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의학박사 학위논문

The Role of Lateral Hypothalamic Leptin Receptor Neurons in Eating Behaviours

가측 시상하부 렙틴 수용체 발현 신경 세포의 섭식행동에 미치는 영향에 관한 연구

2023 년 8 월

서울대학교 대학원

의과학과 의과학전공

이 영 희

The Role of Lateral Hypothalamic Leptin Receptor Neurons in Eating Behaviours

지도 교수 최 형 진

이 논문을 의학박사 학위논문으로 제출함
2023년 8월

서울대학교 대학원
의과학과 의과학전공
이 영 희

이영희의 의학박사 학위논문을 인준함
2023년 8월

위 원 장 _____ (인)

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위 원 _____ (인)

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Abstract

It is crucial for eating behaviours to be sequenced through two distinct seeking and consummatory phases for survival. Heterogeneous lateral hypothalamus (LH) neurons are known to regulate motivated behaviours, yet which subpopulation drives food seeking and consummatory behaviours have not been fully addressed. Here, fibre photometry recordings demonstrated that LH leptin receptor (LepR) neurons are correlated explicitly in both voluntary seeking and consummatory behaviours. Further, micro-endoscope recording of the LH^{LepR} neurons demonstrated that one subpopulation is time-locked to seeking behaviours and the other subpopulation time-locked to consummatory behaviours. Seeking or consummatory phase specific paradigm revealed that activation of LH^{LepR} neurons promotes seeking or consummatory behaviours and inhibition of LH^{LepR} neurons reduces consummatory behaviours. These results identify neural populations that mediate seeking and consummatory behaviours and may lead to therapeutic targets for maladaptive food seeking and consummatory behaviours.

주요어 : Lateral Hypothalamus, Leptin Receptor, Food-seeking, Food-consumption
학 번 : 2016-21998

PREFACE

The majority of this thesis is a part of the paper previously published in a scientific journal by me and other authors (Young Hee Lee, Yu-Been Kim, Kyu Sik Kim, Mirae Jang, Ha Young Song, Sang-Ho Jung, Dong-Soo Ha, Joon Seok Park, Jaegeon Lee, Kyung Min Kim, Deok-Hyeon Cheon, Inhyeok Baek, Min-Gi Shin, Eun Jeong Lee, Sang Jeong Kim, and Hyung Jin Choi, Lateral Hypothalamic Leptin Receptor Neurons Drive Hunger-gated Food-seeking and Consummatory Behaviours in male mice, Nature Communications, 14, 1486 (2023). Text and figures of the publication partially modified.

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Introduction

Eating, without a doubt, stands as the most fundamental and conserved behaviour across all animals(1-3). The specific behaivours we engage in when it comes to having a meal: We embark on a quest to find a suitable place to dine, navigate towards the food, relish the delightful flavors, and finally consume it. Throughout this sequential process, our brains orchestrate the activation of distinct neurons to facilitate appropriate actions in a coordinated manner.

1) What is eating behaviour?

Since the early 1900s, Craig has advocated for eating behaviours into two distinct phases(4): Appetitive behaviour (seeking behaviour) refers to the actions and behaviours exhibited by an animal in order to obtain a desired goal or reward. It encompasses the searching, and preparatory actions that occur before the goal is achieved. Consummatory behaviour, on the other hand, involves behaviours displayed by an animal after the goal has been attained. This phase typically includes the food evaluation and ingestion.

For a long period of time, previous researchers have predominantly used quantitative measures of how much food is consumed, or how much time is spent in an area with food(5-7). This was largely due to the absence of tools that could provide scientific significance from series of short actions involved in the process. Recent advances in neuroscience now allow us to measure neural activity with remarkable temporal resolution, down to milliseconds. This breakthrough opened new avenues for addressing fundamental questions: which specific neurons drive these behaviours?

2) What is boundary between appetitive and consummatory behaviour?

Within the literature, there has been debates regarding the precise boundary between the appetitive and consummatory phases. Some studies define the appetitive phase as the period when the animal samples the reward (2, 8, 9) while others define the consummatory phase as the time when the reward is in proximity (10, 11). I thought latter definition is more logical. Brain receives and processes

sensory information to determine the most appropriate subsequent behaviour. Taste is just one component of this sensory information such as sight, smell, and touch. When do we begin to motivate to eat? It is not merely when we place the food in our mouths, but rather when we are tantalized by a delightful favor and visually perceive the appealing sight of the food.

3) How to measure the food seeking and consummatory phase?

To measure food-seeking behaviour, researchers have traditionally utilized lever-pressing behaviour in an operant conditioning chamber as a method(12-14). However, this test has two limitations. Firstly, the proximity between the food port and the lever results in the temporally overlapping response patterns of the neural populations responsible for seeking and consummatory behaviours. Secondly, as a cue-based experimental tool, it primarily assesses cue-response behaviour rather than voluntary actions. To overcome these limitations, I endeavored to develop an experimental test capable of measuring voluntary, non-cue-dependent behaviour in mice. This device was designed with the following features: Firstly, mice were randomly exposed to electric shocks, presenting them with a choice between the fear of being shocked and hunger. Secondly, a lengthy corridor was constructed to long distance between shelter and food port that prolonged behavioural observations in mice. Through this approach, I successfully correlated the voluntary seeking behaviours and relevant neuronal activity during seeking phase. To measure consummatory behaviour without exhibiting any other exploring behaviour, I should have considered the experimental condition that mice were not a head fixed position. Head fixed test could facilitate to regulates its movement by restricting the head, but rather a freely moving mice; consequently, it was challenging to control other behaviours (exploring or rearing movement) rather than consummatory behaviour. To minimize other behaviours, I developed a small chamber with a window and placed the food in the window, so that the mice could continuously look at the food closely.

4) The role of lateral hypothalamus

The lateral hypothalamus area (LH) is a vast region that makes up 3% of the total brain and has been studied in relation to eating (15). LH has been investigated as the critical regulator in feeding for over 70 years(15-19). Electrical stimulation of LH neurons enhances feeding behaviour(18, 20), whereas lesioning of the LH decreases feeding in rodents(1) and monkeys(3). Interestingly, in primate studies, one study has identified neuronal clusters in the LH that show an increase in action potentials when seeing or smelling food(21), but specific neuronal populations and their molecular phenotype has not been fully investigated.

5) The role of LH^{GABA} neurons and LH^{VGLUT2} neurons

The LH consists of a wide range of genetically and functionally distinct types of cells(19, 22-24). The LH^{GABA} neuronal population has attracted significant research attention since the 2000s due to its involvement in various motivated behaviours (15, 24). Studies have demonstrated that activation of LH^{GABA} neurons can immediately increase time spent towards new surroundings or investing intruder(23). In eating behaviour, activation of LH^{GABA} neurons drives amount of food consuming(14). However, activation of LH^{GABA} neurons also induces aberrant chewing, licking behaviour such as wood or floor(25). Notably, a recent study discovered a one subpopulation of these neurons increase during appetitive behaviour, the other population increase consummatory behaviour(14). These findings suggest that there could be two distinct eating specific subpopulations of LH^{GABA} neurons which are responsible for appetitive and consummatory behaviours. To elucidate which subpopulations in LH^{GABA} neurons exclusively contribute to seeking and /or consummatory behaviours, several studies have been dedicated to identifying subpopulations and neural circuits(13, 23, 24, 26). The other specific marker for glutamate neurons, called vesicular glutamate transporter type (Vglut2), is highly expressed in the LH(27). LH^{VGLUT2} neurons have known to inhibitory effect on food intake and be considered aversive valence. The effects of LH^{VGLUT2} neurons on eating behaviour vary depending on their projection targets and duration of palatable stimuli they are exposed to. LH^{VGLUT2} neurons projecting DMH^{LepR} neurons, inhibit agouti-related peptide (AGRP) neurons (28). Inhibition of LH^{VGLUT2} neurons projecting lateral habenula showed an immediate increase in the intake of a pleasurable liquid high in calories. LH^{VGLUT2} neurons were sensitive

to the exposure of high fat diet (HFD) and genetic association with human body mass index (BMI) (29).

6) The role of LH^{LepR} neurons

LH leptin receptor expressing (LH^{LepR}) neurons are subpopulation of LH^{GABA} neurons and has been reported to be associated with eating (12, 13, 26, 30, 31) (Fig.1). However, the role of LH^{LepR} neurons is controversial; no effect on eating (26), decreased eating (13), decreased eating after leptin treatment in the LH(30). A recent study demonstrated that increased activity of LH^{LepR} neurons correlates with sustained binge eating in early-life trauma (ELT) mice(32). Activation ventrolateral periaqueductal gray (vlPAG)-projecting of LH^{LepR} neurons drives HFD consumption only after repeated cycles of Re-HFD condition. During the first exposure to HFD, (vlPAG)-projecting of LH^{LepR} neurons did not drive food consumption. In addition, activation of proenkephalin-expressing ventrolateral periaqueductal gray neurons, which receive inhibitory inputs from LH^{LepR} neurons, ameliorates maladaptive eating behaviours induced by ELT. Although the study demonstrated LH^{LepR} neurons neural activity during stress evoked binge eating, it did not prove the cause-and-effect relationship of these neural changes in general eating conditions in terms of precise temporal resolution.

Employing *in vivo* calcium imaging and phase specific behavioural tasks, I identified two distinct LH^{LepR} neural populations that are separately activated during seeking and consummatory behaviours, respectively. Further, neural activation results clearly demonstrated that LH^{LepR} neurons are sufficient for driving seeking behaviours and consummatory behaviours. Also, neural inhibition results clearly showed that LH^{LepR} neurons are necessary for driving consummatory behaviours. Collectively, these data highlight the orchestration of seeking and consummatory phases within the LH circuitry.

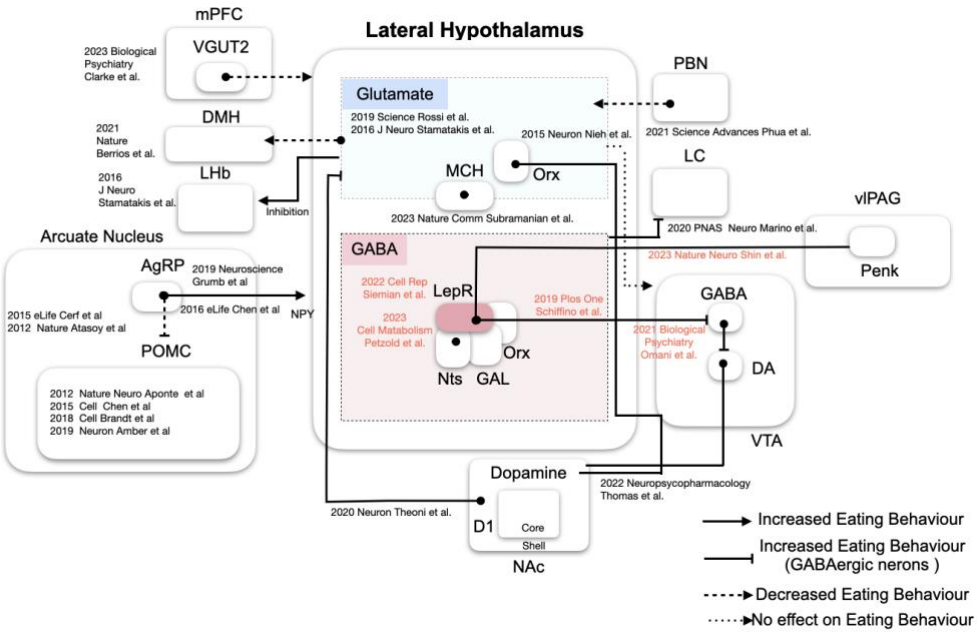


Fig. 1 | LH subpopulation and circuitry in eating behaviour.

Recent studies of eating behaviour in lateral hypothalamus. The dotted lines represent the eating inhibition circuits. The solid lines represent the eating activation circuits.

Methods

Animals

All experimental protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals from the Seoul National University,and approved by the Seoul National University Institutional Animal Care and Use Committee. Mice were housed on a 08:00 to 20:00 light cycle (temperature 22±1 °C, humidity 50±10%) with standard mouse chow (38057, Purina Rodent chow) and water provided ad libitum, unless otherwise noted. Behavioural tests were conducted during the light cycle. Adult male mice (at least 8-weeks-old) of the following strains were used: LepR-Cre (JAX stock no. 008320), Ai-14 Td-Tomato (JAX stock no. 007914), Vgat-Cre (JAX stock no. 028862)

Stereotaxic virus injection

Mice were anaesthetised with xylazine (20 mg/kg) and ketamine (120 mg/kg). A pulled-glass pipette was inserted into the LH (400 nl total; AP, -1.5 mm; ML, ±0.9 mm; DV, 5.25 mm from the bregma) based on the 2D LH^{LepR} distribution (Figure 2). The GCaMP6 virus (AAV1.Syn.Flex.GCaMP6s.WPRE.SV40, Addgene; titre: 1.45×10^{13} genome copies per ml with 1:2 dilution) was utilised for calcium imaging. The AAV5.EF1α.DIO.hChR2(H134R).EYFP (Addgene 20298; titre: 2.4×10^{13} genome copies per ml) or AAV5.EF1α.DIO.eNpHR3.0.EYFP (Addgene 26966; titre: 1.1×10^{13} genome copies per ml) or AAV5.EF1α.DIO.EYFP (Addgene 27056; titre: 2.6×10^{13} genome copies per ml) was utilised for optogenetic experiments.

Optical fibre/GRIN lens insertion

For fibre photometry experiments, a ferrule-capped optical cannula (400 µm core, NA 0.57, Doric Lenses, MF2.5, 400/430–0.57) was unilaterally placed 0–50 µm above the virus injection site and attached to the skull with Metabond cement (C&B Super Bond). For optogenetic manipulation, optic fibres (200 µm core, NA 0.37, Doric Lenses or Inper) were bilaterally implanted 100–200 µm above the LH injection site at a 10° angle from the vertical in the lateral-to-medial direction. For

micro-endoscope imaging, a GRIN lens (500 µm core, 8.4 length, Inscopix #1050-004413) was inserted after 3 weeks of recovery following virus injection. Dexamethasone, ketoprofen, and cefazolin were administered for postoperative care.

Calcium imaging using fibre photometry and micro-endoscope

For bulk calcium imaging, I used a Doric Lenses fibre photometry system. In the experiment, 465 nm and 405 nm LED light sources (Doric LED driver) were delivered continuously through a rotary joint (Doric Lenses, FRJ_1X1_PT-400/430/LWMJ-0.57_1m) connected to the patch cord (Doric Lenses, MFP_400/430/1100-0.57_1m), and the GCaMP6 signal was collected back through the same fibre into the photodetector (Doric Lenses). For single-cell calcium imaging, I used nVoke (Inscopix).

Optogenetics

Laser stimulation (473-nm for activation and 594 nm or 532 nm for inhibition, Shanghai DPSS Laser) was delivered through an FC-FC fibre patch cord (Doric Lenses) connected to the rotary joint, following which the FC-ZF 1.25 fibre patch cord delivered stimulation to the cannula (200 µm core, NA 0.37, Doric Lenses or Inper). The laser intensity was approximately 10 mW at the tip.

Eating behavioural tests

Animal condition. Prior to the experiments, all mice were habituated to the experimental cages, and fibre handling was conducted for at least 3 days. Chocolate-flavoured snack (Oreo O's, 1/8 aliquot: 0.2g) was utilised during eating behavioural tests.

Multi-phase test 1. The multi-phase test is a behavioural paradigm test with seeking and consummatory phases designed to provide sufficient temporal distinction between seeking and consummatory behaviours. To measure neuronal activity before and after conditioning with food, fasted (80~90% of the body weight in the ad libitum state) mice received a chocolate-flavoured snack at the edge of an L-shaped chamber (60cm x 8.5cm) with a shelter (6cm x 12cm x 18cm

triangle box). Conditioning sessions (day 1-2) were performed for 15 trials in 2 days to provide sufficient experience for the mice to learn the location of the food by providing a chocolate-flavoured snack. The test session (day 3) was also performed for 15 trials. Each trial started when a door was removed ("accessibility moment") with scheduled timing from the experimenter. 'Proximate to food' was analysed when the mouse arrived at the top of bridge. 'Food contact' was defined as the moment when the mouse physically contacted the food. Mice usually entered the shelter spontaneously after each trial (end of consumption). Otherwise, the experimenter closed the door after gently pushing the mice to the shelter.

Multi-phase test 2. The multi-phase test 2 is a behavioural paradigm test which mimicked the natural environment of mice in a cave, running to seek and consume food despite the risk of outdoor threats. To measure the temporal onset of LH^{LepR} neural activity in voluntary behaviour, I eliminated all reward-associated cues (e.g., door open, sound) in the experiment. I placed a shelter as cave and delivered an electrical shock as punishment in a square chamber (30cm x 30 cm square chamber with narrow corridors sized 6 cm). Electrical shock was given at a mean of 0.2 mA/shock for 7s with a 10-s interval. I adjusted the total duration of shock delivery to maximise the performance of mice. During conditioning sessions, fasted (80-90% of the body weight in the ad libitum state) mice received a chocolate-flavoured snack at the edge of chamber. During the test session, I exclude the shock and analysed the moment when the mouse's whole body came out of the shelter (onset of seeking behaviours). Trials that were successful in consuming food were analysed. For micro-endoscope experiments, food and no-food trials were conducted randomly during the test session without shock.

Multi-phase test 3. The multi-phase test 3 is a behavioural paradigm test which was designed to provide ad libitum accessibility to both seeking and consummatory behaviours, simultaneously. To measure seeking and consummatory behaviours during photostimulation, sucrose agarose gel (30% sucrose in 3% agarose gel) was placed in a food tray (3 cm height) at one side of the open-field box (33x33x33 cm). Condition of mice were as followed; ad libitum (ChR2)/ fasted (NpHR). The food zone was defined as the zone that included the food tray. The size of food zone was defined as approximately 10 cm x 10 cm.

Seeking behaviour test 1. The seeking behaviour test 1 is a seeking specific

behavioural paradigm test which was designed to evoke only seeking behaviours without any consummatory behaviours. To measure the neural activity during seeking termination, I randomly presented food cue (vertical stripe) and no-food cue (horizontal stripe). During conditioning, fasted (80-90% of the body weight in the ad libitum state) mice received chocolate-flavoured snacks only when the food cue was presented. The success rate ([S2/W], S1 = number of seeking termination, S2 = number of consumptions after food cue, W = S1+S2) was recorded during training until it reached 80%. The duration and amplitude of shocks during training were optimised for each mouse to achieve the best success rate. During experiment, fasted mice initiated seeking after presentation of the food cue, but eventually terminated voluntarily, when the mice realised there was no food.

Seeking behaviour test 2. The seeking behaviour test 2 is a seeking specific behavioural paradigm test which was designed to evoke sustained seeking behaviours without any consummatory behaviours. To solely measure seeking behaviours during photostimulation, I conditioned mice to conduct seeking behaviours but removed food at the test day. For the conditioning sessions, chocolate-flavoured snacks or raisins were hidden under the wooden bedding at each edge of the open field box. Twice a day for 3 consecutive days, the ad libitum mice (ChR2/Control) or fasted mice (NpHR) were allowed to seek the box for hidden food during the 10 min of the experiment. For the test session, there was only wooden bedding without food in which ad libitum mice (ChR2/Control/NpHR) were put to test. Food zone was defined as four corners divided into 16 zones. Seeking behaviours were analysed in three behaviours manually: digging with nose, digging with paw, and digging after floor exposure in food zone.

Consummatory behaviour test 1. The consummatory behaviour test 1 is a consummatory specific behavioural paradigm test which was designed pfc

to evoke consummatory behaviours with or without swallowing. To measure neural activity during consummatory behaviours, a chocolate-flavoured snack was placed in the tray on one side of the wall. During obtainable height (8 cm) sessions, the fasted (80-90% of the body weight in the ad libitum state) mice engaged in sequential consummatory behaviours such as rearing toward visible food, biting, licking, and swallowing. I analysed the moment the mice made physical contact

with the hanging food. During the unobtainable height (11 cm) sessions, the fasted mice initiated consummatory behaviour, rearing toward the visible food, but eventually terminated consummatory behaviours when the mice realised that the mice could not eat it. I analysed the moment the mice voluntarily terminated the consummatory behaviours to the hanging food.

Consummatory behaviour test 2. The consummatory behavioural test 2 is a consummatory behavioural paradigm test which was designed to determine whether the neural activity is food specific. To measure neural activity during consummatory behaviour for edible food or inedible non-food objects, fasted (80-90% of the body weight in the ad libitum state) mice performed chewing behaviour toward food (chocolate-flavoured snack) or an inedible object (a Lego brick). I analysed the moment when the mice made physical contact with the food or inedible object.

Consummatory behaviour test 3. The consummatory behavioural test 3 is a consummatory specific behavioural paradigm test which was designed to evoke consummatory behaviours without any seeking behaviours. To solely measure consummatory behaviours during photostimulation, I minimised chamber size (17 × 6 × 30 cm). Ad-libitum mice (ChR2/Control) or fasted mice (NpHR) were placed in the chamber with sucrose agarose gel (30% sucrose, 3% agarose). During photostimulation, consummatory behaviours were measured; food contact, biting, and chewing.

Consummatory behaviour test 4. The consummatory behavioural test 4 is a consummatory specific behavioural paradigm test which was designed to evoke consummatory behaviours without any seeking behaviours.

Consummatory behaviour test 5. The consummatory behavioural test 5 is a consummatory specific behavioural paradigm test which was designed to evoke discrete short consummatory bouts using small food portions without any seeking behaviours. To solely measure consummatory behaviours during photostimulation, I conducted experiment in a minimised chamber size (13 × 17 × 30 cm). The mice (fasted 16-24hrs) were given ad libitum chocolate-flavoured snacks. On the test day, laser stimulation was delivered for 20 min at 2-min intervals.

Water test. Mice were dehydrated for 2 days. The water test was performed using an open field chamber where a water bottle was placed. I analysed the moment

when mice licked the spout of the water bottle.

3D clearing

Fixed tissue was incubated in reflective index matching solution (C Match, Cat.50-3011) at 37℃ for 2 days. Images were obtained using SPIM (LaVision Biotech, Bielefeld, Germany) and analysed using IMARIS 9.5 (Bitplane AG, Zürich, Switzerland).

Histology, immunohistochemistry, and imaging

Animals were deeply anesthetized by a mixture of ketamine and xylazine. Transcranial perfusion was performed using phosphate-buffered saline, followed by 4% neutral-buffered paraformaldehyde (T&I, BPP-9004). The brains were extracted, post-fixed in 4% paraformaldehyde at 4°C, and transferred to 10% sucrose, followed by 30% sucrose for cryoprotection. Cryoprotected brains were sectioned coronally on a cryostat (Leica Biosystems, CM3050) at 50 µm, and their sections were stained with 4',6-diamidino-2-phenylindole (DAPI) to visualise the nuclei. To verify the scientific exactitude, images of viral fluorescence and fibre/cannula placement were captured using a confocal microscope (Olympus, FV3000).

Analysis

Single-cell RNA-sequence analysis

scRNA-sequence data with the LH (GSE125065) were analysed[25]. Of the initial 7,232 cells (3,439 male and 3,793 female), 598 cells with less than 500 unique molecular identifiers (UMIs) or >40% of mitochondrial reads were discarded. The R package Monocle3 was used to classify the cells[54]. Using Monocle 3, we subjected single-cell gene expression profiles to uniform manifold approximation and projection (UMAP) visualisation. Altogether, I identified 4,091 cells as neural clusters on the basis of cell type–specific marker gene expression(33-35). These neural clusters containing 4,091 cells were extracted for further clustering using Monocle 3 as above, which yielded 37 clusters. Clusters were classified as GABAergic when the median expression of Slc32a1 was greater than that of

Slc17a6 in each cluster and glutamatergic when the median expression of Slc17a6 was greater than that of Slc32a1. Consistent with previous result (92%[21], 80%[23]), most of LH^{LepR} neurons were GABAergic (70%; 100/141).

Simulated distribution of food-specific LH LepR neurons among LH GABA neurons

Simulated results of 1,000 LH^{GABA} neurons. I assumed that 10% of LH^{GABA} neurons are LH^{LepR} neurons, given that our result and a previous result(12) indicated that LH^{LepR} neurons constitute 4-20% of LH^{GABA} neurons. In our result, among LH^{GABA} neurons, 8% of LH^{GABA} neurons were food specific (80 neurons). Among LH^{LepR} neurons (10% of 1,000 LH^{GABA} neurons, 100 neurons), 63% of LH^{LepR} neurons were food specific (63 neurons). Therefore, LH^{LepR} neurons comprise the majority of food-specific LH GABA neurons (79%; 63/80) in this simulation results.

Behavioural tests. All data analyses were performed using custom-written MATLAB (MathWords, Natick, MA) and Python codes. Behavioural experiments were analysed using Observer XT 13 or EthoVision 14 or DeepLabCut.

Fibre photometry imaging. Fibre photometry signal data were acquired using the Doric Studio software. Two signals from fibre photometry, 465 nm calcium and 405 nm isosbestic signals (for artifact correction), were obtained for correction before performing any analysis. Signals from fibre photometry were corrected as follows to minimise artifact recordings: corrected 465 nm signal= (465 nm signal − 405 signal) / 405 signal(36). Signals were decimated to obtain approximately 25 data points in 1s. For photometry experiments, all corrected signals shown were initially computed to Z-scores before further normalisation. The baseline was designated as –10 s to –5 s before recording the initiation of behaviour (t=0). The mean of the baseline (m) and standard deviation (σ) of the baseline were computed to normalise the corrected signals into Z-scores (Z = (corrected 465 nm − m) / σ). The behaviour time point for each test was manually annotated. For the heatmap, each trial was normalised before visualisation (normalised Z = (Z − minimum Z) / (max Z − min Z)). Trials were excluded if the trial length exceeded the optimal trial length (15 s for the multi-phase, 10 s for the rest).

Micro-endoscopic imaging. All data from the micro-endoscope experiments were recorded using nVoke (Inscopix). The raw signal output from CNMF-E (Craw) was

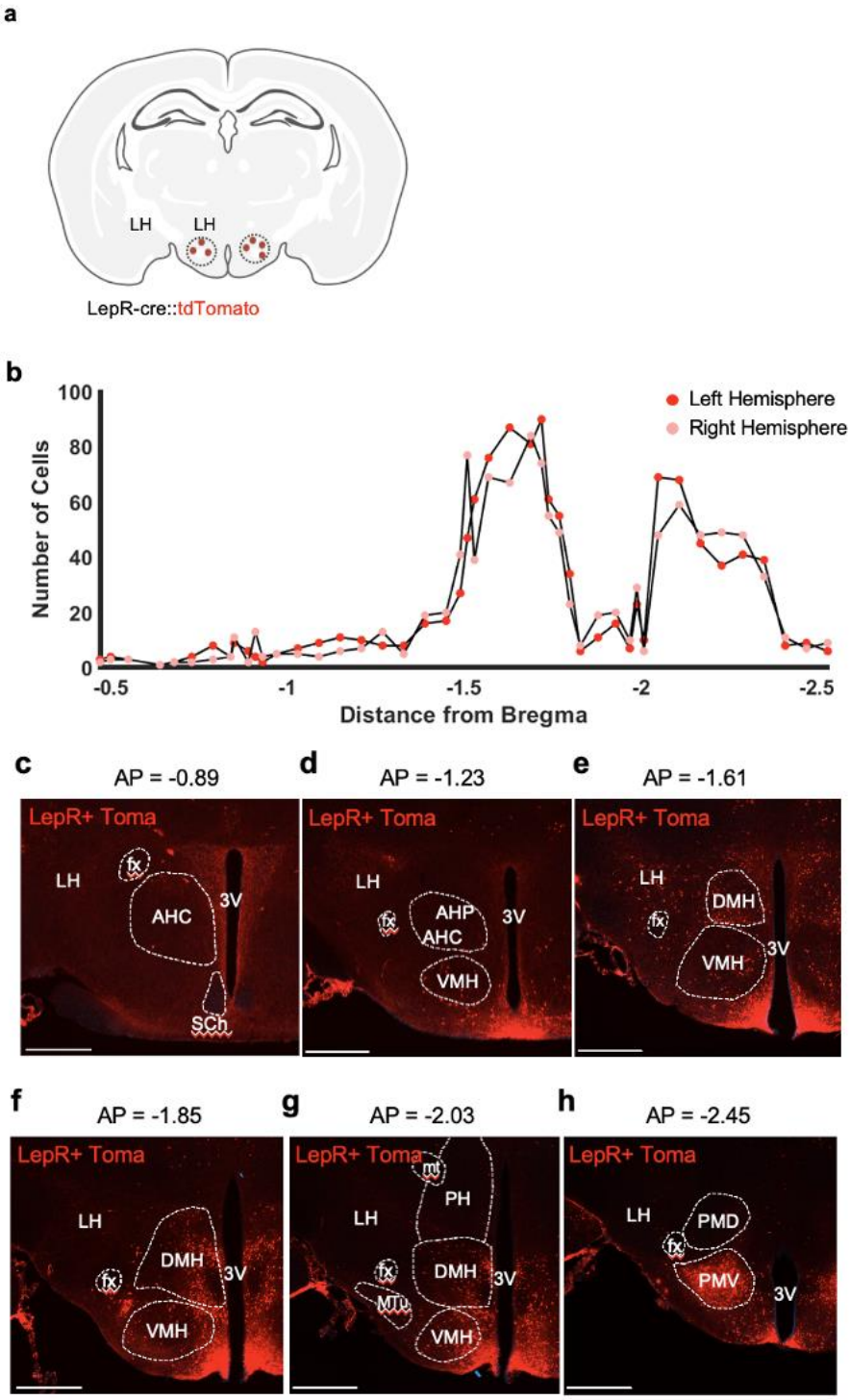
converted into Z-scores (Z= (Craw- m)/σ), according to the mean (m) and standard deviation (σ) of the baseline (-10 s to -5 s before behavioural initiation).

To discriminate food-specific neurons in Figure 1, I applied the following criteria. I defined neurons as food-specific responsive(yellow) when they were activated during all three eating behavioural tests (>4σ) and not activated during a non-food behavioural test (<4σ). I defined neurons as non-specific-responsive (grey) when they were both activated during three eating behavioural tests (>4σ) and a non-food behavioural test (>4σ). I defined neurons as non-food-specific responsive (blue) when they were not activated during all three eating behavioural tests (<4σ) but, activated during a non-food behavioural test (>4σ). I defined neurons as no responsive (white) when they were neither activated during three eating tests nor a non-food behavioural test (<4σ).

To distinguish the distinct populations of LH^{LepR} neurons, the neural activity of LH^{LepR} neurons was recorded in multi-phase test 2 and processed as described above. Trials that exceeded 25 s of total trial length were excluded from the test (seeking moment – food consumption end [food trial] or food zone exit [no food trial]). Activated neurons were defined as cells with Z-scores of >4σ. Otherwise, I defined non-responsive neurons if neural activity was Z-scores of <4σ. Neural activity was then normalised as follows: (NF0) = (Craw − minimum Craw) / (max Craw − minimum Craw). Further analysis was performed with the average normalised activity of the group of trials that had sufficient length. Seeking-score-1 was defined as NF0 at the food contact moment in the seeking with consummatory behaviour session. Seeking-score-2 was defined as NF0 at the food contact moment in the seeking without consummatory session.

Statistical analysis

All statistical data were analysed using MATLAB or IBM SPSS 25.0 (IBM Corp., Armonk, NY). Data in the figures are reported as the mean ± standard error of the mean. Paired t-tests were used to compare data between two groups. Two-way repeated-measures analyses of variance (ANOVA) were used for multiple comparisons. P-values for comparisons across multiple groups were corrected using the Greenhouse–Geisser method in IBM SPSS 25.0. Levels of significance were as follows: $*p < 0.05$. $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.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LepR-cre::tdTomato
b
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60
40
20
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-0.5
-1
-1.5
-2
-2.5
Distance from Bregma
Left Hemisphere
Right Hemisphere
c
AP = -0.89
LepR+ Toma
LH
fx
AHC
3V
SCh
d
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LH
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3V
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PH
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LepR+ Toma
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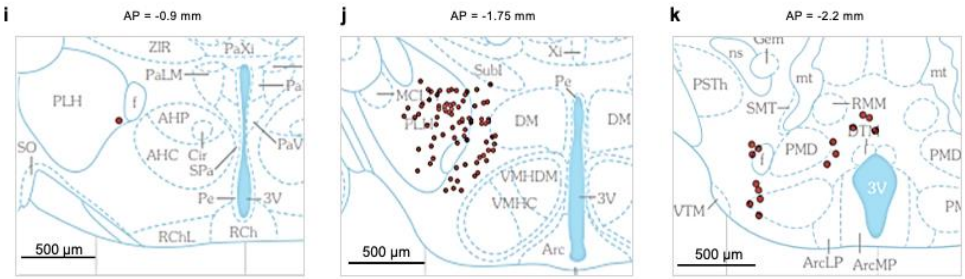


Fig. 2 | 2D Distribution of LepR neural population in the lateral hypothalamus.

a, b, Distribution profile and average quantification of LepR-positive cells in the LH along the anterior-posterior axis of LepR-tdTomato mice (n = 3 mice). **c-h**, Coronal brain sections showing tdTomato+ cells. Scale bar: 500µm. The experiment was repeated 3 times independently with similar results. fx, fornix; 3V, the 3rd ventricle; AHC, anterior hypothalamus central; SCh, suprachiasmatic nucleus; AHP, anterior hypothalamus posterior; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; PH, posterior hypothalamus; MTu, medial tuberal nucleus; mt, mamillothalamic tract; PMD, premamillary nucleus dorsal part; PMV, premamillary nucleus ventral part. **i-k**, Representative images depicting the distribution of LH^{LepR} tdTomato+ cell bodies (red dot).

Results

Overview of multiphasic experimental paradigms

To investigate seeking and consummatory behaviours, I developed phase specific tests to dissect two phases via temporal distinctions. I developed seeking phase specific tests, which minimised consummatory behaviours (manipulating, licking, biting, chewing, and swallowing). To exclusively measure consummatory behaviours, I developed consummatory phase specific tests, which minimised seeking behaviours (searching, and digging) (Fig.3).

LH^{LepR} neurons are the food-specific subpopulation of LH^{GABA} neurons

To investigate the heterogeneous LH^{GABA} neurons and test if LH^{LepR} neurons are part of food-specific LH^{GABA} subpopulation, I first investigated the anatomical distribution of LH^{LepR} neurons via whole-LH three-dimensional (3D) tissue clearing (Fig.3) and 2D histological mapping using LepR-tdTomato mice (Fig.2). As a result, LH^{LepR} neurons were mainly distributed in the middle region (-1.5 mm from bregma).

According to mapping results (Fig.2), vesicular GABA transporter (Vgat)-cre (Fig.5) and LepR-cre mice (Fig.6), were injected with cre-dependent adeno-associated virus (AAV) carrying GCaMP6s and implanted a gradient index (GRIN) lens in the middle LH. Using micro-endoscopic imaging of calcium dynamics, I analysed three eating behaviours in fasted mice; running toward expected food (Fig.7,8 left in the food test), approach toward proximate food (consummatory behaviour, Fig.7,8 middle in food test,) and chewing the proximate food (consummatory behaviour, Fig.7,8 right in the food test). I first measured individual LH^{GABA} neural activity (Fig. 7) during these tests compared to non-food behavioural test (chewing behaviour towards inedible Lego brick, Fig.7,8, non-food test). I defined neurons as food-specific responsive (yellow) when they were activated during all three eating behavioural tests and not activated during a non-food behavioural test. Non-food-specific responsive neurons (blue), non-specific responsive neurons (grey), and no responsive neurons (white) were defined based on neural activity patterns during the tests (see methods).

Among LH^{GABA} neurons, most neurons (64%) were activated in non-food

behavioural tests (Fig.7, grey and blue panels). Instead, only a small subpopulation of neurons (8%) was food-specific responsive neurons (activated only in eating behaviour related tests) (Fig.7, yellow panel), suggesting that only a small food-specific subpopulation exists within the vast total population of LH^{GABA} neurons (Fig.9a-c). Of note, when LH^{LepR}-cre mice conducted the same experiments (Fig. 8), most LH^{LepR} neurons (63%) were food-specific responsive (Fig. 9 e-f)

Based on the previous single-cell RNA sequencing data for the LH(33), LH^{LepR} neurons are mostly GABAergic (Fig. 10a), consistent with previous results(12, 31). Further, LH^{LepR} neurons constitute only 4% of LH^{GABA} neurons (VGAT positive cells) (Fig.10a). A previous study has reported that LH^{LepR} neurons constitute less than 20% of LH^{GABA} neurons[21]. Although LH^{LepR} neurons represented only a minor portion (4–20%) of LH^{GABA} neurons (Fig.10a), my results indicate that most (79%; 63/80) of food-specific responsive LH^{GABA} neurons are LH^{LepR} neurons (Fig.12). Furthermore, LH^{LepR} neurons were not activated to non-food investigation (Fig.13). Compared to the robust response to food, only a minor response was observed to water (Fig.11). These results suggest that LH^{LepR} neurons are food-specific population among LH^{GABA} neurons.

LH^{LepR} neurons are activated during seeking and consummatory behaviours

Next, to investigate temporal dynamics of LH^{LepR} neural activity during eating behaviour, neural activity was measured using fibre photometry at the population level (Fig. 14a-b). LH^{LepR} neural activity significantly increased at each eating bout with time-locked temporal dynamics in fasted mice (Fig. 14d-i). Interestingly, LH^{LepR} neural activity increased even before physical contact with food, implying that LH^{LepR} neurons may also be involved in seeking behaviours. suggesting that LH^{LepR} neural activity is associated with voluntary behaviours.

To dissect seeking and consummatory phase, I developed a multi-phase test to provide sufficient temporal distinction between seeking and consummatory behaviours (Fig.15 a,b). In the L-shaped chamber, fasted mice sequentially explored an empty corridor and arrived proximate to food. Before conditioning, mice explored the whole maze since mice were not aware of the food location (Fig.15 d). LH^{LepR} neural activity did not increase during this non-goal-directed locomotion (Fig.15 f). LH^{LepR} neural activity significantly started to increase when

mice conducted consummatory behaviours at the end of the corridor. However, after conditioning (Fig.15 e), the mice moved directly to the food at the end of the corridor (goal-directed seeking; significantly shorter time from accessibility to food contact) (Fig.15c). When compared with the neural activity results before conditioning, LH^{LepR} neural activity started to increase significantly when the mice initiated seeking, and there was an additional activity increase in the consummatory phase (Fig.15 g). Additional tests revealed that LH^{LepR} neural activity decreased when mice voluntarily terminated both seeking or consummatory behaviours (Fig. 16).

Two distinct subpopulations of LH^{LepR} neurons individually encode seeking and consummatory behaviours

My photometry data showed that LH^{LepR} neural population is activated sequentially at seeking and consummatory behaviours. I thought that two hypotheses could be possible; 1) one homogenous LH^{LepR} neuronal population encodes both seeking and consummatory behaviours, or 2) two distinct LH^{LepR} neuron populations encode seeking or consummatory behaviours, respectively. However, individual neural dynamics is not accurately reflected in the fibre photometry. To prove this hypothesis, I investigated changes in LH^{LepR} neural activity using micro-endoscope during seeking and consummatory behaviours (Fig. 17 a,b). To distinguish between seeking and consummatory behaviours, I modified the multi-phase test described above (Fig7a left in food test). During food sessions, fasted mice sequentially performed seeking and consummatory behaviours (Fig. 17c left). In contrast, during no-food sessions, mice performed seeking, but not consummatory behaviours since food was not present in food zone (Fig. 17c right). I identified two distinct neural populations that specifically responded to seeking or consummatory behaviours (Fig. 17d, Fig.18), which was robustly consistent across numerous trials (Fig.17e).

One population of neurons were activated only during seeking and not during consummatory behaviours (seeking LH^{LepR} neurons) (Fig.19a-c). Another population of neurons were activated only during consummatory and not during seeking behaviours (consummatory LH^{LepR} neurons) (Fig.19d-f). Geographic location of seeking and consummatory encoding neurons are intermingled within

field of view. Among the population of LH^{LepR} neurons, 25% were seeking neurons, and 39% were consummatory neurons (Fig.17f).

Collectively, my micro-endoscope data showed that seeking LH^{LepR} neurons and consummatory LH^{LepR} neurons; 1) respectively encode seeking or consummatory behaviours 2) are sequentially activated (Fig.18) and are exclusively activated (not simultaneously activated).

LH^{LepR} neurons fail to evoke eating behaviours in experiments with combination of seeking and consummatory phases

Optogenetic stimulation induces simultaneous activation of both seeking and consummatory LH^{LepR} neurons, which are unphysiological in contrast to my physiological micro-endoscope results. These results imply that optogenetic activation of both seeking and consummatory LH^{LepR} neurons will not induce effective behavioural changes if the mice have choice of both seeking and consummatory behaviours due to competition between two distinct behavioural choices.

To examine this hypothesis, LepR cre-mice were injected with cre-dependent channelrhodopsin 2 (ChR2)/halorhodopsin (NpHR) or enhanced yellow fluorescent protein (EYFP) AAV vector, and an optic fibre was implanted in the LH (Fig.20a, b). I conducted a multi-phase test, in which ad-libitum mice had choice of both seeking and consummatory behaviours in a large chamber (33 × 33 × 33cm) (Fig. 20c). As expected, unphysiological simultaneous activation/inhibition of both seeking and consummatory LH^{LepR} neurons failed to show any change in seeking (food zone duration and food zone entry number) or consummatory (food contact number and food intake) behaviours (Fig. 20d-g, Fig.21).

LH^{LepR} neurons evoke seeking or consummatory behaviours in phasic specific conditions

My micro-endoscope data distinguished two distinct subpopulations (seeking and consummatory LH^{LepR} neurons that drive respective behaviours, which are sequentially activated and not simultaneously activated. Therefore, I hypothesised that activation of LH^{LepR} neurons could evoke respective seeking or consummatory behaviours, when the seeking or consummatory phase was isolated so that mice

only had a choice of one specific behaviour.

To isolate the seeking phase, mice were conditioned to seek hidden foods in the four corners of an open-field chamber filled with bedding (Fig.22a). On the photo-stimulation day, ad libitum mice were placed in the same chamber covered with bedding without food to only evoke sustained seeking behaviour. Activation of LH^{LepR} neurons significantly increased seeking behaviours (digging with the nose, digging with the paw, and digging after floor exposure), entry into the food zones, and seeking locomotion compared to no-stimulation (Fig. 22c-g) or control conditions (Fig.23a-d). However, inhibition of LH^{LepR} neurons failed to show significant differences in seeking behaviours (Fig.23e-h). Collectively, these results show that LH^{LepR} neurons are sufficient to drive seeking behaviours when the seeking phase is isolated.

To isolate the consummatory phase, ad-libitum mice were placed in a chamber of minimised size (17 × 6 × 30cm) and were provided with ad-libitum food at proximate range. Of note, activation of LH^{LepR} neurons significantly increased the number and total duration of consummatory behaviours and food intake when compared to no stimulation (Fig. 24a-e). EYFP control mice did not show any significant change in consummatory behaviour (Fig.24f-j). I further performed closed-loop stimulation of LH^{LepR} neurons when mice were proximate to food significantly increased consummatory behaviours compared to no stimulation conditions (Fig.25).

Next, I hypothesised that inhibition of LH^{LepR} neurons decreases consummatory behaviours. Fasted mice were tested in a small chamber where the mice could perform only consummatory behaviours rather than seeking (Fig 26a left). To quantify the consummatory behaviour, multiple small snacks were presented during several interleaved photoinhibition blocks (Fig. 26b right). During the session, the mice exhibited consummatory behaviours (sniffing, biting and chewing). NpHR mice, but not EYFP control mice, significantly decreased consummatory behaviours (total duration and bout duration) (Fig.26, Fig.27). Collectively, these results show that LH^{LepR} neurons are sufficient and necessary for driving consummatory behaviours when the consummatory phase is isolated.

References

1. Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale J Biol Med. 1951;24(2):123-40.
2. Alcantara IC, Tapia APM, Aponte Y, Krashes MJ. Acts of appetite: neural circuits governing the appetitive, consummatory, and terminating phases of feeding. Nat Metab. 2022;4(7):836-47.
3. Anand BK, Dua S, Shoenberg K. Hypothalamic control of food intake in cats and monkeys. J Physiol. 1955;127(1):143-52.
4. Craig W. Appetites and aversions as constituents of instincts. Biol Bull-Us. 1918;34(2):91-107.
5. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, et al. Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci. 2005;8(10):1289-91.
6. Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nat Neurosci. 2011;14(3):351-5.
7. Cansell C, Denis RG, Joly-Amado A, Castel J, Luquet S. Arcuate AgRP neurons and the regulation of energy balance. Front Endocrinol (Lausanne). 2012;3:169.
8. Keen-Rhinehart E, Ondek K, Schneider JE. Neuroendocrine regulation of appetitive ingestive behavior. Front Neurosci. 2013;7:213.
9. Zhang SX, Lutas A, Yang S, Diaz A, Fluhr H, Nagel G, et al. Hypothalamic dopamine neurons motivate mating through persistent cAMP signalling. Nature. 2021;597(7875):245-9.
10. Watts AG, Kanoski SE, Sanchez-Watts G, Langhans W. The physiological control of eating: signals, neurons, and networks. Physiol Rev. 2022;102(2):689-813.
11. Burghardt GM. A place for emotions in behavior systems research. Behav Processes. 2019;166:103881.
12. Schiffino FL, Siemian JN, Petrella M, Laing BT, Sarsfield S, Borja CB, et al. Activation of a lateral hypothalamic-ventral tegmental circuit gates motivation. PLoS One. 2019;14(7):e0219522.
13. de Vrind VAJ, Rozeboom A, Wolterink-Donselaar IG, Luijendijk-Berg MCM, Adan RAH. Effects of GABA and Leptin Receptor-Expressing Neurons in the Lateral Hypothalamus on

Feeding, Locomotion, and Thermogenesis. Obesity (Silver Spring). 2019;27(7):1123-32.
14. Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, et al. Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell. 2015;160(3):516-27.
15. Stuber GD, Wise RA. Lateral hypothalamic circuits for feeding and reward. Nat Neurosci. 2016;19(2):198-205.
16. Rolls ET, Burton MJ, Mora F. Hypothalamic neuronal responses associated with the sight of food. Brain Res. 1976;111(1):53-66.
17. Rolls ET, Sanghera MK, Roper-Hall A. The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey. Brain Res. 1979;164:121-35.
18. Aou S, Takaki A, Karadi Z, Hori T, Nishino H, Oomura Y. Functional heterogeneity of the monkey lateral hypothalamus in the control of feeding. Brain Res Bull. 1991;27(3-4):451-5.
19. Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA, Wichmann R, et al. Decoding neural circuits that control compulsive sucrose seeking. Cell. 2015;160(3):528-41.
20. Morgane PJ. Distinct "feeding" and "hunger motivating" systems in the lateral hypothalamus of the rat. Science. 1961;133(3456):887-8.
21. Burton MJ, Rolls ET, Mora F. Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. Exp Neurol. 1976;51(3):668-77.
22. Petrovich GD. Lateral Hypothalamus as a Motivation-Cognition Interface in the Control of Feeding Behavior. Front Syst Neurosci. 2018;12:14.
23. Nieh EH, Vander Weele CM, Matthews GA, Presbrey KN, Wichmann R, Leppla CA, et al. Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. Neuron. 2016;90(6):1286-98.
24. Qualls-Creekmore E, Yu S, Francois M, Hoang J, Huesing C, Bruce-Keller A, et al. Galanin-Expressing GABA Neurons in the Lateral Hypothalamus Modulate Food Reward and Noncompulsive Locomotion. J Neurosci. 2017;37(25):6053-65.

25. Navarro M, Olney JJ, Burnham NW, Mazzone CM, Lowery-Gionta EG, Pleil KE, et al. Lateral Hypothalamus GABAergic Neurons Modulate Consummatory Behaviors Regardless of the Caloric Content or Biological Relevance of the Consumed Stimuli. Neuropsychopharmacology. 2016;41(6):1505-12.
26. Siemian JN, Arenivar MA, Sarsfield S, Borja CB, Russell CN, Aponte Y. Lateral hypothalamic LEPR neurons drive appetitive but not consummatory behaviors. Cell Rep. 2021;36(8):109615.
27. Stamatakis AM, Van Swieten M, Basiri ML, Blair GA, Kantak P, Stuber GD. Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula Regulate Feeding and Reward. J Neurosci. 2016;36(2):302-11.
28. Berrios J, Li C, Madara JC, Garfield AS, Steger JS, Krashes MJ, et al. Food cue regulation of AGRP hunger neurons guides learning. Nature. 2021;595(7869):695-700.
29. Rossi MA, Basiri ML, McHenry JA, Kosyk O, Otis JM, van den Munkhof HE, et al. Obesity remodels activity and transcriptional state of a lateral hypothalamic brake on feeding. Science. 2019;364(6447):1271-4.
30. Leinninger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. Cell Metab. 2009;10(2):89-98.
31. Shin S, You IJ, Jeong M, Bae Y, Wang XY, Cawley ML, et al. Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus-brainstem pathway. Nat Neurosci. 2022.
32. Shin S, You IJ, Jeong M, Bae Y, Wang XY, Cawley ML, et al. Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus-brainstem pathway. Nat Neurosci. 2023;26(1):79-91.
33. Mickelsen LE, Bolisetty M, Chimileski BR, Fujita A, Beltrami EJ, Costanzo JT, et al. Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. Nat Neurosci. 2019;22(4):642-56.

34. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, Hill AJ, et al. The single-cell transcriptional landscape of mammalian organogenesis. Nature. 2019;566(7745):496-502.
35. Hanchate NK, Lee EJ, Ellis A, Kondoh K, Kuang D, Basom R, et al. Connect-seq to superimpose molecular on anatomical neural circuit maps. Proc Natl Acad Sci U S A. 2020;117(8):4375-84.
36. Lerner TN, Shilyansky C, Davidson TJ, Evans KE, Beier KT, Zalocusky KA, et al. Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. Cell. 2015;162(3):635-47.
37. Shang C, Liu A, Li D, Xie Z, Chen Z, Huang M, et al. A subcortical excitatory circuit for sensory-triggered predatory hunting in mice. Nat Neurosci. 2019;22(6):909-20.
38. Li Y, Zeng J, Zhang J, Yue C, Zhong W, Liu Z, et al. Hypothalamic Circuits for Predation and Evasion. Neuron. 2018;97(4):911-24 e5.
39. Lee YH, Kim M, Lee M, Shin D, Ha DS, Park JS, et al. Food Craving, Seeking, and Consumption Behaviors: Conceptual Phases and Assessment Methods Used in Animal and Human Studies. J Obes Metab Syndr. 2019;28(3):148-57.
40. Yang B, Karigo T, Anderson DJ. Transformations of neural representations in a social behaviour network. Nature. 2022;608(7924):741-9.
41. Omrani A, de Vrind VAJ, Lodder B, Stoltenborg I, Kooij K, Wolterink-Donselaar IG, et al. Identification of Novel Neurocircuitry Through Which Leptin Targets Multiple Inputs to the Dopamine System to Reduce Food Reward Seeking. Biol Psychiatry. 2021;90(12):843-52.
42. Kennedy A, Asahina K, Hoopfer E, Inagaki H, Jung Y, Lee H, et al. Internal States and Behavioral Decision-Making: Toward an Integration of Emotion and Cognition. Cold Spring Harb Symp Quant Biol. 2014;79:199-210.
43. Song C, Knopfel T. Optogenetics enlightens neuroscience drug discovery. Nat Rev Drug Discov. 2016;15(2):97-109.
44. Euston DR, Gruber AJ, McNaughton BL. The role of medial prefrontal cortex in memory and decision making. Neuron. 2012;76(6):1057-70.
45. Livneh Y, Ramesh RN, Burgess CR, Levandowski KM, Madara JC, Fenselau H, et al. Homeostatic circuits selectively

gate food cue responses in insular cortex. Nature. 2017;546(7660):611-6.
46. Marino RAM, McDevitt RA, Gantz SC, Shen H, Pignatelli M, Xin W, et al. Control of food approach and eating by a GABAergic projection from lateral hypothalamus to dorsal pons. Proc Natl Acad Sci U S A. 2020;117(15):8611-5.
47. Hao S, Yang H, Wang X, He Y, Xu H, Wu X, et al. The Lateral Hypothalamic and BNST GABAergic Projections to the Anterior Ventrolateral Periaqueductal Gray Regulate Feeding. Cell Rep. 2019;28(3):616-24 e5.
48. Clarke RE, Verdejo-Garcia A, Andrews ZB. The role of corticostriatal-hypothalamic neural circuits in feeding behaviour: implications for obesity. J Neurochem. 2018;147(6):715-29.
49. Giardino WJ, Eban-Rothschild A, Christoffel DJ, Li SB, Malenka RC, de Lecea L. Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci. 2018;21(8):1084-95.
50. Lee D, Hyun JH, Jung K, Hannan P, Kwon HB. A calcium- and light-gated switch to induce gene expression in activated neurons. Nat Biotechnol. 2017;35(9):858-63.
51. Hyun JH, Nagahama K, Namkung H, Mignocchi N, Roh SE, Hannan P, et al. Tagging active neurons by soma-targeted Cal-Light. Nat Commun. 2022;13(1):7692.
52. Betley JN, Xu S, Cao ZFH, Gong R, Magnus CJ, Yu Y, et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature. 2015;521(7551):180-5.
53. Sternson SM, Eiselt AK. Three Pillars for the Neural Control of Appetite. Annu Rev Physiol. 2017;79:401-23.

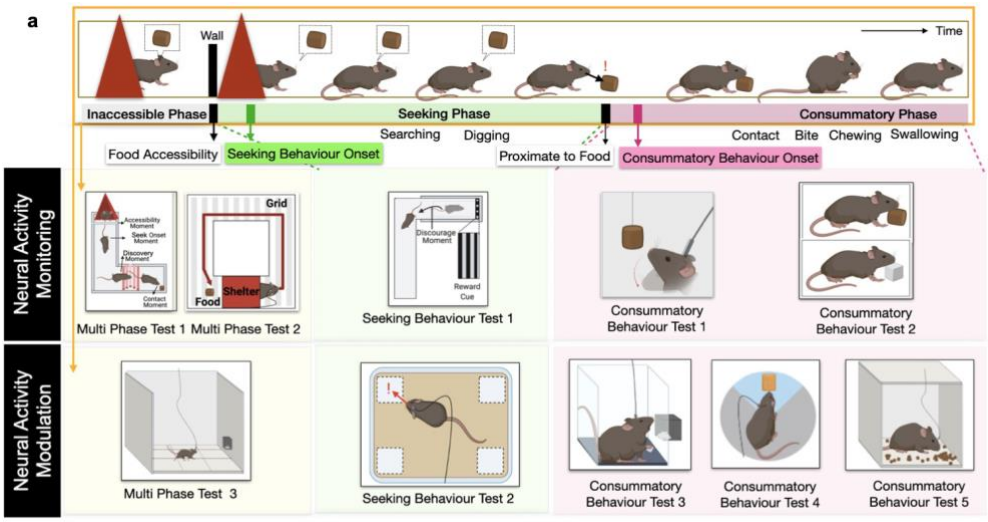


Fig. 3 | Experimental paradigms for seeking and consummatory behaviours.

a, Comprehensive summary of behavioural experiments to observe specific phases of feeding behaviours

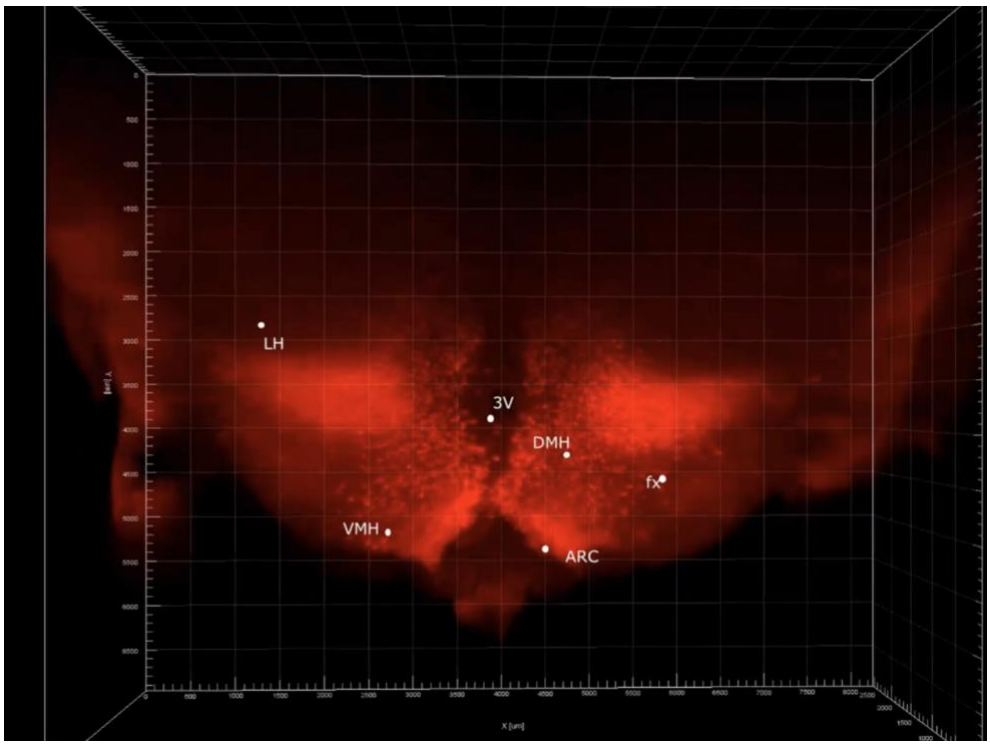


Fig. 4 | 3D Distribution of LepR neural population in the lateral hypothalamus.

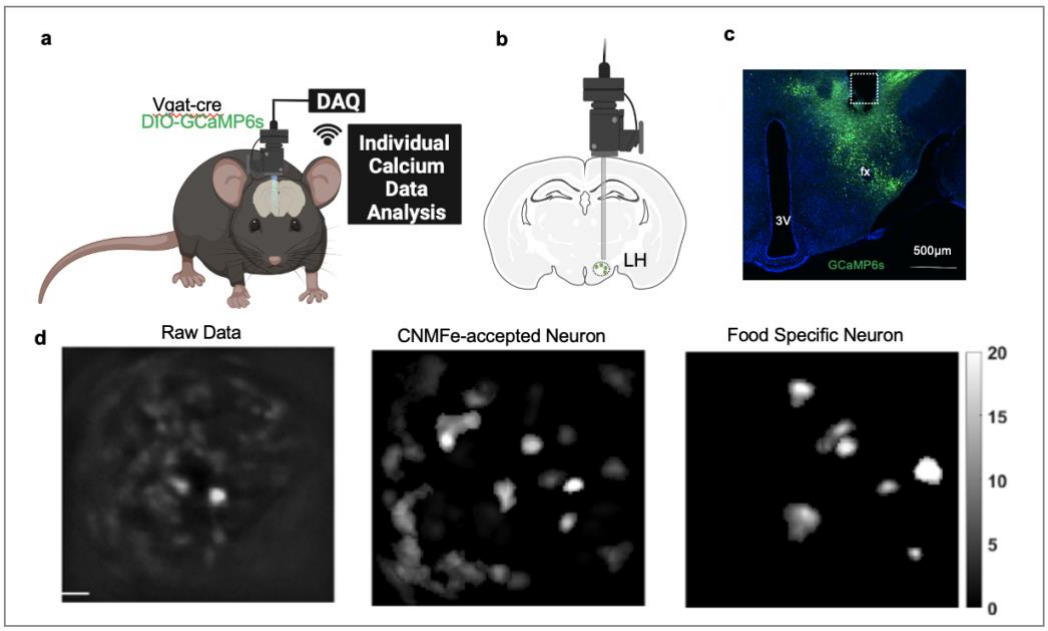


Fig. 5 |Micro-endoscopic imaging of GABAergic neurons in the lateral hypothalamus.

a, b, Schematic of micro-endoscopic calcium imaging. **c,** image of GCaMP6s expression in the LH from Vgat-cre mice. The experiment was repeated 6 times independently with similar results. fx, fornix; 3V, the 3rd ventricle **d,** Spatial map of raw data (left), accepted cells using CNMFe (middle), and cells that only respond to food-related behaviour (right) from LH^{GABA} neurons. Cells are coloured according to the maximum Z-score. Scale bar: 50μm. Yu-been Kim jointly contributed to micro-endoscope surgery.

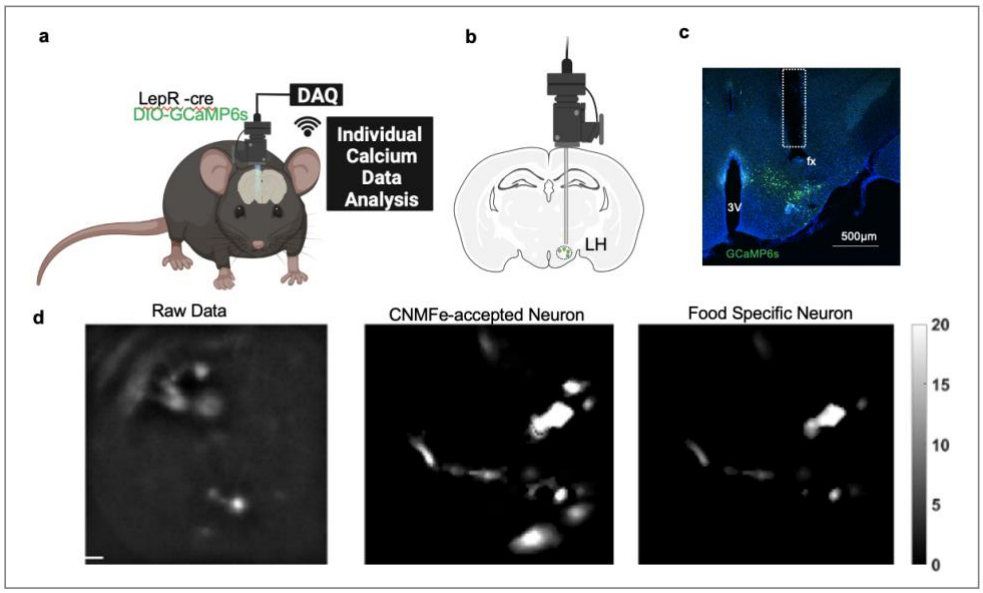


Fig. 6 |Micro-endoscopic imaging of LepR neurons in the lateral hypothalamus.

a, b, Schematic of micro-endoscopic calcium imaging. **c,** image of GCaMP6s expression in the LH from LepR-cre mice. The experiment was repeated 4 times independently with similar results. fx, fornix; 3V, the 3rd ventricle **d,** Spatial map of raw data (left), accepted cells using CNMFe (middle), and cells that only respond to food-related behaviour (right) from LH^{LepR} neurons. Cells are coloured according to the maximum Z-score. Scale bar: 50μm. Yu-been Kim jointly contributed to micro-endoscope surgery.

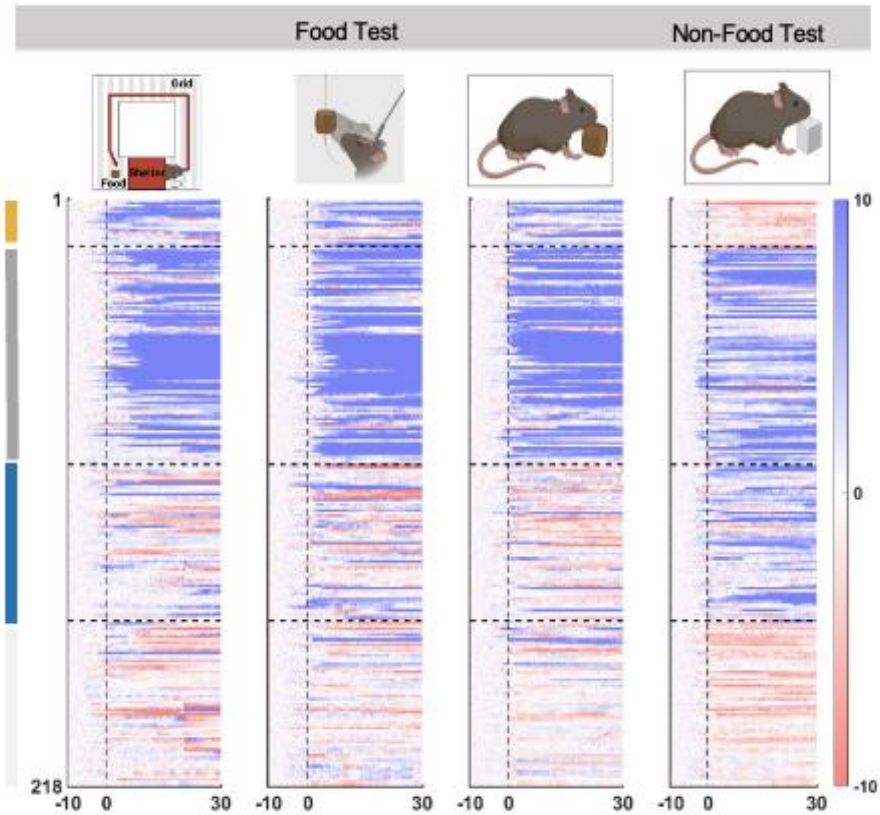


Fig. 7 | Heatmap of LH^{GABA} neurons related to micro-endoscopic imaging

Schematic of the multi-phase test 2, consummatory behaviour test 1, consummatory behaviour test 2 (food and non-food) (top). Heatmap depicting calcium signals aligned to the onset of eating behaviours (running to food, rearing to food, contact with food, contact with edible object) (below). Four populations are discriminated: food-specific responsive (yellow), non-specific responsive (grey), non-food-specific responsive (blue), and non-responsive (white) cells. (LH^{GABA} neurons 218 cells, 6 mice). Kyu Sik Kim contributed to data visualization.

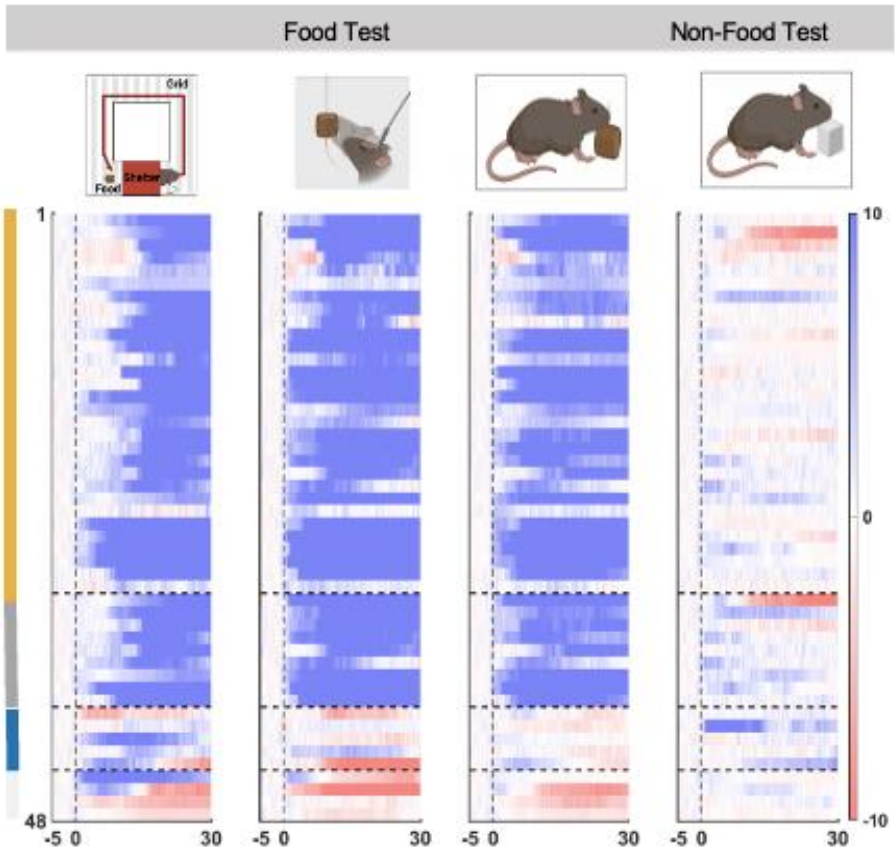
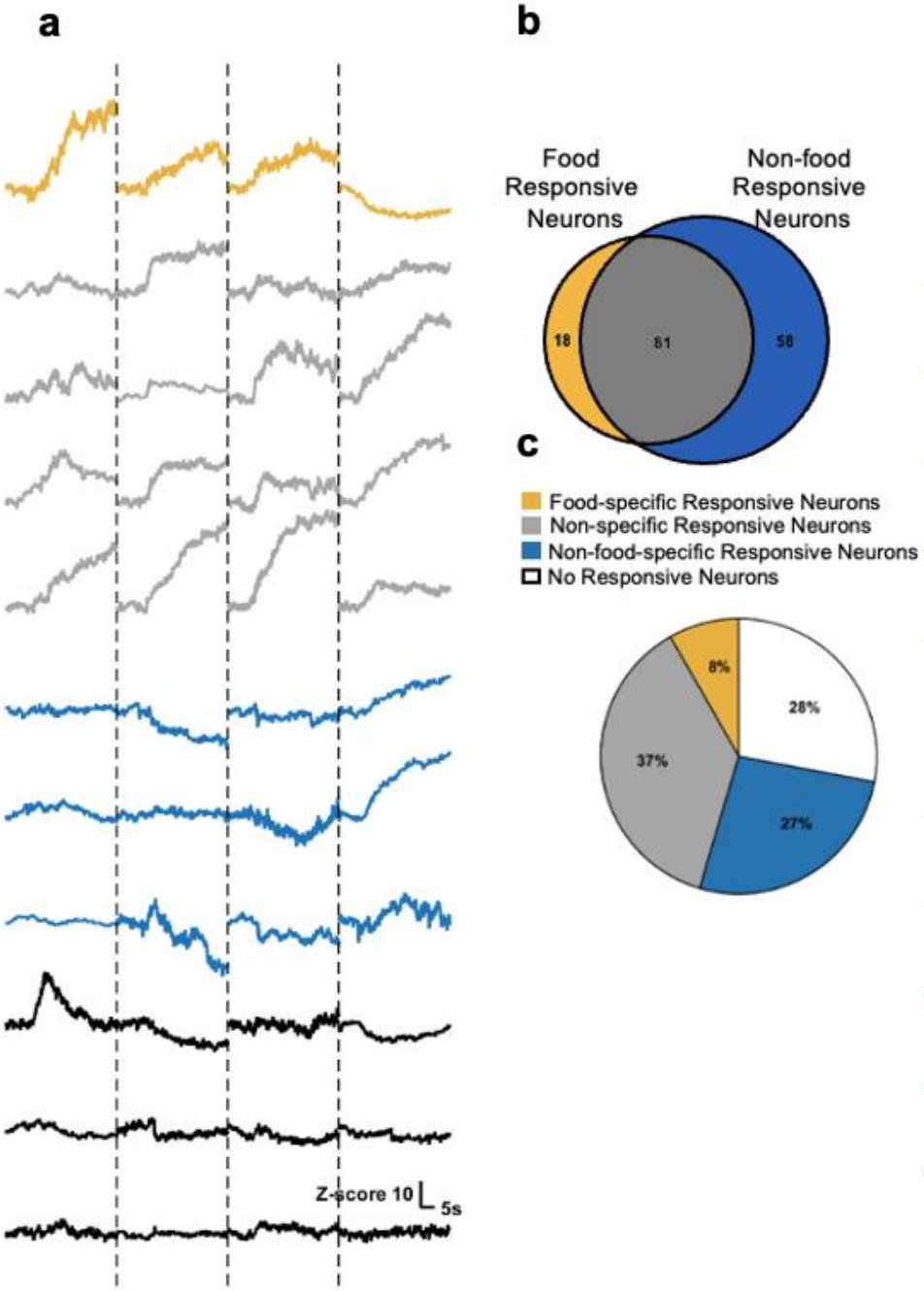


Fig. 8 | Heatmap of LH^{LepR} neurons related to micro-endoscopic imaging

a, Schematic of the multi-phase test 2, consummatory behaviour test 1, consummatory behaviour test 2 (food and non-food) (top). Heatmap depicting calcium signals aligned to the onset of eating behaviours (running to food, rearing to food, contact with food, contact with edible object) (below). Four populations are discriminated: food-specific responsive (yellow), non-specific responsive (grey), non-food-specific responsive (blue), and non-responsive (white) cells. (LH^{LepR} neurons 48 cells, 4 mice). Kyu Sik Kim contributed to data visualization.


a
b
Food Responsive Neurons
Non-food Responsive Neurons
18
81
58
c
Food-specific Responsive Neurons
Non-specific Responsive Neurons
Non-food-specific Responsive Neurons
No Responsive Neurons
8%
28%
37%
27%
Z-score 10
5s

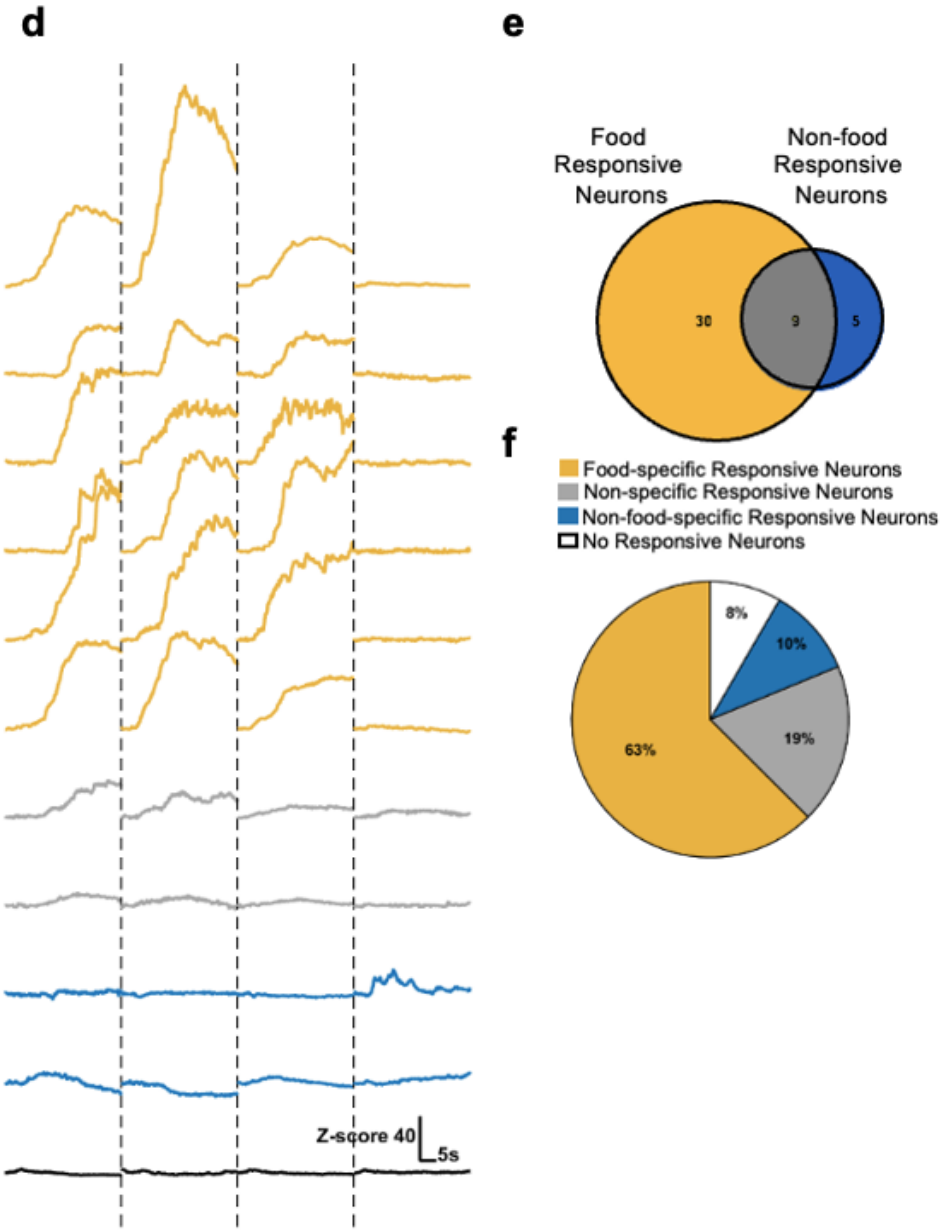


Fig. 9 | Individual neuronal trace and proportion of LH^{GABA} neurons and LH^{LepR} neurons related to micro-endoscopic imaging

a,d, Representative traces of four populations from LH^{GABA} neurons (**a**) and LH^{LepR} neurons (**d**). The dotted line separates each behavioural experiment. **b,e,** Venn diagram of food responsive and non-food responsive neurons. Percentage of food-responsive neurons are as follows (LH^{GABA} neurons 8% (18 / 218 cells) (**b**), LH^{LepR}

neurons 63% (30 / 48 cells) (**e**). **c, f,** Proportion of food-specific responsive (yellow), non-specific responsive (grey), non-food-specific responsive (blue), and non-responsive (white) cells from LH^{GABA} neurons (**c**) and LH^{LepR} neurons (**f**). Yu-been Kim and Kyu Sik Kim contributed to data visualization and behavioural experiment.

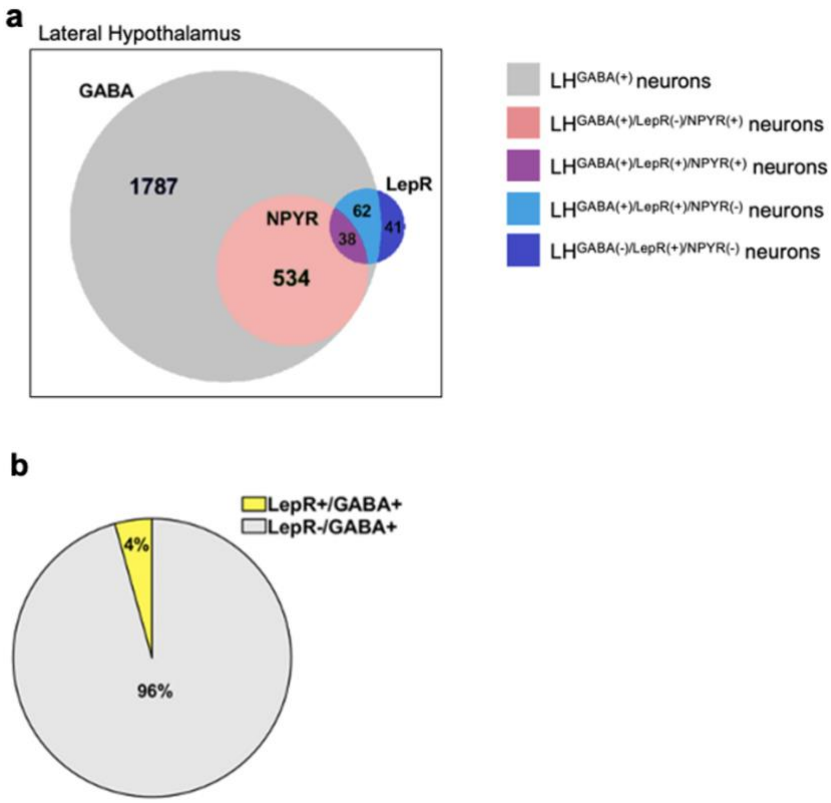


Fig. 10 | Molecular identity of LepR neural population in the lateral hypothalamus.

a, Venn diagram of molecular characteristics (GABA, NPYR, LepR) of the LH neurons based on single-cell RNA sequencing data. **b**, Proportion of LepR-positive (yellow) and LepR-negative (grey) neurons among GABA-positive neurons based on single-cell RNA sequencing data.

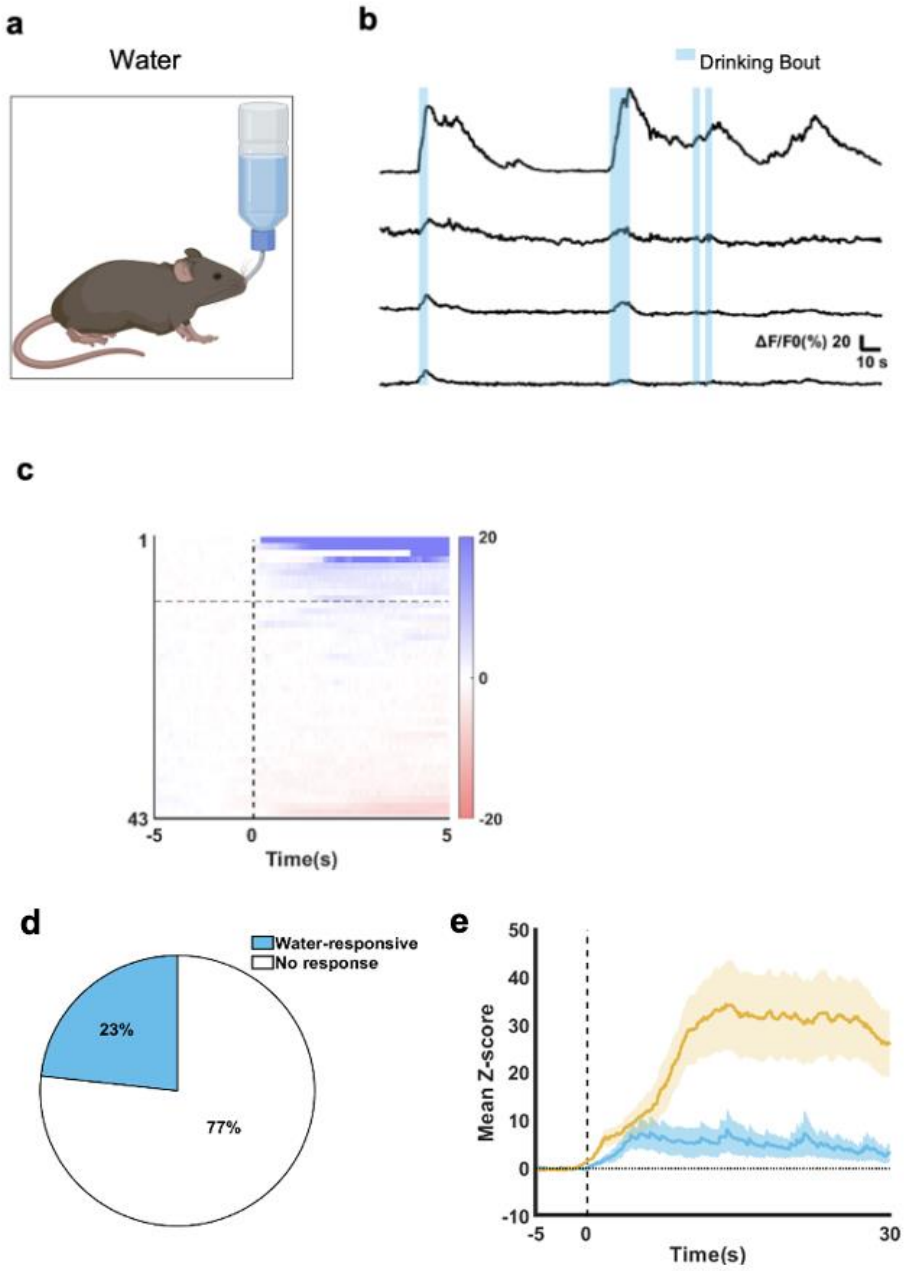


Fig. 11 | Activity of LH^{LepR} neurons is food-specific.

a, Schematic of the behavioural test for drinking water in the dehydration state. **b**, Representative single-cell traces of LH^{LepR} neurons. The blue shaded box indicates each bout of drinking. **c**, Heatmap depicting calcium signals aligned with drinking behaviour. Water-responsive cells (blue) activated (>4σ) during drinking water.

(LH^{LepR} neurons 48 cells, 4 mice). **d**, Proportion of water-responsive cells (blue, 23%) and no responsive cells (white, 77%). **e**, Average Z-score from the LH^{LepR} calcium signal aligned to drinking behaviour for water (blue) and feeding behaviour for food (yellow). Kyu Sik Kim contributed to data visualization.

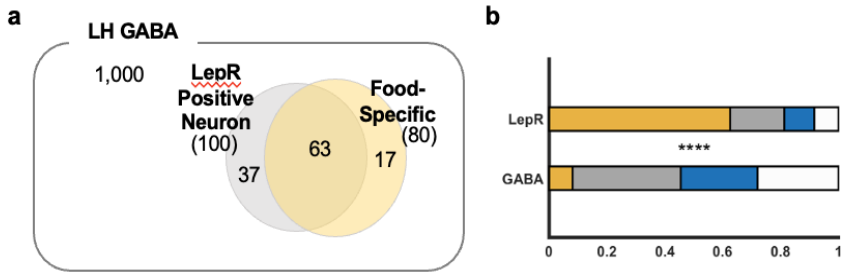


Fig. 12 | Comparison neuronal activity of LH^{GABA} neurons and of LH^{LepR} neurons related to micro-endoscopic imaging

a, Venn diagram simulating the number of LH^{LepR} positive (yellow) and food-specific (grey) neurons when the total number of LH ^{GABA} neurons is simulated as 1,000. **b**, Comparison between the proportion of LH ^{GABA} neurons and proportion of LH ^{LepR} neurons for food-specific neurons responsive (yellow), non-specific responsive (grey), non-food-specific responsive (blue), and non-responsive (white) cells. Chi-square statistic of cross-tabulation table; **** $p < 0.0001$

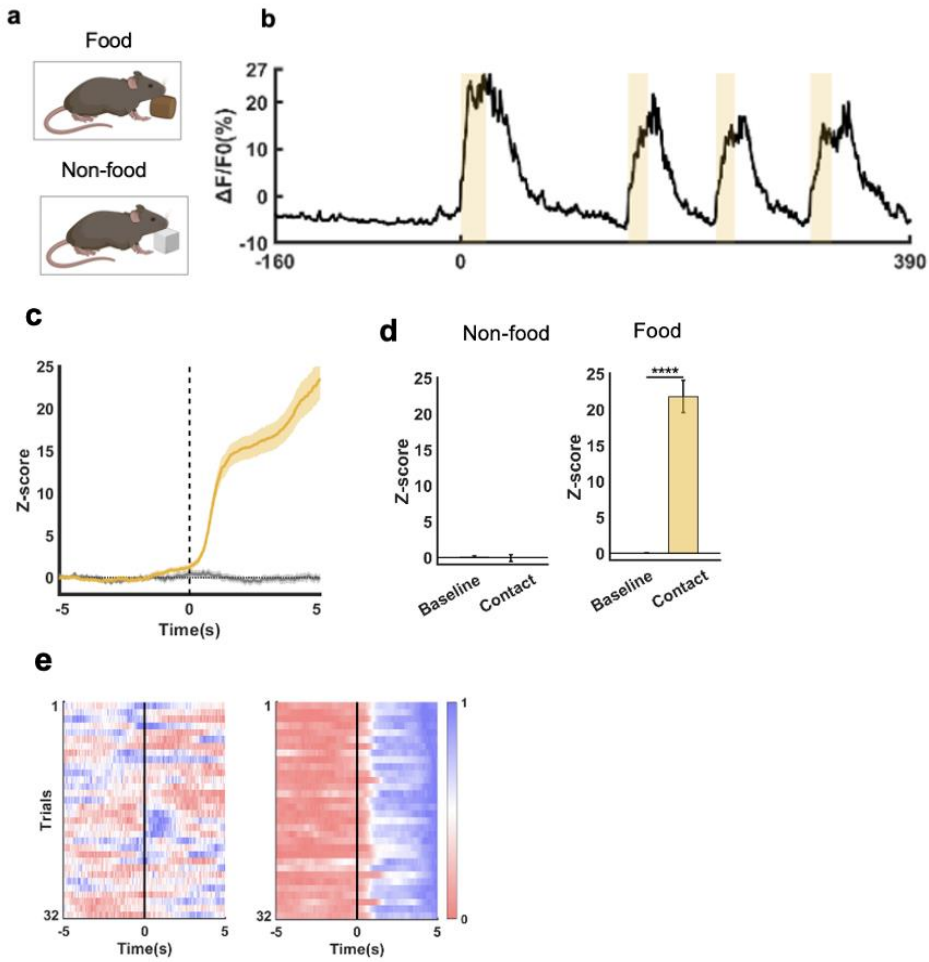


Fig. 13 | LH^{LepR} neurons are response to food.

a, Schematic of consummatory behaviour test 2 (food/non-food). **b**, Representative calcium traces from the LH^{LepR} calcium signal during (**a**). The yellow shaded box indicates behaviour from food contact to end of consumption, and the grey line indicates contact with non-food. **c**, Average Z-score from the LH^{LepR} calcium signal aligned to contact with food (yellow) and non-food (grey). **d**, Quantification of the Z-score in calcium signal changes from (**c**). Comparison between baseline (-8 to -7s) and after contact (4 to 5s). (4 mice; 32 trials). **e**, Heatmap depicting the normalised LH^{LepR} calcium signal aligned to contact with non-food and food. Yu-been Kim and Kyu Sik Kim jointly performed behavioural tests.

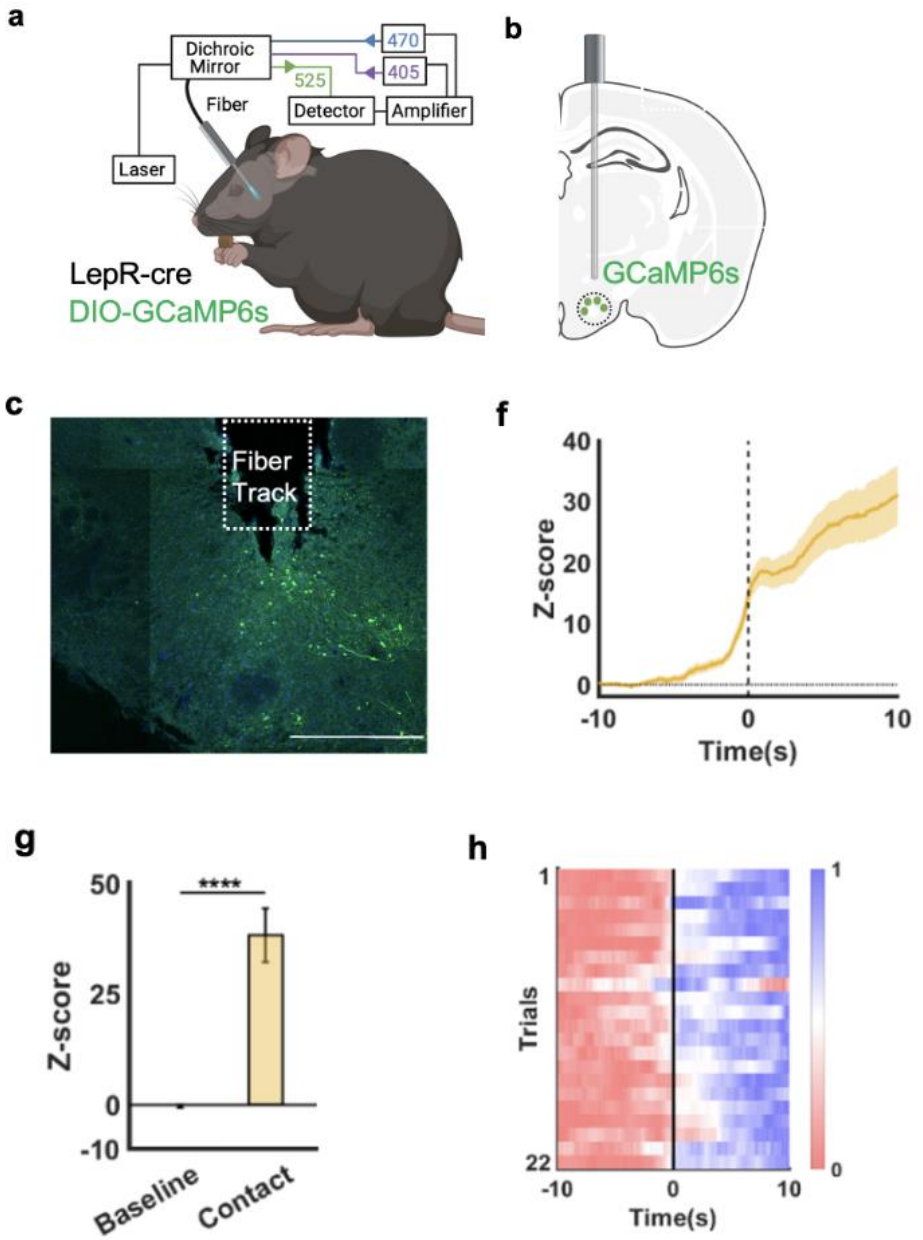
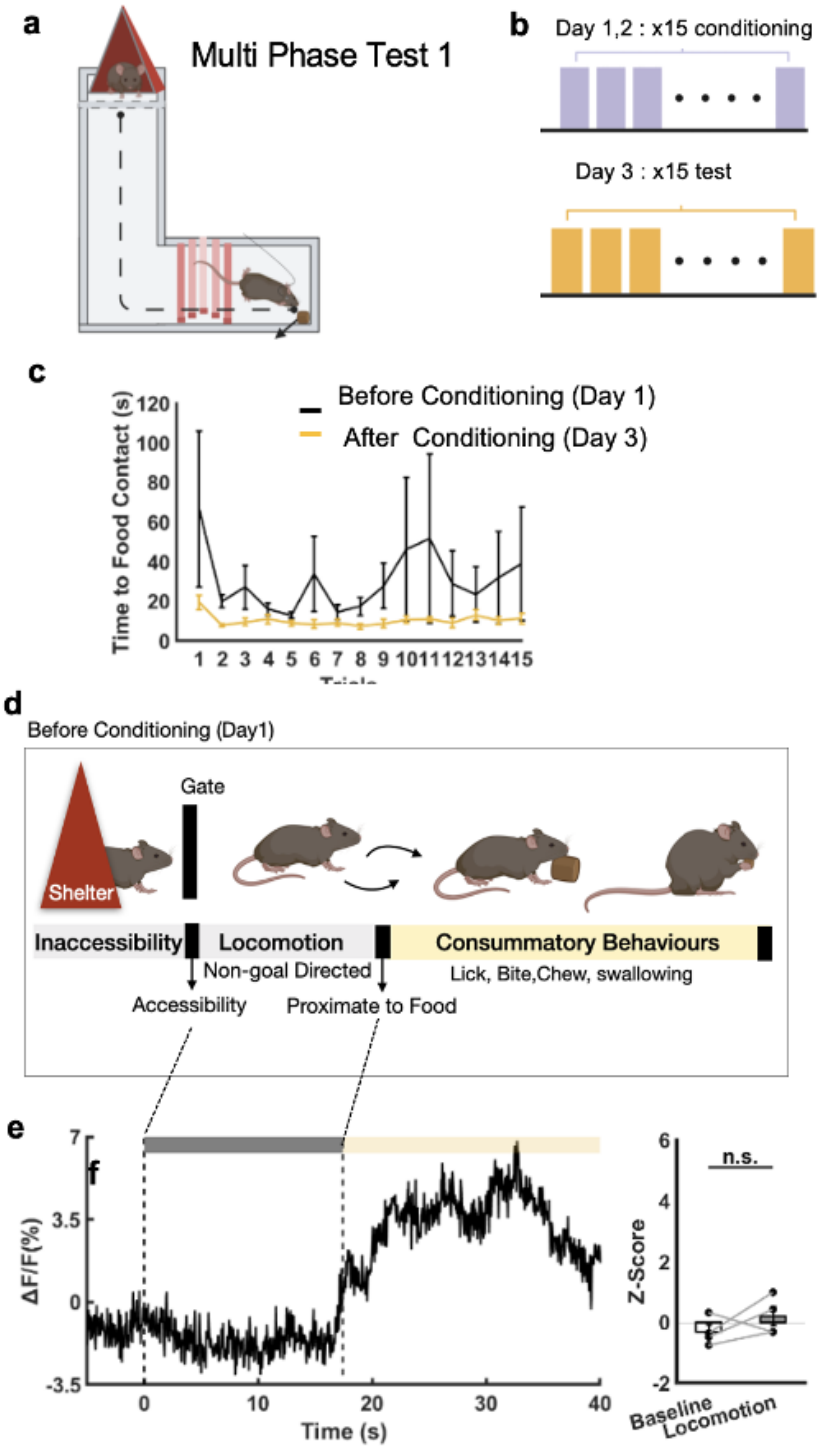
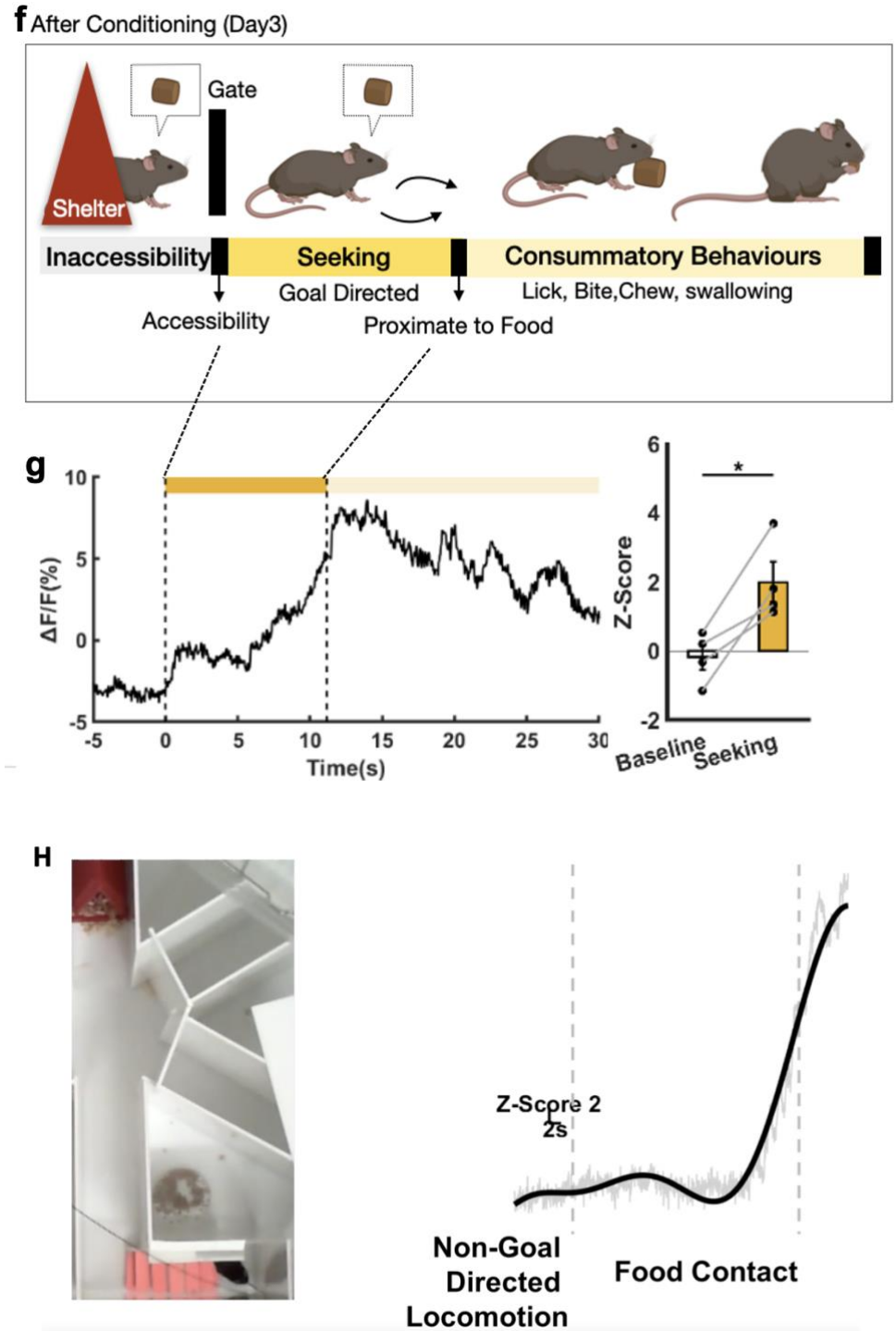


Fig.14 | Activity of LH^{LepR} neurons is time locked to eating behaviours.

a,b, Schematic of virus injection/fibre insertion for fibre photometry in LH from LepR-cre mice. **c,** A representative image validates GCaMP6s expression in LepR neurons and optical fibre tract above the LH. Scale bar: 500μm. The experiment was repeated 5 times independently with similar results. fx, fonix. **d**, Schematic of the consummatory behaviour test 1 (obtainable). **e,** Representative calcium traces from LH^{LepR} neurons. Yellow shaded box: from the moment of food contact to the

end of food consumption. **f,** Average Z-score from LH^{LepR} calcium response aligned to contact with food (5 mice, 22 trials). **g,** Quantification of Z-score in calcium signal change from **(e)**. Comparison between baseline (-8 to -7s) and after contact (9 to 10s). **h**, Heatmap depicting normalised LH^{LepR} neural activity aligned to the moment of contact with food. **i**, Representative behavioural test and calcium traces from LH^{LepR} neurons. Yu-been Kim and Kyu Sik Kim contributed to data visualization.


a
Multi Phase Test 1
b
Day 1,2 : x15 conditioning
Day 3 : x15 test
c
Before Conditioning (Day 1)
After Conditioning (Day 3)
Time to Food Contact (s)
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
d
Before Conditioning (Day1)
Gate
Shelter
Inaccessibility
Locomotion
Consummatory Behaviours
Non-goal Directed
Lick, Bite,Chew, swallowing
Accessibility
Proximate to Food
e
f
ΔF/F(%)
7
3.5
0
-3.5
0
10
20
30
40
Time (s)
Z-Score
6
4
2
0
-2
n.s.
Baseline
Locomotion


f After Conditioning (Day3)
Gate
Shelter
Inaccessibility
Seeking
Consummatory Behaviours
Goal Directed
Lick, Bite,Chew, swallowing
Accessibility
Proximate to Food
g
ΔF/F(%)
Time(s)
Z-Score
Baseline
Seeking
H
Z-Score 2
2s
Non-Goal Directed Locomotion
Food Contact

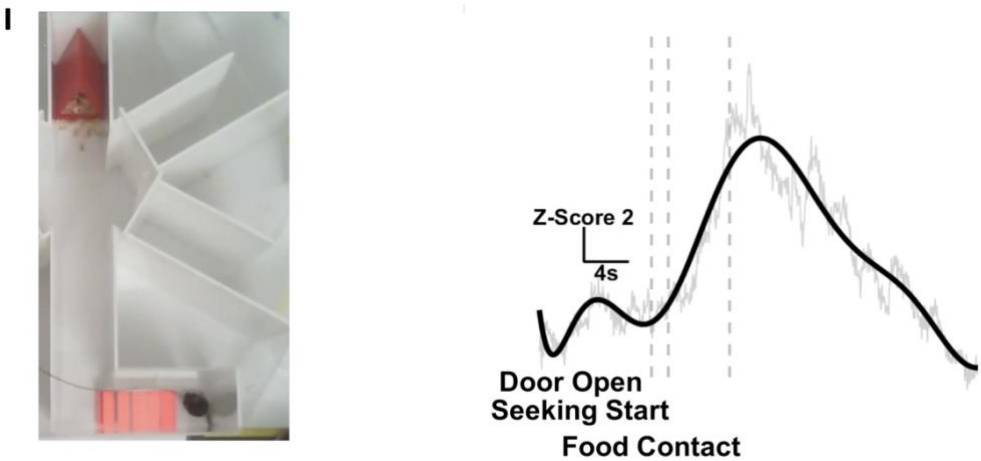


Fig.15 | Activity of LH^{LepR} neurons is time-locked to seeking and consummatory behaviours.

a,b, Schematic and schedule of the multi-phase test 1. **c**, Time from food accessibility to food contact before and after conditioning (n = 4 mice). **d, e,** Eating phase before conditioning (**d**) and after conditioning (**e**). **f,g,** Representative calcium signal of LH^{LepR} neurons aligned to food accessibility (left) and quantification of Z-score in calcium signal change (right) before (**d**) and after (**e**) conditioning. Comparison between baseline (-2 to -1s) and after locomotion or seeking behaviour (1 to 2s). Two-sided paired t-test; n.s., $p > 0.5$ (**f**), * $p = 0.02$ (**g**). Data are mean ±s.e.m. Yu-been Kim and Kyu Sik Kim contributed to data visualization.

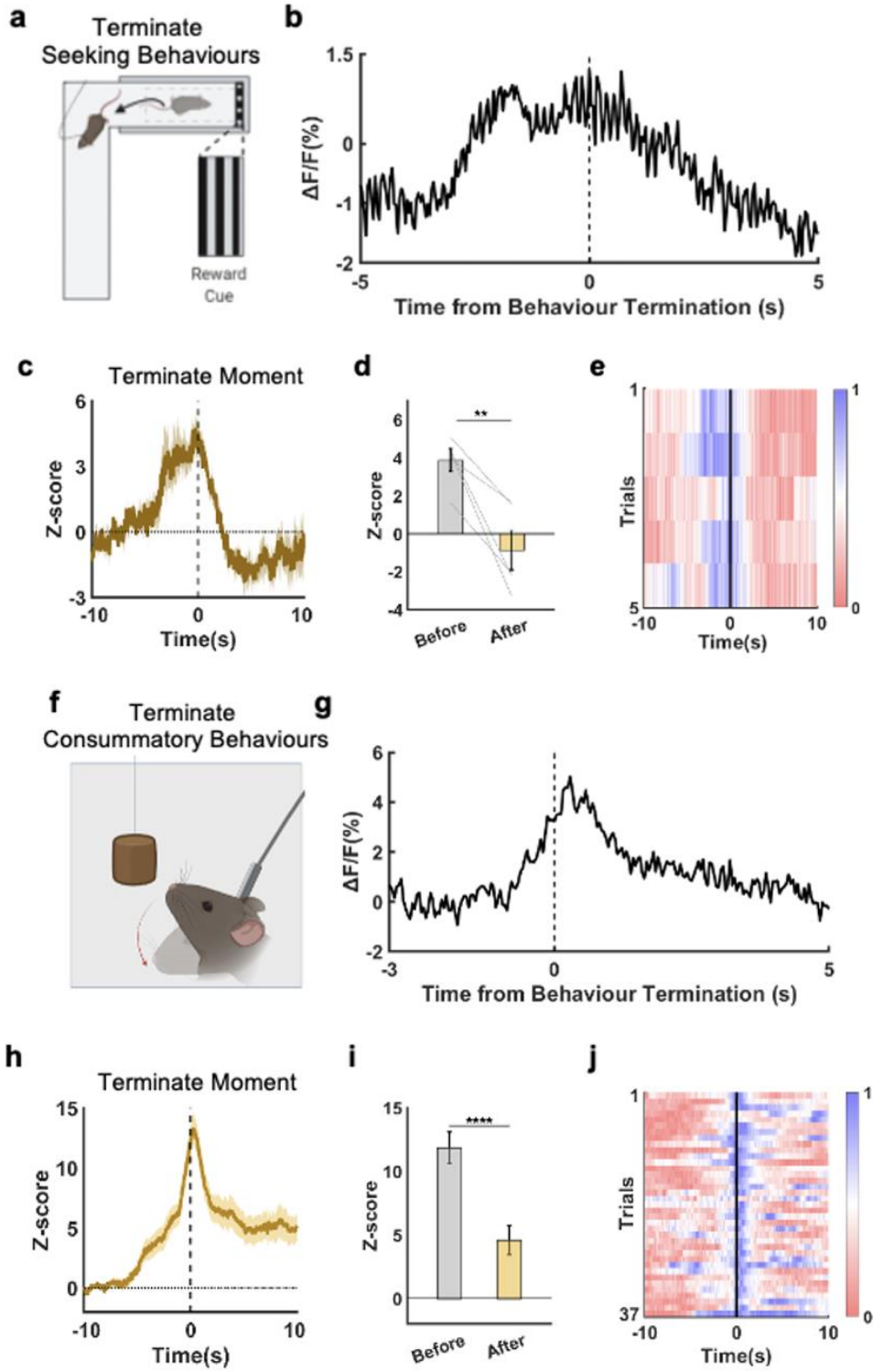


Fig.16 | LH^{LepR} neurons are inactivated at termination moment of seeking and

consummatory behaviours.

a, **f**, Schematic of the seeking behaviour test 1 (**a**) and consummatory behaviour test 1 (unobtainable) (**f**). **b**, **g**, Representative calcium signal of LH^{LepR} neurons aligned to the termination moment of seeking (**b**) and consummatory (**g**) behaviours. **c**, **h**, Average Z-score from LH^{LepR} neurons aligned to the termination moment of seeking (**c**) and consummatory (**h**) behaviours. **d**, **i**, Quantification of the Z-score from (**c**, **h**). Comparison between the before (0 to 1s) and (9–10s) after behavioural termination. (**d:** 1 mouse, 5 trials) (**i:** 5 mice, 37 trials). **e**, **j**, Heatmap depicting the normalised LH^{LepR} calcium signal aligned to the termination moment of seeking (**e**) and consummatory (**j**) behaviours. Data are mean ± s.e.m. Source data are provided as a Source Data file. The schematics in a and f were created using BioRender. Kyu Sik Kim performed behavioural tests.

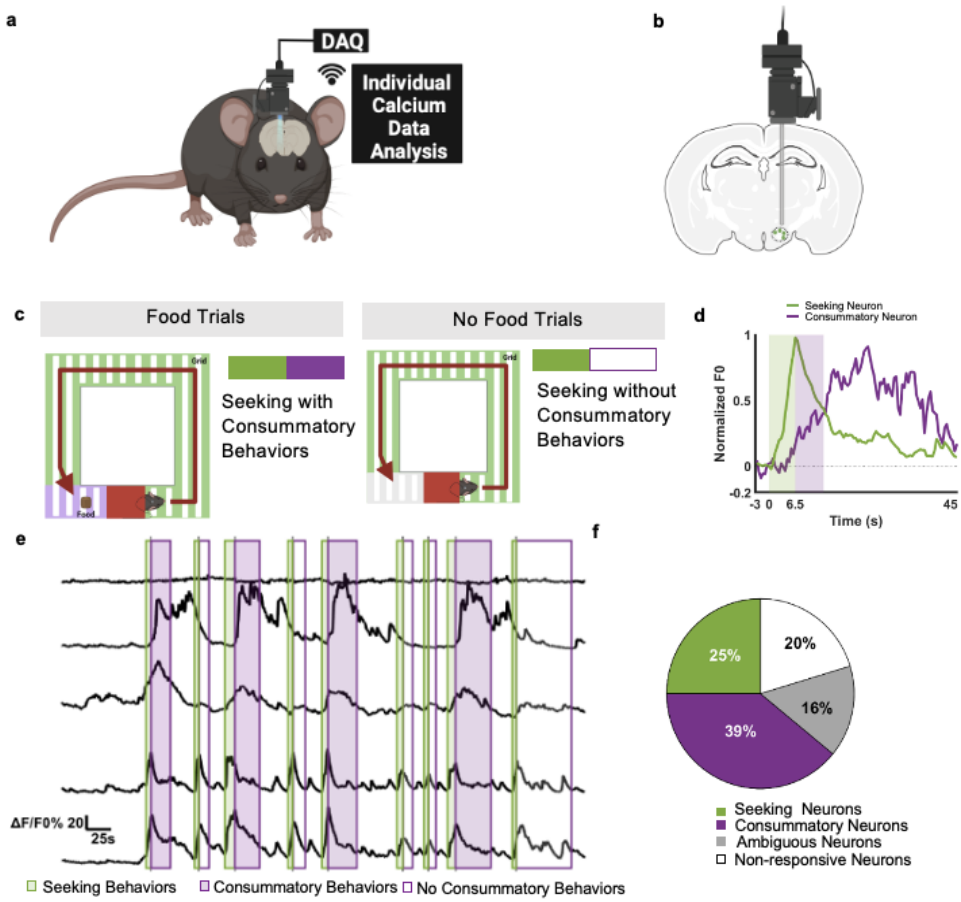


Fig. 17 | Two distinct populations of LH^{LepR} neurons encode seeking and consummatory behaviours.

a,b, Schematic of virus injection/GRIN lens insertion for micro-endoscopic calcium imaging in the LH from LepR-cre mice. **c**, Schematic of the multi-phase test 2. Seeking with consummatory behaviours, in the presence of food (left). Seeking without consummatory behaviours, in the absence of food (right). **d,e,**Representative single cell traces of LH^{LepR} neurons within one trial (**d**) and several trials (**e**). Green shaded box indicated seeking behaviours and purple shaded box indicated consummatory behaviours. **e**, Proportion of cell populations. Yu-been Kim and Kyu Sik Kim jointly contributed to data visualization.

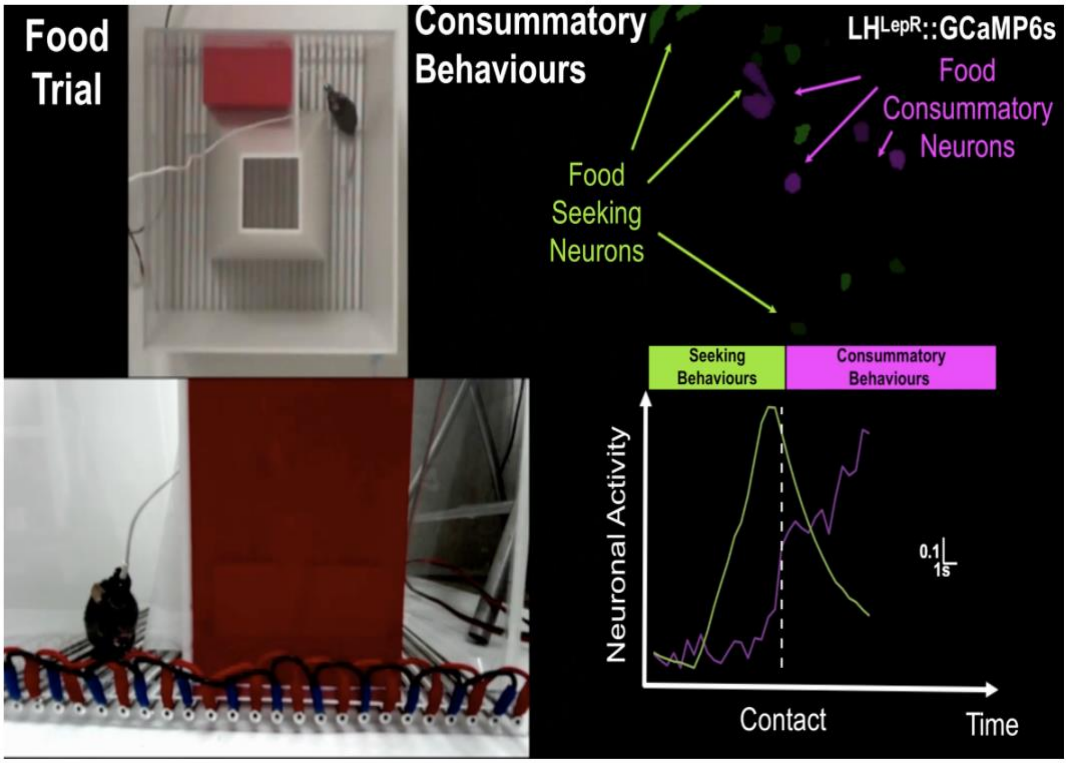
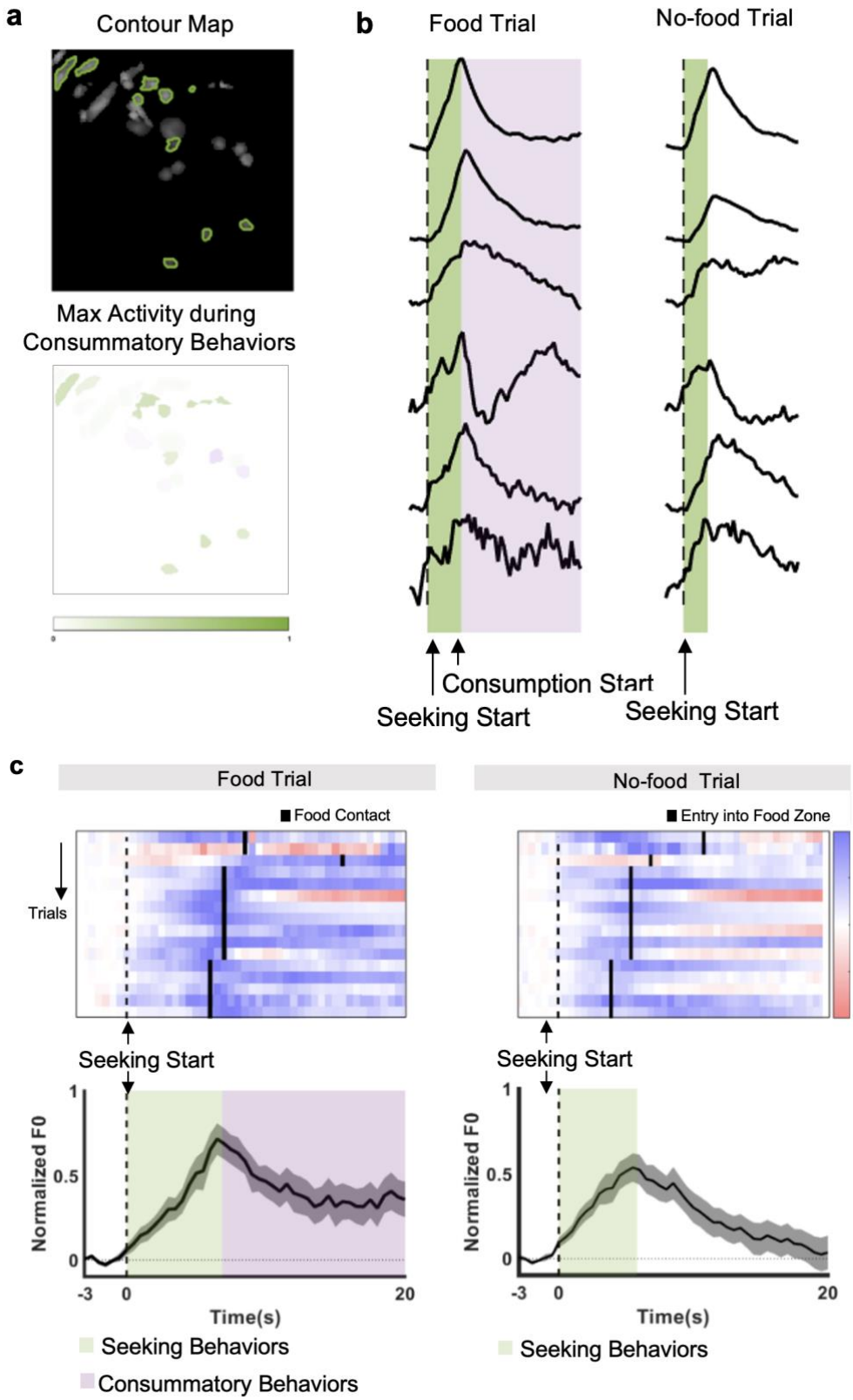


Fig. 18 | Two distinct populations of LH^{LepR} neurons encode seeking and consummatory behaviours. Representative video of multi-phase test 2. Yu-been Kim and Kyu Sik Kim jointly contributed to data visualization.


a
Contour Map
Max Activity during
Consummatory Behaviors
b
Food Trial
No-food Trial
Consumption Start
Seeking Start
Seeking Start
c
Food Trial
No-food Trial
Food Contact
Entry into Food Zone
Trials
Seeking Start
Seeking Start
Normalized F0
Time(s)
Seeking Behaviors
Consummatory Behaviors
Seeking Behaviors

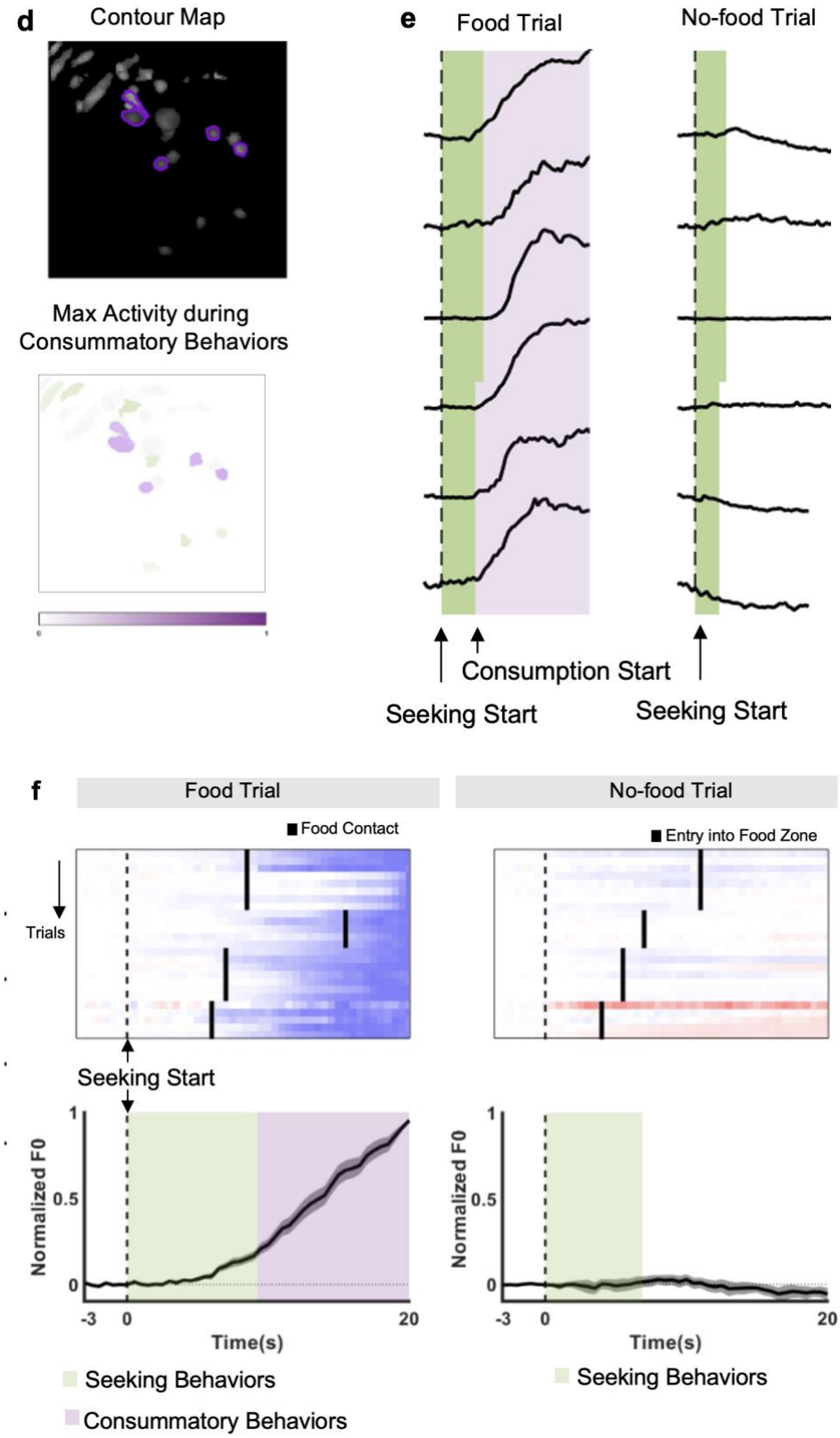

d
Contour Map
Max Activity during
Consummatory Behaviors
e
Food Trial
No-food Trial
Consumption Start
Seeking Start
Seeking Start
f
Food Trial
No-food Trial
Food Contact
Entry into Food Zone
Trials
Seeking Start
Normalized F0
1
0.5
0
-3
0
20
Time(s)
Seeking Behaviors
Consummatory Behaviors
Normalized F0
1
0.5
0
-3
0
20
Time(s)
Seeking Behaviors

Fig. 19 | Two distinct populations of LH^{LepR} neurons encode seeking and consummatory behaviours.

Representative contour map of seeking (**a,** green) and consummatory (**d,** purple) neurons of an accepted cell (top). The degree of colour brightness represents the cell activity degree (max Z-score) (bottom). **b, e**, Representative single cell traces of LH^{LepR} neurons of seeking (**b**) and consummatory (**e**) neurons during food and no-food trials. **c,f,** Heatmap depicting the calcium signals (top) and average Z-scores (bottom) of seeking neurons (**c**) or consummatory neurons (**f**). The magnitude of the calcium signals corresponds to its colour density. (4 mice, 15 cells (**h,i**), 4 mice, 25 cells (**l,m**)). Data are mean ± s.e.m. Source data are provided as a Source Data file. The schematics in a and b were created using BioRender. Yu-been Kim and Kyu Sik Kim contributed to data visualization.

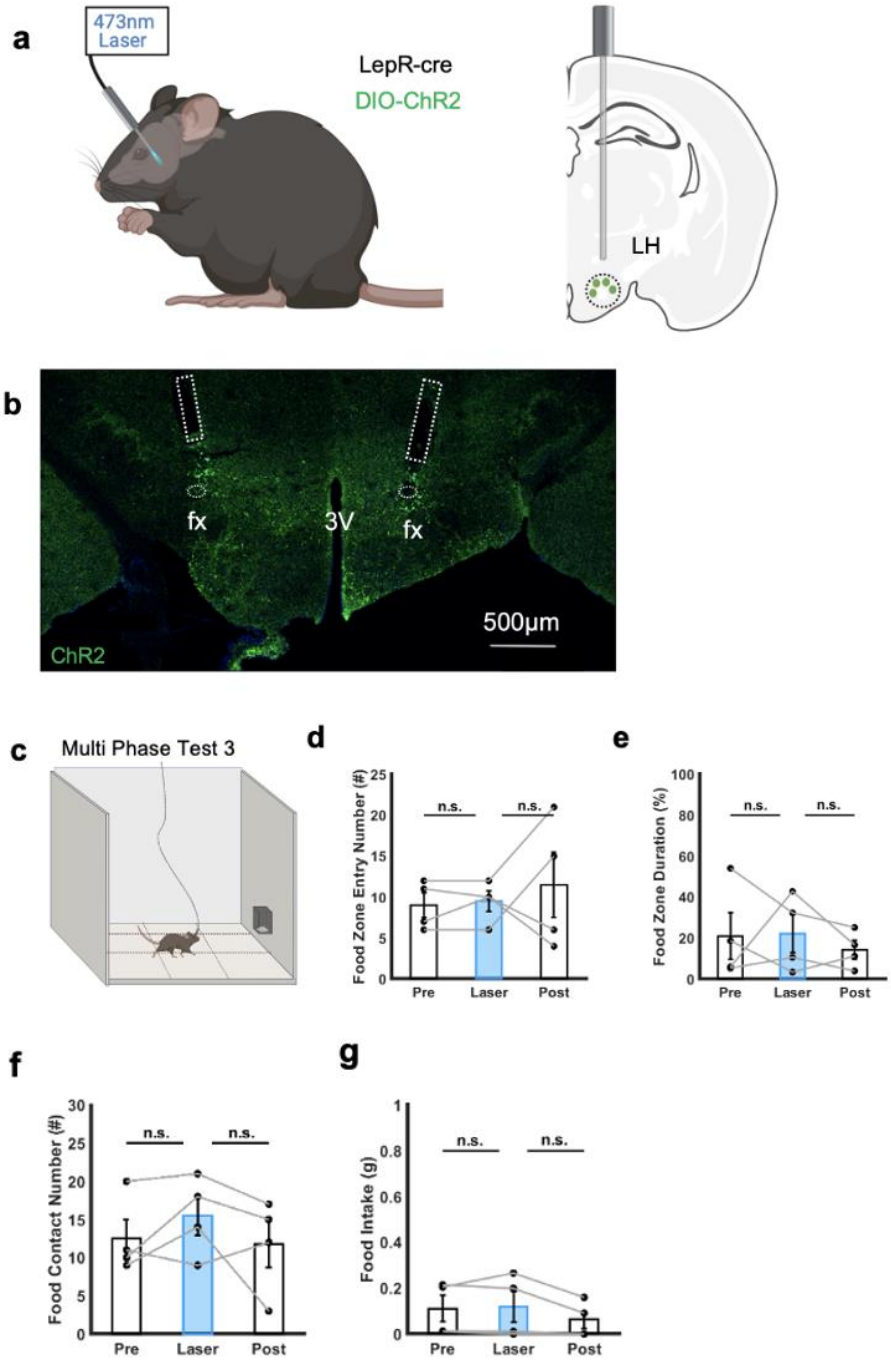


Fig. 20 | Activation of LH^{LepR} neurons did not drive eating behaviours during phase combination tests

a, b, Schematic of optogenetic activation and image of ChR2 expression in LH^{LepR} neurons. The experiment was repeated at least 4 times independently with similar results. fx, fornix; 3V, the 3rd ventricle. **c**, Schematic of the multi-phase test 3. **d-g**,

Number of food zone entries (**d**), duration in the food zone (**e**), number of food contacts (**f**) and food intakes (**g**) (n = 4 mice). Two-sided paired t-test; n.s., $p > 0.5$. Kyu Sik Kim jointly performed behavioural tests.

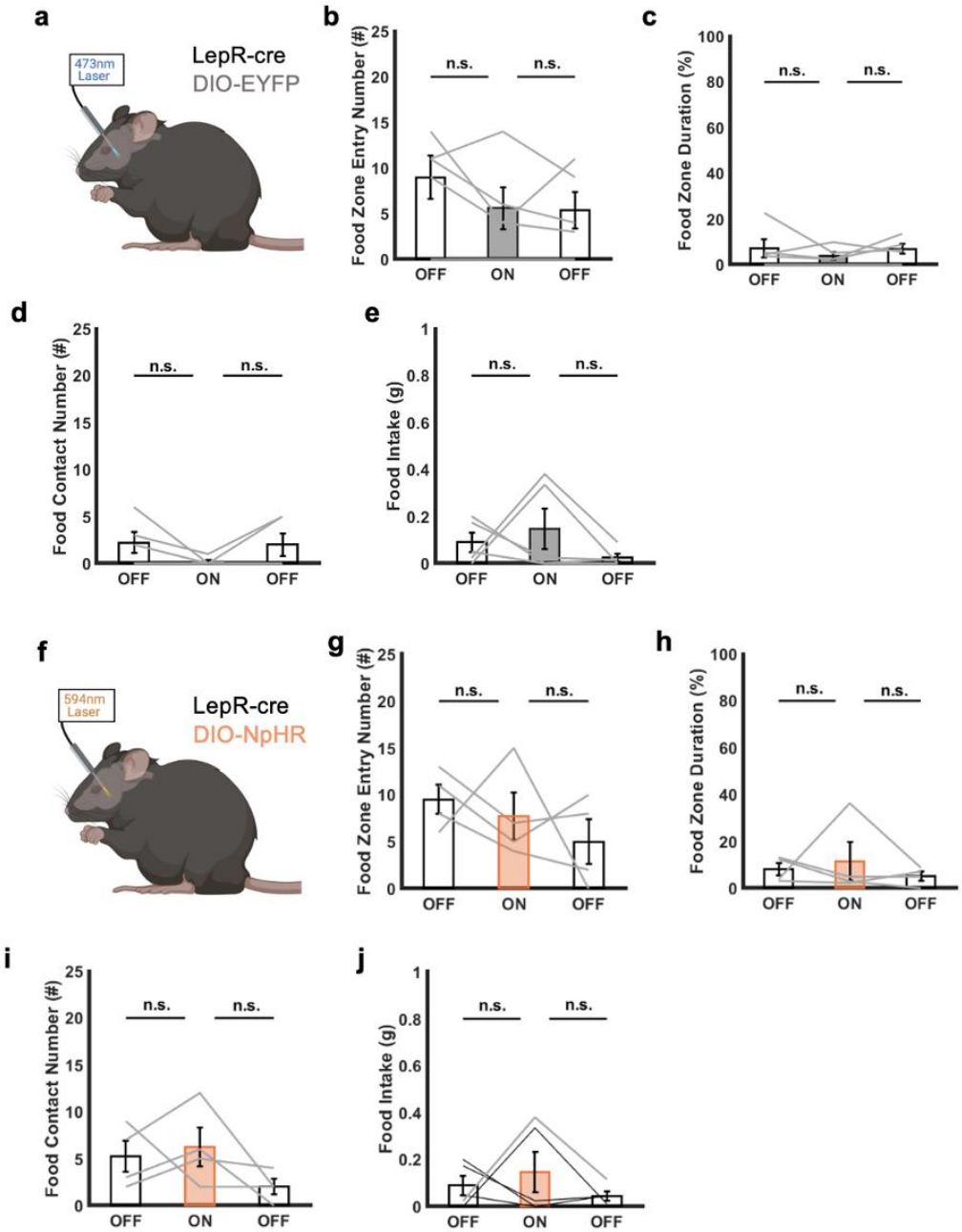
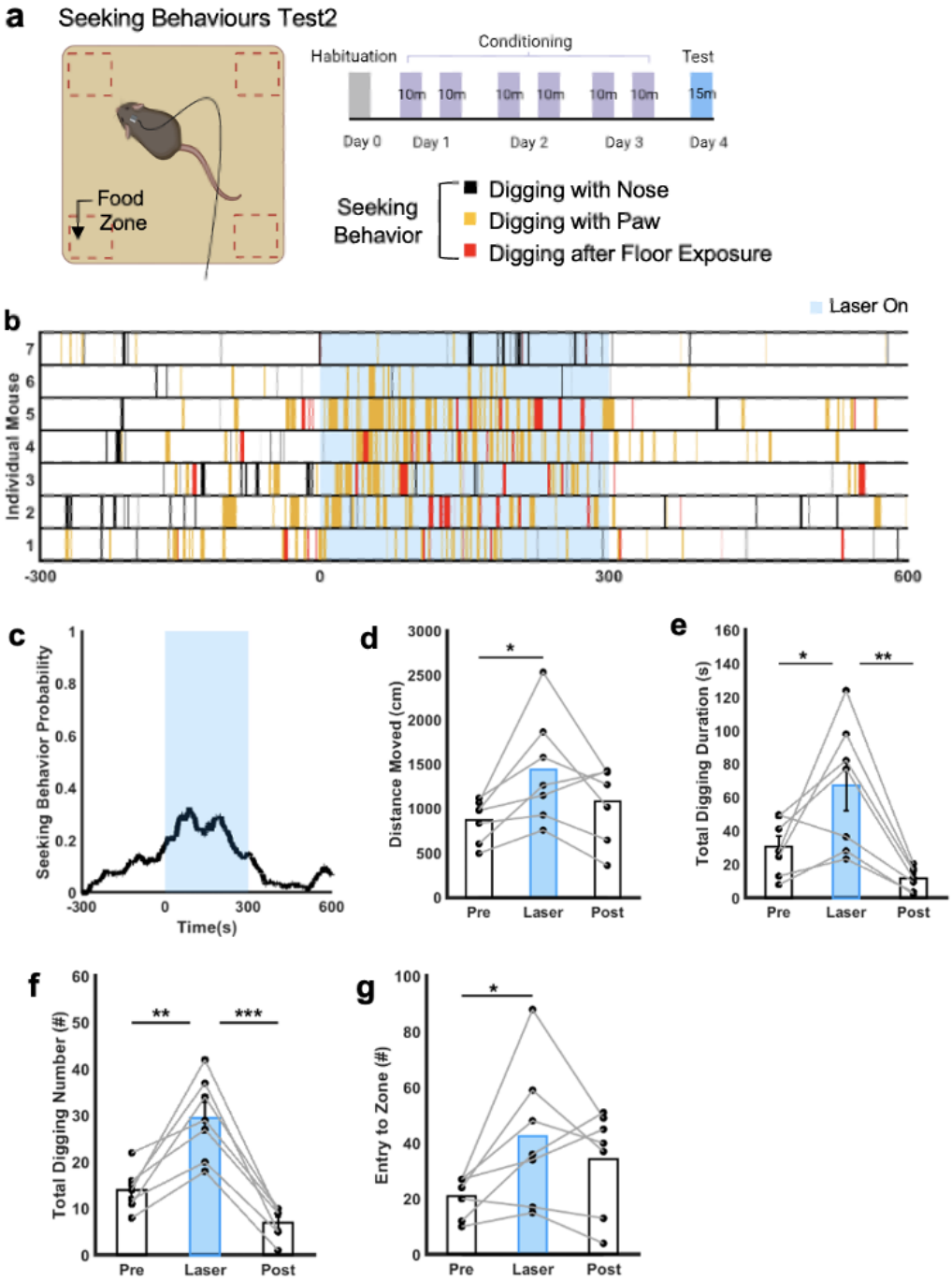


Fig. 21 | Control test of Figure 20.

a, f, Schematic of optogenetic control (**a**) and inhibition (**f**) of LH^{LepR} neurons during multi-phase test 3. **b-e**, Number of food zone entries (**b**), duration in the food zone (**c**), number of food contacts (**d**) and food intakes (**e**) from EYFP-injected mice (n = 4 mice). Two-sided paired t-test; n.s., $p > 0.05$. **g-i**, Number of

food zone entries (**g**), duration in the food zone (**h**), number of food contacts (**i**) and food intakes (**j**) from NpHR-injected mice (n = 4 mice). Two-sided paired t-test; n.s., $p > 0.5$.


a
Seeking Behaviours Test2
Food Zone
Habituation
Conditioning
Test
10m
15m
Day 0
Day 1
Day 2
Day 3
Day 4
Seeking Behavior
Digging with Nose
Digging with Paw
Digging after Floor Exposure
b
Laser On
Individual Mouse
-300
0
300
600
c
Seeking Behavior Probability
Time(s)
d
Distance Moved (cm)
Pre
Laser
Post
e
Total Digging Duration (s)
f
Total Digging Number (#)
g
Entry to Zone (#)

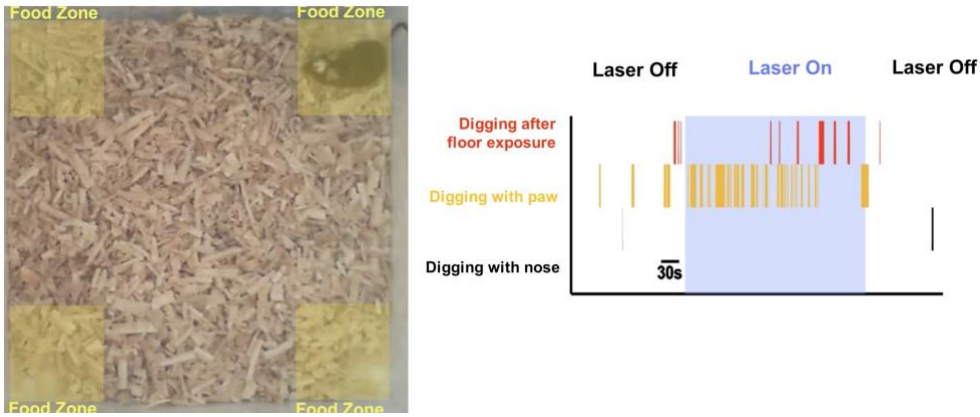


Fig. 22 | Activation of LH^{LepR} neurons drives seeking behaviours

a, Schematic and schedule of the seeking behaviour test 2. **b**, Raster plot during (**a)**. **c**, Behavioural probability from (**b**). **d-g**, Quantification of distance moved (**d**), total digging duration (**e**), number of digging behaviours (**f**) and frequency of food zone entries (**g**) (n = 7 mice). Two-sided paired t-test; * p = 0.02 (**d**, pre vs laser), * p = 0.04 (**e**, pre vs laser), ** p = 0.003 (**e,** laser vs post), ** p = 0.002 (**f**, pre vs laser), *** p = 0.00018 (**f**, laser vs post), * p = 0.04 (**g**, pre vs laser).

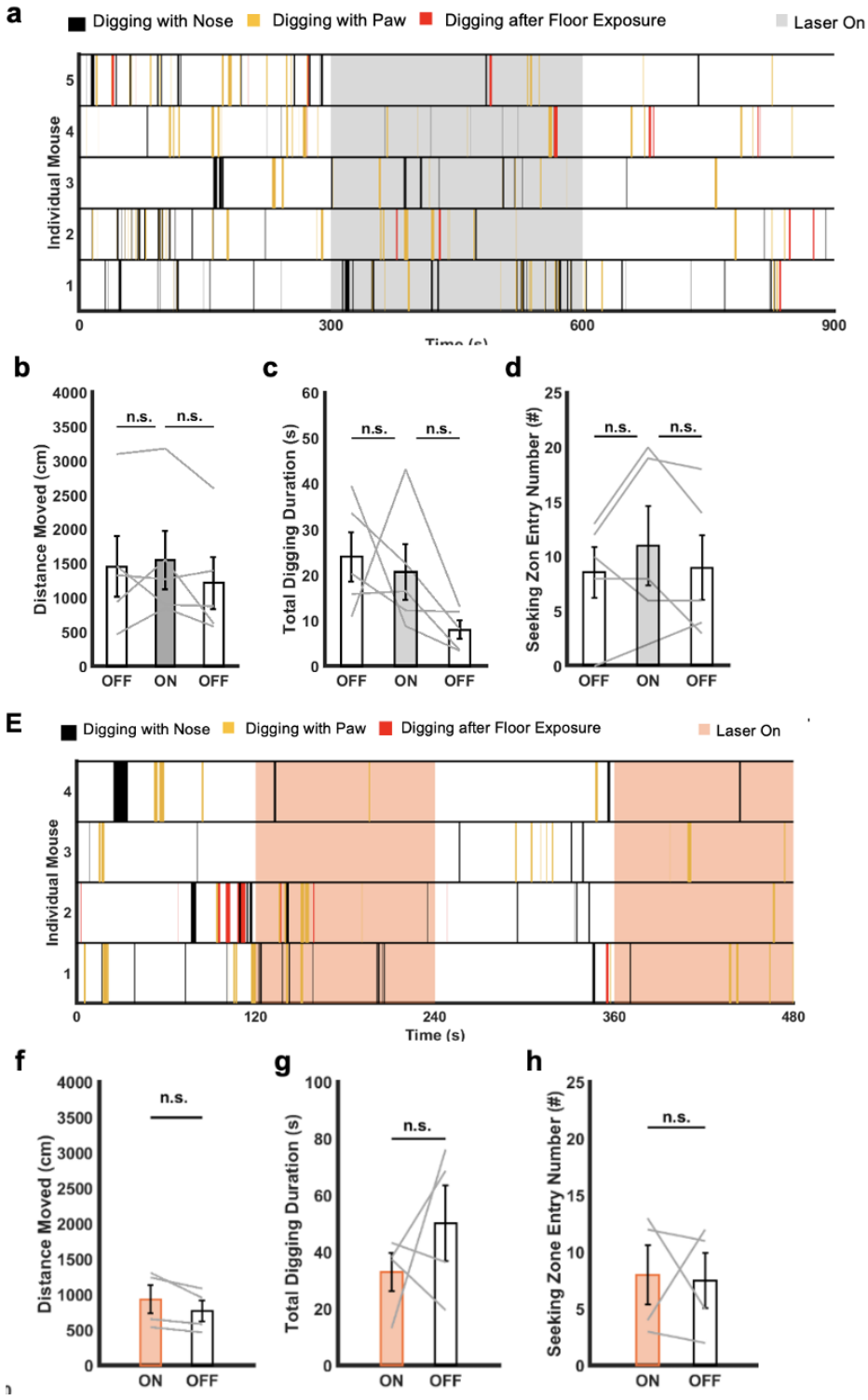
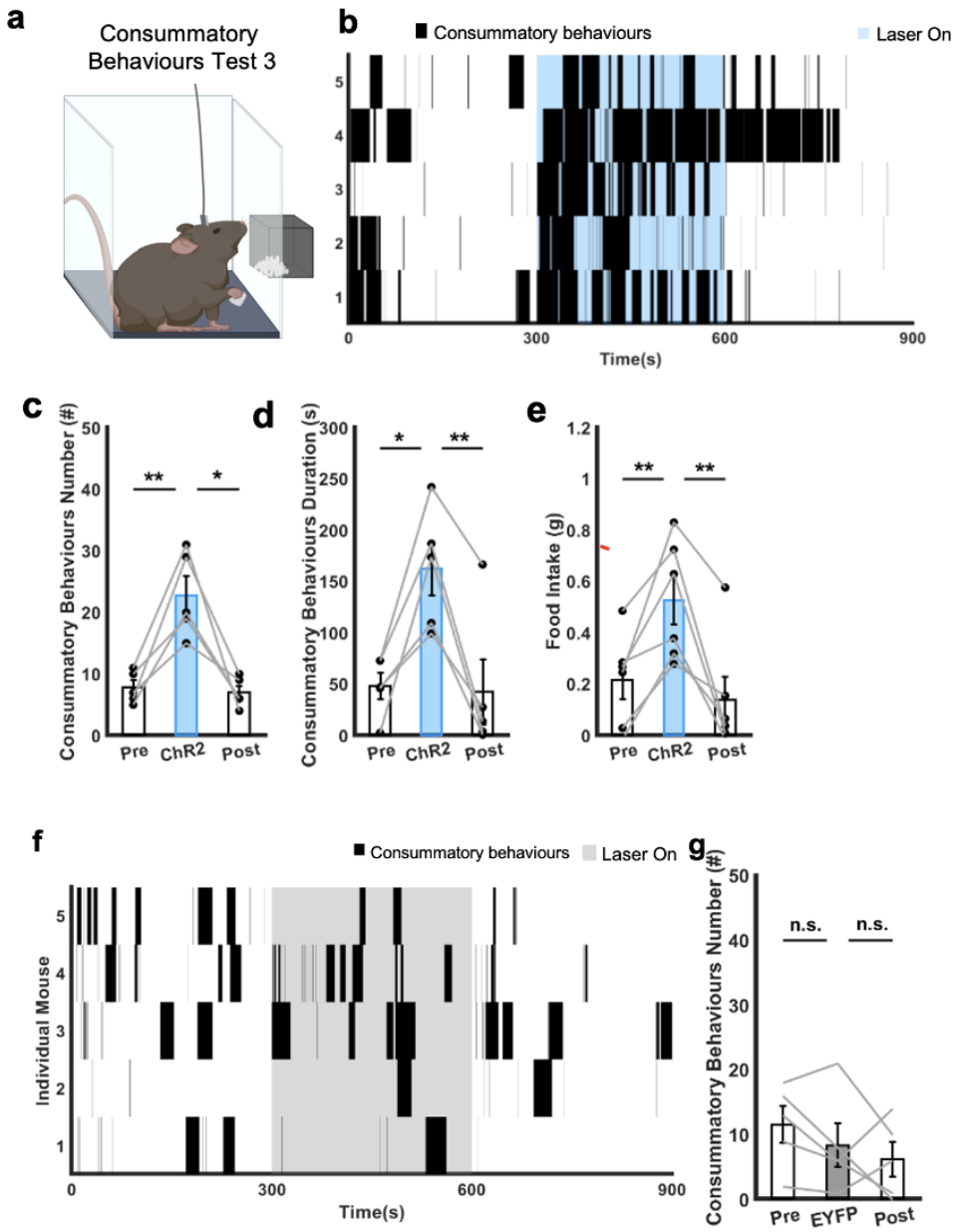


Fig. 23 | Control test of Figure 22.

a, e, Raster plot from EYFP-injected mice (**a**) and NpHR-injected mice (**e**) during

the seeking behaviour test 2. (n = 5 mice ; n = 4 mice). **b-d**, Quantification of distance moved (**b**), total digging duration (**c**), and frequency of food zone entries (**d**) from EYFP-injected mice. Two-sided paired t-test; n.s., $p > 0.05$. **f-h**, Quantification of distance moved (**f**), total digging duration (**g**), and frequency of food zone entries (**h**) from NpHR-injected mice. Two-sided paired t-test; n.s., $p > 0.05$


a
Consummatory Behaviours Test 3
b
Consummatory behaviours
Laser On
Time(s)
c
Consummatory Behaviours Number (#)
Pre
ChR2
Post
d
Consummatory Behaviours Duration (s)
e
Food Intake (g)
f
Individual Mouse
g
n.s.
EYFP

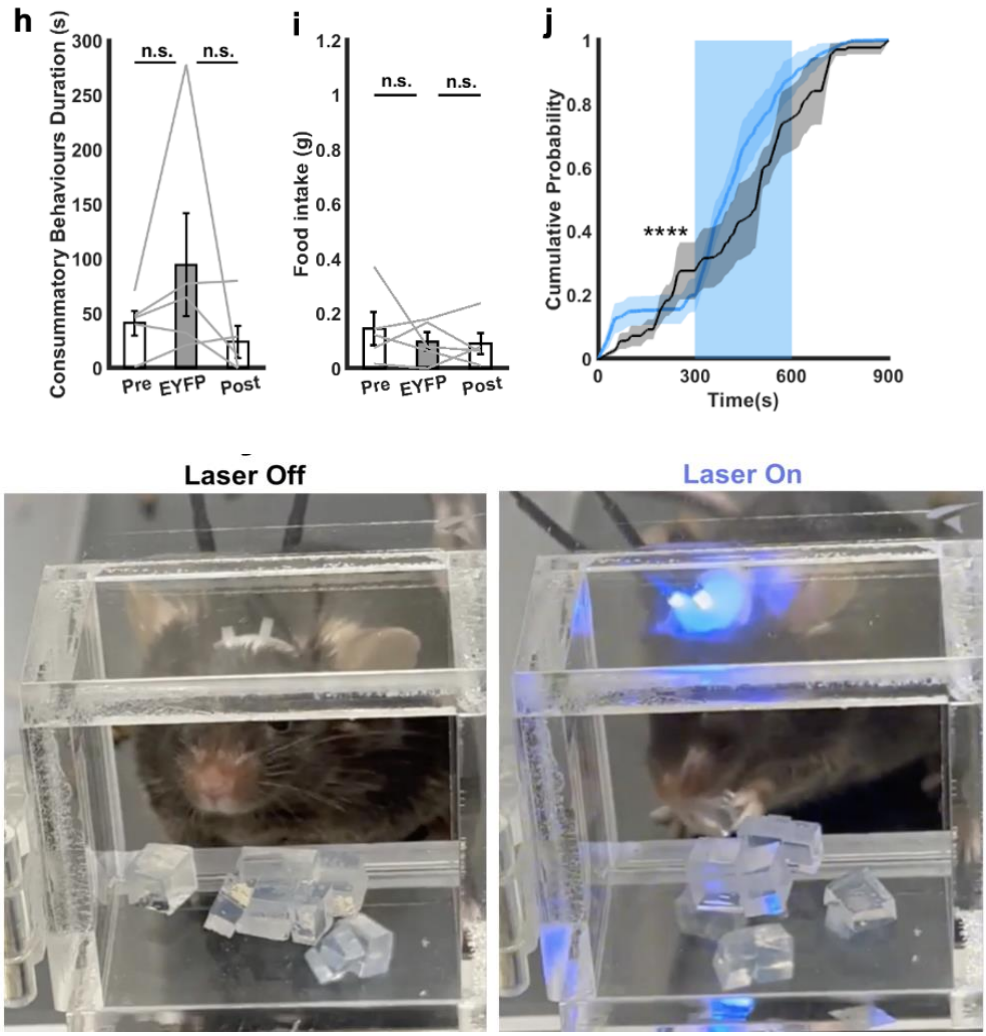


Fig. 24 | Activation of LH^{LepR} neurons drives consummatory behaviours

a, Schematic of the consummatory behaviour test 3. **b**, Raster plot during (**a**). **c-e**, Number (**c**) and duration (**d**) of consummatory behaviours, and food intake (**e**) (n = 5 mice). Two-sided paired t-test; ** p = 0.007 (**c**, pre vs laser), * p = 0.0105 (**c**, laser vs post), * p = 0.0105 (**d**, pre vs laser), ** p = 0.005 (**d**, laser vs post), * p = 0.015 (**e**, pre vs laser), * p = 0.017 (**e**, laser vs post). Yu-been Kim and Kyu Sik Kim jointly contributed data visualization.

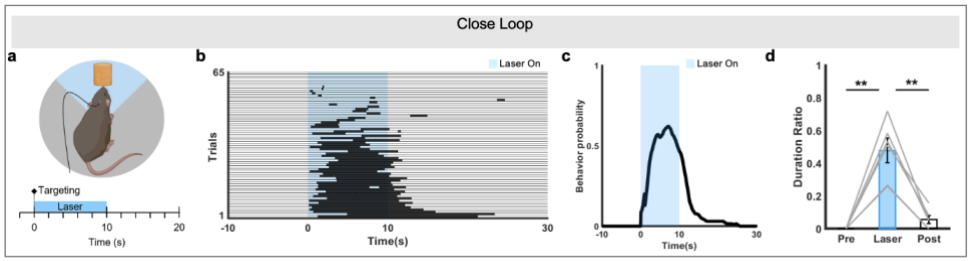
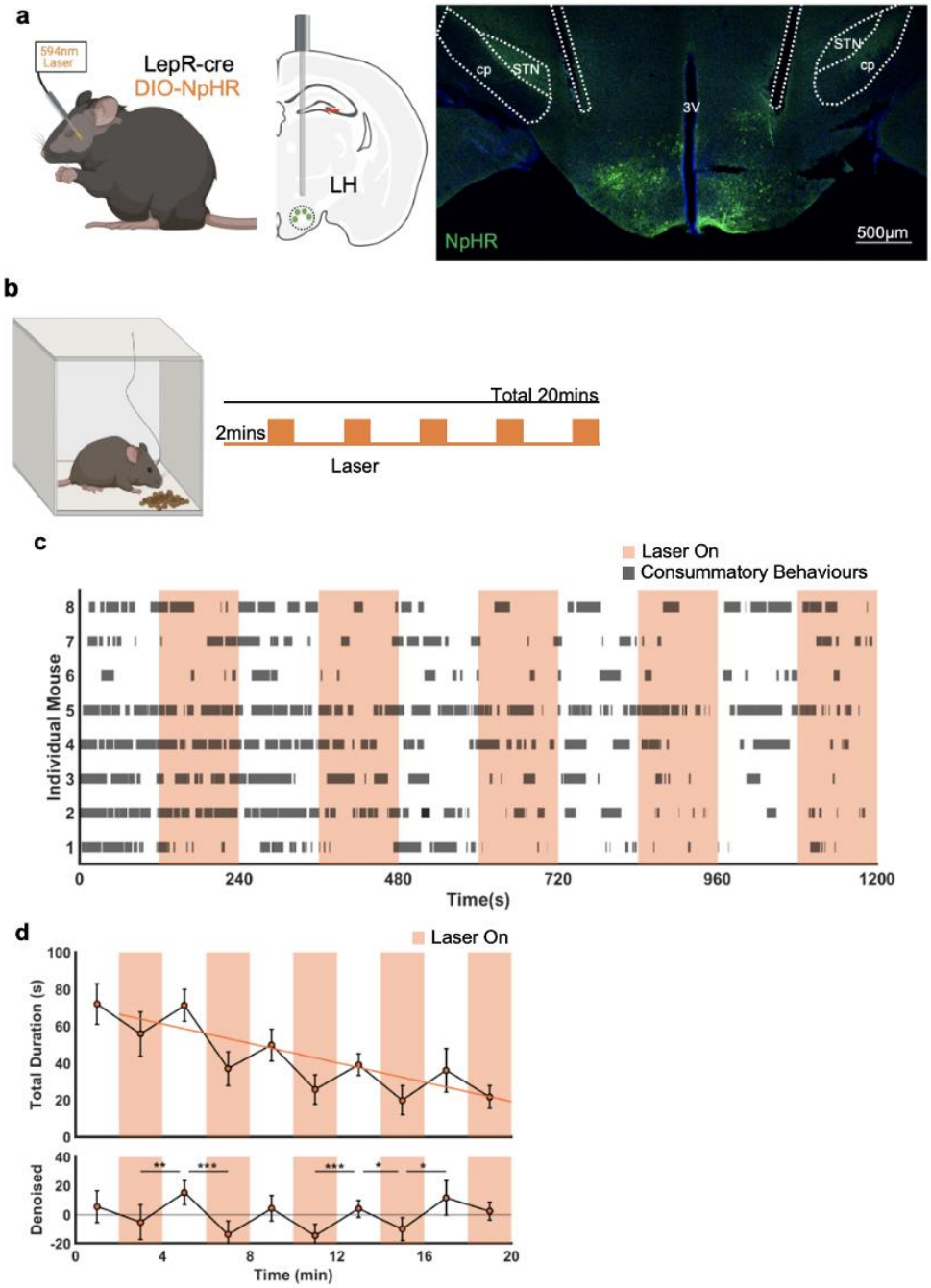


Fig. 25 | Activation of LH^{LepR} neurons drives consummatory behaviours in closed loop test

a, Schematic of the consummatory behaviour test 4. The laser is stimulated when the head of the mouse is in the food zone (blue). **b**, **c**, Raster plot (**b**) and behavioural probability (**c**) of consummatory behaviours (n = 6 mice, 65 trials). **d**, Quantification of consummatory behaviours from (**c**). Two-sided paired t-test; ** p = 0.0014 (pre vs laser), ** p = 0.0013 (laser vs post). Yu-been Kim and Kyu Sik Kim jointly contributed data visualization and behavioural tests.


a
594nm Laser
LepR-cre
DIO-NpHR
LH
cp
STN
3V
STN
cp
NpHR
500μm
b
Total 20mins
2mins
Laser
c
Laser On
Consummatory Behaviours
Individual Mouse
Time(s)
d
Laser On
Total Duration (s)
Denoised
Time (min)

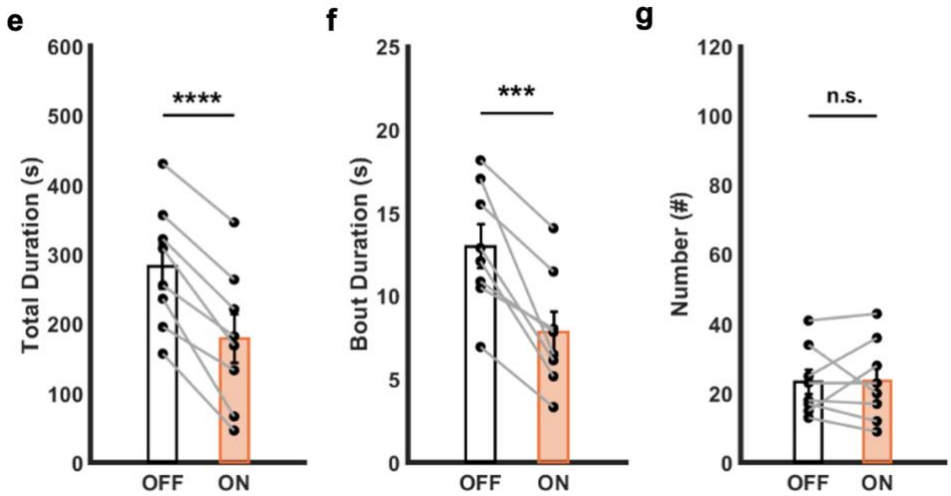


Fig.26 | Inhibition of LH^{LepR} neurons decreases consummatory behaviours.

a, Schematic of optogenetic inhibition (left, middle) and image of NpHR expression in LH^{LepR} neurons (right). The experiment was repeated 8 times independently with similar results. 3V, the 3rd ventricle; STN, subthalamic nucleus; cp, cerebral peduncle. **b,** Schematic of the consummatory behaviour test 5 and schedule of laser stimulation. **c,** Raster plot of consummatory behaviours during (**b**) (n = 8 mice). **d**, Average duration of consummatory behaviours (top). Calibrated graph (bottom) of the top. Two-sided paired t-test; ** p = 0.006 (time bin 2-4 min vs 4-6 min), *** p = 0.0007 (time bin 4-6 min vs 6-8 min), *** p = 0.0009 (time bin 10-12 min vs 12-14 min), * p = 0.02 (time bin 12-14 min vs 14-16 min), * p = 0.019 (time bin 14-16 min vs 16-18 min), **e-j**, Total duration (**e**), bout duration (**f**), and number (**g**) of consummatory behaviours (**e-g**). Two-sided paired t-test; **** p < 0.0001 (**e**), *** p = 0.0009 (**f**), n.s., p = 0.93 (**g**). Data are mean ± s.e.m. Source data are provided as a Source Data file.The schematics in a left and b were created using BioRender.

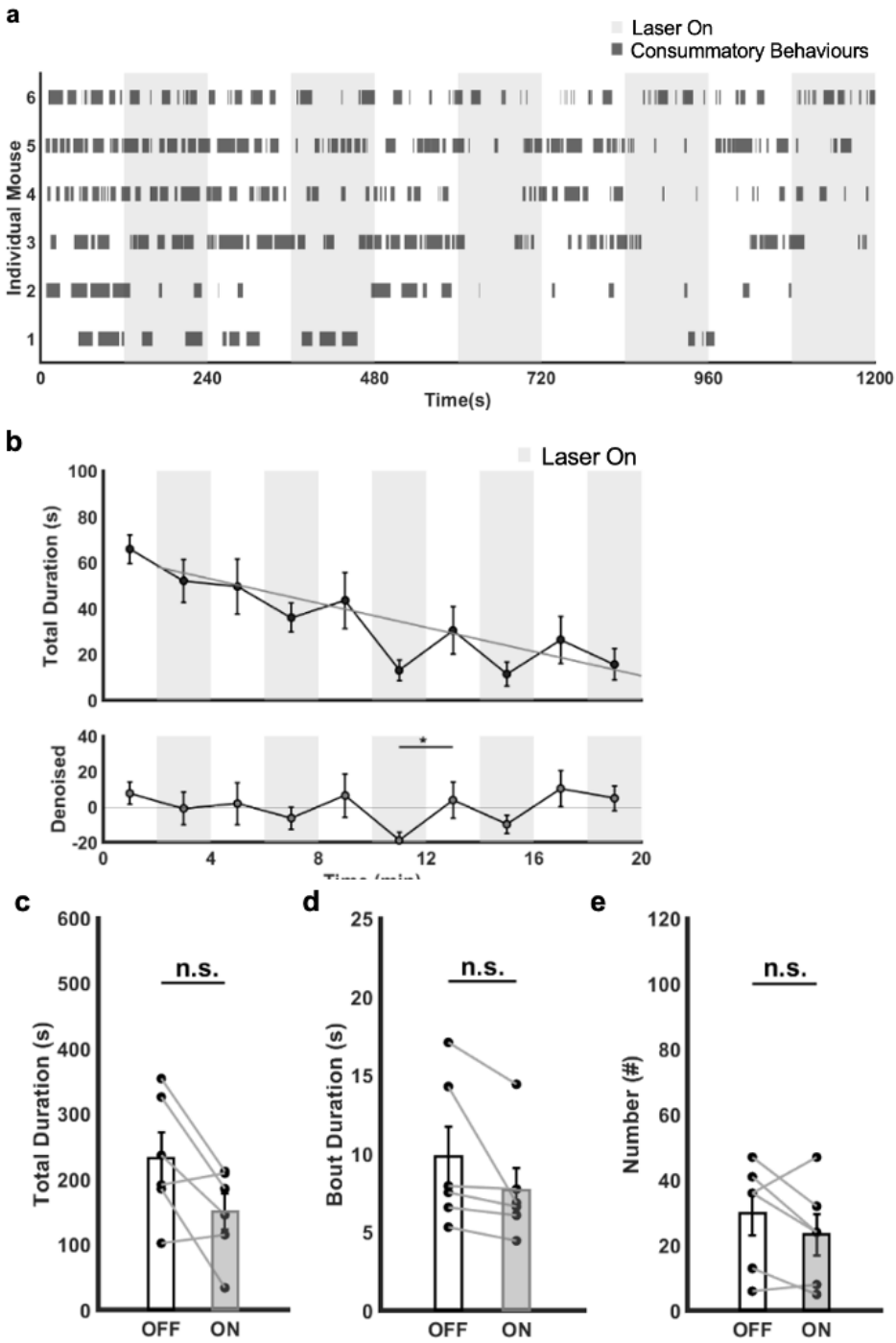


Fig.27 | Control of Figure 26

a, Raster plot of consummatory behaviours from EYFP-injected mice during consummatory behaviour test 5 (n = 6 mice). **b**, Average duration of consummatory

behaviours (top). Calibrated graph (bottom) of top. **c-e**, Quantification from (**a**). Total duration (**c)**, bout duration (**d**), number (**e**) of consummatory behaviours. Two-sided paired t-test (**b-e**); * p = 0.04 (**b**, time bin 10-12 min vs 12-14 min), n.s., p = 0.0502 (**c**), p = 0.12 (**d**), p = 0.2 (**e**). Data are mean ± s.e.m.

Discussion

I demonstrated that two distinct LH^{LepR} neuronal populations are activated sequentially and exclusively during the seeking and consummatory eating phases. Further, activation of LH^{LepR} neurons evoked seeking or consummatory behaviours. Collectively, I suggest that two distinct LH^{LepR} neuronal populations drive seeking and consummatory behaviours.

Previous studies have attempted to investigate seeking or consummatory behaviour. To investigate seeking behaviour, several studies have been recently published regarding the prey related hunting behaviours(37, 38). During hunting, superior colliculus to the zona incerta (ZI) neurons temporally correlates with predatory attacks but not the prey consumption phase. Activation of ZI GABAergic neurons strongly increase hunting of both live and artificial prey(37). However, these behaviours are more of predatory chasing behaviour rather than food-seeking behaviour. To investigate consummatory behaviour, several studies usually had been measured by the amount of food (or liquids) that has been consumed during certain amount of time (seconds, minutes, and hours). The difference in the weight of food (or food bowel) before and after the consummatory behaviour is most frequently used method to measure the quantity of food consummatory behaviour. However, these methods are intermingled with consummatory (licking, biting, chewing and swallowing) and seeking (searching, and digging) behaivours. Therefore, to solely investigate seeking and consummatory behaviours, I developed eating phase specific tests. Using in phase specific tests and vivo micro-endoscope imaging, I clearly demonstrated that LH^{LepR} neurons comprise 1) two distinct populations (seeking and consummatory LH^{LepR} neurons), which are sequentially activated during seeking and consummatory behaviours and 2) encode the voluntary drive for eating behaviours. I discovered findings compared to previous literature, as follows. A previous micro-endoscope study concluded that LH^{LepR} neurons are one specific population that discriminates between reward cues and non-reward cues(26). However, this paper did not provide a conclusion regarding different subpopulations among LH^{LepR} neurons. Another previous micro-endoscope study on LH^{LepR} neurons did not classify subpopulation heterogeneity of

LH^{LepR} neurons and did not differentiate the different phases of eating(31). In contrast, applying my comprehensive eating behavioural paradigm, I could successfully distinguish the two distinct populations. In my study, one population of LH^{LepR} neurons (seeking LH^{LepR} neurons) were only activated during seeking behaviours, not with those for consummatory behaviours. Further, the LH^{LepR} neural activity onset precedes the voluntary seeking behaviour initiation onset, which suggests that LH^{LepR} neurons are drivers of seeking behaviour rather than the consequence of seeking behaviour. The other population of LH^{LepR} neurons (consummatory LH^{LepR} neurons) is only activated during consummatory behaviours, not during seeking behaviours. This population robustly starts to be activated when animals are proximate to food and sustain its activity during consummatory behaviours. These two distinct populations are sequentially activated within the two distinct behavioural phases in eating. For survival, it is crucial for eating behaviours to be correctly sequenced and successfully executed through two distinct phases: seeking (appetitive) and consummatory phases(10, 39). This is equivalent to other motivated behaviours such as social or mating behaviours(9, 40).

Optogenetics results clearly demonstrated that the causal role of LH^{LepR} neurons in driving seeking and consummatory behaviours via eating phase specific paradigms. Previous studies reported controversial results that activation of LH^{LepR} neurons decrease eating(13) or fails to drive eating(26). Another study showed that activation of LH^{LepR} -vlPAG neurons drive eating(31). To investigate the underlying mechanism of these controversial results, I conducted the following experiments with three phase specific designs. Since my single-cell resolution results of LH^{LepR} neuron using micro-endoscope robustly distinguished two distinct subpopulations (seeking and consummatory behaviours), I hypothesised that optogenetic stimulation of LH^{LepR} neurons should be conducted during each phase specific design. As expected, in seeking phase-specific experiments (when only seeking behaviours are possible), LH^{LepR} neurons were sufficient to drive seeking behaviours (searching and digging for expected food). This is consistent with previous results showing that activation of LH^{LepR} neurons increase operant behaviour (lever presses) for food since the operant conditioning test is a one of the seeking-phase-specific experiments(12). Regarding the consummatory phase

specific experiments (when only consummatory behaviours are possible), as expected, LH^{LepR} neurons are sufficient and necessary for consummatory behaviours only in consummatory phase specific experiments. On the other hand, in experiments with combination of seeking and consummatory phases (when both seeking and consummatory behaviours are possible), activation of LH^{LepR} neurons failed to evoke seeking or consummatory behaviours (Fig.20, 21), similar to the previous studies(26, 41). These phase context-specific optogenetics results provide the neural mechanistic explanation why previous research failed to show increase food intake with large chamber (standard rat/mouse housing cage) experiments where both seeking and consummatory behaviours are possible(26, 41). These phase context-specific optogenetics results are consistent with my micro-endoscope results regarding two distinct phase specific activation patterns. These results provide wider understandings of how LH^{LepR} neurons regulate seeking and consummatory behaviours.

Collectively, I clearly showed that LH^{LepR} neurons fulfil the major criteria necessary to identify them as eating phase specific neurons(42, 43); they are sufficient to drive seeking/consummatory behaviour; they are necessary for consummatory behaviours LH^{LepR} neurons are activated during seeking and consummatory behaviours. I suggest that the two distinct types of seeking and consummatory LH^{LepR} neurons could have different molecular or connectivity identities. Since voluntary seeking and consummatory behaviours must precede decision-making through the integration of sensory modality information, the medial prefrontal cortex (mPFC) or insular cortex might mediate this process by communicating with LH^{LepR} neurons(44, 45). Seeking and consummatory LH^{LepR} neurons should have distinct upstream and downstream neurons to specifically drive seeking or consummatory behaviours, respectively. Since LH^{GABA} -Ventral Tegmental Area (VTA)(19, 46), LH^{GABA} -vlPAG(31, 47), and LH^{GABA} -Locus Coeruleus (LC)(46) have been known to mediate eat behaviour, LH^{LepR} seeking or consummatory neurons may innervate VTA, vlPAG or LC. Further, the LH is known to receive input from mPFC, Orbitofrontal Cortex (OFC), Nucleus Accumbens (NAc), Arcuate Nucleus (ARC) and Nucleus Tractus Solitarii (NTS)(48). LH^{LepR} neurons also received monosynaptic input from diverse regions such as intra LH, Anterior Cingulate (ACC), Diagonal Band of Broca (DBB),

Tuberomammillary Nucleus (TMN) and Ventral Premammillary Nucleus (PMV)(49).

Interestingly, there were no spatial differences between seeking and consummatory encoding neurons in single cell resolution.

Future studies involving behaviour dependent neuronal labeling could elucidate neural circuits mechanisms of LH^{LepR} food-seeking and consummatory subpopulations. High temporal precision behavioural tagging(50, 51) would be essential for future studies since the time resolution for food-seeking or consummatory behaviour is a second rather than several hours. In addition, identifying food-seeking and consummatory molecular characteristics would provide a crucial scientific foundation for the development of novel pharmaceuticals tailored to specific eating behaviours in the market for apetite and obesity, which is currently in the spotlight.

Previously, it was believed that AgRP/NPY neurons directly drive the whole phase of eating behaviours(6, 7). However, recent research has indicated that AgRP/NPY neurons deactivate even in response to a food cue(52). This suggests that, after the inactivation of AgRP/NPY neurons, another set of neuron drive seeking or consummatory behaviours(2, 53). I did not investigate the *in vivo* relationship between AgRP/NPY neurons and LH^{LepR} neurons, despite I elucidate the function of two distinct LH^{LepR} neurons drive seeking and consummatory behaviours. Future research should clarify the temporal distinction, the distinct role of eating behaviour, and the cause-and-effect relationship between AgRP/NPY neurons and LH^{LepR} neurons.

I provide insight into the role of two distinct LH^{LepR} neurons in orchestrating seeking and consummatory behaviours. Understanding the neural circuit mechanism for multi-phase eating behaviours may provide specific treatment options for patients with maladaptive food seeking and consummatory behaviours.

References

1. Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale J Biol Med. 1951;24(2):123-40.
2. Alcantara IC, Tapia APM, Aponte Y, Krashes MJ. Acts of appetite: neural circuits governing the appetitive, consummatory, and terminating phases of feeding. Nat Metab. 2022;4(7):836-47.
3. Anand BK, Dua S, Shoenberg K. Hypothalamic control of food intake in cats and monkeys. J Physiol. 1955;127(1):143-52.
4. Craig W. Appetites and aversions as constituents of instincts. Biol Bull-Us. 1918;34(2):91-107.
5. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, et al. Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci. 2005;8(10):1289-91.
6. Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nat Neurosci. 2011;14(3):351-5.
7. Cansell C, Denis RG, Joly-Amado A, Castel J, Luquet S. Arcuate AgRP neurons and the regulation of energy balance. Front Endocrinol (Lausanne). 2012;3:169.
8. Keen-Rhinehart E, Ondek K, Schneider JE. Neuroendocrine regulation of appetitive ingestive behavior. Front Neurosci. 2013;7:213.
9. Zhang SX, Lutas A, Yang S, Diaz A, Fluhr H, Nagel G, et al. Hypothalamic dopamine neurons motivate mating through persistent cAMP signalling. Nature. 2021;597(7875):245-9.
10. Watts AG, Kanoski SE, Sanchez-Watts G, Langhans W. The physiological control of eating: signals, neurons, and networks. Physiol Rev. 2022;102(2):689-813.
11. Burghardt GM. A place for emotions in behavior systems research. Behav Processes. 2019;166:103881.
12. Schiffino FL, Siemian JN, Petrella M, Laing BT, Sarsfield S, Borja CB, et al. Activation of a lateral hypothalamic-ventral tegmental circuit gates motivation. PLoS One. 2019;14(7):e0219522.
13. de Vrind VAJ, Rozeboom A, Wolterink-Donselaar IG, Luijendijk-Berg MCM, Adan RAH. Effects of GABA and Leptin Receptor-Expressing Neurons in the Lateral Hypothalamus on

Feeding, Locomotion, and Thermogenesis. Obesity (Silver Spring). 2019;27(7):1123-32.
14. Jennings JH, Ung RL, Resendez SL, Stamatakis AM, Taylor JG, Huang J, et al. Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. Cell. 2015;160(3):516-27.
15. Stuber GD, Wise RA. Lateral hypothalamic circuits for feeding and reward. Nat Neurosci. 2016;19(2):198-205.
16. Rolls ET, Burton MJ, Mora F. Hypothalamic neuronal responses associated with the sight of food. Brain Res. 1976;111(1):53-66.
17. Rolls ET, Sanghera MK, Roper-Hall A. The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey. Brain Res. 1979;164:121-35.
18. Aou S, Takaki A, Karadi Z, Hori T, Nishino H, Oomura Y. Functional heterogeneity of the monkey lateral hypothalamus in the control of feeding. Brain Res Bull. 1991;27(3-4):451-5.
19. Nieh EH, Matthews GA, Allsop SA, Presbrey KN, Leppla CA, Wichmann R, et al. Decoding neural circuits that control compulsive sucrose seeking. Cell. 2015;160(3):528-41.
20. Morgane PJ. Distinct "feeding" and "hunger motivating" systems in the lateral hypothalamus of the rat. Science. 1961;133(3456):887-8.
21. Burton MJ, Rolls ET, Mora F. Effects of hunger on the responses of neurons in the lateral hypothalamus to the sight and taste of food. Exp Neurol. 1976;51(3):668-77.
22. Petrovich GD. Lateral Hypothalamus as a Motivation-Cognition Interface in the Control of Feeding Behavior. Front Syst Neurosci. 2018;12:14.
23. Nieh EH, Vander Weele CM, Matthews GA, Presbrey KN, Wichmann R, Leppla CA, et al. Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. Neuron. 2016;90(6):1286-98.
24. Qualls-Creekmore E, Yu S, Francois M, Hoang J, Huesing C, Bruce-Keller A, et al. Galanin-Expressing GABA Neurons in the Lateral Hypothalamus Modulate Food Reward and Noncompulsive Locomotion. J Neurosci. 2017;37(25):6053-65.

25. Navarro M, Olney JJ, Burnham NW, Mazzone CM, Lowery-Gionta EG, Pleil KE, et al. Lateral Hypothalamus GABAergic Neurons Modulate Consummatory Behaviors Regardless of the Caloric Content or Biological Relevance of the Consumed Stimuli. Neuropsychopharmacology. 2016;41(6):1505-12.
26. Siemian JN, Arenivar MA, Sarsfield S, Borja CB, Russell CN, Aponte Y. Lateral hypothalamic LEPR neurons drive appetitive but not consummatory behaviors. Cell Rep. 2021;36(8):109615.
27. Stamatakis AM, Van Swieten M, Basiri ML, Blair GA, Kantak P, Stuber GD. Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula Regulate Feeding and Reward. J Neurosci. 2016;36(2):302-11.
28. Berrios J, Li C, Madara JC, Garfield AS, Steger JS, Krashes MJ, et al. Food cue regulation of AGRP hunger neurons guides learning. Nature. 2021;595(7869):695-700.
29. Rossi MA, Basiri ML, McHenry JA, Kosyk O, Otis JM, van den Munkhof HE, et al. Obesity remodels activity and transcriptional state of a lateral hypothalamic brake on feeding. Science. 2019;364(6447):1271-4.
30. Leinninger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. Cell Metab. 2009;10(2):89-98.
31. Shin S, You IJ, Jeong M, Bae Y, Wang XY, Cawley ML, et al. Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus-brainstem pathway. Nat Neurosci. 2022.
32. Shin S, You IJ, Jeong M, Bae Y, Wang XY, Cawley ML, et al. Early adversity promotes binge-like eating habits by remodeling a leptin-responsive lateral hypothalamus-brainstem pathway. Nat Neurosci. 2023;26(1):79-91.
33. Mickelsen LE, Bolisetty M, Chimileski BR, Fujita A, Beltrami EJ, Costanzo JT, et al. Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. Nat Neurosci. 2019;22(4):642-56.

34. Cao J, Spielmann M, Qiu X, Huang X, Ibrahim DM, Hill AJ, et al. The single-cell transcriptional landscape of mammalian organogenesis. Nature. 2019;566(7745):496-502.
35. Hanchate NK, Lee EJ, Ellis A, Kondoh K, Kuang D, Basom R, et al. Connect-seq to superimpose molecular on anatomical neural circuit maps. Proc Natl Acad Sci U S A. 2020;117(8):4375-84.
36. Lerner TN, Shilyansky C, Davidson TJ, Evans KE, Beier KT, Zalocusky KA, et al. Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. Cell. 2015;162(3):635-47.
37. Shang C, Liu A, Li D, Xie Z, Chen Z, Huang M, et al. A subcortical excitatory circuit for sensory-triggered predatory hunting in mice. Nat Neurosci. 2019;22(6):909-20.
38. Li Y, Zeng J, Zhang J, Yue C, Zhong W, Liu Z, et al. Hypothalamic Circuits for Predation and Evasion. Neuron. 2018;97(4):911-24 e5.
39. Lee YH, Kim M, Lee M, Shin D, Ha DS, Park JS, et al. Food Craving, Seeking, and Consumption Behaviors: Conceptual Phases and Assessment Methods Used in Animal and Human Studies. J Obes Metab Syndr. 2019;28(3):148-57.
40. Yang B, Karigo T, Anderson DJ. Transformations of neural representations in a social behaviour network. Nature. 2022;608(7924):741-9.
41. Omrani A, de Vrind VAJ, Lodder B, Stoltenborg I, Kooij K, Wolterink-Donselaar IG, et al. Identification of Novel Neurocircuitry Through Which Leptin Targets Multiple Inputs to the Dopamine System to Reduce Food Reward Seeking. Biol Psychiatry. 2021;90(12):843-52.
42. Kennedy A, Asahina K, Hoopfer E, Inagaki H, Jung Y, Lee H, et al. Internal States and Behavioral Decision-Making: Toward an Integration of Emotion and Cognition. Cold Spring Harb Symp Quant Biol. 2014;79:199-210.
43. Song C, Knopfel T. Optogenetics enlightens neuroscience drug discovery. Nat Rev Drug Discov. 2016;15(2):97-109.
44. Euston DR, Gruber AJ, McNaughton BL. The role of medial prefrontal cortex in memory and decision making. Neuron. 2012;76(6):1057-70.
45. Livneh Y, Ramesh RN, Burgess CR, Levandowski KM, Madara JC, Fenselau H, et al. Homeostatic circuits selectively

gate food cue responses in insular cortex. Nature. 2017;546(7660):611-6.
46. Marino RAM, McDevitt RA, Gantz SC, Shen H, Pignatelli M, Xin W, et al. Control of food approach and eating by a GABAergic projection from lateral hypothalamus to dorsal pons. Proc Natl Acad Sci U S A. 2020;117(15):8611-5.
47. Hao S, Yang H, Wang X, He Y, Xu H, Wu X, et al. The Lateral Hypothalamic and BNST GABAergic Projections to the Anterior Ventrolateral Periaqueductal Gray Regulate Feeding. Cell Rep. 2019;28(3):616-24 e5.
48. Clarke RE, Verdejo-Garcia A, Andrews ZB. The role of corticostriatal-hypothalamic neural circuits in feeding behaviour: implications for obesity. J Neurochem. 2018;147(6):715-29.
49. Giardino WJ, Eban-Rothschild A, Christoffel DJ, Li SB, Malenka RC, de Lecea L. Parallel circuits from the bed nuclei of stria terminalis to the lateral hypothalamus drive opposing emotional states. Nat Neurosci. 2018;21(8):1084-95.
50. Lee D, Hyun JH, Jung K, Hannan P, Kwon HB. A calcium- and light-gated switch to induce gene expression in activated neurons. Nat Biotechnol. 2017;35(9):858-63.
51. Hyun JH, Nagahama K, Namkung H, Mignocchi N, Roh SE, Hannan P, et al. Tagging active neurons by soma-targeted Cal-Light. Nat Commun. 2022;13(1):7692.
52. Betley JN, Xu S, Cao ZFH, Gong R, Magnus CJ, Yu Y, et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature. 2015;521(7551):180-5.
53. Sternson SM, Eiselt AK. Three Pillars for the Neural Control of Appetite. Annu Rev Physiol. 2017;79:401-23.

국문초록

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생존을 위해서는 음식을 찾아가는 행동과 찾은 음식을 먹는 행동은 각각 따로 정밀하게 상황에 맞게 조절해야 한다. 하지만, 지금까지의 기존 연구들은 식욕을 단순한 한단계의 행동으로 바라보았기에, 뇌의 어떤 신경들이 이런 다단계 행동들을 각각 지휘하는지 알려지지 않았다. 본 학위 논문에서는 가측 시상하부의 렙틴 수용체 신경군집을 실시간으로 측정하여 해당 신경군이 이러한 섭식의 다단계에 주요 역할을 수행함을 규명하였다. 음식을 찾아가는 행동을 할 때, 가까이 있는 음식을 먹는 행동을 할 때, 서로 다른 가측 시상하부 렙틴 수용체 신경군집은 두 부류로 나뉘어 각각 해당 시기에 활성화되는 것을 발견했다. 또한 가측 시상하부 렙틴 수용체 신경군집을 실시간으로 빛을 이용해 활성화시키는 실험을 수행하였다. 음식을 찾아야하는 상황이 주어졌을 때 해당 신경을 활성화하면, 더 열심히 음식을 찾는 것을 규명하였으며, 음식이 눈앞에 있을 때 해당 신경군을 활성화하면 눈 앞에 있는 음식을 먹게되는 것을 입증하였다. 이를 통해 본 연구자는 해당 신경 신경군들이 각각 섭식의 세부단계인 음식 찾기와 눈앞의 음식을 소비는데 주요 역할을 함을 입증하였다. 이러한 연구를 통하여 본 연구자는 앞으로 섭식 관련한 질병의 치료에 있어서 새로운 관점을 제시하는데 초석을 마련하고자 하였다.

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