Medial prefrontal D1 dopamine neurons control food intake

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Although the prefrontal cortex influences motivated behavior, its role in food intake remains unclear. Here, we demonstrate a role for D1-type dopamine receptor–expressing neurons in the medial prefrontal cortex (mPFC) in the regulation of feeding. Food intake increases activity in D1 neurons of the mPFC in mice, and optogenetic photostimulation of D1 neurons increases feeding. Conversely, inhibition of D1 neurons decreases intake. Stimulation-based mapping of prefrontal D1 neuron projections implicates the medial basolateral amygdala (mBLA) as a downstream target of these afferents. mBLA neurons activated by prefrontal D1 stimulation are CaMKII positive and closely juxtaposed to prefrontal D1 axon terminals. Finally, photostimulating these axons in the mBLA is sufficient to increase feeding, recapitulating the effects of mPFC D1 stimulation. These data describe a new circuit for top-down control of food intake.

The decision of whether or not to eat is critically important for an animal’s survival. For humans, the modern environment with ready access to food biases this decision and helps to contribute to overeating and obesity. In mammals, the prefrontal cortex (PFC) has a crucial function in decision-making and regulation of behavior1–2 and is implicated in control of food intake, although the underlying neural mechanisms remain unclear. Humans with frontotemporal dementia display hyperphagia, whereas patients with generalized dementia do not3. Additionally, human imaging studies have correlated activity in the PFC with hunger in obese patients4 and with the pleasantness of food5. However, in animal studies, lesions of the PFC have varied and opposing effects on intake6–8, and many pharmacological manipulations targeting monoamine systems produce no change at all9. This disparity between human and preclinical studies suggests that there are limitations in the classical pharmacological and inactivation approaches and that manipulation of specific cell types within the PFC is necessary to determine respective contributions to food intake.

Prefrontal dopamine systems represent an attractive target for neural influence over feeding behaviors. Midbrain dopaminergic projections have an important function in food intake, and without dopamine animals become hypophagic and die from starvation10–12. Both nigrostriatal and mesolimbic dopamine systems contribute to feeding13–16, and dopaminergic neurons from the ventral tegmental area also prominently project to the PFC17. Although dopaminergic systems in the PFC are implicated in control over tasks such as working memory, habit and timing18–20, a direct effect of prefrontal dopamine systems in feeding remains unexplored.

Dopamine D1 receptors are highly expressed in the mPFC21, and there is evidence that dopamine D1 receptor–containing neurons in the mPFC have a function in food-related behaviors22,23. However, direct assessment of food intake as a result of prefrontal D1 neuron stimulation has yet to be investigated. Here we first demonstrate that mPFC D1 neurons are activated during feeding. We then use cell type–specific optogenetics to stimulate or inhibit mPFC neurons expressing D1 receptors and directly assess their influence on food intake.

RESULTS

D1 dopamine receptor neurons are activated during feeding

To map prefrontal dopamine circuitry related to feeding, we examined whether feeding activated prefrontal neurons. Mice expressing Cre recombinase in D1 dopamine receptor neurons (Drd1a-cre+; see Supplementary Fig. 1a) were food-deprived for 24 h and then tested for 90 min with free access to chow to isolate a discrete feeding period. Animals that were deprived ate approximately sixfold more than control Drd1a-cre− animals that remained fed ad libitum (n = 2.2 cage averages, control = 0.17 ± 0.04 g, deprived = 1.15 ± 0.20 g, mean ± s.e.m.). Immediately after feeding, animals were sacrificed, and immunohistochemical analyses were performed. Compared to control animals, restricted mice showed significantly increased Fos density in the mPFC (Fig. 1a,b, P = 0.007).

As D1-type dopamine receptors have higher expression in rodent medial prefrontal regions24, we examined whether neurons with increased feeding-related activity expressed D1 dopamine receptors by colabeling with an antibody against Cre recombinase. Restricted animals showed a significant increase in the percentage of D1-expressing (D1+) neurons that were also Fos+, indicating that these neurons were more active during feeding (Fig. 1c,d and Supplementary Fig. 2, respectively).
D1-selective PFC neuronal activation using light
To establish that we could selectively stimulate only prefrontal D1 neurons, we administered a virus containing a double-inverted loxP-flanked (floxed) channelrhodopsin (ChR2) construct (AAV-EF1a-DIO-ChrR2-eYFP) into the mPFC of Drd1a-cre+ mice23 (Fig. 2a and Supplementary Fig. 3a,b). ChR2 was expressed predominantly in medium-sized pyramidal cells (Fig. 2b), and patch clamp experiments on layer 5 D1 ChR2+ neurons showed no Ih current at hyperpolarizing currents (‘voltage sag’; Fig. 2c) and no rebound depolarization as compared to larger, D1-negative pyramidal neurons within layer 5 (Supplementary Fig. 3c,d). This is consistent with previous descriptions of D1 pyramidal neurons in prefrontal cortex24.

Photostimulation of the brain slice with 20-Hz, 473-nm light (frequencies based on preliminary behavioral tests) resulted in highly reliable, frequency-dependent inward currents in these neurons (Fig. 2d), which produced sustained action potentials for at least 3 min (Fig. 2e). These data demonstrate that we are able to selectively activate D1 neurons in the mPFC with high fidelity.

Unilateral activation of D1 PFC neurons drives intake
To assess whether D1 neuronal activation was sufficient to influence feeding, ChR2 was expressed in the left mPFC of Drd1a-cre+ mice, and a fiber optic cannula was implanted to allow for optical illumination during behavior (Fig. 3a). ChR2 expression in layer 5 was verified and photoillumination produced a robust Fos response compared to control, Drd1a-cre− animals, confirming neuronal activation in vivo (Fig. 3b, P = 0.01). Animals were habituated for 1 h to a home cage containing grain pellets in a dish, available ad libitum, for 3 d. Subsequent to habituation, animals were stimulated at 5 Hz or 20 Hz (in 3-min on-off cycles), and the number of pellets consumed in 1 h was recorded. Drd1a-cre− mice ate significantly more pellets than Drd1a-cre− controls when stimulated at 20 Hz but not at 5 Hz (Fig. 3c, P = 0.029). Further, consumption of a highly palatable, high-fat (45%) chow was also significantly increased in Drd1a-cre− animals after 20-Hz photostimulation (Fig. 3d, P = 0.032).

Because these increases in feeding were observed during times when mice do not usually feed (mid-light cycle), we also used an operant paradigm to measure food intake during natural feeding periods. Animals were trained under food restriction to make a nose-poke into an empty port (a ‘response’) for a grain pellet, and once trained, were re-fed until their weights were equal to or greater than pretraining weights. On the testing day, animals were returned to the test chamber before the onset of the dark cycle and coupled to the laser. Each hour, the animals went through two 30-min epochs. In the first epoch there was no photostimulation (‘no-light’), and in the second epoch illumination occurred in a 3-min on-off cycle at 20 Hz (‘light’, Fig. 3e). Drd1a-cre− animals significantly increased the number of pokes only during the light epochs compared to Drd1a-cre− littermates, who were unaffected by illumination (Fig. 3f and Supplementary Fig. 4a,b, P = 0.025). Within the light epoch, there was no significant difference in responding when the laser was on versus when it was off, although there was a trend toward an increase (Supplementary Fig. 4c,d, P = 0.094), suggesting that responses may not be absolutely time-locked to stimulation. Nonetheless, in total the Drd1a-cre− animals had significantly increased pokes during the light epoch compared to the no-light epoch (Fig. 3g, P = 0.0003). Comparing the ratio of light to no-light cumulative responses over each hour showed this increase in light epoch responses to be sustained and linear over 13 h (Fig. 3h, P = 0.04). Drd1a-cre− animals also ate more pellets than controls during this time (Fig. 3i); a total increase

Figure 1 Characterization of prefrontal neurons activated during feeding. (a) Representative micrographs showing prefrontal Fos nuclei in control and deprived Drd1a-cre+ mice after 90 min access to food (scale bar, 200 µm). (b) Quantification of Fos+ nuclei in the PFC of control and deprived–re-fed animals (n = 5,5 animals, t0 = 3.6, P = 0.007, two-tailed t-test; control = 42.0 ± 4.5; deprived = 82.44 ± 10.4, mean ± s.e.m.). (c) Representative single channel and overlay confocal micrographs of Cre (green) and Fos (red) for control and deprived mice. White arrowheads indicate examples of overlap in the deprived condition (scale bar, 20 µm). (d) Quantification of the percentage of Drd1a-cre+ neurons that were also Fos+ (n = 4,4 animals, t0 = 3.1, P = 0.022, two-tailed t-test; control = 22.5 ± 6.5; deprived = 53.3 ± 7.8, mean ± s.e.m.). *P < 0.05; **P < 0.01.

Figure 2 Physiological responses to prefrontal D1 photoactivation. (a) ChR2 expression in prefrontal D1 neurons after viral injection in Drd1a-cre+ mice. Strong staining is seen in layer 2/3 and layer 5 of both the prelimbic (PL) and infralimbic (IL) prefrontal cortex. (b) Representative micrograph of two D1+ layer 5 pyramidal neurons filled with biotin (red; background eYFP in green; scale bar, 60 µm). (c) Physiological properties of a D1 neuron in response to depolarizing and hyperpolarizing currents. Red arrow highlights lack of voltage sag; blue arrow highlights lack of rebound depolarization. (d) Inward currents induced by 20-Hz blue light demonstrates high fidelity over the course of 3 m. (e) Representative voltage trace showing high-fidelity action potentials in response to 20-Hz blue light over the course of 3 m.
of ~1 g, P = 0.049), demonstrating that increased responding corresponded to an increase in intake and was not a nonspecific motor response. Locomotor activity during the light epoch was unchanged, showing that prefrontal D1 stimulation did not influence gross motor movement (Supplementary Fig. 5a, b, P = 0.77). In addition, free overnight consumption of high-fat chow was significantly increased with photostimulation (Supplementary Fig. 5c, P = 0.045), while stimulation did not significantly increase licking for water, suggesting this response is specific to food consumption (Supplementary Fig. 5d, P = 0.38). Finally, nose-poking on an unrefilled port was decreased during photostimulation, suggesting that animals spent less time exploring the environment at times when feeding was increased (Supplementary Fig. 5e, P = 0.05), consistent with limited effects of stimulation on non-specific motor activity. These results demonstrate that sustained photostimulation of prefrontal D1 neurons is sufficient to selectively drive food intake under sated conditions during normal feeding.

**Bilateral inhibition of D1 PFC reduces intake**

To test whether D1 prefrontal inhibition modifies intake, we expressed halorhodopsin (eNpHR 3.0, AAV-EF1a-DIO-eNpHR3.0-EYFP) bilaterally in D1 neurons of the mPFC, and implanted bilateral optical fiber cannula (Fig. 4a). To motivate consumption, animals were restricted to approximately 90% free-feeding body weight while being habituated to ad libitum access to grain pellets. After 4 d of habituation, animals were coupled to the laser and received 590 nm light during two 15-min epochs in a 1-h test (Supplementary Fig. 6a). During the illuminated epochs, Drd1a-cre+ animals ate significantly fewer pellets than when the light was off (P = 0.045) than did Drd1a-cre− animals, whose intake was unchanged by light (Fig. 4b and Supplementary Fig. 6a). Total intake between the groups was not different (Supplementary Fig. 6b, P = 0.76). After this restricted test, animals were re-fed for 1 week, and then retested under sated conditions. Again, compared to consumption on the previous day, the number of test-day pellets consumed was reduced in Drd1a-cre+, but not Drd1a-cre−, mice (Fig. 4c, P = 0.039 and P = 0.73, respectively). This suggests that inhibiting activity of prefrontal D1 neurons can attenuate food intake.

**Downstream targets of mPFC stimulation**

To identify the functional circuit that mediates this feeding behavior, we analyzed mPFC D1 neuronal projections. These could be readily visualized owing to axonal localization of the ChR2-eYFP fusion protein in Drd1a-cre+ animals. By combining tract tracing with Fos responses following D1 stimulation, we surveyed the mouse brain for potential target regions. Animals were fiber-optically coupled to the laser, stimulated at 20 Hz and perfused 90 min later for detection of Fos expression. Axons of prefrontal D1 neurons were prominent in medial nucleus accumbens (shell and core), dorsomedial striatum and caudal-medial basolateral nuclei of the amygdala (mBLA, Fig. 5a, Supplementary Fig. 7a, b), and sparse axons were found in the lateral hypothalamus (Supplementary Fig. 7c). We found that Fos immunoreactivity was increased selectively in the mBLA of Cre+ animals, with the ipsilateral side showing significantly more Fos+ nuclei than the contralateral side or Drd1a-cre− mBLA sections (Fig. 5b, c, P = 0.003). No differences in Fos were observed in the nucleus accumbens or amygdala.
accumbens (Supplementary Fig. 7d,e, P = 0.35). We characterized these mBLA, Fos+ neurons using co-immunolabeling (Fig. 5d,e and Supplementary Fig. 8), and found that ~85% of Fos+ nuclei co-stained with CaMKII, a marker of glutamatergic neurons. This represented ~20% of CaMKII neurons within this region of the mBLA. Only ~5% of Fos+ nuclei were positive for parvalbumin (PV), a major type of interneuron in the mBLA (Fig. 5f).

To verify the presence of prefrontal D1 neuron-terminal synapses in the mBLA, we used a Cre-dependent adeno-associated virus (AAV) encoding an eGFP fluorophore fused to synaptobrevin 2 (AAV-EF1a-DIO-Synb-eGFP), which selectively labels presynaptic vesicles of an infected neuron (Fig. 5g). The resulting fluorescence is highly punctate, and when we injected it into Drd1a-cre+ mice, we observed putative synaptic contacts in the same mBLA region as observed with ChR2-eYFP expression (Fig. 5g). Co-staining showed that these puncta were in close apposition (<5 µm) to CaMKII+ cell bodies, consistent with a direct synaptic connection (Fig. 5h) that corroborates the increases in Fos immunoreactivity.

mBLA terminal stimulation of D1 neurons drives intake

To test whether prefrontal D1 projections to the mBLA are sufficient for the increase in feeding during prefrontal stimulation, we injected AAV-EF1a-DIO-ChR2-eYFP virus in the mPFC of Drd1a-cre+ mice and implanted an optical fiber directed to the mBLA (Fig. 6a). This allowed selective photostimulation of the terminals of these prefrontal D1 afferents and evaluation of the contribution of this specific projection to the feeding response. Examination of mBLA after photostimulation revealed a significant increase in Fos immunoreactivity compared to the unstimulated, contralateral side (Fig. 6b,c, P = 0.008). Using the overnight feeding paradigm, selective terminal stimulation of prefrontal D1 afferents in the mBLA increased responding only in Drd1a-cre+ mice (Fig. 6d, P = 0.007), as measured by the ratio of light to no-light cumulative responses over the course of the 13-h session. This increase in response resembled that seen with direct photostimulation of the PFC (Fig. 3g), although the ratio increased more quickly during the early hours of the test (hours 3–6) and remained relatively constant after hour 7.
Overall, Drd1a-cre+ animals showed increased responses during the illuminated session ($P = 0.018$), whereas Drd1c-cre- animals showed no difference (Fig. 6e). Importantly, the Drd1a-cre+ animals also showed a significant increase in intake, consuming ~0.6 g more food than control animals ($P = 0.002$, One-way ANOVA, Bonferroni post hoc). Error bars, mean ± s.e.m. *$P < 0.05$, **$P < 0.01$.

**DISCUSSION**

In the present study we show that selectively stimulating prefrontal D1 neurons and their afferents within the BLA can increase feeding. This follows the observation that D1 neurons are activated during feeding and provides functional data to support a direct role for D1 prefrontal neuron activity in intake. Notably, activation of these D1 neurons does not result in classical ‘top-down’ inhibition of behavior but rather drives food intake in sated animals. In addition, stimulation of the prefrontal D1 neurons activates a subset of glutamatergic neurons in the BLA, and glutamatergic afferents from the BLA have been previously shown to be important for appetitive behaviors via interactions with the nucleus accumbens (NAc)26, suggesting that cortical control of these neurons could modulate this appetitive drive. The BLA has also been implicated in other food-seeking behaviors such as conditioned potentiation of feeding through its reciprocal connections back to the mPFC27. Finally, these BLA glutamatergic neurons also project to the lateral hypothalamus, another structure important for both food intake and reward27.

Prefrontal neurons also project to the shell region of the NAc28, and interruption of glutamate signaling in the NAc shell has been shown to have strong effects on feeding29. The NAc receives glutamatergic input from a variety of nuclei, including the prefrontal cortex, and we show that projections from D1 neurons are also found there. However, we did not observe a Fos response in the NAc, suggesting that, under the stimulation conditions used, the prefrontal D1 system is not directly influencing the NAc neuronal activity as assessed with Fos.

The sustained effect on feeding after prolonged stimulation periods is notable. We saw no changes in the first hour of photostimulation for either the PFC-targeted or BLA-targeted groups during the overnight
feeding paradigm, but rather gradual increases during the subsequent hours. Because animals began the test during times when they would normally start feeding, the stimulation during the first hours could be obscured by normal intake (for example, Supplementary Fig. 4a,b). This suggests that only once satiety signals were present did prefrontal stimulation drive additional intake. This is further supported by the 1-h free-feeding data, in which increases were observed at times when animals do not typically eat and thus are presumably sated. Also the observation that intake of both grain pellets and more palatable foods are increased as a result of stimulation suggests that palatability does not seem to play a significant role in this stimulated intake.

While we have implicated D1-receptor containing neurons in food intake, how dopamine interacts with these neurons to influence this response is not yet known. Dopamine in the PFC is increased during consumption of food30, so increased firing of VTA neurons could provide increased activation of D1 neurons. It is possible that sustained increases in D1 activity are required for the feeding response, something that could occur with PFC overactivation4, as is seen in some obese humans. However, it is also possible that another common feature to D1 neurons could be mediating this independently of dopamine. The increased intake observed here is nonetheless consistent with previous studies that demonstrate a relationship between dopamine and feeding in nigrostriatal and mesolimbic pathways13.

A recent study has found support for a role of mPFC µ-opioid receptor (MOR) activity in mediating food intake, but the neuronal population responsible was not identified6. It is possible that these MORs are located on interneurons whose inhibition results in a net disinhibition of glutamatergic neurons. However, recent work suggests that MOR and D1 colocalize in prefrontal neurons, and an interaction between the βγ subunit of the MOR G-protein on adenylyl cyclase results in potentiation of D1 agonism31. Although the present study identifies that D1 pyramidal neurons are candidates for mediating this effect, further work will need to examine the contribution of µ-opioids to D1 receptor cellular excitability through either direct or indirect mechanisms.

By selectively limiting our stimulation to the D1 neurons, we focus on a neuronal population that has been previously demonstrated to be necessary for working memory17 and interval timing18 as well as relapse to palatable food seeking22,23. These results extend a role for D1 neurons in the feeding response and suggest a direct interaction with the amygdala to mediate this effect. This circuit also presents new therapeutic opportunities, as future interventions for obesity or eating disorders may consider this prefrontal circuit for pharmacotherapy.

METHODS
Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS
B.B.L., N.S.N. and R.J.D conceived the study; B.B.L., N.S.N., R.-J.L., C.A.G. and G.K.A. conducted the experiments and analyzed the data; C.E.B., D.M.G., M.S., D.J.G. and K.D. provided viral constructs and genotyping support; B.B.L., N.S.N. and R.J.D. wrote the manuscript.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.
ONLINE METHODS

Animals. A total of 64 male or female Dnd1a-cre1 mice and Dnd1a-cre1 littermate controls, strain EY262, Gensat, back-crossed at least ten generations to a C57Bl/6 background) weighing 20–30 g were used for these studies. For behavioral optogenetic studies, males were exclusively used. All animals were housed in groups until optogenetic testing, when individuals were housed singly. Animals were on a 12:6 light-dark cycle and provided standard chow and water ad libitum except during behavioral training, and all animal procedures were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Viral preparation and surgery. Viral production for AAV-EF1α-DIO-Chr2-eGFP and AAV-EF1α-DIO-Synb-eGFP was accomplished using a triple-transfection, helper-free method and purified as described in detail previously33. Additional Chr2, flox-eNhP (AAV-EF1α-DIO-eNhPHR3.0-EYFP) and CaMKII-promoted eNhHR (AAV-CaMKIIa-eNhPHR3.0-EYFP) viruses were purchased from the UNC viral core. To generate the flox-eGFP-synaptobrevin fusion construct, the flox-Chr2 construct was cut inside the asymmetric loxP sites using NheI and Ascl restriction enzymes, and a cassette containing synaptobrevin fused to eGFP was cloned in.

For surgery, animals were anesthetized with 10% ketamine and 1% xylazine and placed in a stereotaxic frame (Stoelting). After craniotomy, mice were injected with AAV-flox-Chr2 or AAV-flox-eNhP into the PFC (AP, +1.8, ML, −0.2, DV, −2.8) and/or CaMKII-promoted eNhHR mBLA (AP, +1.8, ML, −3.0, DV, −4.6), with immediate placement of an optical fiber cannula (200-µm core, 0.22 NA, Doric Lenses) either into the PFC at the same coordinates (unilateral or bilateral) or just dorsal to the caudal-mBLA (AP, −1.8, ML, −3.0, DV, −4.5). Animals receiving AAV-eGFP-synaptobrevin 2 in the PFC were not cannulated. The injection consisted of 0.5 µl of −1011 infectious particles per milliliter. Animals recovered for at least 2 weeks before behavioral or electrophysiological testing.

Electrophysiology. Brain slices were prepared as previously described34. Briefly, mice were anesthetized (chloral hydrate, 400 mg per kilogram of body weight, intraperitoneal (i.p.)) and brains removed and placed in ice cold (4 °C) artificial cerebrospinal fluid (ACSF) in which sucrose (252 mmol/L) was substituted for sodium chloride (sucrose-ACSF). Blocks of tissue containing PFC (400 µm) were cut in sucrose-ACSF with an oscillating-blade tissue slicer (Leica). Slices were placed in a submerged recording chamber; bath temperature was then raised slowly to 32 °C. The standard ACSF (pH 7.35), equilibrated with 95% oxygen and 5% carbon dioxide, contained 128 mmol/L sodium chloride, 3 mmol/L potassium chloride, 2 mmol/L calcium chloride, 2 mmol/L magnesium sulfate, 24 mmol/L sodium bicarbonate, 1.25 mmol/L sodium phosphate and 10 mmol/L d-glucose. There was recovery period of 1–2 h before recording.

ChrR2+ pyramidal neurons in layer 5 were visualized by video microscopy using a microscope (×60 infrared lens; Olympus, Center Valley Pennsylvania) with infrared differential interference contrast and an eYFP filter cube (Olympus). Patch pipettes (3–5 MΩ) were pulled from glass tubing using a Flaming-Brown horizontal Puller (Sutter). The pipette solution contained the following: 115 mmol/L potassium gluconate, 5 mmol/L potassium chloride, 2 mmol/L magnesium chloride, 2 mmol/L magnesium ATP, 2 mmol/L disodium adenosine 5'-triphosphate, 10 mmol/L sodium-phosphocreatine, 0.4 mmol/L disodium guanosine 5'-triphosphate and 10 mmol/L HEPES, pH 7.33 (American Bioanalytical). Neurobiotin (0.3%; Vector Laboratories) was added to the pipette solution to mark selected cells for later processing and imaging.

Whole-cell recordings were done with an Axoclamp-2B amplifier (Molecular Devices). The output signal was low-pass filtered at 3 kHz and digitized at 15 kHz; data were acquired by pClamp 9.2/Digidata 1320 software (Molecular Devices). Series resistance, which was monitored throughout the experiment, was usually between 4 MΩ and 8 MΩ. Postynaptic currents were studied in the continuous single-electrode voltage-clamp mode (3000 Hz low-pass filter) clamped near resting potential (75 mV ± 5 mV). Photostimulation of the slice was performed using a 100-mW, 473-nm laser (OEM optics) driven at 20 Hz by an interval generator (pulse width, 10 ms). The fiber optic was placed just above the slice, outside of the perfusion solution.

Food deprivation–refeeding paradigm. Animals were assigned to deprived or control groups, and the deprived animals' food was removed at 2:00 p.m. Twenty-four hours later, both control and deprived groups were given several small chow pellets in the home cage, and the deprived animals additionally received food in the metal hopper cage-top. After 90 min, food was weighed and animals were immediately intracardially perfused and prepared for immunohistochemistry.

One-hour free-feeding–photostimulation paradigm. After recovery from surgery, animals were placed into a home-cage that lacked bedding but contained a small weigh-boat containing grain pellets. Animals habituated to the home cage for 1 hr before photo-illumination. On day 4, animals were briefly anesthetized using isoflurane, and the incoming fiber optic was joined to the indwelling optical fiber. The animal was placed in the home cage and photoillumination took place in 10 repeating cycles of 3-min, 20-Hz light pulses (pulse width 10 msec) and 3 min of no illumination (1 h). The number of pellets counted at the end of the test was subtracted from the initial amount. After several days of recovery, animals were again tested using 5-Hz light pulses. For the PFC D1 terminal and mBLA stimulation-inhibition experiment, 20-Hz blue light was delivered simultaneously with virtually constant yellow (590 nm; 2,900 ms on, 100 ms off) light from a separate source laser (OEM) using the same 3 min on-off cycling protocol. For the high-fat experiment, animals were exposed to the high-fat diet in their home cage several days before testing. On test day, a high-fat pellet was placed in the testing home cage, and the same protocol was used, with stimulation at 20 Hz.

One-hour free-feeding–photoinhibition paradigm. After recovery from surgery, animals were food restricted to −90% of free-feeding weight and habituated to the home cage as described above for 4 d. On the ‘restricted’ test day, light delivery consisted of four 15-min epochs as follows: no light, yellow light, no light, yellow light. Yellow light was nearly constant (2,900 ms on, 100 ms off) during illuminated epochs. During each change in light, pellets were counted and the number subtracted from the previous amount to calculate amounts eaten during light and no-light epochs. After testing, animals were fed ad libitum for 1 week and then tested again fully sated. For this test, the light epochs were reversed such that yellow light was presented first. Pellets were counted at the end of the session and compared to a pretest session the day before, done without illumination.

Overnight feeding paradigm. After recovering from surgery, animals were food restricted to 85–90% of free-feeding weight. After 3 d of overnight food restriction, animals were trained to make an operant response for a grain pellet (20 mg, BioServ) on a cued, fixed response 1 schedule (one nose-poke into an empty port yielded one pellet) in an apparatus (Med Associates) that had a nose-poke port on one side and a food magazine on the other. After animals reached asymptotic responding (at least 80 pellets in 1 h for 2 d), animals were returned to ad libitum feeding in their home cages for at least 1 week until weights recovered to prerestriction amounts. On test day, animals were taken to the testing apparatus and briefly anesthetized with isoflurane, and the incoming fiber optic was joined to the indwelling optical fiber. The animal was then placed in the apparatus and the overnight feeding program was initiated. This program consisted of repeating 1-h cycles consisting of two 30-min epochs starting at 5:30 p.m., 1.5 h before the onset of the dark cycle. In the first 30-min epoch, the laser was off, and no light was delivered to the animal. In the second 30-min epoch, five 3-min, 20-Hz light trains were delivered, interspersed with 3 min of no laser (Fig. 3e). Throughout the test, animals received a grain pellet after a nose-poke (response). Importantly, after the first response the cue light was turned off and remained off, so there were no cues associated with the response. Animals performed the test for 13 h, and after the animal was removed from the apparatus in the morning, the number of pellets remaining were counted and subtracted from the total to give the exact number of pellets consumed. Animals in the high-fat overnight feeding paradigm were not restricted and were placed in the chamber after laser coupling with two high-fat pellets (1–0.5–0.5 g total). Licks on a water spout and non-reinforced head entries were measured over the 13 h, and initial and final weight of food was recorded.

Immunofluorescence. All animals subject to behavioral tests were briefly anesthetized with isoflurane, coupled to the laser, and placed back in their home cages. After anesthetic recovery, light was delivered at 20 Hz for 5 min in 30 s on-off trains. 90 min after the start of light delivery, animals were deeply anesthetized.

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and intracardially perfused with 4% paraformaldehyde. The brain was removed and post-fixed in paraformaldehyde, and after immersion in sucrose for cryoprotection, 40 µm sections were made on a freezing microtome, and stored in 1× PBS with 0.01% sodium azide to prevent bacterial growth. Immunohistochemistry was performed according to methods described previously19. Staining for Fos (rabbit anti-cFos; Santa Cruz-sc-52; 1:500), CaMKII (mouse anti-camkii; Cayman Chemical-10011437; 1:250), PV (mouse anti-parvalbumin; Swant-PV 235; 1:1,000), Cre (mouse anti-Cre; Millipore-MAB 3120; 1:500) or D1 receptor (Guinea Pig anti-D1R; Frontier Science co-D1R-GP-AF500; 1:200) with secondary antibodies (Alexa 488 or Alexa 555; Invitrogen/Life Sciences A21202, A31570, A31572; Jackson Immunoresearch 706-546-148, 1:500) was performed in 3% normal donkey serum and 0.3% Triton X-100. Tissue was visualized and images were captured using a fluorescent microscope (Zeiss) using standard FITC and TRITC filters or using a confocal microscope (Olympus). Fos labeling was quantified by standard threshold settings in ImageJ (http://rsb.info.nih.gov/ij/) over matched areas on one or more (averaged) sections per animal, and overlap between Fos, PV, CaMKII, D1 and/or Cre was determined by apposition of the separate color channels.

Statistics. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications19,33. Cage littermates were assigned to experimental or control groups on the basis of genotype and were age- and weight-matched. Data distribution was assumed to be normal, but this was not formally tested. Comparisons were made between Drd1a-cre+ and Drd1a-cre− animals using one-way and two-way ANOVA (with light, genotype, side of injection and/or time as factors) or unpaired, two-tailed t-tests for pellet consumption, and within subjects using paired, two-tailed t-tests when appropriate. Differences in means were considered significant when P < 0.05 and were calculated using GraphPad Prism 5.0 and 6.0. Error bars represent s.e.m.

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