Food cue regulation of AGRP hunger neurons guides learning

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Agouti-related peptide (AGRP)-expressing neurons are activated by fasting—this causes hunger^{1–4}, an aversive state that motivates the seeking and consumption of food^{5,6}. Eating returns AGRP neuron activity towards baseline on three distinct timescales: rapidly and transiently following sensory detection of food cues^{6–8}, slowly and longer-lasting in response to nutrients in the gut^{9,10}, and even more slowly and permanently with restoration of energy balance^{9,11}. The rapid regulation by food cues is of particular interest as its neurobiological basis and purpose are unknown. Given that AGRP neuron activity is aversive⁶, the sensory cue-linked reductions in activity could function to guide behaviour. To evaluate this, we first identified the circuit mediating sensory cue inhibition and then selectively perturbed it to determine function. Here, we show that a lateral hypothalamic glutamatergic → dorsomedial hypothalamic GABAergic (γ-aminobutyric acid-producing)¹² → AGRP neuron circuit mediates this regulation. Interference with this circuit impairs food cue inhibition of AGRP neurons and, notably, greatly impairs learning of a sensory cue-initiated food-acquisition task. This is specific for food, as learning of an identical water-acquisition task is unaffected. We propose that decreases in aversive AGRP neuron activity⁶ mediated by this food-specific circuit increases the incentive salience¹³ of food cues, and thus facilitates the learning of food-acquisition tasks.

We hypothesized that the rapid regulation of AGRP neurons by food cues is likely to arise from an afferent neural pathway that is distinct from those that cause sustained inhibition. One such afferent to AGRP neurons is the GABAergic ventral dorsomedial hypothalamic (vDMH) projection that expresses leptin receptor (LEPR) and prodynorphin (PDYN)¹². These neurons (hereafter referred to as DMH^{LEPR} neurons) are activated by the sensory detection of food and their activation is proportional to caloric value and palatability¹². To precisely determine the relationship between food cue detection and changes in neuronal activity, we trained mice to associate an arbitrary cue (light) with access to food. Food-restricted mice were trained on a two-alternative forced-choice task (2AFC) in which the illumination of a light source was randomly presented at one of two active ports, indicating food availability in the illuminated port¹⁴. With training, mice learned to poke the correct port associated with this initially arbitrary visual cue (light) to receive the food reward (Ensure) (Fig. 1a). Using this paradigm in combination with fibre photometry, we found that in trained mice, DMH^{LEPR} neurons are robustly activated and their activation follows visual cue presentation (green line) as opposed to nose poking for ‘ensure’ (black line) (Fig. 1b, Extended Data Fig. 1a). As anticipated, this neural response to the otherwise arbitrary light cue was absent in naive mice—thus it developed over time as the mouse learned to associate the light cue with food availability (Fig. 1c, d, Extended Data Fig. 1a, b).

DMH^{LEPR} neurons are clearly rapidly activated by the sensory detection of food cues¹² (Fig. 1b, Extended Data Fig. 1a). However, it is unknown whether DMH^{LEPR} neurons are also capable of responding to nutrients in the gastrointestinal tract, in a similar way to AGRP neurons^{9,10}. To address this, we infused both caloric (Ensure and glucose) and non-caloric (saline and saccharin) substances directly into the stomach. DMH^{LEPR} neurons were activated by infusions of caloric substances, but not by non-caloric substances (Extended Data Fig. 1c–g). However, compared with sensory detection of food, the DMH^{LEPR} neuron response to gut infusions was slower and smaller in magnitude (approximately 5 min and 5% ΔF_n) compared to the pellet drop response (about 1 s, about 20% ΔF_n) (Extended Data Fig. 1d–f, h). Of note, this regulation appears to not be consequential for satiation, as inhibition of DMH^{LEPR} neurons did not increase the amount of food eaten during a meal (Extended Data Fig. 1i, Supplementary Information).

As DMH^{LEPR} neurons are not necessary for regulating processes that occur over longer timescales—that is, satiation (Extended Data Fig. 1i) or satiety¹²—we next investigated the basis for and function of the rapid regulation of DMH^{LEPR} neurons by food cues. To find the monosynaptic afferents to DMH^{LEPR} neurons, we used EnvA pseudotyped, G-deleted rabies tracing^{15,16} and channelrhodopsin (ChR2)-assisted circuit mapping. Notably, whereas many sites provide GABAergic input, only one site provides glutamatergic input—the lateral hypothalamus (LH) (Extended Data Fig. 2, Supplementary Information). As neurons in

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Fig. 1 | Regulation of DMH^{LEPR} neurons by learned food cues. **a**, Illustration of the 2AFC behavioural task. Randomized cue lights are associated with the appearance of food (beige). **b**, Representative heat map of a well-trained mouse using DMH^{LEPR} neuron fibre photometry to observe cue responses during a single behavioural session. Single trials are sorted by response time. The green line indicates light onset (time = 0), the black line indicates time when the

mouse poked correctly. **c**, Example traces in a mouse when completely naive to the 2AFC task (top) and after it has become proficient at the task (bottom). **d**, Mean peak amplitude of DMH^{LEPR} neuron fibre photometry responses in naive and trained mice in the 2AFC task. Peak amplitude was taken within a 2-s window following cue-light presentation. $n = 4$ mice; unpaired two-tailed t -test, $*P = 0.0241$. Data represent mean $\pm$ s.e.m.

the LH expressing vesicular glutamate transporter 2 (VGLUT2) (LH^{VGLUT2} neurons) are excitatory and would activate DMH^{LEPR} neurons, and as the general LH^{VGLUT2} population has been shown to be involved in sensory cue detection¹⁷, we characterized the LH^{VGLUT2} → DMH^{LEPR} circuit in greater depth.

The LH is a well-known but poorly understood central regulator of motivational drive, reward learning, and feeding behaviour^{18,19}. Previous studies have demonstrated that LH^{VGLUT2} neurons, en masse, potently suppress food intake and are aversive^{20–23}. However, as LH^{VGLUT2} neurons are extremely diverse, both genetically and in their projection targets^{24,25}, we sought to test the effects of activating the DMH-projecting subset of LH^{VGLUT2} neurons on feeding and valence (that is, positive-rewarding versus negative-aversive). As these LH^{VGLUT2} neurons activate DMH^{LEPR} neurons, which in turn inhibit AGRP neurons, we hypothesized that their activation would produce effects consistent with AGRP neuron inhibition—namely, suppress food intake and be appetitive during times of caloric deficiency (when AGRP neurons are active). To test this, we bilaterally injected adeno-associated virus (AAV) expressing Cre-dependent-ChR2 into the LH of *Vglut2-IRES-Cre* mice and placed a single optic fibre within the midline above the DMH to selectively stimulate LH^{VGLUT2} → DMH terminals (Fig. 2a). We then measured food intake while optically stimulating LH^{VGLUT2} → DMH terminals, beginning at the onset of the dark cycle (when AGRP neuron activity is high and mice are inclined to eat). Consistent with our proposed circuit, we found that LH^{VGLUT2} → DMH terminal stimulation significantly decreased food intake (Fig. 2b). To evaluate the valence of the LH^{VGLUT2} → DMH circuit, we used a real-time place-preference assay (RTPP) in which mice were optically stimulated in one side of a behavioural arena and could roam between the stimulation and non-stimulation side. When ad libitum-fed mice were placed in the RTTP arena at the onset of the light cycle (that is, when AGRP activity is low⁸), the mice were agnostic to LH^{VGLUT2} → DMH stimulation (Fig. 2c, Extended Data Fig. 3a). We then repeated the RTTP assay at the onset of the dark cycle (that is, when AGRP activity is high⁸). Under these conditions, ad libitum-fed mice showed a robust preference (about 80%) for LH^{VGLUT2} → DMH stimulation, which is expected to inhibit AGRP neuron activity (Fig. 2d, Extended Data Fig. 3a). Finally, to drive AGRP activity to a maximal level and to control for the time of day, we food-restricted the mice overnight and repeated the RTTP assay at the onset of the light cycle. Remarkably, the mice displayed a robust preference (approximately 80%) for LH^{VGLUT2} → DMH stimulation (Fig. 2e, Extended Data Fig. 3a). Notably, total locomotor activity was not significantly different between X-coloured fluorescent protein (XFP)- and ChR2-expressing groups (Extended Data Fig. 3b–d). Consistent with these LH^{VGLUT2} → DMH neurons not promoting aversion, we found that, unlike other LH^{VGLUT2} neurons, they do not send collaterals to sites known to promote aversive behaviours^{20–23} (Extended Data

Fig. 3e–g). In total, these findings suggest that the DMH-projecting LH^{VGLUT2} neurons are functionally and anatomically distinct from previously studied LH^{VGLUT2} neurons^{20–23,26}. Furthermore, as AGRP neurons promote hunger and aversion^{1–4,6,27,28}, the observed effects (decreased hunger and being appetitive) are consistent with these LH^{VGLUT2} neurons being upstream of the DMH^{LEPR} (GABAergic) → AGRP circuit.

The LH is known to participate in many aspects of feeding behaviour^{18,19,24} and LH^{VGLUT2} → nucleus accumbens (NAc) neurons have been found to be necessary for signalling cue–outcome associations¹⁷. With this in mind, we tested whether the DMH-projecting LH^{VGLUT2} neurons were responsive to food pellet drops. We used axon-fibre photometry to record LH^{VGLUT2} → DMH axonal Ca²⁺ activity in freely moving mice (Fig. 2f) while presenting food pellets of increasing caloric value and palatability^{7,12} (Fig. 2g–k). LH^{VGLUT2} → DMH axons were rapidly activated upon the presentation of a food item, were scalable to caloric content, and the responses were transient (returning to baseline within 10–20 s) (Fig. 2h–k, Extended Data Fig. 3i, j). In addition, we found that the response was mostly specific for food in that a much smaller and much more delayed response was seen when a non-edible object was presented (Extended Data Fig. 3h, i) or when water was presented to water-deprived mice (Extended Data Fig. 3k–m). In sum, our findings suggest that LH^{VGLUT2} → DMH neurons are activated by sensory cues preceding food ingestion, and that they probably provide this information to DMH^{LEPR} neurons.

If the LH does indeed provide food cue information to the DMH^{LEPR} → AGRP pathway, then selectively inhibiting LH^{VGLUT2} → DMH afferents should attenuate the food cue response in DMH^{LEPR} neurons. We used two distinct methods to determine whether LH → DMH afferents were necessary for the cue-evoked response in DMH^{LEPR} neurons. First, we used a retrograde AAV-FlpO virus to selectively express FlpO in LH neurons that project to the DMH. To record Ca²⁺ activity while simultaneously inhibiting LH → DMH afferents, we injected Flp-dependent AAV-hM4Di into the LH and Cre-dependent AAV-GCaMP6s into the DMH of *Lepr-IRES-Cre* mice (Fig. 3a). Flp-dependent AAV-hM4Di efficiently hyperpolarized LH neurons (Extended Data Fig. 4a, b). Notably, inhibiting LH → DMH afferents attenuated the food cue-evoked response in DMH^{LEPR} neurons by about 70% (Fig. 3b, c). Clozapine-*N*-oxide (CNO) injections in GFP-expressing control mice had no effect (Extended Data Fig. 4c, d). To specifically investigate the role of LH^{VGLUT2} neurons in mediating DMH^{LEPR} neuron food cue responses, we used *Vglut2-IRES-S2-FlpO::Lepr-IRES-Cre* mice to express Flp-dependent AAV-hM4Di in LH^{VGLUT2} neurons and Cre-dependent AAV-GCaMP6s in DMH^{LEPR} neurons (Fig. 3d). Selectively inhibiting LH^{VGLUT2} neurons also attenuated the rapid DMH^{LEPR} cue-evoked response by approximately 68% (Fig. 3e, f). Collectively, these studies show that LH^{VGLUT2} neurons drive food cue-evoked responses in DMH^{LEPR} neurons.

Fig. 2 | Functional characterization of a LH^{VGLUT2} → DMH^{LEPR} neuronal circuit.

a, Experimental design schematic and representative image. AAV-DIO-ChR2 was bilaterally injected into LH^{VGLUT2} neurons and a single optic fibre was placed in the midline above the DMH. Scale bar, 500 μ m. *n* = 8 mice. **b**, Optogenetic stimulation of LH^{VGLUT2} → DMH terminals decreases nighttime food intake in ad libitum-fed mice. *n* = 8 mice; repeated-measures two-way ANOVA; Sidak's multiple comparison test; ***P* = 0.0016, ****P* = 0.0002. **c–e**, Real-time place preference: optogenetic stimulation (stim.) of LH^{VGLUT2} → DMH terminals has no effect when mice are calorically replete (*P* = 0.6531) (**c**) and is appetitive when mice are calorically deficient at night (*P* = 0.008) (**d**) and during fasting (*P* = 0.0129) (**e**). *n* = 7 (ChR2) and 7 (XFP). Two-tailed unpaired *t*-test, **P* ≤ 0.05,

P* ≤ 0.01; NS, not significant. **f**, Experimental design (top) and representative image (bottom). AAV-DIO-GCaMP6s was injected into LH^{VGLUT2} neurons. Optic fibre was placed over the DMH to perform fibre photometry in LH^{VGLUT2} terminals. Coloured arrows indicate light wavelengths delivered and collected. Scale bar, 500 μ m. *n* = 9 (mice). **g–j**, LH^{VGLUT2} → vDMH axons are rapidly activated upon the sensory detection of food (**g**) and are scalable by caloric value (**h, i**) and palatability (**j**). Heat map (**g**) represents the trial-by-trial response in a representative mouse from 15 mg pellet presentation (time = 0). Line in **h–j** represents mean $\pm$ s.e.m. **k**, Mean peak response within the first 5 s following pellet presentation. One-way ANOVA; Friedman test, *P* = 0.0007. *n* = 9 mice. Data represent mean $\pm$ s.e.m.

To determine whether LH^{VGLUT2} neurons do indeed cause food cue evoked activation of AGRP neurons, we used *Vglut2-IRES2-FlpO::AGRP-IRES-Cre* mice to restrict expression of Flp-dependent AAV-hM4Di in LH^{VGLUT2} neurons and Cre-dependent AAV-GCaMP6s in AGRP neurons (Fig. 3g). Selective inhibition of LH^{VGLUT2} neurons robustly attenuated the rapid, cue-evoked response in AGRP neurons (approximately 76% decrease in response magnitude) but did not affect sustained AGRP inhibition during food consumption of large food pellets (Fig. 3h, i, Extended Data Fig. 4f). Thus, LH^{VGLUT2} neurons have an essential role in food cue-evoked inhibition of AGRP neurons but appear to not be involved in gut nutrient-mediated regulation of AGRP neurons.

We next explored the function of this food cue-mediated regulation of the LH^{VGLUT2} → DMH^{LEPR} → AGRP circuit. In mice that learned the task, CNO-hM4Di inhibition of LH → DMH neurons had no effect on task performance (Extended Data Fig. 4e, g–j). This lack of effect may be owing to habit-like performance in such highly trained mice²⁹. To determine whether this food cue-regulated circuit has a role in the learning of this task, we expressed Cre-dependent AAV-tetanus toxin (TeNT) in DMH^{LEPR} neurons to eliminate evoked synaptic release within the circuit (Fig. 4a). We then trained naive mice on the 2AFC task (Fig. 1a) until they mastered the task (or a maximum of 21 training days). We calculated the number of correct responses in addition to the errors made, including misses (failing to poke within the response window) and false alarms (poking in the incorrect port). We also assessed the response time (how long after cue presentation the poke was performed). TeNT-mediated DMH^{LEPR} silencing caused a significant delay in task acquisition (Fig. 4b, c). In addition, the TeNT-mediated DMH^{LEPR}-silenced mice took many more days of training to reduce their misses, false alarms and response times (Extended Data Fig. 5a–c). The higher number of misses and false alarms and, in particular, the longer response times suggest that in DMH^{LEPR}-silenced mice, the cue light is

less effective in motivating food-seeking behaviour. Consistent with the view that the DMH^{LEPR} → AGRP neuron circuit is not involved in either satiety¹² or satiation (Extended Data Fig. 1i), body weight (Extended Data Fig. 5d) and post-fast refeeding (Extended Data Fig. 5e) were not affected in TeNT-mediated DMH^{LEPR}-silenced mice.

Although AGRP neuron-regulating DMH^{LEPR} neurons project only to the arcuate nucleus¹² (ARC), the DMH contains other LEPR-expressing neurons that project elsewhere¹². Thus, the impairment in learning caused by TeNT silencing of DMH^{LEPR} neurons could be owing to the silencing of DMH^{LEPR} neurons that project to other parts of the brain (Extended Data Fig. 5f). To address this, we used *Pdyn-IRES-Cre* mice to express TeNT selectively in DMH neurons expressing PDYN (DMH^{PDYN} neurons). It was previously established that PDYN is expressed by a major subset of DMH^{LEPR} neurons and that DMH^{PDYN} neurons send long-range projections exclusively to the ARC, where they inhibit AGRP neurons¹² (Extended Data Fig. 6). These mice were then trained on the 2AFC task. Of interest, silencing DMH^{PDYN} neurons modestly increased body weight in TeNT-expressing mice (Extended Data Fig. 5l) but did not affect post-fast refeeding (Extended Data Fig. 5m). As body weight was not increased when DMH^{LEPR} neurons were silenced (Extended Data Fig. 5d), this body weight effect may be owing to the silencing of LEPR-negative DMH^{PDYN} neurons. Of note, silencing DMH^{PDYN} neurons, similar to silencing DMH^{LEPR} neurons, significantly altered task performance in that it delayed task acquisition, increased mistakes made and increased response time (Extended Data Fig. 5g–k). Given that DMH^{PDYN} neurons project selectively to AGRP neurons, these findings, along with those from the DMH^{LEPR}-silencing study (Fig. 4a–c, Extended Data Fig. 5a–c), strongly support the view that the DMH → AGRP neuronal circuit has a key role in the learning of a sensory cue–food acquisition task.

To specifically test the role of the LH → DMH segment of the circuit in learning the 2AFC task, we selectively silenced DMH-projecting

Fig. 3 | LH afferents to the DMH are necessary for rapid food cue-evoked responses in DMH^{LEPR} and AGRP neurons. **a**, Top, retrograde AAV-FlpO and AAV-DIO-GCaMP6s were injected into the DMH of *Lepr-IRES-Cre* mice and AAV-fDIO-hM4Di was injected into the LH to inhibit LH → DMH neurons. Bottom, sample images of hM4Di expression (left) and GCaMP6s expression and fibre placement (right). Scale bar, 200 μ m. $n = 4$ mice. **b**, Example traces of vehicle and CNO recording session within the same animal. Vehicle and CNO recording sessions were split so that treatment comparisons were done within the same day. Vehicle 1.1 represents the first vehicle recording session and vehicle 1.2 represents the second recording session. Vehicle 2.1 represents vehicle recording session on the second day and CNO 2.2 represents the second recording session with CNO injection. The black vertical line represents cue presentation; the purple box represents the analysed 2-s period; the blue horizontal line represents peak of vehicle control from the first recording session. **c**, LH → DMH inhibition attenuates the cue response in DMH^{LEPR} neurons. Normalized responses are calculated as $\Delta F/F_0$ for the second recording session (R2) divided by $\Delta F/F_0$ for the first recording session (R1). $n = 4$ mice. Two-tailed t -test, $^{**}P = 0.0035$. **d**, Top, AAV-fDIO-hM4Di was injected into the LH and AAV-DIO-GCaMP6s was injected into the DMH of *Vglut2-IRES2-FlpO::Lepr-IRES-Cre* mice. Bottom, sample images of AAV-fDIO-hM4Di expression (left) and AAV-DIO-GCaMP6s expression and fibre placement (right). Scale bar, 500 μ m. $n = 4$ mice. **e**, Example traces from one mouse for experiment described in **d** (labelled as in **b**). **f**, LH^{VGLUT2} inhibition attenuates the cue response in DMH^{LEPR} neurons. $n = 4$ mice; two-tailed t -test, $^{*}P = 0.0009$. **g**, Top, AAV-fDIO-hM4Di was injected into the LH and AAV-DIO-GCaMP6s was injected into the ARC of *Vglut2-IRES2-FlpO::AgRP-IRES-Cre* mice. Bottom, sample images of expression and fibre placement: AAV-fDIO-hM4Di (right) and AAV-DIO-GCaMP6s (left). Scale bar, 500 μ m. $n = 4$ mice. **h**, Example traces in one mouse for vehicle and CNO (labelled as in **b**). **i**, LH^{VGLUT2} inhibition decreases the cue response in AGRP neurons. $n = 4$ mice; two-tailed t -test, $^{*}P = 0.027$. Data represent mean $\pm$ s.e.m.

LH neurons by bilaterally injecting a retrograde AAV-Cre virus into the DMH and a Cre-dependent TeNT AAV into the LH (Extended Data Fig. 5n). Indeed, silencing these neurons significantly impaired the rate of task acquisition and significantly increased misses and response times (Extended Data Fig. 5o–s). Of note, TeNT-mediated silencing of LH → DMH neurons did not affect body weight or post-fast refeeding (Extended Data Fig. 5t, u), suggesting that this LH → DMH projection is not necessary for satiety or satiation as seen when silencing DMH^{LEPR} neurons (Extended Data Fig. 5d, e). Collectively, these data (Fig. 4a–c, Extended Data Fig. 5a–u) show that the LH^{VGLUT2} → DMH^{GABAergic} → AGRP neuron circuit has an important role in promoting the mastery of this caloric-deficiency-driven, sensory cue-initiated food-acquisition task.

To assess the possibility that TeNT expression in DMH^{LEPR} neurons somehow interferes non-specifically with deprivation state-motivated

learning of an operant task, we generated a cohort of TeNT-expressing water-deprived mice and determined their ability to learn the identical task with water, instead of food, as the reward (Fig. 4d). We hypothesized that the principles of learning in these two tasks would be identical, with the sole exception that water cue regulation of aversive thirst neurons^{6,30–32}, instead of food cue regulation of hunger neurons, would promote learning. As the DMH → AGRP circuit is involved in food cue regulation of hunger neurons, and not in water cue regulation of thirst neurons, this experiment controls for nonspecific effects. As shown in Fig. 4e, f and Extended Data Fig. 5v–x, TeNT expression had no effect on learning in the water-oriented version of the task. Thus, the DMH^{LEPR} → AGRP neuronal circuit is specific for learning food cue-initiated food-acquisition tasks, which is congruous with the very specific role of AGRP neurons in regulating food intake but not water intake.

Fig. 4 | Afferent modulation of AGRP neurons is required for learning a cue-initiated food acquisition task. **a**, Top, AAV-DIO-TeNT injection into DMH^{LEPR} neurons. Bottom, example images of TeNT expression. Scale bar, 500 μ m. $n = 5$ (mice). **b**, DMH^{LEPR} neuron silencing attenuates correct responses. $n = 5$ (GFP) and 5 (TeNT) mice. Red line is the line of best fit ($***P < 0.0001$, plateau = 97.49 (GFP), 132.4 (TeNT); tau = 6.928 (GFP), 20.80 (TeNT)). Two-way repeated measures ANOVA; main effect of days, $****P < 0.0001$; main effect of group, $****P < 0.0001$. **c**, Silencing DMH^{LEPR} neurons increases time to reach learning criterion (>80% correct across three consecutive days); 5 out of 5 GFP and 4 out of 5 TeNT mice reached the learning criterion. $n = 5$ (GFP) and 5 (TeNT)

mice. Two-tailed, unpaired t -test; $*P = 0.0238$. **d**, Top, AAV-DIO-TeNT injection into DMH^{LEPR} neurons. Bottom, example images of TeNT expression. Scale bar, 500 μ m. $n = 6$ mice. **e**, Silencing DMH^{LEPR} neurons does not affect behavioural performance in learning to obtain water. $n = 4$ (GFP) and 6 (TeNT) mice. Red line is the line of best fit (plateau = 129.3, tau = 11.53). Two-way repeated measures ANOVA; main effect of days, $****P < 0.0001$; main effect of group, $P = 0.7203$. **f**, Silencing DMH^{LEPR} neurons does not affect learning (>80% correct across three consecutive days) when water-deprived mice are trained to receive water rewards. $n = 4$ (GFP) and 6 (TeNT) mice. Two-tailed, unpaired t -test; $P = 0.4375$. Data represent mean $\pm$ s.e.m.

Discussion

These findings lead us to propose the following model. Caloric deficiency activates AGRP neurons (reviewed in ref.¹¹) and this causes the aversive feeling of hunger⁶. Environmental cues instructive for food acquisition engage the LH^{VGLUT2} $\rightarrow$ DMH^{LEPR} $\rightarrow$ AGRP neuron circuit and this transiently reduces AGRP neuron activity. As AGRP neurons have been proposed to transmit a negative-valence teaching signal⁶, these ‘appetitive’ falls in aversive AGRP neuron activity, over time, increase the incentive salience¹³ of food cues, thereby facilitating the learning of food acquisition tasks. If it holds, this model implies the existence of a very important general neurobiological mechanism for how homeostatic deficiency states promote the learning of tasks directed at acquiring the cognate goals (such as caloric deficiency promoting food acquisition tasks or dehydration promoting water acquisition tasks)—by providing the ‘substrate’—the deficiency-activated aversive drive neurons^{6,30–32}—on which the inhibitory sensory cue-regulated afferent neurons can operate.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-021-03729-3>.

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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Experimental subjects

Vglut2-IRES-Cre (JAX 016963)³³, *Vglut2-IRES2-FlpO* (JAX 030212) (unpublished, donating investigator H. Zeng (Allen Institute for Brain Science)), *AGRP-IRES-Cre* (JAX 012899)³⁴, *Pdyn-GFP*³⁵ and wild-type mice (JAX101045) were obtained through Jackson Laboratories or in house. *Lepr-IRES-Cre*³⁶ mice were maintained as previously described¹². All mice were maintained on a mixed genetic background unless otherwise noted. *Pdyn-IRES-Cre*²⁷ mice were maintained as previously described and kept on a congenic C57BL/6J background. The National Institute of Health and Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee approved all animal care and experimental procedures. Mice were housed at 22–24 °C, 20–30% humidity with a 12:12 light:dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664) and water was provided ad libitum, unless otherwise stated. All diets were provided as pellets. For all behavioural studies, we used male mice between 8 and 20 weeks of age. For electrophysiological recordings, we used male mice between 8 and 12 weeks of age.

Brain tissue preparation

Mice were terminally anaesthetized with chloral hydrate (Sigma-Aldrich C8383) and transcardially perfused with phosphate-buffered saline (PBS) followed by 10% neutral buffered formalin (Fisher Scientific SF100). Brains were extracted then cryoprotected in 20% sucrose. All brains were sectioned coronally on a freezing sliding microtome (Leica Biosystems) at 40 µm and collected in four equal series.

Immunohistochemistry

Tissue sections were washed with 0.1 M phosphate-buffered saline (pH 7.4) then blocked in 5% normal donkey serum/0.2% Triton X-100 in PBS for 1 h at room temperature. Sections were then incubated overnight at room temperature in blocking solution containing: rat anti-mCherry (1:3,000, Invitrogen M11217) and chicken anti-GFP (1:1,000, Invitrogen A10262). Secondary detection was performed with Alexa Fluor 488 or 594 conjugated donkey anti-chicken or donkey anti-rat (1:1,000, Invitrogen) for 1 h at room temperature. After secondary incubation, sections were washed and mounted onto gelatin-coated slides and fluorescent images were obtained with an Olympus VS120 slide scanner microscope. Our inferred Bregma coordinates on all histological images were adopted from a stereotaxic atlas³⁷.

Stereotaxic surgeries and viral injections

For viral injections, six-to-eight week old male mice were anaesthetized with a ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹) cocktail diluted in 0.9% saline and placed into a stereotaxic apparatus (Kopf model 940). Subcutaneous injection of sustained-release meloxicam (4 mg kg⁻¹) was provided as postoperative care. A pulled glass micropipette (20–40 µm diameter tip) was used for stereotaxic injections of AAV. For electrophysiological experiments, bilateral injections (25 nl) of purified AAV (6.24 × 10¹² viral genomes ml⁻¹) were injected into the NAc (from bregma: +1.3 AP, ± 0.5 ML, –4.25 DV), BNST (from bregma: +0.14 AP, ± 0.75 ML, –4.9 DV), MPO (from bregma: +0.4 AP, ± 0.25 ML, –4.75 DV), LH (from bregma: –1.3 AP, ± 0.9 ML, –5.3 DV), VTA (from bregma: –3.15 AP, ± 0.6 ML, –4.74 DV), IPN (from bregma: –3.4 AP, ± 0.0 ML, –4.8 DV), and PAG (from bregma: –4.16 AP, ± 0.3 ML, –2.5 DV). For optogenetic experiments, bilateral injections (15 nl) of AAV9-EF1α-DIO-ChR2(H134R)-eYFP purchased from the University of Pennsylvania School of Medicine Vector Core (donating investigator, K. Deisseroth; AV-9-20298P; Addgene: 20298; 2.7 × 10¹³ viral genomes ml⁻¹) were injected into the LH (coordinates as above). For in vivo fibre photometry experiments,

AAV1-hSyn-DIO-GCaMP6s (University of Pennsylvania Vector core; Addgene 100845-AAV1; 1.6 × 10¹³ viral genomes ml⁻¹) was injected unilaterally into either the LH (25 nl, coordinates as above), DMH (25 nl, from bregma: –1.80 AP, ± 0.3 ML, –5.2 DV), or ARC (150 nl, from bregma: –1.45 AP, ± 0.25 ML, –5.85 DV). For retrograde chemogenetic silencing studies, AAV6-CAG-FlpO (Boston Children's Hospital Viral Vector Core, modified from Addgene 67829; 2.88 × 10¹⁴ viral genomes ml⁻¹) was bilaterally injected into the DMH (15 nl, coordinates as above) and AAV8-nEF-fDIO-hM4Di-mCherry (Boston Children's Hospital Viral Vector Core, modified from Addgene 44362 and 55644; 9.98 × 10¹³ viral genomes ml⁻¹) was bilaterally injected into the LH (25 nl, coordinates as above). For Flp-dependent chemogenetic studies in combination with fibre photometry, AAV8-nEF-fDIO-hM4Di-mCherry was bilaterally injected into the LH (25 nl, coordinates as above) or, the DMH (25 nl, coordinates as above). For tetanus toxin mediated silencing studies, AAVDJ-CMV-DIO-eGFP-2A-TeNT (Stanford; GVVC-AAV-71; 3.6 × 10¹² viral genomes ml⁻¹) was injected bilaterally into the DMH (15 nl, coordinates as above). Finally, for projection specific tetanus toxin mediated silencing studies, AAVDJ-CMV-DIO-eGFP-2A-TeNT was injected bilaterally into the LH (20 nl, coordinates as above) and rAAV2-hSyn-Cre (Addgene 105553; 1.2 × 10¹³ viral genomes ml⁻¹) was injected bilaterally into the DMH (15 nl, coordinates as above). Mice were allowed to recover for a minimum of three weeks before the initiation of any experiments. Following each experimental procedure, accuracy of AAV injections were confirmed via post hoc histological analysis of mCherry, YFP, or GFP fluorescent protein reporters, viral expression of each individual surgery is catalogued in detail within Extended Data Figs. 7–11. All subjects determined to be surgical 'misses' were those with absent or low reporter expression and were removed from the experimental dataset. In addition, mice were excluded from the data set if reporter expression was primarily outside of the area of interest. Anatomical boundaries were drawn by using the DAPI signal so that we could clearly discern landmarks within a histological section. On the basis of the landmarks present, we then inferred the A/P coordinate in the atlas³⁷ and traced the outline of different nuclei and superimposed the outline on our histological images Extended Data Fig. 12.

Optic fibre implantation

Optic fibre implantations were performed during the same surgery as viral injections (above). For optogenetic photostimulation of LH → DMH terminals, ceramic ferrule (Precision Fibre Products) optical fibres (200 µm diameter core, 0.39 NA, multimode; Thorlabs) were implanted within the midline over the DMH (from bregma: –1.8 AP, ± 0 ML, –4.7 DV). For LH^{VGLUT2} → DMH axon fibre photometry, a metal ferrule (Precision Fibre Products) optic fibre (400 µm diameter core, 0.5 NA, multimode; Thorlabs) was implanted unilaterally over the DMH (from bregma: –1.8 AP, ± 0.3 ML, –5.1 DV). For DMH^{LEPR} and ARC^{AGRP} cell body fibre photometry, a stainless steel ferrule optic fibre (same as above) was implanted unilaterally over the DMH (coordinates as above) or the ARC (from bregma: –1.45 AP, ± 0.25 ML, –5.8 DV). Fibres were fixed to the skull using dental acrylic and mice were allowed to recover for three weeks before the start of acclimation to behavioural testing.

Monosynaptic rabies mapping

Lepr-IRES-Cre mice were unilaterally injected with a 1:1 mixture of AAV8-EF1α-DIO-TVA-mCherry (University of North Carolina Vector Core, donating investigator N. Uchida) and AAV8-CAG-FLEX-Rabies G (Stanford; GVVC-AAV-59) into the DMH (15 nl, coordinates as above) (Extended Data Fig. 2a). Mice recovered for three weeks after TVA and RG transduction to ensure adequate levels of TVA and RG viral expression. Following recovery, mice underwent a second surgery in which mice were injected with SADΔG-EGFP (EnvA) rabies (Salk Gene Transfer Targeting and Therapeutics Core) into the DMH (15 nl, coordinates as above). Mice recovered for 7 days to allow for the retrograde transport of rabies virus and EGFP expression before perfusion and histological

processing. Sites of afferent input to DMH^{LEPR} neurons were assessed by the presence of EnvA-eGFP positive neurons.

Rabies collateral mapping

Vglut2-IRES-Cre mice were unilaterally injected with AAV8-EF1α-DIO-TVA-mCherry into the LH (15 nl) and allowed to recover for three weeks (Extended Data Fig. 3e–g). Then, SADΔG-EGFP (EnvA) rabies was unilaterally injected into the DMH (15 nl). Mice were allowed to recover for seven days to allow for the retrograde transport of rabies virus and EGFP transgene expression before perfusion and histological processing. Comprehensive examination of SADΔG-EGFP (EnvA) axonal and retrograde transduction was assayed using immunohistochemistry followed by imaging the entire brain for the presence of EGFP expression.

Electrophysiology

Mice were deeply anaesthetized, and intracardially perfused with ice-cold dissection buffer (in mM: 2.5 KCl, 1.25 NaH₂PO₄, 20 HEPES, 10 MgSO₄·7H₂O, 0.5 CaCl₂·2H₂O, 92 choline chloride, 25 glucose, 2 thiourea, 5 sodium ascorbate, 3 sodium pyruvate, and 20 NaHCO₃) bubbled with 95% O₂, 5% CO₂. Brains were then rapidly removed and immersed in ice-cold dissection buffer. DMH sections were dissected and 300 μm thick coronal slices were prepared using a vibrating microtome (Campden 7000smz 2). Slices recovered for 10 min in a 35 °C submersion chamber filled with oxygenated dissection buffer. Slices were then transferred to a secondary 35 °C submersion chamber filled with oxygenated artificial cerebrospinal fluid (ACSF; in mM: 125 NaCl, 2.5 KCl, 25 NaHCO₃, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 25 glucose) and allowed to recover for an additional 15 min. Slices were then kept at room temperature in oxygenated ACSF for ≥ 30 min until use (Extended Data Fig. 2b–m).

Channelrhodopsin-2 assisted circuit mapping

To isolate optically evoked inhibitory post-synaptic currents (oIPSCs) and excitatory post-synaptic currents (oEPSCs), slices were placed in a submersion chamber and perfused at 4 ml min⁻¹ with oxygenated ACSF. Cells were visualized with a Scientifica SliceScope Pro 1000 microscope equipped with infrared differential interference contrast optics. DMH^{PDYN} GABAergic neurons were identified by GFP fluorescence ventral to the DMC (dorsomedial hypothalamic nucleus compacta). Open-tip resistances for patch pipettes were between 2–4 MΩ and were backfilled with a Cs-based internal solution containing (in mM): 135 CsMeSO₃, 10 HEPES, 1 EGTA, 3.3 QX-314 (Cl⁻ salt), 4 Mg₂-ATP, 0.3 Na₂-GTP, and 8 Na₂-phosphocreatine with pH adjusted to 7.3 with CsOH and osmolarity adjusted to about 295 mOsm by the addition of sucrose. oEPSCs were isolated with membrane potential clamped at V_h = -70 mV and oIPSCs were isolated with membrane potential clamped at V_h = 0 mV. Bath solutions for pharmacological isolation of excitatory or inhibitory currents in whole-cell voltage clamp recordings contained SR95531 (10 μM, gabazine), kynurenic acid (1 mM), tetrodotoxin (TTX, 1 μM), and 4-Aminopyridine (4-AP, 500 μM). To photostimulate ChR2-positive fibres, an LED light source was used (470 nm, Cool LED pE-100). The blue light was focused onto the back aperture of the microscope objective (40×) producing wide-field exposure around the recorded cell of 10–15 mW per mm² as measured using an optical power meter (PM100D, Thorlabs). A programmable pulse stimulator, Master-8 (A.M.P.I.) and pClamp 10.2 and 10.6 software (Molecular Devices, Axon Instruments) controlled the photostimulation output. The oIPSC/oEPSC detection protocol consisted of one blue-light pulse (5 ms pulse length) at 30 s intervals for at least 6 consecutive sweeps. Changes in series and input resistance were monitored throughout the experiment by giving a test pulse every 30 s and measuring the amplitude of the capacitive current. Cells were discarded if series resistance rose above 25 MΩ (Extended Data Fig. 2b–m). All electrophysiology data was analysed using Clampfit 10.2 and 10.6.

Optogenetic behavioural experiments

In vivo photostimulation of LH^{VGLUT2}→DMH terminals was conducted by firmly attaching a fibre optic cable (1.25 m long, 200 μm core diameter, 0.63 NA; Doric Lenses) with ceramic split sleeves (Precision Fibre Products) (Fig. 2, Extended Data Fig. 3a–d). Mice were acclimated by connecting them to a 'dummy' fibre optic cable three days before the initiation of the experiment. Mice were stimulated with blue light (465 nm LED; Plexon) at 20 Hz, 5 ms pulses for 1 s with a 3 s recovery period (LED off) during stimulation trains to avoid ChR2 desensitization, neuronal transmitter depletion, and tissue heating. Light pulse trains were programmed using a waveform generator (National Instruments) that provided TTL input to the blue light LED. The light power exiting the fibre optic cable measured by an optical power meter (Thorlabs) was 7–8 mW in all experiments. After completion of photostimulation experiments, mice were perfused for assessment of surgical accuracy of both ChR2-expression and optic fibre tip location via histological analysis as described in 'Stereotaxic surgeries and viral injections'.

Food intake studies

To test the sufficiency of LH^{VGLUT2}→DMH neurons for satiety, mice were tested under conditions of physiological hunger at the onset of the dark cycle (Fig. 2b). For dark cycle feeding, mice with ad libitum access to food were photostimulated for 5 min before the onset of the dark cycle (a time when mice often eat) and photostimulation continued throughout the duration of the study (three hours). For post-fast refeeding assays (Extended Data Figs. 1, 5), mice were food restricted for 24 h then, were given ad libitum access to food. Food was then weighed each hour to determine the amount consumed during the experimental manipulation.

Real-time place preference assays

Mice were placed in a custom-made behavioural arena (transparent acrylic, 50 × 50 × 25 cm) for 20 min (Fig. 2, Extended Data Fig. 3a–d). One counterbalanced side of the arena was designated as the photostimulation side. The mouse was placed in the stimulation side at the onset of the experiment and each time the mouse crossed to the non-stimulation side of the arena, the photostimulation immediately stopped until the animal crossed back into the stimulation side. Behavioural data was recorded with Ethovision software (Noldus Information Technologies). To test photostimulation preference during different hunger states, ad libitum-fed mice were placed within the arena immediately before the onset of the dark cycle. Following a one-week rest period, ad libitum fed mice were then placed in the same arena and tested for photostimulation preference at the onset of the light cycle. Following a one-week rest period, mice were then fasted overnight then, placed in the arena and tested for photostimulation preference at the onset of the light cycle. Each RTPP assay was counterbalanced within animal and within day.

In vivo fibre photometry

Fibre photometry was performed on a rig constructed as follows: A 465-nm LED (PlexBright LED, Plexon) was used as the excitation source which was passed through a fluorescence mini cube (excitation: 460–490 nm, detection: 500–550 nm; Doric Lenses), and transmitted onto the sample via a fibre optic cable (1 m long, 400 μm diameter, 0.48 NA; Doric Lenses). The optic fibre was coupled to the implanted optic fibre with a ceramic mating sleeve (Precision Fibre Products). Light intensity was measured as 100–200 μW at the end of the patch cord and was kept constant across sessions for each mouse. Emitted light was collected by a photodetector (2151; Newport). The signal was digitized at 1 kHz with a data acquisition card (National Instruments) and collected with a custom MATLAB (MATLAB2016a; MathWorks) script (Figs. 1–3, Extended Data Figs. 1, 3, 4).

For LH^{VGLUT2}→DMH axon fibre photometry recordings (Fig. 2, Extended Data Fig. 3h–j), mice underwent a 25-min recording session within their home cage that consisted of: eight ‘small’ chow (15 mg; Bio-Serv) trials, two ‘large’ chow (500 mg; Bio-Serv) trials, and one peanut butter (Reese’s peanut butter chips; Hershey) trial which were dropped into a Pyrex Petri dish. Each mouse underwent only one session per day and was food restricted (85% of ad libitum body weight) for at least one week before beginning the experiment in which it was habituated to the pellet drops within the Petri dish. All trials were pooled to calculate mean peak response (0–5 s following food presentation) to each food presentation. For object drop experiments, mice underwent a 12-min recording session within their home cage that consisted of 10 total trials of non-edible object drops. Objects consisted of uniform, white plastic marbles (BC Percision, Hungry Hungry Hippos Marbles). After an object drop session, a 500 mg chow pellet was dropped as a positive control. Each mouse underwent a single session per day. All trials were pooled to calculate mean z-score response to each object drop.

For water presentation experiments (Extended Data Fig. 3k–m), mice were water restricted (85% of original body weight) for at least one week before beginning the experiment. Mice were then habituated to receiving water in a ceramic dish within their home cage and were given free access for 5 min. All sessions were pooled to calculate the mean peak response (0–30 s) and the time to peak response.

Data were analysed using a custom MATLAB (MATLAB2016b; MathWorks) script (Figs. 1–3, Extended Data Figs. 1, 3, 4). Fluorescence traces were down-sampled from 1 kHz to 100 Hz and smoothed using a 1-s running average. The fractional change in fluorescence was calculated as $\Delta F/F = (F - F_0)/F_0$, in which F_0 was the mean of all data points from the baseline before each trial. In home cage pellet drop or water presentation experiments, F_0 was the average for 5 s before the food drop/water presentation. In operant chamber silencing experiments, F_0 was the average for 1 s before cue presentation. All trials in a single session were averaged then the mean peak amplitude was taken for quantification.

Two-alternative forced-choice task

To determine if LH^{VGLUT2}→DMH neurons and DMH^{LEPR} neurons are necessary for food cue responses (Figs. 1–3, Extended Data Figs. 1, 4), mice underwent training in a three-nosepoke operant chamber (Bpod r2; Sanworks) controlled by a custom MATLAB (MATLAB2016a; Mathworks) script. In brief, food restricted mice (85% of ad libitum body weight) were trained to associate a light presentation with Ensure (10 μ l; Ensure PLUS, vanilla) and were required to nose poke and hold their snouts within the port for 200 ms before Ensure was delivered. Light delivery was randomized between the left and right nose poke and, mice had a 10 s response window with a 10 s inter-trial interval. The required learning criterion was a success rate of $\geq 80\%$ across three consecutive days. After mice learned the task, mice were then attached to a fibre optic cable as described above. To determine the necessity of LH afferents on food-cue responses (Fig. 3, Extended Data Fig. 4), mice underwent two, 11-min recording sessions on the same day separated by a 10-min ‘break’ period. The first session was always a saline run and mice were injected with saline 10 min before the onset of the recording session. The second session was a clozapine-*n*-oxide (CNO; 1 mg kg⁻¹; 0.5% body weight volume) or saline injection 10 min before the onset of each recording session. Comparisons between vehicle and CNO recordings were made within day; therefore, a mouse received two saline injections or, one saline and one CNO injection in a single recording session. A TTL pulse triggered at the onset of each trial determined cue onset. All trials were pooled to calculate the mean peak response (0–2 s following cue presentation) and were normalized to the first recording session within the same day.

Intragastric catheter surgery

Mice with DMH^{LEPR} photometry neural activity signal larger than 10% $\Delta F/F$ to chow during fast re-feed were implanted with intragastric

catheters³⁸ (Extended Data Fig. 1). During surgery, mice were anaesthetized with isoflurane (1.5–3%) and treated post-operation with buprenorphine (1 mg kg⁻¹ subcutaneously) analgesia. A midline incision on the abdomen was made through skin and muscle layers. Micro-Renathane catheter tubing 6–7 cm in length (Braintree Scientific, MRE-033, 0.033 $\times$ 0.014 in) was anchored with epoxy spheres on each end (Devcon Clear Epoxy Adhesive, 92926, Lowes). The catheter was inserted into the fundus of the stomach through a puncture hole and secured with surgical mesh (5-mm diameter piece, Bard, 0112660). The other end of the catheter was directed out of an intrascapular incision. A metal cap made out of 27G blunt needle was placed in the exposed end for seal. The gastric catheter was flushed with sterile water immediately, and daily, after surgery to prevent blockage. Mice were fed with gel chow diet and given at least 1–1.5 week for recovery before experimentation. Daily body weight was monitored until stable pre-surgical weight was regained.

Gastric infusion

Upon recovery, gastric infusions of liquid substances listed below were performed in a counterbalanced experimental design, under both overnight fasted and sated conditions (Extended Data Fig. 1). The intrascapularly exposed end of the gastric catheters were connected to tubing and a syringe driven by an infusion pump (Harvard Apparatus, 70-3007). At a rate of 0.1 ml min⁻¹, 1-ml infusions were performed³⁸ over the course of 10 min. Fibre photometry recordings were collected via a lock-in amplifier (TDT) and the software Synapse (TDT). Each trial was approximately 27 min (>7 min baseline recording, followed by a 10 min gastric infusion and a 10 min chow refeed). Each mouse underwent infusions of the following infusates: 0.9% isotonic saline, Ensure Original Nutrition Shake (vanilla), 1% saccharine, 25% D-glucose (equal caloric content as Ensure).

Each photometry recording data point was normalized against the average of the last 5 min of baseline period to produce the normalized traces. For normalized and delta ($\Delta F_n/F_n$, %) quantified comparisons, we use 1-min averages at the end of baseline period ($t = -1$ to 0 min), 5 min into infusion ($t = 4$ to 5 min), and 10 min into infusion ($t = 9$ to 10 min). Owing to the transient nature of neural response to food, comparison of delta ($\Delta F_n/F_n$, %) maximum magnitude between ensure infusion and food presentation were made using 30 s averages at the end of ensure infusion ($t = 9.5$ –10 min) and at the beginning of food presentation after saline infusion ($t = 10$ –10.5 min).

TeNT-mediated silencing

AAV-DIO-TeNT was injected into the DMH of *Lepr-IRES-Cre* mice, *Pdyn-IRES-Cre* mice, or wild-type mice (Fig. 4, Extended Data Fig. 5). Littermate controls were used for AAV-DIO-TeNT and AAV-DIO-GFP behavioural groups. Following three weeks, mice were then placed within the Bpod arena and trained everyday as described above. Mice were either food or water restricted and maintained at $\geq 85\%$ ad libitum body weight. Food and water restriction was performed in separate cohorts of mice. For food-learning assays, mice were trained for a total of 21 days. For water-learning assays, mice were trained for a total of 14 days. Mice were excluded if they were non-learners meaning that they did not increase their performance rate for five consecutive days (one GFP-expressing mouse (in water-deprived group) and one TeNT-expressing mouse (in food-deprived group) were removed based on this criterion). Mice were placed in the Bpod for a total of 20 min and allowed to perform as many trials as possible with an intertrial interval of 5 s and a response window of 10 s. Mice were given either 10 μ l of Ensure or 5 μ l of water for food and water-learning assays, respectively. Each day, performance in the task was quantified with a custom MATLAB script (Fig. 4, Extended Data Fig. 5). It should be noted that *Pdyn-IRES-Cre* and wild-type behavioural cohorts (Extended Data Fig. 5–u) were different strains than our *Lepr-IRES-Cre* cohort; this was owing to the limited availability of mice during the COVID-19

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pandemic. As such, they performed slightly differently on the 2AFC task. Therefore, their learning criterion was lowered to >70% correct responses across three consecutive days.

Quantification and statistical analysis

Statistical analyses were performed using Prism 5 and Prism 8 (Graph-Pad) software and are described in the figure legends in all cases. No statistical method was used to predetermine sample size, nor were randomization and blinding methods used. Statistical significance was defined as $P < 0.05$. All data presented met the assumptions of the statistical test employed. As mentioned in sections above, experimental mice were excluded if histological validation revealed poor or absent reporter expression or poor fibre optic placement in the region of interest. These criteria were established before data collection. n values reflect the final number of validated mice per group.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author upon request. Source data are provided with this paper.

Code availability

MATLAB scripts to run and analyse 2AFC behaviour are freely available on Github (https://github.com/jbwallace123/2AFC_BPOD_ANALYSIS).

For more details on installation please visit: <https://sites.google.com/site/bpodddocumentation/installing-bpod>.

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Additional information

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Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | DMH^{LEPR} neuron photometry responses are greater with pellet drops than with gut-infusions of caloric substances and inhibiting DMH^{LEPR} neurons does not affect food intake following an overnight fast. **a**, Heat maps of additional well-trained mice included in Fig. 1d of DMH^{LEPR} neuron fibre photometry responses to cue (light) onset. Each heat map represents a single behavioural session. Single trials have been sorted by response time. Green line indicates light onset (time = 0), black line indicates time when the mouse poked within the correct port. **b**, Heat maps of all naive mice included in Fig. 1d of DMH^{LEPR} neuron fibre photometry responses to cue (light) onset. Each heat map represents a single behavioural session. Single trials have been sorted by response time. Green line indicates light onset (time = 0), black line indicates time when the mouse poked within the correct port. It should be noted that naive mice perform significantly fewer trials than experienced mice. **c**, Gastric infusion experimental design. **d–f**, Plot of fibre

photometry responses from DMH^{LEPR} neurons aligned to gut infusions of Ensure (**d, e**), saline (**d, e**), or caloric and non-caloric sweeteners (**f**). These were also aligned to food pellet drops post-infusion. Mice were either food deprived or fed ad libitum for sated Ensure infusions. Light colour tones indicate S.E.M. **g**, Quantification of fluorescence changes ten minutes post-gut infusions of saline, Ensure, and Ensure when animals were fed ad libitum (sated Ensure). $n = 9$ (mice); Repeated measures one-way ANOVA, $***P = 0.0007$. **h**, Comparison between peak fibre photometry responses during the last 30 s of Ensure infusion (red) and first 30 s of pellet drop response (beige) when animals were given saline infusions. Peak response is significantly larger with pellet drops vs. Ensure infusions. $n = 9$ (animals); Two-tailed, paired t -test, $*P = 0.0241$. **i**, hM4Di-mediated inhibition of DMH^{LEPR} neurons does not affect food intake in a post-fast refeeding assay. $n = 12$ (mice). Two-way ANOVA, $P = 0.9992$. All data represents the mean $\pm$ S.E.M.

Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | LH^{VGLUT2}→DMH neurons preferentially synapse onto DMH^{PDYN-GFP} neurons. **a**, Sample image of rabies labelling of afferents to DMH^{LEPR} neurons. (Top left) Sample of injection site of AAV-DIO-TVA, AAV-DIO-RG, and EnvA-GFP within the DMH. (Right and bottom row) Sample images of rabies labelled afferents. Scale bar = 200 μ M. A/P levels are inferred based on histological landmarks. $n = 3$ (animals). **b**, Monosynaptic connection probability of optically evoked IPSCs and EPSCs to vDMH^{PDYN-GFP} neurons. X-axis = afferent source. **(c, d)** Quantification of oIPSC (a) and oEPSC (b) amplitude from all candidate afferents to DMH^{LEPR} neurons in the presence and absence of TTX and 4AP. Two-tailed, paired t -test, $*P \leq 0.05$. (b) $P = 1.664$ (NAC), $P = 0.0285$ (BNST), $P = 0.6413$ (LH), $P = 0.0165$ (VTA) (c) $P = 0.9366$ (LH). **e**, Experimental design schematic. AAV-DIO-ChR2 injected in LH^{VGLUT2} neurons and oEPSCs were measured in vDMH^{PDYN-GFP} neurons. Bottom: Example current-clamp recordings in vDMH^{PDYN-GFP} neuron during optogenetic activation of LH^{VGLUT2} neurons. **f**, LH^{VGLUT2} neurons preferentially connect to about 80% of DMH^{PDYN-GFP} neurons and have sparse connectivity to DMH^{PDYN-GFP} negative

neurons. $n = 15$ (cells) GFP+, 17 (cells) GFP-. **g**, oEPSC amplitude is unchanged when TTX and 4AP are added. $n = 9$ (ACSF) and 11 (TTX+4AP). Two-tailed unpaired t -test, $P = 0.1666$. **h**, AAV-DIO-ChR2 was injected into the LH of *Vglut2-IRES-Cre* mice. Whole-cell recordings were performed in vDMH^{PDYN-GFP} negative neurons while stimulating LH^{VGLUT2} terminals. Bottom: representative ChR2-assisted circuit mapping traces from vDMH^{PDYN-GFP} negative neurons in the presence and absence of TTX and 4AP. **i**, Summarized postsynaptic current amplitude in DMH^{PDYN-GFP} negative neurons in the presence and absence of TTX and 4AP. $n = 9$ (ACSF) and 8 (TTX, 4AP). Two-tailed, unpaired t -test, $P = 0.26$. **j**, Summarized input resistance in both PDYN-GFP positive and negative neurons. $n = 20$ (GFP+) and 17 (GFP-). Two-tailed, unpaired t -test, $P = 0.14$. **k**, Summarized series resistance in both PDYN-GFP positive and negative neurons. $n = 20$ (GFP+) and 17 (GFP-). Two-tailed, unpaired t -test, $P = 0.24$. **l, m**, Averaged current onset latency (**l**) and jitter (**m**) in PDYN-GFP positive neurons in the absence of TTX and 4AP. $n = 8$ (cells). All data represents the mean $\pm$ S.E.M.

Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | LH^{VGLUT2}→DMH neurons are appetitive, do not send collaterals to the LHb or PAG, have negligible responses to non-edible object presentations, and respond differently to water when compared to food. **a**, Representative wire plots of an XFP and ChR2 expressing animals throughout the entire 20-min RTPP session across three different conditions: lights-on ad libitum, lights-off ad libitum, and lights-on fasted. Blue line represents stimulation side of the RTPP chamber and black represents the non-stimulation side. **b–d**, Total distance travelled is unchanged between XFP and ChR2 expressing animals throughout the RTPP session across all three conditions (lights-on ad libitum, lights-off ad libitum, and lights-on fasted). $n = 7$ (animals) for both XFP and ChR2-expressing groups. Unpaired t -test, $P = 0.7498$ (**b**), $P = 0.5277$ (**c**), $P = 0.3482$ (**d**). **e**, DIO-TVA-mCherry was injected into the LH of *Vglut2-IRES-Cre* mice and pseudotyped EnvA-GFP was injected into the DMH to map collaterals of LH^{VGLUT2}→DMH neurons. **f**, Injection sites within the LH (left) and DMH (right). Scale bar = 500 μ M (LH) and 200 μ M (DMH). **g**, Collaterals were found within the BNST, MPO/LPO, and AHA and were absent in LH targets known to promote aversion such as the LHb and PAG. $n = 3$ (animals). Scale bar = 500 μ M. **h**, Averaged axonal fibre-photometry response

in LH^{VGLUT2}→vDMH terminals across all animals when presented with a non-edible object. Vertical line represents object presentation. $n = 7$ (animals). **i**, Summarized Z-score of LH^{VGLUT2}→vDMH axonal fibre photometry response to pellet ($n = 9$) and object ($n = 7$) drops in fasted mice. One-way ANOVA; Kruskal-Wallis, **** $P < 0.0001$. **j**, Summarized mean peak response in LH^{VGLUT2}→vDMH axonal fibre photometry response to pellet drops when animals were fed ad libitum. $n = 9$; Two-tailed, unpaired t -test, * $P = 0.0129$. **k**, Averaged axonal fibre-photometry response in LH^{VGLUT2}→vDMH terminals across all animals when given a 500 mg food pellet when food restricted (green) or, a bowl of water when water restricted (blue). Vertical line represents food/water presentation. **l, m**, Quantification of mean peak response of food (in food-deprived mice) and water presentations (in water-deprived mice) (**l**, $P = 0.0206$). $n = 9$ (food) and 8 (water). Peak water response occurred at a much later time point than food responses suggesting that the neurons respond differently to water cues when compared to food (**m**, $P = 0.0391$). $n = 9$ (food) and 8 (water); Two-tailed, unpaired t -test, * $P < 0.05$. A/P levels are inferred based on histological landmarks from the referenced mouse atlas. All data represents the mean $\pm$ S.E.M.

Extended Data Fig. 4 | AAV-fDIO-hM4Di efficiently inhibits LH^{VGLUT2} neurons, CNO does not affect DMH^{LEPR} activation, and hM4Di inhibition does not affect sustained AGRP inhibition. **a, Validation of AAV-fDIO-hM4Di construct using whole-cell patch clamp electrophysiology. Upon CNO wash-on (red), the rheobase increase is indicative of a successfully hyperpolarized LH^{VGLUT2} neuron given that a higher injection of current is needed to fire an action potential during CNO-wash on. **b**, CNO wash-on also eliminated spontaneous action potential firing in LH^{VGLUT2} neurons in fDIO-hM4Di expressing neurons. **c**, **d**, XFP controls were used to determine if CNO would affect DMH^{LEPR} activation in the absence of hM4Di. CNO did not affect DMH^{LEPR} neuron photometry responses (c , $P=0.7616$) or behavioural performance (d).**

$n=3$ (mice). **e**, Inhibiting LH $\rightarrow$ DMH afferents does not affect behavioural response time in well-trained mice. $n=4$ (mice). Relates to Fig. 3a–c. Two-tailed t -test, $P=0.7068$. **f**, CNO treatment does not affect the later consummatory response in AGRP neurons when mice are given a large (250 mg) food pellet. $n=4$ (mice); Two-tailed t -test, $P=0.61$. **g**, Inhibiting LH $\rightarrow$ DMH afferents in well-trained mice does not affect behavioural performance. $n=4$ (mice), Two-tailed t -test, $P=0.3819$. Relates to Fig. 3a–c. **h**, Inhibition of LH^{VGLUT2} neurons does not affect behavioural performance. $n=4$ (mice), Two-tailed t -test $P=0.3661$. Relates to Fig. 3d–f. **i**, **j**, Inhibition of LH^{VGLUT2} neurons does not affect behavioural accuracy (**i**) or response time (**j**). $n=4$ (mice), Two-tailed t -test, $P=0.7107$. Relates to Fig. 3g–i. All data represents the mean $\pm$ S.E.M.

Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | TeNT-mediated silencing of DMH^{LEPR}, DMH^{PDYN}, and LH^{DMH} neurons significantly attenuates learning a cue-initiated food acquisition task by increasing mistakes made during the task but TeNT-mediated silencing of DMH^{LEPR} neurons does not affect learning a cue-initiated water acquisition task. a, b, DMH^{LEPR} neuron silencing leads to an increase in response errors in the 2AFC task. TeNT-expressing mice largely miss the response window (a) in the initial phases of training and have an increase in false alarms (b). *n* = 5 (GFP), 5 (TeNT). a, Red dotted line indicates line of best fit ($*P < 0.0001$, plateau = 5.735 (GFP), -2.910 (TeNT); tau = 2.923 (GFP), 12.64 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = 0.05. b, Red dotted line indicates line of best fit ($***P < 0.0001$, plateau = 0.1324 (GFP), -162.8 (TeNT); tau = 6.679 (GFP), 117.6 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = 0.6. c, TeNT-expressing *Lepr-IRES-Cre* mice take significantly longer to nose poke following cue-light presentation. *n* = 5 (GFP), 5 (TeNT). Red dotted line indicates line of best fit ($***P < 0.0001$, plateau = 1.319 (GFP), 0.633 (TeNT); tau = 4.272 (GFP), 9.860 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $**P < 0.01$, main effect of group = 0.08. d, Body weight is unaffected upon DMH^{LEPR} silencing with TeNT (7 weeks post-surgery). *n* = 5 (GFP), 5 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.7043$. e, Food-intake does not significantly differ with DMH^{LEPR} silencing following an overnight fast. *n* = 5 (GFP), 6 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.9495$. f, AAV-DIO-TeNT injection into DMH^{PDYN} neurons. Bottom: Example images of TeNT expression. Scale bar = 500 μ M. *n* = 5 (mice). g, h, Similar to (Fig. 2b) in DMH^{PDYN} neurons. *n* = 7 (GFP), 5 (TeNT). Red line indicates = line of best fit ($***P < 0.0001$, plateau = 82.83 (GFP), 65.52 (TeNT); tau = 5.619 (GFP), 7.337 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = $**P < 0.01$. h, Similar to (Fig. 2c) in DMH^{PDYN} neurons (learning criterion >70% correct across three consecutive days). >20 on the y-axis is to indicate that by day 20, mice had not learned. 7/7 GFP and 1/6 TeNT mice learned. *n* = 7 (GFP), 5 (TeNT). Two-tailed, unpaired *t*-test, $**P = 0.0094$. i, j, DMH^{PDYN} neuron silencing leads to an increase in response errors in the 2AFC task. TeNT-expressing mice largely miss the response window (i) in the initial phases of training and do not have a difference in false alarm rate (j). *n* = 7 (GFP), 5 (TeNT). (i) Red dotted line indicates line of best fit ($***P < 0.0001$, plateau = 7.142 (GFP), 20.89 (TeNT); tau = 0.8597 (GFP), 1.96 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $****P < 0.001$, main effect of group = $*P < 0.05$. j, Line of best fit could not be calculated. Two-way repeated measures ANOVA, main effect of days = $***P < 0.001$, main effect of group = 0.8267. k, TeNT-expressing *Pdyn-Cre* mice take significantly longer to nose poke following cue-light presentation. *n* = 7 (GFP), 5 (TeNT). Red dotted line indicates line of best fit ($***P < 0.0001$, plateau = 1.185 (GFP), 2.748 (TeNT); tau = 2.122 (GFP), 3.939 (TeNT)). Two-way**

repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = $****P < 0.0001$. l, Body weight increases upon long term DMH^{PDYN} silencing with TeNT (20 weeks post-surgery). *n* = 7 (GFP), 5 (TeNT). Two-tailed, unpaired *t*-test, $**P = 0.0086$. m, Food-intake does not significantly differ with DMH^{PDYN} silencing following an overnight fast. *n* = 7 (GFP), 5 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.2668$. n, AAV-DIO-TeNT injection into the LH and rAAV-Cre injection into the DMH in wild-type mice. Bottom: Example images of TeNT expression. Scale bar = 500 μ M. *n* = 6 (mice). o, p, Similar to (Fig. 2b) in LH^{DMH} neurons. *n* = 5 (GFP), 6 (TeNT). Red line = line of best fit ($***P < 0.0001$, slope = 2.033 (GFP), 1.488 (TeNT); Y-intercept = 44.50 (GFP), 30.02 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = $P = 0.0548$. p, Similar to (Fig. 2c) in LH^{DMH} neurons (learning criterion >70% correct across three consecutive days). 5/5 GFP and 2/6 TeNT-expressing mice learned. *n* = 5 (GFP), 6 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.0789$. q, r, LH^{DMH} neuron silencing leads to an increase in response errors in the 2AFC task. TeNT-expressing mice largely miss the response window (q) in the initial phases of training and do not have a difference in false alarm rate (r). *n* = 5 (GFP), 6 (TeNT). q, Red dotted line indicates line of best fit ($***P < 0.0001$, slope = -0.299 (GFP), -0.4169 (TeNT); Y-intercept = 16.75 (GFP), 39.91 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $*P = 0.0416$, main effect of group = $P = 0.1896$. r, Line of best fit is not significantly different for TeNT and GFP-expressing animals ($P = 0.1724$, plateau = -22.34 (GFP), -74.42 (TeNT); Tau = 23.91 (GFP), 83.89 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $*P = 0.0101$, main effect of group = 0.9094. s, TeNT-expression in LH^{DMH} neurons significantly increases nose poke response following cue-light presentation. *n* = 5 (GFP), 6 (TeNT). Red dotted line indicates line of best fit ($***P < 0.0001$, plateau = 1.790 (GFP), 3.314 (TeNT); tau = 1.818 (GFP), 8.042 (TeNT)). Two-way repeated measures ANOVA, main effect of days = $***P = 0.0004$, main effect of group = $****P < 0.0001$. t, Body weight is unaffected upon LH^{DMH} silencing with TeNT (8 weeks post-surgery). *n* = 5 (GFP), 6 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.2820$. u, Food-intake does not significantly differ with LH^{DMH} silencing following an overnight fast. *n* = 5 (GFP), 6 (TeNT). Two-tailed, unpaired *t*-test, $P = 0.8666$. v-x, DMH^{LEPR} neuron silencing does not affect response errors or response times in the 2AFC task when dehydrated mice receive water rewards. *n* = 4 (GFP), 6 (TeNT). v, Red dotted line indicates line of best fit (plateau = 57.74, tau = 4.477). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = 0.942. w, Red dotted line indicates line of best fit (plateau = -15.32, tau = 11.67). Two-way repeated measures ANOVA, main effect of days = $*P < 0.05$, main effect of group = 0.13. x, Red dotted line indicates line of best fit (plateau = 0.7882, tau = 2.929). Two-way repeated measures ANOVA, main effect of days = $****P < 0.0001$, main effect of group = 0.82. All data represents the mean $\pm$ S.E.M.

Extended Data Fig. 6 | PDYN-expressing DMH neurons exclusively project to the ARC. AAV-DIO-ChR2-mCherry was injected into the DMH of *Pdyn-Cre* mice to search for long range projections of DMH^{PDYN} neurons. The ARC was the

only area of the brain that received DMH^{PDYN} innervation. A portion of this data was previously published in Garfield et al. 2016¹².

Extended Data Fig. 7 | Schematic representation of viral spread and fibre placement. **a–c**, Schematic representing the extent of viral spread (transparent shaded regions) and fibre placement (solid ovals) for every animal related to studies presented in Fig. 1b–d, Extended Data Fig. 1a (**a**), Fig. 2a–e, Extended Data Fig. 3a–d (**b**) and Fig. 2f–k, Extended Data Fig. 3h–m (**c**). Please note that different animals are represented by different colours and that “hits” and “misses” are represented on different hemispheres (miss is on the left). GENERAL CRITERION FOR HITS AND MISSES: Animals were deemed a “hit” if

viral expression was within the area of interest and fibre placement was either within or immediately dorsal to the area of interest (that is, the DMH or the LH). Excluded animals are specified by the “miss” label. Reason each animal was considered a “miss”: Animal 5; fibre placement dorso-lateral to DMH (**a**); Animal 10; unilateral viral expression and fibre placement lateral to midline/DMH (**b**); Animal 10; viral expression ventro-medial to LH, fibre placement lateral to the DMH (**c**).

Lepr-Cre::AAV-FlpO::fDIO-hM4Di::DIO-GCaMP6s

Figs. 3a-3c; Extended Data Fig. 4e-4g

Hit

Extended Data Fig. 8 | Schematic representation of viral spread and fibre placement within *Lepr-Cre* animals. Schematic representing the extent of viral spread (transparent shaded regions) and fibre placement (solid ovals) for every animal related to studies presented in Fig. 3a–c and Extended Data Fig. 4e–g. Please note that individual animals are represented by different colours. Bilateral hM4Di injections were targeted to the LH and bilateral AAV6-FlpO injections were targeted to the DMH. GCaMP6s injections into the DMH were unilateral. CRITERION FOR HITS AND MISSES: Animals were deemed a “hit” if viral expression was within the area of interest (that is, hM4Di bilateral

in the LH, GCaMP unilateral in the DMH) and fibre placement was either within or immediately dorsal to the area of interest (that is, the DMH). For clarity, hits are shown on the top and misses on the bottom. Reason each animal was considered a “miss”: Animal 1; viral expression (hM4Di) was medial to DMH, fibre placement ventral to DMH. Animal 2; viral expression (hM4Di) was unilateral, fibre placement ventral to DMH. Animal 3; viral expression (hM4Di) was unilateral, fibre placement dorsal to DMH. Animal 4; hM4Di expression undetected, fibre placement ventral to DMH.

Vglut2-FlpO::Lepr-Cre::fDIO-hM4Di/DIO-GCaMP6s
Figs. 3d-3f; Extended Data Fig. 4h

Extended Data Fig. 9 | Schematic representation of viral spread and fibre placement within *Vglut2-FlpO::Lepr-Cre* animals. Schematic representing the extent of viral spread (transparent shaded regions) and fibre placement (solid ovals) for every animal related to studies presented in Fig. 3d–f and Extended Data Fig. 4h. Please note that individual animals are represented by different colours. Bilateral hM4Di injections were targeted to the LH and GCaMP6s injections were unilateral into the DMH. GENERAL CRITERION FOR HITS AND MISSES: Animals were deemed a “hit” if viral expression was within

the area of interest (that is, hM4Di bilateral in the LH, GCaMP unilateral in the DMH) and fibre placement was either within or immediately dorsal to the area of interest (that is, the DMH). For clarity, hits are shown on the top and misses on the bottom. Reason each animal was considered a “miss”: Animal 1; viral expression of hM4Di was dorsal to LH. Animal 2; hM4Di viral expression was unilateral and dorsal to LH and minimal GCaMP6s expression was detected lateral to DMH.

Vglut2-FlpO::AgRP-Cre::fDIO-hM4Di/DIO-GCaMP6s

Figs. 3g-3i; Extended Data Fig. 4i-4j

Extended Data Fig. 10 | Schematic representation of viral spread and fibre placement within *Vglut2-FlpO::AgRP-Cre* animals. Schematic representing the extent of viral spread (transparent shaded regions) and fibre placement (solid ovals) for every animal related to studies presented in Fig. 3g-i and Extended Data Fig. 4i, j. Please note that different animals are represented by different colours. Bilateral hM4Di injections were targeted to the LH and GCaMP6s injections were unilateral into the ARC. Purple regions indicate areas with no viral expression. GENERAL CRITERION FOR HITS AND MISSES: Animals

were deemed a "hit" if viral expression was within the area of interest (that is, hM4Di bilateral in the LH, GCaMP unilateral in the ARC) and fibre placement was either within or immediately dorsal to the area of interest (that is, the ARC). For clarity, hits are shown on the top and misses on the bottom. Reason each animal was considered a "miss": Animal 1; viral expression of hM4Di was unilateral and dorsal to LH and GCaMP6s expression was undetectable. Animal 2; viral expression of hM4Di was unilateral and dorsal to LH and GCaMP6s expression was undetectable.

Extended Data Fig. 11 | Schematic representation of viral spread.

a–d, Schematic representing the extent of viral spread (transparent shaded regions) for every animal related to studies presented in Fig. 4a–c, Extended Data Fig. 5a–e (**a**), Extended Data Fig. 5f–m (**b**), Extended Data Fig. 5n–u (**c**) and Fig. 4d–f and Extended Data Fig. 5v–x (**d**). Please note that different animals are represented by different colours. GENERAL CRITERION FOR HITS AND MISSES: Animals were deemed a “hit” if viral expression was within the area of interest (that is, the DMH or the LH). Excluded animals are specified by the “miss” label.

Reason each animal was considered a “miss”: Animal 7, viral expression had spread to the DMH and VMH (**a**); Animal 6, viral expression was dorsal to DMH (**b**); Animal 7, viral expression was barely detected (**b**); Animal 7, viral expression was dorsal to LH (**c**); Animal 8, diffuse, widespread viral expression dorsal to LH (**c**); Animal 9, viral expression was dorsal to LH and within zona incerta (**c**); Animal 10, viral expression was dorsal to LH and within zona incerta (**c**); Animal 7; viral expression was dorsal to DMH (**d**); Animal 8, viral expression was barely detectable in a small region within the anterior DMH (**d**).

Extended Data Fig. 12 | Example of method used to draw anatomical boundaries in histological images. DAPI-stained images were taken and contrast was enhanced (left). Images were matched with the appropriate A/P coordinate within the histological atlas (middle) then, boundaries were drawn

using the atlas as a template (right). All anatomical boundaries represented within the figures were drawn using this method. The atlas used for these studies was *The Mouse Brain in Stereotaxic Coordinates* by Paxinos and Franklin, Second Edition.

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Software and code

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Data collection

Electrophysiology data were collected using Molecular Devices pClamp software (version 10.2 & 10.6). Real-time place preference data were collected using Noldus-Ethovision XT7. All photometry and operant behavioral data were collected using a custom MATLAB script on MATLAB-R2016b. Operant behavior data collection code can be found at <https://sites.google.com/site/bpoddokumentation/installing-bpod>. All bodyweight and food-intake studies were collected without the use of any software. Images were acquired using Olympus VS-ASW-FL.

Data analysis

Electrophysiology data were analyzed using Molecular Devices Clampfit (10.2 & 10.6). Real-time place preference data were analyzed using Noldus-Ethovision XT7 and Excel 2016. Photometry data were analyzed using a custom MATLAB script on MATLAB-R2016b. Operant behavioral data were analyzed using a custom MATLAB script on MATLAB-R2016b. All bodyweight and food-intake studies did not require software for analysis. Images were analyzed using Olympus OlyVIA. All data were summarized using Graphpad Prism (V5 & V8).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample sizes used within these studies. Sample sizes were chosen to reliably measure experimental parameters while remaining in compliance with ethical guidelines for minimizing animal use and were similar to those reported in previous publications (Garfield et. al., 2016 and Livneh et. al. 2017).
Data exclusions	Data were excluded if post-hoc histological verification showed that either viral injection or fiber-optic implantation missed the target region. Data were also excluded if animals were considered 'non-learners' (Figure 4), this occurred twice. All exclusion criterion were preestablished.
Replication	Data was collected across multiple animals and, in most cases, in at least two separate cohorts to ensure that findings were not biased towards a particular animal or a single experimental cohort. All of our attempts at replication were successful.
Randomization	Randomization was implemented whenever possible. Our 2AFC behavioral assay was randomized such that the visual stimulus were delivered randomly across the two nosepoke ports (Figs. 1, 3-4). All animals used in this study were randomly chosen for experimental/control groups.
Blinding	Investigators were not blinded during group allocation or analysis due to the nature of the experiments. Comparisons were done within animal or with littermate controls. No prior assumptions were made prior to data collection.

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☒	☐ Palaeontology	☒	☐ MRI-based neuroimaging
☐	☒ Animals and other organisms		
☒	☐ Human research participants		
☒	☐ Clinical data		

Antibodies

Antibodies used	Rat anti-mCherry (1:3000, Invitrogen #M11217, Clone 16D7) and Chicken anti-GFP (1:1000, Invitrogen #A10262). Vectashield with DAPI (H-1200) was used for nuclear staining.
Validation	These antibodies were previously verified and used to amplify native fluorescence signals from fluorescent proteins during post-hoc histological verification of injections. Please see manufacturer's website for validation information (https://www.thermofisher.com/us/en/home/life-science/antibodies.html)

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Experiments were performed on adult male mice (8-20 weeks) of the following strains: Vglut2-IRES-Cre (JAX #: 016963), Vglut2-IRES2-FlpO (JAX #: 030212), AgRP-IRES-Cre (JAX #: 012899), Pdyn-GFP, Pdyn-ires-Cre, wildtype (JAX#:101045), and Lepr-IRES-Cre.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	The National Institute of Health and Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee approved all animal care and experimental procedures. Mice were housed at 22-24°C, 20-30% humidity with a 12:12 light:dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664) and water was provided ad libitum; unless otherwise stated. All diets were provided as pellets. For all behavioral studies, we used male mice between 8 and 20 weeks of age. For electrophysiological recordings, we used male mice between 8 and 12 weeks of age.

Note that full information on the approval of the study protocol must also be provided in the manuscript.