



## Key role for hypothalamic interleukin-6 in food-motivated behavior and body weight regulation

Lorena López-Ferreras<sup>a</sup>, Francesco Longo<sup>a,b</sup>, Jennifer E. Richard<sup>a</sup>, Kim Eerola<sup>a,d</sup>, Olesya T. Shevchouk<sup>a</sup>, Madeleine Tuzinovic<sup>a</sup>, Karolina P. Skibicka<sup>a,b,c,\*</sup>

<sup>a</sup> Institute for Neuroscience and Physiology, University of Gothenburg, Sweden

<sup>b</sup> Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Sweden

<sup>c</sup> Department of Nutritional Sciences, Pennsylvania State University, University Park, PA, USA

<sup>d</sup> Research Centre of Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku, Finland

### ARTICLE INFO

#### Keywords:

Interleukin-6  
Lateral hypothalamus  
Food reward  
Food-motivated behavior  
Body weight regulation

### ABSTRACT

The pro-inflammatory role of interleukin-6 (IL-6) is well-characterized. Blockade of IL-6, by Tocilizumab, is used in patients with rheumatoid arthritis and those diagnosed with cytokine storm. However, brain-produced IL-6 has recently emerged as a critical mediator of gut/adipose communication with the brain. Central nervous system (CNS) IL-6 is engaged by peripheral and central signals regulating energy homeostasis. IL-6 is critical for mediating hypophagia and weight loss effects of a GLP-1 analog, exendin-4, a clinically utilized drug. However, neuroanatomical substrates and behavioral mechanisms of brain IL-6 energy balance control remain poorly understood. We propose that the lateral hypothalamus (LH) is an IL-6-harboring brain region, key to food intake and food reward control. Microinjections of IL-6 into the LH reduced chow and palatable food intake in male rats. In contrast, female rats responded with reduced motivated behavior for sucrose, measured by the progressive ratio operant conditioning test, a behavioral mechanism previously not linked to IL-6. To test whether IL-6, produced in the LH, is necessary for ingestive and motivated behaviors, and body weight homeostasis, virogenetic knockdown by infusion of AAV-siRNA-IL6 into the LH was utilized. Attenuation of LH IL-6 resulted in a potent increase in sucrose-motivated behavior, without any effect on ingestive behavior or body weight in female rats. In contrast, the treatment did not affect any parameters measured (chow intake, sucrose-motivated behavior, locomotion, and body weight) in chow-fed males. However, when challenged with a high-fat/high-sugar diet, the male LH IL-6 knockdown rats displayed rapid weight gain and hyperphagia. Together, our data suggest that LH-produced IL-6 is necessary and sufficient for ingestive behavior and weight homeostasis in male rats. In females, IL-6 in the LH plays a critical role in food-motivated, but not ingestive behavior control or weight regulation. Thus, collectively these data support the idea that brain-produced IL-6 engages the hypothalamus to control feeding behavior.

### 1. Introduction

Interleukin-6 (IL-6) is a key component of the acute phase response during infection. The pro-inflammatory role of IL-6 is well-characterized. Blockade of IL-6, by Tocilizumab, is approved for clinical use in patients with rheumatoid arthritis (Scott, 2017), and is now also investigated in patients with COVID-19 showing signs of the dangerous conditions – cytokine storm (Xu et al., 2020). IL-6 is secreted by several cell types, including leukocytes, hepatocytes and adipocytes

(Andersson et al., 2008); but, it is also produced in the central nervous system (CNS), in both neuronal and glial cells (Cornfield and Sils, 1991; Gadiant and Otten, 1993, 1994a, 1994b; Schobitz et al., 1992, 1993; Yan et al., 1992). Obesity is associated with chronic inflammation, and individuals with this condition often present with elevated systemic levels of interleukin-1 (IL-1) and IL-6 cytokines (Fain, 2006; Roytblat et al., 2000). Elevated IL-6 levels in obesity result from its increased production specifically in the adipose tissue (Roytblat et al., 2000; Stenlof et al., 2003). Surprisingly, in the CNS, in contrast to the changes observed in

\* Correspondence to: Department of Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Medicinargatan 11, PO, Box 434, SE-405 30 Gothenburg, Sweden.

E-mail address: [Karolina.Skibicka@neuro.gu.se](mailto:Karolina.Skibicka@neuro.gu.se) (K.P. Skibicka).

<https://doi.org/10.1016/j.psyneuen.2021.105284>

Received 4 January 2021; Received in revised form 14 May 2021; Accepted 19 May 2021

Available online 23 May 2021

0306-4530/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

the adipose tissue and plasma, studies in rodents and humans report reduced IL-6 levels, potentially indicating a divergent role of peripherally and centrally acting IL-6 in obesity (Mishra et al., 2019; Stenlof et al., 2003). On the other hand, both acute and persistent increases in IL-6 were previously noted in the whole or mediobasal hypothalamus in response to an obesogenic diet (De Souza et al., 2005; Thaler et al., 2012). Furthermore, elevated body weight, resulting from global IL-6 knockout in mice, has been attributed to the actions of the cytokine in the CNS (Timper et al., 2017; Wallenius et al., 2002a, 2002b), indicating that while increased peripheral IL-6 in an obesogenic state is detrimental to the individual, central IL-6 may be beneficial, and acts to reduce food intake and body weight. The neuroanatomical substrates and mechanisms by which central IL-6 mediates these effects are poorly understood.

The lateral hypothalamus (LH) has long been known for its pivotal role in the regulation of feeding behavior: bilateral lesions of this area result in profound aphagia (Anand and Brobeck, 1951; Morrison and Mayer, 1957; Teitelbaum and Stellar, 1954; Van den Pol, 1982), while stimulation results in increased food intake, even in satiated rats (Miller, 1960). Orexin-, melanin-concentrating hormone- and neurotensin-producing neurons all reside within the LH and are critical in feeding and food-reward control (Alon and Friedman, 2006; Cooke et al., 2009; Harris et al., 2005; Haynes et al., 2002; Kim et al., 2008; Leininger et al., 2011; Ludwig et al., 2001; Qu et al., 1996; Sakurai, 1999; Shimada et al., 1998). In addition, we have recently identified the LH as a critical mediator of the effects of the hormone glucagon-like peptide-1 (GLP-1) on food intake and reward (Lopez-Ferreras et al., 2018). It is this newly discovered GLP-1R impact on the LH, that led us to posit that IL-6 production in this area may be a critical element in feeding control, in healthy animals, outside of the context of inflammation or infection. Pursuit of this hypothesis is supported by previous data showing that the anorexigenic and weight loss effects of GLP-1 require intact CNS IL-6 (Shirazi et al., 2013), and intra-LH injection of the GLP-1 agonist exendin-4 (Ex4) leads to a marked increase in IL-6 expression (Lopez-Ferreras et al., 2018). Here, we will confirm the presence of IL-6 in the LH, and also determine which CNS cell type - neurons, astrocytes, or microglia - preferentially expresses IL-6 in healthy rats, and whether the cellular source of IL-6 in the LH differs between males and females. Using virogenetic and pharmacological manipulations, the LH will be evaluated as a potential novel and critical site of the anorexic action of IL-6, capable of producing and responding to IL-6, in a sex specific manner.

In addition to its role in ingestive aspects of feeding behavior, the LH is suggested to act as an interface, linking the homeostatic and hedonic food intake regulating systems, many studies indicate that a variety of LH neuropeptides play a role in motivated behavior control (Davis et al., 2011; Hurlley and Johnson, 2014; Leininger et al., 2009; Lopez-Ferreras et al., 2018). Link between LH and reward-driven behavior was indicated already in 1960s by a row of seminal studies identifying LH as a potent self-stimulation area of the brain (Blundell and Herberg, 1968; Hoebel and Teitelbaum, 1962; Margules and Olds, 1962; Olds, 1962). While little is known about a potential link between IL-6 and motivated behavior control, considering the key role of LH, and specifically GLP-1R in the LH in this behavior (Anesten et al., 2019; Lopez-Ferreras et al., 2018), along with the fact that LH GLP-1R activation increases LH IL-6, it is logical to extend the hypothesis of LH IL-6 role in feeding to food-motivated behavior control. Thus, here we will evaluate the possibility that CNS IL-6 contributes to food-motivated behavior control, and does so specifically release and action on the LH. Since sex-differences have been demonstrated for the regulation of ingestive and motivated behaviors in the LH (Funabashi et al., 2009; Lopez-Ferreras et al., 2019, 2017, 2018; Van den Pol, 1982), the current study is designed to detect potential sex divergence in LH IL-6 behavioral control. Furthermore, since some evidence points to presence of not only IL-6 but also IL-6 receptors (IL-6R) in LH (Schele et al., 2012) we will also evaluate the possibility that LH-produced IL-6 acts locally on the LH

receptors to exert its feeding and reward controls. Considering the partially differential engagement of brain feeding circuits in a lean vs obese state, and, importantly different contribution of CNS IL-6 in health and obesity, both lean rats, and those fed a palatable high-calorie obesogenic diet, will be evaluated here.

## 2. Methods

### 2.1. Animals

Female and male Sprague-Dawley rats (5 weeks of age at arrival, Charles River, Sulzfeld, Germany) were housed in a 12-h light/dark cycle (lights on at 07:00 h) in individual cages. Rats were given ad libitum access to chow and water unless otherwise stated. All studies were carried out with ethical permissions from the Animal Welfare Committee of the University of Gothenburg, in accordance with legal requirements of the European Community (Decree 86/609/EEC). All efforts were made to minimize suffering.

### 2.2. Drugs

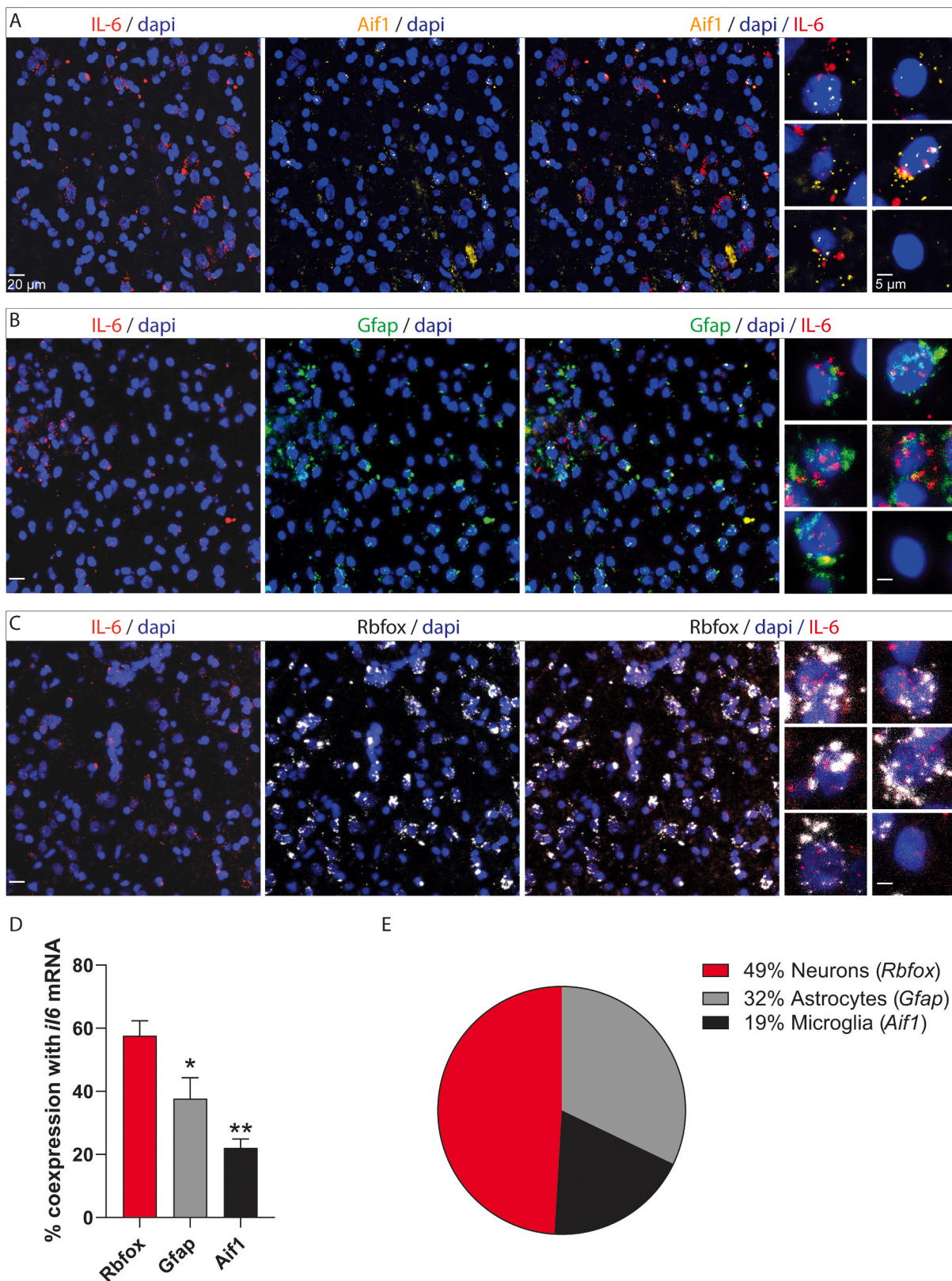
IL-6 was purchased from Peprotech (United Kingdom), and GLP-1R agonist, Ex-4, was purchased from Tocris (Bristol, UK). Both drugs were dissolved in artificial cerebrospinal fluid (aCSF; Tocris; used as vehicle) and stored as aliquots at  $-20^{\circ}\text{C}$ .

### 2.3. Brain cannulation

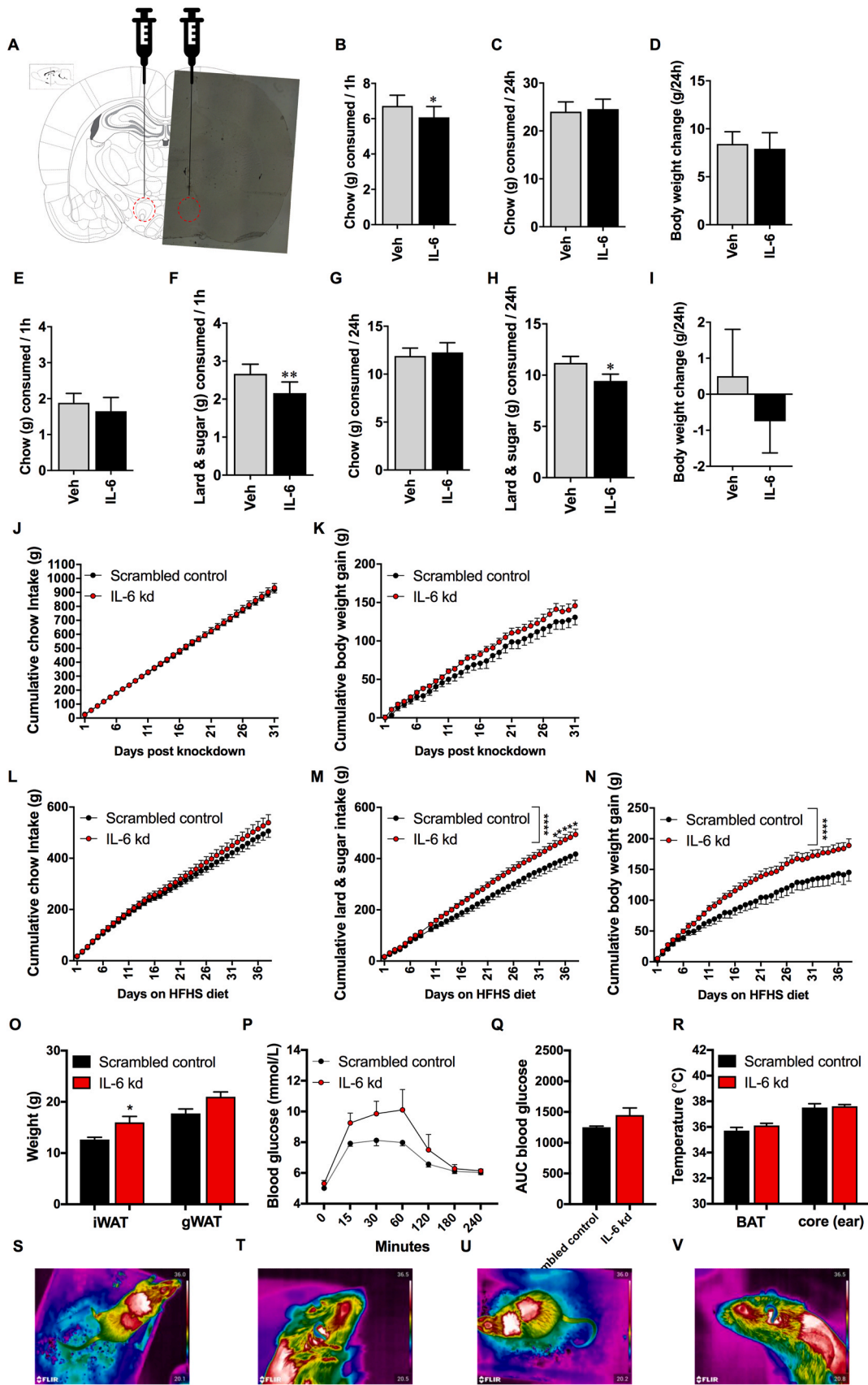
Guide cannulas (26 gauge; Plastics One, Roanoke, VA) were inserted into the LH under ketamine anesthesia as previously described (Lopez-Ferreras et al., 2017, 2018). The following coordinates were used:  $\pm 1.5$  mm from midline, 2.8 mm posterior to bregma, and 6.8 mm ventral from the surface of the skull, with injector aimed 8.8 mm ventral to skull. After positioning the guide cannula, the cannula was secured to the surface of the skull using dental acrylic and jeweler's screws. The incision was then closed using an obturator as described previously (Skibicka et al., 2009). Cannula placement in the LH was confirmed post mortem in cryostat coronal slices of brain tissue throughout the LH. In short, after dissection, the brains were flash frozen in isopentane and stored at  $-80^{\circ}\text{C}$  until further use. Coronal sections ( $10\ \mu\text{m}$ ) were collected from each brain, stained with DAPI and visualized using a confocal microscope (see section on fluorescent *in situ* hybridization for further detail). Representative image of intra-LH injection is presented in Fig. 2A (males) and 4A (females). Only subjects with correctly placed cannulas were included in data analysis.

### 2.4. Operant conditioning

To test food-motivated behavior progressive ratio (PR) operant conditioning was used (Hodos, 1961). Operant conditioning training and testing took place in rat conditioning chambers (Med-Associates, Georgia, VT, USA) as described previously (Dickson et al., 2012; la Fleur et al., 2007). Training consisted of four stages: fixed ratio 1 (FR1), FR3, FR5 and PR. Each FR training session was carried out for 30 min, in ad libitum fed rats. In FR1, one press on the active lever resulted in the delivery of one sucrose pellet (45 mg TestDiet, Richmond, IN, USA), while FR3 and FR5 required 3 and 5 presses, for each delivered pellet, respectively. A minimum of 30 responses on the active lever was needed to advance to the next stage. In the final stage PR (60 min), the amount of work (presses on the active lever) required to obtain one sucrose pellet is progressively increased according to the following equation: response ratio =  $[5e(0.2 \times \text{reward number})] - 5$  through the following series: 1, 2, 4, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328. The amount of active lever presses conducted during the 60-min session is deemed as the breaking point and reflects the amount of work, or motivation, that the rat is willing to put in to receive the



**Fig. 1.** Expression of IL-6 in LH neurons, astrocytes and microglia. To understand the cellular origin of IL-6 in the LH, we used RNAScope to co-localize IL-6 mRNA with microglial (AIF1; orange; A), astrocytic (GFAP; green; B), or neuronal (Rbfox3; gray; C) mRNA markers. Results of approximate quantification of co-expression of cellular markers with IL-6 mRNA in the LH of male and female rats. (D) Diagram illustrating the proportion (by %) of each cell type co-expressing IL-6 mRNA at bregma -3.0 mm level. (E) Proportion (by %) of IL-6 mRNA expressed by neurons, astrocytes, and microglia. For Rbfox and Aif1 experiments, the LH from three male and three female rats was used, Gfap counts are taken from three male and two female rats. Graphs present aggregate data of male and female rats as no literature evidence of expected sex differences at baseline was identified. For sex analysis see Fig. S1. Data represent mean ± SEM. \*P < 0.05, \*\*P < 0.01.



(caption on next page)

**Fig. 2.** Effects of IL-6 receptor stimulation and IL-6 kd on ingestive behavior in male rats. Representative image of injection site (A). Acute injection of IL-6 into the LH of males reduced intake of chow 1 h after injection (B). Food intake (C) and body weight (D) were unaltered 24 h after injection. In males fed a HFHS diet, intra-LH IL-6 injection significantly reduced the intake of lard and sugar 1 (F) and 24 (H) hours after injection, while intake of normal chow was unaffected at both time points (E & G). IL-6 injection did not alter 24-h body weight change (I). IL-6 kd in the LH did not alter cumulative chow intake (J) or body weight (K) in male rats fed a normal chow diet. However, this manipulation resulted in a significant increase in cumulative lard and sugar intake (M) and body weight change (N), while cumulative intake of chow remained unaltered (L). Intra-LH IL-6 kd led to a significant increase in iWAT (O). GWAT weight was unaltered between IL-6 kd and scrambled controls (O). Blood glucose was not significantly altered by an oral glucose challenge in males (P, Q). Core and BAT temperature were unaltered by intra-LH IL-6 kd (R). Representative thermal images for scrambled control BAT (S), ear (core; T) and IL-6 kd BAT (U) and ear (core; V). AUC = area under the curve, iWAT = inguinal white adipose tissue, gWAT = gonadal white adipose tissue, BAT = brown adipose tissue. Data expressed as mean  $\pm$  SEM. For IL-6 injection experiments:  $n = 12$  (chow fed) and  $n = 17$  (HFHS fed) per treatment. For IL-6 kd:  $n = 8$ – $11$  per treatment group. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\*\* $P < 0.001$ .

reward (sucrose pellet). PR was conducted until responding was considered stable (number of pellets earned per session did not differ more than 15% between three consecutive sessions).

### 2.5. Pharmacological intra-LH IL-6 injection effect on food intake, body weight, food-motivated behavior and locomotor activity

To investigate potential effects of IL-6 in the LH on food intake and reward, male and female rats were restricted overnight, to 25% of their normal intake, prior to injection of IL-6 (0.5  $\mu$ g; volume: 0.5  $\mu$ L) or vehicle (0.5  $\mu$ L of aCSF). Reward behavior testing, by PR, was carried out 20 min after injection. Food seeking was assessed as the number of head pokes into the feeding chamber during the 60 min operant session. Locomotor activity was measured using horizontal infrared beams in the operant chambers (Med-Associates, Georgia, VT, USA).

In the rats maintained on an obesogenic diet (6 weeks of high-fat/high-sugar (HFHS) mix available alongside regular chow) intake of chow or lard (70%) and sugar (30%) mix, was measured 1 and 24 h after PR testing (see Table 1 for graphical timeline). Since the food rewards are very small (45 mg = 0.18 kcal) they are unlikely to make a meaningful contribution to the food intake test that follows where rats consume 10–20-fold more than the total consumed over the 1 h of PR testing. Body weight was measured at the 24-h time point. In rats maintained on chow the measurements were done at the same time points. Testing was carried out in a Latin square, counterbalanced design, where each condition was separated by a minimum of 48 h washout period.

### 2.6. Intra-LH IL-6 knockdown (kd)

To investigate the effects of long-term IL-6 reduction in the LH on food intake and food-motivated behavior we utilized a virogenetic knockdown strategy, where an adeno-associated viral vector for delivery of siRNA targeting IL-6 (IL-6 AAV siRNA pooled virus, serotype 2; ABM; Richmond, Canada; Cat# iAAV01087702) is infused specifically into the LH. Scrambled AAV siRNA was used as a control (scrambled AAV siRNA control virus, serotype 2; ABM; Richmond, Canada; Cat# iiAAV01502). This siRNA was previously used to kd CNS IL-6 expression and was shown to reduce IL-6 mRNA by 50% in the parabrachial nucleus (Mishra et al., 2019). Here we confirmed this previously indicated viral kd efficiency, and show that in the current experiment, kd of IL-6 in the LH of males (Fig. 7B) and females (Fig. 7C) was approximately 50%.

Viral injections were carried out after completed operant conditioning training, through surgically implanted LH-directed guide

cannulae (described above). AAVs were infused bilaterally into the LH (0.5  $\mu$ L per hemisphere; 0.1  $\mu$ L/min). Microinjectors were left in place for 10 min after infusion to allow for diffusion away from the injection site. Rats in each treatment group were matched for body weight, food intake and food reinforcement parameters on PR. Body weight and chow intake were measured for 4 weeks. Seven weeks after viral infusion, the rats were challenged with a HFHS diet consisting of chow, and a solid mix of lard and sucrose (70/30). Body weight and food intake were measured daily for 5 weeks. In addition, food motivation was measured once a week. See Table 2 for graphical timeline of all procedures performed in this experiment.

### 2.7. Intra-LH Ex4 injection in control and LH IL-6 kd rats

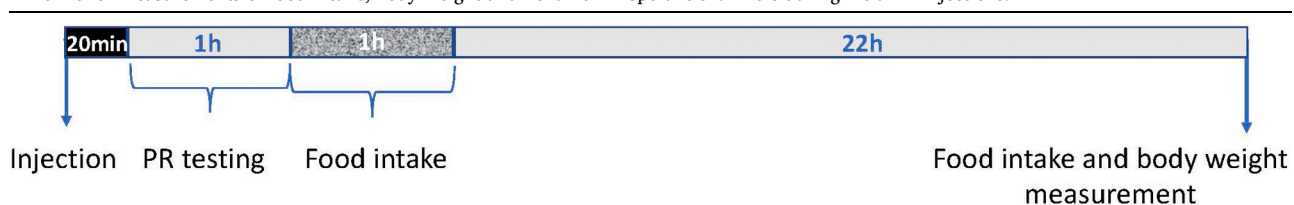
The goal of this experiment was to test whether the partial loss of LH IL-6 attenuates the motivated and ingestive behavior reductions induced by LH GLP-1R activation. To achieve this, male and female kd and control rats were food restricted overnight, to 50% of their usual intake, prior to injection of GLP-1R agonist Ex4 (0.05  $\mu$ g in 0.5  $\mu$ L) or vehicle. Operant conditioning testing commenced 20 min after injection. Following operant testing, rats were given ad libitum access to chow for 1 h after returning to their home cage. Injections were carried out 4–5 weeks after control (scrambled AAV) or IL-6 kd (IL-6 AAV siRNA) intra-LH injection (see Table 2 for timeline).

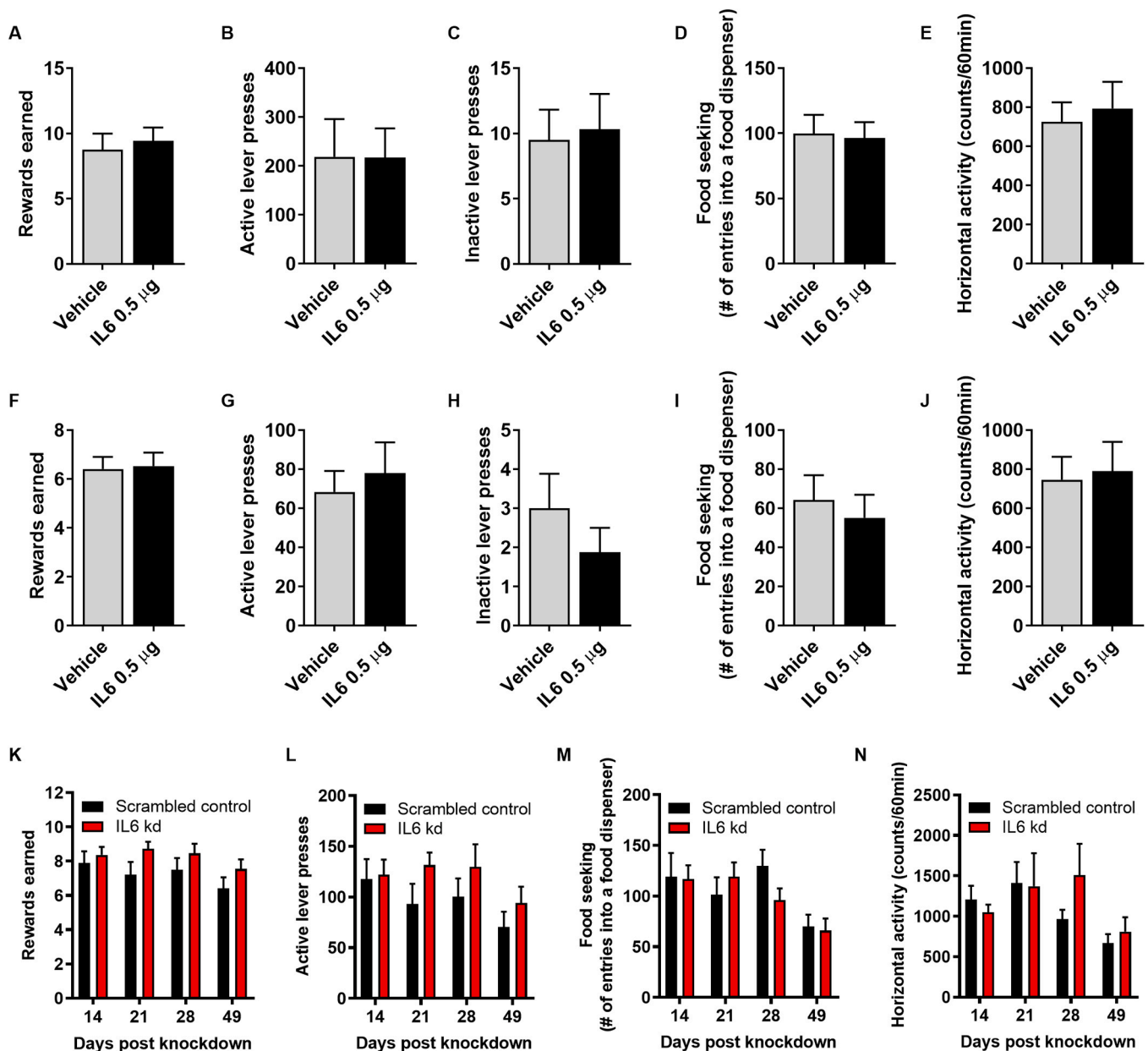
### 2.8. In situ hybridization using RNAscope

*In situ* hybridization for IL-6 mRNA using RNAscope Multiplex Fluorescent kit (Advanced Cell Diagnostics) was utilized to detect IL-6 in LH rat brain tissue. To determine IL-6 presence in neuronal, astrocytic, or microglial cells, IL-6 mRNA and mRNA for established markers of each cell type were assessed using RNAscope. Briefly, 15-week-old male and female rats were sacrificed and their brains were flash frozen. Twelve  $\mu$ m thick LH-containing brain sections were cut and fixed in 4% formalin (ThermoFisher scientific, Waltham, MA) for 15 min at 4°C. Following two quick washes in PBS, brain slices were dehydrated in 50% (5 min), 70% (5 min) and 2  $\times$  100% (5 min each) ethanol and treated with protease solution (pre-treatment IV, ACDBio kit) at room temperature for 30 min. The protease was washed away with PBS for a total time of 15 min. Target probes and negative control probes were applied directly on the sections to cover them completely and incubated at 40 °C for 2 h in the HyBEZ oven. Next, slides were incubated with preamplifier and amplifier probes (AMP1, 40 °C for 30 min; AMP2, 40 °C for 15 min; AMP3, 40 °C for 30 min). Next, slides were incubated with fluorescent

**Table 1**

Timeline for measurements of food intake, body weight and behavior in operant chambers during intra-LH injections.



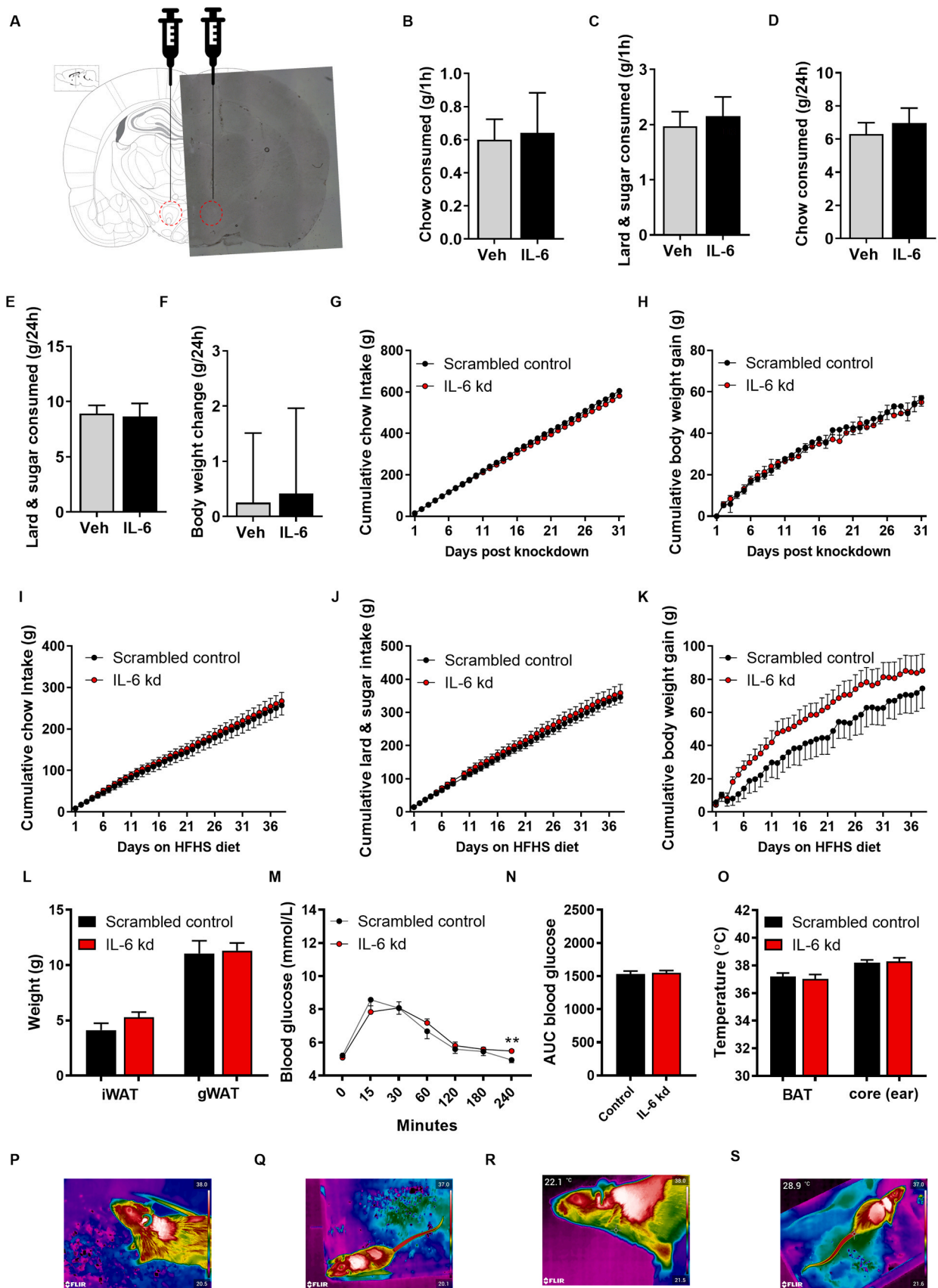


**Fig. 3.** Intra-LH IL-6 is neither sufficient nor necessary for the regulation of food-motivated behavior in male rats. IL-6 injection in the LH did not lead to any effects on active rewards earned (A), lever presses (B), inactive lever presses (C), food seeking (D) or horizontal activity (E) in chow fed males. Likewise, these behaviors were not altered by treatment after exposure to a HFHS diet (F–J). Intra-LH IL-6 kd did not alter rewards earned (K), active lever presses (L), food seeking (M) or horizontal activity (N), in the operant conditioning task 14, 21, 28 or 49 days after viral injection. Data expressed as mean  $\pm$  SEM. For IL-6 injection experiments:  $n = 12$  (chow fed) and  $n = 17$  (HFHS fed) per treatment. For IL-6 kd:  $n = 10$ –11 per treatment group.

labels (AMP4-Alt A or Alt C). Finally, brain sections were incubated for 30 s with DAPI and mounting medium for fluorescence (VECTASHIELD, USA). The following RNAScope probes were used: microglial (targeting AIF1; orange; ID Rn-Aif1-C2; cat# 457731), astrocytic (GFAP; green; ID Rn-GFAP-C1; cat# 407881), neuronal (Rbfox3; gray; ID Rn-Rbfox3-C1; cat# 436351) mRNA marker, and IL-6 targeting (Probe ID Rn-IL-6-C3; cat# 427141), all purchased from Advanced Cell Diagnostics, as previously reported: Mishra et al. (2019). Of note, while GFAP is widely used as an astrocytic marker, and most (not all) astrocytes are labeled by GFAP, it may also label small populations of non-astrocytic cells like tanycytes.

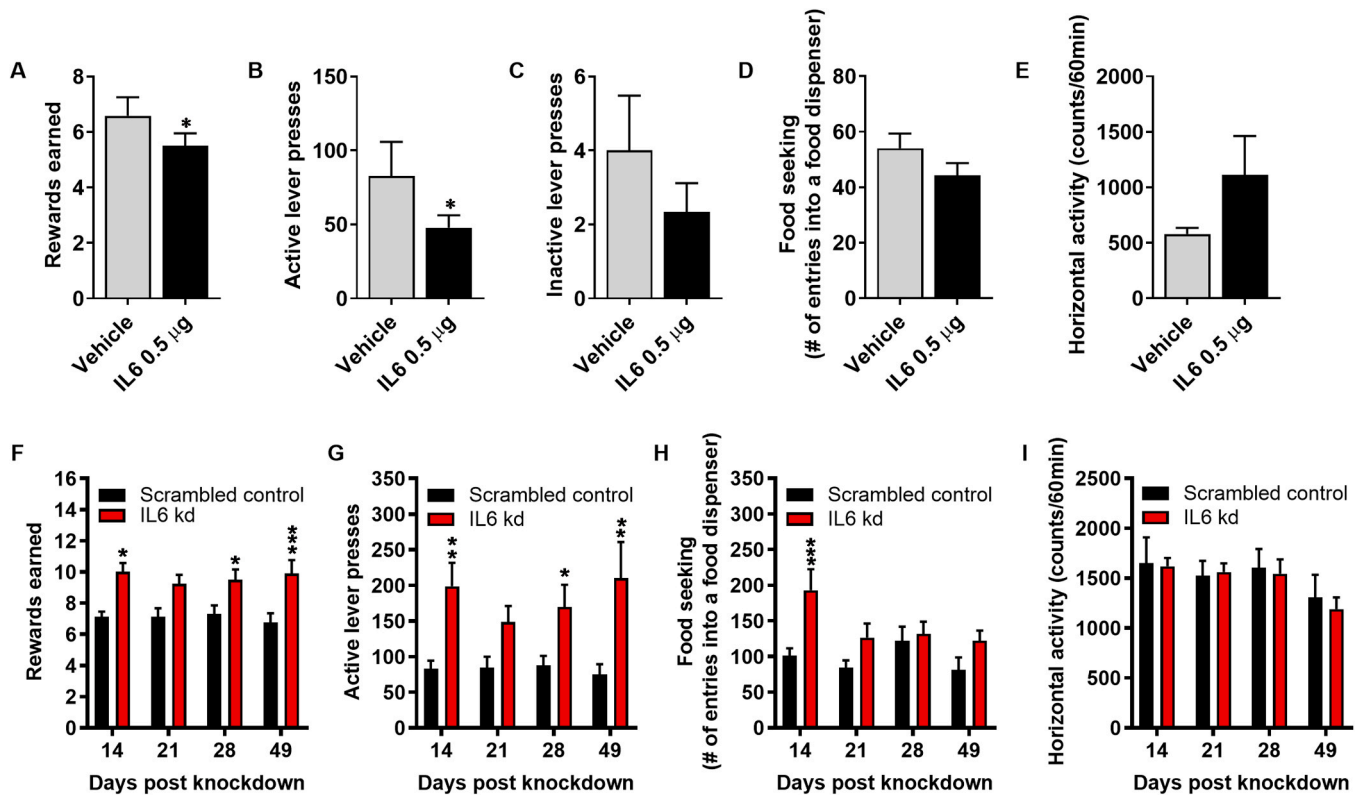
Fluorescent images of the LH were captured with 20X magnification objective lens using LSM700 Zeiss confocal microscope and processed using ImageJ software (NIH, USA). All parameters (pinhole, contrast, gain and offset) were held constant for all sections from the same

experiment. Approximate quantification strategy, was performed on  $320 \times 320 \mu\text{m}$  sections of the LH. Sections for three male and three female rats were processed for each cell type marker and IL-6. In short, 20 regions of interest (ROI) corresponding to a border of  $\sim 2 \mu\text{m}$  drawn around all nuclei were selected from 20x obtained images and the cells outlined in this manner were scored for absence or presence of the *in-situ* signal for IL-6 mRNA, or the three respective neuronal markers (Mishra et al., 2019). For analysis of IL-6 mRNA signal, arbitrary fluorescent units (a.u.) were taken from Z-stack acquired images from the minimum value in the ROI to preserve the dynamic range as the increase in fluorescence between IL-6 kd vs. control rats (Longo et al., 2021; Shrestha et al., 2020).  $n$  refers to the number of rats per group (average of 20 somas per slice, 2 slices per rat).



(caption on next page)

**Fig. 4.** IL-6 does not regulate food intake through actions in the LH of female rats. Representative image of injection site (A). In females, acute injection of IL-6 into the LH did not alter consumption of lard and sugar or chow, 1 (B, C) or 24 (D, E) hours after injection. Body weight was unaffected at the 24-hour time point (F). Intra-LH IL-6 kd did not alter cumulative chow intake (G), or cumulative body weight gain (H) in chow fed females, or females fed a HFHS diet (I–K). Intra-IL6 kd did not alter the terminal weight of iWAT or gWAT (L). IL-6 kd females failed to return to baseline at the 4-hour time point after an oral GTT (M); total blood glucose (measured as the AUC for the 4-hour period) was however not significantly altered (N). Core and BAT temperature were unaltered by intra-LH IL-6 kd (O). Representative thermal images for scrambled control BAT (P), ear (core; Q) and IL-6 kd BAT (R) and ear (core; S). AUC = area under the curve, iWAT = inguinal white adipose tissue, gWAT = gonadal white adipose tissue, BAT = brown adipose tissue. Data expressed as mean  $\pm$  SEM. For IL-6 injection:  $n = 12$  per treatment. For IL-6 kd:  $n = 10$ –11 per treatment group. \*\* $P < 0.01$ .



**Fig. 5.** Intra-LH IL-6 is both sufficient and necessary for the regulation of food-motivated behavior in female rats. Intra-LH IL-6 injection significantly reduced food rewards earned (A) and active lever presses for reward (B), without altering inactive lever presses (C), in the operant conditioning task. Food seeking (D) and horizontal activity (E) were not affected by the manipulation. Chow fed female IL-6 kd rats displayed a significant increase in active lever presses (G), rewards earned (F) and food seeking behavior (H) without altering locomotor activity (I). Data expressed as mean  $\pm$  SEM.  $n = 12$  per treatment. For IL-6/scrambled control injection:  $n = 12$  per treatment. For IL-6 kd:  $n = 31$  per treatment. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

## 2.9. Oral glucose tolerance test

Prior to the oral glucose tolerance test (OGTT), rats were food deprived overnight to ensure complete consumption of the test sucrose meal. Sucrose pellets (2.6 g of sugar/kg body weight; 45 mg TestDiet, Richmond, IN, USA) were then presented for 10 min and any leftover pellets were recorded. Only rats that consumed the full amount were included in the data analysis. Blood glucose was measured from tail blood 15, 30, 60, 120, 180 and 240 min after the sucrose meal. The test was conducted 3 weeks after introduction of the HFHS diet (11 weeks after AAV injection, see Table 2 for timeline). Additionally, 2 weeks following OGTT, basal blood glucose levels were measured in blood collected from the carotid arteries at the time of euthanizing. Food was removed 2 h prior to blood glucose measurements.

## 2.10. Body temperature measurements

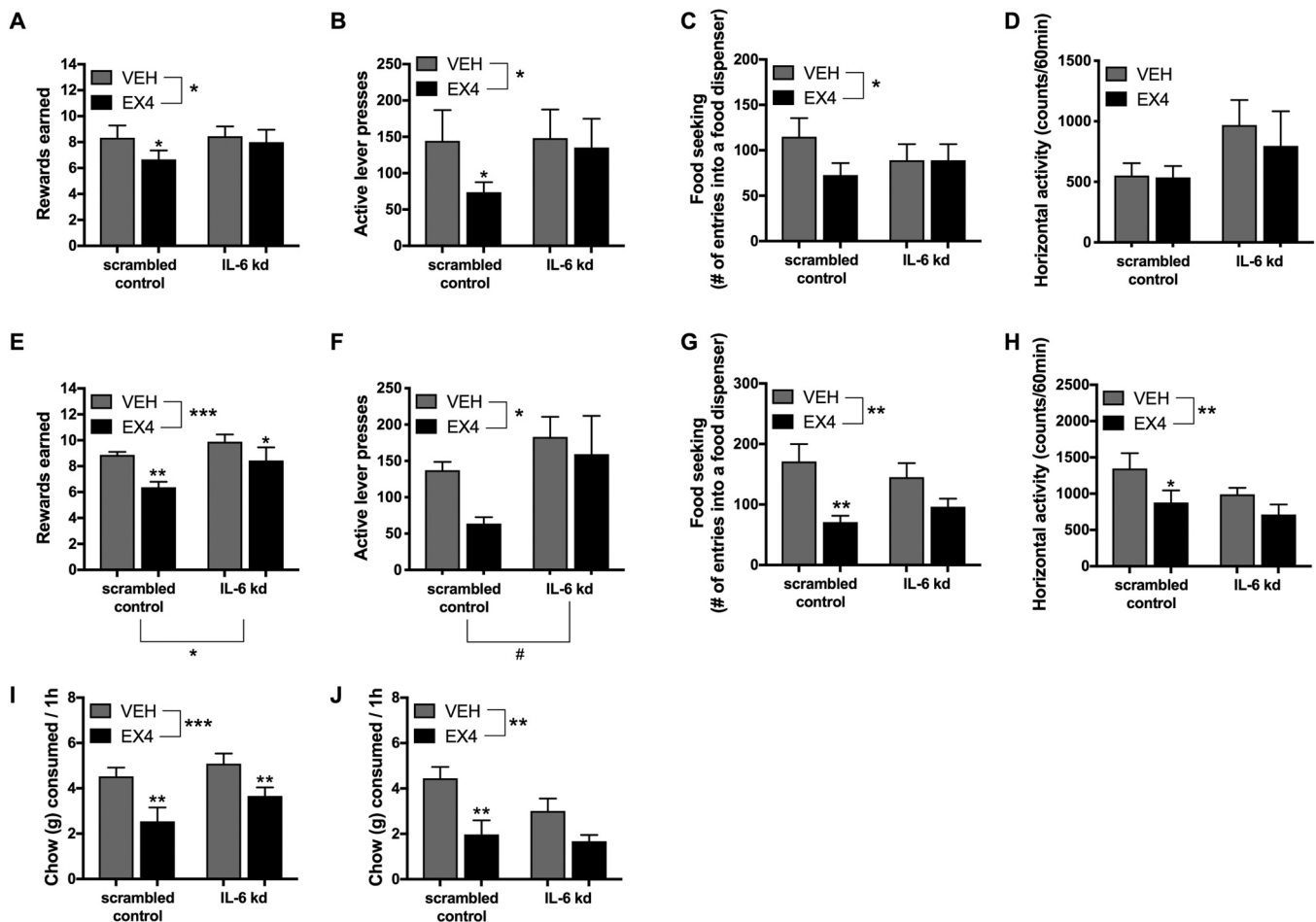
A FLIR T500-Series thermal camera was used to measure the temperature of the area above the brown adipose tissue (BAT; interscapular depot) and core temperature (ear), as previously described (Lee et al., 2018; Martinez-Sanchez et al., 2017; Matesanz et al., 2017). The skin

above BAT was shaved two days prior to the collection of the thermal images to facilitate thermal measurements without confounding effects of fur in the image. Four to five images per rat were taken of each area, in the home-cage environment, from a fixed distance; the average temperature was used for data analysis. Measurements were conducted after 4 weeks on HFHS diet in kd and control rats (see Table 2 for timeline).

## 2.11. Brain dissection and gene expression

Rats were anesthetized briefly in an induction chamber using 4% isoflurane (Baxter AB, Sweden). Brains were rapidly removed and frozen in isopentane cooled over a bed of dry ice; they were then stored on dry ice before being transferred and stored in a  $-80^{\circ}\text{C}$  freezer. Brains were cut in  $80\ \mu\text{m}$  sections using a cryostat (Leica 3050S; Leica Biosystems Nussloch GmbH, Nussloch, Germany), and the LH was dissected using disposable biopsy punches with plungers (INTEGRA, USA). Total RNA was extracted using RNeasy Lipid Tissue Mini kit (QIAGEN). RNA quality and quantity were assessed using Nanodrop 1000 (NanoDrop Technologies) prior to cDNA synthesis which was carried out using the iScript cDNA Synthesis kit (Bio-Rad). Gene expression levels of IL-6 were quantified through RT-PCR using TaqMan gene expression kits from Life





**Fig. 6.** Intra-LH IL-6 is necessary for the food-reward reducing effects of GLP-1R agonist Ex4. Ex4 reduced rewards earned and active lever presses in both males (A, B) and females (E, F). These effects were abolished in IL-6 kd male rats. Intra-LH IL-6 kd also prevented the effects of Ex4 on food seeking in males (C), and to a smaller extent in females (G). Intra-LH Ex4 injection led to a significant reduction in horizontal activity in females (H), but not males (D); this effect was not present in IL-6 kd rodents. Effects of intra-LH Ex4 on chow intake were not prevented by IL-6 kd in males (I). Ex4 did not significantly reduce chow intake in intra-LH IL-6 kd females (J). Data expressed as mean  $\pm$  SEM. Males:  $n = 9$  (scrambled control) and 11 (IL-6 kd). Females:  $n = 8$  (scrambled control) and 9 (IL-6 kd). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$ , #  $P > 0.05$  but  $>0.08$ .

Technologies. Reference numbers for probe and primer sets were as follows: IL6-Rn01410330\_m1; Actb-Rn00667869\_m1 (used as control gene; Applied Biosystems). Comparative threshold cycle method was used to quantify relative mRNA expression (Livak and Schmittgen, 2001).

## 2.12. Adipose tissue dissection

Inguinal white adipose tissue (iWAT) was dissected by creating a wide skin incision on the abdominal side, and the fat was separated from the skin and underlying muscle using scissors. After removing the fat pad, lymph nodes were removed from the tissue. The dorsal subcutaneous pad was not included in this dissection. To remove the gonadal white adipose tissue (gWAT), the abdominal wall was opened and the genitals (ovaries or testes, according to the sex) were extracted. The fat pads were then carefully separated from surrounding tissue by gently pulling the fat.

## 2.13. Statistical analysis

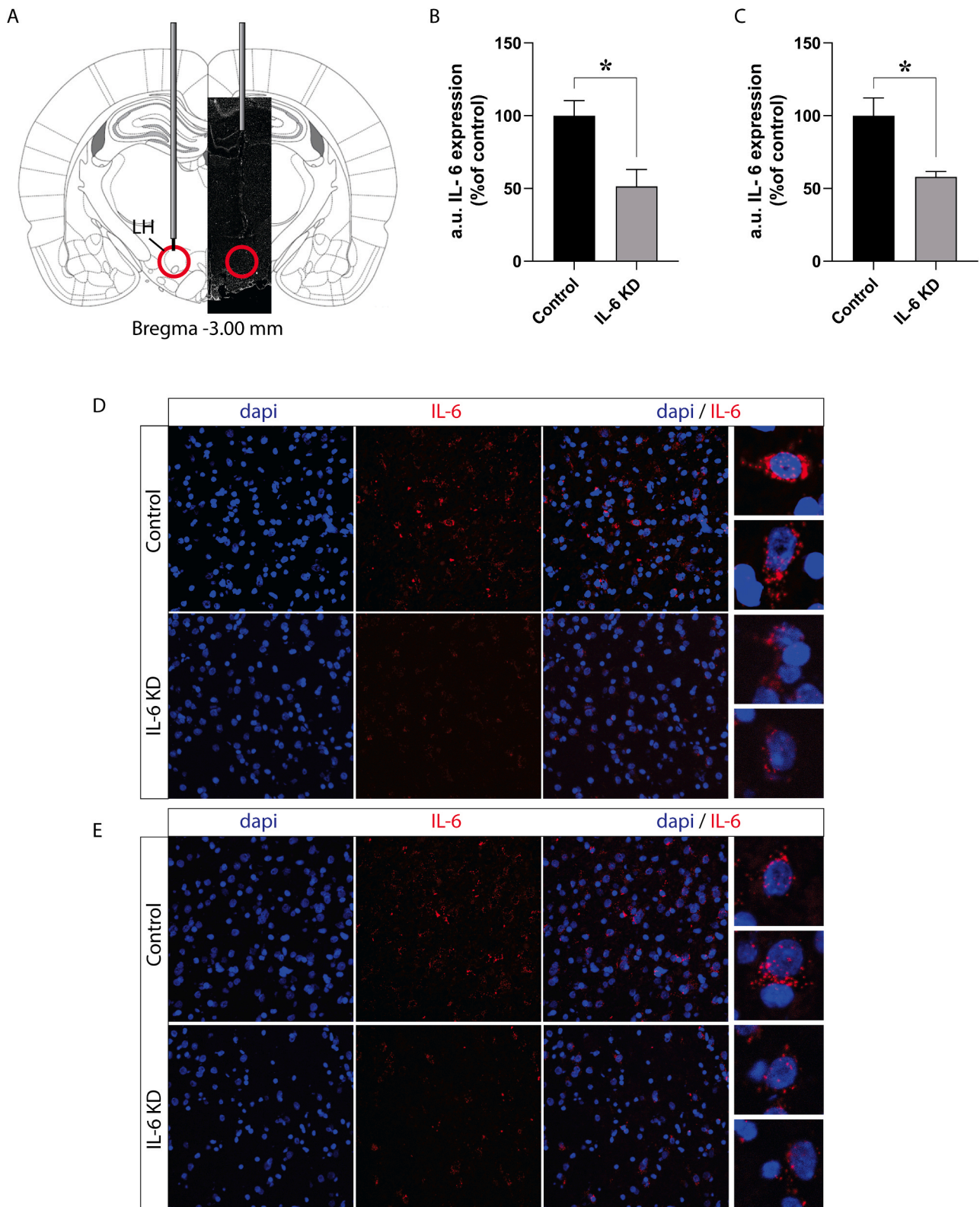
All data are presented as mean  $\pm$  SEM. Statistical significance was analyzed using Student's  $t$ -test for comparisons of two groups, or one- or two-way ANOVA with post-hoc Holm-Sidak's multiple comparison test when appropriate (GraphPad Prism 7 Software, Inc). P-values lower

than 0.05 were considered statistically significant. We elected to present our results for all behavioral and physiological parameters for males and females separately, motivated by the fact that males and females have very different baselines for most of them (as indicated here but also as well-established in the literature), and that showing disaggregated sex data is consistent with the 2015 NIH guidelines on reporting sex in preclinical literature. What follows is that the statistical analysis visualized on the figures reflects separate sex analysis, thus asking the question: is there an effect in females and is there an effect in males. Statistical analysis combining both sexes is presented in [Supplementary Tables S1–S4](#). For *In situ* data in [Fig. 1](#), previous literature indicating any potential sex differences was lacking (both in regard to our own data and other published scientific literature). Results are therefore presented as both aggregate analysis ([Fig. 1](#)) and as images from males and females separately ([Fig. S1](#)).

## 3. Results

### 3.1. Neurons, astrocytes and microglia in the LH express IL-6

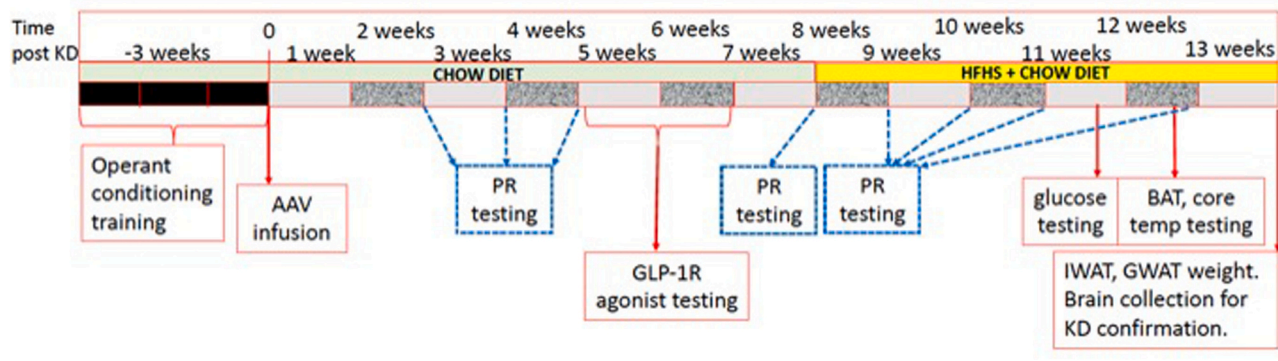
To determine the cellular origin of IL-6 in the LH, we used RNAScope to co-localize IL-6 mRNA with microglial (AIF1; orange; [Fig. 1A](#)), astrocytic (GFAP; green; [Fig. 1B](#)), or neuronal (Rbfox3; gray; [Fig. 1C](#)) mRNA markers. The majority of IL-6-expressing cells are neurons, which



**Fig. 7.** Intra-LH IL-6 kd led to 50% reduction of IL-6 mRNA compared to scrambled controls. Representative image of rat coronal brain atlas drawing and rat tissue section showing LH-targeted AAV infusions (A). Lateral hypothalamus IL-6 mRNA was detected using fluorescent in situ hybridization (RNAScope). IL-6 mRNA signal in fluorescent arbitrary units (a.u.) expressed as % of control in LH of male (B) and female (C) rats. IL-6 mRNA intensity was measured in ImageJ. Statistical significance was determined by using Student's *t*-test (Control vs. IL-6 kd rats; unpaired *t*-test; male, B,  $t(4) = 3.123$ ,  $P = 0.035$ ; C,  $t(4) = 3.285$ ,  $P = 0.030$ ). Data are shown as mean  $\pm$  SEM. of  $n = 3$  rats per group (average of  $n = 20$  cells per slice,  $n = 2$  LH areas per rat) \* $P < 0.05$ . Representative immunofluorescence images of IL-6 mRNA (red) and cell nuclei (blue; DAPI) detected using RNAScope in LH coronal brain slices from male (D) and female (E) control rats and rats with siRNA-mediated knockdown of IL-6 gene.

Table 2

Overview of the timeline of interventions and testing in the IL-6 kd experiment.



make up almost half (49%) of the IL-6-expressing cells in the LH (Fig. 1E). Approximately 32% of IL-6-expressing cells are astrocytes, followed by microglia (19%). Data were collected from six rats, three males and three females, two slices per rat were analyzed. Two-way ANOVA analysis indicates that the LH distribution of IL-6 by cell type did not differ between sexes (Fig. S1).

### 3.2. LH-targeted administration of IL-6 reduces ingestive behavior in males

Acute intra-LH IL-6 injection ( $n = 12$ , within subject experiment) led to a significant reduction in chow intake, 1 h after injection (Fig. 2B). The effect on food intake was no longer present after 24 h (Fig. 2C), and did not result in a change in body weight at this time point (Fig. 2D). In contrast, in male rats maintained on a HFHS/chow choice diet ( $n = 17$ , within subject experiment), intra-LH IL-6 selectively reduced intake of the HFHS food, both 1 h (Fig. 2F) and 24 h (Fig. 2H) after injection, without altering intake of regular chow (Fig. 2E & G). However, body weight at the 24-hour time point was still not altered (Fig. 2I).

### 3.3. Chronic intra-LH virogenetic IL-6 kd increases palatable food intake and body weight in metabolically challenged males

Partial loss of IL-6 in the LH did not alter chow intake (Fig. 2J), or body weight (Fig. 2K), in males fed a normal chow diet ( $n = 8-11$  per treatment grp). However, when challenged with a HFHS alternative, rats with LH IL-6 kd selectively increased intake of the palatable food (Fig. 2M), while their intake of chow remained similar to controls (Fig. 2L). Rats maintained on a HFHS diet option also displayed a significant body weight gain (Fig. 2N). Changes in food intake were not accompanied by changes in the intake of water (Fig. S2). Partial loss of IL-6 in the LH in HFHS-fed males led to increased adiposity, as indicated by increased iWAT mass compared to control rats; gWAT mass was not significantly affected by the treatment (Fig. 2O). Loss of IL-6 in the LH did not affect basal blood glucose level (Fig. S4A), or OGTT (Fig. 2P, Q). While IL-6 manipulation at other CNS regions lead to altered BAT thermogenesis (Mishra et al., 2019), LH-specific IL-6 kd did not affect core or BAT temperature (Fig. 2R). Representative thermal images are displayed in Fig. 2S-V.

### 3.4. Intra-LH IL-6 is neither sufficient nor necessary to alter food-motivated behavior in males

Intra-LH IL-6 injection ( $n = 17$ , within subject experiment) did not affect food-motivated behavior or food seeking in chow-fed males (Fig. 3A-D). Food reward and food seeking parameters were also unaffected in males fed a HFHS diet (Fig. 3F-I). Moreover, horizontal activity did not differ between treatment groups on either diet (Fig. 3E,J), also

diet alone had no effect on locomotor activity during operant testing.

In line with the lack of effect of intra-LH exogenous IL-6 injections, virogenetic LH IL-6 reduction ( $n = 10-11$  per treatment group) did not change motivated behavior for palatable food, or palatable food seeking (Fig. 3K-M), or locomotor activity (Fig. 3N) during any of the four measurement instances performed 2, 3, 4, and 7 weeks after viral IL-6 kd. In contrast to the increased intake of the palatable food after partial LH IL-6 loss, motivated behavior for palatable food was not altered in HFHS diet-maintained rats (Fig. S3A-D).

### 3.5. Food intake or body weight in females is not affected by intra-LH administration of IL-6, or partial LH IL-6 loss

In contrast to the anorexic effect found in males, microinjection of IL-6 in the LH of females ( $n = 12$ , within subject experiment) did not alter the intake of chow, or lard and sugar, measured 1 (Fig. 4B & C) and 24 (Fig. 4D & E) hours after injection in female rats fed a HFHS diet. Also 24 h body weight change was not affected by this treatment in females (Fig. 4F). In line with the lack of effect in females of exogenous IL-6 delivery into the LH, chow intake (Fig. 4G) and body weight (Fig. 4H) were not altered by virogenetic LH IL-6 kd in chow-fed females ( $n = 8-10$  per treatment group). Even the HFHS diet challenge, which was effective at uncovering the necessity of LH-produced IL-6 in feeding control and weight regulation in males, did not produce the same results in female rats. Female rats with virogenetic IL-6 kd ate the same amount of chow and HFHS food as controls (Fig. 4I, J), and while weight gain may appear to slightly diverge between kd and control rats, this difference was not significant (Fig. 4K). Note that given a marginally small effect obtained on chow intake in males, and no indication of ingestive behavior being affected in females (either while on HFHS diet, or on chow during the knockdown experiment), we did not test the effect of LH-targeting IL-6 injection on females presented with chow alone. Adiposity, measured by determined the mass of iWAT and gWAT in kd and control rats, did not differ between treatments (Fig. 4L). Basal blood glucose levels (Fig. S4A) or those resulting from OGTT (Fig. 4M-N) were largely not altered by LH IL-6 kd. However, slightly, but significantly, higher blood glucose level was detected 4 h after sucrose consumption in kd females (Fig. 4M). This still did not result in an overall difference in the glucose area under the curve (AUC; Fig. 4N). As in males, LH IL-6 kd females had normal thermogenesis, as indicated by unaffected core or BAT temperature (Fig. 4O). Representative thermal images are displayed in Fig. 4P-S (4 P, R: ear temperature; 4Q and S: whole body thermal image to capture intrascapular BAT temperature).

### 3.6. IL-6 in the LH is sufficient and necessary to alter food-motivated behavior in females

Exogenous IL-6 microinjection targeting the LH ( $n = 11$ , within

subject experiment) significantly reduced food-motivated behavior in female rats, as indicated by fewer sucrose rewards earned (Fig. 5A) and reduced active lever presses (Fig. 5B) in LH IL-6-treated females. Importantly, the activity at the inactive lever (Fig. 5C), was not altered. Food seeking was not significantly affected, although a small trend to reduced number of entries into the food dispenser was noted (Fig. 5D). While horizontal activity was not significantly altered (Fig. 5E) female rats injected with IL-6 into the LH moved twice as much as control rats on average in the operant box (Fig. 5E). This clearly highlights that the reduced activity toward obtaining a food reward was a food-specific effect and not a result of a general activity/locomotion suppression.

Conversely, partial LH IL-6 loss ( $n = 9-10$  per treatment group) resulted in a drastic disinhibition in food motivated behavior, as indicated by increased lever presses for sucrose and greater number of sucrose rewards earned (Fig. 5F–G). This increase in food wanting was maintained throughout the 7-week period of testing, with an over 40% increase in rewards earned and doubling in the active lever presses at 7 weeks post kd. Interestingly, food seeking was significantly affected only 14 days post knockdown, where the rats doubled the amount of times, from 100 to 200, they entered the food dispenser zone (Fig. 5H). This increase was transient, as it was absent at 3-, 4- or 7-weeks post IL-6 kd. The discrepancy between food motivation and food seeking highlights that these two behaviors are not controlled by the same mechanisms, and indicated that measurement of both is warranted. Horizontal activity was not altered by LH IL-6 kd at all time points (Fig. 5I). For 2-way ANOVA results directly comparing results between males and females see Table S2. Challenging the female rats with a HFHS diet partly attenuated the motivated behavior impact of LH IL-6, where a significant increase in rewards earned was recorded only 2 weeks after the introduction of HFHS diet in females (Fig. S3E). Moreover, the operant task data normalized to compare the effect of IL-6 kd on food-motivated behavior in HFHS- vs chow-fed rats indicate potential devaluation of the food reward in all rats fed the HFHS diet. Interestingly, the food reward was devalued to a greater extent in rats in the IL-6 kd group (Fig S4B–D), abolishing the difference noted in the chow-fed rats and resulting in close to normal performance of these rats in the PR operant task.

Since we observed a clear reduction in operant performance in both sexes after exposure to HFHS diet, we formally asked whether this reduction interacts with kd or sex. For 2-way ANOVA results comparing results between males and females see Table S3. Two-way ANOVA analysis for rewards earned indicated a significant effect of the kd, but not sex, and no interaction (Fig. S4C). Likewise, two-way ANOVA analysis of active lever presses for sugar during the operant conditioning task indicated a significant effect of the kd, but not sex, and no significant interaction between the two (Fig. S4B). However, post hoc analysis points to a larger reduction in active lever presses in the IL-6 kd group compared to controls only in females (Fig. S4B;  $p < 0.05$ ), although this increased effect size may simply reflect the higher chow-fed baseline. Two-way analysis of food-seeking behavior indicated a trend in the effect of the treatment, and no significant differences in sex, or in the interaction between sex and treatment (Fig. S4D). Food-seeking behavior in females was, however, reduced to a larger degree in the IL-6 kd treated group (Fig. S4D,  $p < 0.05$ ). No significant effect of sex, kd or interaction was found on horizontal activity measured during the 60-minute operant conditioning test.

### 3.7. LH IL-6 is necessary for GLP-1R-induced motivated behavior suppression but not hypophagia

Since a wealth of data points to CNS IL-6 as the necessary mediator of the anorexic effect of GLP-1R agonists, we evaluated whether this interaction takes place at the level of the LH. GLP-1R agonist Ex4 was applied directly into the LH of food restricted male and female rats, and reduced the number of sucrose rewards earned and active lever presses for sucrose in both males (Fig. 6A–B) and females (Fig. 6E–F). The reducing effect of Ex4 was significantly attenuated by the kd on some

parameters – for example food seeking, suggesting a functional relationship between hypothalamic IL-6 and GLP-1R. Two-way ANOVA of active lever presses for males revealed a significant effect of Ex4 treatment ( $F(1, 18) = 4.502, p < 0.05$ ). The effect of kd was not significant ( $F(1, 18) = 0.4542, p > 0.05$ ), nor was there an interaction between the two ( $F(1, 18) = 2.150, p = 0.15$ ). Two-way ANOVA of active lever presses for females revealed a significant effect of Ex4 treatment ( $F(1, 15) = 4.41, p < 0.05$ ). There was a trend to effect of kd ( $F(1, 15) = 3.49, p = 0.08$ ), no interaction was detected ( $F(1, 15) = 2.150, p = 0.15$ ). Ex4 treatment significantly decreased rewards earned in males ( $F(1, 18) = 6.6, p < 0.05$ ), but not kd ( $F(1, 18) = 0.4740, p > 0.05$ ), no interaction was detected ( $F(1, 18) = 1.216, p = 0.15$ ). Ex4 treatment also significantly decreased rewards earned in females ( $F(1, 15) = 17.95, p < 0.001$ ), there was a significant effect of kd ( $F(1, 15) = 4.27, p < 0.05$ ), and there was no interaction between the two factors ( $F(1, 15) = 0.97, p > 0.05$ ). IL-6 kd also attenuated the effects of Ex4 on food-seeking behavior in males (Fig. 6C), and to a smaller extent in females (Fig. 6G). Two-way analysis showed a significant effect of Ex4 on food-seeking behavior in females ( $F(1, 18) = 6.312, p < 0.05$ ). The effect of kd was not significant ( $F(1, 18) = 0.04181, p > 0.05$ ); there was a significant interaction between Ex4 and kd ( $F(1, 18) = 6.312, p < 0.05$ ). It also appears that food restriction, like HFHS challenge, reduces the impact of IL-6 kd in female rats, by increasing the food motivation of control rats. Horizontal activity was not altered in males (Fig. 6D). In males, two-way analysis indicated no significant effect of Ex4 treatment on horizontal activity ( $F(1, 18) = 2.72, p > 0.05$ ). There was no significant effect of the kd ( $F(1, 18) = 2.72, p = 0.11$ ), nor an interaction between kd and Ex4 ( $F(1, 18) = 0.1466, p > 0.05$ ). Ex4-treated females moved significantly less than controls during the 60-minute operant conditioning test period (Fig. 6H); this difference was not present in kd females. In females, two-way analysis indicated a significant effect of Ex4 treatment on horizontal activity ( $F(1, 15) = 11.02, p < 0.001$ ). There was no significant effect of the kd ( $F(1, 15) = 1.983, p > 0.05$ ), nor an interaction between kd and Ex4 ( $F(1, 15) = 0.7297, p > 0.05$ ). Kd of IL-6 in this area did not, however, affect the actions of Ex4 on food intake, as a reduction in 1-hour intake was present in both control and IL-6 kd conditions of both sexes (Fig. 6I–J). Two-way analysis showed a significant effect of Ex4 in males ( $F(1, 18) = 22.94, p < 0.001$ ) and females ( $F(1, 15) = 15.90, p < 0.01$ ), but no effect of kd (males:  $F(1, 18) = 2.408, p > 0.05$ ; females:  $F(1, 15) = 2.854, p > 0.05$ ), nor an interaction between Ex4 and kd (males:  $F(1, 18) = 0.6200, p > 0.05$ ; females:  $F(1, 15) = 1.429, p > 0.05$ ) in either sex. For 3-way ANOVA results, conducted to directly compare results between males and females see Table S4. Overall 3-way ANOVAs indicated a significant effect of LH-injected Ex4 on rewards, active lever presses, food seeking, and chow intake, and a strong trend ( $p = 0.06$ ) in the activity parameter. It also detected a significant interaction between kd and sex in activity and chow intake. An interaction between kd and Ex4 was detected for food seeking parameter, and trends towards interaction of  $p = 0.09$  were detected for rewards and active lever presses.

## 4. Discussion

The LH plays an essential role in coordinating food intake and food motivated behavior, and neuropeptides, such as orexin-, melanin-concentrating hormone- and neurotensin act in this area to control hunger and food reward (Alon and Friedman, 2006; Cooke et al., 2009; Harris et al., 2005; Haynes et al., 2002; Kim et al., 2008; Leininger et al., 2011; Ludwig et al., 2001; Qu et al., 1996; Sakurai, 1999; Shimada et al., 1998). In addition, the anorexigenic gut/neuro hormone, GLP-1, acts in the LH to reduce food intake and food motivated-behavior; effects previously linked to IL-6 at a whole brain level (Shirazi et al., 2013). Here we show that neurons represent the most abundant source of IL-6 in the LH, in both male and female rats. We demonstrate that LH IL-6 is a pivotal component of food intake and food reward control systems through actions on IL-6r in the LH, in a sex divergent manner.

While LH IL-6 is necessary and sufficient for intake of high caloric foods (lard and sugar) and body weight regulation in males, in females it is necessary and sufficient for food-motivated behavior control only. Thus, in addition to contributing to the food intake and reward-reducing effects of GLP-1 in this area, IL-6 emerges as an important mediator of energy balance and reinforcement in the LH in its own right. Moreover, to the best of our knowledge this is the first report indicating a role for IL-6 in control of motivated behavior, outside of the context of infection or inflammation, significantly expanding the range of behavioral impact of this cytokine in the CNS.

Previous studies drawing on data from different brain areas or neuronal cell cultures indicate that neurons, astrocytes, and microglia are capable of producing IL-6 during normal physiology for example in response to gut-brain signals (Hidalgo et al., 2010; Le Foll et al., 2015; Lopez-Ferreras et al., 2018; Shirazi et al., 2013). Here we show that similarly to what we have previously noted for the parabrachial nucleus, IL-6 in the LH is primary localized to neurons. Despite that only males showed a response in ingestive behavior to IL-6 kd, while only motivated behaviors were affected in females after IL-6 manipulation, *IL-6* mRNA was found to be co-expressed with neuronal markers in both sexes to the same extent. Moreover, the astrocytic and microglial co-expression was also similar between the sexes. Thus, the behavioral divergence is likely to stem from either a different neurochemical phenotype of the IL-6 expressing neurons and/or divergent downstream molecular and neuroanatomical targets. The current dataset is in line with previous studies suggesting actions on the IL-6r in the paraventricular (PVH) and ventromedial hypothalamus (VMH) (Benrick et al., 2009; Le Foll et al., 2015; Timper et al., 2017). Most hypothalamic nuclei are highly interconnected; thus, since IL-6 is present in LH neurons, possibly capable of carrying IL-6 to sites outside of the LH, LH-produced IL-6 may reach IL-6r populations outside of the LH and including PVH and VMH, in addition to the intra-LH action outlined here.

The LH is well-established in its reward-controlling role (Anand and Brobeck, 1951; Hoebel and Teitelbaum, 1962; Margules and Olds, 1962; Miller, 1960; Olds, 1962). IL-6, however, has not been linked to reward-control previously. Only one study evaluated the effects of IL-6 in the LH on rewarding intra-cranial self-stimulation, yet it found no effect of IL-6 on this behavior (Anisman et al., 1998). This may seemingly contrast with the current results, however, the above cited work tested only males, like great majority of studies in the field. Thus, these results are in fact consistent with the lack of effects of intra-LH IL-6 treatment on food reward behavior in males in the present study. But the conclusion of lack of effect of IL-6 alone in the LH on motivated behavior is not valid for females, where a clear and rather potent increase in food-motivated behavior is noted after reduced expression of IL-6 in the LH. These results are further strengthened by a clear reduction in food-motivated behavior after selective intra-LH delivery of exogenous IL-6. These data also point to a local IL-6-driven circuit, where IL-6 is both produced and exerts its effects within the LH.

With the current results IL-6 joins a prominent group of food intake and food reward-controlling molecules shown to act in a sex-specific manner in the LH. Ghrelin is sufficient to induce hyperphagia and increase food-motivated behavior in both sexes, but only necessary in females (Lopez-Ferreras et al., 2017). In addition, GLP-1 receptor stimulation in the LH reduces food intake and food-motivated behavior more potently in males, and is necessary for the regulation of motivated behaviors in adult males but not females of the same age (Lopez-Ferreras et al., 2018). The effects of GLP-1R stimulation on food intake and reward in the LH were associated with a significant increase in IL-6 in both sexes. However, IL-6 expression was only increased in females in the high estrogen signaling estrus phase, in line with the effects of the GLP-1 analog on food-motivated behavior, which was reduced in females in estrus, but not metestrus or diestrus cycle phases. In the current study increased IL-6 resulted in a reduction of food-motivated behavior in females, indicating that the sex-specific effects of GLP-1 in the LH are most likely due to other down-stream mediators; for example,

neurotensin or orexin, expression of which was reduced in a sex-specific manner in response to GLP-1R activation in the LH (Lopez-Ferreras et al., 2018). Neurotensin neurons reside within the LH; they project to the ventral tegmental area (VTA), and activation of these neurons promotes reward behavior (Kempadoo et al., 2013; Leininger et al., 2009, 2011). Since increased IL-6 expression, in response to GLP-1R stimulation in the LH, is accompanied by a significant reduction in neurotensin; an effect which is specific to females in the high estrogen-signaling phase estrus (Lopez-Ferreras et al., 2018), distinct effects of IL-6 on neurotensin in females could therefore potentially contribute to the sex specific effects of IL-6 alone on food reward behavior in the current study.

Males in the present study present with reduced food intake in response to intra-LH IL-6 injection. Intake of palatable food, consisting of a mix of lard and sugar, was reduced when both chow and palatable mix were presented as a choice. In fact, both acute pharmacological IL-6 injection and chronic IL-6 kd altered HFHS food intake without affecting the intake of regular chow in males. However, when chow was offered as the sole food source, acute IL-6 injections did reduce chow intake, albeit the effect size was very small. In addition, males on a chow diet maintained similar body weight levels as controls. Furthermore, body weight was only affected in males fed an obesogenic diet. These data demonstrate that IL-6 rather selectively reduces the intake of palatable food through its actions in the LH, in males. IL-6 was previously shown to reduce the intake of standard chow after injection into the ventricles (Shirazi et al., 2013), or the lateral parabrachial nucleus (IPBN) (Mishra et al., 2019), indicating that IL-6 may more potently alter the intake of different food types in distinct CNS areas. However, intra-IPBN injection of IL-6 also led to an increase in lard specifically, when rats were given a choice between lard, sucrose and standard chow (Mishra et al., 2019). Thus, IL-6 also has the ability to affect palatable food intake through actions in nuclei outside of the LH. The ability to affect the same behavior from multiple sites in the CNS is a well-established but perhaps underappreciated characteristic of many energy balance controlling neuropeptides (Grill, 2010; Grill and Hayes, 2012).

The effects of intra-LH IL-6 on food-motivated behavior in females were virtually abolished after exposure to a HFHS diet. This is likely due to a drastic change in motivation to work for sucrose displayed by both controls and IL-6 kd rats. Rats on this diet had ad libitum access to a lard/sugar mix in their home cage and current results (Fig. S4), which compare the motivation of rats fed chow vs HFHS indicate what might be interpreted as devaluation of sucrose rewards characterized by a drastic reduction in motivation to work for sucrose pellets in an operant test irrespective of the IL-6 manipulation. Previous studies of the effects of unrestricted HFHS feeding on food-motivated behavior are somewhat conflicted in terms of the impact of this feeding on motivated behavior for the same or similar (e.g. in macronutrient content) type of food, where both increased and reduced motivated behavior has been reported (Glass et al., 1999; Greenwood et al., 1974; la Fleur et al., 2007; Vasselli et al., 1980). Current results suggest that the counterregulatory changes in the LH circuitry in response to overfeeding either may include an upregulation of IL-6 in the LH, which in turn overcomes the kd effect, or represent engaging other circuits which also have the ability to counteract the increases in motivated behavior resulting from loss of IL-6. Considering that at the termination of the experiment a clear reduction of IL-6 gene expression was still detected in females, the latter idea is more probable. However, the fact that previous studies indicate a rapid increase in IL-6 expression in response to obesogenic diet, in addition to that many reports suggest a long-term increase associated with inflammation in the hypothalamus (Thaler et al., 2012, 2013; Thaler and Schwartz, 2010) may support the idea of upregulation of IL-6 in response to diet. On the other hand, most of these studies did not focus on the lateral hypothalamus, mainly investigating the mediobasal hypothalamus (e.g. Thaler et al., 2012), thus it is possible that the inflammatory response may differ by the specific hypothalamic nucleus studied. Interestingly, the effect size of *IL-6* mRNA changes appears to be

quite different in the mediobasal hypothalamus in response to an obesogenic diet (50% increase (Thaler et al., 2012)) vs GLP-1 agonists in the whole or lateral hypothalamus (5–10-fold increase (Lopez-Ferreras et al., 2018)). Impact of IL-6 on behavior is likely to vary based on the region of interest, cell type producing IL-6 and presence or absence of other cytokines in the same microenvironment. Here considering the proximity of the mediobasal hypothalamus to the circumventricular median eminence it is likely that this area may respond differently to an obesogenic diet, and potentially more like the changes found in the plasma of obese individuals and obesity animal models. Also, most of the previous studies investigated only male rats or mice (for example De Souza et al., 2005; Thaler et al., 2012).

Consistent with our previous results, Ex4 injection in the LH led to a significant reduction in food intake and food-motivated behavior in both males and females (Lopez-Ferreras et al., 2018). Interestingly, while IL-6 kd attenuated the effects of Ex4 on food-motivated behavior in both sexes, effects on food intake persisted. These results indicate that while IL-6 is necessary for the effects of GLP-1R stimulation on food reward, food intake control by Ex4 does not require IL-6. Furthermore, while direct IL-6R stimulation or kd in the LH acts in a sex-divergent manner, IL-6 as a downstream mediator of GLP-1 receptor stimulation is necessary for the regulation of food-motivated behavior in both sexes.

In conclusion, our data identify the LH as a novel CNS area through which IL-6 regulates food intake and food-motivated behavior outside of the context of infection or inflammation. We highlight that food-motivated behavior appears to be under LH-IL-6 control in females, while ingestive behavior is under LH IL-6 control in males. These data therefore demonstrate the importance of testing both sexes in experiments exploring the actions of centrally acting molecules on food intake and food reward behavior, and uncover the ability of IL-6 to specifically reduce the drive to consume or work for palatable food, which may aid in the development of future therapeutics against obesity.

## Acknowledgements

This research was funded by the Swedish Research Council (2014-2945 to K.P.S) and 2013-7107 to Patrik Rorsman), the Novo Nordisk Foundation Excellence project grant (to K.P.S.), the Ragnar Söderberg Foundation (to K.P.S.), Harald Jeansson's Stiftelse and Greta Jeansson's Stiftelse (to K.P.S.), Magnus Bergvalls Stiftelse (to K.P.S.), the Wallenberg Foundation and the Center for Molecular and Translational Medicine (to K.P.S.).

## Conflict of interest

All the authors declare no conflict of interest.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.psyneuen.2021.105284](https://doi.org/10.1016/j.psyneuen.2021.105284).

## References

- Alon, T., Friedman, J.M., 2006. Late-onset leanness in mice with targeted ablation of melanin concentrating hormone neurons. *J. Neurosci.* 26, 389–397.
- Anand, B.K., Brobeck, J.R., 1951. Localization of a “feeding center” in the hypothalamus of the rat. *Proc. Soc. Exp. Biol. Med.* 77, 323–324.
- Andersson, C.X., Gustafson, B., Hammarstedt, A., Hedjazifar, S., Smith, U., 2008. Inflamed adipose tissue, insulin resistance and vascular injury. *Diabetes Metab. Res. Rev.* 24, 595–603.
- Anesten, F., Dalmau Gasull, A., Richard, J.E., Farkas, I., Mishra, D., Taing, L., Zhang, F., Poutanen, M., Palsdottir, V., Liposits, Z., Skibicka, K.P., Jansson, J.O., 2019. Interleukin-6 in the central amygdala is bioactive and co-localised with glucagon-like peptide-1 receptor. *J. Neuroendocr.* 31, e12722.
- Anisman, H., Kokkinidis, L., Borowski, T., Merali, Z., 1998. Differential effects of interleukin (IL)-1beta, IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. *Brain Res.* 779, 177–187.
- Benrick, A., Schele, E., Pinnock, S.B., Wernstedt-Asterholm, I., Dickson, S.L., Karlsson-Lindahl, L., Jansson, J.O., 2009. Interleukin-6 gene knockout influences energy balance regulating peptides in the hypothalamic paraventricular and supraoptic nuclei. *J. Neuroendocr.* 21, 620–628.
- Blundell, J.E., Herberg, L.J., 1968. Relative effects of nutritional deficit and deprivation period on rate of electrical self-stimulation of lateral hypothalamus. *Nature* 219, 627–628.
- Cooke, J.H., Patterson, M., Patel, S.R., Smith, K.L., Gbatei, M.A., Bloom, S.R., Murphy, K.G., 2009. Peripheral and central administration of xenin and neurotensin suppress food intake in rodents. *Obesity* 17, 1135–1143.
- Cornfield, L.J., Sills, M.A., 1991. High affinity interleukin-6 binding sites in bovine hypothalamus. *Eur. J. Pharmacol.* 202, 113–115.
- Davis, J.F., Choi, D.L., Schurdak, J.D., Fitzgerald, M.F., Clegg, D.J., Lipton, J.W., Figlewicz, D.P., Benoit, S.C., 2011. Leptin regulates energy balance and motivation through action at distinct neural circuits. *Biol. Psychiatry* 69, 668–674.
- De Souza, C.T., Araujo, E.P., Bordin, S., Ashimine, R., Zollner, R.L., Boschero, A.C., Saad, M.J., Velloso, L.A., 2005. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology* 146, 4192–4199.
- Dickson, S.L., Shirazi, R.H., Hansson, C., Bergquist, F., Nissbrandt, H., Skibicka, K.P., 2012. The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors. *J. Neurosci.* 32, 4812–4820.
- Fain, J.N., 2006. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam. Hormon.* 74, 443–477.
- Funabashi, T., Hagiwara, H., Mogi, K., Mitsushima, D., Shinohara, K., Kimura, F., 2009. Sex differences in the responses of orexin neurons in the lateral hypothalamic area and feeding behavior to fasting. *Neurosci. Lett.* 463, 31–34.
- Gadient, R.A., Otten, U., 1993. Differential expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat hypothalamus. *Neurosci. Lett.* 153, 13–16.
- Gadient, R.A., Otten, U., 1994a. Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. *Brain Res.* 637, 10–14.
- Gadient, R.A., Otten, U., 1994b. Identification of interleukin-6 (IL-6)-expressing neurons in the cerebellum and hippocampus of normal adult rats. *Neurosci. Lett.* 182, 243–246.
- Glass, M.J., O'Hare, E., Cleary, J.P., Billington, C.J., Levine, A.S., 1999. The effect of naloxone on food-motivated behavior in the obese Zucker rat. *Psychopharmacology* 141, 378–384.
- Greenwood, M.R., Quartermain, D., Johnson, P.R., Cruce, J.A., Hirsch, J., 1974. Food motivated behavior in genetically obese and hypothalamic-hyperphagic rats and mice. *Physiol. Behav.* 13, 687–692.
- Grill, H.J., 2010. Leptin and the systems neuroscience of meal size control. *Front. Neuroendocr.* 31, 61–78.
- Grill, H.J., Hayes, M.R., 2012. Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. *Cell Metab.* 16, 296–309.
- Harris, G.C., Wimmer, M., Aston-Jones, G., 2005. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437, 556–559.
- Haynes, A.C., Chapman, H., Taylor, C., Moore, G.B., Cawthorne, M.A., Tadayyon, M., Clapham, J.C., Arch, J.R., 2002. Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul. Pept.* 104, 153–159.
- Hidalgo, J., Florit, S., Giral, M., Ferrer, B., Keller, C., Pilegaard, H., 2010. Transgenic mice with astrocyte-targeted production of interleukin-6 are resistant to high-fat diet-induced increases in body weight and body fat. *Brain Behav. Immun.* 24, 119–126.
- Hodos, W., 1961. Progressive ratio as a measure of reward strength. *Science* 134, 943–944.
- Hoebel, B.G., Teitelbaum, P., 1962. Hypothalamic control of feeding and self-stimulation. *Science* 135, 375–377.
- Hurley, S.W., Johnson, A.K., 2014. The role of the lateral hypothalamus and orexin in ingestive behavior: a model for the translation of past experience and sensed deficits into motivated behaviors. *Front. Syst. Neurosci.* 8, 216.
- Kempadoo, K.A., Tourino, C., Cho, S.L., Magnani, F., Leininger, G.M., Stuber, G.D., Zhang, F., Myers, M.G., Deisseroth, K., de Lecea, L., Bonci, A., 2013. Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. *J. Neurosci.* 33, 7618–7626.
- Kim, E.R., Leckstrom, A., Mizuno, T.M., 2008. Impaired anorectic effect of leptin in neurotensin receptor 1-deficient mice. *Behav. Brain Res.* 194, 66–71.
- la Fleur, S.E., Vanderschuren, L.J., Luijendijk, M.C., Kloeze, B.M., Tiesjema, B., Adan, R.A., 2007. A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int. J. Obes.* 31, 1286–1294.
- Le Foll, C., Johnson, M.D., Dunn-Meynell, A.A., Boyle, C.N., Lutz, T.A., Levin, B.E., 2015. Amylin-induced central IL-6 production enhances ventromedial hypothalamic leptin signaling. *Diabetes* 64, 1621–1631.
- Lee, S.J., Sanchez-Watts, G., Krieger, J.P., Pignatola, A., Norell, P.N., Cortella, A., Pettersen, K.G., Vrdoljak, D., Hayes, M.R., Kanoski, S.E., Langhans, W., Watts, A.G., 2018. Loss of dorsomedial hypothalamic GLP-1 signaling reduces BAT thermogenesis and increases adiposity. *Mol. Metab.* 11, 33–46.
- Leininger, G.M., Jo, Y.H., Leshan, R.L., Louis, G.W., Yang, H., Barrera, J.G., Wilson, H., Opland, D.M., Faouzi, M.A., Gong, Y., Jones, J.C., Rhodes, C.J., Chua Sr, Jr, Diano, S., Horvath, T.L., Seeley, R.J., Becker, J.B., Münzberg, H., Myers MG Jr, 2009. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab.* 10, 89–98.

- Leinninger, G.M., Opland, D.M., Jo, Y.H., Faouzi, M., Christensen, L., Cappellucci, L.A., Rhodes, C.J., Gnegy, M.E., Becker, J.B., Pothos, E.N., Seasholtz, A.F., Thompson, R. C., Myers MG, Jr, 2011. Leptin action via neurotensin neurons controls orexin, the mesolimbic dopamine system and energy balance. *Cell Metab.* 14, 313–323.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–408.
- Longo, F., Mancini, M., Ibraheem, P.L., Aryal, S., Mesini, C., Patel, J.C., Penhos, E., Rahman, N., Mamcarz, M., Santini, E., Rice, M.E., Klann, E., 2021. Cell-type-specific disruption of PERK-eIF2 $\alpha$  signaling in dopaminergic neurons alters motor and cognitive function. *Mol. Psychiatry*.
- Lopez-Ferreras, L., Eerola, K., Mishra, D., Shevchouk, O.T., Richard, J.E., Nilsson, F.H., Hayes, M.R., Skibicka, K.P., 2019. GLP-1 modulates the supramammillary nucleus-lateral hypothalamic neurocircuit to control ingestive and motivated behavior in a sex divergent manner. *Mol. Metab.* 20, 178–193.
- Lopez-Ferreras, L., Richard, J.E., Anderberg, R.H., Nilsson, F.H., Olandersson, K., Kanoski, S.E., Skibicka, K.P., 2017. Ghrelin's control of food reward and body weight in the lateral hypothalamic area is sexually dimorphic. *Physiol. Behav.* 176, 40–49.
- Lopez-Ferreras, L., Richard, J.E., Noble, E.E., Eerola, K., Anderberg, R.H., Olandersson, K., Taing, L., Kanoski, S.E., Hayes, M.R., Skibicka, K.P., 2018. Lateral hypothalamic GLP-1 receptors are critical for the control of food reinforcement, ingestive behavior and body weight. *Mol. Psychiatry* 23, 1157–1168.
- Ludwig, D.S., Tritos, N.A., Mastaitis, J.W., Kulkarni, R., Kokkotou, E., Elmquist, J., Lowell, B., Flier, J.S., Maratos-Flier, E., 2001. Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J. Clin. Investig.* 107, 379–386.
- Margules, D.L., Olds, J., 1962. Identical “feeding” and “rewarding” systems in the lateral hypothalamus of rats. *Science* 135, 374–375.
- Martínez-Sánchez, N., Seoane-Collazo, P., Contreras, C., Varela, L., Villarroya, J., Rial-Pensado, E., Buqué, X., Aurrekoetxea, I., Delgado, T.C., Vázquez-Martínez, R., González-García, I., Roa, J., Whittle, A.J., Gomez-Santos, B., Velagapudi, V., Tung, Y., Morgan, D.A., Voshol, P.J., Martínez de Morentin, P.B., López-González, T., Liñares-Pose, L., Gonzalez, F., Chatterjee, K., Sobrino, T., Medina-Gómez, G., Davis, R.J., Casals, N., Orešič, M., Coll, A.P., Vidal-Puig, A., Mittag, J., Tena-Sempere, M., Malagón, M.M., Diéguez, C., Martínez-Chantar, M.L., Aspichueta, P., Rahmouni, K., Nogueiras, R., Sabio, G., Villarroya, F., López, M., 2017. Hypothalamic AMPK-ER stress-JNK1 Axis mediates the central actions of thyroid hormones on energy balance. *Cell Metab.* 26, 212–229.e12.
- Matesanz, N., Bernardo, E., Acín-Pérez, R., Manieri, E., Pérez-Sieira, S., Hernández-Cosido, L., Montalvo-Romeral, V., Mora, A., Rodríguez, E., Leiva-Vega, L., Lechuga-Vieco, A.V., Ruiz-Cabello, J., Torres, J.L., Crespo-Ruiz, M., Centeno, F., Álvarez, C.V., Marcos, M., Enríquez, J.A., Nogueiras, R., Sabio, G., 2017. MKK6 controls T3-mediated browning of white adipose tissue. *Nat. Commun.* 8, 856.
- Miller, N.E., 1960. Motivational effects of brain stimulation and drugs. *Fed. Proc.* 19, 846–854.
- Mishra, D., Richard, J.E., Maric, I., Porteiro, B., Häring, M., Kooijman, S., Musovic, S., Eerola, K., López-Ferreras, L., Peris, E., Grycel, K., Shevchouk, O.T., Micallef, P., Olofsson, C.S., Wernstedt Asterholm, I., Grill, H.J., Nogueiras, R., Skibicka, K.P., 2019. Parabrachial interleukin-6 reduces body weight and food intake and increases thermogenesis to regulate energy metabolism. *Cell Rep.* 26, 3011–3026.e5.
- Morrison, S.D., Mayer, J., 1957. Adipsia and aphagia in rats after lateral subthalamic lesions. *Am. J. Physiol.* 191, 248–254.
- Olds, J., 1962. Hypothalamic substrates of reward. *Physiol. Rev.* 42, 554–604.
- Qu, D., Ludwig, D.S., Gammeltoft, S., Piper, M., Pelleymounter, M.A., Cullen, M.J., Mathes, W.F., Przypek, R., Kanarek, R., Maratos-Flier, E., 1996. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 380, 243–247.
- Roytblat, L., Rachinsky, M., Fisher, A., Greemberg, L., Shapira, Y., Douvdevani, A., Gelman, S., 2000. Raised interleukin-6 levels in obese patients. *Obes. Res.* 8, 673–675.
- Sakurai, T., 1999. Orexins and orexin receptors: implication in feeding behavior. *Regul. Pept.* 85, 25–30.
- Schéle, E., Fekete, C., Egri, P., Füzesi, T., Palkovits, M., Keller, É., Liposits, Z., Gereben, B., Karlsson-Lindahl, L., Shao, R., Jansson, J.O., 2012. Interleukin-6 receptor alpha is co-localised with melanin-concentrating hormone in human and mouse hypothalamus. *J. Neuroendocr.* 24, 930–943.
- Schobitz, B., de Kloet, E.R., Sutanto, W., Holsboer, F., 1993. Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Eur. J. Neurosci.* 5, 1426–1435.
- Schobitz, B., Voorhuis, D.A., De Kloet, E.R., 1992. Localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Neurosci. Lett.* 136, 189–192.
- Scott, L.J., 2017. Tocilizumab: a review in rheumatoid arthritis. *Drugs* 77, 1865–1879.
- Shimada, M., Tritos, N.A., Lowell, B.B., Flier, J.S., Maratos-Flier, E., 1998. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 396, 670–674.
- Shirazi, R., Palsdottir, V., Collander, J., Anesten, F., Vogel, H., Langlet, F., Jäschke, A., Schürmann, A., Prévot, V., Shao, R., Jansson, J.O., Skibicka, K.P., 2013. Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6. *Proc. Natl. Acad. Sci. USA* 110, 16199–16204.
- Shrestha, P., Ayata, P., Herrero-Vidal, P., Longo, F., Gastone, A., LeDoux, J.E., Heintz, N., Klann, E., 2020. Cell-type-specific drug-inducible protein synthesis inhibition demonstrates that memory consolidation requires rapid neuronal translation. *Nat. Neurosci.* 23, 281–292.
- Skibicka, K.P., Alhadeff, A.L., Grill, H.J., 2009. Hindbrain cocaine- and amphetamine-regulated transcript induces hypothermia mediated by GLP-1 receptors. *J. Neurosci.* 29, 6973–6981.
- Stenlof, K., Wernstedt, I., Fjallman, T., Wallenius, V., Wallenius, K., Jansson, J.O., 2003. Interleukin-6 levels in the central nervous system are negatively correlated with fat mass in overweight/obese subjects. *J. Clin. Endocrinol. Metab.* 88, 4379–4383.
- Teitelbaum, P., Stellar, E., 1954. Recovery from the failure to eat produced by hypothalamic lesions. *Science* 120, 894–895.
- Thaler, J.P., Guyenet, S.J., Dorfman, M.D., Wisse, B.E., Schwartz, M.W., 2013. Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? *Diabetes* 62, 2629–2634.
- Thaler, J.P., Schwartz, M.W., 2010. Minireview: inflammation and obesity pathogenesis: the hypothalamus heats up. *Endocrinology* 151, 4109–4115.
- Thaler, J.P., Yi, C.X., Schur, E.A., Guyenet, S.J., Hwang, B.H., Dietrich, M.O., Zhao, X., Sarraf, D.A., Izgur, V., Maravilla, K.R., Nguyen, H.T., Fischer, J.D., Matsen, M.E., Wisse, B.E., Morton, G.J., Horvath, T.L., Baskin, D.G., Tschöp, M.H., Schwartz, M.W., 2012. Obesity is associated with hypothalamic injury in rodents and humans. *J. Clin. Investig.* 122, 153–162.
- Timper, K., Denson, J.L., Steculorum, S.M., Heilinger, C., Engstrom-Ruud, L., Wunderlich, C.M., Rose-John, S., Wunderlich, F.T., Bruning, J.C., 2017. IL-6 improves energy and glucose homeostasis in obesity via enhanced central IL-6 signaling. *Cell Rep.* 19, 267–280.
- Van den Pol, A.N., 1982. Lateral hypothalamic damage and body weight regulation: role of gender, diet, and lesion placement. *Am. J. Physiol.* 242, R265–R274.
- Vasselli, J.R., Cleary, M.P., Jen, K.L., Greenwood, M.R., 1980. Development of food motivated behavior in free feeding and food restricted Zucker fatty (fa/fa) rats. *Physiol. Behav.* 25, 565–573.
- Wallenius, K., Wallenius, V., Sunter, D., Dickson, S.L., Jansson, J.O., 2002a. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem. Biophys. Res. Commun.* 293, 560–565.
- Wallenius, V., Wallenius, K., Ahren, B., Rüdiger, M., Carlsten, H., Dickson, S.L., Ohlsson, C., Jansson, J.O., 2002b. Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* 8, 75–79.
- Xu, X., Han, M., Li, T., Sun, W., Wang, D., Fu, B., Zhou, Y., Zheng, X., Yang, Y., Li, X., Zhang, X., Pan, A., Wei, H., 2020. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc. Natl. Acad. Sci. USA* 117, 10970–10975.
- Yan, H.Q., Banos, M.A., Herregodts, P., Hooghe, R., Hooghe-Peters, E.L., 1992. Expression of interleukin (IL)-1 beta, IL-6 and their respective receptors in the normal rat brain and after injury. *Eur. J. Immunol.* 22, 2963–2971.