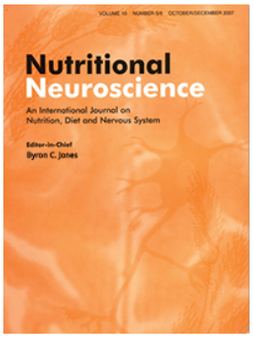

Taylor & Francis
Taylor & Francis Group

Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

Effects of Neuropeptide Y administration into the lateral hypothalamus on intake of free-choice high-fat high-sucrose diet components of the male Wistar rat

Myrtille C.R. Gumbs , Leslie Eggels , Anna H. Vuuregge , Unga A. Unmehopa , Joram D. Mul & Susanne E. la Fleur

To cite this article: Myrtille C.R. Gumbs , Leslie Eggels , Anna H. Vuuregge , Unga A. Unmehopa , Joram D. Mul & Susanne E. la Fleur (2020): Effects of Neuropeptide Y administration into the lateral hypothalamus on intake of free-choice high-fat high-sucrose diet components of the male Wistar rat, Nutritional Neuroscience, DOI: 10.1080/1028415X.2020.1788774

To link to this article: https://doi.org/10.1080/1028415X.2020.1788774

NUTRITIONAL NEUROSCIENCE
https://doi.org/10.1080/1028415X.2020.1788774

Taylor & Francis
Taylor & Francis Group

Effects of Neuropeptide Y administration into the lateral hypothalamus on intake of free-choice high-fat high-sucrose diet components of the male Wistar rat

Myrtille C.R. Gumbs [a,b], Leslie Eggels[a,b], Anna H. Vuuregge[b], Unga A. Unmehopa[b], Joram D. Mul [a,b†] and Susanne E. la Fleur [a,b]

[a]Department of Endocrinology and Metabolism & Laboratory of Endocrinology, Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; [b]Metabolism and Reward group, Netherlands Institute for Neuroscience, An institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), Amsterdam, The Netherlands

ABSTRACT

Introduction: Neuropeptide Y (NPY) signaling in the brain plays an important role in energy regulation, and is altered during diet-induced obesity. Yet, NPY function during the consumption of specific diet components remains to be fully determined. We have previously demonstrated that consumption of a saturated fat component (free-choice high-fat; fcHF), a sucrose solution (high-sugar; fcHS), or both (fcHFHS) combined with a standard diet (chow and water) has diverse effects on *Npy* expression in the arcuate nucleus and the sensitivity to intraventricular NPY administration. Arcuate NPY neurons project to the lateral hypothalamus (LHA), and NPY administration in the LHA potently promotes chow intake in rats on a standard diet. However, it is currently unclear if short-term consumption of a palatable free-choice diet alters NPY function in the LHA. Therefore, we assessed the effects of intra-LHA NPY administration on intake in rats following one-week consumption of a fcHF, fcHS, or fcHFHS diet.

Methods: Male Wistar rats consumed a fcHF, fcHS, fcHFHS, or control (CHOW) diet for one week before NPY (0.3 µg / 0.3 µL) or phosphate-buffered saline (0.3 µL) was administered into the LHA. Intake was measured 2h later. fcHFHS-fed rats were divided into high-fat (fcHFHS-hf) and low-fat (fcHFHS-lf) groups based on differences in basal fat intake.

Results: Intra-LHA NPY administration increased chow intake in fcHFHS- (irrespective of basal fat intake), fcHF- and CHOW-fed rats. Intra-LHA NPY infusion increased fat intake in fcHF-, fcHFHS-hf, but not fcHFHS-lf, rats. Intra-LHA NPY infusion did not increase caloric intake in fcHS-fed rats.

Discussion: Our data demonstrate that the effects of intra-LHA NPY on caloric intake differ depending on the consumption of a fat or sugar component, or both, in a free-choice diet. Our data also indicate that baseline preference for the fat diet component modulates the effects of intra-LHA NPY in fcHFHS-fed rats.

KEYWORDS
Neuropeptide; diet; lateral hypothalamus; diet-induced obesity; orexigenic

Introduction

Chronic consumption of palatable high-caloric diets, enriched with fat and sugar, is an important driver for the development of obesity. In addition to their impact on peripheral energy stores, these diets also dysregulate central processes involved in energy homeostasis, including the function of the Neuropeptide Y (NPY) brain circuitry [1,2]. NPY neurons in the arcuate nucleus of the hypothalamus (Arc) sense and process peripheral signals of energy balance [3], and contribute to the regulation of energy balance by relaying this information to intra- and extrahypothalamic projection areas [4–8].

Consumption of the preclinical free-choice high-fat high-sucrose (fcHFHS) diet, in which animals can choose from four separate diet components (*i.e.* chow, saturated fat, sucrose solution, and water) increases *Npy* expression in the Arc, compared to rats on a free-choice high-fat diet (fcHF; with access to chow, water, and saturated fat), a free-choice high-sucrose diet (fcHS; with access to chow, water, and a sucrose solution), or a control diet (CHOW) [1]. In addition, intraventricular NPY administration increases intake of chow and saturated fat in fcHFHS-fed rats, whereas this dose does not increase caloric intake in fcHF-, fcHS-, or CHOW-fed rats [2]. We have previously shown that

CONTACT Susanne E. la Fleur s.e.lafleur@amsterdamumc.nl Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Meibergdreef 9, K2-283, Amsterdam-Zuidoost, AZ 1105, The Netherlands
[†]Current address: Center for Neuroscience, Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, Amsterdam, The Netherlands
Supplemental data for this article can be accessed https://doi.org/10.1080/1028415X.2020.1788774

Arc NPY neurons project to the nucleus accumbens (NAc), a key brain region in the reward-related brain circuitry, and that NPY administration into the NAc increases intake of fat, but not of chow or sucrose solution in fcHFHS-fed rats [5]. Thus, it is currently unclear via which brain region NPY administration increases the intake of chow in fcHFHS-fed rats.

Arc NPY neurons project to the lateral hypothalamic area (LHA) [8,9], and NPY peptide levels in this brain region are affected by the metabolic state of the animal [10]. Furthermore, intra-LHA administration of NPY can potently increase chow intake. For example, intra-LHA NPY administration or virus-mediated overexpression of *Npy* in the LHA increases intake [11,12]. Interestingly, virus-mediated overexpression of *Npy* in the LHA promotes long lasting increases in caloric intake, as compared to the temporary increases observed after viral-mediated overexpression of *Npy* in the paraventricular nucleus of the hypothalamus (PVN) [12,13]. Nonetheless, it is currently unclear if NPY function is altered in the LHA of fcHFHS-fed rats.

In this study, we determined the effects of intra-LHA NPY administration on caloric intake in fcHFHS-fed rats. As a comparison, we also determined the effects of intra-LHA NPY administration on caloric intake in fcHF-, fcHS- and CHOW-fed rats. Male Wistar rats were given *ad libitum* access to a fcHFHS-, fcHF-, fcHS- or a control CHOW diet. After one week of diet consumption, NPY or saline was administered into the LHA in a crossover design and caloric intake was measured two hours following infusion. The fcHFHS-fed group was divided into fcHFHS-high fat (fcHFHS-hf) and fcHFHS-low fat (fcHFHS-lf) subgroups based on baseline fat intake.

Materials and Methods

Animals and housing

The experimental procedures described below are standard procedures that have been used in a separate experiment described in a prior publication [14]. All experiments were performed in male Wistar rats (Charles River Breeding Laboratories, Sulzfeld, Germany) weighing 270–300 grams at arrival to the animal facility of The Netherlands Institute for Neuroscience (Amsterdam, The Netherlands). Rats were housed in temperature- (21 ± 2̊C), humidity- (60 ± 5%) and light-controlled (12:12 hr light/dark; lights on 07:00–19:00) rooms with background noise (radio) during the entire experiment. Before the start of the diet, rats had *ad libitum* access to a container with a nutritionally-complete high-carbohydrate diet (chow; Teklad global diet 2918; 24% protein, 58% carbohydrate, and 18% fat, 3.1 kcal/g, Envigo, Horst, The Netherlands) and a bottle of tap water. The animal ethics committees of the Amsterdam UMC and The Netherlands Institute for Neuroscience approved all experiments according to Dutch legal ethical guidelines.

Stereotaxic surgery

Details for surgical procedures are described in [14]. After one week of acclimatization, rats were implanted with bilateral cannulas aimed at the lateral hypothalamus for infusion of NPY. Rats were anesthetized before stereotaxic placement of permanent cannulas (C315G-SPC 9 mm; Plastics One, Bilaney Consultants GmbH, Düsseldorf Germany) with Bregma coordinates A/P: −2.64 mm, L: ±3.44 mm, and D/V: −8.2 mm below the surface of the skull in an angle of 10° in the frontal plane. Cannulas were secured to the skull and were occluded by stainless steel dummy's (C315-D; Plastics One). After surgery, rats received an analgesic and were housed individually. Rats recovered from surgery until they reached pre-surgical body weight before continuation of the experiments. After recovery, rats received a saline infusion (see Infusion parameters) to habituate to the handling procedures, which occurred at least one week before the start of the diet intervention.

Diet interventions

Rats were divided into four experimental groups with *ad libitum* access to their respective dietary components [1]: a control group (CHOW; chow diet and tap water; $N = 14$), a free-choice high-fat high-sucrose group (fcHFHS; chow, a bottle of tap water, a dish of saturated beef tallow [Ossewit/Blanc de Boeuf, Vandemoortele, Belgium; 9 kcal/g] and a bottle of 30% sucrose solution [mixed from commercial grade sugar and tap water; 1.2 kcal/g]; $N = 13$), a free-choice high-fat group (fcHF; chow, a bottle of tap water, a dish of saturated beef tallow; $N = 8$), and a free-choice high-sucrose group (fcHS; chow, a bottle of tap water, a bottle of 30% sucrose solution; $N = 8$). Assignment to a diet was done in a balanced manner taking into account basal food intake, body weight, and body weight gain after surgery. Food intake was measured at least five times per week and all components were refreshed twice a week.

Infusion parameters

Detailed infusion parameters are described in [14]. After seven days of diet consumption, all food components were removed from the cage during the early

light phase at 09:00. Intra-LHA infusions were performed between 09:30 and 11:00. Bilateral intra-LHA infusions of 0.3 µg/ 0.3 µL NPY (H6375, Bachem, Germany) in 0.1 mol PBS (PBS; M090001.02NL; Fresenius Kabi GmbH, Zeist, The Netherlands) or 0.3 µL PBS (vehicle) were performed at a rate of 0.3 µL/min using an injector that extended 1 mm below the end of the cannula (C315I, Plastics One, Bilaney Consultants GmbH, Düsseldorf, Germany) and a Hamilton syringe placed in an infusion pump (Harvard Apparatus, Massachusetts, United States of America). Upon completion of all infusions, all diet components were returned to the animal cage and weighed two hours after the intra-LHA infusion of NPY. Infusions were repeated in a crossover design with three days between consecutive infusions.

Perfusion parameters

At the end of the experiment, rats were deeply anesthetized with an i.p. injection of pentobarbital and the left epididymal white adipose tissue (EWAT) was quickly isolated and weighed. Brains were removed after transcardial perfusion, sectioned and thionine-stained according to the procedures described in [14] to determine whether cannulas were placed in the LHA.

Statistics and analyses

Only data from rats with correct uni- and bilateral intra-LHA placements were included in the data analysis according to the Paxinos rat brain atlas [15] and the criteria described in [14]. See Figure 1 for a schematic representation of the infusion sites.

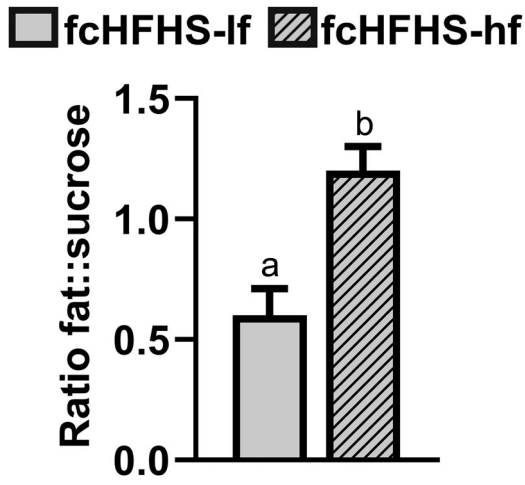

Figure 2. Fat::sucrose solution intake ratio for fcHFHS-lf and fcHFHS-hf groups. The fcHFHS-fed group was separated into two groups based on their basal fat::sucrose solution intake ratio. Different letters indicate a statistically significant difference at $p < 0.05$.

A previous study from our lab indicated that a low or high ratio of fat::sucrose solution intake can lead to differential changes in the dopamine-related brain circuitry [16]. Furthermore, baseline differences in dietary intake modulate the orexigenic effects of NPY [17]. Therefore, the fcHFHS-fed group was divided into a fcHFHS-high fat (fcHFHS-hf; $N = 7$, ratio < 1) and a fcHFHS-low fat (fcHFHS-lf; $N = 6$, ratio > 1) group based on their fat::sucrose solution intake ratio (see Figure 2).

Caloric intake after infusion was calculated for each diet item and summed to determine total caloric intake after the infusions. Group differences were analyzed using a one-way ANOVA followed by Fishers' LSD *post hoc* analysis, and group differences in the response to NPY infusion were analyzed using a mixed-effects model followed by

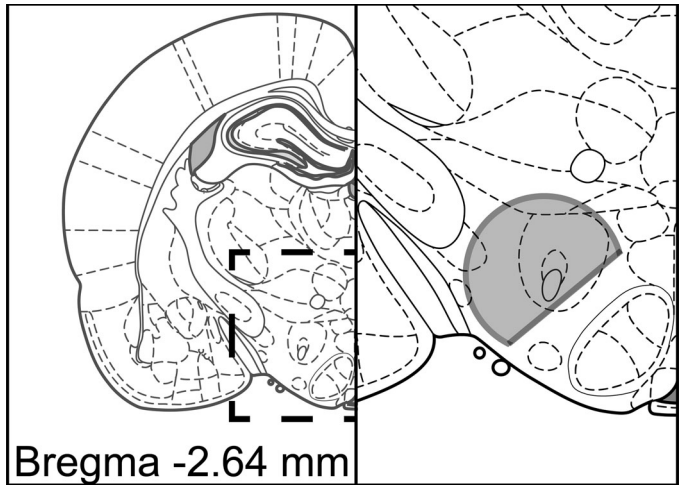

Figure 1. Lateral hypothalamic area (LHA) infusion sites. Atlas illustration indicating the general site for NPY or saline infusion in the LHA. *Left:* overview of a coronal rat brain section based on the Paxinos and Watson rat brain atlas [15]. *Right:* inset from the left figure showing the schematic infusion site in gray.

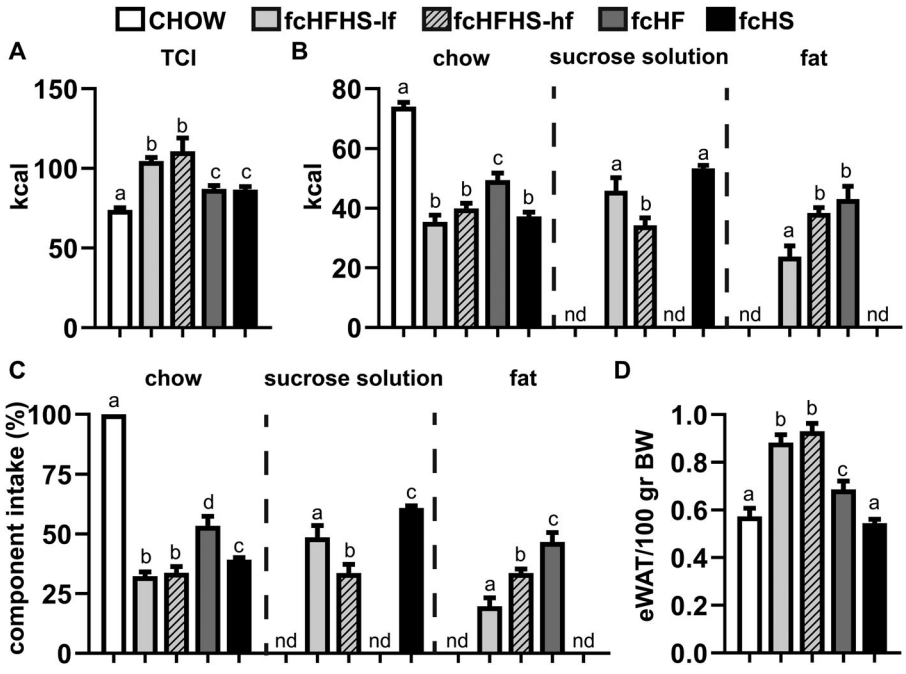

Figure 3. Characteristics of the dietary intervention. (A) Mean daily total caloric intake (TCI) per diet group. (B) Mean daily caloric intake of chow, sucrose solution, and fat components per diet group. (C) Mean daily percentage of component intake per diet group. (D) Epididymal white adipose tissue (eWAT) mass per diet group normalized for body weight (BW). nd = no data, TCI = total caloric intake. Different letters indicate significant differences at $p < 0.05$. Details for statistics are provided in Table S1 and S2.

Fishers' LSD *post hoc* analysis. All statistical analyses were performed using Graphpad Prism 8 [version 8.0.2 (263), 30 January 2019] and are detailed in supplemental Tables 1 and 2. For all cases, $p < 0.05$ was considered significant. All data are presented as mean ± *SEM*.

Results

Effects of obesogenic diet consumption

Absolute caloric intake

One-way ANOVA analysis of daily total caloric intake (TCI) revealed a main effect of *Diet* ($F_{4,38} = 24.40$, $p < 0.0001$; Figure 3(A), Table 1). *Post hoc* analysis demonstrated that TCI was higher in the fcHFHS-lf and fcHFHS-hf groups compared to the CHOW-fed group (both $p < 0.01$), with the fcHF- and fcHS-fed groups showing intermediate elevations in TCI compared to the fcHFHS-lf and fcHFHS-hf groups and the CHOW-fed group (all $p < 0.01$).

One-way ANOVA analysis of diet component intake revealed a main effect of *Diet* for daily caloric intake of chow ($F_{4,38} = 97.9$, $p < 0.0001$), the sucrose solution ($F_{2,18} = 11.3$, $p = 0.0007$), and fat ($F_{2,18} = 8.16$, $p = 0.0003$; Figure 3(B), Table 1). For daily caloric intake from chow, *post hoc* analysis demonstrated that all diet groups consumed less calories from chow compared to the CHOW-fed group (all $p < 0.01$), with the fcHF group consuming more daily calories from chow than the fcHFHS-lf, fcHFHS-hf and fcHS-fed groups (all $p < 0.05$; Figure 3(B), *left*). For daily caloric intake from the sucrose solution, *post hoc* analysis demonstrated that the fcHFHS-

Table 1. Characteristics of the dietary intervention.

	CHOW	fcHFHS-lf	fcHFHS-hf	fcHF	fcHS
Daily TCI¶	74.0 ± 1.4^{a}	104.7 ± 2.1^{b}	110.8 ± 8.3^{b}	87.3 ± 2.0^{c}	86.7 ± 1.8^{c}
chow¶	74.0 ± 1.4^{a}	35.4 ± 2.3^{b}	40.0 ± 1.7^{b}	49.4 ± 2.4^{c}	37.2 ± 1.4^{b}
sucrose water¶	nd	45.9 ± 4.3^{a}	34.2 ± 2.6^{b}	nd	53.4 ± 0.9^{a}
fat¶	nd	23.7 ± 3.6^{a}	38.4 ± 1.8^{b}	43.1 ± 4.2^{b}	nd
Component %					
chow	100^{a}	32.2 ± 1.9^{b}	33.7 ± 2.6^{b}	53.4 ± 4.0^{d}	39.2 ± 0.89^{c}
sucrose water	nd	48.6 ± 4.9^{a}	33.6 ± 3.6^{b}	nd	60.8 ± 0.89^{c}
fat	nd	19.8 ± 3.4^{a}	33.7 ± 1.7^{b}	46.6 ± 4.0^{c}	nd
eWAT§	0.57 ± 0.03^{a}	0.88 ± 0.03^{b}	0.93 ± 0.03^{b}	0.69 ± 0.04^{c}	0.55 ± 0.02^{a}

¶ = presented as mean daily caloric intake in kcal, § = epididymal fat mass per 100 gram body weight, nd = no data, TCI = total caloric intake. Different letters indicate $p < 0.05$, mean ± *SEM*.

hf group consumed fewer daily calories from the sucrose solution compared to the fcHFHS-lf and fcHS-fed groups (all $p < 0.01$; Figure 3(B), *middle*). For daily caloric intake from fat, *post hoc* analysis demonstrated that the fcHFHS-lf group consumed fewer daily calories from fat compared to the fcHFHS-hf and fcHF-fed groups (all $p < 0.01$; Figure 3(B), *right*).

Relative component intake

One-way ANOVA analysis of the percentage of caloric intake per diet component revealed a main effect of *Diet* for chow ($F_{4,38} = 265.5$, $p < 0.0001$), the sucrose solution ($F_{2,\ 18} = 15.69$, $p = 0.0001$), and fat ($F_{2,18} = 16.61$, $p < 0.0001$; Figure 3(C), Table 1). For percentage caloric intake from chow, *post hoc* analysis demonstrated that all diet groups consumed significantly less chow compared to the CHOW-fed group (all $p < 0.0001$), with the fcHF-fed group consuming a higher percentage intake from chow than the fcHFHS-lf, fcHFHS-hf and the fcHS-fed groups consuming an intermediate percentage of daily caloric intake from chow (all $p < 0.05$; see Figure 3(C), *left*). For percentage caloric intake from the sucrose solution, *post hoc* analysis demonstrated that the fcHFHS-lf group consumed a higher percentage of intake from sucrose solution than the fcHFHS-hf group, and fcHS-fed rats consumed a higher percentage of sucrose solution than both fcHFHS groups (all $p < 0.05$; Figure 3(C), *middle*). For percentage intake from fat, *post hoc* analysis demonstrated that the fcHFHS-lf group consumed a lower percentage of intake from fat compared to the fcHFHS-hf group, with the fcHF-fed group consuming the highest percentage intake from fat (all $p < 0.05$; Figure 3(C), *right*).

Epididymal fat mass

One-way ANOVA analysis of eWAT normalized for body weight revealed a main effect of *Diet* ($F_{4,37} = 25.53$, $p < 0.0001$; Figure 3(D); Table 1). *Post hoc* analysis demonstrated that fcHFHS-hf and fcHFHS-lf rats had greater eWAT mass compared to the CHOW-fed group, with fcHF-fed rats showing intermediate eWAT mass levels (all $p < 0.05$; Figure 3(D)). Lastly, eWAT mass did not differ between fcHS- and CHOW-fed rats ($p > 0.05$, Figure 3(D)).

Effects of intra-LHA NPY infusion on caloric intake

A mixed effects model analysis of total caloric intake 2 h following intra-LHA NPY infusion revealed a main effect of *Infusion* ($F_{1,38} = 37.22$, $p < 0.0001$), no main effect of *Diet* ($F_{4,38} = 0.67$, $p > 0.05$), and a *Diet* x *Infusion* interaction effect ($F_{4,38} = 2.89$, $p = 0.04$; Figure 4(A) and Table 2). To assess which diets drive the main effect of *Infusion*, we performed *post hoc* t-tests, indicating that the main effect of *Infusion* was driven by significant increases in total caloric intake in the CHOW-fed group ($t_{14,14} = 3.97$, $p = 0.0003$), fcHFHS-lf group ($t_{7,7} = 3.46$, $p = 0.001$), and fcHFHS-hf group ($t_{6,6} = 4.29$, $p = 0.0001$). For the fcHF-fed group, TCI showed a trend to be increased ($t_{8,8} = 1.78$, $p = 0.08$), whereas NPY infusion had no effect on caloric intake in the fcHS-fed group ($t_{8,8} = 0.27$, $p > 0.05$; Figure 4(A), Table 2).

A mixed effects model analysis of caloric intake of chow after intra-LHA NPY infusion revealed a main effect of *Infusion* ($F_{1,25} = 13.96$, $p = 0.001$), but no main effect of *Diet* ($F_{3,25} = 0.61$, $p > 0.05$), and no *Diet* x *Infusion* interaction effect ($F_{3,25} = 0.63$, $p > 0.05$; Figure 4(B), Table 2). As our data indicate that the effect of intra-LHA NPY administration produces variable effects on caloric intake in the different diet groups, we explored whether *post hoc* analysis provides additional information despite not observing a significant interaction effect. *Post hoc* testing suggested that the main effect of *Infusion* was driven by significant increases in chow intake in the fcHFHS-lf group ($t_{7,7} = 2.32$, $p = 0.03$), the fcHFHS-hf group ($t_{6,6} = 2.06$, $p = 0.05$), and the fcHF-fed group ($t_{8,8} = 2.29$, $p = 0.03$). Chow intake after NPY infusion was not different from saline controls in the fcHS-fed group ($t_{8,8} = 0.77$, $p > 0.05$).

A mixed effects model analysis of caloric intake from the sucrose solution after intra-LHA NPY infusion revealed a main effect of *Diet* ($F_{2,36} = 24.83$, $p < 0.0001$), but no main effect of *Infusion* ($F_{1,36} = 1.49$, $p > 0.05$), and no *Diet* x *Infusion* interaction effect ($F_{2,36} = 1.50$, $p > 0.05$; Figure 4(C), Table 2). *Post hoc* analysis showed that the fcHS-fed group consumed more sucrose solution independent of NPY or saline infusion, compared to the fcHFHS-lf ($t_{16,14} = 5.08$, $p < 0.0001$) and the fcHFHS-hf groups ($t_{16,12} = 6.64$, $p < 0.0001$).

A mixed effects model analysis of caloric intake from fat after intra-LHA NPY infusion revealed a main effect of *Infusion* ($F_{1,18} = 9.27$, $p = 0.007$), a trend for a *Diet* x *Infusion* interaction effect ($F_{2,18} = 3.08$, $p = 0.07$), and no main effect of *Diet* ($F_{2,18} = 0.77$, $p > 0.05$; see Figure 4(D), Table 2). As our data indicate that the effect of intra-LHA NPY administration produces variable effects on caloric intake in the different diet groups, we explored whether *post hoc* analysis provides additional information despite observing only a trend for a significant interaction effect. *Post hoc* analysis suggests that the main effect of *Infusion* wasdriven by a significant increase in fat intake after intra-LHA NPY infusion in the fcHFHS-hf group ($t_{6,6} = 3.42$, $p = 0.003$), as the fcHFHS-lf ($t_{7,7} = 1.47$, $p > 0.05$) and the fcHF-fed group ($t_{8,8} = 0.16$, $p > 0.05$) showed no increase in fat intake following intra-LHA NPY infusion.

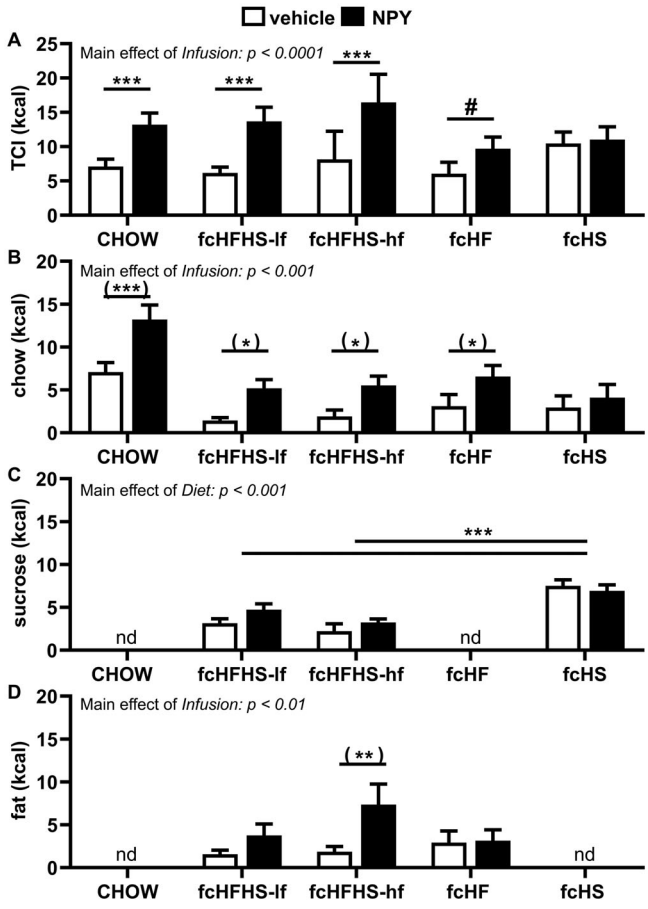

Figure 4. Intra-lateral hypothalamic area (LHA) NPY infusion affects intake in a diet composition dependent manner. (A) Total caloric intake (TCI) after intra-LHA NPY infusion. (B) Caloric intake of chow after intra-LHA NPY infusion. (C) Caloric intake from the sucrose solution after intra-LHA NPY infusion. (D) Caloric intake from fat after intra-LHA NPY infusion. nd = no data, *$p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, # trend for statistical significance at $p < 0.08$. Details for statistics are provided in the Results section.

Table 2. Caloric intake after NPY or vehicle infusion.

	Vehicle	NPY
CHOW		
TCI	7.1 ± 1.1	13.2 ± 1.7[a]
fcHFHS-lf		
TCI	6.2 ± 0.9	13.7 ± 2.0[a]
Chow	1.4 ± 0.3	5.2 ± 1.0[a]
Sucrose water	3.2 ± 0.5	4.7 ± 0.7
Fat	1.6 ± 0.5	3.8 ± 1.3
fcHFHS-hf		
TCI	6.0 ± 1.2	16.2 ± 4.1[a]
Chow	1.9 ± 0.7	5.5 ± 1.1[a]
Sucrose water	2.2 ± 0.9	3.3 ± 0.4
Fat	1.9 ± 0.6	7.4 ± 2.4[a]
fcHF		
TCI	6.1 ± 1.7	9.7 ± 1.7
Chow	3.1 ± 1.3	6.6 ± 1.3[a]
Fat	2.9 ± 1.4	3.2 ± 1.3
fcHS		
TCI	10.5 ± 1.7	11.0 ± 1.9
Chow	3.0 ± 1.4	4.1 ± 1.5
Sucrose water	7.5 ± 0.7	6.9 ± 0.7

[a] = $p < 0.05$ compared to vehicle control, TCI = total caloric intake. Data is presented in kilocalories and mean ± *SEM*.

Discussion

Summary

Here, we demonstrate that intra-LHA NPY administration increases caloric intake in fcHF-, fcHFHS-lf, fcHFHS-hf, and CHOW-fed rats, but not in fcHS-fed rats. Taken together with our previous finding that NPY administration in the nucleus accumbens of fcHFHS-fed rats specifically increases fat intake [5], our data indicate that NPY has region-specific effects on dietary component intake in rats that have consumed a free-choice diet containing high levels of fat and sucrose.

In addition, our data might suggest that previous consumption of a free-choice diet as well as dietary preference can modulate the orexigenic effects of intra-LHA NPY administration in the male Wistar rat. As in CHOW-fed control rats, intra-LHA NPY increases caloric intake in the fcHFHS-hf, fcHFHS-lf and fcHF-fed

rats, but not in fcHS-fed rats. Intra-LHA NPY additionally increased intake of the fat component in rats that have a high-fat::sucrose solution ratio (fcHFHS-hf group), but not in the fcHFHS-lf group with a low fat:: sucrose solution intake ratio. Both fcHFHS-fed groups showed similar daily intake of calories and had a similar body fat percentage, suggesting that the differential effects of NPY on intake are a result of differences in dietary preference, which lead to differences in diet component intake and subsequent adaptations in the brain. In fcHF-fed rats, that consumed high levels of fat, but did not have access to a liquid sucrose component, intra-LHA NPY administration did not increase fat intake. This suggests that access to and consumption of relatively high levels of fat together with sucrose can lead to changes in the NPY circuitry such that intra-LHA NPY promotes fat intake. Moreover, intra-LHA NPY infusion in fcHS-fed rats did not increase consumption of chow or of the sucrose solution. The effects of intra-LHA NPY on food intake are thus altered compared to the control CHOW-condition when a sucrose component is present in the diet. Altogether, our observations thus suggest that consumption of a sucrose solution changes the LHA NPY circuitry, and that these changes are different depending on whether the sucrose solution is consumed together with chow and fat (fcHFHS-hf *vs.* fcHFHS-lf), or together with chow only (fcHF). Our findings thus indicate an important interaction between the consumption of a sucrose solution and fat on the responsivity of the LHA NPY circuity.

The role of LHA NPY in the regulation of energy homeostasis

Several studies evaluating the effects of NPY on food intake have mostly focussed on its effects within the paraventricular nucleus of the hypothalamus (PVN), as this hypothalamic area receives dense NPY projections [18,19] and peptide levels in the PVN fluctuate with the energetic status of the animal [20–22]. Intra-PVN injection of NPY specifically increases consumption of carbohydrates [23,24]. Nonetheless, intra-PVN injection of NPY can also increase fat intake in rats with high baseline intake of fat, and these effects depend on the presence of choice between different food components [23,24]. The findings from our study suggest that LHA NPY, as has been shown for PVN NPY, predominantly increases intake of complex carbohydrates. Moreover, we find that when fcHFHS rats have a preference for fat (*i.e.* a high fat::sucrose solution intake ratio), intra-LHA NPY also promotes fat intake. Finally, we also find that intra-LHA NPY does not promote the consumption of simple sugars such as sucrose in a solution.

In contrast to NPY in the PVN, LHA NPY is not regulated by changes in diet or energy levels. For example, intra-PVN NPY infusion leads to carbohydrate intake, which itself also increases NPY peptide in the PVN [25,26]. For the LHA, we find that NPY infusion promotes the intake of chow, yet a high baseline intake of carbohydrates or fat does not increase LHA NPY content [26,27]. Regulation of NPY peptide levels by the energetic status of the animal is also different in the LHA compared to the PVN. For example, LHA NPY peptide levels do not increase during fasting, but they do after re-feeding, whereas PVN NPY peptide levels increase during fasting and remain high after re-feeding [10]. Moreover, viral overexpression of *Npy* in the PVN and LHA both increase food intake, but LHA *Npy* overexpression has long-lasting effects on *meal size*, whereas PVN *Npy* overexpression temporarily increases *meal frequency* [12]. Thus, the LHA and PVN NPY circuitries appear to play different roles in feeding behavior, even though the orexigenic effects of intra-LHA NPY appear similar to the orexigenic effects of intra-PVN infusion.

In addition to the regulation of energy homeostasis, the LHA is also important for (food) reward-related behaviors [28,29]. However, the role of intra-LHA NPY in mediating reward-related behaviors is currently unclear. For example, intra-LHA NPY can elicit a conditioned place preference, but does not always increase operant responding for sucrose pellets [30–32]. In relation to our observations, if the effects of intra-LHA NPY on component choice were mediated through reward-related mechanisms, it would be more likely that intra-LHA NPY predominantly increases intake of the palatable components, such as fat or the sucrose solution as opposed to chow. Therefore, we hypothesize that the intra-LHA NPY circuitry is primarily involved in the homeostatic regulation of energy homeostasis. Future studies should investigate the role of LHA NPY and if it relates to the regulation of energy homeostasis, reward processing, or both.

Intra-LHA NPY infusion does not modulate caloric intake in fcHS-fed rats

We find that fcHS-fed rats are most likely insensitive to a dose of intra-LHA that is effective in the rats consuming the other experimental diets, suggesting decreased NPYR function in order to limit intake. Changes in sensitivity to NPY are not solely a result of drinking the sucrose solution, as both the fcHS-fed and fcHFHS-lf groups consume equal amounts of sucrose

solution at baseline, and fcHFHS-lf rats do increase chow intake in response to intra-LHA NPY infusion. To date, little is known about the mechanisms underlying changes in NPYR sensitivity. It is therefore difficult to explain why consumption of a sucrose solution without concurrent fat consumption reduces NPY sensitivity in the LHA. Both the NPY1R and NPY5R are expressed in the LHA, and only simultaneous deletion of both receptors elicits a change in consumption behavior, suggesting that the presence of one receptor type can compensate for the loss of the other receptor type [33]. It is therefore possible that consumption of a sucrose solution reduces activity of both receptors, whereas consumption of fat increases the function of one or both receptors, maintaining sensitivity when animals are concurrently consuming fat and a sucrose solution. Possibly these nutrients, that can cross the blood barrier, elicit direct effects in the hypothalamus to affect NPY sensitivity. Alternatively, it might be that consumption of a fcHS diet exerts different hormonal or substrate release patterns compared to rats consuming a fcHFHS diet that can lead to differences in NPY sensitivity. Differences in leptin-, insulin- or glucose levels due to dietary consumption may be involved in changes in the sensitivity of these neurons to NPY. For example, the LHA contains insulin- and leptin-responsive neurons [29], which can be either *pro-melanin concentrating hormone (mch)*- or *orexin/hypocretin*-expressing neurons and are known to play a role in the regulation of food intake and energy balance [34,35]. However, from earlier studies in our lab, we have no indication that fcHS diet consumption leads to changes in insulin- or leptin- concentrations in such a short period of consumption that could explain a negative feedback to the LHA [36,37]. We cannot exclude the involvement of other plasma factors that are altered by the consumption of a fcHS diet. Future studies could take these considerations into account and measure plasma factors to determine whether these could clarify the mechanisms of decreased LHA NPY sensitivity fcHS-fed rats.

Strengths and limitations

This is the first study to investigate if the orexigenic effects of intra-LHA NPY administration is altered in male Wistar rats that consumed a fcHFHS-, fcHF-, or a fcHS-diet compared to rats that consumed a control CHOW diet. As NPY in the LHA has strong effects on caloric intake and the LHA NPY circuitry is dysregulated during diet-induced obesity [11], understanding the role of the LHA NPY circuitry in the regulation of energy balance is important.

The free-choice diets employed in our study, in particular the fcHFHS-diet, are valid models of diet-induced obesity, whereas other studies on the interrelationship of diet, preference and the central NPY system generally do not use diets that result in changes that liken those in obesity [38,39]. In addition, our goal was not to assess the effects of dietary history on food *choice*. Our choice diets therefore do not include a separate protein source, nor provide all choice options at the time of infusion. Intra-PVN infusion of NPY does not modulate intake of a separate protein source [27]. As the effects of NPY on caloric intake are highly similar to those described for the PVN, this suggests that the absence of a separate protein sources does not affect dietary choice. Also, studies with sugar provided in solid form, find that intra-cerebral or intra-LHA NPY infusion can increase consumption of sucrose under specific circumstances, such as limited access to sugar or limited choice options [30,40]. In our study, sucrose was provided in liquid form as a 30% solution and *ad libitum*, which may explain the different observations. In particular, as intra-LHA NPY may not stimulate drinking behavior [24]. To exclude the effects of orosensory properties and protein availability on dietary selection, future studies can provide separate pelleted dietary components containing variable levels of fat, sucrose and protein, and systematically determine the effect of solid *vs.* fluid diet component options

Importantly, this study only included male Wistar rats. As the NPY system shows sex-specific characteristics, future studies should investigate the effects of dietary composition on the brain NPY system in female rats.

Implications for future research

The NPY neurons in the Arc are recognized as important regulators of energy homeostasis, and efforts to elucidate the downstream circuitry of Arc NPY neurons have focused predominantly on Arc-PVN communication. However, the LHA is nowadays recognized as another important target of Arc NPY neurons. Our study indicates similarities as well as differences between the NPY circuitries in the PVN and LHA in the regulation of feeding behavior and energy balance. We also demonstrate that dietary choice and/or composition can modulate the sensitivity of the LHA NPY circuitry in the male Wistar rat. The LHA is optimally located in the brain to process and relay information regarding energy homeostasis and reward, yet the role of LHA NPY in the development of obesity is still unclear. Our study provides insight into the role of the LHA NPY circuitry in male Wistar rats during certain obesogenic dietary conditions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Academisch Medisch Centrum (AMC), via an AMC PhD scholarship grant awarded to MCRG; and by the Netherlands Organization of Scientific Research under Grant Nederlandse Organisatie voor Wetenschappelijk Onderzoek NWO-VICI grant 016.160.617.

Notes on contributors

M.C.R. Gumbs is a PhD student in the field of Neuroscience at the Department of Endocrinology & Metabolism at the Amsterdam UMC. Her research is focussed on elucidating the role of the Neuropeptide Y system in food intake regulation and how it is dysregulated by dietary factors and during the development of diet-induced obesity. She is also a teacher in the bachelor Psychobiology at the University of Amsterdam. Her teaching responsibilities include lecturing courses in academic skills, neuroanatomy and neurophilosophy, as well as the mentorship of first year students. She is also a developer and innovator for the curriculum for Psychobiology and higher education in general.

L. Eggels has been a research technician since 2010 at the Department of Endocrinology & Metabolism at the Amsterdam UMC. She has been working on several research projects focussed on glucose metabolism, obesity and the brain.

A.H. Vuuregge is currently working as a (junior) lecturer for bachelor students in Pharmaceutical Sciences and Mechanical Engineering at the Vrije Universiteit and University of Twente. I am involved in courses concerning academic skills, biochemistry and basic physics as a lecturer and innovator in Pharmaceutical Sciences. For the bachelor Mechanical Engineering I am involved as a lecturer, developer and innovator of academic skills throughout the curriculum.

U.A. Unmehopa is a research technician in the field of Neuroscience, with an expertise in histotechnology, molecular biology, image analysis and neuroanatomy. She has worked at the Netherlands Institute for Neuroscience, group neuropsychiatric Diseases from 1996 to 2014 and has been working at the research laboratory for Endocrinology & Metabolism since 2014.

J.D. Mul is a senior researcher at the Department of Endocrinology & Metabolism, Amsterdam UMC, location AMC, and at the Netherlands Institute of Neuroscience. He is also an assistant professor at the Swammerdam Institute for Life Sciences at the University of Amsterdam. His research is focussed on understanding brain mechanisms underlying the beneficial effects of exercise training on stress resilience as well as energy homeostasis. He teaching responsibilities include several bachelor and master courses at the University of Amsterdam.

S.E. la Fleur is a professor of Neurobiology of Energy Metabolism in the laboratory of Endocrinology and the Department of Endocrinology and Metabolism at Amsterdam UMC. She is also honorary group leader at the Netherlands Institute for Neuroscience in Amsterdam in the Netherlands. She obtained her PhD in Neuroscience from the Faculty of Medicine, University of Amsterdam in 2001. She worked at UCSF, San Francisco, USA and the Rudolf Magnus Institute of Neuroscience in Utrecht in the Netherlands before starting her own independent research group in Amsterdam in 2008.

ORCID

Myrtille C.R. Gumbs http://orcid.org/0000-0003-4347-6300
Joram D. Mul http://orcid.org/0000-0003-0453-2568
Susanne E. la Fleur http://orcid.org/0000-0002-4298-7451

References

[1] la Fleur SE, van Rozen AJ, Luijendijk MC, Groeneweg F, Adan RA. A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia. Int J Obesity (2005). 2010;34(3):537–46.
[2] van den Heuvel JK, Eggels L, van Rozen AJ, Luijendijk MC, Fliers E, Kalsbeek A, et al. Neuropeptide Y and leptin sensitivity is dependent on diet composition. J Neuroendocrinol. 2014;26(6):377–85.
[3] Kohno D, Yada T. Arcuate NPY neurons sense and integrate peripheral metabolic signals to control feeding. Neuropeptides. 2012;46(6):315–9.
[4] Gumbs MCR, Vuuregge AH, Eggels L, Unmehopa UA, Lamuadni K, Mul JD, et al. Afferent neuropeptide Y projections to the ventral tegmental area in normal-weight male Wistar rats. J Comp Neurol. 2019;527(16):2659–2674. doi:10.1002/cne.24698.
[5] van den Heuvel JK, Furman K, Gumbs MC, Eggels L, Opland DM, Land BB, et al. Neuropeptide Y activity in the nucleus accumbens modulates feeding behavior and neuronal activity. Biol Psychiatry. 2015;77(7):633–41.
[6] Sim LJ, Joseph SA. Arcuate nucleus projections to brainstem regions which modulate nociception. J Chem Neuroanat. 1991;4(2):97–109.
[7] Michel MC, Beck-Sickinger A, Cox H, Doods HN, Herzog H, Larhammar D, et al. XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol Rev. 1998;50(1):143–50.
[8] Broberger C, De Lecea L, Sutcliffe JG, Hokfelt T. Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. J Comp Neurol. 1998;402(4):460–74.
[9] Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, et al. Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. J Comp Neurol. 1998;402(4):442–59.
[10] Beck B, Jhanwar-Uniyal M, Burlet A, Chapleur-Chateau M, Leibowitz SF, Burlet C. Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. Brain Res. 1990;528(2):245–9.
[11] Stanley BG, Magdalin W, Seirafi A, Thomas WJ, Leibowitz SF. The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). Brain Res. 1993;604(1–2):304–17.

[12] Tiesjema B, Adan RA, Luijendijk MC, Kalsbeek A, la Fleur SE. Differential effects of recombinant adeno-associated virus-mediated neuropeptide Y overexpression in the hypothalamic paraventricular nucleus and lateral hypothalamus on feeding behavior. J Neurosci Off J Soc Neurosci. 2007;27(51):14139–46.
[13] Tiesjema B, la Fleur SE, Luijendijk MC, Adan RA. Sustained NPY overexpression in the PVN results in obesity via temporarily increasing food intake. Obesity (Silver Spring, Md). 2009;17(7):1448–50.
[14] Gumbs MCR, Eggels L, Kool T, Unmehopa UA, van den Heuvel JK, Lamuadni K, et al. Neuropeptide Y signaling in the lateral hypothalamus modulates diet component selection and is dysregulated in a model of diet-induced obesity. Neuroscience. (in press). doi.org/10.1016/j.neuroscience.2019.12.014.
[15] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. London: Elsevier; 2007.
[16] van de Giessen E, la Fleur SE, Eggels L, de Bruin K, van den Brink W, Booij J. High fat/carbohydrate ratio but not total energy intake induces lower striatal dopamine D2/3 receptor availability in diet-induced obesity. Int J Obesity (2005). 2013;37(5):754–7.
[17] Stanley BG, Anderson KC, Grayson MH, Leibowitz SF. Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. Physiol Behav. 1989;46(2):173–7.
[18] Chronwall BM, DiMaggio DA, Massari VJ, Pickel VM, Ruggiero DA, O'Donohue TL. The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience. 1985;15(4):1159–81.
[19] Sawchenko PE, Swanson LW, Grzanna R, Howe PR, Bloom SR, Polak JM. Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. J Comp Neurol. 1985;241(2):138–53.
[20] Dube MG, Sahu A, Kalra PS, Kalra SP. Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an in vitro study. Endocrinology. 1992;131(2):684–8.
[21] Sahu A, Kalra PS, Kalra SP. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides. 1988;9(1):83–6.
[22] Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc Natl Acad Sci U S A. 1991;88(23):10931–5.
[23] Stanley BG, Daniel DR, Chin AS, Leibowitz SF. Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides. 1985;6(6):1205–11.
[24] Smith BK, Berthoud HR, York DA, Bray GA. Differential effects of baseline macronutrient preferences on macronutrient selection after galanin, NPY, and an overnight fast. Peptides. 1997;18(2):207–11.
[25] Beck B, Stricker-Krongrad A, Burlet A, Cumin F, Burlet C. Plasma leptin and hypothalamic neuropeptide Y and galanin levels in long-Evans rats with marked dietary preferences. Nutr Neurosci. 2001;4(1):39–50.
[26] Jhanwar-Uniyal M, Beck B, Jhanwar YS, Burlet C, Leibowitz SF. Neuropeptide Y projection from arcuate nucleus to parvocellular division of paraventricular nucleus: specific relation to the ingestion of carbohydrate. Brain Res. 1993;631(1):97–106.
[27] Wang J, Akabayashi A, Dourmashkin J, Yu HJ, Alexander JT, Chae HJ, et al. Neuropeptide Y in relation to carbohydrate intake, corticosterone and dietary obesity. Brain Res. 1998;802(1–2):75–88.
[28] Bonnavion P, Mickelsen LE, Fujita A, de Lecea L, Jackson AC. Hubs and spokes of the lateral hypothalamus: cell types, circuits and behaviour. J Physiol. 2016;594(22):6443–62.
[29] Berthoud HR, Munzberg H. The lateral hypothalamus as integrator of metabolic and environmental needs: from electrical self-stimulation to opto-genetics. Physiol Behav. 2011;104(1):29–39.
[30] Brown CM, Coscina DV, Fletcher PJ. The rewarding properties of neuropeptide Y in perifornical hypothalamus vs. nucleus accumbens. Peptides. 2000;21(8):1279–87.
[31] Brown CM, Fletcher PJ, Coscina DV. Neuropeptide Y-induced operant responding for sucrose is not mediated by dopamine. Peptides. 1998;19(10):1667–73.
[32] Pandit R, Luijendijk MC, Vanderschuren LJ, la Fleur SE, Adan RA. Limbic substrates of the effects of neuropeptide Y on intake of and motivation for palatable food. Obesity (Silver Spring, Md). 2014;22(5):1216–9.
[33] Nguyen AD, Mitchell NF, Lin S, Macia L, Yulyaningsih E, Baldock PA, et al. Y1 and Y5 receptors are both required for the regulation of food intake and energy homeostasis in mice. PloS One. 2012;7(6):e40191.
[34] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998;92(5):1 page following 696.
[35] Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, et al. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. Nature. 1996;380(6571):243–7.
[36] la Fleur SE, Luijendijk MC, van Rozen AJ, Kalsbeek A, Adan RA. A free-choice high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity. Int J Obesity (2005). 2011;35(4):595–604.
[37] Diepenbroek C, Eggels L, Ackermans MT, Fliers E, Kalsbeek A, Serlie MJ, et al. Differential effects of hypercaloric choice diets on insulin sensitivity in rats. J Endocrinol. 2017;232(1):49–57.
[38] la Fleur SE, Vanderschuren LJ, Luijendijk MC, Kloeze BM, Tiesjema B, Adan RA. A reciprocal interaction between food-motivated behavior and diet-induced obesity. Int J Obesity (2005). 2007;31(8):1286–94.
[39] Slomp M, Belegri E, Blancas-Velazquez AS, Diepenbroek C, Eggels L, Gumbs MCR, et al. Stressing the importance of choice: Validity of a preclinical free-choice high-caloric diet paradigm to model behavioural, physiological and molecular adaptations during human diet-induced obesity and metabolic dysfunction. J Neuroendocrinol. 2019;31(5):e12718.
[40] Glass MJ, Cleary JP, Billington CJ, Levine AS. Role of carbohydrate type on diet selection in neuropeptide Y-stimulated rats. Am J Physiol. 1997;273(6):R2040–5.