contribute to a complete meal episode that was composed of eight or more feeding bouts (B1 to B8), whereby each feeding bout is composed of foraging (S1), prebout (S2), initiation (S3), engagement (S4), pretermination (S5), and interbout (S6) stages (Fig. S3). Through optrode recording in vivo (Fig. 3A and fig. S4, A to C), putative DA neurons were identified by a basal firing rate of ≤10.0 Hz and spike width of ≥1.5 ms (Fig. S4D) (44, 45). We isolated ChR2-expressing DA<sup>cVTA</sup> neurons that exhibited reliable light-evoked responses, indicating that these neurons projected to the LPBN (putative DA<sup>cVTA→LPBN</sup> neurons) (Fig. S5) (46). The amount of food intake per each bout showed a gradual decrease from B1 to B8 in the first eight bouts of a meal (Fig. 3B). The amount of food intake per each bout showed a gradual decrease from B1 to B8 in the first eight bouts of a meal (Fig. 3B). The amount of food intake per each bout showed a gradual decrease from B1 to B8 in the first eight bouts of a meal (Fig. 3B).

To further characterize the physiological role of DA<sup>cVTA→LPBN</sup> neurons in the context of meal cessation, we simultaneously treated Slc6a3<sup>Cre</sup> mice with retrograde CAV-Flp injected into the LPBN and AAV9<sub>Cav2</sub>-<i>F<sub>ret</sub></i>-ChR2-EYFP and AAV9<sub>Cav2</sub>-<i>F<sub>ret</sub></i>-NpHR3.3-EYFP into the cVTA, thereby allowing expression of ChR2 and NpHR3.3 within DA<sup>cVTA→LPBN</sup> neurons (Fig. 3K). As before, DA<sup>cVTA→LPBN</sup> neurons were identified with the capacity to be photostimulated by ChR2 or photoinhibited by NpHR3.3 (fig. S6). To manipulate the activity of DA<sup>cVTA→LPBN</sup> neurons during the S5 stage, we programmed the pulse generator to exert photoinhibition based on the real-time monitor of firing rates from DA<sup>cVTA→LPBN</sup> neurons. To suppress the activity of DA<sup>cVTA→LPBN</sup> neurons during S5, the inhibitory opsin was activated in the cVTA 3 s after entering S5 (Fig. 3L). Optogenetic inhibition of DA<sup>cVTA→LPBN</sup> neurons during S5 significantly decreased the probability of meal termination and significantly prolonged bout duration (Fig. 3, M and N). The increase in food intake was strongly associated with the duration of optogenetic inhibition in S5 (Fig. 3O). These results demonstrate that the DA<sup>cVTA→LPBN</sup> neurons play a pivotal role in the control of the satiation response to food.

Infusion of the DRD1-selective agonists SKF38393 or SKF81297 into the LPBN suppressed food intake in mice after a fast (Fig. 4A and fig. S7). In contrast, administration of SKF81297 into the two brain regions close to the PBN, the laterodorsal tegmental nucleus (LDTg) or the supratrigeminal nucleus (Su5), had no effect on food intake (fig. S8). Chemogenetic activation of the DRD1<sup>LPBN</sup> neurons by intraperitoneal injection of clozapine N-oxide (CNO)
chemogenetic manipulations of DRD1 LPBN neuronal activity had no effect on locomotion activity (Fig. 4E).

To examine whether Drd1 signaling contributes to feeding and body weight control, we inactivated the receptors by bilateral injection of AAV2-CreGFP into the LPBN of Drd1−/−/Cre mice (Fig. 4F and fig. S11). This treatment led to a significant and progressive increase in food intake and body weight, without affecting locomotion (Fig. 4, G to J). Deletion of Drd1 within the LPBN affected multiple aspects of consummatory feeding, including a significant increase in cumulative food intake, bout numbers, time in bouts and meals, and average meal size, without affecting meal numbers (Fig. 4, K to Q). These data reveal that DRD1LPBN signaling regulates food intake through meal size and duration.

We investigated the real-time firing characteristics of DRD1LPBN neurons in vivo throughout a complete feeding bout by expressing ChR2 in genetically identified DRD1 neurons and inserting an optrode. We isolated ChR2-expressing DRD1LPBN neurons that exhibited reliable light-evoked responses, indicating that these neurons were DRD1LPBN neurons (fig. S12). DRD1LPBN neurons were activated by applying 20-Hz laser pulses, while non-DRD1 neurons were unaffected (Fig. 4R). Compared to other recorded LPBN neurons, the DRD1LPBN neurons fired at significantly higher rates during S5, which is consistent with the peak activity of afferent DA<sub>VTA</sub>-LPBN neurons during S5 (Fig. 4, S to V).

MPH is a clinically approved medication for the mitigation of attention deficit hyperactivity disorder (ADHD) and narcolepsy by...
Fig. 4. The DRD1LPBN signaling regulates consummatory feeding by increasing firing rate before meal cessation. (A) Refeeding test after bilateral infusion of the selective DRD1 agonist SKF38393 (1 μg per side) or SKF81297 (0.5 μg per side) into the LPBN (n = 7 per group; *P < 0.05, saline versus SKF38393; #P < 0.05, saline versus SKF81297; two-way ANOVA followed by Bonferroni post hoc test). (B) Chemogenetic manipulation of DRD1LPBN neurons by injection of AAV2-DIO-hM3Dq-mCherry or AAV2-DIO-hM4Di-mCherry within the LPBN of Drd1lox/lox mice. (C to E) Food intake and locomotion with CNO (0.1 mg/kg, ip) (n = 8 per group; *P < 0.05; two-way ANOVA followed by Bonferroni post hoc test). (F) Injection of AAV2-CreGFP within the LPBN of Drd1lox/lox mice. (G to J) Food intake (G), body weight (H), refeeding (I), and locomotor activity (J) (n = 8 per group; *P < 0.05; two-way ANOVA followed by Bonferroni post hoc test). (K to Q) Cumulative food intake (K) and meal structure analysis (L to Q) [n = 8 per group; *P < 0.05; two-way ANOVA followed by Bonferroni post hoc test (K) and unpaired t test (L to Q)]. (R) Firing rate of representative DRD1 neurons in Drd1lox/lox mice with an injection of AAV2-DIO-Chr2-GFP into the LPBN and implantation of optrode above the LPBN (n = 12 trials from 12 DRD1LPBN neurons and n = 15 trials from 15 non-DRD1LPBN neurons). (S to T) The firing rate and normalized firing rate of DRD1 neurons during B8 in the isolated meals [n = 12 per group in (U); *P < 0.05; two-way ANOVA followed by Bonferroni post hoc test]. (V) The percentage of DRD1LPBN neurons and non-DRD1LPBN neurons of all recorded neurons (12 DRD1LPBN neurons identified from 57 recorded neurons). Error bars represent means ± SEM.

blocking dopamine and norepinephrine reuptake in the forebrain (47). The anorexigenic effect of MPH was notable in clinical studies, although there has yet to be any knowledge of its cellular targets or its underlying neural mechanism (48). To determine whether MPH regulates feeding through this cVTA→LPBN neural circuit, the LPBN-projecting VTA neurons were ablated by targeted expression of caspase-3 (fig. S13A). Immunostaining showed the DA cVTA→LPBN neurons were effectively ablated (fig. S13, B to D). Ablation of these neurons enhanced food intake and led to a 20% increase in body weight 4 weeks after viral transduction (Fig. 5, A to C). Administration of MPH caused a significant reduction in food intake and body weight, which was prevented by the ablation of VTA neurons projecting to the LPBN (Fig. 5, A to C). Consistently, chronic inactivation of the cVTA→LPBN neural circuit abolished MPH-induced hypophagia and loss of body weight (Fig. 5, D to G). Genetic inactivation of Drd1 within the LPBN abrogated MPH-induced hypophagia and weight loss in a progressive manner with an eventual reversion to hyperphagia and normalized body weight (Fig. 5, H to J). These results indicate that MPH suppresses feeding and causes weight loss by acting through this cVTA→LPBN→LPBN→DA→cVTADA pathway.

Electrophysiological recordings in vivo showed that MPH significantly increased the firing rate of DRD1LPBN neurons throughout a feeding bout with the strongest elevation of firing rate in S5 as compared to non-DRD1LPBN neurons (Fig. 5, K to M). These results indicate that the DA transmission onto the DRD1 LPBN neurons underlies the feeding and body weight control by MPH. Furthermore, microinjection of MPH into the PBN potently inhibited feeding on high-fat diet (HFD) and abolished HFD-induced obesity (Fig. 5, N to P). Together, these results suggest that MPH promotes a satiety response to HFD, thereby eliminating hyperphagia and obesity by augmenting DAergic transmission within the cVTA→LPBN→DA→cVTADA circuit.
Fig. 5. MPH mediates hypophagia and body weight loss through the cVTA DA→LPBN DRD1 circuit. (A to C) Food intake (A), body weight (B), and refeeding (C) with an injection of CAV-Cre into the LPBN and AAV2-DIO-Casp3 into the cVTA with or without MPH (1.25 mg/kg, ip) (n = 12 per group; *P < 0.05, vehicle versus MPH; #P < 0.05, vehicle versus Casp3; tP > 0.05, Casp3 versus Casp3 + MPH). (D) Diagram showing chronic infusion of CNO into the LPBN by the osmotic minipump in Slc6a3Cre mice after an injection of AAV2-DIO-hM4Di-mCherry into the cVTA with or without MPH (intraperitoneally). (E to G) Food intake (E), body weight (F), and feeding test in ad lib fed mice (G) (n = 8 per group; *P < 0.05, vehicle versus CNO; tP > 0.05, CNO versus CNO + MPH). (H to J) Food intake (H), body weight (I), and refeeding test (J) with or without MPH (intraperitoneally) in Drd1Lpbn−/− mice with AAV2-Cre-GFP into the LPBN (n = 8 per group; *P < 0.05, vehicle + vehicle versus vehicle + MPH; #P < 0.05, vehicle + vehicle versus AAV-Cre + vehicle; tP > 0.05, AAV-Cre + vehicle versus AAV-Cre + MPH). (K to M) Representative firing raster (K) and firing rate (L and M) from DRD1 during feeding bout stage. Optogenetic inhibition of DRD1LPBN neurons sends DA projections to DRD1LPBN neurons in the brainstem to reinforce meal satiety and reduce consummatory feeding, through which MPH exerts anti-obesity effects. Error bars represent means ± SEM. Two-way ANOVA followed by Bonferroni post hoc test was used in (A) to (C), (E) to (J), and (L) to (P).

DISCUSSION

Here, we showed that a subpopulation of DA neurons within the cVTA elicits precise control of bout duration and meal termination by acting through hindbrain DRD1LPBN signaling (Fig. 5Q). Our present studies established that these DA<sup>cVTA</sup> neurons and their postsynaptic DRD1<sup>LPBN</sup> neurons organize a functional neural circuit where DA<sup>cVTA</sup>→LPBN neurons suppress consummatory feeding by showing robustly enhanced neural activities immediately before the termination of a feeding bout. Optogenetic inhibition of DA<sup>cVTA</sup>→LPBN neurons is sufficient to decrease the probability of meal termination, prolong bout duration, and increase food intake. Consistently, postsynaptic DRD1<sup>LPBN</sup> neurons suppress feeding by governing the meal termination response. In contrast to the CGRP PBN neurons playing a role in control of adverse feeding (12), we demonstrated a previously unknown physiological role of DRD1<sup>LPBN</sup> neurons in the regulation of the satiety response during consummatory feeding. Our results further suggested that the DRD1LPBN signaling pathway centrally mediates the hypogastic effect of MPH. Overall, our study indicated that the cVTA<sup>DA</sup>→LPBN<sup>DRD1</sup> neural circuit, functionally segregated from other DA circuits, plays a pivotal role in meal regulation.
role in control of consummatory feeding by reinforcing the satiation response, thereby effectively reducing total food intake and body weight.

To reveal the anatomical structure of this DA circuit, we used a herpes simplex virus (HSV)–based retrograde-tracing method. Our results showed a total of ~500 DA\textsubscript{VTA}→Acb neurons in the unilateral side, which is consistent with a previous DA circuit mapping study (49). Comparably, we observed ~300 DA\textsubscript{VTA} neurons in the unilateral side that sent projections to the LPBN, suggesting that DA\textsubscript{VTA}→LPBN neurons account for a sizable population of the DA\textsubscript{VTA} neurons. Furthermore, our results showed that most of the DA neurons that project to the PBN are located within the caudal region of the VTA. While the DA\textsubscript{VTA} neurons do innervate D2R neurons in the Acb, our electrophysiological recording and behavioral data indicated that the D1R signaling within the VTA→PBN pathway contributes to the regulation of meal satiation and consummatory feeding (50, 51). Although we observed that the LPBN neurons received direct, monosynaptic, DAergic innervations from the VTA, whether glutamate co-releases from DA\textsubscript{VTA} neurons onto the PBN target neurons and whether the glutamate\textsubscript{VTA} signaling plays a role in control of meal satiation need further investigation. In our study, optogenetic activation of DA\textsubscript{VTA}→LPBN neurons was sufficient to suppress feeding but not affect locomotion, whereas activation of DA\textsubscript{VTA}→Acb neurons enhanced locomotion without obvious effects on feeding (33, 52, 53). These results suggest that the DA\textsubscript{VTA} neurons projecting to the LPBN and Acb are anatomically and functionally segregated.

We detected notably enhanced activities of the DA\textsubscript{VTA}→LPBN neurons in response to consummatory feeding. In our electrophysiological recording, we observed that more than 65% of meals contain eight or more feeding bouts. Hence, to reveal the significance of DA\textsubscript{VTA}→LPBN neurons in contribution to the satiation response in a typical meal, the electrophysiological data from those meals that comprised of eight or more bouts were isolated. Our results showed that the firing rate in S5 was progressively increased from B1 and reached the peak level in the B8 (Fig. 3C). We further characterized the firing activities of DA\textsubscript{VTA} neurons (LPBN-projecting versus others) during B8 where each behaviorally defined feeding stage lasted no less than 10 s (Fig. 3, D to J). Nevertheless, the neuronal firing activities of this DAergic circuit in response to meals that contain either fewer than eight bouts or with any stage shorter than 10 s need further investigation. Our results strongly suggested that, for a typical meal, the DA\textsubscript{VTA}→LPBN neurons exhibited obviously elevated activity before the meal termination. Moreover, optogenetic inhibition of DA\textsubscript{VTA}→LPBN neurons resulted in a robustly reduced probability of meal termination. On the basis of these studies, we conclude that the LPBN-projecting DA neurons, but not other DA neurons, play a vital role in encoding meal satiation.

We functionally characterized a unique group of DRD1 neurons within the PBN. Chemogenetic activation of the DRD1\textsubscript{LPBN} neurons reduced food intake, while silencing of DRD1\textsubscript{LPBN} neurons played an exact opposite role. However, manipulation of DRD1\textsubscript{LPBN} neuronal activity had no effect on locomotion activity. Genetic deletion of Drd1\textsubscript{LPBN} neurons in response to consummatory feeding. In our electrophysiological recording results implicated that the DRD1\textsubscript{LPBN} neurons fired at significantly higher rates during the pretermination stage of the feeding bout. Therefore, we conclude that postsynaptic DRD1\textsubscript{LPBN} neurons play a role in DA\textsubscript{VTA}-mediated control of food satiation. Clinical application of MPH in the treatment of ADHD reliably exhibits a reduced appetite and weight loss. We revealed that MPH suppresses feeding and body weight by strengthening the DAergic neural activity within the cVTA→LPBN→DRD1\textsubscript{LPBN} circuit, a new perspective that endorses a potential off-label application of a class of MPH and derivatives in tackling obesity and the future development of circuity-based precision medicine that can deliver higher safety and effectiveness.

**MATERIALS AND METHODS**

**Animals**

All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committees at Baylor College of Medicine (protocol #AN-6598). Mice used for data collection were at least 8 weeks old males and kept in temperature- and humidity-controlled rooms, in a 12-hour light/12-hour dark cycle, with lights on from 7:00 a.m. to 7:00 p.m. Health status was normal for all animals. Slc6a3\textsuperscript{Cre} mice (stock no: 006660 in The Jackson Laboratory) (54), Drd1\textsuperscript{Cre} mice (stock no: 37156-JAX in MMRRC) (55), and Drd1\textsuperscript{lox/lox} mice (stock no: 025700 in The Jackson Laboratory) (56) were produced as previously described. All mice are on a C57BL/6 background with at least eight generations backcrossed.

**General surgical procedures**

All the mice with brain surgery performed were provided the same preoperative and postoperative care. In all cases, for preoperative analgesia, buprenorphine (1.0 mg/kg, sc; 1 hour before the start of anesthesia) was administered. Animals were anesthetized with ~2% isoflurane anesthesia and placed on a stereotaxic frame (David Kopf, Tujunga, CA). A line block of local anesthetics (50/50 mix of lidocaine and bupivacaine in 1:20 dilution) (0.1 ml/25 g of mouse) was made before making an incision. For postoperative analgesia, buprenorphine (1.0 mg/kg, sc) was administered once 72 hours after surgery. All surgeries were performed having the animals placed on a heating pad and allowed to recover in a heating cage until they chose to reside in the unheated side of the cage.

**Stereotaxic viral injections**

After anesthetization, a circular craniotomy was drilled at the location of different experiments. In all experiments, virus was loaded into a needle (Hamilton Small Hub RN 33-gauge, Reno, NV) connected with a 10-μl syringe (Hamilton 700 Microliter, Reno, NV). Injections were performed with an Ultra MicroPump (World Precision Instruments, Sarasota, FL) and Micro4 controller (Heidenhain Corporation, Schaumburg, IL), at a rate of 0.1 μl/min. For adenovirus-associated virus (AAV), a total of 0.5-μl volume was delivered into brain regions. For CAV and HSV, a total of 0.2 μl was delivered into the LPBN or Acb. The relevant stereotaxic coordinates for the injections are described in the following according to a standardized atlas of the mouse brain (57). To target the cVTA, the coordinates used were as follows: anteroposterior (AP), −3.52 mm; mediolateral (ML), ±0.4 mm; dorsoventral (DV), −4.3 mm; the viral aliquots [AAV2-hSyn-DIO-hM3D(Gq)-mCherry, AAV2-hSyn-DIO-hM4D(Gi)-mCherry, and AAV2-EF1a-DIO-hChR2(E123T/T159C)-GFP from UNC were used and diluted to a final working titer of 7 × 10^12 viral genomes/ml; AAV9-Con-Fon-ChR2-EYFP and AAV9-Con-Fon-NpHR3.3-EYFP were synthesized by GenScript, Piscataway, NJ, USA, and packaged by the Optogenetics and Viral Design/Expression
Core at Baylor College of Medicine and diluted to a final working titer of 3.6 × 10^{12} viral genomes/ml; AAV9-DIO-WGA-zsGreen was packaged by the Optogenetics and Viral Design/Expression Core at Baylor College of Medicine and diluted to a final working titer of 3.8 × 10^{12} viral genomes/ml; AAV2-Flex-taCas3-TEVP from UNC was used and diluted to a final working titer of 1.8 × 10^{12} viral genomes/ml were used for injection. To target the LPBN, the coordinates used were as follows: AP, −5.20 mm; ML, ±1.3 mm; DV, −3.4 mm; the viral aliquots [CAV-Cre and CAV-Flp from CNRS were used and diluted to a final working titer of 4.4 × 10^{12} viral genomes/ml; AAV2-hSym-DIO-hM3D(G1)-mCherry, AAV2-hSym-DIO-hM4D(Gi)-mCherry; AAV2-GFP, and AAV2-CreGFP] were packaged by the Optogenetics and Viral Design/Expression Core at Baylor College of Medicine and diluted to a final working titer of 5 × 10^{12} viral genomes/ml; HSV hEF1α-mCherry from Harvard) were respectively injected. To target the Acb, the coordinates used were as follows: AP, +1.10 mm; ML, ±0.85 mm; DV, −4.6 mm; the viral aliquots (HSV hEF1α-EYFP from Harvard) were injected.

Double retrograde labeling
For the HSV tracing, HSV hEF1α-EYFP and HSV hEF1α-mCherry were unilaterally injected into the Acb and LPBN, respectively, in wild-type (WT) mice. The whole VTA, PBN, and Acb were sectioned with 20 μm thickness by a microtome (Thermo Fisher Scientific, Waltham, MA). The fluorescent images of green fluorescent protein (GFP) and mCherry neurons along the rostral to caudal axis of the VTA were obtained by an Axio Observer microscope (Zeiss, Thornwood, NY) and further analyzed using ImageJ software [National Institutes of Health (NIH)].

Ablation of DA^{VTA} neurons projecting to the LPBN
To ablate DA^{VTA} neurons, WT mice were bilaterally injected with CAV-Cre into the LPBN, and Cre-inducible AAV expressing caspase-3 (AAV2-Flex-taCas3-TEVP) was injected within the cVTA. Twenty-eight days after injection, the mice were euthanized, and the brain was harvested. The whole VTA was sectioned with 20 μm thickness by a microtome (Thermo Fisher Scientific, Waltham, MA). Immunostaining for TH (tyrosine hydroxylase) was performed in all VTA sections. Fluorescent images of Cy3-labeled TH neurons along the rostral to caudal axis of the VTA were obtained by an Axio Observer microscope (Zeiss, Thornwood, NY) and further analyzed using ImageJ software (NIH) in which Abercrombie’s correction of overcounting of profiles in sections was applied (58).

Drug administration
The mice received a general administration of CNO intraperitoneally at a dose of 0.1 mg/kg 30 min before feeding assay. The guide cannula (23-gauge steel, Plastics One) was used for injecting MPH, SKF38393, or SKF81297 into the LPBN or infusing SKF81297 into the LDTg or Su5. A circular craniotomy (diameter, 0.5 mm) was drilled at the locations of the LPBN, LDTg, or Su5. The guide cannula was installed on the holder and guided into the target brain region. To deliver the drug, the internal cannula was inserted onto the top of the guide cannula and extended below the guide cannula at 0.5 mm. To target the LPBN, the cannula was implanted within the LPBN with the following coordinates: AP, −5.2 mm; ML, ±1.3 mm; DV, −2.9 mm. To target the LDTg, the cannula was implanted within the LDTg with the following coordinates: AP, −5.02 mm; ML, ±0.7 mm; DV, −2.9 mm. To target the Su5, the cannula was implanted within the Su5 with the following coordinates: AP, −5.02 mm; ML, ±1.4 mm; DV, 4.0 mm. For some experiments, MPH (2 μg per side; Spectrum Chemical, New Brunswick, NJ), SKF38393 (1 μg per side; Tocris Bioscience, Minneapolis, MN), or SKF81297 (0.5 μg per side; Tocris Bioscience, Minneapolis, MN) was infused intracranially. For the experiments of systemic administration of MPH, the MPH was intraperitoneally injected with a dose of 1.25 mg/kg.

To chronically deliver CNO into the LPBN, a cannula was implanted within the LPBN, and an Alzet 14-day minipump (model 1002, Durect, Cupertino, CA) was used in this study. The minipump that dispensed at a speed of 0.25 μl/hour was loaded with 100 μl of CNO (0.12 μg/ml in saline; Enzo Life Sciences, Farmingdale, NY) and was implanted subcutaneously on the back of anesthetized mice for 14 days.

Food intake
For the acute feeding studies, food intake was measured (from the start of the “lights off” cycle, 6:00 p.m. to 7:00 p.m.) 1 hour during and after 1 hour of photostimulation with chow diet (5V5R, LabDiet, St. Louis, MO). For the refeeding test, food intake (1, 2, and 4 hours) was monitored from 12:00 p.m. to 4:00 p.m. with chow diet after 18 hours of fasting from 6:00 p.m. to 12:00 p.m. The food intake (1, 2, and 4 hours) in well-fed mice was monitored from 6:00 p.m.
to 10:00 p.m. with chow diet. The acute feeding and refeeding test were performed 3 weeks after virus injection. For the chronic feeding studies, food intake and body weight were measured daily between 9:00 a.m. and 10:00 a.m. for up to 4 weeks.

To study meal structure, the BioDAQ (Research Diets, NJ) was used 4 weeks after viral injection. Mice were habituated to BioDAQ cages for at least 10 days before feeding recordings. Feeding recordings were analyzed using BioDAQ Viewer (software v.2.3.07). A meal was defined if the food ingested in a bout feeding was ≥0.02 g and if it was separated from another meal by ≥5 min.

Feeding bouts were isolated by an interbout interval of 5 s, and meals consisted of one or more bouts separated by an intermeal interval of 5 min (60, 61). A typical meal consists of ~8 bouts, and a single feeding bout is divided into six stages: foraging stage (S1); prebout stage (S2), 10 s before ingestion begins; initiation stage (S3), 10 s after ingestion begins; engagement stage (S4); pretermination stage (S5), 10 s before bout cessation; interbout stage (S6). The S1 to S6 stages were determined by the location of mice in the cage and the time spent in a specific arena (fig. S2B). The S1 to S6 stages (10 s per stage) were extracted from an integrated meal. The recording data from the bouts with all the stages lasting 10 s or more were extracted and binned to 10 s for presentation and statistical analysis, while those bouts with any of the stages lasting less than 10 s were discarded. An automated video-tracking system (EthoVision by Noldus) was used to track animals. Specifically, the home cage was divided into three arenas. The food was located on the middle of one side that was defined as a food zone. The size of the premeal zone (half circle, ~5 cm diameter) was adopted as half of the body length so as to match the center-point detection method. The reminder region in the home cage was defined as the foraging zone.

**Measurement of locomotor activities**

To assess locomotor activity, animals were gently removed from the home cage and placed singly into an open-field arena (Plexiglas cage, 50 cm (length) by 50 cm (width) by 40 cm (height)) 3 weeks after virus injection. An automated video-tracking system (EthoVision by Noldus) was used to track multiple subjects simultaneously. After the experiment, we gently returned each mouse to the respective home cage. To avoid olfactory cues, the arena (usually 50% ethanol) was carefully wiped after every running. The distance traveled within the chamber during a session of 5 min was calculated.

**In vitro electrophysiology**

The brains of adult mice were sectioned in coronal plane (250 to 300 μm). The brains and slices were handled and kept in aCSF as described recently (62). Animals were subjected to anesthesia, and the handling protocol of the local committee was followed. In most cases, we have used the same mouse first for the in vivo experiments and immediately after for combined electrophysiology and optogenetics in vitro to minimize the number of mice. Mice were deeply anesthetized with isoflurane and transcardially perfused with a modified ice-cold sucrose-based cutting solution (pH 7.3) containing 10 mM NaCl, 25 mM NaHCO3, 195 mM sucrose, 5 mM glucose, 2.5 mM KCl, 1.25 mM sodium phosphate buffer, 2 mM Na-pyruvate, 0.5 mM CaCl2, and 7 mM MgCl2, bubbled continuously with 95% O2 and 5% CO2. The mice were then decapitated, and the entire brain was removed and immediately submerged in the cutting solution. Slices were cut with a Microm HM 650V vibratome (Thermo Fisher Scientific). Slices containing the PBN were recovered for 1 hour at 34°C and then maintained at room temperature (RT) in aCSF (pH 7.3) containing 126 mM NaCl, 2.5 mM KCl, 2.4 mM CaCl2, 1.2 mM sodium phosphate buffer, 1.2 mM MgCl2, 11.1 mM glucose, and 21.4 mM NaHCO3, saturated with 95% O2 and 5% CO2 before recording. Slices were superfused at 34°C in oxygenated aCSF at a flow rate of 1.8 to 2 ml/min. GFP- or mCherry-labeled neurons in the LPBN were visualized using epifluorescence and infrared differential interference contrast imaging on an upright microscope (Eclipse FN-1, Nikon) equipped with a moveable stage (MP-285, Sutter Instrument). Patch pipettes with resistances of 3 to 5 megohms were filled with intracellular solution (pH 7.3) containing 128 mM K-glucosone, 10 mM KCl, 10 mM Hapes, 0.1 mM EGTA, 2 mM MgCl2, 0.05 mM Na-GTP, and 0.05 mM Mg-ATP. Recordings were made using a MultiClamp 700B amplifier (Axon Instruments), sampled using Digidata 1440A, and analyzed offline with pClamp 10.3 software (Axon Instruments). Series resistance was monitored during the recording, and the values were generally <10 megohms and were not compensated. The liquid junction potential was +12.5 mV and was corrected after the experiment. Data were excluded if the series resistance increased markedly during the experiment or without overshoot for action potential. Currents were amplified, filtered at 1 kHz, and digitized at 20 kHz. Current clamp was engaged to test neural firing frequency and resting membrane potential (Vm) at the baseline. The aCSF solution contained 1 μM tetrodotoxin (TTX) and a cocktail of fast synaptic inhibitors, AP-5 (30 μM; an NMDAR antagonist) and CNQX (30 μM; an AMPA receptor antagonist), to block most of the presynaptic inputs. For the light-evoked excitatory postsynaptic current (EPSC) recordings, the internal recording solution contained 125 mM CsCH3SO3, 10 mM CsCl, 5 mM NaCl, 2 mM MgCl2, 1 mM EGTA, 10 mM Hapes, 5 mM (Mg)ATP, and 0.3 mM (Na)GTP (pH 7.3 with NaOH). mEPSC within the LPBN neurons was measured in the voltage clamp mode with a holding potential of −60 mV in the presence of 1 μM TTX and 50 μM bicuculline. 4AP (4-aminopyridine) and TTX were used to confirm the evoked EPSC currents are monosynaptic currents. Depolarization was defined as a >2-mV increase of the resting membrane potential, whereas hyperpolarization was defined as a >-2-mV decrease of the resting membrane potential; values between a 2-mV decrease and a 2-mV increase were defined as “unchanged” (63).}

**In vivo tetrode recording**

We used the microdrive model that enabled delivery of the laser or drug into the brain and recorded neural activities simultaneously (64, 65). The microdrives were modified on the basis of tetrode microdrives from Neuralynx, which were loaded with one optic fiber in the center and seven nichrome tetrodes consisting of four thin wires twined together (Stablohm 675, California Fine Wire Co., Grover Beach, CA). The optic fiber was positioned 0.1 mm from the tetrode and was glued to the middle of the bundle of tetrodes. Tetrode tips were gold-plated to reduce impedance to 0.3 to 0.4 M (tested at 1 kHz). The microdrive was implanted into the cVTA in Slec6a3 Cre mice with an injection of CAV-Flp within the LPBN and an injection of AAV-Con-Fon-Chr2-EYFP and AAV-Con-Fon-NphHR3.3-EYFP within the cVTA. After recovery from microwire implantation, the mouse was connected to a 32-channel preamplifier headstage. All signals recorded from each tetrode were amplified, filtered between 0.3 and 6 kHz, and digitized at 32 kHz. The local field potentials...
were amplified and filtered between 0.1 Hz and 1 kHz. The tetrodes were slowly lowered in quarter turns of a screw on the microdrive (~60-µm steps). Spikes were sorted using Offline Sorter software (Plexon). Units were separated by the T-distribution E-M method, and cross-correlation and autocorrelation analyses were used to confirm unit separation. Clustered waveforms were subsequently analyzed by using NeuroExplorer (Nex Technologies, Colorado Springs, CO) or MATLAB (MathWorks, Natick, MA). The firing rates were presented with spikes per bin with 1-s intervals or spikes per second. Single units that had a basal firing rate of ≤10.0 Hz and spike width of ≥1.5 ms were assigned to the putative dopamine neurons (44, 45). The ChR2+ neurons were identified by the short latencies of evoked spikes accurately following high-frequency photostimulation, as well as the identical waveforms of evoked and spontaneous spikes (66).

According to our experimental data, the majority of the meals (>65%) recorded thus far by us contain eight or more feeding bouts, whereas a small number of meals contain few bouts. Thus, we isolated those meals comprised of eight or more feeding bouts for data analysis. The electrophysiological data derived from the first eight feeding bouts were analyzed to show the progressive increase of firing rate in S5 of bouts in the isolated meals. The firing activities during B8 in bouts were analyzed to show the progressive increase of firing rate (44, 45). The ChR2+ neurons were identified by the short latencies of evoked spikes accurately following high-frequency photostimulation, as well as the identical waveforms of evoked and spontaneous spikes (66).

To inhibit the DA^cVTA–LPBN neurons during the S5 stage, the neural firing of DA^cVTA–LPBN neurons during feeding was continuously monitored, and the yellow light was given only when the firing increased to twofold of the basic firing rate and stayed for 3 s by real-time online firing rate analysis. To achieve an automatic, firing event–triggered photostimulation, we built a platform by integrating three major equipment, including the Digital Lynx SX recording system (Neuralynx), the RZ5P programmable real-time signal processor (Tucker-Davis Technologies), and the Spectralynx optogenetic box (Neuralynx). The analog outputs of the Digital Lynx SX were connected with onboard analog inputs of the RZ5P processor, while the N1/O channel of the RZ5P was connected with the TTL I/O ports of the Spectralynx box. During a recording session, the sorted spike signals from DA^cVTA–LPBN neurons recorded by the Digital Lynx SX were fed to and processed by the RZ5P in a real-time manner under the control of Synapse Suite software (Tucker-Davis Technologies). In our experimental design, following upward crossing of the threshold for photoinhibition to twofold of the basic firing rate and staying for 3 s (during the S5) as preprogrammed in the Synapse Suite, the RZ5P generated and output a TTL signal to the Spectralynx optogenetic box and triggered laser stimulation (589 nm) for 10 s. The probability of meal termination, feeding bout duration, and food intake was calculated.

**Histology**

Immunostaining was performed as described with modification (19). Mice were euthanized and perfused transcardially with ice-cold phosphate-buffered saline buffer (pH 7.4) containing 3% (w/v) paraformaldehyde (Alfa Aesar) and 1% glutaraldehyde (Sigma-Aldrich, St. Louis, MO). Brains were collected and postfixed overnight under 4°C in a fixation buffer containing 3% paraformaldehyde. Free-floating sections (25 µm) were cut by a microtome (Thermo Fisher Scientific, Waltham, MA) and then blocked with 5% (w/v) normal donkey serum in 0.1% Triton X-100 [TBST buffer (pH 7.2)] for overnight.

Rabbit anti-TH (1:1500 dilution; ABN60, EMD Millipore, Burlington, MA) was applied to the sections for overnight incubation under 4°C, followed by 4× 15-min rinses in the TBST buffer. Last, sections were incubated with Alexa Fluor Cy5-conjugated secondary antibody (1:1000 dilution; 711-175-152, Jackson ImmunoLab, West Grove, PA) or Alexa Fluor Cy3-conjugated secondary antibody (1:1000 dilution; 711-585-152, Jackson ImmunoLab, West Grove, PA) for 2 hours at RT, followed by 4× 15-min rinses in TBST buffer. For mounted sections, fluorescent images were captured by a digital camera mounted on an Axio Observer microscope (Zeiss, Thornwood, NY).

Fluorescence in situ hybridization (FISH) was performed to examine the expression of Drd1 in the LPBN. All reagents and the Drdr1 probe are commercially available from Advanced Cell Diagnostics (catalog no. 320293, Newark, CA). The RNAscope Fluorescent Assay is one of the FISH techniques to visualize cellular RNA targets in fresh-frozen tissues. All FISH procedures were performed following the protocol provided by the manufacturer. Briefly, fresh-frozen brain tissues were sectioned with a cryostat at 20 µm thickness and mounted onto SuperFrost Plus slides. Chilled slides were fixed with 4% paraformaldehyde for 15 min at 4°C. Then, the sections were dehydrated with grade ethanol. Slides were air-dried for 5 min at RT, and ~5 drops of RNAscope Protease IV were added to each section for 30 min at RT and then washed three times with PBN. The sections were hybridized with the Drd1 probe (catalog no. 461908, Advanced Cell Diagnostics) for 2 hours at 40°C. After two washes, the slides were hybridized with Amp1FL for 30 min, Amp-2FL for 15 min, Amp-3FL for 30 min, and Amp-4FL for 15 min at 40°C followed by washing three more times with PBN. Lastly, the glass covers were mounted onto glass slides. The fluorescent images were captured by an Axio Observer microscope.

**Statistical analyses**

Data were analyzed by unpaired t test, paired t test, and one- or two-way analysis of variance (ANOVA) with appropriate post hoc testing. A Wilcoxon signed-rank test was used when the data were not normally distributed. Statistical analyses were performed using Prism software (GraphPad Software, San Diego, CA). Results were considered significantly different at P < 0.05. All data are presented as means ± SEM.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/22/eabf8719/DC1

“View request a protocol for this paper from Bio-protocol.”

**REFERENCES AND NOTES**

1. C. J. Burnett, C. Li, E. Webber, E. Tsousidou, S. Y. Xue, J. C. Brüning, M. J. Krashes, Hunger-driven motivational state competition. *Neuron* 92, 187–201 (2016).
2. G. J. Morton, T. H. Meeke, M. W. Schwarz, Neurobiolology of food intake in health and disease. *Not. Rev. Neurosci.* 15, 367–378 (2014).
3. J. W. Sohn, J. K. Elmqvist, K. W. Williams, Neuronal circuits that regulate feeding behavior and metabolism. *Trends Neurosci.* 36, 504–512 (2013).
4. M. A. Rossi, G. D. Stuber, Overlapping brain circuits for homeostatic and hedonic feeding. *Cell Metab.* 27, 42–56 (2018).
5. R. C. Ritter, Gastrointestinal mechanisms of satiation for food. *Physiol. Behav.* 81, 249–273 (2004).
6. G. P. Smith, The direct and indirect controls of meal size. *Neurosci. Biobehav. Rev.* 20, 41–46 (1996).
7. H. J. Grill, A role for GLP-1 in treating hyperphagia and obesity. *Endocrinology* 161, bqa093 (2020).
and protein, but not melanin concentrating hormone, in heterogeneity of Neuron of Phasic firing in B. 2C receptors in P. H. J. receptor blockade within nucleus accumbens subregions on and S. and mutant mice have higher “wanting” but not “liking” for F. R. G. Zhu, G. Luquet, F. Norgren, C. D. Wu, M. Wu, M. feeding in Wolterink-Donselaar, G. involving in metabolism. 'Liking' 3645–3650 (2016). Berridge, J. 150, 2654–2659 (2009). Endocrinology 1, 2233–2243 (2014). Neuroscience 57, 161–169 (2002). 160, 161–169 (2002). 1129–1137 (1991). 71, 537–550 (2009). 110, 75–85 (2013). 71, 1646–1660 (1994). 13, 1225–1234 (2009). 36, 336–342 (2013). 18, 73–85 (2017). 25, 403–404 (2009). 36, 1028–1030 (2014). 23, 8935–9402 (2003). 3, 439–459 (2007). 191, 439–459 (2007). 3, 893–901 (1986). 173, 1136–1139 (2007). 19, 85–88 (2012). 15, 181–187 (2012). 15, 3rd Edn. 160, 2654–2659 (2009). 251, R977–R977 (1986). 1. A. L. Alhadeff, J. P. Baird, J. C. Swick, M. R. Hayes, J. H. Grill, Glucagon-like peptide-1 receptor signaling in the lateral parabrachial nucleus contributes to the control of food intake and motivation to feed. Neuropeptides 39, 2233–2243 (2014). Han et al., Sci. Adv. 2021; 7 : eabf8719 26 May 2021
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