

RESEARCH ARTICLE

Dietary fat exacerbates postprandial hypothalamic inflammation involving glial fibrillary acidic protein-positive cells and microglia in male mice

Céline Cansell¹ | Katharina Stobbe¹ | Clara Sanchez¹ | Ophélie Le Thuc¹ | Coralie-Anne Mosser² | Selma Ben-Fradj³ | Joris Leredde¹ | Cynthia Lebeau¹ | Delphine Debayle¹ | Lucile Fleuriot¹ | Frédéric Brau¹ | Nadège Devaux¹ | Alexandre Benani³ | Etienne Audinat⁴ | Nicolas Blondeau¹ | Jean-Louis Nahon¹ | Carole Rovère¹ 

¹IPMC, CNRS, Université Côte d'Azur, IPMC, CNRS, Valbonne, France

²Laboratory of Neurophysiology and New Microscopies, INSERM, Université Paris Descartes, Paris, France

³CSGA, AgroSup Dijon, CNRS, INRA, Université Bourgogne Franche-Comté, Dijon, France

⁴IGF, CNRS, INSERM, Université de Montpellier, Montpellier, France

Correspondence

Carole Rovère, IPMC-CNRS UMR 7275, Université Côte d'Azur, 660 Route des Lucioles, 06560 Valbonne, France.
Email: rovere@ipmc.cnrs.fr

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Abstract

In humans, obesity is associated with brain inflammation, glial reactivity, and immune cells infiltration. Studies in rodents have shown that glial reactivity occurs within 24 hr of high-fat diet (HFD) consumption, long before obesity development, and takes place mainly in the hypothalamus (HT), a crucial brain structure for controlling body weight. Here, we sought to characterize the postprandial HT inflammatory response to 1, 3, and 6 hr of exposure to either a standard diet (SD) or HFD. HFD exposure increased gene expression of astrocyte and microglial markers (glial fibrillary acidic protein [GFAP] and Iba1, respectively) compared to SD-treated mice and induced morphological modifications of microglial cells in HT. This remodeling was associated with higher expression of inflammatory genes and differential regulation of hypothalamic neuropeptides involved in energy balance regulation. DREADD and PLX5622 technologies, used to modulate GFAP-positive or microglial cells activity, respectively, showed that both glial cell types are involved in hypothalamic postprandial inflammation, with their own specific kinetics and reactivity to ingested foods. Thus, recurrent exacerbated postprandial inflammation in the brain might promote obesity and needs to be characterized to address this worldwide crisis.

Abbreviations: ACN, acetonitrile; AgRP, agouti-related peptide; ARC, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; CCL, chemokine (C–C motif) ligand; ChCl3, chloroform; CNO, clozapine *N*-oxide; CSF1R, colony stimulating factor 1 receptor; DREADD, designer receptors exclusively activated by designer drugs; GAPDH, glyceraldehyde phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; HFD, high-fat diet; HT, hypothalamus; Iba1, ionized calcium binding adaptor molecule 1; IL, interleukin; INF, interferon; IPA, isopropanol; MBH, medial basal hypothalamus; MCH, melanin-concentrating hormone; MeOH, methanol; NPY, neuropeptide Y; ORX, orexine; PBS, phosphate buffer saline; POMC, Pro-Opioid Melanocortin; SD, standard diet; TG, triglyceride; TNF, tumor necrosis factor.

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KEYWORDS

chemokines, cytokines, energy balance, GFAP-positive cells, hypothalamus, inflammation, microglia, neuropeptides, nutritional lipids, obesity, postprandial

1 | INTRODUCTION

Obesity is a major public health problem that has been highlighted by the World Health Organization. In 2016, almost 40% of the worldwide population was overweight and 13% was obese (<http://www.who.int/en/>). The main driving force of this body weight disturbance is an energy balance dysregulation, due to kilocalorie consumption overriding energy expenditure (Gao & Horvath, 2008). Therefore, chronic exposure to the Western diet, which is rich in saturated fat (commonly known as the high-fat diet [HFD]) is now recognized as a significant contributor to the development of obesity and its associated comorbidities, such as insulin resistance, Type 2 diabetes mellitus and other chronic illnesses such as cardiovascular disease or cancer (Hotamisligil, 2006).

Excessive intake of dietary fat induces chronic low-grade inflammation in many metabolic organs including pancreas, adipose tissue, muscle, and liver, as well as in the brain (Lumeng & Saltiel, 2011). HFD consumption increases the expression of inflammatory mediators (De Souza et al., 2005; Thaler & Schwartz, 2010), especially the pro-inflammatory cytokines Interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF- α), and interleukin 6 (IL-6), and disrupts insulin and leptin signaling in murine and primate hypothalamus (HT), a brain area involved in energy balance regulation (Arruda et al., 2011; Grayson et al., 2010; Milanski et al., 2009; Zhang et al., 2008). HFD-induced inflammatory modifications of brain signals also converge to alter levels of hypothalamic neuropeptides, suggesting that hypothalamic neuropeptides could act as a link between central inflammation, dysregulation of feeding behavior and energy expenditure, and indicating that these peptides could be potential therapeutic targets for the treatment of obesity (Timper & Bruning, 2017).

Since 2012, several studies have shown that HFD consumption induces astrogliosis and microgliosis in the arcuate nucleus (ARC) of the HT (Andre et al., 2017; Balland & Cowley, 2017; Baufeld, Osterloh, Prokop, Miller, & Heppner, 2016; Gao et al., 2014; Guillemot-Legris et al., 2016; Kim, Yoon, Jin, & Diano, 2019; Thaler et al., 2012; Valdearcos et al., 2014; Valdearcos et al., 2017). The glial activation that is induced by lipids and connects dietary fat and brain inflammatory signaling is begging to be seen as the missing link between the hypothalamic response to HFD and susceptibility to obesity (Andre et al., 2017; Douglass, Dorfman, Fasnacht, Shaffer, & Thaler, 2017; Gao, Layritz, et al., 2017; Gao, Vidal-Itriago, et al., 2017; Kim et al., 2019; Valdearcos et al., 2014; Valdearcos et al., 2017). Nevertheless, it has recently been shown that HFD-induced inflammation in astrocytes may have a protective effect against the development of obesity (Buckman et al., 2015). Interestingly, hypothalamic inflammation has been found to occur very early in response to HFD, before the onset of obesity (Balland & Cowley, 2017; Buckman et al., 2015; Kim et al., 2019; Thaler

et al., 2012; Waise et al., 2015). Exposure to HFD for a 24 hr period increases gene expression of inflammatory astrocyte and microglia markers as well as astrogliosis in the HT (Buckman et al., 2015; Dalvi et al., 2017; Thaler et al., 2012). Microgliosis has also been found in the ARC after a 72-hr exposure to HFD (Thaler et al., 2012). These findings tempt us and others to speculate that hypothalamic inflammation may not be limited to chronic exposure to dietary fat, but may occur after consumption of a single meal as observed at the periphery (Emerson et al., 2017; Ghanim et al., 2009; Herieka & Erridge, 2014; Kelly, Colgan, & Frank, 2012; Khor et al., 2014). Therefore, we investigated cellular and molecular hypothalamic remodeling in mice that were exposed for the first time to a HFD for short periods of time (1, 3, or 6 hr) and we compared them to mice exposed to standard diet (SD).

2 | METHODS

2.1 | Animals

An 8-week-old C57Bl/6J male and female mice (20–25 g, Janvier Labs, France) and 9–10 week-old CX3CR1eGFP/eGFP male mice (Jung et al., 2000) were group housed (two animals/cage) in a room maintained at $22 \pm 1^\circ\text{C}$ with a 12 hr light/12 hr reversed dark cycle and were acclimatized for 2–3 weeks before experiments were performed. Animals had access to water and SD ad libitum (3,395 kcal/kg with 25.2% from proteins, 13.5% fat, and 61.3% from carbohydrates; Safe #A03). All protocols were carried out in accordance with French standard ethical guidelines for laboratory animals and with approval of the Animal Care Committee (Nice-French Riviera, registered number 04042.02; University Paris Descartes registered number CEEA34.EA.027.11 and CEEA16-032; APAFIS#14072-2018030213588970v6). The heterozygous CX3CR1eGFP/+ mice used for electrophysiology were obtained by crossing CX3CR1eGFP/eGFP (Jung et al., 2000) with C57BL/6J (Janvier Labs) wild-type mice.

2.2 | Short-term high-fat feeding studies

All mice were food-deprived for 2 hr prior the onset of the dark cycle to synchronize groups. The 2-hr food deprivation at the end of the light period does not alter metabolic state as evidenced by hormonal examination (Nuzzaci et al., 2020), and maintains natural neuroendocrine control of food intake, based on synergic action of peripheral hormones (Matarazzo et al., 2012). Before food deprivation, bedding was changed to remove any leftover food in the bottom of the cage. At the beginning of the dark cycle ($T = 0$ hr) mice were fed either SD (Safe #A03) or HFD (4,494.5 kcal/kg with 16.1% from proteins, 40.9% from fat, and

43% from carbohydrates; Safe #U8954P V0100). The non-fed group used for normalization was killed on the same day at the beginning of the dark cycle ($T = 0$ hr) and had unlimited access to SD from their arrival to the laboratory until the beginning of experiments. Then, 1, 3, and 6 hr after food exposure, food intake was measured and brain and blood samples were collected. Retro-orbital bleeding was performed on mice anesthetized with isoflurane. Blood samples were kept on ice before centrifugation. After centrifugation, serum samples were collected and immediately frozen at -80°C and kept for further analyses. After blood sampling, mice were sacrificed and brain samples collected were immediately frozen in liquid nitrogen and kept at -80°C for further analyses. Animals from the 1, 3, and 6 hr groups were different.

2.3 | PLX5622 treatment

Microglia were depleted by administering the colony stimulating factor 1 receptor (CSF1R) inhibitor PLX5622 (Plexxikon), formulated in AIN76A (3,827.7 kcal/kg with 18.2% from proteins, 12.6% fat, and 69.2% from carbohydrates; Research Diet) at a dose of 1.2 g/kg for 2 weeks. The control group received AIN76A without PLX5622 for 2 weeks as well. After 2 weeks of treatment, animals underwent short-term high-fat feeding. Animals from the 1, 3, and 6 hr groups were different.

2.4 | Stereotaxic virus injections and clozapine N-oxide injection

Here, 2 weeks before short-term high-fat feeding studies, mice were anesthetized by intraperitoneal (i.p.) injection of a ketamine–xylazine mix (80–12 mg/kg). They were then placed on a stereotaxic frame. Adeno associated virus 8 (AAV8)/glial fibrillary acidic protein (GFAP)-HA-hM4G(Gi)-mCitrine (Translational Vector Core, France) virus was injected bilaterally into the medial basal HT (MBH). Stereotaxic coordinates relative to bregma were: $x: \pm 0.3$ mm, $y: -1.5$ mm, and $z: +6$ mm. Injections were applied at a rate of 0.5 $\mu\text{l}/\text{min}$ for 1 min per side. At the end of the surgical procedures, mice received 1 mg/kg i.p. atipamezole and 5 mg/kg subcutaneous (s.c.) ketoprofen. The clozapine N-oxide (CNO) (Sigma, France) was prepared in water at 1 mg/kg and injected i.p. 30 min before food delivery during short-term high-fat feeding studies. The control group was injected i.p. 30 min before food delivery during short-term high-fat feeding studies with saline solution (NaCl). Animals from the 1, 3, and 6 hr groups and treated with CNO or NaCl were different.

2.5 | RNA isolation and quantitative PCR

Total RNA from frozen HT samples were isolated using Fast Prep apparatus (Q-Biogene, France) as previously described (Le Thuc et al., 2016). First-strand cDNAs were synthesized from 2 μg of total RNA with 200 U of SuperScript III reverse transcriptase (SuperScript III, Invitrogen, France) in the appropriate buffer in the presence of

25 ng/ μl oligo-dT primers, 0.5 mM desoxyribonucleotide triphosphate mix, 5 mM dithiothreitol, 40 U RNasin (Promega, France). The reaction was incubated 5 min at 25°C , then 50 min at 50°C then inactivated for 15 min at 70°C . Real-time PCR was performed for amplification of mouse IL-1 β , IL-6, TNF- α , chemokines CCL2 and CCL5 (chemokine [C–C motif] Ligands 2–5), ionized calcium binding adaptor molecule 1 (Iba1), GFAP, melanin-concentrating hormone (MCH), OREXine (ORX), Pro-OpioMelanoCortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), agouti-related peptide (AgRP), and GlycerAldehyde Phosphate Dehydrogenase (GAPDH) mRNA. GAPDH was used as housekeeping gene for normalization. Primers (detailed in Supplementary data, Supplementary Table S1) were purchased from Eurogentec (France).

2.6 | Cytokine and chemokine quantification

A V-Plex multiplex assay (Meso Scale Discovery) and a mouse CCL5 ELISA Ready-SET-Go (eBiosciences, France) were used to measure the levels of inflammatory mediators in mice serum according to the manufacturer's protocol.

2.7 | Immunohistochemical analysis

Mice are first i.p. injected with 100 mg/kg pentobarbital sodium for terminal anesthesia. Brains were harvested from mice perfused with 4% paraformaldehyde in phosphate buffer saline (PBS) and postfixed in the same fixative overnight at 4°C . Brain coronal sections (30 μm) were cut on a vibratome, blocked for 1 hr with 3% normal goat serum in PBS containing 0.1% Triton X-100 and incubated with primary antibodies overnight at 4°C . Primary anti-rabbit antibodies were against Iba1 (1:500, CP290A, B, Biocare Medical) and GFAP (1:300, Z0334, Dako, Denmark). Adequate Alexa Fluor 488 conjugated secondary antibodies were used for immunofluorescence microscopy. Sections at -1.70 mm relative to Bregma were mounted in VECTASHIELD solution (H-1000, Vector Laboratories). 3D mosaics of 1024×1024 images were acquired with a TCS SP5 laser-scanning confocal microscope (Leica Microsystems, Nanterre, France) through a $\times 40/1.4$ oil immersion objective for GFAP staining and $\times 63/1.4$ oil immersion objective for Iba1 staining, with a z-step of 2 μm . GFAP staining analysis, Iba1 staining analysis and soma size measurements were done on ImageJ (Halg, Besserer, & Schneider, 2012) on maximal intensity projections of these z-stacks of images. After the z-projection, a region of interest (ROI) corresponding to the ARC of the HT was manually drawn on these images. GFAP and Iba1 staining areas were selected and measured (in μm^2) in these ROIs by intensity thresholding and measurement above this threshold. The same process was applied to measure the soma size of microglial cells. The activity states of the astrocytes and microglia were assessed using the graded scoring system described in Harrison, Pfuhlmann, Schriever, and Pfluger (2019). Regarding the GFAP staining, we were not able to distinguish each cell individually. We therefore assessed the overall activation status of the ROI.

2.8 | Acute HT slices preparation for microglia recordings

Male mice aged between 9 and 10 weeks were anesthetized with isoflurane before the brain was harvested and coronal slices through the HT were cut using a Leica VT1200 vibratome in an oxygenated (5% CO₂ and 95% O₂) ice-cold protective extracellular solution containing (in mM): 93 NMDG, 2.5 KCl, 1.2 NaH₂PO₄, 30 NaHCO₃, 20 HEPES, 2 thiourea, 25 D-glucose, 5 sodium ascorbate, 3 sodium pyruvate, 10 MgCl₂, and 0.5 CaCl₂ (pH 7.3, 320 mOsm). Slices were then incubated in the same protective extracellular solution for 7 min at 34°C and then incubated for 30 min in artificial cerebrospinal fluid (aCSF; pH 7.3, 310 mOsm) at 34°C containing (in mM): 135 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 2.5 D-glucose, 1 sodium pyruvate, 1 MgCl₂, and 2 CaCl₂. The slices were then maintained at room temperature (RT, 22°C) for 0.5–5 hr in the regular oxygenated aCSF. After incubation, individual slices selected for best visibility of the ARC were transferred to a recording chamber on the stage of an Olympus microscope (BX50WI) with a ×40 water immersion objective, equipped with a CCD camera (Hamamatsu ORCA2-AG, France). Slices were constantly perfused at RT with oxygenated aCSF (5 ml/min).

2.9 | Electrophysiological recordings

Visually identified eGFP-expressing microglial cells, located at least 50 μm below the slice surface, were patched in whole-cell configuration in the ARC. Micropipettes (6–7 mV) were filled with a solution containing (in mM): 132 K-gluconate, 11 HEPES, 0.1 EGTA, 4 MgCl₂ (pH 7.35, osmolarity 300 mOsm). All potential values given in the text were corrected for a junction potential of 10 mV. Voltage-clamp recordings were performed using an Axopatch 200B (Molecular Devices). Series resistance (R_s), cell membrane resistance (R_m), and capacitance (C_m) were determined from the current response to a 10 ms depolarizing pulse of 10 mV that was performed at the beginning of each recording. These currents were filtered at 10 kHz and collected using PClamp 10 (Molecular Devices) at a frequency of 100 kHz. To measure the I/V relationship, we used hyperpolarizing and depolarizing steps (from –150 to +40 mV for 50 ms), and currents were low-pass filtered at 5 kHz and collected at a frequency of 20 kHz. Currents were analyzed off line using Clampfit 10.7 (Molecular Devices). To measure capacitance, currents were first fitted with a double exponential function in order to determine the time constant tau ($\tau_{weighted}$) defined as $\tau_{weighted} = (A1 \times \tau1 + A2 \times \tau2)/(A1 + A2)$ and then capacitance was calculated as $C_m = (\tau_{weighted}(R_m + R_s))/((R_m \times R_s))$.

2.10 | Lipopolysaccharide mass quantitation by LC-MS/MS

The detailed protocol is provided in the Supplementary data, Supplementary Materials and Methods. Lipopolysaccharide (LPS) mass concentration was determined by direct quantitation of 3-hydroxytetradecanoic

acid (or 3 hM) by LC/MS/MS as previously described by Pais de Barros et al. (2015).

2.11 | Triglyceride analysis

The detailed protocol is provided in the Supplementary data, Supplementary Materials and Methods. Briefly, lipids from mouse serum were extracted according to a modified Bligh and Dyer protocol with a mixture of methanol/chloroform (2:1). The lipid extract was then separated on a C18 column in an appropriate gradient. Mass spectrometry data were acquired with a Q-exactive mass spectrometer (ThermoFisher, France) operating in data-dependent MS/MS mode (dd-MS2). Finally, lipids were identified using LipidSearch software v4.1.16 (ThermoFisher) in product search mode. We quantified the serum triglycerides (TG), as well as the degree of saturation of the fatty acids contained in the TG. We then isolated TG containing only saturated fatty acids (TG0) and TG containing only monounsaturated fatty acids (TG1). In other words, TG0 is an ester derived from glycerol and three saturated fatty acids, and TG1 is an ester derived from glycerol and one to three monounsaturated fatty acids and zero to two saturated fatty acids.

2.12 | Statistical analysis

Values are displayed as box extending plots: the box extends from the 25th to 75th percentiles; the line in the box is the median; the “+” in the box is the mean; the whiskers span the smallest and largest values.

To test if the data set was well modeled, a Kolmogorov–Smirnov normality test was conducted (with Dallal–Wilkinson–Lillie for p value). The ROUT method (robust regression and outlier removal) was used to identify outliers with a Q coefficient equal to 1%. Variance equality was tested using an F -test. If samples fulfilled normal distribution and variance equality criteria, comparisons between groups were carried out using an unpaired t test for single comparison. If samples did not follow a normal distribution nor had different variances, comparisons between groups were carried out using a non-parametric Mann–Whitney U test for a single comparison. Multiple comparisons and interactions were carried out using two-way analysis of variance (ANOVA). Analysis of covariance (ANCOVA) were performed using R script and method described in Speakman et al. (2013) (Jha, Jo, Kim, & Suk, 2019). Because mice were group housed for “short-term high-fat feeding studies,” covariance between gene expression and food intake was run on the mean of each cage. When necessary, before running two-way ANOVA or ANCOVA, data were transformed to normalize them using the log₁₀ conversion. After transformation, data were retested for normality using Kolmogorov–Smirnov normality (with Dallal–Wilkinson–Lillie for p value) or D’Agostino and Pearson omnibus normality test. When appropriate, comparison with the theoretical mean control (1 or 100%) was done using a non-parametric Wilcoxon signed-rank test. A p -value of .05 was considered statistically significant. All tests were performed using R, GraphPad Prism 7.02 and Microsoft Office Excel. Numbers of

animals and cells are given in the legends. Numbers of outliers are given in Supplementary Table S3.

3 | RESULTS

3.1 | A single high-fat meal increases serum levels of endotoxins, but not those of cytokines, chemokines and TGs

Obesity and metabolic syndromes are concomitant with a state of chronic systemic inflammation (Lumeng & Saltiel, 2011) and the degree to which TGs increase after HFD consumption correlates with the

probability of developing metabolic syndrome (Herieka & Erridge, 2014; Nogaroto et al., 2015). Because the main difference between SD and HFD is lipid content, we measured TG serum levels after short exposure to the diet. By mass spectrometry, we found that HFD consumption did not change the total TG serum levels (Figure 1a) compared to SD-fed mice. Previous studies have linked HFD consumption to postprandial systemic inflammation and endotoxemia (Cani et al., 2007; Emerson et al., 2017; Ghanim et al., 2009; Herieka & Erridge, 2014; Kelly et al., 2012; Khor et al., 2014). Therefore, we measured the serum levels of the pro-inflammatory cytokines IL-1 β , IL-6, TNF α , and pro-inflammatory chemokines CCL2, CCL5, as well as endotoxin (LPS, 3OH C14:0) after SD and HFD short-term exposure (1, 3, and 6 hr). Interestingly, HFD consumption did not increase pro-

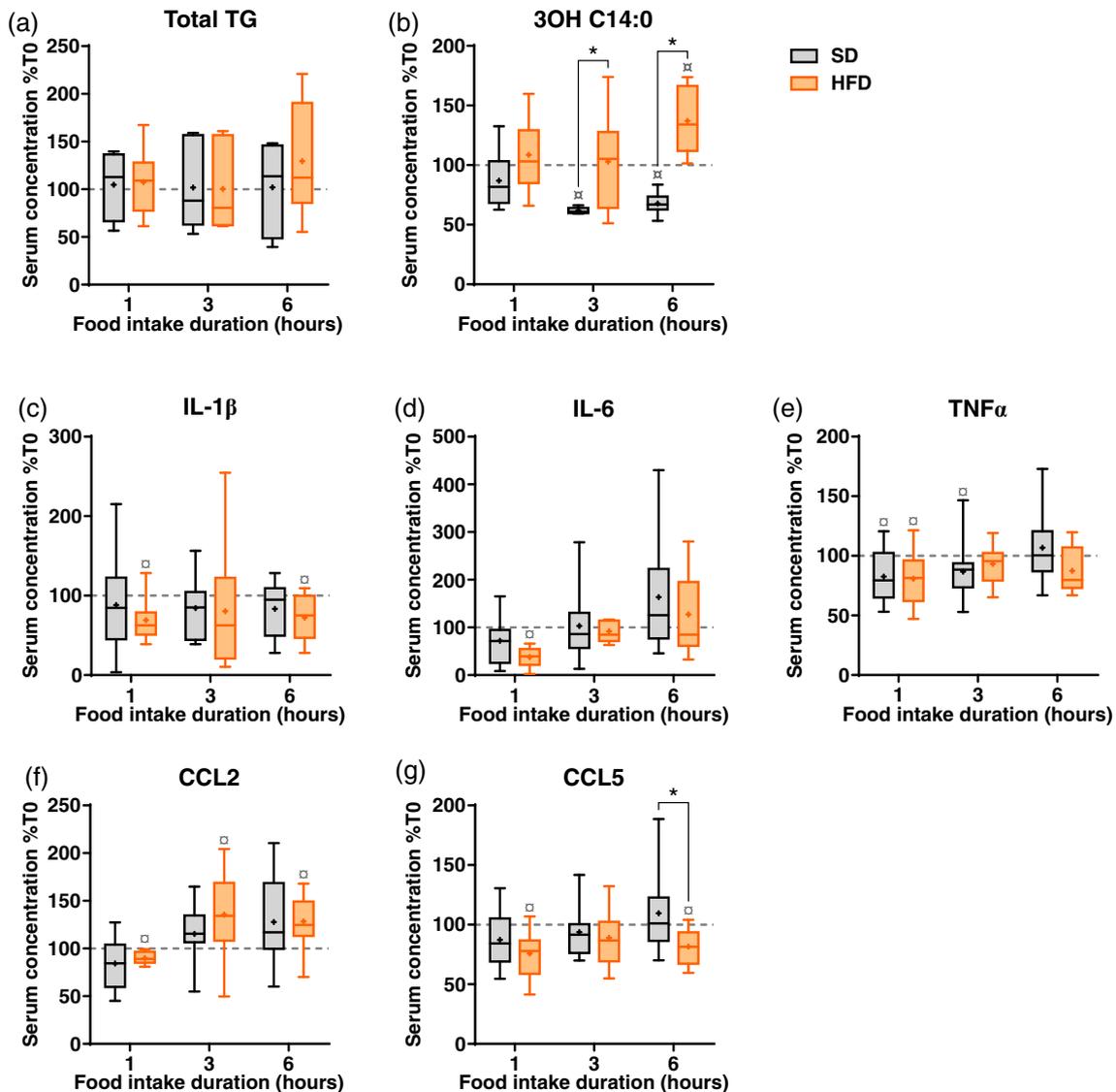


FIGURE 1 Short-term high-fat diet (HFD) exposure increases endotoxin levels without affecting triglycerides (TGs), cytokine, and chemokine levels in serum. Serum levels of (a) total TGs, (b) endotoxin (3OH C14:0), pro-inflammatory cytokines, (c) interleukin-1 beta (IL-1 β), (d) interleukin 6 (IL-6), (e) tumor necrosis factor alpha (TNF α), chemokines (f) CCL2, (g) CCL5 of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either standard diet (SD) or HFD. Results are expressed in % compared to baseline prior to diet exposure (T0, dashed line = initial level). * $p < .05$ Mann-Whitney test or unpaired *t*-test SD versus HFD; $\#p \leq .05$ Wilcoxon signed-rank test SD/HFD $\neq 100$; box extends from the 25th to 75th percentiles; line in the box is the median; "+" at the mean; whiskers go down to the smallest value and up to the largest. $N = 6-12$ [Color figure can be viewed at wileyonlinelibrary.com]

inflammatory cytokine or chemokine serum levels compared to SD-fed mice over the three time points (Figure 1c–g). However, 3 and 6 hr of HFD consumption increased endotoxin serum levels compared to SD-fed mice (Figure 1b). As anticipated (Benani et al., 2012; Buckman et al., 2015), an increase in kilocalories consumed was noticeable in mice fed for 6 hr with HFD compared to SD-fed mice (Supplementary Figure S1a), while the total quantity in grams of ingested food was similar between groups (Supplementary Figure S1b). Altogether, these observations indicate that short-term HFD exposure does not always affect systemic levels of inflammatory markers, but increases endotoxin concentrations in the serum compared to SD-fed mice.

3.2 | A single high-fat meal induces an exacerbated inflammatory-like gene response in the HT

We next investigated the impact of a HFD on hypothalamic inflammatory markers. Consistent with previous studies (Andre et al., 2017;

Baufeld et al., 2016; Buckman et al., 2015; Gao et al., 2014; Gao, Bielohuby, et al., 2017; Guillemot-Legris et al., 2016; Nadjar, Leyrolle, Joffre, & Laye, 2017; Thaler et al., 2012; Thaler & Schwartz, 2010; Valdearcos et al., 2014; Valdearcos et al., 2017) we found that gene expression of the pro-inflammatory cytokine genes IL-1 β , IL-6, and TNF α , as well as chemokine genes CCL2 and CCL5 was significantly increased in HFD-fed mice compared to SD-fed mice (Figure 2a–e). In addition, we found that an inflammatory-like gene response in the HT is also induced by SD and arises within the first hours of food consumption. Moreover, this inflammatory-like gene response exhibits a diet-specific profile according to cytokine/chemokine type, amplitude and kinetic of the response. In fact, increased IL-1 β and TNF α gene expression appeared 1 hr after food introduction (Figure 2a,c) with a greater increase in HFD-fed mice for IL-1 β (Figure 2a). Moreover, the increase in CCL2 and CCL5 gene expression also appeared within 1 hr of food exposure, but was observed exclusively in HFD-fed mice (Figure 2d,e). After 3 hr of food exposure, IL-1 β , IL-6, TNF α , and CCL2 gene expressions were increased only in HFD-fed mice (Figure 2a–d). Finally, after

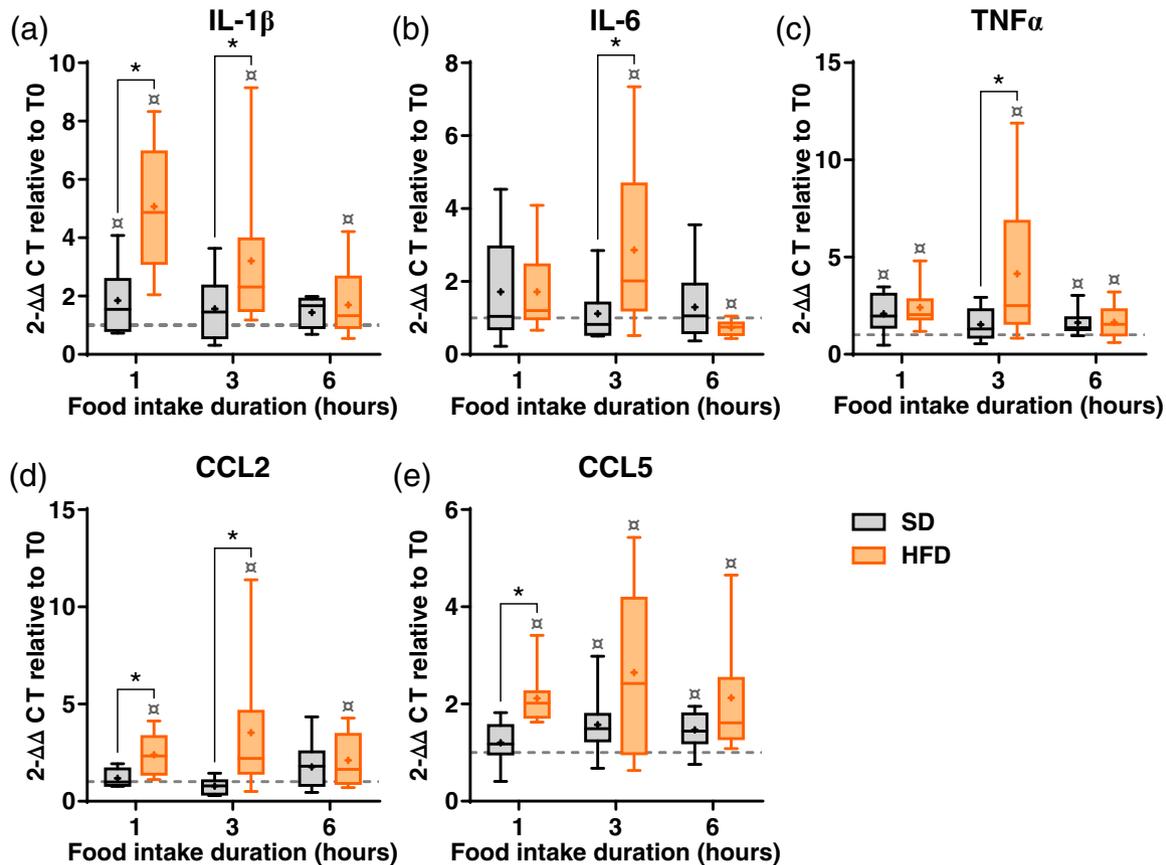


FIGURE 2 Short-term high-fat diet (HFD) exposure exacerbated postprandial inflammatory-like gene response in the hypothalamus (HT). Quantification of mRNA encoding pro-inflammatory cytokines (a) interleukin-1 beta (IL-1 β), (b) interleukin 6 (IL-6), (c) tumor necrosis factor alpha (TNF- α), and chemokines (d) CCL2, (e) CCL5 in HT of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either standard diet (SD) or HFD. All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta CT$ method and presented as fold-change relative to baseline prior to diet exposure (T0, dashed line = initial level). * $p < .05$ Mann-Whitney test or unpaired t -test SD versus HFD; $\#p < .05$ Wilcoxon signed-rank test SD/HFD versus 1; box extends from the 25th to 75th percentiles; line in the box is the median; "+" at the mean; whiskers go down to the smallest value and up to the largest. $N = 10$ –14 [Color figure can be viewed at wileyonlinelibrary.com]

6 hr of food exposure, pro-inflammatory cytokine and chemokine gene expression in HFD-fed mice tend to return to SD-mice levels. Taken together, these results indicate that short-term HFD exposure induces a specific and exacerbated hypothalamic inflammatory-like gene response, different from the one observed in SD-fed mice.

3.3 | A single high-fat meal induces a specific acute neuropeptide gene expression profile in the HT

In addition, we investigated postprandial regulation of several genes encoding energy balance regulating neuropeptides in the HT. As before, we examined expression of genes after 1, 3, and 6 hr exposure to SD and HFD (Gao & Horvath, 2008). Food consumption induces changes in both SD- and HFD- fed mice. However, hypothalamic gene expression levels of orexigenic peptides MCH, AgRP, and ORX

(Figure 3c,d,f) and anorexigenic peptide CART (Figure 3e) were higher in HFD-fed mice compared to SD-fed mice while NPY levels remained equivalent in the two groups (Figure 3a). In contrast, a decrease in anorexigenic peptide POMC gene expression, was observed at 1 hr only in HFD-fed mice (Figure 3b). Interestingly, the time course of those changes was very specific for each diet. For example, SD-fed mice presented a transient and modest increase in MCH gene expression after 3 hr of food exposure compared to basal level (Figure 3c) while it increased within the first hour of food exposure in HFD-fed mice and remained high for 6 hr (Figure 3c). AgRP and ORX gene expression levels increased within the first hours of food exposure in both diet groups, with an exacerbated response in HFD-fed mice that lasted for 6 hr (Figure 3d,f), unlike the increase in CART gene expression, which did not occur until 3 hr of food exposure and was only observed in HFD-fed mice (Figure 3e). Taken together, our results show that short-term HFD exposure is

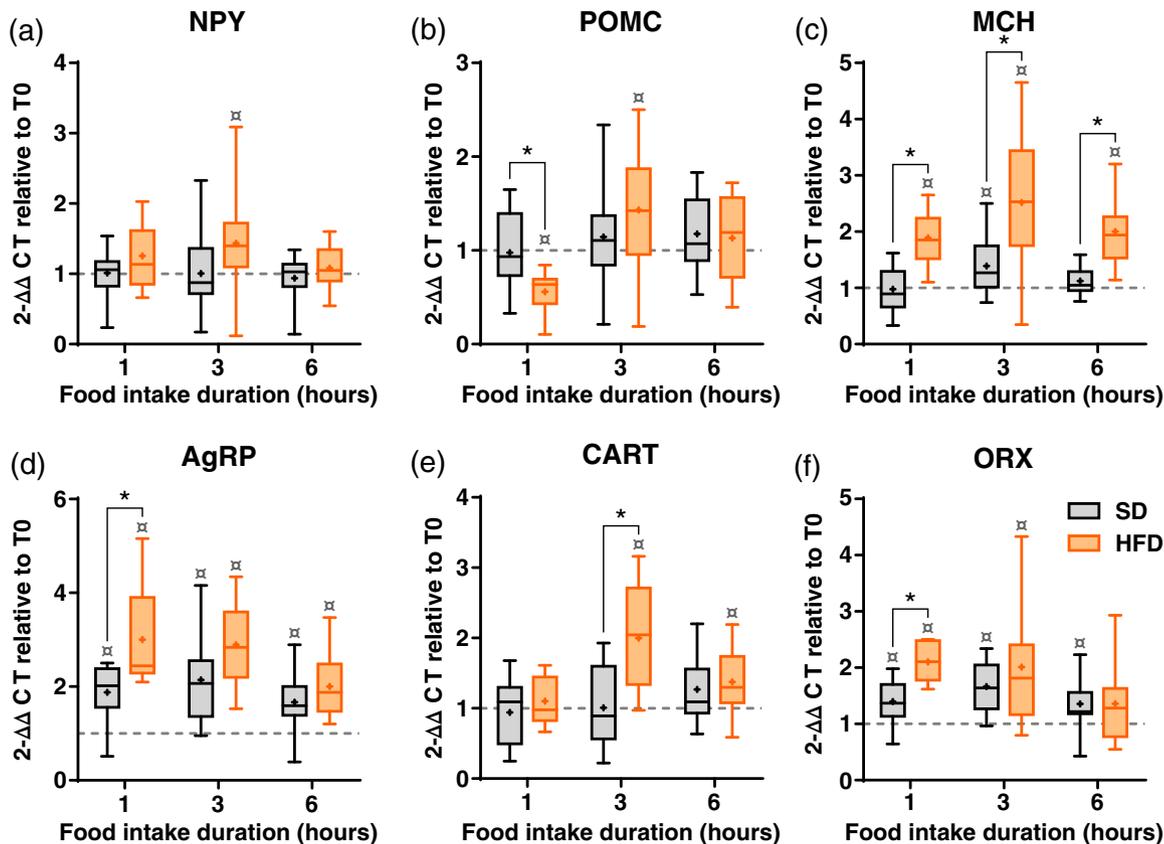


FIGURE 3 Short-term high-fat diet (HFD) exposure induces a specific acute neuropeptide gene expression profile in the hypothalamus (HT). Quantification of mRNA encoding orexigenic neuropeptides (a) neuropeptide Y (NPY), (d) agouti-related peptide (AgRP), (c) melanin-concentrating hormone (MCH), (f) OREXine (ORX) and anorexigenic neuropeptides (b) Pro-Opiomelanocortin (POMC), (e) cocaine- and amphetamine-regulated transcript (CART) in HT of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either standard diet (SD) or HFD. All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta CT$ method and presented as fold change relative to baseline prior to diet exposure (T0, dashed line = initial level). * $p < .05$ Mann-Whitney test or unpaired t -test SD versus HFD; $\#p < .05$ Wilcoxon signed-rank test SD/HFD versus 1; box extends from the 25th to 75th percentiles; line in the box is the median; "+" at the mean; whiskers go down to the smallest value and up to the largest. $N = 11-14$ [Color figure can be viewed at wileyonlinelibrary.com]

sufficient to specifically modulate hypothalamic neuropeptide gene expression involved in energy balance regulation, differently from what is observed in SD-fed mice.

3.4 | A single high-fat meal induces an acute increase of GFAP expression in the HT

Hypothalamic inflammation after medium-term (1–10 days) and long-term (2–32 weeks) HFD exposure is associated with astrogliosis (Balland & Cowley, 2017; Baufeld et al., 2016; Buckman et al., 2015; Thaler et al., 2012). It has yet to be determined whether the same is true at earlier time points. Given that our results showed an exacerbated hypothalamic inflammatory-like gene response in HFD-fed

mice, we tested whether short-term exposure (within 1, 3, and 6 hr) to HFD might affect astrocytes in the HT. To this end, we used the astrocyte marker GFAP for genetic and histological analysis. One hour of food exposure increased GFAP gene expression in the HT of HFD-fed mice but not in SD-fed mice (Figure 4a). Moreover, the increase in GFAP gene expression appeared to be transient as expression levels returned to baseline at 3 and 6 hr (Figure 4a). We then tested if the increase in GFAP gene expression was associated with changes in astrocyte remodeling in the ARC, as described previously for medium-term and long-term HFD exposure (Balland & Cowley, 2017; Buckman et al., 2015; Thaler et al., 2012). SD and HFD-fed mice presented similar GFAP staining in the ARC, as shown in the photomicrographs displayed in Figure 4c and corroborated by fluorescence quantification in the ARC of mice fed for 1, 3, and 6 hr (Figure 4b). Moreover, the

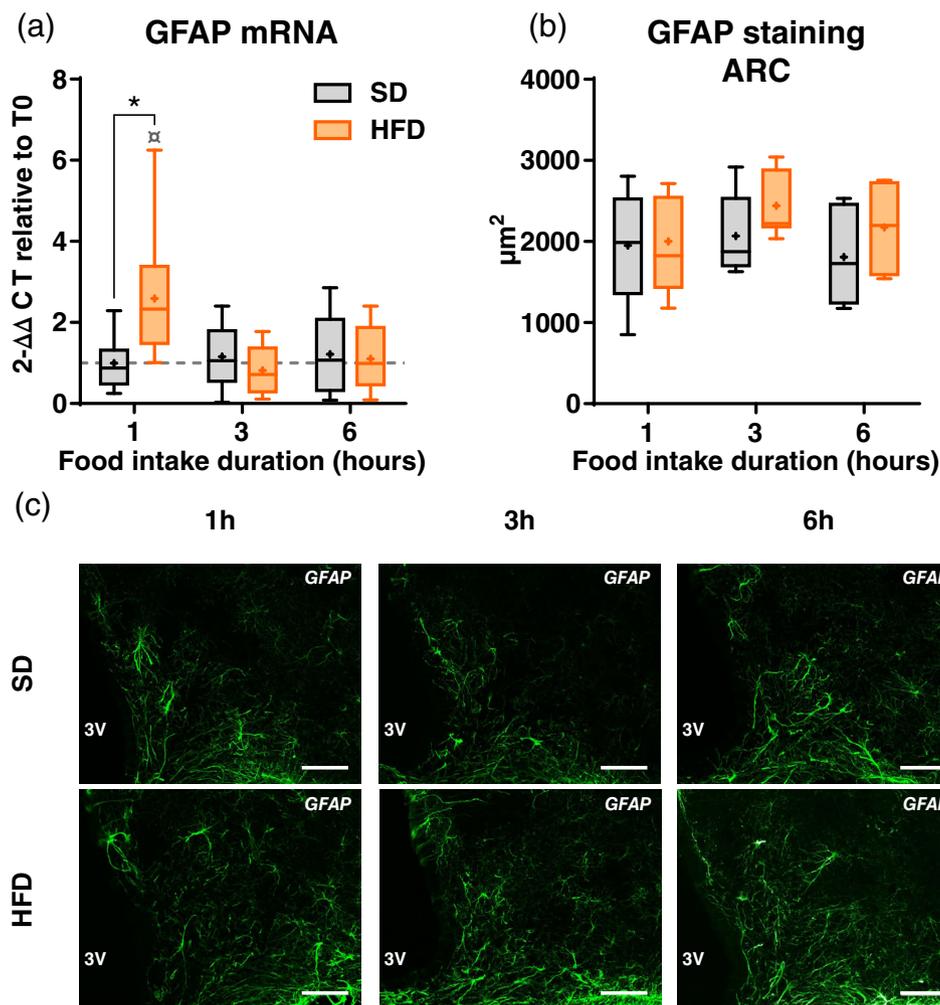


FIGURE 4 Short-term high-fat diet (HFD) exposure induces an acute increase of glial fibrillary acidic protein (GFAP) gene expression in the hypothalamus (HT). (a) Quantification of mRNA encoding the astrocyte marker GFAP in HT of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either standard diet (SD) or HFD $N = 11-14$. (b) Quantification of immunohistochemical detection of GFAP protein (stained area, μm^2) in coronal sections of the arcuate nucleus of HT (ARC; $30 \mu\text{m}$, -1.70 mm relative to Bregma) from mice fed for 1, 3, and 6 hr with either SD or HFD $N = 5-7$. (c) Immunohistochemical detection of GFAP protein in ARC of mice fed for 1, 3, and 6 hr with either SD or HFD. All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta\text{CT}$ method and presented as fold-change relative to baseline prior to diet exposure (in A, T0, dashed line = initial level). Scale bar in (c): $50 \mu\text{m}$, 3 V in (c): third ventricle $*p < .05$ Mann-Whitney test or unpaired t -test SD versus HFD; $\#p < .05$ Wilcoxon signed-rank test SD/HFD versus 1; box extends from the 25th to 75th percentiles; line in the box is the median; “+” at the mean; whiskers go down to the smallest value and up to the largest [Color figure can be viewed at wileyonlinelibrary.com]

activation state of astrocytes, assessed using the graded scoring system described in Harrison et al. (2019), was not different between SD and HFD-fed mice (Supplementary Figure S1c). These findings demonstrate that short-term HFD exposure, specifically and transiently, upregulates hypothalamic GFAP gene expression.

3.5 | A single high-fat meal induces acute microglial remodeling in the ARC of the HT

In addition to astrogliosis, hypothalamic inflammation after medium-term (3–7 days) and long-term (2–20 weeks) HFD exposure is also associated with microgliosis (Andre et al., 2017; Baufeld et al., 2016; Dorfman et al., 2017; Gao et al., 2014; Gao, Bielohuby, et al., 2017; Thaler et al., 2012; Valdearcos et al., 2014; Valdearcos et al., 2017). As for astrocytes, we tested whether short-term exposure (1, 3, and 6 hr) to HFD might affect microglial cells in the HT. HFD exposure increased microglial marker Iba1 gene expression in the HT at 3 and 6 hr compared to SD-fed mice (Figure 5a). We then tested whether Iba1 gene upregulation impacted Iba1 protein levels in the ARC. No change in Iba1 immunofluorescence staining was observed (Figure 5b, d). We also considered whether Iba1 gene expression modification might be associated with a change in microglial activity. The activation state of the microglia, assessed using the graded scoring system described in Harrison et al. (2019), was not different between SD and HFD-fed mice (Supplementary Figure S1d). Furthermore, we examined cell morphology. While the histological approach may have had insufficient resolution to detect changes in soma size after 3 hr of HFD exposure (Figure 5c,d), we did observe an increase in soma size in 6 hr HFD-fed mice (Figure 5c). Membrane properties of microglial cells were finally determined in using patch clamp recording, in 3 hr SD- and 3 hr HFD-fed mice. We did not observe changes in the current–voltage relationship (Figure 5e), but a significant increase in the cell capacitance in HFD-fed mice (Figure 5f), confirming changes in cell morphology upon HFD. Collectively, these results indicate that short-term HFD exposure induces fast microglial cells remodeling in the ARC.

3.6 | Modulation of GFAP-positive cells changes postprandial hypothalamic neuropeptide and inflammatory-like gene responses to SD and HFD exposure

We observed an acute and diet-specific hypothalamic gene response to food exposure for neuropeptides involved in energy balance regulation and pro-inflammatory markers. This response is associated with an increase in GFAP mRNA levels and microglial remodeling in the HT. We subsequently tested whether modulation of GFAP-positive cells and microglial activities could change the postprandial hypothalamic gene response. In order to modulate GFAP-positive cell activity we used designer receptors exclusively activated by designer drugs (DREADD) technology (Chen et al., 2016; Sweeney,

Qi, Xu, & Yang, 2016; Yang, Qi, & Yang, 2015). An AAV8 with a GFAP promoter controlling DREADD Gi coupled with mCitrine gene sequence expression in the MBH of mice was used to target GFAP-positive cells. Analysis of mCitrine staining by immunohistochemistry confirmed successful injection (Supplementary Figure S2a–c). Two weeks after virus injection, we treated mice with the specific agonist CNO at a dose of 1 mg/kg to activate the DREADD Gi and 30 min later we exposed the mice to SD or HFD for 1 hr. CNO injection did not change 1 hr food consumption in both SD- and HFD-treated mice (Supplementary Figure S2d,e). Using this tool, we observed differential inflammatory-like and neuropeptide gene responses to SD and HFD in vehicle (NaCl) group (Figure 6) compared to our initial observation (Figures 2 and 3). This likely relies on different basal inflammatory states between animals. Nevertheless, pharmacogenetic manipulation altered postprandial transcriptional response in the HT. Precisely, CNO injection did not alter POMC, MCH, and ORX gene expression response to SD and HFD exposure (Figure 6b, c,f). In contrast, CNO injection decreased AgRP and CART hypothalamic gene expression regardless of diet (Figure 6d,e). Only NPY gene expression in response to food exposure differed due to the activation of DREADD Gi in GFAP-positive cells in a diet-specific manner (Figure 6a). Interestingly, CNO injection decreased IL-1 β , TNF α , CCL2, CCL5, and Iba1 gene expression in the HT regardless of diet (Figure 6g,i,j,k,l). Finally, CNO injection decreased IL-6 gene expression specifically after 1 hr of HFD (Figure 6h). Collectively, these results show that modulation of GFAP-positive cell activity in the MBH alters hypothalamic neuropeptide and inflammatory-like gene responses to both SD and HFD exposure. Interestingly, our results also demonstrate that specific NPY and IL-6 gene responses to food exposure are altered by the modulation of GFAP-positive cells activity in a diet-dependent manner.

3.7 | Modulation of microglial activity changes postprandial hypothalamic neuropeptide and inflammatory-like gene responses to SD and HFD exposure

To assess whether microglia is involved in the neuropeptide and inflammatory gene responses induced by food exposure, we used PLX5622, which has previously been used to remove microglia from brain (Elmore et al., 2014). PLX5622 is a CSF1R inhibitor known to regulate microglial density in the brain. Two weeks after PLX5622 treatment, we assessed its efficiency to deplete microglia. PLX5622 almost completely ablated the microglial population as shown by the absence of Iba1 staining in the MBH (Supplementary Figure S3b,c) and the increase of Δ CT for Iba1 (Supplementary Figure S6d) in the PLX5622-treated group compared to the nontreated group. We exposed PLX5622-treated and nontreated mice to SD or HFD for 1 or 3 hr. Using this tool, we observed differential inflammatory-like and neuropeptide gene responses to SD and HFD in the nontreated group (Figure 7) compared to our original observation (Figures 2 and 3). This may be due to AIN76A diet exposure, which might affect

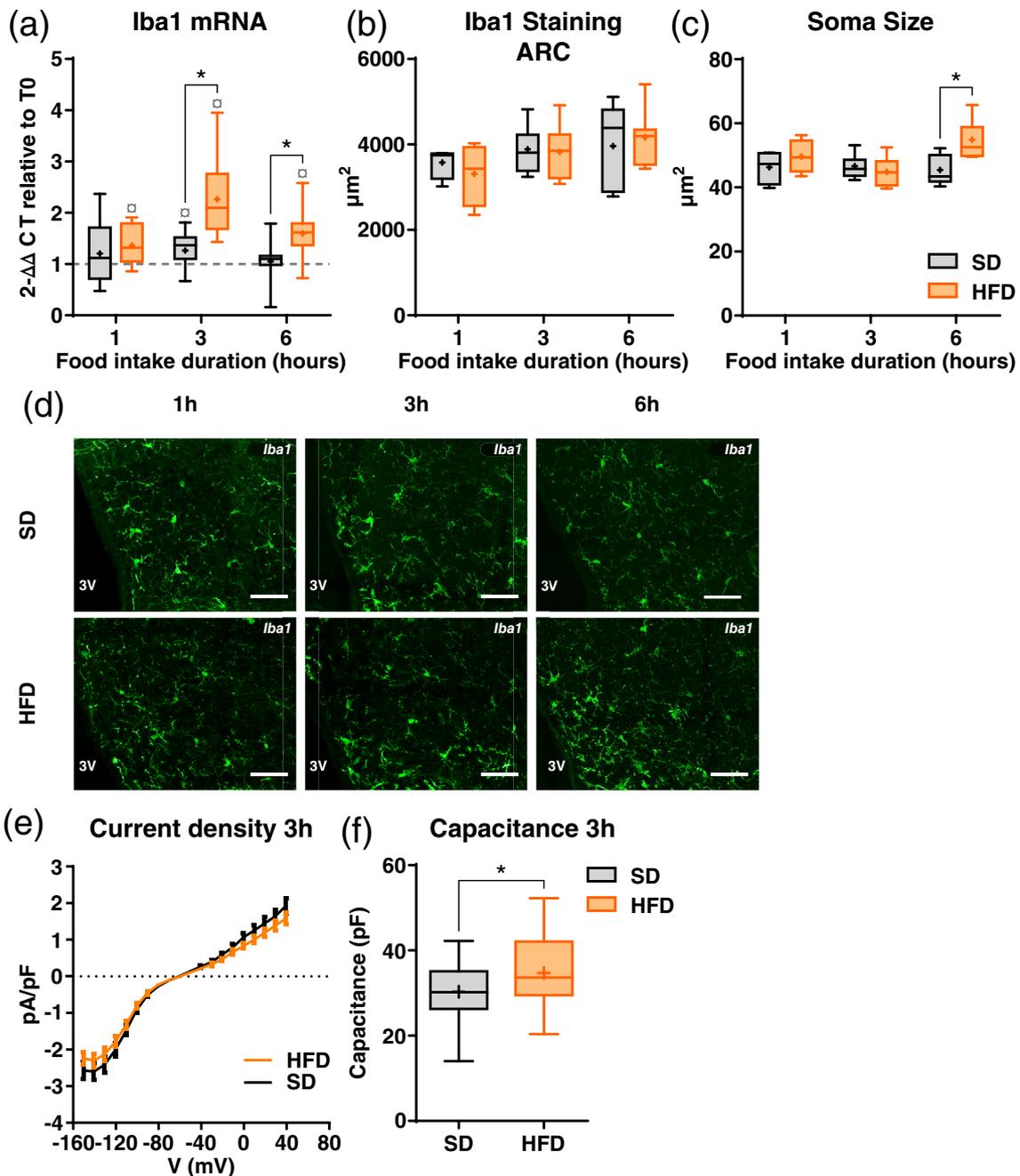


FIGURE 5 Short-term high-fat diet (HFD) exposure induces acute microglial remodeling in the arcuate nucleus of the hypothalamus (HT). (a) Quantification of mRNA encoding the microglial marker *Iba1* in the HT of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either standard diet (SD) or HFD $N = 11-14$. (b) Quantification of immunohistochemical detection of *Iba1* protein (stained area, μm^2) in coronal sections of the arcuate nucleus of the HT (ARC) ($30 \mu\text{m}$, -1.70 mm relative to Bregma) from 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either SD or HFD $N = 5-7$. (c) Quantification of average microglial soma size in ARC of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either SD or HFD $N = 5-7$, $n = 8-21$. (d) Immunohistochemical detection of *Iba1* protein in ARC of 8-week-old C57Bl/6J male mice fed for 1, 3, and 6 hr with either SD or HFD. (e) Electrophysiological recording of current-voltage relationships and (f) membrane capacitance of microglia cells whole-cell recorded in ARC of 9–10 week-old CX3CR1eGFP/eGFP male mice fed for 3 hr with either SD ($N = 3$, $n = 30$) or HFD ($N = 4$, $n = 26$). All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta\text{CT}$ method and presented as fold-change relative to baseline prior to diet exposure (in (a), T0, dashed line = initial level). Scale bar in (d): $50 \mu\text{m}$, 3V in (d): third ventricle. * $p < .05$ Mann-Whitney test or unpaired t -test SD versus HFD; $\#p < .05$ Wilcoxon signed-rank test SD/HFD versus 1; box extends from the 25th to 75th percentiles; line in the box is the median; “+” at the mean; whiskers go down to the smallest value and up to the largest [Color figure can be viewed at wileyonlinelibrary.com]

inflammatory status as explained in the discussion but does not undermine our results. Body weight, SD or HFD intake (Supplementary Figure S3a,d,e), and hypothalamic GFAP gene

expression (Supplementary Figure S3f) were not modified by PLX5622-induced microglial depletion. Moreover, PLX5622 treatment did not affect the hypothalamic neuropeptide NPY, CART, MCH, and

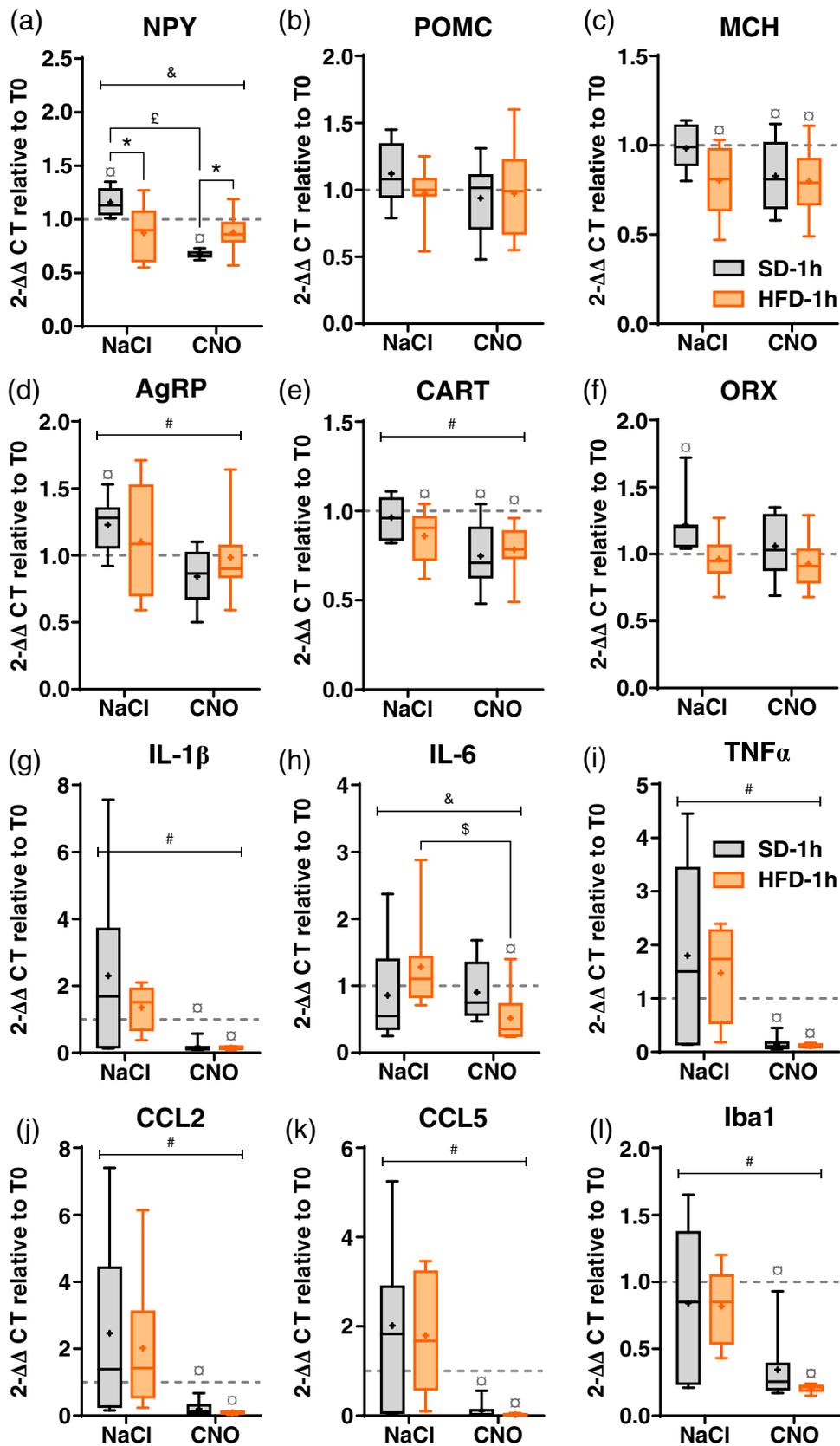


FIGURE 6 Legend on next page.

ORX gene response induced by 1 hr of food exposure (Figure 7a,e,c,f). However, POMC and AgRP gene expressions seem to be slightly increased by the PLX5622 treatment regardless of diet (Figure 7b,d). After 3 hr of food exposure, PLX5622 treatment increased NPY, AgRP, POMC, and CART gene expression regardless the diet (Figure 7a,b,d,e). Interestingly, PLX5622 treatment changed MCH and ORX gene expression response to 3 hr food exposure in a diet-dependent manner (Figure 7c,f).

With respect to the impact of microglia depletion on inflammatory gene responses, PLX5622 treatment affected IL-1 β , IL-6, and TNF α mRNA levels in response to 1 hr of food exposure in a diet-dependent manner (Figure 7g,h,i) while CCL2 and CCL5 were not affected by the treatment (Figure 7j,k). Moreover, IL-6 and CCL2 mRNA levels tended to be higher after 3 hr of feeding regardless of the diet (Figure 7h,j) while TNF α mRNA levels seemed lower (Figure 7i) in PLX5622-treated groups. PLX5622 treatment did not affect IL-1 β or CCL5 gene expression in 3 hr SD and HFD-fed mice (Figure 7g,k).

Therefore, the results show that microglia depletion affects hypothalamic neuropeptide and inflammatory-like gene responses to both SD and HFD exposure. More interestingly, our results also demonstrate that specific MCH, ORX, IL-1 β , IL-6, and TNF α gene responses to food exposure are affected by the absence of microglial cells in a diet-dependent manner.

4 | DISCUSSION

Several studies have shown a link between HFD consumption, inflammation, and risk factors associated with obesity. It seems certain that long-term exposure to HFD induces chronic low-grade systemic inflammation, which is responsible for the development of insulin resistance, Type 2 diabetes mellitus along with other chronic illnesses such as cardiovascular disease or cancer (Cani et al., 2007; Emerson et al., 2017; Ghanim et al., 2009; Herieka & Erridge, 2014; Kelly et al., 2012; Lumeng & Saltiel, 2011; Thaler & Schwartz, 2010). More recently, hypothalamic inflammation, a process involving neurons, astrocytes and microglia, has been correlated with obesity and chronic HFD consumption (Balland & Cowley, 2017; Baufeld et al., 2016;

Buckman et al., 2015; Douglass et al., 2017; Gao et al., 2014; Gao, Layritz, et al., 2017; Gao, Vidal-Itriago, et al., 2017; Guillemot-Legris et al., 2016; Kim et al., 2019; MacDonald, Holmes, Beall, Pickering, & Ellacott, 2020; Milanova et al., 2019; Thaler et al., 2012; Thaler & Schwartz, 2010; Valdearcos et al., 2014; Valdearcos et al., 2017), underlining the effect of chronic exposure to HFD on the central inflammatory status. Interestingly, brain inflammatory responses in the HT were also observable after only 1 day of HFD consumption (Buckman et al., 2015) or 24 hr of lipid perfusion to the brain (Dalvi et al., 2017), long before the development of obesity. Moreover, inhibition of brain inflammatory responses to HFD leads to energy balance dysregulation (Buckman et al., 2015; Fernandez-Gayol et al., 2019). In humans, studies have described acute postprandial inflammation associated with increased levels of circulating inflammatory markers in response to HFD (Emerson et al., 2017; Herieka & Erridge, 2014). Altogether, these data suggest that HFD-induced inflammation is a physiological response to cope with excess lipids, which could occur at the peripheral or central levels.

In our study, the calories from fat and sugar in HFD were equivalent (40% each), in contrast to numerous studies where fat content accounts for 60% kilocalories (kcal) while carbohydrate content was only 20% kcal. Therefore, the increase in caloric proportion represented by the lipids is more realistic in our model, and comparable to the typical human diet, where the occurrence of lipids in the presence of elevated carbohydrate content contributes to brain inflammation (Andre et al., 2017; Gao, Bielohuby, et al., 2017; Kim et al., 2019).

According to previous studies about systemic postprandial inflammation, HFD consumption promotes LPS trafficking over the intestinal epithelium through transcellular and paracellular transport (Ghanim et al., 2009; Herieka & Erridge, 2014; Kelly et al., 2012). In our study, according to investigated time points, biochemical analysis of serum and molecular measurements in the HT suggest that postprandial inflammation appears very early in the HT and might precede the rise in periphery. Above all, our study established, for the first time, the existence of a specific postprandial hypothalamic inflammation response to both SD and HFD exposure which is closely associated with a modulation of mRNA levels of neuropeptides involved in the regulation of energy balance (Gao & Horvath, 2008).

FIGURE 6 Modulation of glial fibrillary acidic protein (GFAP)-positive cells changes postprandial hypothalamic neuropeptide and inflammatory-like gene responses to 1 hr standard diet (SD) or high-fat diet (HFD) exposure. Quantification of mRNA encoding orexigenic neuropeptides (a) neuropeptide Y (NPY), (d) agouti-related peptide (AgRP), (c) melanin-concentrating hormone (MCH), (f) ORXine (ORX), anorexigenic neuropeptides (b) Pro-Opiomelanocortin (POMC), (e) cocaine- and amphetamine-regulated transcript (CART), pro-inflammatory cytokines, (g) interleukin-1 beta (IL-1 β), (h) interleukin 6 (IL-6), (i) tumor necrosis factor alpha (TNF- α), chemokines (j) CCL2, (k) CCL5, and (l) microglial marker Iba1 in hypothalamus (HT) of mice fed for 1 hr with either SD or HFD. Two weeks prior to the experiment, 8-week-old C57Bl/6J male mice were injected with the adeno associated virus 8 (AAV8)-GFAP-designer receptors exclusively activated by designer drugs (DREADD)-Gi virus in the mediobasal HT. To specifically modulate GFAP-positive cell activity, a selective DREADD-Gi receptor agonist, clozapine-N-oxide (CNO), was injected intraperitoneally 30 min before food delivery (CNO). Control mice were injected with vehicle solution (NaCl). All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta$ CT method and presented as fold-change relative to NaCl injected-condition prior to diet exposure (T0, dashed line = initial level). Interaction and treatment effects with multiple comparisons were carried out using two-way analysis of variance (ANOVA): $\&p \leq .05$ for Interaction effect (diet SD-HFD \times treatment NaCl-CNO), $\#p \leq .05$ for treatment effect (NaCl-CNO), $*p \leq .05$ SD versus HFD, $\pounds p \leq .05$ NaCl-SD versus CNO-SD, $\$p \leq .05$ NaCl-HFD versus CNO-HFD. Box extends from the 25th to 75th percentiles; line in the box is the median; "+" at the mean; whiskers go down to the smallest value and up to the largest. $N = 7-10$ [Color figure can be viewed at wileyonlinelibrary.com]

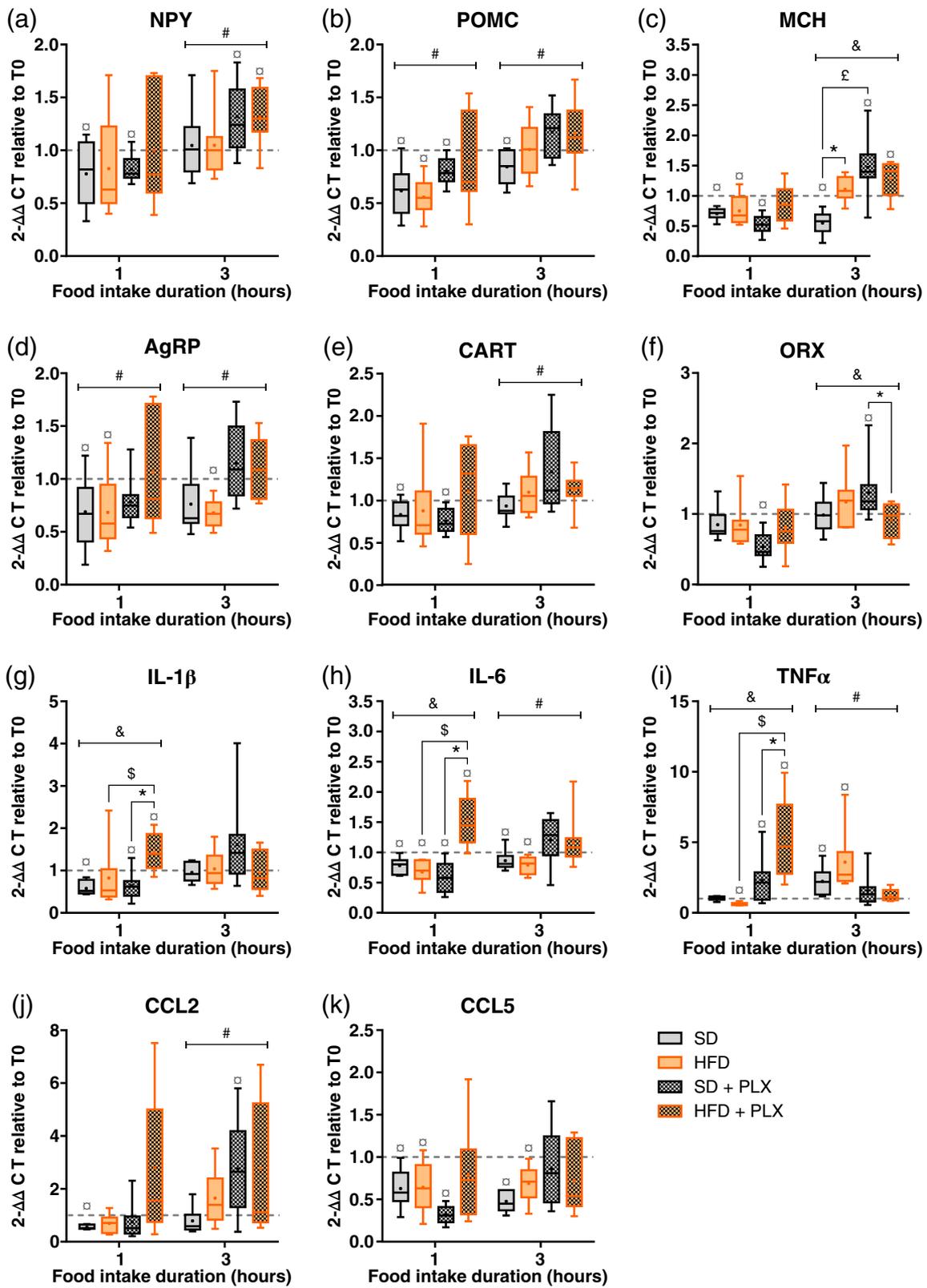


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In this study, we focused only on males. Other analyses performed in females revealed that the inflammatory response in the HT is weaker in females exposed to HFD compared to mice fed SD (Supplementary Figure S4). Furthermore, the neuropeptide gene response in females was not as marked as in males (Supplementary Figure S5). Although the origin of the sex specificity remains to be elucidated (Dorfman et al., 2017), this result suggests a coordinated regulation between cytokines and neuropeptides. However, in males, while both SD and HFD consumption increased hypothalamic gene expression of the orexigenic neuropeptides AgRP and ORX, as well as inflammatory markers, IL-1 β and TNF α , within the first hours of diet exposure, the amplitude of the response was higher in HFD-fed mice. Moreover, compared to SD exposure in the same time frame, 1 hr exposure to HFD induced selective expression of MCH orexigenic neuropeptide, as well as pro-inflammatory cytokines, CCL2 and CCL5. The sustained expression of pro-inflammatory cytokines IL-1 β , IL-6, TNF α , chemokine CCL2, and the orexigenic neuropeptide MCH was still observable after 3 hr of food exposure in HFD-fed mice. Finally, after 6 hr of food exposure, the differences observed between SD- and HFD-treated mice tended to disappear, except for the MCH orexigenic neuropeptide mRNA levels, which remained higher in the HFD group. This suggests that short-term exposure to a lipid-rich diet triggers hypothalamic gene expression of orexigenic neuropeptides leading to a behavioral response correlated to increased kilocalorie intake observed 6 hr post HFD exposure (Gao & Horvath, 2008). It is not yet known how these genetic changes occur in orexigenic neurons, although we believe that they may be the consequence of lipid-induced inflammation. We have yet to define the role of the inflammatory brain cells in this response over such a short time frame in this study. Nevertheless, a recent study demonstrated that 24 hr of lipid perfusion to the brain is sufficient to activate an inflammatory response in NPY/AgRP neurons through TNF α and ER stress signaling (Dalvi et al., 2017). We could therefore expect such a mechanism to appear even earlier in response to HFD to modulate the neuropeptide expression involved in energy balance regulation, as we observed in our study.

As previously described, HFD-induced hypothalamic inflammation is associated with glial reactivity (Balland & Cowley, 2017; Baufeld et al., 2016; Buckman et al., 2015; Douglass et al., 2017; Gao et al., 2014; Guillemot-Legris et al., 2016; Kim et al., 2019; MacDonald

et al., 2020; Milanova et al., 2019; Thaler et al., 2012; Valdearcos et al., 2014; Valdearcos et al., 2017; Waise et al., 2015). First, we focused on GFAP-positive cells. Targeting of cell activity decreases the postprandial pro-inflammatory cytokines IL-1 β , IL-6, TNF α , and the chemokines CCL2 and CCL5 gene response to both SD and HFD exposure. Interestingly AAV8-DREADD-Gi-mCitrine injection exclusively in MBH is powerful enough to downregulate pro-inflammatory marker mRNA levels measured in the whole HT, suggesting that MBH GFAP-positive cells play a critical role in hypothalamic postprandial inflammation. Moreover, we provided novel evidence that acute exposure to HFD specifically increases GFAP mRNA levels in HT within the first hours of food consumption. This mechanism might contribute to the postprandial astrocytic remodeling that happens after a balanced meal (Nuzzaci et al., 2020). Another observation worth highlighting is that the IL-6 gene response to food exposure is modulated by GFAP-positive cells in a differential way, depending on the diet. In fact, modulation of GFAP-positive cellular activity decreases IL-6 mRNA hypothalamic levels only in HFD-treated mice. This is consistent with recent discoveries demonstrating that IL-6 produced by astrocytes is a major actor in central energy balance regulation and plays a protective role against obesity development during HFD exposure (Fernandez-Gayol et al., 2019; Quintana et al., 2013; Timper et al., 2017). On the other hand, modulation of GFAP-positive cellular activity does not dramatically affect hypothalamic neuropeptide mRNA levels and does not change HFD intake. This is consistent with previously published results and indicates that modulation of NPY/AgRP neuron activity by GFAP-positive cells in response to HFD occurs over a different time frame from our study (Chen et al., 2016; Yang et al., 2015).

Because glial reactivity to HFD consumption is also characterized by microglial reactivity, we then focused on microglia. In accordance with previous studies, we did not observe any effect on HFD intake when microglia was depleted (Waise et al., 2015). We found that 3 hr of HFD exposure increases hypothalamic mRNA levels of the microglial marker Iba1 compared to SD-fed mice. This rise was associated with soma size enlargement of microglial cells in the ARC of HT only in HFD-treated mice. For microglial cells, the association of a specific activity state with morphological aspects remains controversial (Kettenmann, Hanisch, Noda, & Verkhratsky, 2011; Tremblay et al.,

FIGURE 7 Modulation of microglial activity changes postprandial hypothalamic neuropeptide and inflammatory-like gene responses to 3 hr standard diet (SD) or high-fat diet (HFD) exposure. Quantification of mRNA encoding orexigenic neuropeptides (a) neuropeptide Y (NPY), (d) agouti-related peptide (AgRP), (c) melanin-concentrating hormone (MCH), (f) ORExine (ORX), anorexigenic neuropeptides (b) Pro-Opiomelanocortin (POMC), (e) cocaine- and amphetamine-regulated transcript (CART), pro-inflammatory cytokines, (g) interleukin-1 beta (IL-1 β), (h) interleukin 6 (IL-6), (i) tumor necrosis factor alpha (TNF- α), chemokines (j) CCL2, and (k) CCL5 in hypothalamus of mice fed for 1 hr and 3 hr with either SD or HFD. Two weeks prior to the experiment, 8-week-old C57Bl/6J male mice were treated with the colony stimulating factor 1 receptor (CSF1R) inhibitor, PLX5622, formulated in the AIN76A food to remove microglia in the brain (SD + PLX and HFD + PLX). Control mice received AIN76A food without PLX5622 for 2 weeks prior to the experiment (SD and HFD). All mRNA levels were quantified relative to GAPDH housekeeping gene expression by the $\Delta\Delta$ CT method and presented as fold-change relative to AIN76A-fed \pm PLX5622 mice baseline prior to SD or HFD diet exposure (T0, dashed line = initial level). Interaction and treatment effects with multiple comparisons were carried out using two-way analysis of variance (ANOVA): &#p < .05 for Interaction effect (diet SD-HFD \times treatment AIN76A \pm PLX5622), #p \leq .05 for treatment effect (AIN76A \pm PLX5622), *p \leq .05 SD versus HFD, \pounds p \leq .05 SD versus SD + PLX, $\$$ p \leq .05 HFD versus HFD + PLX. Box extends from the 25th to 75th percentiles; line in the box is the median; "+" at the mean; whiskers go down to the smallest value and up to the largest. N = 7–9 [Color figure can be viewed at wileyonlinelibrary.com]

2011). However, a change in microglia state initiated by HFD consumption is clearly indicated by their soma size enlargement and the modification of Iba1 gene expression. Such postprandial microglial plasticity might enable protective mechanisms to deal with lipid excess or to adjust future food intake. Similar to the astrocytic response, the postprandial microglial plasticity induced by dietary fat appears within the first hours on HFD. Moreover, the pharmacological approach clearly shows that microglial cells participate in the postprandial hypothalamic inflammation induced by both SD and HFD, though it is too early to state to what extent and to pinpoint the diet-dependent effects. Nevertheless, microglial depletion seems to exacerbate the hypothalamic inflammatory-like response to 1 hr HFD exposure. Moreover, our experiments showed that microglial depletion altered the postprandial transcriptional response for neuropeptides involved in energy balance regulation. Not only did it increase NPY, AgRP, POMC, and CART hypothalamic mRNA levels in both SD- and HFD-treated mice, but it also affected MCH and ORX gene responses according to the nature of ingested diet, suggesting that postprandial microglial responses could specifically act on neurons involved in energy balance regulation in a diet-dependent manner. Despite the destruction of microglia cells that is induced by PLX5622 treatment, we still observed cytokine and chemokine gene expression and regulation. This result leads us to question the identity of cells hosting these regulations. In the literature, the source of production of these molecules is still debated because of the involvement of several cell types including microglia, astrocytes, oligodendroglia, neurons, cerebrovascular cells, and circulating immune cells (Pokorna Formanova et al., 2019; Ramesh, MacLean, & Philipp, 2013). The decrease of cytokine gene expression after targeted pharmacogenetic manipulation of GFAP-positive cells makes us assume that astrocytes play an important role in the postprandial upregulation of cytokines expression in the HT. However, due to complex cell interactions in the brain, it is possible that astrocytes are not directly the source of these cytokines.

Up until now, astrocytic or microglial reactive gliosis in the ARC of the HT in response to chronic HFD exposure and their participation in HFD-induced inflammatory processes and functional impact remains controversial and may depend on the nutritional lipid composition of each diet (Harrison et al., 2019; Kim et al., 2019; McLean et al., 2019; Valdearcos et al., 2014). Our study offers new insight into the impact of HFD consumption on astrocytic or microglial populations located in areas involved in energy balance regulation. Both cell types may be activated by short-term food exposure and orchestrate inflammatory-like processes in the ARC modulating neuronal function, but in differential time frames and in a diet-dependent manner. Indeed, during short-term HFD exposure, GFAP gene expression is acute and transient, whereas Iba1 gene expression and microglial reactivity occurs after 3 hr of diet exposure and lasts over 6 hr. This raises question about the nature of the signal from HFD that initiates the hypothalamic inflammatory response. According to the literature, the caloric difference per gram of food between SD and HFD could be perceived as a signal (Harrison et al., 2019). In addition, as described above, the increase in plasma endotoxin concentrations following ingestion of HFD could also act as a pro-inflammatory signal.

Finally, analysis of the composition of circulating TGs shows that the proportion of TG containing only saturated fatty acids (pTGO) as well as the proportion of TG enriched in monounsaturated fatty acids (pTG1), relative to the total amount of TG, increases within 6 hr following HFD ingestion compared to mice fed SD (Supplementary Figure S1e,f). This observation is consistent with previous studies showing that hypothalamic inflammation induced by saturated fatty acids requires microglial activation (Kim et al., 2019; Valdearcos et al., 2014). In order to determine whether a particular factor could be responsible for the exacerbated inflammatory response to HFD and the changes in hypothalamic neuropeptide expression, we analyzed the correlations between these different factors and the resulting gene expression of inflammatory markers and neuropeptide (Supplementary Table S2) (Jha et al., 2019). The amount of kilocalories (Kcal) seemed to impact the expression of certain genes; however, except for GFAP and IL-6, there is always an effect of the diet on top of this effect (Supplementary Table S2). Regarding LPS plasma concentration, we found that it is not sufficient to explain the variation in the gene expression of inflammatory markers and neuropeptides. However, the positive interaction between LPS and diet on the expression of IL-1 β , IL-6, and CCL2 demonstrates that LPS effects are diet dependent. Total TG plasma concentration was not associated with a specific effect on gene expression. On the other hand, the nature of the TG may have a slightly greater impact. Indeed, changes in pTGO and pTG1 can explain the variations in MCH and CART gene expression, respectively (Supplementary Table S2). To conclude, gene expression of the astrocyte marker GFAP in response to HFD may depend only on a diet's caloric value, as already described in the literature (Harrison et al., 2019). LPS alone is not sufficient to cause the exacerbated response to HFD while TG quality seems to play a specific role in the gene response for MCH and CART. It is thus difficult to conclude on the specific role of each factor. Additional "design of experiments" plans are required to explain how each factor influences these variations. Nevertheless, our results indicate that dietary fat exacerbates postprandial hypothalamic inflammation via different signals that remain to be discovered.

These original findings have certain limitations. While we focused on the HT because it is a well-known area involved in energy balance regulation (Gao & Horvath, 2008), we do not exclude the possibility that other brain areas may also display postprandial inflammation associated with neuropeptide modulation. Indeed, several studies have shown that chronic HFD consumption leads to hippocampal inflammatory responses and affects behavioral disorders associated with obesity (Abbink, van Deijk, Heine, Verheijen, & Korosi, 2019; Guillemot-Legris et al., 2016; Gziel, Kielbinski et al. 2017; Spencer, Basri et al. 2019; Tsai, Wu et al. 2018; Waise et al., 2015). Moreover, astrocytes in the brainstem dorsal vagal complex responds to acute nutritional excess (MacDonald et al., 2020). In addition, the methodology we used was not designed for a detailed analysis of regional changes that might occur in the functionally distinct nuclei of the HT. Finally, to gain insight on the involvement of each cell type, we used pharmacological and pharmacogenetic tools to manipulate activities of astrocytes and microglial cells, and some issues inherent to

these technics should be pointed out. DREADD technology has already been used to target GFAP-positive cells of the ARC glia in the regulation of neuronal subtype-specific modulation of energy homeostasis (Chen et al., 2016; Sweeney et al., 2016; Yang et al., 2015). Given this context, we initiated our study using DREADD Gi as this strategy was proven to achieve cellular inhibition—at least in neuronal cell types (Roth 2016). However, a 2019 study has demonstrated that Gi/o protein-coupled receptor may differentially affect brain cell type, inhibiting neurons and activating astrocytes stimulating gliotransmitter release (Durkee, Covelo et al. 2019). Thus, at this stage, while it seems difficult to definitely conclude whether GFAP-positive glial cell activation or inhibition mediates HFD-induced HT remodeling, our results clearly support GFAP-positive glial cell involvement in this process. In a recent work, we reported efficiency of this tool to alter cAMP levels in astrocytes (Nuzzaci et al., 2020). Thus, postprandial changes in cytokines expression involve cAMP signaling. Concerning microglial cells, although new tools using DREADD technology to target microglia have been recently reported (Grace, Wang et al. 2018), we chose PLX5622 as it was the only tool available at the time to selectively modulate microglia. PLX5622 has been shown to act mainly on microglia in adult whole brains (Elmore et al., 2014; Oosterhof, Kuil et al. 2018). Since this compound ablates all microglial cells, further studies are required to specify involvement of hypothalamic microglia in postprandial inflammation. Another important point is that, using those two approaches we observed differential inflammatory and neuropeptide gene responses in respective control groups for AAV8-DREADD-Gi-mCitrine or AIN76A diet experiments compared to our initial observation in naïve animals without any experimental treatment. This could reflect different basal inflammatory states in animals. Apparently, sham and nontreated animals differ from injected mice with viral particles or mice fed with AIN76A diet. While it is obvious that stereotaxic injection of virus has immunological implications that could affect brain inflammatory status, it is worth noting that 76.9% of carbohydrates contained in AIN76A are sucrose, known to play a critical role in diet-induced hypothalamic inflammation (Andre et al., 2017; Gao, Bielohuby, et al., 2017). In this work, WT mice, DREADD injected mice and mice exposed to AIN76A should be therefore considered as three independent conditions in which we analyzed hypothalamic postprandial inflammation and glial reactivity.

Overall, while the effect of medium-term and long-term nutritional lipid exposure has been extensively demonstrated to promote hypothalamic inflammation involving astrocytic and microglial cells (Andre et al., 2017; Bolland & Cowley, 2017; Baufeld et al., 2016; Douglass et al., 2017; Gao et al., 2014; Gao, Bielohuby, et al., 2017; Guillemot-Legris et al., 2016; Nadjar et al., 2017; Thaler et al., 2012; Valdearcos et al., 2014; Valdearcos et al., 2017), little was known about short-term exposure. Here we demonstrate that a moderate increase of nutritional lipid content in diet – mimicking current human food—can exacerbate hypothalamic postprandial inflammation in a short time and that this response involves hypothalamic GFAP-positive cells and microglial cells. Although this primary response is probably aimed at regulating energy balance, continual nutritional lipid excess may contribute to dysregulating those signals and contribute

to the hypothalamic disturbances leading to obesity (Thaler & Schwartz, 2010). Because the amplitude of systemic postprandial inflammation has been correlated with Type 2 diabetes and cardiovascular diseases (Emerson et al., 2017; Milan et al., 2017), our study leads us to believe that exacerbated hypothalamic postprandial inflammation might predispose individuals to obesity and needs to be characterized in order to address this worldwide crisis.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Céline Cansell, Jean-Louis Nahon, Nicolas Blondeau, and Carole Rovère conceived and supervised the study, designed experiments, and wrote the manuscript. Céline Cansell performed the majority of the experiments, interpreted results, and generated figures. Katharina Stobbe performed EIA multiplex assays. Clara Sanchez and Joris Leredde performed most of the quantitative PCR experiments and contributed to data analysis. Ophélie Le Thuc initiated immunohistochemistry experiments and quantitative PCR experiments with Cynthia Lebeaupin. Coralie-Anne Mosser performed and analyzed results from electrophysiology experiments. Selma Ben-Fradj performed food intake experiments. Nadège Devaux participated to management of mice lines. Delphine Debayle and Lucile Fleuriot performed TG measurements. Frédéric Brau participated to the quantification of astrocytes and microglia morphology changes. Etienne Audinat and Alexandre Benani contributed to data analysis and paper writing. All the authors discussed the results and/or reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data available within the article or its supplementary materials. The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

ORCID

Carole Rovère  <https://orcid.org/0000-0001-9400-7972>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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