Dynamic GABAergic afferent modulation of AgRP neurons

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Agouti-related peptide (AgRP) neurons of the arcuate nucleus of the hypothalamus (ARC) promote homeostatic feeding at times of caloric insufficiency, yet they are rapidly suppressed by food-related sensory cues before ingestion. Here we identify a highly selective inhibitory afferent to AgRP neurons that serves as a neural determinant of this rapid modulation. Specifically, GABAergic projections arising from the ventral compartment of the dorsomedial nucleus of the hypothalamus (vDMH) contribute to the preconsummatory modulation of ARCAgRP neurons. In a manner reciprocal to ARCAgRP neurons, ARC-projecting leptin receptor-expressing GABAergic vDMH neurons exhibit rapid activation upon availability of food that additionally reflects the relative value of the food. Thus, leptin receptor-expressing GABAergic vDMH neurons form part of the sensory network that relays real-time information about the nature and availability of food to dynamically modulate ARCAgRP neuron activity and feeding behavior.

The sensory processing of caloric deficiency is critical to prevent starvation and ensure survival.1 The fidelity of such need detection and response enactment is defined by an evolutionarily conserved homeostatic system that links the detection of this deficiency with the instinctual drive to consume food. ARCAgRP neurons have been classically viewed as a first-order interoceptive population fundamental for this counter-regulatory response2–4. Indeed, increasing ARCAgRP neuron activity with mounting energy deficit reflects caloric need and promotes a hardwired anabolic program that drives feeding behavior.3,6. Experimentally, activation of ARCAgRP neurons during times of caloric repletion engenders a state of artificial hunger7 that promotes motivated food seeking8 and consumption2,3. However, recent investigation of the endogenous activity of ARCAgRP neurons has revealed that while a high firing rate during times of caloric depletion permits overall feeding behavior, these neurons exhibit a rapid and robust decrease in activity, the onset of which is coincident with the detection or expectation of available food before consumption (and maintained throughout the feeding bout)5,7,8. At present, the functional significance of this preconsummatory suppression remains uncertain, with numerous nonexclusive hypotheses proposed, including (i) its role as a preparatory or predictive signal of future satiety that prevents overconsumption and primes the celiac response for ingestion, (ii) its requirement for the transition from food-seeking behavior to food consumption and (iii) its purpose as a negative teaching signal that facilitates a learning-based association between detected food items and future relief from hunger following food ingestion.9 Notwithstanding this issue, the rapidity of the ARCAgRP neuron response to the detection of food strongly suggests that the input responsible is neuronal in origin. As such, an important first step in understanding the nature and significance of the polysynaptic connections that link food-related sensory input with this rapid modulatory event is identifying the presynaptic population(s) that directly regulate ARCAgRP neuron activity at fast timescales. Here we identify an inhibitory afferent arising from the vDMH that is sufficient to robustly inhibit ARCAgRP neurons and suppress homeostatic feeding. These presynaptic GABAergic vDMH neurons, identified by their expression of the leptin receptor (LepR) and of prodynorphin (pDYN), exhibit rapid preconsummatory activation upon detection of food, in a manner reciprocal to ARCAgRP neurons. We conclude that this population plays an important role in sensory cue-mediated regulation of ARCAgRP neuron activity.

RESULTS

vDMHlepR neurons are ARCAgRP neuron inhibitory afferents

GABAergic modulation of ARC melanocortin neurons play a well-established role in the regulation of energy homeostasis10,11. Previous monosynaptic rabies mapping12 from genetically defined ARCAgRP neurons identified the ARC, DMH and, to a much lesser extent, the lateral hypothalamus (LH) as potential anatomic sources of presynaptic input13,14. To validate these observations and determine their valence we employed channelrhodopsin-assisted circuit mapping (CRACM). Using an Slc32a1 (vGAT)-ires-cre mouse to selectively transduce putative presynaptic GABAergic neurons, we recorded

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postsynaptic currents on ARC<sup>AgRP</sup> neurons, which were demarked by an Npy-GFP transgene that labels all ARC<sup>AgRP</sup> neurons<sup>13,16</sup>. All recorded ARC<sup>AgRP</sup> neurons exhibited picrotoxin-sensitive, light-evoked inhibitory postsynaptic currents arising from distal DMH<sup>vGAT</sup> neurons (25 of 25 neurons Fig. 1a and Supplementary Fig. 1a) and local ARC<sup>GAT</sup> neurons (10 of 10 neurons; Supplementary Fig. 1b,d) but not from LH<sup>GAT</sup> neurons (0 of 13 neurons; Supplementary Fig. 1c,e). However, ARC-projecting DMH<sup>GAT</sup> and ARC<sup>GAT</sup> neurons were also synaptically connected to counteracting satiety-promoting ARC pro-opiomelanocortin (POMC) neurons (demarked by a Pomp-hrGFP transgene; Fig. 1b, Supplementary Fig. 1f,g and Online Methods), negating the utility of the vGAT-ires-cre mouse as a selective marker of inhibitory ARC<sup>AgRP</sup> neuron afferents.

We subsequently identified the leptin receptor (labeled by a Lepr-ires-cre mouse line) as a marker of GABAergic DMH afferents to ARC<sup>AgRP</sup> neurons. Specifically, CRACM analysis demonstrated that 100% of ARC<sup>AgRP</sup> neurons recorded (31 of 31 neurons; Fig. 1c) but only 9% of ARC<sup>POMC</sup> neurons recorded (4 of 45 neurons; Fig. 1d) and 5% of all non-AgRP ARC neurons recorded (1 of 20 neurons; Supplementary Fig. 1h) received monosynaptic inhibitory input from vDMH<sup>LepR</sup> neurons (and no glutamatergic input). Furthermore, and consistent with their dense axosomatic innervation of ARC<sup>AgRP</sup> cell bodies (Fig. 1e), pulsed light-evoked GABA release from vDMHLepR→ARC terminals was sufficient to robustly suppress ARC<sup>AgRP</sup> neuron action potential firing (Fig. 1f). In contrast to this selectivity, ARC<sup>LepR</sup> neurons engaged 100% of recorded ARC<sup>AgRP</sup> (10 of 10 neurons; Supplementary Fig. 1i) and ARC<sup>POMC</sup> neurons (21 of 21 neurons; Supplementary Fig. 1j,k), while LHLepR neurons did not engage either population (Supplementary Fig. 1l,m). Thus, GABAergic vDMH<sup>LepR</sup> neurons represent a highly preferential and potent source of presynaptic inhibitory input to ARC<sup>AgRP</sup> neurons.

As revealed by Stat3 immunoreactivity, GABAergic leptin-responsive DMH neurons were largely restricted to the ventral compartment (Supplementary Fig. 2a), while the glutamatergic subpopulation was localized to the dorsal regions (Supplementary Fig. 2b). Consistent with this and the GABAergic nature of vDMHLepR→ARC<sup>AgRP</sup> neurons (Fig. 1c), the majority of vDMH ARC<sup>AgRP</sup> afferents are leptin-responsive (71 ± 1.6%, n = 3; Supplementary Fig. 2c,d). Together, these data suggest that the vDMH is the principle source of GABAergic vDMH LepR-expressing ARC<sup>AgRP</sup> neuron afferents. In addition, although as a population vDMH<sup>LepR</sup> neurons are widely ramifying (Supplementary Fig. 3a), the ARC-projecting axons do not collateralize to send projections to other neuroanatomical targets (Supplementary Fig. 3b,c), as demonstrated by rabies collateral mapping<sup>17</sup>.

**vDMH<sup>LepR</sup>→ARC neurons are sufficient to inhibit feeding**

Since direct inhibition of ARC<sup>AgRP</sup> neurons suppresses food consumption<sup>13,18</sup>, we anticipated that the in vivo activation of vDMH<sup>LepR</sup>→ARC projections would similarly reduce food intake during times of physiological hunger, thus confirming behaviorally the inhibitory nature of the circuit. In vivo optogenetic stimulation of vDMH<sup>LepR</sup>→ARC terminals facilitated the functional isolation of this noncollaterlizing circuit from the broader DMH<sup>LepR</sup> population (Fig. 2a). Photostimulation of ChR2-mCherry-expressing vDMH<sup>LepR</sup>→ARC efferents (Supplementary Fig. 4a) before the initiation of consumption (10 min or 10 s before consumption), using the same pulsed-light protocol that successfully silenced ex vivo ARC<sup>AgRP</sup> neuron firing (Fig. 1f and Supplementary Fig. 4b), significantly decreased (by ~88%) dark-cycle food intake (Fig. 2b); this was not observed in photostimulated GFP controls (Supplementary Fig. 4c). Optogenetic activation also attenuated hypermotivated food consumption following an overnight fast (Fig. 2c), while cessation of photostimulation rapidly reestablished normal refeeding behavior (Fig. 2c and Supplementary Video 1). Notably, photostimulation of this circuit was also sufficient to halt food intake 10 s after the initiation of consumption following an overnight fast (Fig. 2d) or during the dark cycle (Supplementary Fig. 4d). Photostimulation in the dark cycle (Supplementary Fig. 4e) or light cycle (Supplementary Fig. 4f) revealed no overt changes in locomotor activity. No changes in anxiety-like behaviors were evident in an open-field experiment (Supplementary Fig. 4g–i). Photostimulation in the fasted state increased the time spent grooming to a level comparable to that following food intake (Supplementary Fig. 4j), consistent with an induction of satiety-like behavior<sup>19</sup>. Chemogenetic silencing of vDMH<sup>LepR</sup> neurons did not increase light-cycle food consumption (Supplementary Fig. 5), indicating that this population is not required for maintaining physiological satiety. Together these data demonstrate that vDMH<sup>LepR</sup>→ARC neurons are sufficient, but not necessary, to robustly suppress homeostatic feeding through the inhibition of ARC<sup>AgRP</sup> neurons and the induction of artificial satiety.
vDMHLepR→ARC neurons are activated by food availability

Given these functional observations, and the inhibitory capacity of the vDMHLepR→ARC projections, we considered whether vDMHLepR neurons contribute to the rapid and transient modulation of ARC\(\text{AgRP}\) neurons upon sensory detection of food.\(^7\)\(^8\) We therefore employed in vivo fiber photometry to study the endogenous calcium activity of populations of vDMHLepR neurons during food presentation. Virally-mediated Cre-dependent expression of the genetically encoded calcium indicator GCaMP6s (ref. 20) in vDMHLepR neurons enabled within-subject fluorometric analysis of real-time neuronal activity.

We first assessed the population response of vDMHLepR cell bodies (Fig. 3a) to repeated presentation of small chow pellets (14 mg). In food-restricted mice (85% of free-feeding body weight), we observed a rapid and robust increase in calcium activity upon pellet detection and approach (Fig. 3b–d), as compared to detection of a similarly sized nonfood object. This effect preceded the initiation of consumption (Fig. 3e). The absence of a significant calcium response to a nonfood item also confirmed that the observed effect was not due to a startle response. In the ad libitum fed state, when mice were calorically replete, calcium responses to presentation of these pellets were significantly attenuated as compared to the food-restricted state (Fig. 3c,d). No calcium responses to food or object presentation were evident from vDMHLepR neurons transduced with Cre-dependent GFP (Supplementary Fig. 6a,b) or in validated ‘misses’ (no GCaMP6s expression in vDMH; Supplementary Fig. 6c,d). Thus, vDMHLepR neurons exhibit a preconsummatory response that is similar in nature but opposite in sign to AgRP neurons: specifically, a decrease in activity upon food presentation, the magnitude of which correlates with the animal’s hunger state.

Larger chow pellets (500 mg) also elicited a calcium response that exhibited energy-state dependence (Fig. 3f,g), but this response was of greater magnitude than that observed with small pellets (Fig. 3h), suggesting that vDMHLepR neuron activity conveys information not only about the presence but the nature of discovered food items. ARC\(\text{AgRP}\) neurons exhibit exaggerated preconsummatory suppression upon the presentation of chocolate, a highly palatable food that is more calorically dense and rewarding than chow.\(^8\) As predicted, presentation of chocolate fragments (approximately 14 mg) elicited an increase in GCaMP6 fluorescence in vDMHLepR cell bodies. In contrast to chow presentation, responses to chocolate presentation did not vary across fasted compared to fed states (Fig. 3i), possibly due to sustained food-seeking for chocolate compared to chow pellets (Fig. 3k).

Thus, the preconsummatory activation of vDMHLepR neurons is potentiated by the nutritive value of detected food in a manner that reflects both food quantity and quality.

To isolate the vDMHLepR→ARC projecting neurons from the broader vDMHLepR population, we assessed calcium activity specifically in vDMHLepR→ARC axons (Fig. 4a). As observed in population activity from vDMHLepR cell bodies, axonal calcium activity in food-restricted mice rapidly increased upon presentation of a small chow pellet (Fig. 4b–d) before consumption (Fig. 4e–g) but not in reaction to a nonfood object or in the ad libitum fed state. Larger chow pellets elicited larger calcium responses than small pellets (Fig. 4h and Supplementary Fig. 7a,b), similar to responses in vDMHLepR cell bodies. The vDMHLepR→ARC axon responses were larger to presentation of chocolate versus small pellets and did not depend on hunger state (Fig. 4i and Supplementary Fig. 7c,d). In sum, vDMHLepR→ARC neurons respond to availability of food in a manner opposite to that of ARC\(\text{AgRP}\) neurons, relaying real-time sensory information regarding the availability and quality of food.

A subset of vDMHLepR→ARC neurons are dynorphinergic

In light of the heterogeneity of vDMHLepR neurons,\(^2\)–\(^23\) we sought to further specify the neurochemical identity of GABAergic vDMHLepR→ARC\(\text{AgRP}\) afferents. Recent analysis of hypothalamic LepR neurons has indicated that a subset of those in the vDMH express the inhibitory neuropeptide pDYN (ref. 24). Quantitative PCR analysis of individual manually isolated vDMHLepR neurons revealed that 14 of 25 (56%) of those expressing Slc32a1 (vGAT) also expressed Pdyn (Supplementary Fig. 8a,b). Consistent with the localization of vDMHLepR→ARC\(\text{AgRP}\) neurons (Supplementary Fig. 2), the preponderance of leptin-responsive vDMHLepR neurons (as defined by pStat3 immunoreactivity) were within the vDMH (Supplementary Fig. 8c–e).\(^18\) Furthermore, projection profiling from vDMHLepR neurons identified the mediobasal ARC as their only long-range target (Supplementary Fig. 8f-h).

CRACM analysis demonstrated that almost all recorded ARC\(\text{AgRP}\) neurons (20 of 21 neurons; Fig. 5a) but no ARC\(\text{non-AgRP}\) neurons upon sensory detection of food 5,7,8. We therefore employed Feeding 5,7,8. We therefore employed...
neurons (Supplementary Fig. 8i; including ARCPOMC neurons, Fig. 5b) received direct GABAergic input from vDMHPDYN neurons. vDMHPDYN→ARCPGR inhibitory postsynaptic currents were of smaller amplitude than those derived from vDMHLepR afferents (Supplementary Fig. 8j) which led to less effective light-evoked inhibition of ARCPGR neuron spiking (Supplementary Fig. 8k). This suggests that vDMHPDYN neurons are only a proportion of the total GABAergic vDMHLepR→ARCPGR population. In vivo optogenetic activation of vDMHPDYN→ARC terminals suppressed food consumption during the dark cycle (Fig. 5c) and following an overnight fast (Fig. 5d). The magnitude of feeding suppression was less than that observed in the vDMHLepR→ARC circuit (Fig. 2), especially during

**Figure 3** vDMHLepR neurons are rapidly activated upon sensory detection of food. (a) The real-time activity of vDMHLepR cell bodies was determined using in vivo fiber photometry. (b–d) vDMHLepR neurons were rapidly activated upon presentation of a small chow pellet (t = 0), compared to a nonfood object, in an energy-state dependent manner. (b) Individual trials in one representative mouse on one day in the calorie restricted and ad libitum fed state. (c) Mean effects from all mice across time, n = 6. (d) Mean response from 0–10 s after food presentation, repeated-measures ANOVA, F(5,15) = 7.2, P = 0.02; post hoc: fasted, nonfood object (obj) vs. chow, *P = 0.028; fed, obj vs. chow, P = 0.064. (e) Responses of vDMHLepR to small pellet availability occurred before the initiation of consumption and was not increased further once consumption began (n = 15; repeated-measures ANOVA, F(14,28) = 12.16, P = 0.0002; post hoc: appro vs. base, ****P = 0.0026; consum vs. base, ***P = 0.001). (f,g) vDMHLepR neurons were rapidly activated upon presentation of a large chow pellet, compared to a nonfood object, in an energy-state dependent manner. (f) Mean effects from all mice across time, n = 6; (g) mean response from 0–10 s after food presentation, repeated-measures ANOVA, F(5,15) = 24.15, P = 0.0001; post hoc: fasted, obj vs. chow, **P = 0.004; fed, obj vs. chow, *P = 0.027; fasted, chow vs. fed, chow, *P = 0.019). (h) Response of vDMHLepR neurons to large chow pellets was more potentiated than that elicited by small chow pellets in the same mouse (n = 7; paired t-test,  \( t_{(6)} = 3.88, **P = 0.0081 \)). (i,j) Presentation of chocolate produced stronger activation in vDMHLepR neurons than did a nonfood object, and the activation in food-restricted mice was comparable to responses in ad libitum chow-fed mice. (i) Mean effects from all mice across time, n = 5; (j) mean response from 0–10 s after food presentation; repeated-measures ANOVA, F(5,15) = 24.21, P = 0.0003; post hoc: fasted, obj vs. chocolate (choc), *P = 0.015; fed, obj vs. choc, *P = 0.018. (k) Responses to chocolate were greater than responses to chow (n = 6; paired t-test,  \( t_{(5)} = 4.58, **P = 0.006 \)). Appro., approach; base., baseline; consum., consumption; lg, large chow pellet; sm, small chow pellet; ns, nonsignificant. All data presented as mean ± s.e.m.
a refeed after fasting, likely reflecting the weaker inhibitory potency of this circuit.

Subsequent in vivo GCamp6s photometry demonstrated that vDMHLepR neurons showed similar functional properties to vDMHlepR neurons and vDMHLepR→ARC axons. Small pellets presented to hungry mice elicited a significant increase in calcium activity before consumption, which was not observed upon detection of a nonfood item or in ad libitum fed mice (Fig. 5c–g). Calcium responses in food-restricted mice were potentiated by presentation of larger chow pellets (Fig. 5h–j) and chocolate (Fig. 5k–m), with chocolate responses being independent of energy state. Together, these data suggest that vDMHlepR neurons represent a subpopulation of GABAergic vDMHlepR→ARCAgRP afferents.

DISCUSSION

Using a combination of in vivo techniques for the manipulation and monitoring of genetically defined neuronal populations, we identified a source of inhibitory input to ARCAgRP neurons that contributes to their rapid sensory regulation.\(^5,7,8\) This population of GABAergic vDMHlepR→ARC agRP neurons exhibits a highly circumscribed efferent field within the ventromedial ARC with dense perisomatic innervation of ARCAgRP somata. As such, when photostimulated they provide a highly selective inhibitory input sufficient to robustly silence ARCAgRP neuron action potential firing and suppress homeostatic feeding. It is important to note that the complete inhibition of ARCAgRP neurons by way of the optogenetic activation of GABAergic vDMHlepR→ARC terminals (Fig. 1f) represents a supraphysiological paradigm that exceeds the level of suppression induced by food availability.\(^2\) Thus, while providing behavioral validation for the nature of the circuit, such optogenetic manipulation does not speak to the physiological role of ARCAgRP neurons (or vDMHlepR→ARC neurons) in the regulation of homeostatic feeding. Indeed, as observed by others,\(^22,23\) vDMHlepR neurons were not necessary for the maintenance of homeostatic satiety, indicating that they are not a source of tonic ARCAgRP neuron inhibition contributing to feeding suppression during times of caloric sufficiency. This circuit may, however, offer a highly tractable experimental approach for real-time temporal control of ARCAgRP neurons.

**Figure 4** vDMHlepR→ARC axons are rapidly activated upon sensory detection of food. (a) Real-time activity of vDMHlepR→ARC axons was determined using in vivo fiber photometry. (b–d) vDMHlepR→ARC axons were rapidly activated upon presentation of a small chow pellet (time, \(t = 0\)), compared to a nonfood object, in an energy-state dependent manner. (b) Individual trials in one representative mouse on one day in the calorie-restricted and ad libitum fed state; (c) mean effects from all mice across time, \(n = 6\); (d) mean response from 0–10 s after food presentation. Repeated measures ANOVA \((F(5,15) = 36.08, P < 0.0001)\); post hoc: fasted, obj vs. chow, \(P = 0.0005\); fed, obj vs. chow, \(P = 0.136\); fasted + chow vs. fed + chow, \(**P = 0.012\). (e) Activation of vDMHlepR→ARC axons in response to small pellet availability occurred before the initiation of consumption (\(n = 36\); repeated-measures ANOVA, \(F(35,70) = 35.30, P < 0.0001\); post hoc: appro vs. base, \(* * * P = 0.0003\); consum vs. base, \(* * * * P < 0.0001\); appro vs. consum, \(* * * P = 0.0002\). (f,g) Mean responses to small pellet presentation aligned to (f) onset of consumption and (g) individual trial responses aligned to food availability (onset of consumption denoted with vertical black bar on each trial) demonstrating activity rising before consumption. (h,i) Calcium response of vDMHlepR→ARC axons to (h) large chow pellets (\(n = 6\); paired \(t\)-test, \(t(6) = 3.61, **P = 0.015\)) and (i) chocolate (\(n = 6\); paired \(t\)-test, \(t(6) = 3.13, *P = 0.026\)) were potentiated compared to that elicited by small chow pellets in the same mouse. Appro., approach; base., baseline; choc, chocolate; consum., consumption; igL, large chow pellet; sm, small chow pellet. All data presented as mean ± s.e.m.
Chow (sm)

post hoc

( obj vs. chow, ****

before the initiation of consumption and did not increase further once consumption began (n = 3, repeated-measures ANOVA, main effect of time: F(3, 18) = 21.49, P < 0.0001; main effect of time: F(3, 18) = 12.69, P = 0.0002; post hoc: 1 h, P = 0.062; 2 h, ***P = 0.0006; 3 h, ***P = 0.0002) and (d) following an overnight fast (n = 3, repeated-measures ANOVA, main effect of treatment: F(3, 18) = 193.60, P < 0.0001; post hoc: 1 h, ***P = 0.0002; 2 h, ***P < 0.0001; 3 h, ****P < 0.0001; main effect of time: F(3, 18) = 111.90, P < 0.0001; interaction: F(3, 18) = 22.63, P = 0.0003). (e,f) In vivo fiber photometry demonstrated that vDMH^POMC neurons were rapidly activated upon presentation of a small chow pellet (t = 0) compared to a nonfood object, in an energy-state dependent manner. (e) Mean effects from all mice across time, n = 5 or 6; (f) mean response from 0–10 s after food presentation; one-way ANOVA, F(3, 18) = 19.56, P < 0.0001; post hoc: fasted, obj vs. chow, ****P < 0.0001; fed, obj vs. chow, P = 0.998; fasted + chow vs. fed + chow, ***P = 0.0001). (g) Activation of vDMH^PDMN in response to small pellet availability occurred before the initiation of consumption and did not increase further once consumption began (n = 44; repeated-measures ANOVA, F(3, 180) = 40.61, P < 0.0001; post hoc: appro vs. base, ****P < 0.0001; consum vs. base, ****P < 0.0001; appro vs. consum, P = 0.304). (h,l) vDMH^PDMN neurons were more rapidly activated upon presentation of a large chow pellet than to a nonfood object, in an energy-state dependent manner. (h) Mean effects from all mice across time, n = 5 or 6; (i) mean response from 0–10 s after food presentation; one-way ANOVA, F(3, 18) = 13.43, P < 0.0001; post hoc: fasted, obj vs. chow, ****P < 0.0001 fed, obj vs. chow, P = 0.98; fasted + chow vs. fed + chow, ***P = 0.0028). (j) Calcium response of vDMH^PDMN neurons to large chow pellets was potentiated compared to that elicited by small chow pellets in the same mouse (n = 6; paired t-test, t(5) = 3.56, *P = 0.016). (k,l) Presentation of chocolate activated vDMH^PDMN neurons more than a nonfood object did, and was comparable in food-restricted mice to the response elicited in ad libitum chow-fed mice. (k) Mean effects from all mice across time, n = 5 or 6; (l) mean response from 0–10 s after food presentation; one-way ANOVA, F(3, 18) = 18.03, P < 0.0001; post hoc: fasted, obj vs. chow, ****P < 0.0001; fed, obj vs. choc, **P = 0.001; fasted + chow vs. fed + choc, P > 0.99). (m) vDMH^PDMN neuron calcium responses to chocolate were increased compared to Chow (n = 6, paired t-test, t(5) = 5.09, ****P = 0.0038). Appro, approach; base, baseline; choc, chocolate; consum, consumption; lg, large chow pellet; sm, small chow pellet; ON, with photostimulation; OFF, without photostimulation. All data presented as mean ± s.e.m.
Recent investigations of the endogenous activity of ARC<sup>AgRP</sup> neurons has revealed their preconsummatory suppression upon food presentation or expectation<sup>5,7,8</sup>. The rapidity of this response strongly suggests that it is synaptically, rather than hormonally, mediated. Indeed, our finding that all ARC<sup>AgRP</sup> neurons recorded received GABAergic input from vDMHL<sup>LepR</sup>/pDYN neurons is consistent with the majority of ARC<sup>AgRP</sup> neurons exhibiting preconsummatory suppression<sup>5,7</sup>. Thus, in light of the specificity and potency of the vDMHL<sup>LepR</sup>→ARC circuit, we asked whether it contributed to the dynamic modulation of ARC<sup>AgRP</sup> neurons during food discovery. We found that, reciprocal to ARC<sup>AgRP</sup> neurons, vDMHL<sup>LepR</sup> cell bodies and vDMHL<sup>LepR</sup>→ARC axons both exhibited rapid and reproducible preconsummatory activation upon food detection. Chow presentation elicited fluorescent responses with both cue- and energy state-dependency, indicating that some level of neural gating upstream of these neurons is important for attributing salience to the sensory input in a manner that considers the animal’s broader external and internal environment. Furthermore, as observed of ARC<sup>AgRP</sup> neurons<sup>8</sup>, the magnitude of calcium responses in vDMHL<sup>LepR</sup> neurons and their ARC projections increased with presentation of more palatable food. Thus, in the fasted state, the potentiation of the vDMHL<sup>LepR</sup>→ARC response to increased nutritive content (both quality and quantity) may signal the greater value of the food item as a source of relief from hunger. However, as reflected by vDMHL<sup>LepR</sup>→ARC neuron activity (and feeding behavior), in the calorically replete state, food quantity loses incentive value but food quality retains incentive value, possibly suggesting a switch in value processing from the homeostatic to the hedonic in the absence of a physiological hunger drive.

Other populations of neuronal afferents also contribute to the sensory regulation of ARC<sup>AgRP</sup> neurons. Indeed, although vDMHL<sup>LepR</sup> neuron activity peaked upon food approach before consumption, we observed a delay in the peak amplitude of the calcium response before the termination of feeding. This contrasts with the sustained reduction in ARC<sup>AgRP</sup> neuron activity throughout consumption<sup>5,7,8</sup>, the magnitude of calcium responses in vDMHL<sup>LepR</sup>→ARC neuron activity (and feeding behavior), in the calorically replete state, food quantity loses incentive value but food quality retains incentive value, possibly suggesting a switch in value processing from the homeostatic to the hedonic in the absence of a physiological hunger drive.

The significance of LepR expression on GABAergic vDMH<sup>LepR</sup> neurons also remains to be determined. In acute electrophysiological slices, leptin depolarizes GABAergic vDMH<sup>LepR</sup> neurons (data not shown). This raises the possibility that low leptin levels, by decreasing the basal activity of these neurons, may increase their dynamic range and facilitate their response to food-related sensory cues. Alternatively, it is plausible that LepR signaling at these neurons is involved in a slower transcriptional modulation, potentially related to synaptic restructuring. In this way, LepR signaling at vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> neurons may concern longer-term regulation reflecting the chronic nutritional state, such as might underlie maladapted associations between sensory cues and feeding behavior in obesity or eating disorders. Real-time monitoring of vDMHL<sup>LepR</sup> neuron activity in diet-induced or genetically obese mice will prove informative in this regard.

As a population, vDMHL<sup>LepR</sup> neurons have been implicated in a number of physiologies, including autonomic regulation of energy expenditure and cardiovascular tone<sup>11,22,23</sup>. Although the specific networks underlying these functions are yet to be defined, it is likely that they are independent of the vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> circuit. DMHL<sup>LepR</sup> neurons that regulate energy expenditure are glutamatergic and located in the dorsal DMH (refs. 22,26) and thus spatially and neurochemically distinct from GABAergic vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> neurons. Furthermore, the thermogenic effect of DMHL<sup>LepR</sup> neurons has been demonstrated to be melanocortin independent<sup>24</sup>. For a number of reasons it is also unlikely that the vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> circuit is involved in cardiovascular control. First, DMH mediated regulation of blood pressure is predicted to proceed via more direct projections to preautonomic neurons in the rostral ventrolateral medulla<sup>27</sup>. Second, leptin-mediated or chemogenetic activation of DMHL<sup>LepR</sup> neurons only influences blood pressure after 3 d of chronic simulation<sup>21,28</sup>, inconsistent with the acute modulatory function of vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> neurons. Third, no feeding suppression was observed during chemogenetically induced hypotension<sup>24</sup>, as would be expected of activation of the vDMHL<sup>LepR</sup>→ARC<sup>AgRP</sup> circuit. It is therefore likely that vDMHL<sup>LepR</sup>→ARC neurons represent a functionally specific subpopulation involved in transitory sensory modulation of ARC<sup>AgRP</sup> neurons. Notably, vDMHL<sup>LepR</sup>/pDYN neurons are distinct from the non-LepR expressing DMHpDYN neurons within the central compartment (cDMH), implicated in the attenuation of food consumption during intense feeding bouts<sup>29</sup>

The rapid preconsummatory inhibition of ARC<sup>AgRP</sup> neurons and their sustained suppression during consumption represents a striking aspect of their physiological function<sup>5,7,8</sup>, although the significance of this phenomenon for feeding behavior remains controversial. Our data now expand an understanding of the nature and source of this modulation. Specifically, we identified GABAergic vDMHL<sup>LepR</sup>/pDYN<sup>Nes</sup> neurons as potent inhibitory afferents to ARC<sup>AgRP</sup> neurons that, like their postsynaptic targets, are rapidly regulated by food detection. As expected, the directionality of this modulation is reciprocal to ARC<sup>AgRP</sup> neurons but occurs on a comparable timescale. Furthermore, like ARC<sup>AgRP</sup> neuron activity, vDMHL<sup>LepR</sup>/pDYN neuron activity reflects not only the presence but also the quality of the food item. These observations strongly support the hypothesis that vDMHL<sup>LepR</sup>/pDYN neurons are a physiologically relevant source of inhibitory input to ARC<sup>AgRP</sup> neurons and provide an entry point into the upstream circuitry that underlies rapid evaluation of sensory food cues during homeostatic feeding.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in [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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**ONLINE METHODS**

**Animals.** Slc32a1(LvGAT)-ires-cre<sup>11</sup>, Lepr-ires-cre<sup>9</sup>, Pdyn-ires-cre<sup>13</sup>, Npy-humanized renilla (hr)GFP<sup>13</sup>, Pmcn-hrGFP<sup>13</sup> and Rosa26-loxSTOPlox-L10-GFP<sup>22</sup> mice were generated and maintained as previously described. All mice are on a mixed background. All animal care and experimental procedures were approved by the National Institute of Health and Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee. Mice were housed at 22–24 °C with a 12-h light:12-h dark cycle with standard mouse chow (Teklad F6 Rodent Diet 8664; 4.05 kcal g<sup>−1</sup>, 3.3 kcal g<sup>−1</sup> metabolizable energy, 12.5% kcal from fat; Harlan Teklad) and water provided ad libitum, unless otherwise stated. All diets were provided as pellets. For all behavioral studies, male mice between 6 and 10 weeks of age were used. For electrophysiological studies we used male mice between 4 and 8 weeks.

**Brain tissue preparation.** Mice were terminally anesthetized with chloral hydrate (Sigma-Aldrich) and transcardially perfused with phosphate-buffered saline (PBS) followed by 10% neutral buffered formalin (Fisher Scientific). Brains were extracted, cryoprotected in 20% sucrose, and sectioned coronally on a freezing sliding microtome (Leica Biosystems) at 30 μm and collected in four equal series.

**Immunohistochemistry.** Brain sections were washed in 0.1 M phosphate-buffered saline pH 7.4, blocked in 3% normal donkey serum/0.25% Triton X-100 in PBS for 1 h at room temperature and then incubated overnight at room temperature in blocking solution containing primary antiserum (rabbit anti-DsRed, Cloneget #632496, 1:1,000; chicken anti-GFP, Life Technologies #A10262). The next morning sections were extensively washed in PBS and then incubated Alexa fluorophore secondary antibodies (donkey anti-rabbit 594 and donkey anti-chicken 488; Molecular Probes, 1:1,000) for 2 h at room temperature (20–22 °C). After several washes in PBS, sections were mounted onto gelatin-coated slides and fluorescent images were captured with Olympus VS120 slide scanner microscope. All primary antibodies used are validated for species and application (1DEgreeBio and Antibody Registry).

**pStat3 immunohistochemistry.** Mice were injected with 5 mg/kg recombinant leptin 2 h before perfusion (as above). Brain sections were washed in 0.1 M phosphate-buffered saline, pH 7.4, followed by incubation in 5% NaOH and 0.3% H<sub>2</sub>O<sub>2</sub> for 2 min, then with 0.3% glycine (10 min), and finally with 0.3% SDS (10 min), all made up in PBS. Sections were blocked in 3% normal donkey serum/0.25% Triton X-100 in PBS for 1 h at room temperature and then incubated overnight at room temperature in blocking solution containing 1/250 rabbit anti-pStat3 (Cell Signaling, #9145) and 1/1,000 chicken anti-GFP (Life Technologies, #A10262). The next morning sections were extensively washed in PBS and then incubated in 1/250 donkey anti-rabbit 594 (Molecular Probes, #R37119) and 1/1,000 donkey anti-chicken 488 (Jackson ImmunoResearch, 703-545-155) for 2 h at room temperature. After several washes in PBS, sections were mounted onto gelatin-coated slides and fluorescent images were captured with Olympus VS120 slide scanner microscope.

**Single cell quantitative PCR.** vDMH was acutely dissected from adult LepR-ires-cre-:: L10-GFP mice (n = 2), then enzymatically dissociated and manually sorted for GFP+ cells as described previously<sup>32</sup>. Isolated GFP-positive cells and negative control samples (cell-picking buffer) were concurrently processed into cDNA libraries using Smart-Seq2 (ref. 33), except that the amplified cDNA was eluted in 30 μl volumes. Gene expression was analyzed by probe-based qPCR on a 7500 Fast Real-Time PCR System (Applied Biosystems) using Brilliant II qPCR Low ROX Master Mix (Agilent Technologies) according to the manufacturer’s instructions. Each 20 μl qPCR reaction contained 2 μl of eluted cDNA and 1 μl of a custom primer/probe set (sequences below; 1:1 ratio of primer:probe; default FAM/ZEN modifications; IDT). Cells showing relatively little to no expression of Gfp, Actb, or Slc32a1(LvGAT) were excluded from further analysis. Remaining cells were analyzed for expression of Pdyn. A heat map of Ct values was generated using GenePattern software (Broad Institute), with a ‘global’ color scale for cross-gene comparisons. Note that in order to include cells for which no signal was detected in 40 cycles of qPCR, a ‘pseudocount’ of 40 was entered as the Ct. Primers (5′–3′): 

**Actb.** (L, AAAAGGGAGGCTCAGACCTGG; R, TCACCCTCCTCCAAA GCACC; probe, GCCCTGTGTCACCCGGG; 

**Gfp.** (L, ATTCGACACACCGCGCAACT and R, ATTCGACCACCG CGCAACT; 

**probe, GGGCCCGTTCGCCACCGTGG; 

**Slc32a1.** L, AGGAGCACTACACGCAACA and R, ATTTCGGGCGGGCG GACCTCA; 

**probe, GGGGCGCTTCGCGGGCGCAT.**

**Viral injections.** Stereotaxic injections were performed as previously described. Mice were anesthetized with xylazine (5 mg per kg) and ketamine (75 mg per kg) diluted in saline (350 mg per kg) and placed into a stereotaxic apparatus (KOPF Model 963 or Stoelting). For postoperative care, mice were injected intraperitoneally with meloxicam (5 mg/kg). After exposing the skull via small incision, a small hole was drilled for injection. A pulled-glass pipette with a 20–40 nm tip diameter was inserted into the brain, and virus was injected by an air pressure system. A micromanipulator (Grass Technologies, Model 548 Stimulator) was used to control injection speed at 25 nl min<sup>−1</sup> and the pipette was withdrawn 5 min after injection. For electrophysiology and in vivo optogenetic experiments, AAV8-hSyn-DIO-ChR2(H134R)-mCherry (University of North Carolina Vector Core; titer 1.3 × 10<sup>12</sup> genome copies per ml) was injected into the ARC (15–50 nl, AP: −1.50 mm, DV: −5.2 mm, ML: ±0.3 mm from bregma), VMH (50–80 nl, AP: −1.80 mm, DV: −5.5 mm, ML: ±0.2 mm from bregma), LHA (50–100 nl, AP: −1.50 mm, DV: −5.00 mm, ML: ±1.00 mm from bregma). For electrophysiology and in vivo chemogenetic experiments, AAV8-hSyn-DIO-hM4Di-mCherry (University of North Carolina Vector Core; titer 1.7 × 10<sup>12</sup> genome copies per ml) was bilaterally injected into the vDMH (15–40 nl, coordinates as above). For ex vivo and in vivo calcium imaging experiments, AAV1-hSyn-DIO-GCaMP6s (University of Pennsylvania Vector Core) was injected into the vDMH (50 nl, coordinates as above). Mice were given a minimum of 2 weeks for recovery and 1 week for acclimation before being used in any experiments.

**Electrophysiology.** To prepare brain slices for electrophysiological recordings, brains were removed from anesthetized mice (4–8 weeks old) and immediately submerged in ice-cold, carbogen-saturated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) high-solute sucrose (238 mM sucrose, 26 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 3.5 mM MgCl<sub>2</sub>, 10.0 mM CaCl<sub>2</sub>, 11 mM glucose). Then, 300-μm thick coronal sections were cut with a Leica VT1000S Vibratome and incubated in oxygenated aCSF (126 mM NaCl, 21.4 mM NaHCO<sub>3</sub>, 2.5 mM KCl, 1.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.0 mM MgCl<sub>2</sub>, 2.4 mM CaCl<sub>2</sub>, 10 mM glucose) at 34 °C for 30 min. Slices were maintained and recorded at room temperature (20–24 °C). For most voltage-clamp recordings, intracellular solution contained the following (in mM): 140 CsCl, 1 BAAPT, 10 HEPES, 5 MgCl<sub>2</sub>, 5 Mg-ATP, 0.3 NaGTP, and 10 lidocaine N-ethyl maleamide (XQ-314), pH 7.35 and 290 mOsm. The intracellular solution for current clamp recordings contained the following (in mM): 128 k-glutamate, 10 KCl, 10 HEPES, 1 EGTA, 1 MgCl<sub>2</sub>, 0.3 CaCl<sub>2</sub>, 2 NaATP, 0.3 NaGTP, adjusted to pH 7.3 with KOH.

Light-activated IPSCs and EPSCs during CRACM experiments<sup>34,35</sup> were recorded in the whole-cell voltage-clamp mode, with membrane potential clamped at −60 mV. In a subset of voltage-clamp CRACM experiments it was necessary to detect light-evoked GABAergic synaptic currents in ChR2-mCherry-expressing neurons (Supplementary Fig. 2d,h,i). As such, to negate the movement of monovalent cations, a Cs<sup>+</sup>-based low-C<sub>i</sub> internal solution was used (129 mM CsMeSO<sub>4</sub>, 16 mM CsCl, 8 mM NaCl, 10 mM HEPES, 0.25 mM EGTA, 3 mM Mg-ATP, 0.3 mM NaGTP and light-sensitive IPSCs recorded at −20 mV. All recordings were made using a Multiclamp 700B amplifier, and data was filtered at 2 kHz and digitized at 10 kHz. To photostimulate channelrhodopsin2-positive fibers, a laser or LED light source (473 nm: Opto Engine LLC; ThorLabs) was used. The blue light was focused on to the back aperture of the microscope objective, producing a wide-field exposure of 1 mW around the recorded cell. The light power at the specimen was measured using an optical power meter, PM100D (ThorLabs). The light output was controlled by a programmable pulse stimulator, Master-8 (AMPI Co. Israel) and pClamp 10.2 software (AXON Instruments). Photostimulation-evoked EPSC/IPSC detection protocol comprised 4 blue light laser pulses (pulse duration, 2 ms) administered 1 s apart, repeating for a total of 30 sweeps. When
recording light-evoked changes in membrane potential in AgRP neurons, current
((~5 pA) was injected into cells to maintain continuous action potential firing.
Number of animals used per study (all male): vDMDH→ARCAgRP = 2; vDMHvGAT→ARCPOMC = 2; ARCAgRP = 3; ARCPOMC = 2; vDMHbGAT→ARCAgRP = 2; vDMH→LHvGAT = 6; vDMH→ARCAgRP = 2; DMDH→ARCAgRP = 2; vDMH-LeprR→ARCPOMC = 4; vDMH→ARCPOMC = 2; LFvGAT→ARCAgRP = 2; vDMH→ARCPOMC = 4; LFvGAT→ARCPOMC = 2; vDMH→ARCAgRP = 2; vDMH→ARCPOMC = 4; LFvGAT→ARCPOMC = 2; vDMH→ARCPOMC = 4.

**Optical fiber implantation.** Optical fiber implantations were performed during the same surgery as viral injection (above). For optogenetic stimulation of vDMH→ARCA terminals, ceramic ferrule optical fibers (200-μm diameter core, BHF37-200 Multimode, NA 0.37; Thor Labs) were implanted bilaterally over the ARC (AP: −1.55 mm, DV: −5.5 mm, ML: ±0.3 mm from bregma). For vDMH→ARCPOMC, a glass fiber (1 m long, 400-μm diameter core, BHF37-400 Multimode; NA 0.37; Thor Labs) was implanted uni laterally over the ARC (AP: −1.8 mm, DV: −5.0 mm, ML: ±0.3 mm from bregma). Fibers were fixed to the skull using dental acrylic and mice were allowed 2 weeks for recovery before acclimatizing to home cages customized for optogenetic stimulation or photometry recording (12-h light-dark cycle starting at 6 am) for 1 week. After the completion of the experiments, mice were killed and the approximate locations of fiber tips were identified based on the coordinates of Franklin and Paxinos.36

**Food intake studies.** Food intake studies on chow were performed as previously described. All animals were singly housed for at least 2.5 weeks following surgery and handled for 10 consecutive days before the assay to reduce stress response. Studies were conducted in a home-cage environment with ad libitum food access. A full trial consisted of assessing food intake from the study subjects after they received injections of saline (chemogenetics) or pseudo-photostimulation (optogenetics) on day 1 and 1 mg/kg CNO (chemogenetics) or photostimulation (optogenetics) on day 2. Animals were allowed to recover for one week between trials before another trial was initiated. The food intake data from all days were then averaged and combined for analysis. Any mice with ‘missed’ injections, incomplete ‘hits’ or expression outside the area of interest were excluded from analysis after post hoc examination of mCherry expression (n = 7 for in vivo optogenetic experiments; n = 11 for photometry experiments). In this way, all food intake measurements were randomized and blind to the experimenter.

Dark-cycle feeding studies were conducted from 6:00 pm to 9:00 pm and intake was monitored for 3 h. For post-fast refedding studies, animals were fasted overnight from 5:00 pm and food returned the following morning at 9:00 am. Food intake was monitored for 5 h after photostimulation. Light-cycle feeding studies were conducted from 9:00 am to 12:00 pm and intake was monitored for 3 h.

**In vivo optogenetic studies.** In vivo photostimulation was conducted as previously described.35 Fiber optic cables (1.25 m long, 200-μm diameter core, 0.37 NA; Doric Lenses) were firmly attached to the implanted fiber optic cannulae with zirconia sleeves (Doric Lenses). Animals were stimulated with blue light (473 nm) at 10 Hz, 5 ms pulses for 5 s with a 1-s recovery period (laser off) during stimulation trains to avoid neuronal transmitter depletion and tissue heating. Photostimulation was provided using a waveform generator (PCGU100; Vallemen Instruments or Arduino electronics platform) that provided TTL input to a blue light laser (Laserglow). We adjusted the power of the laser such that the light power exiting the fiber optic cable was at least 10 mW. Using an online light transmission calculator for brain tissue (http://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php), we estimated the light power at the ARC to be 18.35 mW/mm². Mice were tethered to the patch cords at least 1 h before the commencement of any experiment.

To test the sufficiency of vDMH→ARCPOMC for satiety, mice were tested under two conditions of physiological hunger: at the onset of the dark cycle and prior to refeeding following an overnight fast. For dark-cycle feeding analysis, mice with ad libitum access to food were photostimulated for 10 min before the onset of the dark cycle (which serves as a natural cue for the initiation of feeding behavior) and photostimulation was maintained for the duration of the study. For post-fast refedding analysis, mice were photostimulated 10 min before food presentation (which served as an experimental cue for the initiation of feeding behavior) and photostimulation was maintained for the duration of the study. In the case of the ‘ON (After)’ group in Figure 2d, mice were allowed to consume food freely for 5 min and then photostimulated for the duration of the experiment.

**Behavioral profiling.** Open field testing was conducted in ad libitum fed mice during the light cycle. Mice were placed in a large arena (40 cm × 40 cm), which they were allowed to freely explore for 20 min. Trials were recorded via a CCD camera interfaced with Ethovision software for offline analysis of distance moved and time spent at the edge and center of the arena. Animals were run in a counterbalanced order of laser-on versus laser-off to avoid acclimation.

For assessment of home-cage behavior, mice were tested in the fasted state with photostimulation and in the ad libitum fed state during the light cycle in the absence of food. Trials lasted 10 min and were recorded via a CCD camera interfaced with Ethovision software for offline analysis of time spent grooming and total distance moved. Animals were run in a counterbalanced order of laser-on versus laser-off to avoid acclimation. Locomotor activity during the dark cycle was also assessed in ad libitum fed mice with and without laser stimulation, in the presence of food.

**In vivo fiber photometry.** All photometry experiments were conducted as within-subject tests, with animals tested in both the fed and fasted state. Studies were conducted in the animal’s home cage. Beginning 2 weeks after surgery (details above) mice were food-restricted to 85–90% of starting body weight. Over a 1-week period, mice were acclimated to the large and small chow pellets used in subsequent photometry experiments. Mice were habituated to the model for 1–2 days before the first recording day. In vivo fiber photometry was conducted as previously described. Fiber optic cables (1 m long, metal ferrule, 400-μm diameter; Doric Lenses) were firmly attached to the implanted fiber optic cannulae with zirconia sleeves (Doric Lenses). Laser light (473 nm) was focused on the opposite end of the fiber optic cable such that a light intensity of 0.1–0.2 mW entered the brain; light intensity was kept constant across sessions for each mouse. Emission light was passed through a dichroic mirror (Di02-R488-25x36, Semrock) and GFP emission filter (FF03-525/50-25, Semrock), before being focused onto a sensitive photodetector (2151, Newport). The signal was passed through a low-pass filter (50 Hz) and digitized with a National Instruments data acquisition card and collected using a custom Matlab script. Photobleaching over the course of each 30-min run was negligible, most likely due to the very low laser power used for excitation (0.1 mW) and the short duration of each run. Although we continued to observe clear responses to food presentation at the end of each run (in the food-restricted state), we did note an average 37% decrease between first and last responses. It is possible that minor photobleaching contributed to this effect, though it was likely predominantly due to reduced novelty of food and some level of caloric repletion.

Each 30-min session consisted of 4–6 trials of chow (14 mg pellets) or cholate (14 mg pellets) and 4–6 trials of a similarly sized nonfood object (bedding), in an alternating fashion. Large pellets (500 mg) required up to 15 min to consume and therefore only had 1–2 presentations per run, with alternating nonfood item presentation. Only one food type was used in a given run. Up to 4 runs were performed in a single day for each mouse and mice were run multiple days, with large pellet and chocolate runs never preceding small pellet runs. All trials across days (14 mg pellet: 12 ± 1 presentations per mouse; 500 mg pellet: 6 ± 0.7; chocolate: 7 ± 0.6) were pooled to calculate mean response to food/object in each mouse. After fasted runs mice were given ad libitum access to chow for 5–7 d and the above studies repeated in the fed state. Within-run responses to the same food stimulus showed a trend toward a decrease in response magnitude (decreasing by 37% from the first to the last instance of food availability; data not shown); this may reflect decreasing novelty or increasing satiety (as mice had consumed food throughout the run before the last instance of food presentation).

For data analysis, fluorescent traces were down-sampled to 1 Hz, ∆F/F (F-off/F-on, where F-off was the 20 s before food presentation) was calculated for each presentation of food. Small pellets (per 14 mg pellet: 0.01 kcal from protein, 0.007 kcal from fat and 0.03 kcal from carbohydrates; Bio-Serv). Large pellets (per 500 mg pellet: 0.38 kcal from protein, 0.25 kcal from fat and 1.18 kcal from carbohydrate;
Bio-Serv) or 14 mg chocolate (Hershey’s) or control (cob bedding of size comparable to food). In a subset of mice both time of food availability and the moment when the mouse first made contact with the food item were recorded. For analysis differentiating approach from consumption, the 10 s before food availability was compared to the time between food availability and consumption and to the 10 s following contact with the food item.

**Monosynaptic rabies mapping.** AgRP-ires-cre::RABV-gp4-TVA mice expressing the avian TVA receptor and rabies glycoprotein selectively in ARC_AgRP neurons were injected with SADΔG–EGFP (EnvA) rabies (Salk Gene Transfer Targeting and Therapeutics Core; titer 7.5 × 10⁸ infectious units per ml) unilaterally into the ARC (n = 3). Animals were allowed 6 d for retrograde transport of rabies virus and EGFP expression before they were killed for tissue collection. Sites of afferent input to ARC_AgRP neurons were assessed by the presence of EnvA–EGFP-positive neurons and the slides imaged on an Olympus VS120 slide scanner microscope.

**Rabies collateral mapping.** Three weeks after unilateral injection of AAV8-EFlc-DIO-TVA-mCherry (University of North Carolina Vector Core; titer 1.1 × 10¹² genomes per ml) into the vDMH of LepR-ires-cre mice, SADΔG–EGFP (EnvA) rabies (Massachusetts General Hospital Vector Core; titer 10⁷ infectious units per ml) was unilaterally injected into the ARC. Animals were allowed 6 d for retrograde transport of rabies virus and EGFP transgene expression before being killed for tissue collection. Comprehensive examination of SADΔG–EGFP (EnvA) axonal and retrograde transductions were obtained using 10–15 confocal images of vDMH→ARC boutons along the neuraxis using an Zeiss LSM-510 confocal microscope.

**Statistical analysis.** Statistical analyses were performed using Origin Pro 8.6 and Prism 6.0 (GraphPad) software. Details of statistical tests employed can be found in the relevant figure legends and the Supplementary Methods Checklist. Power analyses were calculated to estimate sample size using statistical conventions for 80% power, assuming an s.d. of change of 1.0, a difference between the means of 1.5-fold and an alpha level of 0.05. In all statistical tests, normal distribution and equal variance was established. The data presented met the assumptions of the statistical test employed. No randomization of animals was conducted since all behavioral tests were within-subject comparisons. Exclusion criteria for experimental animals were (i) sickness or death during the testing period or (ii) if histological validation of the injection site demonstrated an absence of reporter gene expression. These criteria were established before data collection. N numbers represent final numbers of healthy/validated animals.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Corrigendum: Dynamic GABAergic afferent modulation of AgRP neurons

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In the version of this article initially published online, ref. 7 was cited in the sentence “Furthermore, and as observed of ARC<sup>AgRP</sup> neurons<sup>7</sup>, vDMH<sup>LepR</sup> neuron fluorometric responses to chocolate were significantly greater than to similarly sized chow pellets”; this should have been ref. 8. The error has been corrected for the print, PDF and HTML versions of this article.