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Galanin-expressing GABA neurons in the lateral hypothalamus modulate food reward and non-compulsive locomotion

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Galanin-expressing GABA neurons in the lateral hypothalamus modulate food reward and non-compulsive locomotion

Abbreviated title: Galanin-expressing LHA^{GABA} neurons in feeding behavior

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Abstract

The lateral hypothalamus (LHA) integrates reward and appetitive behavior and is composed of many overlapping neuronal populations. Recent studies associated LHA GABAergic neurons (LHA^{GABA}), that densely innervate the ventral tegmental area (VTA), with modulation of food reward and consumption; yet, LHA^{GABA}>VTA projections exclusively modulated food consumption, not reward. We identified a subpopulation of LHA^{GABA} neurons that co-express the neuropeptide galanin (LHA^{Gal}). These LHA^{Gal} neurons also modulate food reward, but lack direct VTA innervation. We hypothesized that LHA^{Gal} neurons may represent a subpopulation of LHA^{GABA} neurons that mediates food reward independent of direct VTA innervation. We used chemogenetic activation of LHA^{Gal} or LHA^{GABA} neurons in mice to compare their role in feeding behavior. We further analyzed locomotor behavior to understand how differential VTA connectivity and transmitter release in these LHA neurons influences this behavior. LHA^{Gal} or LHA^{GABA} neuronal activation both increased operant food seeking behavior, but only activation of LHA^{GABA} neurons increased overall chow consumption. Additionally, LHA^{Gal} or LHA^{GABA} neuronal activation similarly induced locomotor activity, but with striking differences in modality. Activation of LHA^{GABA} neurons induced compulsive-like locomotor behavior; while LHA^{Gal} induced locomotor activity without compulsivity. Thus, LHA^{Gal} neurons define a subpopulation of LHA^{GABA} neurons without direct VTA innervation that mediate non-compulsive food seeking behavior. We speculate that the striking difference in compulsive-like locomotor behavior is also based on differential VTA innervation. The downstream neural network responsible for this behavior and a potential role for galanin as neuromodulator remains to be identified.

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64 **Significance Statement**

65 The lateral hypothalamus (LHA) regulates motivated feeding behavior via GABAergic
66 LHA neurons. The molecular identity of LHA^{GABA} neurons is heterogeneous and largely
67 undefined. Here we introduce LHA^{Gal} neurons as a subset of LHA^{GABA} neurons that lack direct
68 innervation of the ventral tegmental area (VTA). LHA^{Gal} neurons are sufficient to drive
69 motivated feeding and locomotor activity similar to LHA^{GABA} neurons, but without inducing
70 compulsive-like behaviors, which we propose to require direct VTA innervation. Our study
71 integrates galanin-expressing LHA neurons into our current understanding of the neuronal
72 circuits and molecular mechanisms of the LHA that contribute to motivated feeding behaviors.

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Introduction

The lateral hypothalamic area (LHA) is implicated in feeding, food reward, and other motivated behaviors. Early research established that the lateral hypothalamus is necessary to maintain homeostatic feeding behaviors (Anand and Brobeck, 1951). Furthermore, rodents show impressive motivation to self-administer electric stimulations to the LHA (Olds and Milner, 1954) and the LHA is well established for its interaction with the mesolimbic dopamine reward circuitry in the brain (Leinninger et al., 2009; Peyron et al., 1998; Korotkova et al., 2003; Kempadoo et al., 2013). The LHA is a large region, with a heterogeneous molecular makeup and a diverse pattern of inputs and outputs (Swanson et al., 2005; Hahn and Swanson, 2010; Bonnavion et al., 2016) and much research has focused on defining the molecular signature of these neurons and their neural projections which regulate feeding and food reward (Berthoud and Münzberg, 2011).

Recent studies have focused on GABAergic LHA (LHA^{GABA}) neurons and associated neural circuits for their involvement in feeding and especially food reward (Nieh et al., 2015; Jennings et al., 2015). Optogenetic and chemogenetic stimulation and inhibition of LHA^{GABA} neurons had opposing effects on operant food seeking and food consumption. Interestingly, in vivo visualization of LHA^{GABA} neuronal activity highlighted that food seeking versus food consumption behaviors are represented by activation of anatomically distinct GABA populations (Jennings et al., 2015). Similarly, activation of all VTA projecting LHA neurons enhanced sucrose seeking, while selective activation of VTA projecting GABAergic LHA neurons promotes food consumption but not sucrose seeking (Nieh et al., 2015).

We recently described a subpopulation of LHA neurons that express the neuropeptide galanin (LHA^{Gal}) together with leptin receptors and neurotensin (Laque et al., 2013; Berthoud

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97 and Munzberg, 2011). These LHA^{Gal} neurons modulate food reward without promoting overall
98 food consumption (Laque et al., 2015). Also, LHA^{Gal} neurons do not project to the VTA, but
99 instead innervate the locus coeruleus and local LHA neurons (Laque et al., 2015). Galanin often
100 acts as an inhibitory neuropeptide, and many galanin neurons co-express the inhibitory
101 neurotransmitter GABA (Sherin et al., 1998; Melander et al., 1986). Thus, we hypothesized that
102 LHA^{Gal} neurons may represent a distinct subset of LHA^{GABA} neurons that selectively modulates
103 food reward, but not overall food consumption.

104 To test this hypothesis, we compared operant food seeking behavior and overall chow
105 consumption following chemogenetic activation of LHA^{Gal} neurons or LHA^{GABA} neurons. Here
106 we show that LHA^{Gal} neurons are sufficient to promote operant food seeking, while overall chow
107 consumption remained unchanged. In addition, we discovered that activation of LHA^{Gal} or
108 LHA^{GABA} neurons robustly enhanced locomotor activity, but that GABA-induced hyperactivity
109 was associated with a striking, compulsive-like behavior that was not observed with LHA^{Gal}
110 neuron-induced hyperactivity.

111 Thus, our data suggest that LHA^{Gal} neurons represent a subpopulation of LHA^{GABA}
112 neurons that promote food reward independent of direct VTA innervation, while VTA
113 innervating LHA^{GABA} neurons may be required to induce overall chow consumption.
114 Furthermore, we show that targeting exclusively galanin-expressing LHA neurons attenuates
115 compulsive-like locomotor behavior, while maintaining food seeking behavior. The clear
116 behavioral distinction of molecularly defined subsets of LHA^{GABA} neurons could have important
117 implications for treatment strategies of feeding and psychiatric disorders.

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Materials and Methods

Animals.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center. Mice were bred and housed at 22^{0}C on a 12-hour light/dark cycle. Food and water were available ad libitum unless otherwise specified. *Gal*^{Cre} mice were generated in house as previously described (Laque et al., 2015) and *Vgat*^{Cre} mice were generously provided by Dr. Bradford B. Lowell, Beth Israel Deaconess Medical Center and Harvard Medical School, MA (Vong et al., 2011). Experimental animals were bred and used from homozygous breeding colonies. *Gal*^{TgGFP}/ *Vgat*^{Tom} mice were generated by crossbreeding *Gal*^{TgGFP} mice (Stock Tg(*Gal*-EGFP)109Gsat, #0163420UCD), Mutant Mouse Regional Resource Center; described detailed in (Laque et al., 2013), with *Vgat*^{Cre} mice and Rosa-tomato$^{f/f}$ mice (B6.Cg-Gt(ROSA)26Sor<tm14(CAG-tdTomato)Hze>/J, Jackson Laboratory, stock #007914) and were used for histology and cell count experiments as hemizygous *Gal*^{TgGFP}, double heterozygous *Vgat*^{Tom} mice.

Genotyping.

Genotype for experimental animals was determined by PCR using the following primers: *Gal*^{Cre}: 5'-CCT CTC CAC CCA AGC GGC CGG AGA ACC-3' (fwd-cre), 5'-CCG GCT CCG TTC TTT GGT GGC CCC TTC GCG-3' (rev-cre); 5'-TCC TGA GAC CAT GTC CAC TG-3' (fwd- wildtype), 5'-CTG CCA CTC CTG TGA TCT GA-3' (rev- wildtype); *Vgat*^{Cre} : 5'-CTT CGT CAT CGG CGG CAT TCT G-3'(fwd), vGatIRESSR4, 5'-CAG GGC GAT GTG GAA TAG AAA-3' (rev-wildtype), CreFR1, 5'-ATC GAC CGG TAA TGC AGG CAA-3' (rev-cre);

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Gal^{TgGFP} : 5'-TAT ATC ATG GCC GAC AAG CA-3' (fwd), 5'-GAA CTC CAG CAG GAC CAT GT-3' (rev); Rosa-tomato$^{f/f}$: 5'-AAG GGA GCT GCA GTG GAG TA-3' (fwd-wildtype), 5'-CCG AAA ATC TGT GGG AAG TC-3' (rev-wildtype), 5'-GGC ATT AAA GCA GCG TAT CC-3' (fwd- loxP), 5'- CTG TTC CTG TAC GGC ATG G-3' (rev-loxP).

Stereotaxic Surgery.

Viral injections were done as described previously (Rezai-Zadeh et al., 2014); briefly, mice were anesthetized using inhaled isofluorane (2% isofluorane/1L O2/min) and mounted into a stereotaxic frame (Stereotaxic Alignment System, Kopf Instruments) to enhance anatomically reproducible injections into the LHA (coordinates ±0.9mm lateral, -1.35mm caudal and -5.4mm ventral to BREGMA based on the Paxinos mouse brain atlas (Paxinos and Franklin, 2004). Either AAV5-hSyn-DIO-hM3Dq-mCherry (DREADD-Gq, titer $6x10^{12}$ cfu/ml) or AAV5-hSyn-DIO-mCherry (control, titer $6x10^{12}$ cfu/ml) were injected bilaterally (200nl per site at a rate of 20nl/30sec) into the LHA. Guide cannula and injector remained in place for 5min following injection to allow for diffusion into the parenchyma and to prevent backflow up the cannula tract. Skull access holes were filled using bone wax (Lukens, #901) and the incision was closed with stainless steel wound clips (CellPoint Scientific, #203-1000) and post-operative care was given (Bupivicaine/lidocaine: 2.5-12.5mg/kg once at incision; carprofen: 5mg/kg once daily for 72hr; once warm saline i.p). Mice recovered for 2-3 weeks to allow sufficient viral expression before further experimental testing.

Two cohorts of virus-expressing mice were generated. Cohort 1 had exclusively male mice (*Gal*^{Cre}: n=4 control, n=9 DREADD-Gq; *Vgat*^{Cre}: n=4 control, n=8 DREADD-Gq virus),

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Cohort 2 had mixed male and female mice (Gal^{Cre}: control virus, n=3 males, 3 females; DREADD-Gq virus, n=4 males, 5 females).

Behavioral Experiments.

Clozapine-N-Oxide (CNO) Dose Selection. Initial repeatable measurements such as locomotor activity, energy expenditure, and food intake were performed with multiple doses of the designer drug CNO (ligand for the DREADD-Gq receptor) to identify the most efficient CNO dose. Non-repeatable behavioral experiments (operant conditioning, ensure intake, marble-burying) were performed with a single CNO dose (1mg/kg), which has been also used by several other studies (Jennings et al., 2015; Nakajima et al., 2016). For LHA^{Vgat} operant testing experiments 1mg/kg CNO induced excessive compulsive behaviors (gnawing) in all animals and interfered with the mouse's ability to perform the operant task. Thus, all LHA^{Vgat} mice were tested at multiple CNO doses (0.1-1mg/kg) to identify individual CNO doses where mice were able to properly perform the operant task. For the marble burying which can be only performed once we chose the lowest CNO dose (0.1mg/kg) tested, to minimize excessive compulsive behavior.

Metabolic parameters. Animals from cohort 1 were adapted to metabolic chambers (TSE system) for 1 week before data were collected for locomotor activity (beam breaks) and oxygen consumption (VO2 ml/kg/h) in 30min intervals. For stimulation experiments animals were i.p. injected with PBS or CNO (0.1mg/kg or 1mg/kg) at approximately 1000h.

Locomotor activity was also measured with subcutaneously implanted telemetric radio transmitters (G2 E-Mitter, Starr Life Sciences) and detected with individual receivers (Series 4000, Starr Life Sciences) and recorded with VitalView software (Starr Life Sciences).

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Telemetric radio transmitters were surgically implanted on the dorsal surface of the mouse in the interscapular area. Mice recovered for at least 1 week prior to measurement of locomotor activity via telemetry. Measurements occurred in home cage and for neuronal stimulation experiments mice were i.p. injected with PBS or CNO (0.1mg/kg or 1mg/kg, i.p.) at approximately 1000h and locomotor activity was recorded continuously (every minute) for 24h.

Motivated food intake: DREADD-Gq animals from cohort 1 (n=8 per genotype) were food restricted during the training phase of the experiment. This greatly facilitates operant conditioning learning by encouraging exploratory behavior and increasing motivation to work for food. Food restriction started 2 weeks prior to the operant training session with to 2.5-3.5g/day standard laboratory chow (Lab diet 5001) (distributed at 1200h) until animals reached 90% of their baseline body weight. Operant conditioning chambers (Med Associates) were approximately 15.9 cm x 14.0 cm x 12.7 cm, with a house light, wire bar floor, two lighted nose poke holes with infrared beams, and a pellet dispenser which released reward pellets into a hopper located between the nose poke holes. Mice were acclimated to sucrose reward pellets in their home cage two days prior to operant training to prevent neophobia. Training occurred in four steps with illuminated nose poke holes marking the "correct" (rewarded) hole: Step 1) autoshaping: 1 reward per nose poke, both holes are marked correct; Step 2) fixed ratio 1 (FR1): 1 reward per 1 correct nose poke, only one hole is marked correct; Step 3) fixed ratio 5 (FR5): 1 reward per 5 consecutively correct nose pokes, only one hole is marked correct; Step 4) progressive ratio (PR): 1 reward per increasing consecutive correct nose pokes on an exponential scale (i.e. 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118…) (Richardson and Roberts, 1996). Autoshaping was performed for one day in a 30 minute session to acclimate the mice to the chambers and reinforce nose poke behavior; FR1, FR5, and PR training were each performed

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daily for 5-7 days until mice achieved stable performance (≤10% variation in number of correct nose pokes and a 3:1 ratio of correct: incorrect responses over a 3d period). Fixed ratio training consisted of 30min sessions and progressive ratio sessions were 60min or ended when the mouse did not complete any nose poke for a 5min period (break point). Once training was completed, mice were returned to ad libitum feeding until returning to baseline bodyweight. PR testing occurred at 1200h during the light cycle. Stimulation of virally infected neurons was done with i.p. injections of PBS or CNO at doses from 0.05-1 mg/kg body weight 1h prior to PR testing. Data used for analysis included the total number of correct nose pokes (collectively leading to reward), total number of incorrect nose pokes (not leading to reward), and total number of rewards achieved during the PR testing session.

Palatable food intake: Mice from cohort 1 had access to a liquid palatable diet (Ensure, 97kcal/ml) for 1h daily over 5 days. On day 4 and 5, mice received a counterbalanced i.p. injections of either PBS or CNO (0.1 or 1mg/kg body weight) 1h prior to Ensure access; intake (g) was measured following each training or testing session.

Chow intake: Intake (g) of standard laboratory chow (Purina 5001) was measured in metabolic cages (TSE Systems) and analyzed as cumulative food intake for 3h and 24h following i,p. injection of either PBS or CNO (dose range of 0.1-1mg/kg body weight).

Marble burying test: Standard mouse cages were filled with fresh bedding to a depth of 5 cm and 16 marbles (~1cm diameter) were evenly spaced across the bedding. *Vgat*^{Cre} mice from cohort 1 (n=4 control, n=8 DREADD-Gq) and *Gal*^{Cre} mice from cohort 2 (n=6 control, n=9 DREADD-Gq) were placed in the cage for 30min and allowed to ambulate freely. Injection of CNO (1mg/kg in *Gal*^{Cre} mice, 0.1mg/kg in *Vgat*^{Cre} mice) was administered 1h prior to beginning

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the test. Please note, the marble burying test in part reflects the animal's response to a novel environment and is therefore only testable once per animal. Thus, in this test we compared DREADD animals to a control virus group also treated with CNO. A repeated PBS control injection would have confounded the novelty of the marble burying test. A photograph was taken of the cage before and after the mouse was in the cage and data were analyzed to determine the percentage of marbles buried while the mouse occupied the cage. Image J (Abramoff et al., 2004) was used to quantify the visible marble area in each photograph prior to and after the test. Data were expressed as % area buried after testing time to reflect changes in compulsive-like behaviors (Angoa-Perez et al., 2013).

Perfusion and Immunohistochemistry.

Two hours prior to perfusion, mice were injected with CNO (0.1mg/kg or 1mg/kg, i.p.) for later histochemical confirmation of CNO-induced neural activation as indicated by induction of the early response gene cFos (Rezai-Zadeh et al., 2014; Yu et al., 2016). Mice were deeply anesthetized and transcardially perfused with ice-cold saline followed by 10% formalin. Brains were harvested, postfixed in 10% formalin for 24 hours, and then transferred into 30% sucrose for cryopreservation until sectioning into 4 representative series (30µm per section) throughout the brain using a sliding microtome. One (out of four) series of brain sections was processed using free-floating immunohistochemistry. Sections were pretreated with 1% H2O2 in methanol, 0.3% glycine, and 0.03% SDS, before blocking in 3% normal donkey serum, followed by incubation in primary antibody, goat anti-c-Fos, (1:200; Santa Cruz). Staining was developed with the VECTASTAIN Elite ABC kit (Vector Laboratories) and 3,3'-diaminobenzidine

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tetrahydrochloride kit (Metal-Enhanced DAB Substrate Kit, Life Technologies) according to the manufacturers specification. After that mCherry was further stained with rabbit anti-dsRed (1:1000, Clontech) and detected with fluorescent-labeled secondary antibody (1:200, Alexa Fluor 594 donkey anti-rabbit, Thermofisher Scientific). Brains from Gal^{TgGFP}/ $Vgat^{Tom}$ mice (n=4) were stained for GFP using, chicken anti-GFP (1:1000, Abcam) and detected with fluorescent-labeled secondary antibody (1:200, Alexa Fluor 488 donkey anti-chicken, Jackson ImmunoResearch Laboratories) and tdTomato was stained for with rabbit anti-dsRed (1:1000, Clontech) which was detected with fluorescent-labeled secondary antibody (1:200, Alexa Fluor 594 donkey anti-rabbit, Thermofisher Scientific).

Cell counts.

For quantification of colocalization of galanin and Vgat (marker for GABA), we counted the number of galanin neurons which were colocalized with Vgat. Within the LHA, galanin is concentrated around the fornix (perifornical area - PFA), and also in the anterior lateral LHA (indicated as LHA). We sampled both of these regions for cell counting which are located in two anatomical sections rostrocaudally. Images of LHA and PFA regions were collected using a Leica SP5 confocal using 488 nm and 594 nm lasers in sequential scanning mode. HyD detector gain was fixed for all samples, z-section thickness was fixed at 2.6 um per step, zoom was fixed at 1.4 and the objective used was a 20x 0.70 NA dry. Brightness and contrast were modified with identical settings (Adobe Photoshop, Adobe Systems) between brains to better visualize GFP and tdTomato staining. We first counted the total galanin-GFP expressing neurons, and performed a second count of galanin-GFP neurons also expressing tdTomato.

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Data analysis.

Statistical differences were analyzed using SigmaPlot (Systat Software, Inc). Analysis included paired and independent t-test, two-way ANOVA, and two-way repeated measures ANOVA as further indicated in according figure legends. For data sets that did not follow a normal distribution, non-parametric analysis were applied using the Mann-Whitney Rank Sum Test. A p-value <0.05 was considered statistically significant and denoted as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Results

Galanin neurons in the lateral hypothalamus are partially GABAergic

The neuropeptide galanin acts mostly inhibitory and galanin-expressing neurons are often GABAergic. In order to directly address the molecular identity of LHA^{Gal} neurons we generated double reporter mice (Gal^{TgGFP}/ $Vgat^{Tom}$ mice, n=4, 1 males, 3 females) with transgenic GFP expression in galanin neurons (Laque et al., 2013) and conditional tdTomato expression in vesicular GABA Transporter (Vgat)-positive neurons (Vong et al., 2011). We found that about half of LHA (45% ± 9%) and about 30% ± 6% of perifornical area (PFA) galanin neurons were GABAergic neurons and that GABAergic galanin neurons represent a small fraction of overall LHA^{GABA} neurons (Fig. 1A-F). Importantly, LHA^{GABA} neurons heavily innervate the VTA (Nieh et al., 2015), while LHA^{Gal} neurons do not directly project to the VTA (Laque et al., 2015). Instead, LHA^{Gal} neurons heavily innervate LHA orexin neurons and the noradrendergic locus

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coeruleus (Laque et al., 2015; Goforth et al., 2014). These results indicate that LHA^{Gal} neurons represent a GABAergic subpopulation with unique molecular identity and previous work also indicates a distinct projection profile that can be distinguished from the larger heterogeneous population of GABAergic neurons in the LHA.

Chemogenetic activation of LHA^{Gal} neurons enhances food seeking behavior

Recent data have highlighted the entire population of LHA^{GABA} neurons as an important regulator of feeding behavior, emphasizing the LHA^{GABA} > VTA projection as particularly important in motivated feeding behaviors. Thus, here we tested if activation of non-VTA projecting LHA^{Gal} neurons would be sufficient to modulate sucrose seeking behavior.

We first targeted DREADD-Gq or control virus into the LHA of *Gal*^{Cre} mice (LHA^{Gal} DREADD-Gq), which allowed us to selectively activate LHA^{Gal} neurons with systemic CNO injections. Viral expression was visualized via the fluorescent marker mCherry to verify correct targeting throughout the LHA area (Fig. 2A, B). We verified neuronal stimulation capability of DREADD-Gq histologically with CNO-induced cFos expression in mCherry positive neurons, which was prominent in the perifornical area (Fig. 2C, left image) and extended through the LHA with some cFos positive neurons in the zona incerta (ZI), while mice injected with control virus (LHA^{Gal} Control) showed little cFos in mCherry+ neurons (Fig. 2C, right image).

To quantify sucrose seeking behavior we trained the mice for operant responding with palatable sucrose pellets (Fig. 2D) and to perform increasing numbers of nose pokes in order to receive a sucrose pellet (PR testing). After stable PR performance we activated LHA^{Gal} neurons with systemic CNO (1mg/kg) compared to PBS injected mice. Activation of LHA^{Gal} neurons

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317 significantly increased the total number of sucrose rewards earned (Fig. 2E, paired *t* test, n=8, $t_{(7)}$
318 = -2.78, p <0.05) and the total number of correct nose pokes was likewise increased following
319 CNO injections (Fig. 2F, paired *t* test, n=8, $t_{(7)}$ = -2.58, p <0.05). However incorrect nose pokes
320 (no sucrose reward, Fig. 2G) were not changed, indicating a true increase of motivated behavior
321 (sucrose seeking), rather than increased impulsive responding.

322 The operant driven PR task focuses on active food seeking and less on food consumption.
323 In order to contrast food seeking with food consumption we exposed animals to liquid Ensure (a
324 palatable complete nutrition beverage) for 1h over 5 days, CNO or PBS injections were
325 administered during the last 2 days in DREADD-Gq expressing mice and mice that received a
326 control virus injection. However, palatable food consumption remained unchanged by activation
327 of LHA^{Gal} neurons (Fig. 2H). This suggests that activation of LHA^{Gal} neurons specifically
328 enhances the motivational aspect of food seeking rather than consumption of palatable food.

329 We further wanted to distinguish feeding of palatable food which is mainly driven by a
330 hedonic mechanism from feeding of regular chow which is mainly driven by homeostatic
331 mechanisms. This was done in metabolic chambers to allow continuous (24h) recording of food
332 intake following CNO (0.3-1mg/kg) and PBS injections in control and LHA^{Gal} DREADD-Gq
333 mice. CNO-induced activation of LHA^{Gal} neurons did not change 3h or 24h chow intake (Fig.
334 2I). We also measured the amount of time that the mice spent in proximity to the feeding
335 container (labeled as food zone, Fig. 2J) over the 3h following CNO or PBS injections, but the
336 CNO did not affect the time spent in the food zone of the cage (Fig. 2K).
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Chemogenetic activation of LHAGal neurons increases locomotor activity and energy expenditure

LHA^{GABA} neurons have also been implicated in the regulation of arousal and wakefulness, and we therefore implanted mice with telemetric radio transmitters to assess the effect of galanin neuron stimulation on locomotor activity. Animals were implanted with telemetric radio transmitters for minute-by-minute measurement of locomotor activity. LHA^{Gal} DREADD-Gq and control mice were injected with PBS or CNO (1mg/kg, I.P.). Indeed CNO robustly induced locomotor activity that peaked at 1h post-injection and returned to the baseline activity levels about 3 hrs post-injections (Fig. 3A). Cumulative locomotor activity (over the first 3h post-injection) showed a significant 2.5-fold increase in locomotor activity in CNO injected LHA^{Gal} DREADD-Gq mice, but not in control mice (Fig. 3B, LHA^{Gal} Control, n = 4, LHA^{Gal} DREADD-Gq, n = 9, ANOVA for the interaction between treatment and group: $F_{(1,22)} = 5.071$ $p < 0.05$).

We also measured locomotor activity by beam breaks in the TSE system which measures 3D movements including up-and-down rearing, in contrast to the telemetric measurements that exclusively captures 2D movements in the cage. We confirmed an increase in locomotor activity following CNO injection which returned to PBS baseline levels at 3h post injection (Fig. 3C, n = 4 LHA^{Gal} Control, n = 9 LHA^{Gal} DREADD-Gq, repeated measures ANOVA for the interaction between group and interval, $F_{(7,77)} = 6.494$; $p < 0.001$). Comparing cumulative locomotor activity we found a doubling of locomotor activity in LHA^{Gal} DREADD-Gq mice at all tested CNO doses compared to control mice (Fig. 3D, repeated measures ANOVA for the interaction between group and level of drug, $F_{(3,33)} = 4.584$; $p < 0.01$).

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We further analyzed VO_2 consumption in these mice as locomotor activity drives VO_2 consumption as a measure for energy expenditure. CNO consistently increased VO_2 mildly compared to PBS treated mice (Fig. 3E, F), but only reached significance at a CNO dose of 1mg/kg (Fig. 3F, repeated measures ANOVA for the interaction between group and level of drug, $F_{(3,33)} = 4.413$, $p<0.01$). This further suggests that increased locomotor activity may also induce locomotion with less effects on oxygen consumption, e.g. like head movements. Thus, activation of LHA^{Gal} neurons showed a clear increase in locomotor activity that is in line with increased wakefulness during the light cycle, where nocturnal mice are typically asleep. Indeed, while CNO injected LHA^{Gal} DREADD-Gq animals were awake and engaged in various behavior, control or PBS injected animals were often asleep. Notably, activation of LHA^{Gal} neurons did not induce compulsive-like behavior, in contrast to the compulsive eating or foraging locomotor behavior induced by activation of LHA^{GABA} neurons as reported by others (Venner et al., 2016; Jennings et al., 2015).

Chemogenetic activation of LHA^{GABA} neurons increases food seeking and consumption behavior

In order to allow a more precise comparison of LHA^{GABA} neurons to LHA^{Gal} neurons, we performed chemogenetic activation of LHA^{GABA} neurons using the exact experimental setup as employed for chemogenetic activation of LHA^{Gal} neurons.

LHA^{GABA} neurons were targeted by viral injections into *Vgat*^{Cre} mice (Vong et al., 2011) using the same stereotaxic coordinates as described for *Gal*^{Cre} mice, with correct viral targeting to the LHA validated histochemically (Fig. 4A-C). Functional validation was confirmed by

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comparing CNO-induced cFos in mCherry+ neurons in LHA^{Vgat} DREADD-Gq mice with LHA^{Vgat} Control animals. Only LHA^{Vgat} DREADD-Gq mice showed robust CNO-induced cFos in mCherry+ neurons, while CNO injected control mice showed only few cFos+ neurons (Fig. 4B-C.).

Food seeking behavior in LHA^{Vgat} DREADD-Gq mice was examined using the same operant conditioning schedule as for *Gal*^{Cre} mice (Fig. 2D). Initially, when testing operant responding with a CNO dose of 1mg/kg, all mice exhibited an erratic, compulsive-like locomotor behavior (see below) and failed to perform any significant number of nose pokes (data not shown). In an effort to moderate this compulsive-like locomotor behavior we tested all animals with decreasing CNO doses and measured PR responding for all animals. In 7 out of 8 mice we found a CNO dose that increased operant responding, however, the CNO dose sufficient to enhance operant responding and low enough to moderate compulsive locomotor activity was highly variable, possibly due to minute differences in viral expression levels and anatomical locations. In order to visualize CNO-induced operant behavior, we plotted the maximal CNO-induced operant response for each animal in Figure 4D and E. Note that the individual data in Figure 4E and D were induced by varying CNO doses, for clarity we have color coded the individual response data for the used CNO-dose in the figure legend. With this analysis we confirm earlier reports (Jennings et al., 2015) and show that activation of LHA^{GABA} neurons increased total number of sucrose rewards earned (Fig. 4E, paired *t* test, n=8, $t_{(7)} = -2.73$, $p < 0.05$) and correct nose pokes (Fig. 4E, paired *t* test, n=8, $t_{(7)} = -2.75$, $p < 0.05$), but did not alter the number of incorrect nose pokes (not leading to sucrose pellet, Figure 4F). Neither CNO dose induced incorrect nose pokes, indicating that the erratic behavior did not induce impulsive responding but rather prevented the animals to engage in the learned task at all.

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Activation of LHA^{GABA} neurons did not change hedonic food consumption in a paradigm with 1h access to liquid ensure (Fig. 4G), but chow consumption over 3h but not 24h was increased by CNO (Fig. 4H, n=4 LHA^{Vgat} Control, n=8 LHA^{Vgat} DREADD-Gq, for 0.1mg/kg CNO - $U_{(3,7)} = 3.0$, $T = 13.0$, $p<0.05$; for 0.3mg/kg CNO - $U_{(3,7)} = 4.0$, $T = 14.0$, $p<0.05$; 3h chow intake at 1mg failed to reach significance - $U_{(3,7)} = 6.0$, $T = 16.0$, $p = 0.10$). Note, that these data were generated using decreasing doses of CNO (0.1-1mg/kg, I.P.) as used for LHA^{Gal} neuronal activation, again to moderate compulsive locomotor behavior. There was high variability among individual mice in this experiment, with several of the mice demonstrating more robust increases in food intake during the 3h period following CNO injection. Despite the increase in food intake, there was no increase in time spent in the food zone during the 3h following CNO injection for the LHA^{Vgat} DREADD-Gq mice (Fig. 4I).

Chemogenetic activation of LHA^{GABA} neurons increases locomotor activity more robustly than activation of LHA^{Gal} neurons

We further measured locomotor activity following CNO injection in LHA^{Vgat} DREADD-Gq and LHA^{Vgat} Control mice using telemetry in their home cages and beam breaks in metabolic chambers. We found both measures showed a strong increase in locomotor activity which continued to be elevated for approximately 6h following injection. Using telemetric monitoring of locomotor activity, LHA^{Vgat} DREADD-Gq and LHA^{Vgat} Control mice were injected with PBS or CNO (0.1mg/kg, I.P.). We observed a CNO-induced increase in locomotor activity that peaked at 1h post-injection until 5 hours post-injection before returning to the baseline activity levels (Fig. 5A). Cumulative locomotor activity (over the first 3h post-injection) was measured during the 3h following CNO or PBS injection showing a significant 2.5-fold increase in locomotor activity in LHA^{Vgat} DREADD-Gq mice, but not in LHA^{Vgat} Control mice (Fig. 5B,

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LHA^{Vgat} Control, n = 4, LHA^{Vgat} DREADD-Gq, n =8, ANOVA for the interaction between treatment and group: $F_{(1,20)} = 18.663$, $p < 0.001$). These data were similar to locomotor activation of LHA^{Gal} neurons. Contrarily, locomotor activity measured by beam breaks (adding up-and-down movements like rearing) in the TSE system reflected a stronger locomotor behavior after LHA^{GABA} neuron activation compared to activation of LHA^{Gal} neurons. Locomotor activity increased quickly following CNO injection and returned to PBS baseline levels at 6h post injection (Fig. 5C, LHA^{Vgat} Control, n = 4, LHA^{Vgat} DREADD-Gq, n = 8, repeated measures ANOVA for the interaction between group and interval, $F_{(8,80)} = 2.771$; $p < 0.01$). Comparing cumulative increases (sum of beam breaks during 3h following injection) in locomotor activity, LHA^{GABA} neuronal activation caused a 4-fold increase in locomotor activity at multiple CNO dosages (Fig. 5D, LHA^{Vgat} Control, n = 4, LHA^{Vgat} DREADD-Gq, n = 8, repeated measures ANOVA for the interaction between group and level of drug, $F_{(3,47)} = 8.389$; $p<0.001$), in contrast to the 2-fold induction after LHA^{Gal} neurons activation (Fig. 3D).

Energy expenditure (VO_2) also increased with activation of LHA^{GABA} neurons, which extended approximately 4h following CNO injection (Fig. 5E, LHA^{Vgat} Control, n = 4, LHA^{Vgat} DREADD-Gq, n = 8, repeated measures ANOVA for the interaction between group and interval, $F_{(8,80)} = 4.428$; $p < 0.001$). Mean VO_2 was likewise increased following CNO injection at each dosage of drug tested (0.1-1mg/kg), (Fig. 5F, LHA^{Vgat} Control, n = 4, LHA^{Vgat} DREADD-Gq, n = 8, repeated measures ANOVA for the interaction between group and level of drug, $F_{(3,30)} = 12.026$, $p<0.001$). LHA^{GABA} neuronal activation induced a stronger and more prolonged effect on locomotor activity and VO_2 than did activation of LHA^{Gal} neurons, suggesting that galanin neurons in the LHA represent a molecularly distinct subset of the overall GABA population in

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451 the LHA. It may also suggest a role for a different transmitter system which coexpresses with
452 GABA, but is distinct from galanin neurons.
453

454 **LHA^{GABA} neurons, but not LHA^{Gal} neurons, induce compulsive-like behavior**

455 LHA^{Vgat} DREADD-Gq mice showed CNO-induced hyperactive compulsive-like behavior
456 (i.e. digging in bedding, gnawing on inedible objects in the cage); and was clearly distinct from
457 CNO-induced locomotor activity in LHA^{Gal} DREADD-Gq mice. To further examine the nature
458 of the hyperactive behavior following LHA^{GABA} neuronal activation, we employed the marble
459 burying test. The marble burying test is a measure of stereotypical behavior and is accepted as an
460 indicator of psychiatric conditions such as anxiety and obsessive-compulsive disorder. Rodent
461 behavior in the marble burying test can be manipulated with common pharmacological
462 modulators of these conditions such as anxiolytics and 5-HT compounds (Deacon, 2006;Angoa-
463 Perez et al., 2013). LHA^{Gal} DREADD-Gq and LHA^{Vgat} DREADD-Gq mice demonstrated a
464 striking difference in CNO-induced marble burying behavior. While activation of LHA^{Gal}
465 neurons (CNO dose1mg/kg) resulted in significantly less buried marbles than control mice (Fig.
466 6A and C, n= 6 LHA^{Gal} Control, n = 9 LHA^{Gal} DREADD-Gq, $T_{(13)} = 4.126$, $p<0.001$), activation
467 of LHA^{GABA} neurons (CNO dose 0.1mg/kg) resulted in significantly more buried marbles than
468 control mice (Fig. 6B and C, n= 4 LHA^{Vgat} Control, n = 8 LHA^{Vgat} DREADD-Gq, $T_{(10)} = -2.273$,
469 $p<0.05$). These results provide strong support for a physiological distinction between LHA^{Gal}
470 and the larger LHA^{GABA} neuron population.
471
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Discussion

The LHA modulates food reward and consumption behaviors; however, the exact role of anatomically and molecularly distinct regions of LHA neurons and their contribution to these behaviors still remain unclear. Our data confirm that LHA neurons and specifically LHA^{GABA} neurons modulate food reward and consumption behaviors. In addition, we distinguish the larger population of LHA^{GABA} neurons from LHA^{Gal} neurons, which represents a subpopulation of LHA^{GABA} neurons that does not innervate the VTA directly. Overall our data demonstrate that activation of LHA^{Gal} neurons is sufficient to enhance the motivation to work for food and thus does not require direct VTA innervation. In contrast, activation of LHA^{Gal} neurons did not modulate overall food consumption and thus may require direct VTA interaction. This further suggests that VTA projecting LHA^{GABA} neurons may mediate the homeostatic food consumption independently of motivational food seeking. While our study did not directly address the possible role of the neuropeptide galanin *per se*, our study is the first to use genetically targeted approaches to investigate the contribution of LHA^{Gal} circuitry to modulate behavioral output.

Modulation of food seeking versus food consumption

Recent studies demonstrated that LHA^{GABA} neurons are potent modulators of feeding (Jennings et al., 2015; Nieh et al., 2015; Barbano et al., 2016) where activation of overall LHA^{GABA} neurons induced food reward and consumption (Jennings et al., 2015), while select activation of LHA^{GABA} > VTA circuits exclusively induced food consumption, but not food reward (Nieh et al., 2015). These data suggested that non-VTA projecting LHA^{GABA} neurons are responsible to mediate food reward. We show that activation of LHA^{Gal} neurons is sufficient to

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selectively enhance food reward, but not food consumption. Collectively our data provide evidence that non-VTA projecting LHA^{Gal} neurons represent LHA^{GABA} neurons that mediate food reward behavior and can be contrasted to VTA projecting LHA^{GABA} neurons that promote food consumption.

It should be noted that LHA^{Gal} neurons strongly innervate LHA orexin neurons as well as noradrenergic locus coeruleus neurons (Laque et al., 2015), which modulate both goal directed and motivated behavior (Harris et al., 2005; Hofmeister and Sterpenich, 2015). Orexin mediated food reward involves at least in part projections to the VTA (Zheng et al., 2007; Peyron et al., 1998; Korotkova et al., 2003; Kempadoo et al., 2013), nevertheless, the dense orexin input to the locus coeruleus that regulates arousal (Bourgin et al., 2000) could certainly contribute to the overall effect on motivated behavior and food reward and will need to be addressed in future studies.

We also tested palatable food intake after stimulation of either LHA^{Gal} or LHA^{GABA} neurons, but we were unable to confirm increased consumption of palatable, fat-rich Ensure solution with LHA^{GABA} neurons (Jennings et al., 2015). However, due to the large consumption of Ensure (2-3ml) per test session, we cannot rule out that this is due to a ceiling effect that prevents detection of increased consumption. Together our data suggest that motivated behavior and overall chow intake is regulated by distinct LHA neuronal populations and that motivated feeding does not require direct interaction with VTA neurons.

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LHA^{Gal} and LHA^{GABA} neuronal activation both increase locomotor activity, with differing behavioral characteristics

Chemogenetic activation of both LHA^{Gal} and LHA^{GABA} neurons robustly increased locomotor activity which was most pronounced in the LHA^{GABA} DREADD-Gq mice. Locomotor behavior was not evaluated in recent studies using chemo- and optogenetic methods of LHA^{GABA} (Jennings et al., 2015; Nieh et al., 2015). Locomotor activity may depend on a variety of behavioral changes. While physical activity and exercise is tightly bound to changes in energy expenditure, other locomotor behaviors may rather indicate arousal, grooming, anxiety, as well as compulsive behaviors. Yet, routine locomotor assessment (beam breaks, telemetry) does not reflect the cause for changes in locomotor activity.

Indeed, activation of LHA^{Gal} or LHA^{GABA} neurons similarly induced locomotor activity, even though we observed visually distinct behavior. Activation of LHA^{Gal} neurons increased a range of typical mouse behavior like grooming, digging in the bedding, and walking with frequent transitions from one behavior to the other. In contrast, activation of GABA neurons resulted in repetitive behavior that was dependent on the environment (e.g. availability of marbles), but was characterized by compulsive maintenance of one behavior over a long time (e.g. digging, gnawing).

We speculate that the difference in locomotor behaviors is also based on the presence of LHA>VTA projections that seem to promote compulsive-like locomotor behavior, while the absence of LHA>VTA projections diminish compulsive locomotor behavior. This hypothesis is further supported by numerous publications linking increased dopamine release in the nucleus accumbens to obsessive compulsive and repetitive behavior as well as overall locomotor activity

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(Berridge et al., 2005; Molloy and Waddington, 1987; White et al., 1988; Colle and Wise, 1991; Kim et al., 2015; Aliane et al., 2009). Indeed, chemogenetic activation of GABAergic LHA neurotensin neurons that predominantly act via VTA projections induced dopamine dependent locomotor activity (Patterson et al., 2015) and also increases food reward behavior (Kempadoo et al., 2013; Leinninger et al., 2011; Opland et al., 2013), even though compulsivity was not tested in these studies.

Increased locomotor activity may also reflect arousal as recently suggested by data showing that LHA^{GABA} neurons innervate the ventral medial preoptic area (VLPO) (Venner et al., 2016). Future studies will have to dissect if LHA^{Gal} >orexin versus LHA^{GABA}>VLPO circuits contribute to different aspects of arousal and locomotor activity and if this may also differentially effect repetitive compulsive behavior. Several studies mentioned induction of compulsive-like behaviors following chemogenetic or optogenetic activation of LHA^{GABA} or LHA^{GABA} >VTA projections (Venner et al., 2016; Nieh et al., 2015; Navarro et al., 2016), so that an LHA>VTA mediated compulsive behavior is strongly suggested.

Anatomical considerations and other neurotransmitters

Our data collected from the *Gal*^{TgGFP}/ *Vgat*^{Tom} mice show that only half of LHA^{Gal} neurons co-express GABA, so that other neurotransmitters and/or neuropeptides may contribute to the observed effects. LHA^{Gal} and LHA^{GABA} neurons do not express hypocretin/orexin (Hcrt/Ox) or melanin-concentrating hormone (MCH), but do innervate Hcrt/Ox neurons (Laque et al., 2015; Jennings et al., 2015). A portion of LHA^{Gal} neurons co-express neurotensin and

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559 cocaine-and-amphetamine-regulated-transcript (CART), as well as the long form leptin receptor
560 (LepRb) (Laque et al., 2013); which are all mediators of anorexigenic action.

561 Our previous work suggested that LepRb expressing LHA^{Gal} neurons mediate food
562 reward via inhibitory inputs to Hcrt/Ox (Laque et al., 2015), either via inhibitory GABA or
563 galanin signaling (Goforth et al., 2014). However, activation of LHA^{Gal} neurons induced food
564 reward so that indirect actions of GABAergic LHA^{Gal} neurons are likely. Indeed, activation of
565 LHA^{Gal} or LHA^{Vgat} both seemed to induce cFos in hcrt/ox neurons (data not shown), suggesting
566 indirect actions– involving disinhibition via interneurons or feed-forward mechanisms via effects
567 on the first order projection sites in other brain regions (e.g. LC or VTA). We further observed
568 strong cFos induction in non-hcrt/ox neurons, with some of these likely reflecting primary
569 activated LHA^{Gal} or LHA^{Vgat} neurons. However, given the extent of cFos-positive non-hcrt/ox
570 neurons we cannot rule out that other, unidentified LHA populations are equally involved in
571 mediating reward behavior.

572 Thus, the role of Hcrt/Ox neurons to mediate LHA^{Gal} signals requires further
573 investigation. LHA^{Gal} neurons, similar to Hcrt/Ox neurons, strongly innervate the locus
574 coeruleus (LC) (Laque et al., 2015; Carter et al., 2012). The Hcrt/Ox > LC axis mediates arousal
575 (Carter et al., 2012) and the LC is generally associated in the modulation of goal directed
576 behavior (Usher et al., 1999; Bouret and Richmond, 2015). Thus further studies will have to
577 clarify if the LC is a relevant mediator of the observed effects in food reward and locomotor
578 activity.

579 Recent studies have shown that within the LHA^{GABA} population, discrete groups of
580 GABA neurons are differentially activated during select feeding behaviors (Jennings et al.,

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581 2015); suggesting the existence of functionally distinct neuronal populations. Indeed, discrete
582 populations of LHA^{GABA} >VTA terminals respond differentially to varying degrees of
583 optogenetic parameters such as laser frequency and duration (Barbano et al., 2016). While
584 LHA>VTA projections generally enhanced food reward and consumption, LHA^{GABA} >VTA
585 projection selectively modulated food consumption, indicating that non-VTA projecting
586 LHA^{GABA} neurons are required for LHA^{GABA} induced food reward.

587 Our data indicate that LHA^{Gal} neurons represent these non-VTA projecting LHA^{GABA}
588 neurons that selectively modulate food reward. Furthermore, activation of LHA^{GABA} neurons, but
589 not LHA^{Gal} neurons, induced compulsive locomotor activity. We further propose that
590 compulsive locomotor behavior require LHA^{GABA} innervation of the VTA, which is supported by
591 Nieh and colleagues (2015) reporting that activation of LHA^{GABA} > VTA terminals increased
592 maladaptive gnawing behavior (Nieh et al., 2015).

593

594 In summary, we show that LHA^{Gal} neurons represent a population of LHA^{GABA} neurons
595 that do not directly innervate the VTA and are sufficient to modulate food reward. We propose
596 that the effect of LHA^{Gal} stimulation on motivated behavior relies on direct or indirect
597 projections to Hcrt/Ox and/or the locus coeruleus (Fig. 7). Further, LHA^{Gal} neurons are able to
598 induce motivated feeding and locomotor activity without changes in daily caloric intake or
599 compulsive/repetitive behaviors, which could have important implications for treatment
600 strategies for feeding disorders and compulsive behaviors.

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603 **Reference List**

604

605 Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image Processing with ImageJ. pp 36-42.

606 Aliane V, Perez S, Nieoullon A, Deniau JM, Kemel ML (2009) Cocaine-induced stereotypy is
607 linked to an imbalance between the medial prefrontal and sensorimotor circuits of the basal
608 ganglia. Eur J Neurosci 30:1269-1279.

609 Anand BK, Brobeck JR (1951) Hypothalamic control of food intake in rats and cats. Yale J Biol
610 Med 24:123-140.

611 Angoa-Perez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM (2013) Marble burying and
612 nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. J Vis Exp50978.

613 Barbano MF, Wang HL, Morales M, Wise RA (2016) Feeding and Reward Are Differentially
614 Induced by Activating GABAergic Lateral Hypothalamic Projections to VTA. J Neurosci
615 36:2975-2985.

616 Berridge KC, Aldridge JW, Houchard KR, Zhuang X (2005) Sequential super-stereotypy of an
617 instinctive fixed action pattern in hyper-dopaminergic mutant mice: a model of obsessive
618 compulsive disorder and Tourette's. BMC Biol 3:4.

619 Berthoud HR, Munzberg H (2011) The lateral hypothalamus as integrator of metabolic and
620 environmental needs: from electrical self-stimulation to opto-genetics. Physiol Behav 104:29-39.

621 Bonnavion P, Mickelsen LE, Fujita A, de LL, Jackson AC (2016) Hubs and spokes of the lateral
622 hypothalamus: cell types, circuits and behaviour. J Physiol 594:6443-6462.

623 Bouret S, Richmond BJ (2015) Sensitivity of locus ceruleus neurons to reward value for goal-
624 directed actions. J Neurosci 35:4005-4014.

625 Bourgin P, Huitron-Resendiz S, Spier AD, Fabre V, Morte B, Criado JR, Sutcliffe JG, Henriksen
626 SJ, de LL (2000) Hypocretin-1 modulates rapid eye movement sleep through activation of locus
627 coeruleus neurons. J Neurosci 20:7760-7765.

628 Carter ME, Brill J, Bonnavion P, Huguenard JR, Huerta R, de LL (2012) Mechanism for
629 Hypocretin-mediated sleep-to-wake transitions. Proc Natl Acad Sci U S A 109:E2635-E2644.

630 Colle LM, Wise RA (1991) Circling induced by intra-accumbens amphetamine injections.
631 Psychopharmacology (Berl) 105:157-161.

632 Deacon RM (2006) Digging and marble burying in mice: simple methods for in vivo
633 identification of biological impacts. Nat Protoc 1:122-124.

JNeurosci Accepted Manuscript

634 Goforth PB, Leinninger GM, Patterson CM, Satin LS, Myers MG, Jr. (2014) Leptin acts via
635 lateral hypothalamic area neurotensin neurons to inhibit orexin neurons by multiple GABA-
636 independent mechanisms. J Neurosci 34:11405-11415.

637 Hahn JD, Swanson LW (2010) Distinct patterns of neuronal inputs and outputs of the
638 juxtaparaventricular and suprafornical regions of the lateral hypothalamic area in the male rat.
639 Brain Res Rev 64:14-103.

640 Harris GC, Wimmer M, Aston-Jones G (2005) A role for lateral hypothalamic orexin neurons in
641 reward seeking. Nature 437:556-559.

642 Hofmeister J, Sterpenich V (2015) A Role for the Locus Ceruleus in Reward Processing:
643 Encoding Behavioral Energy Required for Goal-Directed Actions. J Neurosci 35:10387-10389.

644 Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, Veleta K, Kantak PA,
645 Aita M, Shilling-Scrivo K, Ramakrishnan C, Deisseroth K, Otte S, Stuber GD (2015) Visualizing
646 hypothalamic network dynamics for appetitive and consummatory behaviors. Cell 160:516-527.

647 Kempadoo KA, Tourino C, Cho SL, Magnani F, Leinninger GM, Stuber GD, Zhang F, Myers
648 MG, Deisseroth K, de LL, Bonci A (2013) Hypothalamic neurotensin projections promote
649 reward by enhancing glutamate transmission in the VTA. J Neurosci 33:7618-7626.

650 Kim IH, Rossi MA, Aryal DK, Racz B, Kim N, Uezu A, Wang F, Wetsel WC, Weinberg RJ, Yin
651 H, Soderling SH (2015) Spine pruning drives antipsychotic-sensitive locomotion via circuit
652 control of striatal dopamine. Nat Neurosci 18:883-891.

653 Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE (2003) Excitation of ventral
654 tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci
655 23:7-11.

656 Laque A, Yu S, Qualls-Creekmore E, Gettys S, Schwartzenburg C, Bui K, Rhodes C, Berthoud
657 HR, Morrison CD, Richards BK, Munzberg H (2015) Leptin modulates nutrient reward via
658 inhibitory galanin action on orexin neurons. Mol Metab 4:706-717.

659 Laque A, Zhang Y, Gettys S, Nguyen TA, Bui K, Morrison CD, Muenzberg-Gruening H (2013)
660 Leptin receptor neurons in the mouse hypothalamus are co-localized with the neuropeptide
661 galanin and mediate anorexigenic leptin action. Am J Physiol Endocrinol Metab.

662 Leinninger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, Wilson H, Opland DM,
663 Faouzi MA, Gong Y, Jones JC, Rhodes CJ, Chua S Jr, Diano S, Horvath TL, Seeley RJ, Becker
664 JB, Munzberg H, Myers MG, Jr. (2009) Leptin acts via leptin receptor-expressing lateral
665 hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. Cell
666 Metab 10:89-98.

JNeurosci Accepted Manuscript

667 Leinninger GM, Opland DM, Jo YH, Faouzi M, Christensen L, Cappellucci LA, Rhodes CJ,
668 Gnegy ME, Becker JB, Pothos EN, Seasholtz AF, Thompson RC, Myers MG, Jr. (2011) Leptin
669 action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy
670 balance. Cell Metab 14:313-323.

671 Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986)
672 Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine,
673 GABA and neuropeptides in the rat CNS. J Neurosci 6:3640-3654.

674 Molloy AG, Waddington JL (1987) Pharmacological characterization in the rat of grooming and
675 other behavioural responses to the D1 dopamine receptor agonist R-SK&F 38393. J
676 Psychopharmacol 1:177-183.

677 Nakajima K, Cui Z, Li C, Meister J, Cui Y, Fu O, Smith AS, Jain S, Lowell BB, Krashes MJ,
678 Wess J (2016) Gs-coupled GPCR signalling in AgRP neurons triggers sustained increase in food
679 intake. Nat Commun 7:10268.

680 Navarro M, Olney JJ, Burnham NW, Mazzone CM, Lowery-Gionta EG, Pleil KE, Kash TL,
681 Thiele TE (2016) Lateral Hypothalamus GABAergic Neurons Modulate Consummatory
682 Behaviors Regardless of the Caloric Content or Biological Relevance of the Consumed Stimuli.
683 Neuropsychopharmacology 41:1505-1512.

684 Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA, Wichmann R, Neve R, Wildes
685 CP, Tye KM (2015) Decoding neural circuits that control compulsive sucrose seeking. Cell
686 160:528-541.

687 Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area
688 and other regions of rat brain. J Comp Physiol Psychol 47:419-427.

689 Opland D, Sutton A, Woodworth H, Brown J, Bugescu R, Garcia A, Christensen L, Rhodes C,
690 Myers M, Jr., Leinninger G (2013) Loss of neurotensin receptor-1 disrupts the control of the
691 mesolimbic dopamine system by leptin and promotes hedonic feeding and obesity. Mol Metab
692 2:423-434.

693 Patterson CM, Wong JM, Leinninger GM, Allison MB, Mabrouk OS, Kasper CL, Gonzalez IE,
694 Mackenzie A, Jones JC, Kennedy RT, Myers MG, Jr. (2015) Ventral tegmental area neurotensin
695 signaling links the lateral hypothalamus to locomotor activity and striatal dopamine efflux in
696 male mice. Endocrinology 156:1692-1700.

697 Paxinos G, Franklin KBJ (2004) The Mouse Brain in Stereotaxic Coordinates. Elsevier Science
698 (USA).

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699 Peyron C, Tighe DK, van den Pol AN, de LL, Heller HC, Sutcliffe JG, Kilduff TS (1998)
700 Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci
701 18:9996-10015.

702 Rezai-Zadeh K, Yu S, Jiang Y, Laque A, Schwartzenburg C, Morrison CD, Derbenev AV,
703 Zsombok A, Munzberg H (2014) Leptin receptor neurons in the dorsomedial hypothalamus are
704 key regulators of energy expenditure and body weight, but not food intake. Mol Metab
705 2014/10/30:681-693.

706 Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration
707 studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66:1-11.

708 Sherin JE, Elmquist JK, Torrealba F, Saper CB (1998) Innervation of histaminergic
709 tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic
710 nucleus of the rat. J Neurosci 18:4705-4721.

711 Swanson LW, Sanchez-Watts G, Watts AG (2005) Comparison of melanin-concentrating
712 hormone and hypocretin/orexin mRNA expression patterns in a new parceling scheme of the
713 lateral hypothalamic zone. Neurosci Lett 387:80-84.

714 Usher M, Cohen JD, Servan-Schreiber D, Rajkowski J, Aston-Jones G (1999) The role of locus
715 coeruleus in the regulation of cognitive performance. Science 283:549-554.

716 Venner A, Anaclet C, Broadhurst RY, Saper CB, Fuller PM (2016) A Novel Population of
717 Wake-Promoting GABAergic Neurons in the Ventral Lateral Hypothalamus. Curr Biol 26:2137-
718 2143.

719 Vong L, Ye C, Yang Z, Choi B, Chua S Jr, Lowell BB (2011) Leptin action on GABAergic
720 neurons prevents obesity and reduces inhibitory tone to POMC neurons. Neuron 71:142-154.

721 White FJ, Bednarz LM, Wachtel SR, Hjorth S, Brooderson RJ (1988) Is stimulation of both D1
722 and D2 receptors necessary for the expression of dopamine-mediated behaviors? Pharmacol
723 Biochem Behav 30:189-193.

724 Yu S, Qualls-Creekmore E, Rezai-Zadeh K, Jiang Y, Berthoud HR, Morrison CD, Derbenev AV,
725 Zsombok A, Munzberg H (2016) Glutamatergic Preoptic Area Neurons That Express Leptin
726 Receptors Drive Temperature-Dependent Body Weight Homeostasis. J Neurosci 36:5034-5046.

727 Zheng H, Patterson LM, Berthoud HR (2007) Orexin signaling in the ventral tegmental area is
728 required for high-fat appetite induced by opioid stimulation of the nucleus accumbens. J
729 Neurosci 27:11075-11082.

730

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Figure Legends

Figure 1. Galanin neurons in the lateral hypothalamus coexpress GABA.

A, B. Overview of galanin- (green) and Vgat (red)-expressing neurons in the hypothalamus of Gal^{TgGFP}/ $Vgat^{Tom}$ mice (scale bar = 200µm). **C, D.** Confocal image of the lateral hypothalamus depicting the perifornical area (PFA, C.) and the lateral hypothalamus dorsal of the PFA(D) in Gal^{TgGFP}/ $Vgat^{Tom}$ mice. Sampled regions are indicated by white boxes in overview images A and B. Galanin-expressing neurons are shown in green (middle image) and Vgat- expressing neurons are shown in red (right image). The merged image (left image) shows co-localization of galanin- and Vgat-expressing neurons (yellow, open arrow), even though several non-Vgat expressing galanin neurons (green, striped arrow) and non-galanin-expressing Vgat neurons (red, filled arrow) were also present. **E, F.** Estimates of cell counts for total galanin ^{GFP} neurons and double-labeled galanin^{GFP}/tdTomato neurons in the LHA and PFA of Gal^{TgGFP}/ $Vgat^{Tom}$ mice expressed as number of cells (E) or as percentage of total galanin neurons (F) (n = 4). Mean ± SEM. Third ventricle (3V), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC), ventromedial hypothalamus (VMH), fornix (fx), lateral hypothalamus (LHA).

Figure 2. Activation of LHA^{Gal} neurons increases motivated feeding behavior.

A. Illustration of viral injection sites to the LHA in Galanin^{Cre} mice with AAV5-hSyn-DIO-hM3Dq-mCherry (DREADD-Gq) or AAV5-hSyn-DIO-mCherry (control). **B.** Representative overview of hM3Dq-mCherry expression in the LHA of Galanin^{Cre} mice. **C.** Representative images of cFos (green) induction in mCherry (red) neurons following CNO (1mg/kg) in LHA^{Gal} DREADD-Gq (left panel) and LHA^{Gal} Control (right panel) mice. **D.** Experimental procedure scheme for operant conditioning using fixed ratio (FR) and progressive ratio (PR) training. **E-G.**

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In the operant conditioning PR-test, LHA^{Gal} DREADD-Gq mice injected with CNO (1mg/kg, i.p.) significantly increases the total number of sucrose pellet rewards earned (**E.**) and total number of correct nose pokes (**F.**), while the total number of incorrect nose pokes was unchanged (**G.**) compared to PBS injected LHA^{Gal} DREADD-Gq mice. **H.** Total 1h intake of palatable fat solution (Ensure) was unchanged in CNO (1mg/kg) injected LHA^{Gal} DREADD-Gq or LHA^{Gal} Control mice. **I.** Acute (3h) chow intake was unchanged in CNO (0.3-1mg/kg, i.p.) injected LHA^{Gal} DREADD-Gq or LHA^{Gal} Control mice. Also 24h chow intake (inset, 1mg/kg CNO) was unchanged. **J, K.** In the food zone experiment (**J.**) we confirmed that CNO (1mg/kg) had no effect on the time spent in the food zone during 3h post-injections (**K.**). **E-G**, $*p < 0.05$, n = 8, Mean ± SEM, paired t-test. **H-K**, n = 4 control, n = 9 DREADD-Gq, Two-way ANOVA or Two-way repeated measures ANOVA for dose response experiments, Third ventricle (3V), median eminence (ME), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC), ventromedial hypothalamus (VMH), fornix (fx), lateral hypothalamus (LHA).

Figure 3. Activation of LHA^{Gal} neurons increases locomotor activity

A. Locomotor activity before and after injection of PBS or CNO (1mg/kg) in LHA^{Gal} DREADD-Gq mice (n = 9) as measured by telemetry in 1min increments. **B.** Cumulative locomotor activity (3hrs post-injection, measured by telemetry) was significantly increased following CNO (1mg/kg) injection in LHA^{Gal} DREADD-Gq mice (n = 9), but not in LHA^{Gal} Control mice (n = 4) (two-way ANOVA, pairwise comparisons by Holm-Sidak method). **C.** Locomotor activity as measured by beam breaks was increased in LHA^{Gal} DREADD-Gq mice following CNO (1mg/kg) injection compared to LHA^{Gal} Controls. **D.** Different CNO doses (0.3-1mg/kg, i.p.) were tested for CNO-induced locomotor activity measured by beam breaks and no reduction in

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777 behavioral effects was observed with the tested doses. **E, F.** Oxygen consumption measurement
778 in metabolic chambers showed only mild effects of CNO (1mg/kg)-induced locomotor activity
779 on energy expenditure (**E.**), that was significant only at the highest CNO-dose tested (1mg/kg)
780 (F). C. - F. (n = 4 LHA^{Gal} Control, n = 9 LHA^{Gal} DREADD-Gq), two-way repeated measures
781 ANOVA, pairwise comparisons by Holm-Sidak method. All data are represented as mean ±
782 SEM, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.
783
784 **Figure 4.** Activation of LHA^{GABA} neurons increases motivated feeding and chow intake.
785 **A.** Illustration of viral injection sites to the LHA in *Vgat*^{Cre} mice with AAV5-hSyn-DIO-hM3Dq-
786 mCherry (DREADD-Gq) or AAV5-hSyn-DIO-mCherry (control). **B.** Representative overview of
787 hM3Dq-mCherry expression in the LHA of *Vgat*^{Cre} mice. **C.** Representative images of cFos
788 (green) induction in mCherry (red) neurons following CNO (1mg/kg) in DREADD-Gq (left
789 panel) and control (right panel) injected *Vgat*^{Cre} mice. **D-F.** In the operant conditioning PR-test,
790 LHA^{Vgat} DREADD-Gq mice injected with CNO (0.05-0.2mg/kg, i.p.) significantly increases the
791 total number of sucrose pellet rewards earned (**D.**) and total number of correct nose pokes (**E.**)
792 (CNO dose-response experiment was performed and data are presented as maximal effective
793 CNO dose per individual, green line = 0.05mg/kg, blue = 0.1mg/kg, red = 0.2mg/kg), while the
794 total number of incorrect nose pokes was unchanged (**F.**) compared to PBS injected LHA^{Vgat}
795 DREADD-Gq mice. **G.** Total 1h intake of palatable fat solution (Ensure) was unchanged in CNO
796 (0.1mg/kg) injected LHA^{Vgat} DREADD-Gq or LHA^{Vgat} Control mice. **H.** Acute (3h) chow intake
797 was increased in CNO (0.1 and 0.3mg/kg, i.p.) injected LHA^{Vgat} DREADD-Gq compared to
798 LHA^{Vgat} Control mice and PBS injected LHA^{Vgat} DREADD-Gq mice. However, 24h chow intake
799 (inset, 0.1mg/kg CNO) was unchanged. **I.** In the food zone experiment, we confirmed that CNO

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(0.1mg/kg) had no effect on the time spent in the food zone during 3h post-injections (**K.**). **D-F**, *$p < 0.05$, n = 8, Mean ± SEM, paired t-test. **G-I**, n = 4 LHA^{Vgat} Control, n = 8 LHA^{Vgat} DREADD-Gq, Two-way ANOVA or Mann-Whitney Rank Sum Test for dose response experiments, Third ventricle (3V), median eminence (ME), dorsomedial hypothalamus (DMH), arcuate nucleus (ARC), ventromedial hypothalamus (VMH), fornix (fx), lateral hypothalamus (LHA).

Figure 5. Activation of LHA^{GABA} neurons robustly increases locomotor activity. **A.** Locomotor activity before and after injection of PBS or CNO (1mg/kg) in LHA^{Vgat} DREADD-Gq mice (n = 8) as measured by telemetry in 1min increments. **B.** Cumulative locomotor activity (3hrs post-injection, measured by telemetry) was significantly increased following CNO (0.1mg/kg) injection in LHA^{Vgat} DREADD-Gq mice (n = 8), but not in LHA^{Vgat} Control mice (n = 4) (two-way ANOVA, pairwise comparisons by Holm-Sidak method). **C.** Locomotor activity as measured by beam breaks was increased in LHA^{Vgat} DREADD-Gq mice following CNO (1mg/kg) injection compared to LHA^{Vgat} Controls. **D.** Different CNO doses (0.1-1mg/kg, i.p.) were tested for CNO-induced locomotor activity measured by beam breaks and no reduction in behavioral effects was observed with the tested doses. **E, F.** Oxygen consumption measurement in metabolic chambers showed significant effects of CNO (0.1mg/kg)-induced locomotor activity on energy expenditure (**E.**), that was significant at all CNO doses tested (0.1-1mg/kg)(**F.**). **C. - F**. (n = 4 LHA^{Vgat} Control, n = 8 LHA^{Vgat} DREADD-Gq), two-way repeated measures ANOVA, pairwise comparisons by Holm-Sidak method. All data are represented as mean ± SEM, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

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Figure 6. Activation of LHA^{Gal} neurons reduces compulsive burying, in contrast, LHA^{GABA} activation increases burying.

A. Marble burying behavior following CNO injection (1mg/kg) in LHA^{Gal} DREADD-Gq mice (n = 9) is decreased compared to LHA^{Gal} Control mice (n = 6). **B.** Marble burying behavior following CNO injection (0.1mg/kg) LHA^{Vgat} DREADD-Gq mice (n = 8) is increased compared to LHA^{Vgat} Control mice (n = 4). All data are represented as mean ± SEM, paired t-test, *p < 0.05, ***p < 0.001. **C.** Representative images of marble placement at test start (far left image), and at the end of 30min testing period for LHA^{Gal} control (left image), LHA^{Gal} DREADD-Gq (middle image), LHA^{Vgat} control (right image), and LHA^{Vgat} DREADD-Gq (far right image).

Figure 7. Proposed circuit for LHA^{Gal} and LHA^{GABA} behavioral phenotypes. We propose that LHA$^{GABA/galanin}$ positive neurons modulate motivated feeding via non-VTA projections such as the locus coeruleus or hypocretin/orexin population within the LHA. Non-galaninergic LHA^{GABA} neurons modulate consummatory behavior along with compulsive behaviors via VTA projections.

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Figure 1

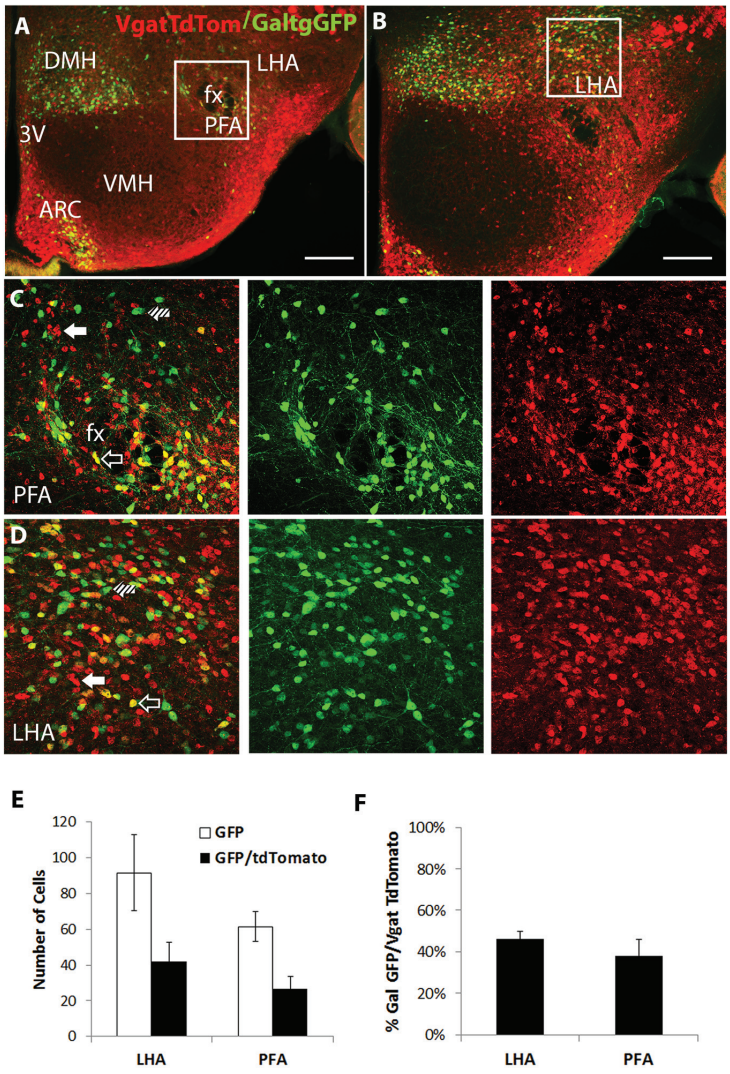

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Figure 2

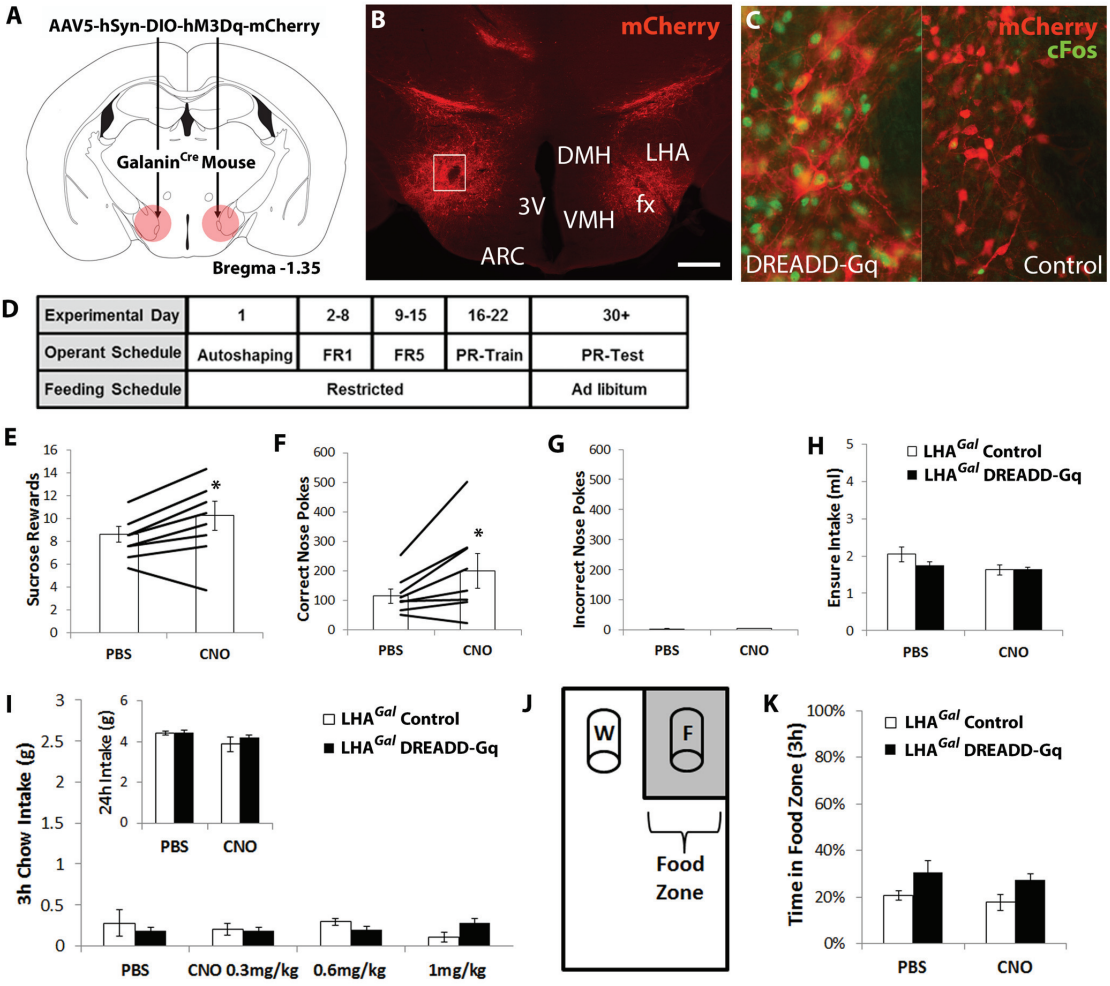

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Figure 3

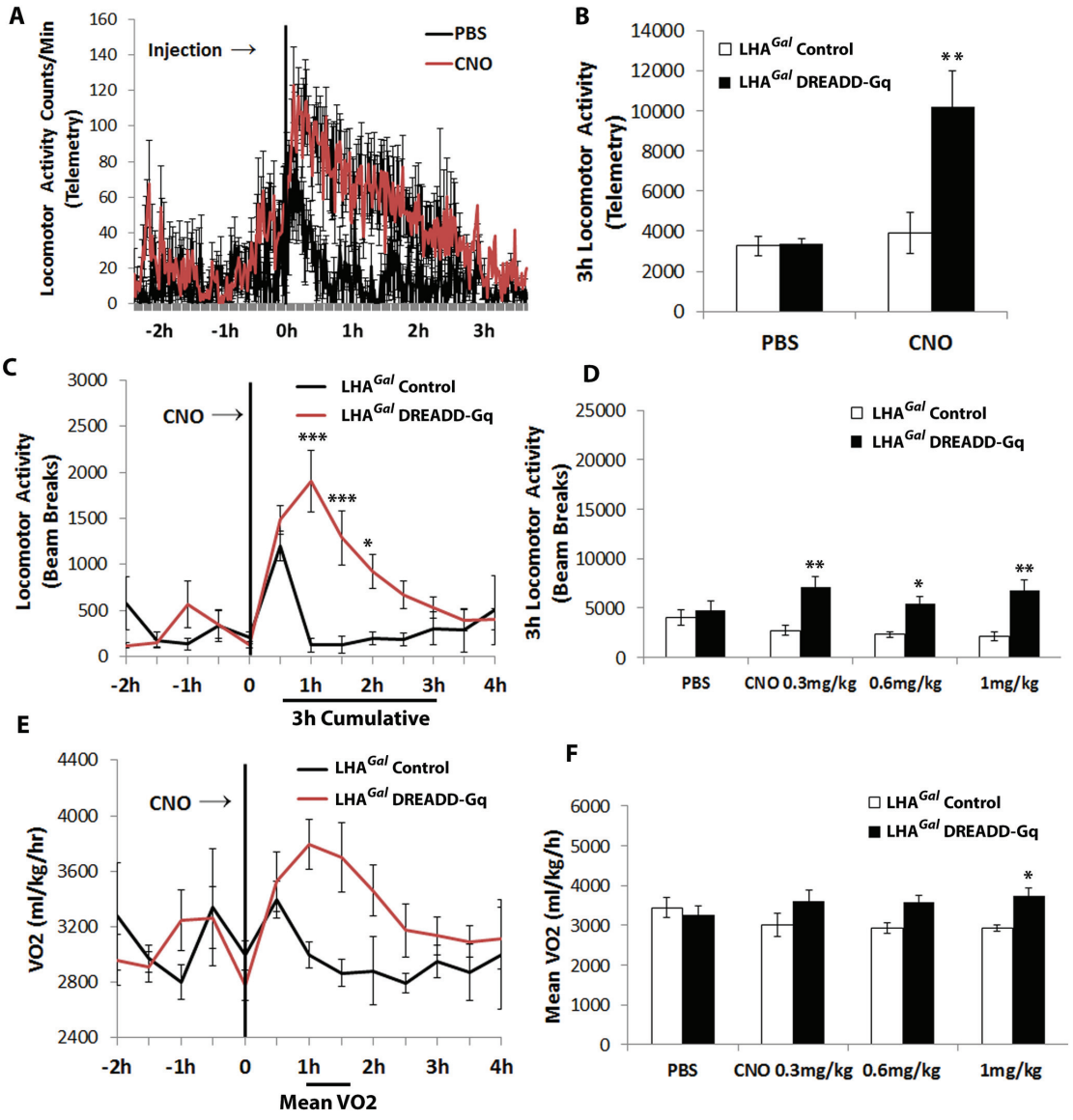

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Figure 4

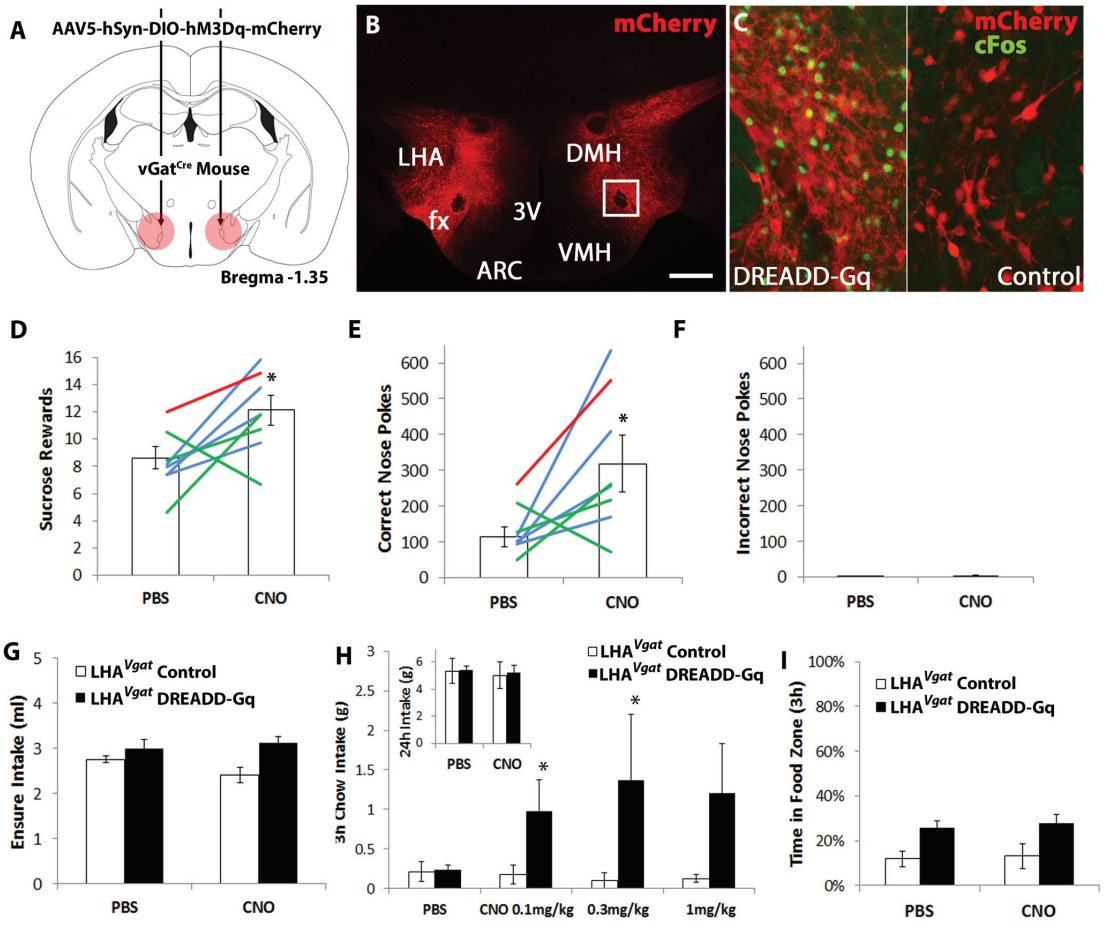

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Figure 5

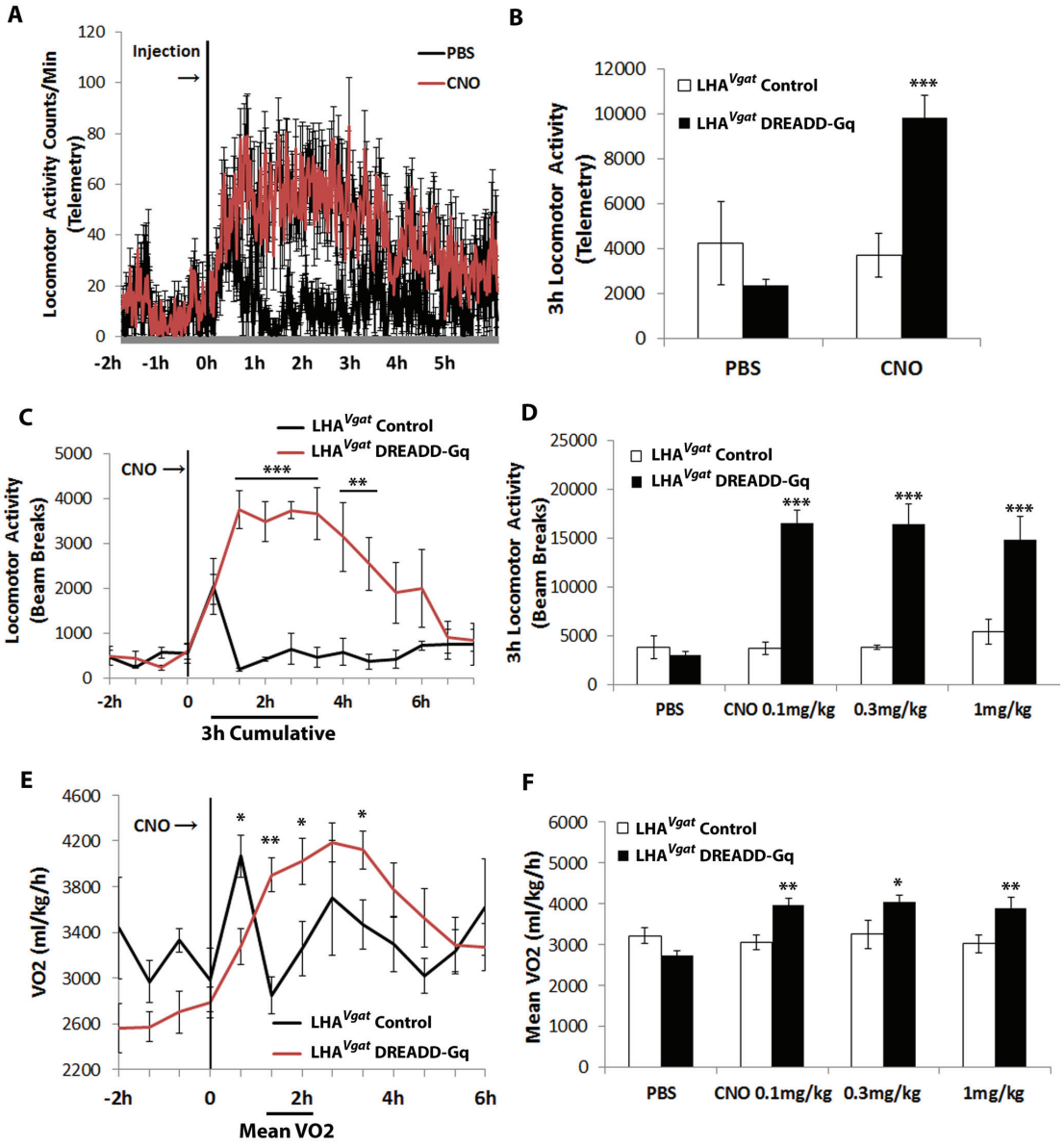

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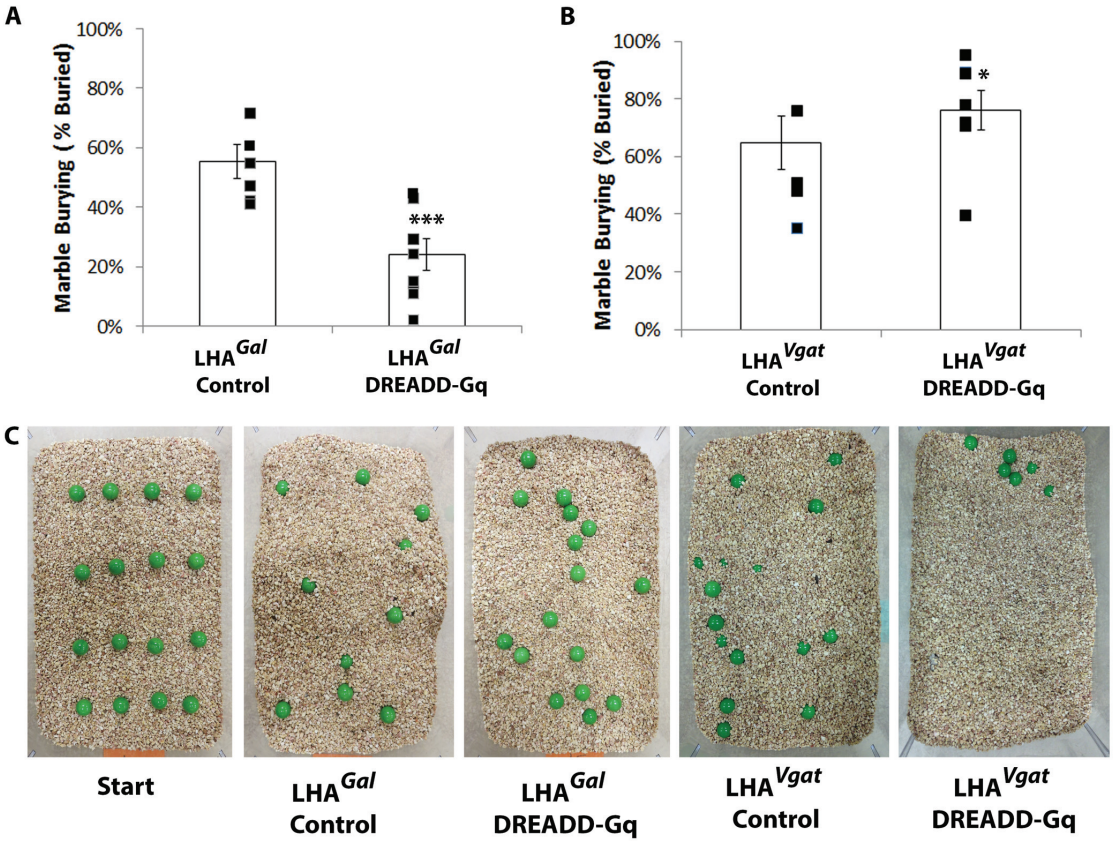
Figure 6



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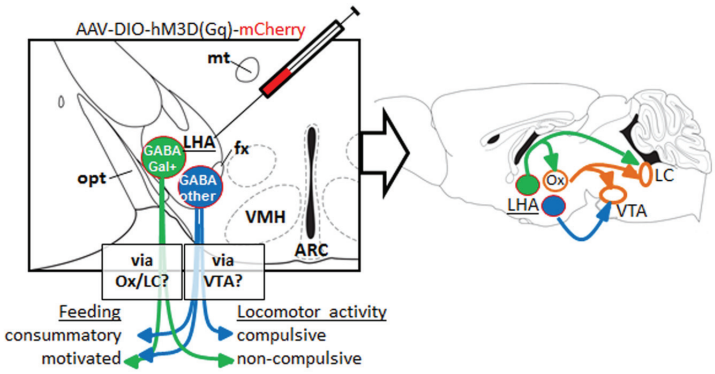
Figure 7