Brain circuitries in control of feeding behaviors

Focus on Neuropeptide Y

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Publication date
2020

Document Version
Other version

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Chapter VII.
Dietary choice and composition modulate the orexigenic effects of Neuropeptide Y in the lateral hypothalamus of the male Wistar rat

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Submitted

Abstract

Central Neuropeptide Y (NPY) signaling plays an important role in energy regulation, and the NPY brain circuitry is affected during diet-induced obesity. NPY receptors are widely distributed throughout the brain and have been extensively studied within the paraventricular nucleus (PVN) of the hypothalamus, where NPY modulates feeding dependent on dietary intake of fat or carbohydrates. Although NPY within the lateral hypothalamus (LHA) elicits stronger effects on caloric intake compared to the PVN, it is unknown if the intra-LHA NPY effects on caloric intake are modulated by dietary choice and/or composition. Here, we determined if dietary choice and/or composition modulate the orexigenic effects of intra-LHA NPY.

Male Wistar rats were fed a free-choice high-fat high-sucrose (fcHFHS), free-choice high-fat (fcHF), free-choice high-sucrose (fcHS) or control (CHOW) diet for one week before NPY (0.3 µg / 0.3 µL phosphate-buffered saline [PBS]) or PBS (0.3 µL) was infused into the LHA. Food intake was measured 2 hours later. fcHFHS-fed rats were divided into fcHFHS-high fat (fcHFHS-hf) and fcHFHS-low fat (fcHFHS-lf) groups based on differences in basal fat intake.

Intra-LHA NPY infusion increased intake of chow and fat in fcHFHS-hf rats, but only chow intake in fcHFHS-lf, fcHF- and CHOW-fed rats. Intra-LHA NPY infusion did not affect caloric intake in fcHS-fed rats.

Intra-LHA NPY infusion preferentially increases the intake of chow, which contains complex carbohydrates and protein. Intra-LHA NPY also increases fat intake in fcHFHS-fed rats that consume at least 21 % of basal intake from fat. Thus, intra-LHA infusion modulated dietary intake similar to the effects described previously for intra-PVN NPY infusion. Furthermore, the effects of intra-LHA NPY on fat intake appear to be dependent on both baseline fat intake as well as the availability of a sucrose solution, as fcHF-fed rats did not increase fat intake after intra-LHA NPY infusion. Finally, the absence of an orexigenic NPY response in fcHS-fed rats suggests that sucrose solution consumption affects LHA NPY sensitivity. Taken together, our findings demonstrate that dietary choice and composition modulate the orexigenic effects of intra-LHA NPY in the male Wistar rat.

Introduction

Chronic consumption of palatable high-calorie foods, enriched with fats and sugars, is an important driver for the development of obesity. In addition to the impact of their energy-dense content on bodily energy stores, such diets also dysregulate peripheral and central processes involved in energy homeostasis, including the Neuropeptide Y (NPY) brain circuitry (la Fleur et al., 2010; van den Heuvel, Eggels, van Rozen, et al., 2014). NPY neurons in the arcuate nucleus of the hypothalamus (Arc) sense and process peripheral signals of energy balance (Kohno & Yada, 2012), and contribute to the regulation of energy balance by relaying this information to other hypothalamic and extra-hypothalamic projection areas through four NPY receptor (NPYR) subtypes (Broberger, De Lecea, et al., 1998; M. C. R. Gumbs et al., 2019; Michel et al., 1998; Sim & Joseph, 1991; van den Heuvel et al., 2015). Arc NPY gene and protein expression fluctuates with the energetic state of the animal (*i.e*. fed versus fasting), and is also modulated by diet composition (M. C. Gumbs et al., 2016; Hahn et al., 1998; Marks et al., 1992; J. Wang et al., 1999). NPY projections from the Arc to the paraventricular nucleus of the hypothalamus (PVN) and the lateral hypothalamic area (LHA) are important for the orexigenic effects of NPY. For example, NPY infusion or virus-mediated overexpression of *Npy* in either the PVN or LHA increases intake (Stanley, Daniel, et al., 1985; Tiesjema et al., 2007; Tiesjema et al., 2009). In addition, NPY levels and exogenous NPY application in the PVN are primarily associated with carbohydrate intake, though intra-PVN NPY infusion can also promote the intake of fat, depending on prior dietary preference and/or the availability of dietary choice options (Beck et al., 2001; Smith et al., 1997; Stanley et al., 1989; Stanley, Chin, et al., 1985; J. Wang et al., 1999). Intra-LHA NPY infusion induces strong orexigenic effects, and LHA NPY peptide levels are affected by the energetic state of the animal (Beck, Jhanwar-Uniyal, et al., 1990; Stanley et al., 1993). In addition, virus-mediated overexpression of *Npy* in the LHA promotes long-lasting increases in caloric intake, as compared to the temporary increases observed after virus-mediated overexpression of *Npy* in the PVN (Tiesjema et al., 2007). Nonetheless, it is currently not known if the orexigenic effects of intra-LHA NPY are modulated by dietary choice and/or composition.

Here, we determined if the orexigenic effects of intra-LHA NPY are modulated by dietary choice and/or composition by giving male Wistar rats *ad libitum* access to a free-choice high-fat high-sucrose (fcHFHS), a free-choice high-fat (fcHF), a free-choice high-sucrose (fcHS) or a control (CHOW) diet. After one week of diet consumption, NPY or saline was infused into the LHA in a crossover design and 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 basal fat intake.

Experimental procedures

Animals and housing

All experiments were performed in male Wistar rats (Charles River Breeding Laboratories, Sulzfeld, Germany) weighing 270-300 g at arrival to the animal facility of The Netherlands Institute for Neuroscience (Amsterdam, The Netherlands). Rats were housed in temperature- (21 ± 2 ˚C), humidity- (60 ± 5%) and light-controlled (12:12hr 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.

Stereotactic surgery

After one week of acclimatization, rats were implanted with bilateral cannulas aimed at the LHA for infusion of NPY. Rats were anesthetized with an i.p. injection of 80 mg/kg ketamine (Eurovet Animal Health, Bladel, The Netherlands), 8 mg/kg xylazine (Bayer Health Care, Mijdrecht, The Netherlands) and 0.1 mg/kg atropine (Pharmachemie B.V., Haarlem, The Netherlands), and fixed in a stereotactic frame. Permanent 26-gauge stainless steel guide cannulas (C315G-SPC 9 mm; Plastics One, Bilaney Consultants GmbH, Düsseldorf, Germany) were placed in an angle of 10° in the frontal plane with Bregma coordinates A/P: -2.64 mm, L: ±3.44 mm, and D/V: -8.2 mm below the surface of the skull. Cannulas were secured to the skull using four anchor screws and dental cement, and were occluded by stainless steel dummy's (C315-D; Plastics One). After surgery, rats received an analgesic subcutaneously (Carprofen, 0.5 mg/100g body weight) 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: a control group (CHOW; chow diet and a bottle of 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), or 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 5x/week and all components were refreshed 2x/week.

Infusion parameters

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 using an injector that extended 1 mm below the end of the cannula (C315I, Plastics One, Bilaney Consultants GmbH, Düsseldorf, Germany), and was connected to a 10 µL Hamilton syringe placed in an infusion pump (Harvard Apparatus, Massachusetts, United States of America). Volumes were infused at a rate of 0.3 µL/min and infusion was confirmed by monitoring fluid movement in the tubing via a small air bubble. After infusion, the injector was left in place for one minute to allow for fluid diffusion. 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 cross-over 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. Rats were then transcardially perfused with saline (4 °C) followed by 4% PFA in 0.1 mol/L PBS (pH 7.6; 4 °C). Brains were removed and, after 24 hours postfixation in 4% PFA at 4 °C, cryoprotected in 30% sucrose in PBS at 4 ˚C. Brains were then frozen on dry ice and stored at -80 ˚C until sectioning. Brains were sectioned coronally on a cryostat and 35 µm sections were mounted on Superfrost ++ slides (Merck), stained with thionine (0.5 % w/v), and studied with a light microscope to determine if cannulas were placed in the LHA.

Statistics and analyses

Only data from rats with correct uni- or bilateral intra-LHA placements were included in the data analysis. Correct placements were spaced from Bregma -2.28 till -3.72 mm, and were contained within an area ventral to the zona incerta, medial of the internal capsula, and lateral to the dorsomedial and ventromedial hypothalamic nuclei according to the Paxinos rat brain atlas (Paxinos & Watson, 2007); Figure 1).

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 (van de Giessen et al., 2013). Furthermore, baseline differences in dietary intake can modulate the orexigenic effects of NPY (Stanley et al., 1989). 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 (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 *post hoc* or *a posteriori* Fishers' LSD tests. All statistical analyses were performed using Graphpad Prism 8 [version 8.0.2 (263), January 30, 2019] and are detailed in supplemental Tables 1-2. For all cases, $p < 0.05$ was considered significant. All data are presented as mean ± *SEM*.

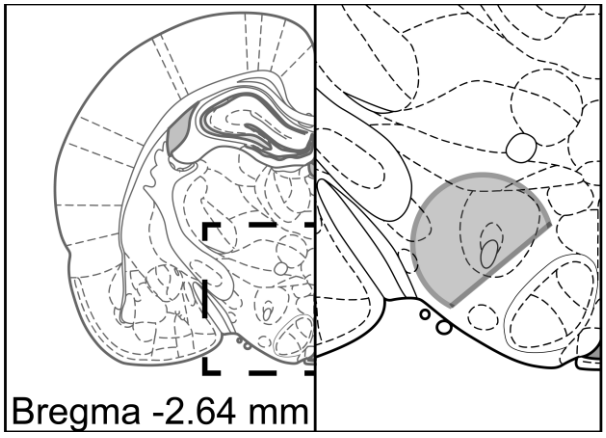

Figure 1. LHA infusion sites. Atlas illustration indicating the general site for NPY or saline infusion in the LHA. Left: overview of coronal rat brain section based on the Paxinos and Watson rat brain atlas (Paxinos & Watson, 2007). Right: inset from left figure showing schematic infusion site in gray.

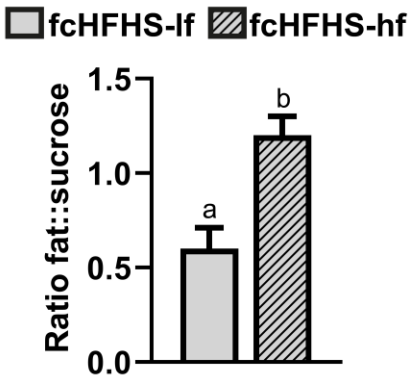

Figure 2. Fat::sucrose solution intake ratio for the fcHFHS-lf and fcHFHS-hf groups. The fcHFHS-fed group was separated into two groups based on their basal fat::sucrose solution intake ratio. Different letters indicate a statistically significant difference at $p < 0.05$.

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* ($F_{4,38} = 24.40$, $p < 0.0001$; see Figure 3A, and 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 ($F_{2,18} = 8.16$, $p = 0.0003$; see Figure 3B, and 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 $p < 0.05$; see Figure 3B, *left*). For daily caloric intake from the sucrose solution, *post hoc* analysis demonstrated that the fcHFHS-hf group consumed fewer daily calories from the sucrose solution compared to the fcHFHS-lf and fcHS-fed groups (all $p < 0.01$; see Figure 3B, *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 $p < 0.01$; see Figure 3B, *right*).

Table 1. Characteristics of the dietary intervention.

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

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

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 ($F_{2,18} = 16.61$, $p < 0.0001$; see Figure 3C, and 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 of intake from chow than the fcHFHS-lf, and fcHFHS-hf groups, and the fcHS-fed group consuming an intermediate percentage of daily caloric intake from chow (all $p < 0.05$; see Figure 3C, *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 the sucrose solution than the fcHFHS-hf group, and fcHS-fed rats consumed a higher percentage of the sucrose solution than both fcHFHS groups (all $p < 0.05$; see Figure 3C, *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 $p < 0.05$; see Figure 3C, *right*).

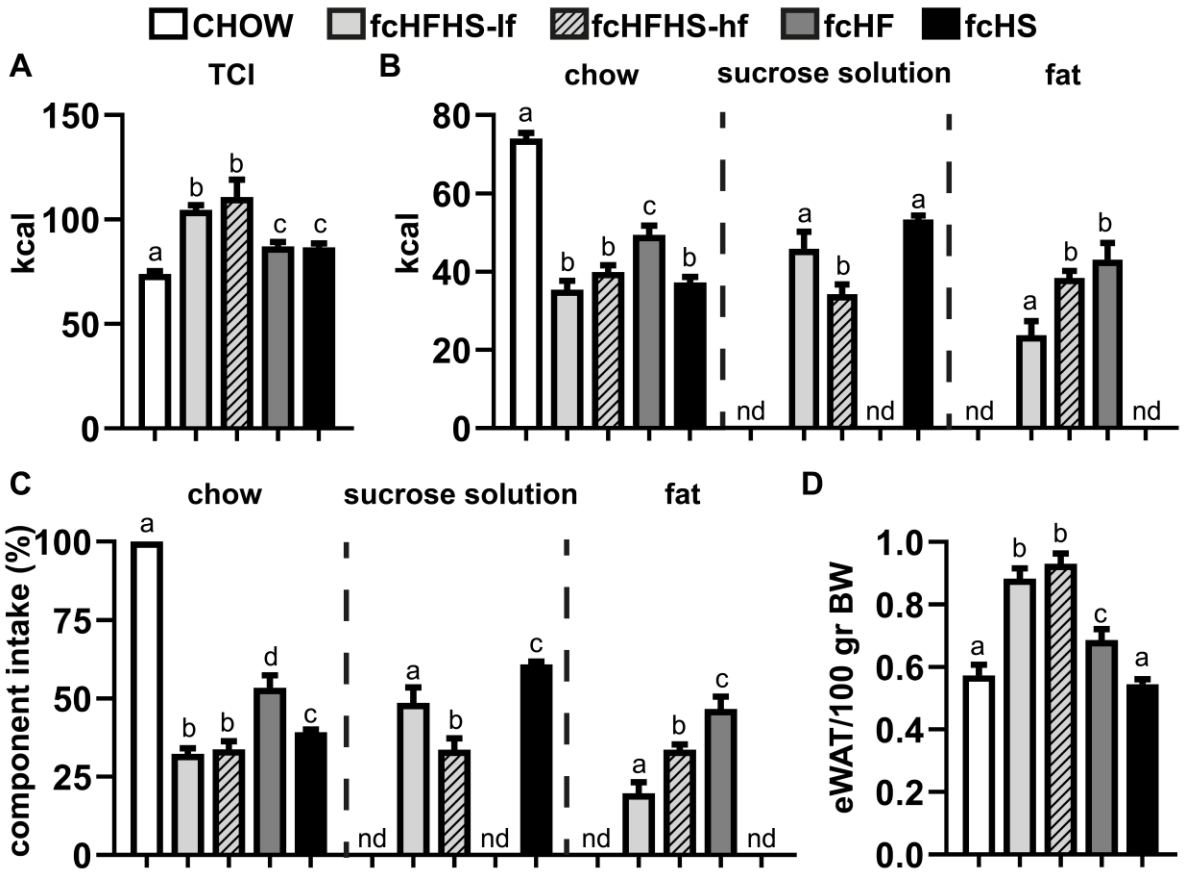

Figure 3. Characteristics of dietary intervention. A) Mean daily total caloric intake per diet group. **B)** Mean daily caloric intake of the chow, sucrose solution, and fat component per diet group. **C)** Mean daily percentage of component intake per diet group. **D)** Epididymal white adipose tissue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

Epididymal fat mass

One-way ANOVA analysis of eWAT normalized for body weight revealed a main effect of *Diet* ($F_{4,37} = 25.53$, $p < 0.0001$; see Figure 3D, and 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 $p < 0.05$; see Figure 3D, and Table 1). Lastly, eWAT mass did not differ between fcHS- and CHOW-fed rats ($p > 0.05$; see Figure 3D, and Table 1).

Effects of intra-LHA NPY infusion on food intake and dietary selection

A mixed effects model analysis of total caloric intake (TCI) 2 hours following intra-LHA NPY infusion revealed a main effect of *Infusion* ($F_{1,38} = 35.81$, $p < 0.0001$), no main effect of *Diet* ($F_{4,38} = 0.67$, $p > 0.05$), and a trend towards a *Diet* x *Infusion* interaction effect ($F_{4,38} = 2.48$, $p = 0.06$; see Figure 4A, and Table 2). To assess which diets drive the main effect of *Infusion*, we performed *a posteriori* t-tests, indicating that the main effect of *Infusion* was driven by significant increases in total caloric intake in the CHOW-fed group ($t_{14,14} = 4.16$, $p = 0.0002$), fcHFHS-lf group ($t_{7,7} = 3.62$, $p = 0.0009$), and fcHFHS-hf group ($t_{6,6} = 3.70$, $p = 0.0007$). For the fcHF-fed group, TCI showed a trend for an increase ($t_{8,8} = 1.87$, $p = 0.08$), whereas NPY infusion had no effect on caloric intake in the fcHS-fed group ($t_{8,8} = 0.28$, $p = 0.89$; see Figure 4A, and Table 2).

A mixed effects model analysis of caloric intake of chow after intra-LHA NPY infusion revealed a main effect of *Infusion* ($F_{1,25} = 14.00$, $p = 0.001$), but no main effect of *Diet* ($F_{3,25} = 0.61$, $p > 0.05$), and no *Diet* x *Infusion* interaction effect ($F_{3,25} = 0.63$, $p > 0.05$; see Figure 4B and Table 2). Similar to the TCI findings, *a posteriori* t-test analyses indicated 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 intake after a saline control infusion 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 ($F_{2,36} = 1.50$, $p > 0.05$; see Figure 4C, and Table 2). *Post hoc* analysis showed that the fcHS-fed group consumed more of the 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 the fat component 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 4D, and Table 2). *A posteriori* t-tests indicated that the main effect of *Infusion* was mainly driven 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.

Table 2. Caloric intake after vehicle or NPY infusion.

Diet group	Intake measure	Vehicle	NPY
CHOW	TCI	7.1 ± 1.1	13.2 ± 1.7*
fcHFHS-lf	TCI	6.2 ± 0.9	13.7 ± 2.0*
	Chow	1.4 ± 0.3	5.2 ± 1.0*
	Sucrose water	3.2 ± 0.5	4.7 ± 0.7
	Fat	1.6 ± 0.5	3.8 ± 1.3
fcHFHS-hf	TCI	8.2 ± 4.1	16.5 ± 4.1*
	Chow	1.9 ± 0.7	5.5 ± 1.1*
	Sucrose water	2.2 ± 0.9	3.3 ± 0.4
	Fat	1.9 ± 0.6	7.4 ± 2.4*
fcHF	TCI	6.1 ± 1.7	9.7 ± 1.7†
	Chow	3.1 ± 1.3	6.6 ± 1.3*
	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

* = $p < 0.05$ compared to vehicle control, † = trend towards statistical significance ($p < 0.08$), TCI = total caloric intake. All data are presented in kilocalories and mean ± *SEM*.

Discussion

Summary

In this study, we demonstrate that previous consumption of a certain diet as well as dietary choice can modulate the orexigenic effects of intra-LHA NPY infusion in the male Wistar rat. Intra-LHA NPY increases chow intake in CHOW-, fcHFHS-hf, fcHFHS-lf and fcHF-fed rats, but not in fcHS-fed rats. Moreover, intra-LHA NPY increases fat intake in rats that have a high fat::sucrose solution intake ratio (fcHFHS-hf group), but not in fcHFHS-lf 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 from differences in diet component intake and the subsequent adaptations in the brain. It has to be noted, however, that the relatively high intake levels of fat are not the only requirement for intra-LHA NPY to drive fat intake, as fcHF-fed rats did not increase fat intake after NPY infusion. Together, these observations indicate that both relatively high intake levels of fat as well as consumption of a sucrose solution are required for intra-LHA NPY to promote fat intake. In contrast, intra-LHA NPY infusion in fcHS-fed rats did not increase consumption of chow or of the sucrose solution. Altogether, our observations suggest that consumption of a sucrose solution disrupts the LHA NPY circuitry, and that this disruption is different depending on whether the sucrose solution is consumed together with chow and fat, or together with chow only. Our findings thus indicate an important interaction between sucrose solution and fat consumption on the responsivity of the LHA NPY circuitry.

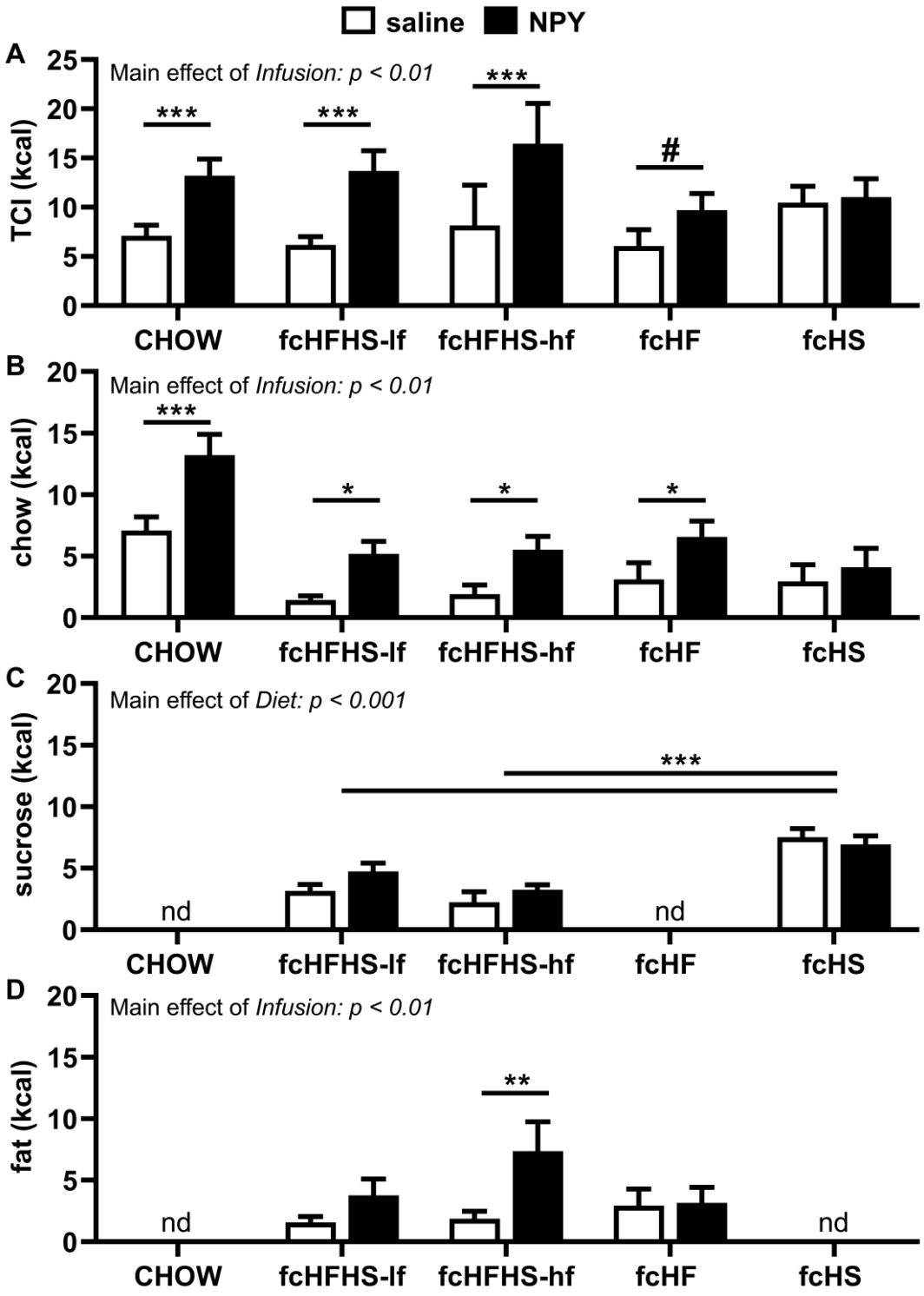

Figure 4. Intra-LHA NPY infusion affects intake in a diet composition dependent manner. A) Total caloric intake (TCI) after intra-LHA NPY or saline infusion. **B)** Caloric intake of chow after intra-LHA NPY infusion. **C)** Caloric intake from the sucrose solution after intra-LHA NPY infusion. **D)** Caloric intake from the fat component 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.

The role of LHA NPY in the regulation of energy homeostasis

Studies evaluating the effects of NPY on food intake have mostly focussed on its effects within the PVN, as this hypothalamic area receives dense NPY projections (Chronwall et al., 1985; Sawchenko et al., 1985) and peptide levels in the PVN fluctuate with the energetic status of the animal (Dube et al., 1992; S. P. Kalra et al., 1991; Sahu et al., 1988). Intra-PVN injection of NPY specifically increases consumption of carbohydrates (Smith et al., 1997; Stanley, Daniel, et al., 1985). 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 (Smith et al., 1997; Stanley, Daniel, et al., 1985). The findings from our study suggest that LHA NPY, as has been shown for PVN NPY, predominantly modulates intake of complex carbohydrates. Moreover, we find that when fcHFHS-fed rats have 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 the diet or negative energy levels. For example, intra-PVN NPY infusion leads to carbohydrate intake, which itself also increases NPY peptide in the PVN (Beck et al., 2001; Jhanwar-Uniyal et al., 1993). 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 (Jhanwar-Uniyal et al., 1993; J. Wang et al., 1998). 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 (Beck, Jhanwar-Uniyal, et al., 1990). 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* (Tiesjema et al., 2007). Thus, the LHA and PVN NPY circuitries appear to play different roles in feeding behaviour, even though the orexigenic effects of intra-LHA NPY appear similar to the orexigenic effects of intra-PVN NPY infusion.

In addition to the regulation of energy homeostasis, the LHA is also important for (food) reward-related behaviours (Berthoud & Munzberg, 2011; Bonnavion, Mickelsen, Fujita, de Lecea, & Jackson, 2016). However, the role of intra-LHA NPY in mediating reward-related behaviours is currently unclear. For example, intra-LHA NPY infusion can elicit a conditioned place preference, but does not always increase operant responding for sucrose pellets (C. M. Brown et al., 2000; C. M. Brown et al., 1998; Pandit et al., 2014a). In relation to our observations, if the effects of intra-LHA NPY infusion on component choice were mediated through reward-related mechanisms, it would be more likely that intra-LHA NPY infusion predominantly increases intake of the palatable food 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 the fcHS-fed rats are insensitive to a dose of intra-LHA that is effective in rats consuming the other experimental diets, suggesting decreased NPYR function in order to limit intake in fcHFS-fed rats. 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 behaviour, suggesting that the presence of one receptor type can compensate for the loss of the other receptor type during development (Nguyen et al., 2012). 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 LHA neurons to NPY. For example, the LHA contains insulin- and leptin-responsive neurons (Berthoud & Munzberg, 2011), which can be either *pro-melaninconcentrating hormone (mch)*- or *orexin*/*hypocretin*-expressing neurons, and are known to play a role in the regulation of food intake and energy balance (Qu et al., 1996; Sakurai et al., 1998). However, from earlier studies in our lab, we have no indication that fcHS diet consumption leads to changes in insulin or leptin concentration after such a short period of consumption that could explain a negative feedback to the LHA (Diepenbroek et al., 2017; la Fleur et al., 2011). We cannot, however, 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 underlying decreased LHA NPY sensitivity in fcHS-fed rats.

Strengths and limitations

This is the first study to investigate if dietary choice and/or dietary composition modulate the orexigenic effects of intra-LHA NPY infusion. As NPY in the LHA has strong effects on caloric intake and the LHA NPY circuitry is dysregulated during diet-induced obesity (M.C.R. Gumbs et al., *accepted;* Stanley et al., 1993), 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 (la Fleur et al., 2007; Slomp et al., 2019). It should be noted that our choice diets therefore do not include a separate protein source. However, intra-PVN infusion of NPY does not modulate intake of a separate protein source (J. Wang et al., 1998). As the effects of NPY on caloric intake are highly similar to those described for the PVN, this may suggest that the absence of a separate protein sources does not affect dietary choice. 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 (C. M. Brown et al., 2000; Glass, Cleary, Billington, & Levine, 1997). In our study, sucrose was provided in liquid form as a 30% solution and *ad libitum*, which may explain the different observations. 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.

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 now 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 behaviour and energy balance. We also demonstrate that dietary choice and/or composition can modulate the sensitivity of the LHA NPY circuitry. 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 during certain obesogenic dietary conditions.

Supplemental results

Supplemental Table 1. Statistical details of daily caloric intake analyses.

Mean daily caloric intake	
Total caloric intake (kcal)	$F_{4,38} = 24.40$, $p < 0.0001$* (One-way ANOVA)
CHOW *vs.* fcHFHS-lf	$t_{14,7} = 7.43$, $p < 0.0001$*
CHOW *vs.* fcHFHS-hf	$t_{14,6} = 8.44$, $p < 0.0001$*
CHOW *vs.* fcHF	$t_{14,8} = 3.35$, $p = 0.002$*
CHOW *vs.* fcHS	$t_{14,8} = 3.2$, $p = 0.003$*
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 1.22$, $p > 0.05$
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 3.77$, $p = 0.0006$*
fcHFHS-lf *vs.* fcHS	$t_{7,8} = 3.89$, $p = 0.0004$*
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 4.87$, $p < 0.0001$*
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 4.99$, $p < 0.0001$*
fcHF *vs.* fcHS	$t_{8,8} = 0.13$, $p > 0.05$
Caloric intake from chow	$F_{4,38} = 97.9$, $p < 0.0001$* (One-way ANOVA)
CHOW *vs.* fcHFHS-lf	$t_{14,7} = 15.47$, $p < 0.0001$*
CHOW *vs.* fcHFHS-hf	$t_{14,6} = 12.97$, $p < 0.0001$*
CHOW *vs.* fcHF	$t_{14,8} = 10.29$, $p < 0.0001$*
CHOW *vs.* fcHS	$t_{14,8} = 15.39$, $p < 0.0001$
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 1.50$, $p > 0.05$
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 5.03$, $p < 0.0001$*
fcHFHS-lf *vs.* fcHS	$t_{7,8} = 0.66$, $p > 0.05$
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 3.27$, $p < 0.01$*
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 0.91$, $p > 0.05$
fcHF *vs.* fcHS	$t_{8,8} = 4.42$, $p < 0.0001$*
Caloric intake from sucrose water	$F_{2,18} = 11.3$, $p = 0.0007$* (One-way ANOVA)
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 2.81$, $p < 0.05$*
fcHFHS-lf *vs.* fcHS	$t_{7,8} = 1.93$, $p = 0.07$†
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 4.74$, $p < 0.01$*
Caloric intake from fat	$F_{2,18} = 8.16$, $p = 0.0003$* (One-way ANOVA)
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 2.77$, $p = 0.01$*
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 3.93$, $p < 0.01$*
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 0.91$, $p > 0.05$

Post hoc analysis was performed with Fishers' LSD. *$p < 0.05$, † $p < 0.07$

Supplemental Table 2. Statistical details dietary composition analyses.

Dietary composition	
Percentage intake from chow	$F_{4,38} = 265.5$, $p < 0.0001$* (One-way ANOVA)
CHOW *vs.* fcHFHS-lf	$t_{14,7} = 25.23$, $p < 0.0001$*
CHOW *vs.* fcHFHS-hf	$t_{14,6} = 23.40$, $p < 0.0001$*
CHOW *vs.* fcHF	$t_{14,8} = 18.12$, $p < 0.0001$*
CHOW *vs.* fcHS	$t_{14,8} = 23.65$, $p < 0.0001$*
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 0.47$, $p > 0.05$
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 7.05$, $p < 0.0001$*
fcHFHS-lf *vs.* fcHS	$t_{7,8} = 2.31$, $p < 0.05$*
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 6.23$, $p < 0.0001$*
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 1.73$, $p = 0.09$
fcHF *vs.* fcHS	$t_{8,8} = 4.91$, $p < 0.0001$*
Percentage intake from sucrose	$F_{2, 18} = 15.69$, $p = 0.0001$ (One-way ANOVA)
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 23.00$, $p = 0.0008$*
fcHFHS-lf *vs.* fcHS	$t_{8,7} = 2.63$, $p = 0.02$*
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 5.60$, $p < 0.0001$*
Percentage intake from fat	$F_{2,18} = 16.61$, $p < 0.0001$* (One-way ANOVA)
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 2.78$, $p < 0.05$*
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 5.76$, $p < 0.0001$*
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 2.67$, $p < 0.05$*
Epididymal fat mass/ 100 gr body weight	$F_{4,37} = 25.53$, $p < 0.0001$* (One-way ANOVA)
CHOW *vs.* fcHFHS-lf	$t_{14,7} = 6.85$, $p < 0.0001$*
CHOW *vs.* fcHFHS-hf	$t_{14,6} = 7.49$, $p < 0.0001$*
CHOW *vs.* fcHF	$t_{14,8} = 2.61$, $p < 0.05$*
CHOW *vs.* fcHS	$t_{14,8} = 0.65$, $p > 0.05$
fcHFHS-lf *vs.* fcHFHS-hf	$t_{7,6} = 0.88$, $p > 0.05$
fcHFHS-lf *vs.* fcHF	$t_{7,8} = 3.94$, $p < 0.01$*
fcHFHS-lf *vs.* fc*HS*	$t_{7,8} = 6.76$, $p < 0.0001$*
fcHFHS-hf *vs.* fcHF	$t_{6,8} = 4.68$, $p < 0.0001$*
fcHFHS-hf *vs.* fcHS	$t_{6,8} = 7.39$, $p < 0.0001$*
fcHF *vs.* fc*HS*	$t_{8,8} = 2.93$, $p < 0.01$*

Post hoc analysis was performed with Fishers' LSD. *p < 0.05, # p < 0.07