A role for the locus coeruleus in the modulation of feeding

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SUMMARY: Noradrenergic neurons of the locus coeruleus (LC-NE) are known to play an important role in arousal, emotion, memory and cognition. In the present study, we use fiber photometry combined with chemogenetic and optogenetic approaches to demonstrate a previously unrecognized role for LC-NE neurons in the modulation of feeding. We show that endogenous activity of LC-NE neurons is enhanced while approaching food and suppressed during feeding. These changes in LC activity during feeding behavior are attenuated as mice approach satiety, demonstrating that nutritional state modulates LC responses to food. Direct activation of LC-NE neurons results in the suppression of feeding. Further, activation of an LC projection to the lateral hypothalamus also suppresses feeding. Together, these findings demonstrate a direct causal role for LC-NE activity in the modulation of feeding.

KEYWORDS: locus coeruleus, norepinephrine, feeding, metabolism, photometry, lateral hypothalamus, optogenetics, chemogenetics, anxiety, and aversion.

INTRODUCTION

Evidence that noradrenergic neurons of the locus coeruleus (LC-NE) play a role in arousal was first established in a series of electrophysiology experiments conducted over four decades ago. These studies consistently showed that LC neurons in several different species are phasically activated during high-arousal behavioral states (e.g., orienting, waking, startle) and in response to salient sensory stimuli[1-5] (reviewed in [6,7-9]). More recent studies using opto- and chemogenetic approaches have demonstrated that LC-NE activity is both necessary and sufficient for promoting behavioral arousal, including sleep-to-wake transitions and emergence from anesthesia[10,11].

In contrast, much less is known about the contribution of LC-NE activity to low-arousal behavioral states (e.g. sleeping, grooming, feeding). One early electrophysiology study noted that LC-NE activity is transiently decreased in rats during sleeping, grooming, and drinking a sucrose reward[2]. In a second study, decreased LC activity was observed in two monkeys eating apples[12]. While these observations suggest that LC-NE activity may play a role in modulating feeding, this possibility has not previously been tested. In the present study, we used fiber photometry combined with chemogenetic and optogenetic approaches to test the hypothesis that activity of LC-NE neurons is involved in the modulation of feeding.

RESULTS

To identify the natural activity patterns of LC-NE neurons during feeding behavior, we used fiber photometry to monitor fluorescence of the calcium sensor GCaMP6f in LC-NE neurons. We selectively target these neurons by co-injecting Flp-dependent GCaMP6f and tdTomato AAVs into the LC of *Dbh*^{Flpo} mice (LC$^{GCaMP/tdT}$) **(Fig. 1a)**. To control for changes in fluorescence due to movement, GCaMP6f signal was normalized to tdTomato to yield a fluorescent ratio using a spectral unmixing approach[13]. To verify our recording conditions were sensitive to detect LC activity, we presented mice a visual flash known to activate LC

neurons[2,14]. We observed the expected increase in LC-NE activity in response to the visual stimulus **(Supplementary Fig. 1)**, validating that we can reliably detect changes in LC-NE activity in the behaving mouse.

To determine if LC activity is altered during feeding-related behaviors, $LC^{GCaMP/tdT}$ mice were fasted overnight and photometry recordings were collected during a 1-hr session while mice approached and consumed food pellets from the feeding experimentation device (FED)[15] **(Fig. 1a)**. To minimize the effects of stress and novelty during the experiments, mice were habituated for several days to eat from the FED while in the testing arena. To determine if nutritional status influences LC responses during approach and consumption, we compared LC activity early and late in the session as mice approached satiety. Assessment of approach revealed an increase in activity of LC-NE neurons during approach early in the session. This approach-related LC response was abolished later in the session when mice had consumed more food **(Fig. 1b-e)**, suggesting that energy levels modulate LC activity during appetitive behavior.

Assessment of consummatory responses revealed that LC-NE activity was rapidly suppressed during feeding **(Fig. 1b-d)**. This decrease in LC activity was observed during consumption of pellets throughout the session **(Fig. 1d)**. To determine if the transition from hunger to satiety had a gradual impact on food-related LC activity across the trial, we next performed a linear regression analysis. We found the suppression of LC-NE activity during feeding was significantly attenuated as fasted mice ate more within the trial (R^2=0.049) **(Fig. 1e),** suggesting energy levels gradually modulate LC activity during consumption. Importantly, there was no significant change in baseline LC-NE activity **(Supplementary Fig. 2)** or movement velocity **(Supplementary Fig. 3**) as mice consumed more pellets across the session. Taken together, these findings suggest nutritional state-related changes in LC-NE activity during feeding-related behavior was not driven by alterations in movement.

To determine whether activation of LC-NE neurons would directly influence feeding behavior, we employed an intersectional chemogenetic approach in which the excitatory Gq-coupled receptor hM3Dq is selectively expressed in noradrenergic neurons of the LC complex[16] (LC^{hM3Dq}) **(Fig. 2a)**. To test whether our chemogenetic strategy activates LC-NE neurons *in vivo*, we performed fiber photometry recordings in LC^{hM3Dq} mice and LC^{GFP} littermate controls following administration of clozapine N-oxide (CNO) or vehicle. Recordings revealed a sustained increase in LC-NE activity following CNO treatment in LC^{hM3Dq} mice compared to controls **(Fig. 2b)**. To compare the magnitude of LC^{hM3Dq} activation to a well-known stimulator of LC activity[17-22] and behaviors associated with stress and threat[23-25], we next administered the inhibitory Gi-coupled alpha-2 adrenergic receptor antagonist yohimbine (3 mg/kg i.p.). As expected, the "pharmacological stressor" yohimbine increased LC-NE activity relative to vehicle control **(Fig. 2b)**, and this effect was comparable in magnitude to LC^{hM3Dq} activation. Importantly, no effect of CNO or vehicle was observed in LC^{GFP} controls **(Fig. 2b**), suggesting the dynamics observed were unrelated to mouse movement. Together, the findings establish that our chemogenetic approach is effective in stimulating LC-NE activity *in vivo*.

To test if activation of LC-NE neurons would suppress feeding in hungry mice, LC^{hM3Dq} mice and littermate controls were overnight fasted and then treated with CNO or vehicle before placement in a novel arena containing standard chow **(Fig. 2c)**. In CNO-treated LC^{hM3Dq} mice, we found activation of LC-NE neurons suppressed feeding **(Fig. 2c)**. Importantly, CNO had no effect on the behavior of littermate controls **(Fig. 2c)**. These findings suggest that, despite the influence of hunger, activation of LC-NE neurons suppresses feeding in fasted mice.

To identify precisely how LC activation influences energy balance, we simultaneously measured feeding and metabolism of LC^{hM3Dq} and littermate control mice given drinking water with CNO (30 or 100 µg/mL) or plain water on alternate days **(Fig. 2d)**. In CNO-treated LC^{hM3Dq} mice, activation of LC-NE neurons dose-dependently suppressed feeding by reducing the amount of meals consumed**,** without affecting meal size **(Fig. 2d)**. Circadian analysis revealed

suppression of feeding occurred during lights-off when LC^{hM3Dq} mice drank the most CNO **(Supplementary Fig. 4).** This suppression of feeding was not observed during lights-on **(Supplementary Fig. 4)**, indicating that LC-mediated suppression of feeding was specific and reversible. Using indirect calorimetry to measure energy expenditure and respiratory exchange rate, we observed LC-NE activation had no effect on these metabolic endpoints **(Fig. 2d)**. Together these changes resulted in weight loss in CNO-treated LC^{hM3Dq} mice **(Fig. 2d)**. CNO had no effect on measures of feeding or metabolism in littermate controls **(Fig. 2d** and **Supplementary Fig. 4)**. These findings collectively demonstrate that activation of LC-NE neurons suppresses feeding without altering metabolism. Given our finding that LC activation selectively altered energy intake, in subsequent experiments we focused our observations to measures of feeding.

Since it is well known that LC-NE neurons co-express several neuropeptides[26-29], we next tested whether LC-mediated suppression of feeding depends on NE signaling. To selectively disrupt NE synthesis in LC neurons, we crossed our dopamine β-hydroxylase (*Dbh)* conditional knockout mice with $En1^{cre}$ (LC^{Dbh} mutants; unpublished reagent, NWP and PJ) **(Fig. 2e).** To increase LC activity[17-19] **(Fig. 2b)** and NE release[20-22], we pretreated LC^{Dbh} mutants and littermate controls with yohimbine (3 mg/kg i.p.) or vehicle and measured food intake. We found that baseline food intake was similar between LC^{Dbh} mutants and controls treated with vehicle **(Fig. 2f).** Food intake was significantly reduced in controls treated with yohimbine, but in contrast, yohimbine had no effect on feeding in LC^{Dbh} mutants **(Fig. 2f).** Because lack of LC-NE is the only difference between the mutants and controls, these findings suggest that LC neurons require norepinephrine for the observed suppression of feeding evoked by yohimbine. Measures of body weight revealed similar weight gain in adult LC^{Dbh} mutants and controls **(Fig. 2e)**, consistent with the full *Dbh* mutant that show deficits in NE does not produce excessive weight gain[30,31].

Prior studies have shown that NE has a strong inhibitory effect when applied directly in the lateral hypothalamus area (LHA)[32-34]. To determine if this effect is mediated by the LC, we measured Fos immunoreactivity in LC^{hM3Dq} mice following treatment of CNO or vehicle. Activation of LC-NE neurons resulted in a significant reduction in Fos expression in the LHA **(Supplementary Fig. 5a)**. To determine if this response was specific to the LHA, we measured Fos expression in two additional feeding-related targets of the LC, the dorsal medial and ventromedial hypothalamic nuclei. We observed no change in Fos expression in either nucleus **(Supplementary Fig. 5b)**.

To directly test whether enhanced activity in the LC-LHA circuit suppresses feeding, we injected AAVs expressing cre-dependent channelrhodopsin-2 (ChR2)[35,36] or eYFP in the LC of *Dbh*^{cre} mice **(Fig. 3a)**. Consistent with prior observations[37-39], we observed LC derived axons in the LHA that were labeled by eYFP **(Fig. 3b-c)**. $ChR2^{LC\text{-}LHA}$ and eYFP mice were next fasted overnight and food intake was measured in the presence or absence of photostimulation (**Fig. 3d)**. Given that high-power (20-mW total power) and high-frequency (>5 Hz) photostimulation of LC-NE soma has been shown to elicit reversible behavioral arrest[10], we used a photostimulation protocol (10-Hz, 10-ms pulses, ~7-mW total power) that has been shown to elicit high tonic rates of LC firing without behavioral arrests[38]. We revealed that $ChR2^{LC\text{-}LHA}$ mice had suppressed feeding during photostimulation compared to eYFP controls (**Fig. 3e)**. Importantly, photostimulation had no significant effect in eYFP controls and none of the manipulations affected locomotor activity (**Fig. 3e-g)**. Using this photostimulation protocol, we never observed a mouse that was immobilized in the recorded video or non-responsive to the experimenter directly after testing, confirming behavioral arrests were not observed in the present study. Furthermore, photostimulation of ChR2-expressing LHA terminals did not induce antidromic activity of LC-NE neurons as measured by Fos **(Supplementary Fig. 6).**

To determine if a shorter duration of stimulation would suppress feeding, we next used an epoch-based design wherein photostimulation was presented in alternating epochs or

withheld for the entire experiment in fasted mice (**Fig. 3h)**. When photostimulation was withheld, we observed a similar amount of feeding between ChR2$^{LC\text{-}LHA}$ and eYFP groups, wherein food intake was gradually decreased across the experiment as mice transitioned from hunger to satiety **(Fig. 3i)**. Similar feeding was also observed between ChR2$^{LC\text{-}LHA}$ and eYFP during the initial photostimulation epoch **(Fig. 3i)**, indicating stimulation of the LC-LHA pathway has no effect when hunger levels are at peak following a fast. In contrast, ChR2$^{LC\text{-}LHA}$ mice had suppressed feeding during the subsequent photostimulation epoch when hunger dampened compared to controls **(Fig. 3i-j)**. Taken together, the findings demonstrate stimulation of the LC-LHA pathway has a suppressive effect on feeding that may be modulated by hunger levels.

Since direct activation of LC-NE neurons enhances aversion and anxiety-like behaviors[16,38,40], we next tested if activation of the LC-LHA pathway would also enhanced these emotion-related responses. We therefore measured anxiety-like behavior of ChR2$^{LC\text{-}LHA}$ and eYFP mice in the open field test (OFT) and elevated plus maze (EPM) during photostimulation **(Supplementary Fig. 7a-b)**. We observed ChR2$^{LC\text{-}LHA}$ mice spent significantly less time in the center of the OFT **(Supplementary Fig. 7c)** and tended to spend less time in the open arms of the EPM **(Supplementary Fig. 7e)**, demonstrating that stimulating the LC-LHA pathway is anxiogenic. To assess if stimulation of the LC-LHA pathway has a negative or positive valence, we employed a real-time place preference test (RTPT) that triggers photostimulation upon entry into a designated side of the arena. We found ChR2$^{LC\text{-}LHA}$ mice spent less time in the stimulation-paired side compared to eYFP controls **(Supplementary Fig. 7g)**, indicating an aversive behavioral response resulting from LC-LHA circuit activation. Assessment of locomotor activity revealed ChR2$^{LC\text{-}LHA}$ mice had reduced ambulation in the OFT, but no change in the EPM and RTPT compared to eYFP control **(Supplementary Fig. 7d, f, h)**, suggesting the LC-LHA circuit does not have an overall impact on locomotion. Together, the findings demonstrate that activation of the LC-LHA pathway suppresses feeding, potentially due to an enhancement of negative valence.

To test if inhibition of the LC-LHA circuit enhances feeding, we injected AAVs expressing cre-dependent halorhodopsin (eNpHR3.0-eYFP) or eYFP in the LC of *Dbh*^{cre} mice **(Supplementary Fig. 8a-b)**. We next measured food intake in free-feeding eNpHR$^{LC\text{-}LHA}$ mice and controls during optical illumination. We observed similar food intake between eNpHR$^{LC\text{-}LHA}$ mice and eYFP controls (**Supplementary Fig. 8c)**. These findings, together with our prior optogenetic results, suggest that activation of the LC-LHA pathway suppresses feeding, but inhibition of the pathway does not promote feeding.

DISCUSSION

Collectively, we have demonstrated that endogenous activity of LC-NE neurons is suppressed during feeding in a manner modulated by nutritional state. We also found that activation of LC-NE neurons resulted in suppression of feeding through release of norepinephrine. Our experiments demonstrate that endogenous activity of LC-NE neurons is dynamically modulated during the consummatory sequence, with initial activation during food approach followed by a suppression of LC activity during consumption. This native pattern of LC-NE activity during consumption is unlikely attributed to changes in sleep, learning, stress or anxiety as our experimental mice were habituated to all aspects of the assay and they were tested during the portion of the circadian cycle when they are the most active. Instead, the approach-related LC response we observed likely reflects an appetitive response to food, in agreement with prior electrophysiological studies that show burst firing of LC neurons is associated with Pavlovian appetitive behavior in monkeys (e.g., lipping)[41]. Given LC neurons are well-known to modulate arousal[6,7,42,43], the decrease in LC activity we observed during feeding might reflect a decrease in arousal and/or disengagement with external sensory inputs to facilitate consummatory behavior.

Interestingly, we uncovered that suppression of LC-NE activity during consumption was gradually attenuated as mice approached satiety, indicating a previously unrecognized influence

of nutritional status on endogenous LC responses. Attenuation of the consummatory-related LC response could serve to direct attentional arousal from internal-to-external environment when energy levels transition from hunger to satiety, although additional research is needed to test this possibility. We also observed a loss of the approach-related LC response as hunger levels diminished, which may contribute to the decrease in salience of food as energy balance is restored. Such potential interpretation is congruent with integrative theories of LC-NE neurons which describe that this modulatory system is designed to optimize behavioral performance to a changing environment (as reviewed in [6,7,9]). These nutritional-state-related changes in LC-NE activity during feeding are not likely involved in coordinating some motor response, as locomotor activity remained stable across the trial, even as mice approached satiety (Supplementary Fig. 3).

While there have been hints that LC-NE neurons could be involved in the suppression of feeding[2,12,30,44-46], this possibility had not been directly tested. Experimentally determining a causal role for the LC-NE activity in feeding remained elusive using traditional lesion and genetic approaches. Physical lesions of the LC area had not produced a consistent change in feeding behavior[46-49], probably due to unreliable and nonselective effects. Further, although genetic ablation of dopamine beta-hydroxylase (*Dbh*) in mice had been linked to an elevation in food intake during cold stress[30], the contribution of the LC had remained unclear as the full *Dbh* knockout impacts all noradrenergic/adrenergic neurons in both the central and peripheral nervous systems. In the present study, we used chemogenetics to reveal a causal role for LC-NE activity in the suppression of feeding. Further, using our LC^{Dbh} mutants, we found that loss of LC-NE prevents the suppression of feeding evoked by yohimbine, which activated LC neurons **(Fig. 2b)**. Optogenetic stimulation of the LC→LHA pathway suppressed feeding while also enhancing aversion and anxiety-like behavior. In contrast, optogenetic inhibition of the LC-LHA pathway did not lead to an enhancement of feeding in freely feeding mice tested in a familiar cage. Together, our findings suggest that enhanced activity in LC-NE neurons suppresses

feeding but inhibition does not promote feeding. In context of the boarder LC literature, our findings suggest that LC-NE neurons are involved in the modulation of feeding by integrating both external cues (e.g., anxiogenic environmental cues) and internal drives (e.g., nutritional state).

Over four decades ago, a series of seminal studies established that direct delivery of NE agonists into the LHA suppressed feeding[50-54]. Using optogenetics, we have now revealed two previously unrecognized roles for a LC to LHA circuit: (1) the suppression of feeding and (2) enhancement of negative valence behavior (i.e., anxiety, aversion). This dual function is coherent with the well-established literature that shows LHA cell-types drive a variety of complex behaviors, including feeding, anxiety, and arousal[55-59]. An important future direction will be to determine whether the feeding and anxiety-related changes following LC-LHA pathway stimulation are mediated by separate or overlapping processes (e.g., target cell-types, receptor signaling mechanisms). Many cell-types may be involved, as prior electrophysiological studies have shown that NE has an inhibitory effect on the majority of LHA neurons[32-34,60,61].

Given the widespread projections of LC-NE neurons throughout the brain[37], it is likely that projections beyond hypothalamus are also involved in LC-NE modulation of feeding. It will be important to test this possibility using newly available tools to map, monitor and manipulate specific LC noradrenergic circuits. In sum, our findings expand upon established theories of LC function, which classically focus on arousal, emotion, memory and sleep[6,7,42,43,62], to include the modulation of feeding.

METHODS

Methods and associated references are available in the ***Supplemental information***.

SUPPLEMENTAL INFORMATION

Supplemental information can be found with this article.

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AUTHOR CONTRIBUTIONS

NRS and PJ conceived, designed and supervised the project. Photometry experiments were performed by NRS and CMM under the guidance of GC and supervision of JDC. Feeding and metabolic experiments were performed by NRS, and analyzed by CAM, JA, JMP and NRS under the guidance of AVK and MJK. Optogenetic experiments were performed by NRS under the guidance of MRB, MJK, and JDC. NP created the *Dbh*^{cre} and *Dbh*^{cKO} alleles and Flp-dependent viral vectors. IE characterized the *Dbh*^{cKO} allele. Immunohistochemistry, *in situ* hybridization, and image acquisition was performed by NRS, KGS, JA, CYK and JP. Cell counts were performed by SAF and CXY. NRS and PJ wrote the manuscript with input from coauthors.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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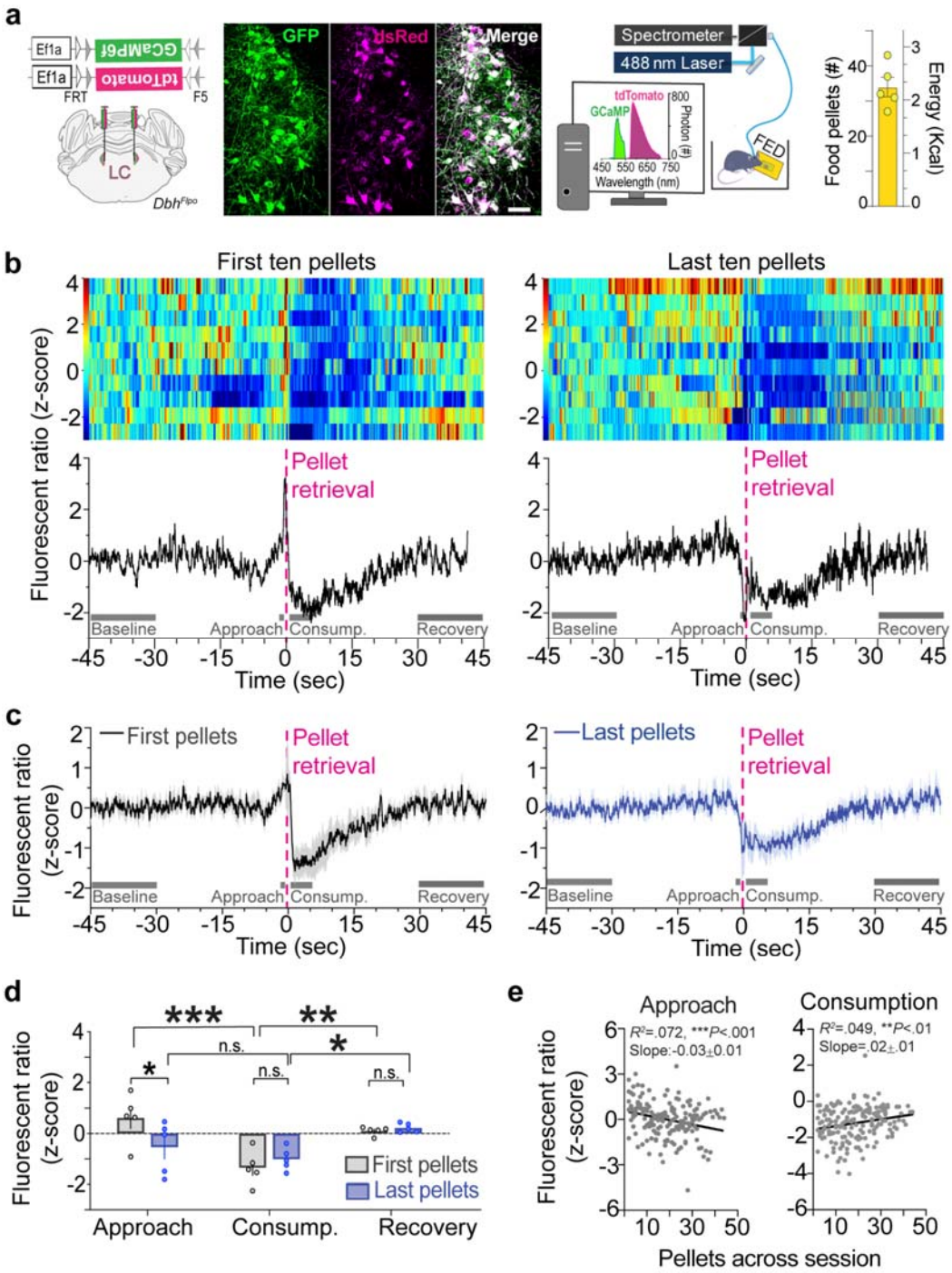

Figure 1. Activity of LC-NE neurons is increased during food approach and suppressed during feeding in a manner influenced by nutritional state. (a) *Top.* Flp-dependent viral genetic strategy for co-expression of GCaMP6f and tdTomato in LC-NE neurons. *Middle.* Coronal view of the locus coeruleus from a LC$^{GCaMP/tdT}$ mouse immunostained for GCaMP6f

(GFP antibody) and tdTomato (dsRed antibody). Schematic of *in vivo* fiber photometry setup and feeding experimentation device (FED). *Right.* Average energy intake across the 1-hr session. Data are mean ± SEM. n=5 fasted $LC^{GCaMP/tdT}$ mice. **(b)** Example response around pellet retrieval of a fasted mouse expressing a fluorescent ratio of GCaMP6f/tdTomato in LC neurons during the first ten pellets (*left*) and last ten pellets of the session (*right*). Single trials are represented in the heat map, whereas the average z-score fluorescent ratio is represented in the trace below. **(c)** Average z-score fluorescent ratio aligned-to-pellet retrieval in fasted mice during the first ten pellets (*left*) and last ten pellets of the session (*right*). Data are mean ± SEM. n=5 $LC^{GCaMP/tdT}$ mice. **(d)** Average z-score fluorescent ratio during feeding-related behaviors. Two-way repeated measures ANOVA, nutritional state x behavior interaction: $F_{2,\ 8}$= 7.018, P=0.0174. Bonferroni post-hoc test ***P<0.001, **P<0.01, and *P<0.05. Data are mean ± SEM. n=5 $LC^{GCaMP/tdT}$ mice. **(e)** Linear regression of the average fluorescent ratio during food approach and consumption in relation to pellet events across the session in $LC^{GCaMP/tdT}$ mice following an overnight fast. Approach (R^2=0.072; $F_{1,168}$=13.06, P<0.001) and Consumption (R^2=0.049; $F_{1,168}$=8.05, **P<0.01).

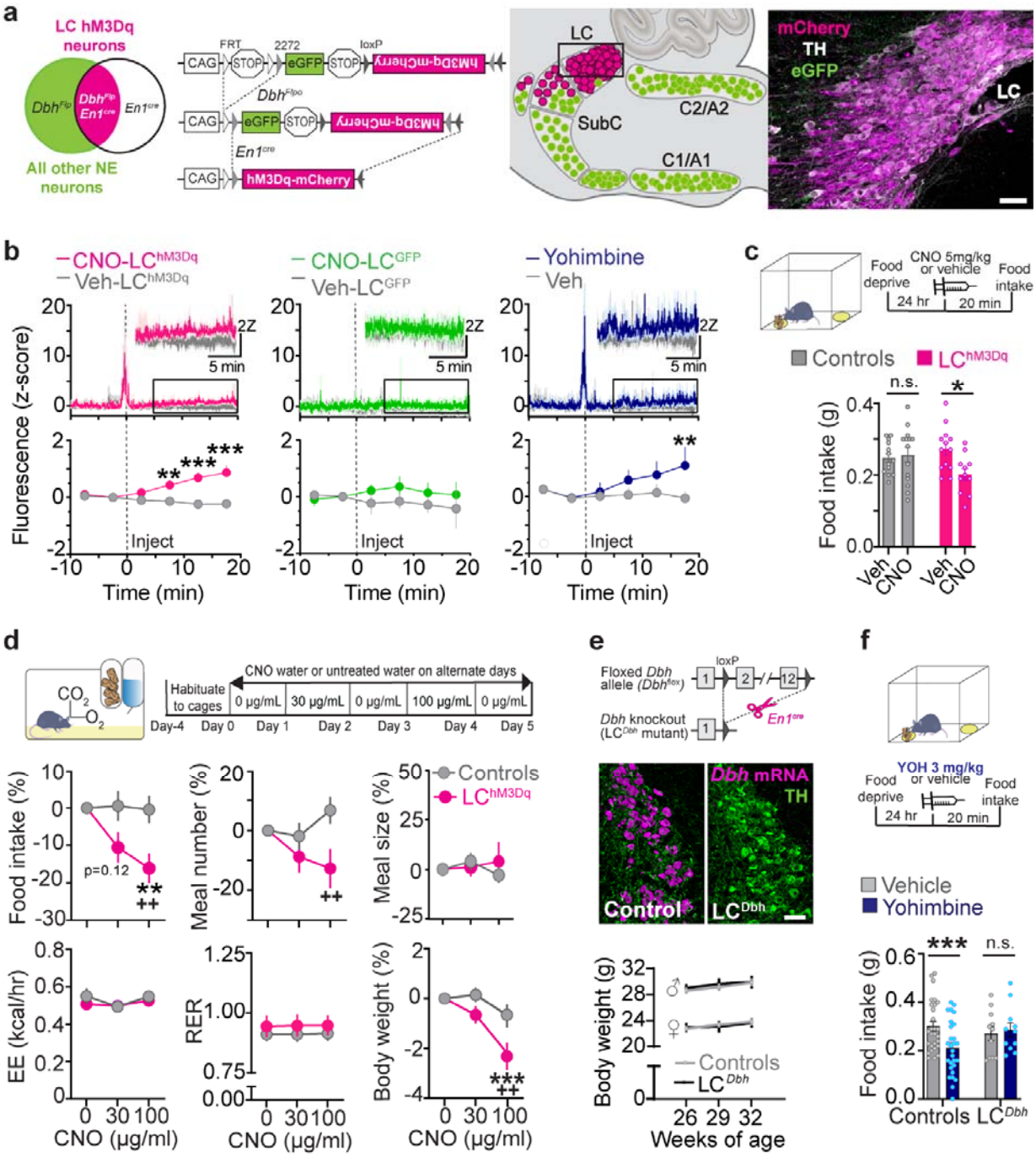

Figure 2. Activation of LC-NE neurons suppresses feeding in a manner dependent on norepinephrine signaling. (a) *Left.* Schematic illustration of intersectional genetic strategy. Recombination of *RC::FL-hM3Dq* allele by *Dbh*^{Flpo} and *En1*^{cre} results in LC-selective hM3Dq-mCherry expression. Recombination by *Dbh*^{Flpo} alone leads to eGFP expression. *Right.* Schematic shows a mouse hindbrain compressed across the sagittal axis. Parasagittal section

from LC^{hM3Dq} brain reveals hM3Dq-mCherry expression in LC-NE neurons. Scale, 50 µm. **(b)** *Top.* Average z-score fluorescence aligned to injection time in LC^{hM3Dq} mice expressing either GCaMP6f or GCaMP6f/tdTomato treated with clozapine n-oxide (CNO; magenta), yohimbine (blue) or vehicle (gray), and in LC^{GFP} mice treated with CNO (green) or vehicle (gray). In all mice, except LC^{GFP} controls, we detected a transient increase in LC-NE activity in response to an experimenter approaching for injection, demonstrating the sensitivity of our recording conditions to detect an established visual threat response characterized previously[3,4,14]. *Bottom.* Quantification of average z-score fluorescence following CNO (5 mg/kg i.p.) or yohimbine (3 mg/kg i.p.) treatment. Two-way repeated measures ANOVA, drug x time interaction: LC^{hM3Dq} ($F_{5, 35}$= 9.157, P=0.0001), LC^{GFP} ($F_{5, 20}$= 0.4994, P=0.7730), LC-Yohimbine ($F_{5, 20}$= 2.592, P=0.0579). Bonferroni post-hoc, ***P<0.01, **P<0.01 vs. vehicle, n=8 LC^{hM3Dq} mice. n=5 LC^{GFP} mice. n=5 mice for yohimbine-LC experiment. Data are mean ± SEM. **(c)** *Top.* Timeline of acute food intake experiments in fasted mice. *Bottom.* Average food intake in fasted mice. Two-way between subject's ANOVA, drug x genotype interaction: $F_{1, 47}$= 5.2, P=0.0272. Bonferroni posthoc test, *P<0.05. n=13 vehicle-treated and n=14 CNO-treated littermate controls. n=13 vehicle-treated and n=11 CNO-treated LC^{hM3Dq} mice. **(d)** *Top.* Timeline of CNO water dosing at 30 and 100 µg/mL. Note that this method delivered a daily total dose of approximately 4 or 10 mg/kg CNO. *Bottom.* Measures in the automated homecage test. Two-way repeated measures ANOVA, drug x genotype interaction: food intake ($F_{2, 54}$= 3.59, P=0.0343), meal number ($F_{2, 54}$= 3.44, P=0.0393), meal size ($F_{2,54}$= 0.864, P=0.4271), energy expenditure (EE, $F_{2, 54}$= 0.22, P=0.8036), respiratory exchange rate (RER, $F_{2,54}$= 0.0509, P=0.9504), and body weight ($F_{2, 70}$= 3.65, P=0.031). Bonferroni posthoc test, ***P<0.001, **P<0.01 vs. vehicle; $^{++}$P<0.01 vs. littermate controls. n=20 littermate controls. n=9 LC^{hM3Dq} mice for all measures (expect body weight wherein n=17 LC^{hM3Dq} mice). Data are mean ± SEM. **(e)** *Top.* Schematic diagram of *Dbh cKO* allele. Recombination by *En1*^{cre} leads to disruption of norepinephrine synthesis in LC-NE neurons. *Middle*. Coronal brain sections stained for *Dbh* (riboprobe; magenta) and tyrosine

hydroxylase (TH antibody; green). Scale, 50-µm. *Bottom.* Two-way repeated subject's ANOVA, time x genotype interaction: $F_{6,114}$= 0.77, P=0.5925. n=18-19 littermate controls, n=11-13 LC^{Dbh} mutants. Data are ± SEM. **(f)** Food intake in fasted mice treated with yohimbine (3 mg/kg, i.p.). Two-way repeated measures ANOVA, drug x genotype interaction: $F_{1,37}$= 10.8, P<0.0022. Bonferroni posthoc test, ***P<0.001 vs vehicle. n.s., non-significant. n=28 littermate controls, n=11 LC^{Dbh} mutants. Data are ± SEM.

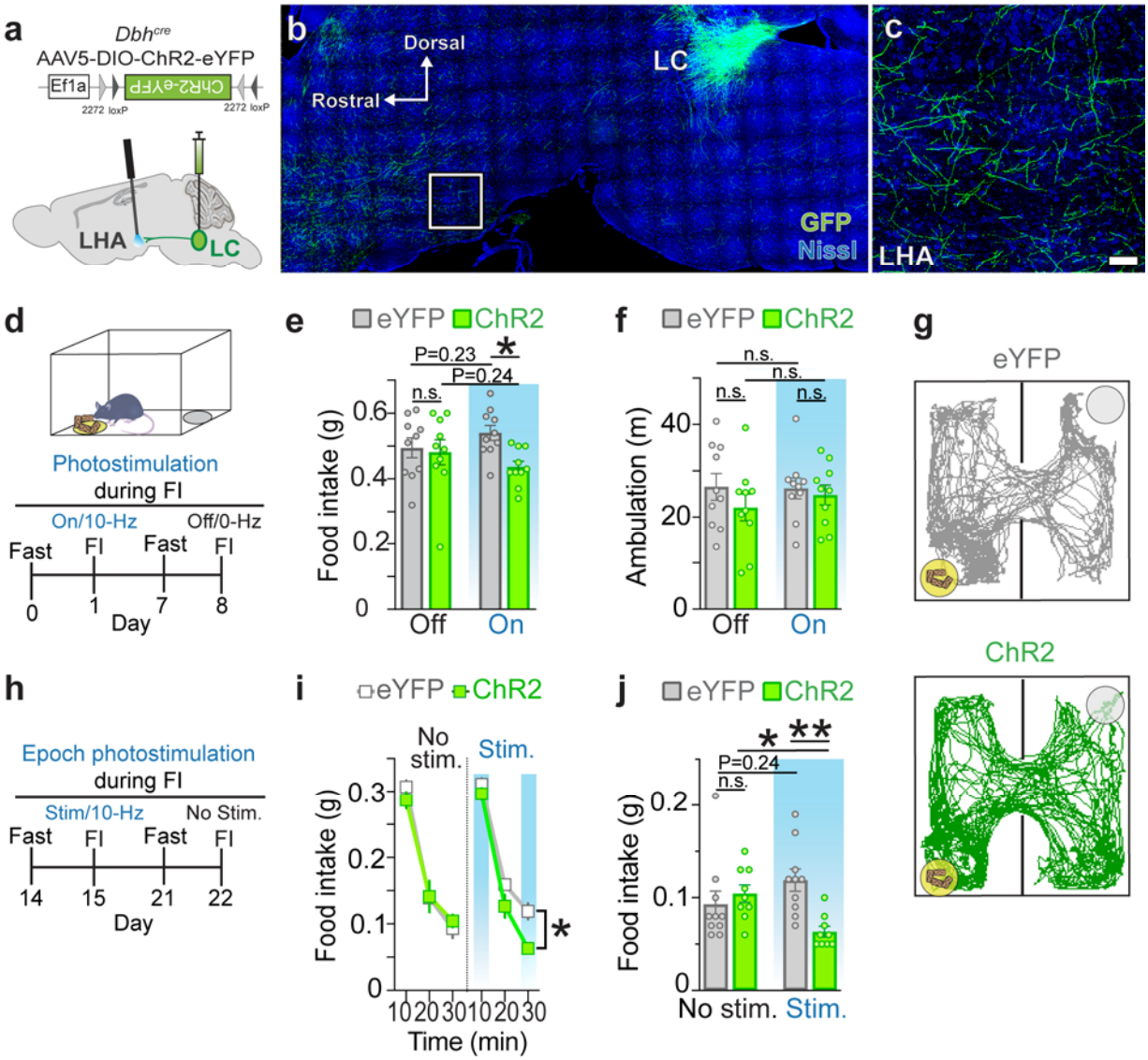

Figure 3. Stimulation of LC-LHA noradrenergic circuit suppresses feeding. (a) Schematic illustration of sagittal mouse brain shows location of cre-dependent AAV used to drive ChR2-eYFP expression and location of fiberoptic probes. **(b-c)** Parasagittal brain section from a *Dbh*^{cre}; eYFP$^{LC\rightarrow LHA}$ mouse shows restricted eYFP expression in LC-NE neurons. High magnification image shows eYFP-expressing LC-NE axonal projections in the LHA. Scale is 400-µm (brain) and 150-µm (LHA). **(d)** Timeline of photostimulation (10-Hz, 10-ms pulses) during food intake (FI) test. **(e-f)** Feeding-related behaviors in fasted mice. Two-way repeated measure ANOVA, Stimulation x Virus interaction: food intake (**e**, $F_{1,\ 18}$= 5.44, P=0.0314) and

ambulation (**f**, $F_{1, 18}$= 0.655, P=0.4290), Bonferroni posthoc test, *P<0.05. n=10 eYFP$^{LC\text{-}LHA}$ mice, n=10 ChR2$^{LC\text{-}LHA}$ mice. Data are mean ± SEM. **(g)** Representative traces shows ambulation in the feeding task during photostimulation. Yellow circle indicates the location of the food cup and gray circle indicates location of the empty cup. **(h)** Timeline of epoch photostimulation (10-Hz, 10-ms pulses) during food intake. **(i-j)** Food intake in fasted mice during entire epoch experiment (i) and last epoch bin (j). Three-way repeated measure ANOVA, Stimulation x Virus x Time interaction: (**i**, $F_{2,34}$= 3.646, P=0.037) and Time main effect (**i**, $F_{2,34}$= 295.996, P<0.001). Two-way repeated measure ANOVA, Stimulation x Virus interaction (**j**, $F_{1,17}$= 8.57, P=0.0094). Bonferroni posthoc test, **P<0.01, *P<0.05 ChR2 vs. eYFP during second photostimulation epoch. n=10 eYFP$^{LC\text{-}LHA}$ mice, n=9 ChR2$^{LC\text{-}LHA}$ mice. Data are mean ± SEM. n.s., non-significant.