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TLR4-dependent neuroinflammation mediates LPS-driven food-reward alterations during high-fat exposure

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Abstract

Background Obesity has become a global pandemic, marked by significant shifts in both the homeostatic and hedonic/reward aspects of food consumption. While the precise causes are still under investigation, recent studies have identified the role of gut microbes in dysregulating the reward system within the context of obesity. Unravelling these gut–brain connections is crucial for developing effective interventions against eating and metabolic disorders, particularly in the context of obesity. This study explores the causal role of LPS, as a key relay of microbiota component-induced neuroinflammation in the dysregulation of the reward system following exposure to high-fat diet (HFD).

Methods Through a series of behavioural paradigms related to food-reward events and the use of pharmacological agents targeting the dopamine circuit, we investigated the mechanisms associated with the development of reward dysregulation during HFD-feeding in male mice. A Toll-like receptor 4 (TLR4) full knockout model and intraventricular lipopolysaccharide (LPS) diffusion at low doses, which mimics the obesity-associated neuroinflammatory phenotype, were used to investigate the causal roles of gut microbiota-derived components in neuroinflammation and reward dysregulation.

Results Our study revealed that short term exposure to HFD (24 h) tended to affect food-seeking behaviour, and this effect became significant after 1 week of HFD. Moreover, we found that deletion of TLR4 induced a partial protection against HFD-induced neuroinflammation and reward dysregulation. Finally, chronic brain diffusion of LPS recapitulated, at least in part, HFD-induced molecular and behavioural dysfunctions within the reward system.

Conclusions These findings highlight a link between the neuroinflammatory processes triggered by the gut microbiota components LPS and the dysregulation of the reward system during HFD-induced obesity through the TLR4 pathway, thus paving the way for future therapeutic approaches.

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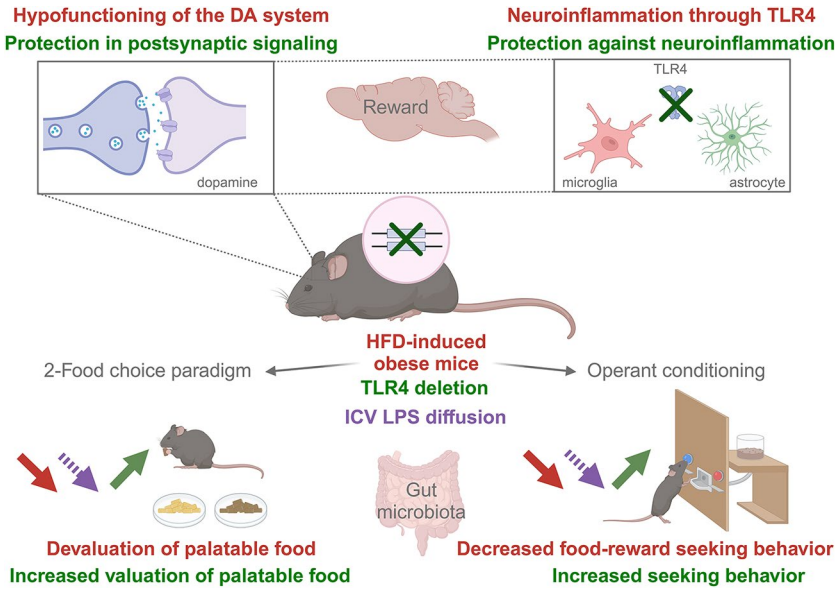
Graphical abstract



Keywords Food-reward, Obesity, Dopaminergic activity, Gut microbiota, Neuroinflammation, Lipopolysaccharides, Toll-like receptor 4, Dopamine receptor 2

Introduction

The increasing prevalence of obesity has become a significant challenge worldwide [1]. Excessive food intake, including palatable and high-calorie foods, is a prominent cause of obesity. Feeding behaviours are regulated by the hypothalamic–brainstem network, along with the hedonic aspects of food which mobilize the mesolimbic dopaminergic reward system [2]. Dopamine (DA), released by the neurons located in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc) and the dorsal striatum (DS) [3, 4] is a critical neural substrate for encoding the tropism towards palatable food and food-seeking behaviors [5, 6]. Initially, food-reward was crucial for ensuring the survival of our ancestors through the ingestion of calorie-dense foods. In modern food environment, highly palatable food consumption has been pointed out as primary culprit in reward-driven eating habits that surpasses the body's energy requirements [7]. Indeed, persistent overeating results in altered DA release, dopamine D1 and D2 receptors (DRD1 and DRD2) signalling, and elevated expression of the dopamine transporter (DAT), all leading to (mal)adaptive changes within the reward system [7–11]. Consequently, these DA-associated alterations lead to reduced hedonic responses to palatable food in both humans and rodents, resulting in compensatory overconsumption in humans [12–15]. Yet, the molecular components linking the consumption of high-fat foods and the (mal)adaptative changes occurring within dopaminergic/dopaminoceptive circuits are still elusive.

Neuroinflammatory processes and compulsive/addictive feeding are clear pathological features associated with obesity and in general with high-fat diet (HFD) consumption even independently from body weight gain [16]. Hypothalamic inflammation is evident in individuals with obesity or even after 24 h of a HFD [17, 18]. In both obese humans and rodents, this inflammation involves increased activation of inflammatory pathways in microglia and astrocytes, along with the disruption of the blood–brain barrier (BBB) [19, 20]. In obesity, inflammation impacts various brain regions and is positively correlated with cognitive impairments [21]. Recent findings have clearly indicated that obesity mediates neuroinflammatory events within the structures of the reward system [22–25], thus prompting us to investigate the mechanisms of neuroinflammation and its inflammatory mediators in the dysregulation of the reward system following exposure to obesogenic diet.

Over the past two decades, the gut microbiota has gained recognition as a pivotal regulator of the host metabolism, by influencing the hypothalamic regulation of food intake through the gut–brain axis [26–28]. However, recent studies support the role of gut microbes also in influencing the hedonic aspects of food intake [29–33]. Their causal role in obesity-related dysregulation of the food-reward system has been clearly demonstrated

through faecal material transplantation from obese mice [34, 35]. Moreover, the effects of specific bacteria have also been recently highlighted in food addiction, a controversial concept characterized by a loss of control over food intake that may promote obesity and which is associated with the dysregulation of the reward system, supporting the role of gut microbes in the regulation of food-reward events [36]. However, the mechanisms linking gut microbes to food-reward dysregulations remain unknown. In obesity, changes in the composition of the gut microbiota are associated with increased gut permeability, and high circulating levels of bacterial components such as lipopolysaccharides (LPS), which leads to metabolic endotoxaemia [37, 38]. This triggers metabolic alterations and inflammation in several organs, including the brain, through the activation of the toll-like receptor 4 (TLR4). Therefore, by using genetic strategies to delete TLR4, we investigated whether and how the activation by LPS had a causal role in HFD-induced neuroinflammation and food-reward dysregulation.

Methods

Mice

The mouse experiments were approved by the UCLouvain Health Sector's ethical committee (approval numbers: 2022/UCL/MD/05, 2023/UCL/MD/A5, and 2023/UCL/MD/A12), conducted in accordance with the local ethics committee guidelines and were compliant with the Belgian Law of May 29, 2013, concerning the protection of laboratory animals (agreement numbers: LA1230314 and LA2230641). All the experiments are summarized in additional file 1.

Specific-opportunistic and pathogen-free (SOPF) C57BL/6J male mice (Janvier laboratories, Le Genest-Saint-Isle, France) and TLR4 knockout (KO) male mice (B6(Cg)-Tlr4tm1.2Karp/J, Jackson Laboratory, Bar Harbor, Maine, USA) were bred to obtain wild-type (WT) and TLR4-KO littermates. The mice were housed in a controlled environment (22±2 °C, 12 h daylight cycle from 6:00 AM until 6:00 PM) in pairs, with access to an irradiated control diet (CT indicates control diet, 3.85 kcal/g with 9% fat and 76% carbohydrates (kcal/100 g), AIN93Mi, Research Diet, New Brunswick, NJ, United States) and sterile water. Six different experiments were performed: Experiment 1 (HFD, total $N=40$), Experiment 2 (HFD Kinetic, total $N=12$), Experiment 3 (HFD TLR4 KO, total $N=62$), Experiment 4 (HFD TLR4 KO in Dopaminergic system, total $n=24$), Experiment 5 (LPS, total $N=25$) and Experiment 6 (LPS in TLR4 KO, total $N=24$). Ten-week-old male mice were randomly divided into groups and fed with CT or HFD (5.24 kcal/g with 60% fat and 20% carbohydrates (kcal/100 g) D12492i, Research Diet, New Brunswick, NJ, USA). One week before the behavioural tests, some of the mice, representative of the mean body weight selected for the behavioural tests ($n=6$), were acclimatized in Phenotyper chambers (Noldus, Wageningen, The Netherlands). Body weight and food intake were recorded weekly.

Stereotaxic surgery

NaCl 0.9% (B. Braun) or LPS (562.6 pg LPS/h, *Escherichia coli* 055:B5; Sigma, Darmstadt, Germany), with concentration optimized based on the literature to mimic metabolic endotoxaemia during obesity [37], was infused via osmotic mini-pumps (Alzet pumps 2006, Alzet from Charles River Laboratories, St Germain-Nuelles, France) for 42 days at a rate of 0.15 µl/h. Before implantation, the pumps were connected to a cannula (Brain Infusion Kit I, Alzet, Charles River Laboratories, St. Germain Nuelles, France) and incubated for 48 h at 37 °C. The mice were anaesthetized with isoflurane (2.7%), received a 5 mg/kg subcutaneous injection (s.c.) of an analgesic (tramadol), and then placed on a stereotactic frame (Model 504926, World Precision Instruments, Hertfordshire, United Kingdom). The cannula was implanted into the right lateral ventricle (from bregma in mm: L=+0.9; AP=0.2; V=−2.5) and secured with dental cement. The pump was placed subcutaneously [39, 40]. After surgery, the mice were individually housed and received 5 mg/kg s.c. of tramadol. Their body weights were monitored daily.

2-Food choice paradigm

As previously described, *ad libitum*-fed mice were exposed to two diets during the end of the light phase: a low-fat, CT diet (3.85 kcal/g with 9% fat (kcal/100 g), AIN93Mi, Research diet, New Brunswick, NJ, USA) and a previously unknown high-fat high-sucrose diet (HFHS, 4.7 kcal/g with 45% fat and 27.8% sucrose (kcal/100 g) D17110301i, Research Diet, New Brunswick, NJ, USA) in Phenotyper chambers (Noldus, Wageningen, The Netherlands) [22]. HFHS and CT diets intake were recorded manually after a 3-hour session in daylight. Excessive food wasters were excluded from the measurement.

Operant conditioning

To assess food reward-seeking behaviour, we used an operant conditioning test as previously described [35]. Sessions occurred during the end of the light phase in Phenotyper chambers (Noldus, Wageningen, the Netherlands) and were analysed via Ethovision XT 17 software. The mice had intermittent access to an operant wall in their home cages, which included two levers, lights and a pellet dispenser. One active lever, associated with a light on, triggered the delivery of a sucrose pellet (20 mg pellet with 3.4 kcal/g, 5-TUT peanut butter flavoured sucrose pellet, TestDiet, St. Louis, MO, USA), whereas another inactive lever, associated with a light off, did not. During the first phase, the mice were trained overnight on a

fixed-ratio 1 (FR) schedule (one lever press on the active lever corresponding to one reward) and then underwent 4 FR sessions of 1 h 30 m. This phase was validated when the ratio of active over total lever presses was above 0.75. To assess food-seeking behaviour, the mice were shifted to 4 progressive ratio (PR) sessions of 2 h. The number of active lever presses used to obtain a reward was incrementally increased ($n+3$) for every pellet. The breakpoint corresponded to the number of responses to obtain the last reward [12]. Mice were food restricted to maintain 85% of their initial body weight, except during HFD feeding for the kinetic experiment (access to food *ad libitum*) [41].

Locomotor activity and catalepsy tests

For two days before any procedure, the mice were intraperitoneally (i.p.) injected with 0.9% NaCl (B. Braun) in their home cages [42, 43]. Locomotor activity was measured in Phenotyper chambers (Noldus, Wageningen, The Netherlands) with Ethovision XT 17 software after i.p. injection of 10 µl/g DRD1 agonist (SKF81297, 5 mg/kg, #1447, Tocris Biosciences, Bristol, United Kingdom) or DAT blocker (GBR12909, 10 mg/kg, #D052, Sigma, Darmstadt, Germany) dissolved in NaCl 0.9% solution (B. Braun).

Catalepsy was scored every 18 min, one hour after the i.p. injection of 10 µl/g DRD2 antagonist (Haloperidol, 0.5 mg/kg, #0931; Tocris Biosciences, Bristol, United Kingdom) [42]. The animals were positioned in front of a 4 cm elevated bar, with their forelegs on the bar while their hind legs remained on the ground and their immobility time was measured. Animals unable to stay on the bar for a minimum of 10 s were retested a maximum of five times. A maximal behavioural threshold of 240 s was established.

RNA preparation and real-time RT-qPCR analysis

To stimulate the reward system, the mice were exposed to 5 sucrose pellets for one hour before being anaesthetized with 2.7% isoflurane (Forene, Abbott, Maidenhead, England) [34]. Tissues (NAc and DS) were accurately dissected, promptly submerged in liquid nitrogen, and stored at -80 °C. TriPure reagent was used to extract total RNA (Roche, Bale, Switzerland). cDNA was synthesized from 1 µg of total RNA via the GoScript Reverse Transcriptase Kit (Promega, Madison, WI, USA), followed by real-time PCR using QuantStudio 3 (Thermo Fisher, Waltham, MA, USA). *Rpl19* RNA was used as a housekeeping gene for the relative quantification of gene expression because of its stable expression across conditions in the brain, essential role in protein synthesis, and minimal variability, making it a reliable reference for normalization of gene expression. All samples were run in duplicate, and data analysis was conducted via the $2^{-\Delta\Delta CT}$ method. Melting curve analysis was conducted to evaluate the identity and purity of the amplified product. The primer sequences used for real-time qPCR were previously described (Additional file 2) [22].

Lipopolysaccharide Assay

LPS levels were measured in serum collected from the portal vein using a competitive inhibition enzyme immunoassay (Cloud-Clone Corp, Houston, TX). Samples were diluted (1:10) with the dispersing agent (PYROSPERSE, Lonza, Bales, Switzerland) to disperse endotoxin molecules during sample preparation, and heated 15 min at 70 °C to inactivate nonspecific inhibitors of endotoxin. The endotoxin concentration was determined spectrophotometrically at 450 nm and calculated from the standard curve.

Statistical analysis

Statistical analyses were performed via GraphPad Prism version 8.0.2 for Windows (GraphPad Software, San Diego, CA, United States). The data are shown as the means ± SEMs. Differences between two groups were assessed via unpaired Student's t test. If the data did not follow a normal distribution according to the Shapiro–Wilk test and Q-Q plot, a nonparametric (Mann–Whitney) test was performed. Equal standard deviations or sphericities were assumed. Differences between more than 2 groups were assessed via one-way ANOVA followed by the Holm–Sidak post hoc test. Differences between different groups at different time points were assessed via two-way repeated-measures ANOVA, followed by Bonferroni post hoc correction. RT–qPCR outliers were excluded after the Grubbs test.

Results

HFD-fed mice show alterations of food-reward behaviours, dopaminergic signalling, and neuroinflammatory responses in the NAc and DS

To investigate food-reward dysregulations elicited by exposure to HFD, mice were fed with CT or HFD and subjected to food-related behavioural tests (Fig. 1A). In Experiment 1 (HFD), as expected, the body weight gain in HFD-fed mice was greater than that of CT-fed mice (Fig. 1B). During the 2-food-choice paradigm, in which mice were given the choice between CT and HFHS diets, HFD-fed mice showed a reduced spontaneous tropism towards palatable food (HFHS) as compared to control mice since the HFD-fed mice ate less palatable (HFHS diet) food than the CT-fed mice (Fig. 1C). Compared to control mice, HFD-fed mice did not present reductions in CT food consumption during the 2-food-choice paradigm, that was even increased compared to CT-fed mice (Additional file 3). These results indicate that the reduction in food consumption observed in HFD-fed mice

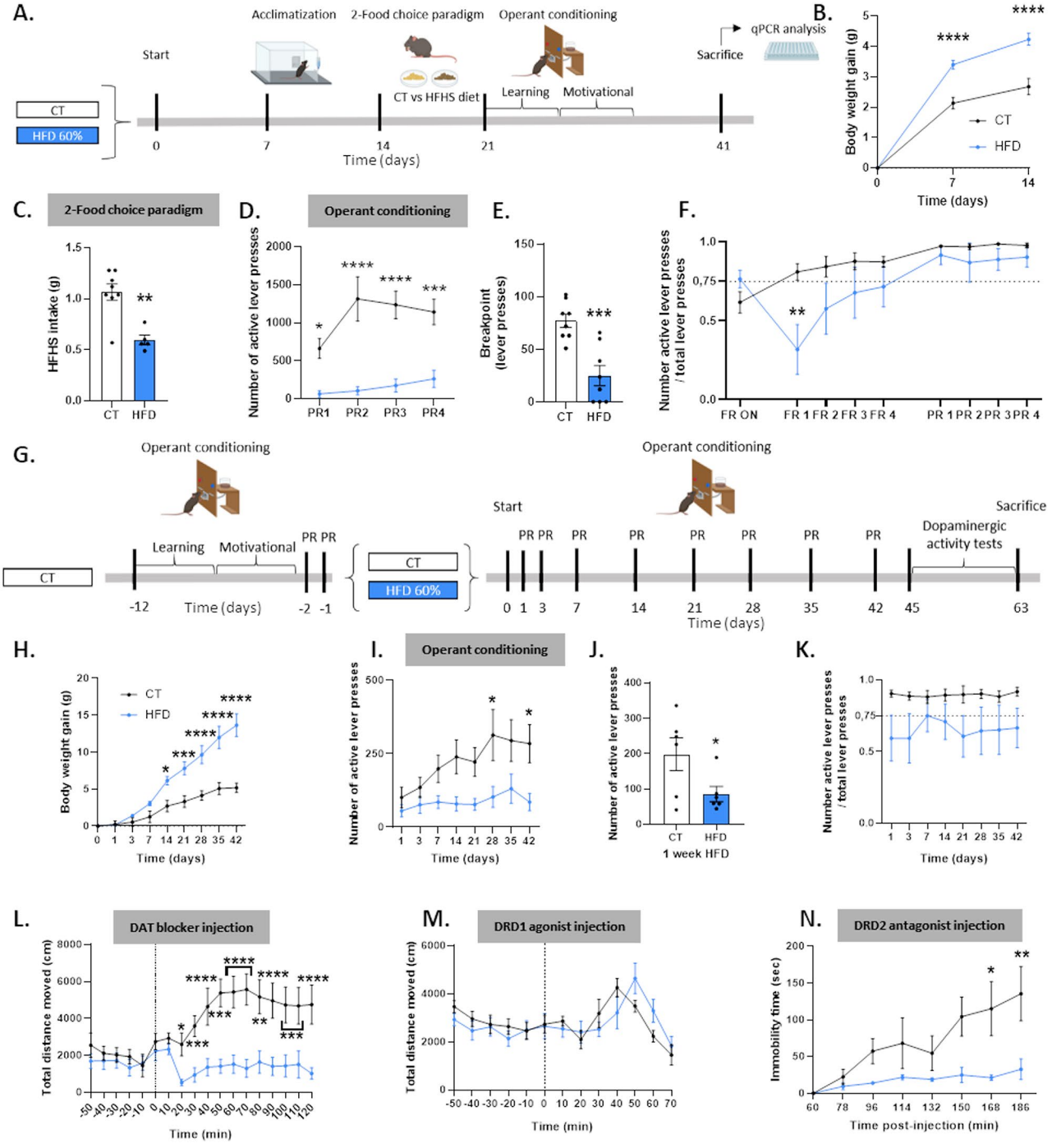


Fig. 1 HFD-induced dysfunctions of food-reward behaviours are associated with hypofunctioning of the dopaminergic system. The mice were monitored during 6 weeks of CT or HFD. (**A**) Experimental plan of Experiment (1) (**B**) Body weight gain evolution in grams before behavioural tests ($n = 20$/group). (**C**) 2-Food choice paradigm: HFHS diet-based food intake in grams by CT and HFD-fed mice ($n = 8$/group). (**D**) Operant conditioning test showing the number of active lever presses during the four progressive ratio (PR) sessions and (**E**) the breakpoint during the PR4 session by CT and HFD-fed mice ($n = 8$/group). (**F**) Ratio of active lever presses during the learning and wanting phases of operant conditioning by CT and HFD-fed mice ($n = 8$/group). In a second experiment, mice were monitored for 12 days on CT diet followed by 9 weeks on a CT or HFD. (**G**) Experimental plan of Experiment (2) (**H**) Body weight gain evolution in grams during PR sessions ($n = 5$–6/group). (**I**) Operant conditioning test showing the number of active lever presses after 1, 3, 7, 14, 21, 28, 35 and 42 days of HFD feeding and (**J**) the number of active lever presses after 1 week of HFD feeding by CT and HFD-fed mice ($n = 5$–6/group). (**K**) Ratios of active lever presses by CT and HFD-fed mice after 2 days on the CT diet, and after 1, 3, 7, 14, 21, 28, 35, and 42 days on the HFD ($n = 5$–6/group). (**L**) GBR12909-induced locomotor activity in CT and HFD-fed mice ($n = 5$–6/group). (**M**) SKF81297-induced locomotor activity in CT and HFD-fed mice ($n = 5$–6/group). (**N**) Immobility time induced by the administration of haloperidol in CT and HFD-fed mice ($n = 5$–6/group). The data are shown as the means ± SEMs. P values were obtained after two-way repeated-measures ANOVA followed by Bonferroni post hoc correction (**B**, **D**, **F**, **H**, **I**, **K**, **L**, **M**, **N**) and after unpaired Student's t-test (**C**, **E**, **J**). *: p value < 0,05; **: p value < 0,01; ***: p value < 0,001; ****: p value < 0,0001 between CT and HFD

was specific to HFHS consumption, thereby supporting the reduced tropism for such palatable food in HFD-fed mice. This dampened tropism towards HFHS diet may result from alterations of reward-associated pathways, as previously reported [7–11]. During the operant conditioning test, we observed that HFD-fed mice pressed significantly less on the active lever to obtain a food-reward than CT-fed mice (Fig. 1D and E), reflecting their impaired food-seeking behaviour. Interestingly, during the operant conditioning training phase, HFD-fed mice showed a lower discriminatory index (ratio of active lever presses over total lever presses) than CT-fed mice, indicating impaired acquisition of the task (Fig. 1F). To accurately evaluate the motivational drive without the confounding factor of an altered learning process, we performed an experiment where the training phase occurred under CT diet feeding before exposure to an HFD (Fig. 1G). In Experiment 2 (HFD Kinetic), after validation of the training phase (discriminatory index ≥ 0.75, Additional file 4), half of the mice were fed with HFD. Interestingly, our results revealed that 24 h exposure to HFD was sufficient to dampen the discriminatory threshold (< 0.75) (Fig. 1K), even before any difference in body weight gain occurred (Fig. 1H). Consistently, we observed that 24 h of HFD feeding led to a downward trend, which become significant at 1 week, in the number of lever presses in HFD-fed mice as compared to CT-fed mice (Fig. 1I-J). These results indicate that a single day of HFD is sufficient to trigger some trends in the alterations of conditioning performance and food-seeking behaviour, with more robust and significant effects after 1 week of exposure to HFD.

To further explore the consequences of HFD-feeding on DA signalling, we measured the locomotor activity induced by the DAT blocker GBR12909 or the DRD1 agonist SKF81297 as well as the immobility time (catalepsy) elicited by the DRD2 antagonist haloperidol in Experiment 2 (HFD Kinetic) (Fig. 1L-N). Compared to CT mice, HFD-fed mice were characterized by a reduction in GBR-induced locomotor activity, suggesting maladaptive changes within the dopaminergic system of HFD-fed mice (Fig. 1L). No locomotor difference was observed between CT and HFD-fed mice following administration of the DRD1 agonist (Fig. 1M), potentially excluding an involvement of DRD1. Thus, we tested the involvement of DRD2. Interestingly, HFD-fed mice showed an impaired cataleptic response to the DRD2 antagonist haloperidol as compared to CT-fed mice (Fig. 1N), thereby indicating potential dysfunction at the level of DRD2 signalling. To investigate whether dysfunction of DRD2 was associated to reduced genetic expression, we performed RT-qPCR experiments to analyse the expression of *Drd1*, *Drd2*, *Dat* and *Th* in the NAc and DS of mice from Experiment 1 (HFD). As previously reported [22], our results showed a tendency towards reduced expression of DA receptor transcripts (*Drd1*, *Drd2*) as well as a reduced expression of DA synthesis enzyme transcript (*Th*) in HFD-fed mice compared to control mice, mainly in the striatum (Fig. 2A-B). These results suggest that (mal)adaptations of DA signalling during exposure to HFD and alterations in DA-dependent behaviours such as food-seeking may be a consequence of these molecular changes.

To gain insights in HFD-induced alterations at cellular level, we investigated the expression of inflammatory markers in the NAc and DS of HFD-fed mice from Experiment 1 (HFD) (Fig. 2). Compared to CT mice, in HFD-fed mice we observed an increased expression of the ionized calcium-binding adaptor protein-1 (*Iba1*), a marker of microglia, whereas the expression of glial fibrillary acidic protein (*Gfap*), a marker of astrocytes, did not differ in the NAc. We also observed an increase in the expression of cluster of differentiation 45 (*Cd45*, a marker of infiltrating immune cells) and the proinflammatory cytokines interleukin–1β (*Il1b*) and tumour necrosis factor α (*Tnfa*) in the NAc of HFD-fed mice compared to CT-fed mice (Fig. 2C). To link food-reward behaviours under an HFD to gut microbe interactions with the host immune system, we measured the expression of specific host receptors involved in the recognition of pathogen-associated molecular patterns (PAMPs): *Tlr2* for peptidoglycan, *Tlr4* for LPS and *Tlr5* for flagellin. The expression of *Tlr4* was greater in the NAc of HFD-fed mice than in the NAc of CT-fed mice, and a positive trend in the expression of *Tlr5* was observed (Fig. 2C). In terms of BBB markers in the NAc, HFD-fed mice presented decreased expression of claudin-1 (*Cldn1*) and occludin (*Ocln*), whereas the expression of claudin–5 (*Cldn5*) and zonula occludens 1 (*Zo1*) was not affected (Fig. 2D). An increase in the expression of *Cd45*, *Tlr4* and *Cldn5* was also observed in the DS of HFD-fed mice (Fig. 2E-F). These findings indicate that HFD-fed mice exhibit neuroinflammation and dysregulation of BBB markers in dopaminoceptive brain regions.

Taken together, these results indicate that HFD-induced alterations of food-reward behaviours may be linked with alterations in DRD2 signalling associated with increased expression of pathogen recognition receptors (PRRs), notably TLR4, and inflammation in the NAc and DS.

TLR4 deletion offers partial protection against HFD-induced food-reward dysregulation and neuroinflammation

Since increased levels of *Tlr4* expression were observed in the NAc and DS of HFD-fed mice (Fig. 2), in Experiment 3 (HFD TLR4 KO) we investigated the causal role of immune system activation through TLR4 on food-reward behaviours during exposure to HFD by using

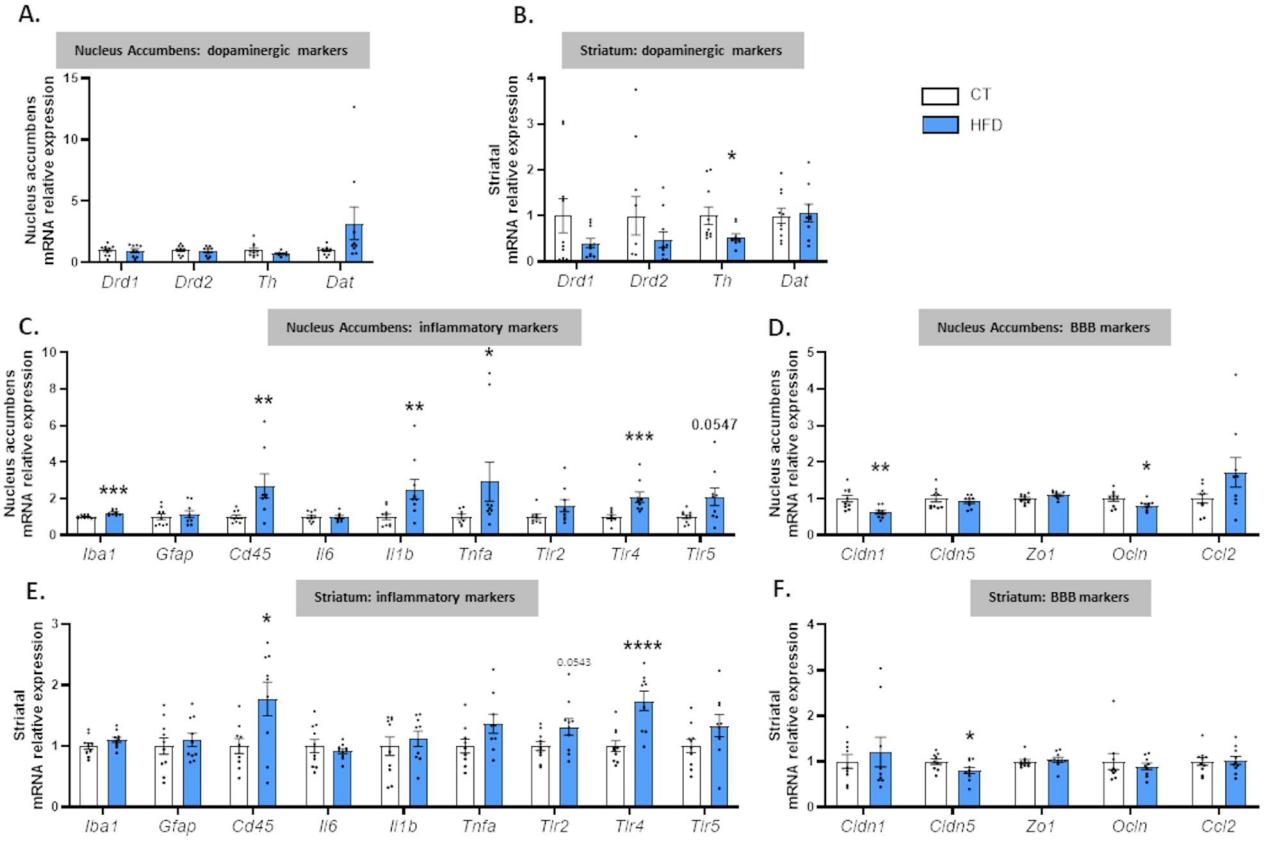


Fig. 2 Exposure to HFD is associated with inflammation and blood–brain barrier alterations in the nucleus accumbens and striatum. (**A**) NAc and (**C**) DS relative mRNA expression of dopamine receptor 1 (*Drd1*), dopamine receptor 2 (*Drd2*), tyrosine hydroxylase (*Th*) and the dopamine transporter (*Dat*). (**C**) NAc and (**D**) DS relative mRNA expression of ionized calcium-binding adapter (*Iba1*), glial fibrillary acidic protein (*Gfap*), cluster of differentiation 45 (*Cd45*), interleukin 6 (*Il6*), interleukin 1 beta (*Il1b*), tumour necrosis factor alpha (*Tnfa*), toll-like receptor 2 (*Tlr2*), toll-like receptor 4 (*Tlr4*) and toll-like receptor 5 (*Tlr5*) and (**E**) NAc and (**F**) DS relative mRNA expression of claudin-1 (*Cldn1*), claudin-5 (*Cldn5*), zonula occludens 1 (*Zo1*), occludin (*Ocln*) and C-C chemokine ligand 2 (*Ccl2*) measured by real-time qPCR in CT and HFD-fed mice. The data are shown as the means ± SEMs. P values were obtained after unpaired Student's t-test or the nonparametric Mann–Whitney test. ($n = 8$–10/group). *: p value < 0,05; **: p value < 0,01; ***: p value < 0,001 and ****: p value < 0,0001 between CT and HFD

TLR4-deleted mice (TLR4 KO) fed either with a CT or HFD (Fig. 3A). Both WT HFD-fed and TLR4 KO HFD-fed mice presented greater body weight gain than CT-fed mice (Fig. 3B) and consumed the same amount of HFD food during *ad libitum* exposure (Fig. 3C). During the 2-food-choice paradigm, similar palatable food intakes were observed in WT and TLR4 KO CT-fed mice. However, HFD-feeding led to enhanced propensity for palatable food intake in TLR4 KO mice as compared to WT mice, whereas no differences in CT food consumption was observed (Additional file 3), highlighting the partial restoration of tropism for the HFHS diet in TLR4 KO HFD-fed mice compared to WT HFD-fed mice. In addition, in the operant conditioning test, TLR4 KO HFD-fed mice showed a higher active lever performance than WT HFD-fed mice (Fig. 3E, $p = 0.0354$ after a t test between TLR4 KO and WT HFD-fed mice in PR4) and a greater breakpoint than WT HFD-fed mice (Fig. 3F). These results suggest that genetic deletion of TLR4 induces a partial protection against HFD-induced behavioural dysregulation of the food-reward events. To explore a potential mechanism associating inflammation and TLR4, we analysed the expression of inflammatory markers in the NAc and DS of TLR4 KO mice in Experiment 3 (HFD TLR4 KO). We observed that compared to WT HFD-fed mice, TLR4 KO HFD-fed mice showed a decrease in the expression of *Iba1* in the NAc and a decrease in the expression of *Gfap* in the NAc and DS (Fig. 3G-H).

Taken together, these results suggest that deletion of TLR4 partially protects against HFD-induced food-reward dysfunctions possibly by reducing microglia and astrocyte activation in the NAc and DS.

TLR4-deleted mice are protected against HFD-induced dysfunctions in DRD2 signalling

To determine whether the activation of TLR4 during HFD exposure was associated to changes in the DA pathway, we challenged the DA circuit with pharmacological

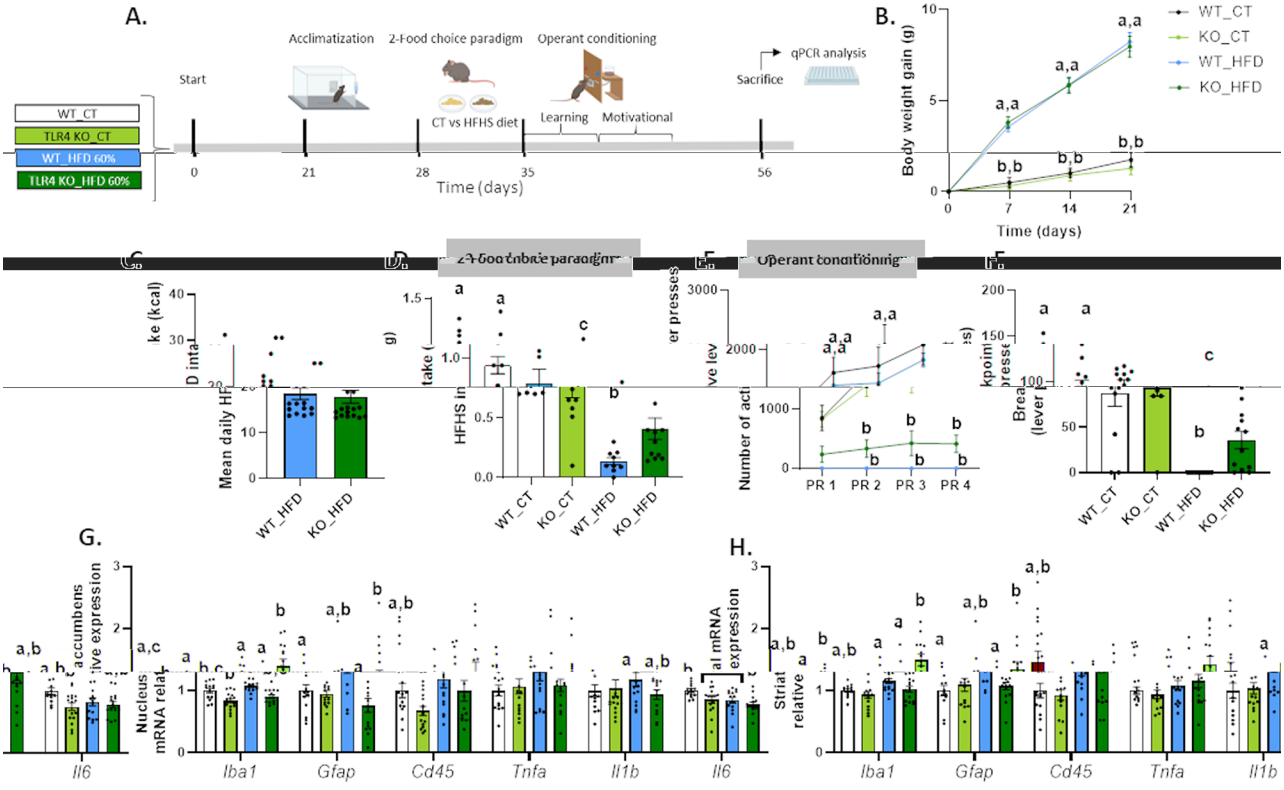


Fig. 3 TLR4-deleted mice are partially protected against HFD-induced food-reward behavioural dysregulations and neuroinflammation. Wild-type and TLR4 KO mice were monitored for 8 weeks on CT or HFD. (**A**) Experimental plan of Experiment 3. (**B**) Body weight gain evolution in grams before behavioural tests ($n = 15–17$/group). (**C**) Mean daily HFD intake in kcal before behavioural tests in WT_HFD and KO_HFD-fed mice ($n = 10–12$/group). (**D**) 2-Food-choice paradigm: HFHS diet-based food intake in grams in WT_CT, KO_CT, WT_HFD and KO_HFD-fed mice ($n = 10–12$/group). (**E**) Operant conditioning test showing the number of active lever presses during the four progressive ratio (PR) sessions and (**F**) the breakpoint during the PR4 session by WT_CT, KO_CT, WT_HFD and KO_HFD-fed mice ($n = 8–12$/group). (**G**) NAc relative mRNA expression of ionized calcium-binding adapter (*Iba1*), glial fibrillary acidic protein (*Gfap*), cluster of differentiation 45 (*Cd45*), tumour necrosis factor alpha (*Tnfa*), interleukin 1 beta (*Il1b*) and interleukin 6 (*Il6*) measured by real-time qPCR in CT and HFD-fed mice ($n = 10–16$/group). (**H**) DS relative mRNA expression of ionized calcium-binding adapter (*Iba1*), glial fibrillary acidic protein (*Gfap*), cluster of differentiation 45 (*Cd45*), tumour necrosis factor alpha (*Tnfa*), interleukin 1 beta (*Il1b*) and interleukin 6 (*Il6*) was measured by real-time qPCR in CT and HFD-fed mice ($n = 10–16$/group). The results were obtained from 2 independent experiments. The data are shown as the means ± SEMs. P values were obtained after two-way repeated-measures ANOVA followed by Bonferroni post hoc correction (**B**, **E**), after unpaired Student's t-test (**C**), and after one-way ANOVA followed by the Holm–Sidak post hoc test (**D**, **F**, **G**, **H**). Different letters indicate significant differences at p values < 0.05 between WT_CT, KO_CT, WT_HFD and KO_HFD

agents in WT and TLR4 KO mice fed with CT or HFD in Experiment 4 (HFD TLR4 KO Dopaminergic system) (Fig. 4A, B). As shown in Fig. 1L–N, compared to CT mice, HFD-fed mice presented reduced locomotor activity after blockade of DAT and a reduced cataleptic response to the DRD2 antagonist (Fig. 4C–E). Interestingly, compared to WT HFD-fed mice, TLR4 KO HFD-fed mice showed a restoration of the haloperidol-induced cataleptic response (Fig. 4E). However, we did not observe major differences in *Drd1*, *Drd2*, *Th* or *Dat* expression in the NAc or in the DS between WT and TLR4 KO HFD-fed mice (Additional file 5), suggesting that additional post-translational processes and intracellular signalling might be at play in the response of DAceptive neurons to HFD in TLR4 KO mice.

These results indicate that TLR4 deletion protects against DRD2 signalling dysfunction under HFD conditions, highlighting a potential connection between TLR4 and DRD2 signalling.

Central diffusion of LPS dysregulates food-reward behaviours

TLR4 can be activated not only by LPS but also by fatty acids [44]. To further investigate the role of LPS in neuroinflammation and food-reward alterations, we quantified the level of LPS in the portal vein of WT and TLR4 KO mice fed with CT or HFD in Experiment 3 (HFD TLR4 KO) and found increased plasma levels in WT HFD-fed mice compared to WT CT-fed mice (p value = 0.02 after unpaired Student's t test), whereas no difference was observed between WT HFD and KO HFD-fed mice (Additional file 6). Since TLR4 deletion does not allow us to discriminate between the action of LPS (microbiota-induced inflammation) and/or fatty acids (diet,

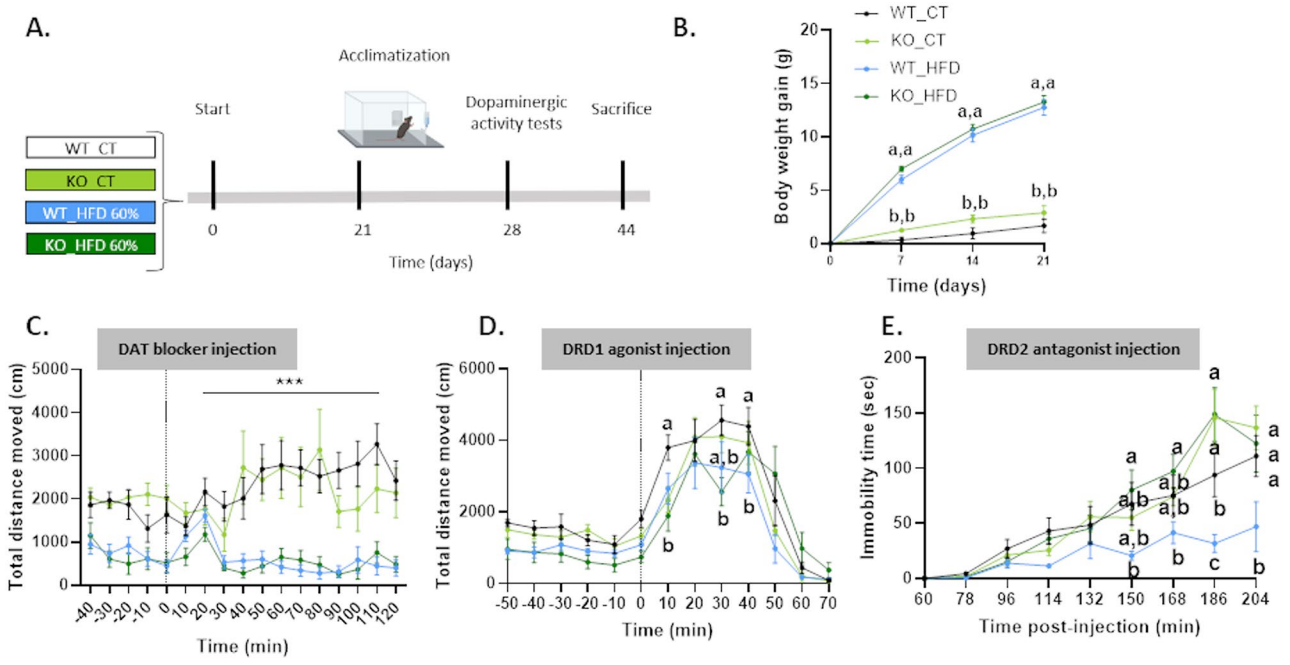


Fig. 4 TLR4-deleted mice fed with a HFD presented intermediate protection against HFD-induced dysfunction of DRD2 postsynaptic signalling. Wild-type and TLR4 KO mice were monitored for 7 weeks on CT or HFD. (**A**) Experimental plan of Experiment 4. (**B**) Body weight gain evolution in grams before behavioural tests ($n = 6$/group). (**C**) GBR12909-induced locomotor activity in WT_CT, KO_CT, WT_HFD and KO_HFD-fed mice ($n = 6$/group). (**D**) SKF81297-induced locomotor activity in WT_CT, KO_CT, WT_HFD and KO_HFD-fed mice ($n = 6$/group). (**E**) Immobility time induced by the administration of haloperidol in WT_CT, KO_CT, WT_HFD and KO_HFD-fed mice ($n = 6$/group). The data are shown as the means ± SEMs. P values were obtained after two-way repeated-measures ANOVA followed by Bonferroni post hoc correction (**B**, **C**, **D**, **E**). Different letters indicate significant differences at p values < 0.05 between WT_CT, KO_CT, WT_HFD and KO_HFD

endogenous metabolites), we decided to chronically diffuse a low concentration of LPS (562,6 pg/h) in the brain of CT mice in Experiment 5 (LPS) (Fig. 5A). We validated the selected LPS dose by comparing its capacity to mimic increased inflammatory markers as observed in HFD-induced neuroinflammation (Additional file 7). During the 2-food choice paradigm, we observed that the LPS-treated mice consumed less palatable food than saline-treated mice but more food than HFD-treated mice under saline conditions, suggesting that LPS partially replicates HFD-induced devaluation of palatable food (Fig. 5B). During the operant conditioning test, LPS-treated mice pressed significantly less on the lever than saline-treated mice during PR2 and PR4 but pressed more than HFD-treated mice receiving saline (Fig. 5C). Similar results were obtained for the breakpoint (Fig. 5D). Taken together, these results indicate that LPS partially contribute in altering food-reward behaviour in the context of obesity. While this is unlikely the sole factor contributing to these effects, it is nevertheless a significant aspect to consider. To further validate the causal role of LPS-induced inflammation through TLR4, we centrally administered low doses of LPS (or saline) in TLR4 KO mice fed with CT diet (Experiment 6 (LPS in TLR4 KO) in Additional file 8 A). In the absence of TLR4, LPS was not able to alter neither the food-reward tropism for a palatable diet during the 2-food-choice paradigm nor the motivational drive during the operant conditioning test (Additional file 8 B-D).

Taken together, these results reveal that TLR4 mediates LPS-induced food-reward dysregulations.

Discussion

Impairments in the reward system during food consumption significantly contribute to overeating and to the escalation of metabolic disorders. Therefore, identifying the mechanisms and factors involved in HFD-feeding and obesity-associated reward dysregulation is highly important. In this study, we provide evidence supporting the role of the LPS-TLR4 pathway in the behavioural (mal) adaptations observed during HFD exposure. Moreover, we highlight the involvement of TLR4 in both inflammatory responses and alterations in the dopaminergic pathway within the NAc and DS in the context of HFD exposure (graphical abstract).

Consistent with the literature [12–16, 45], we found that both short- and long-term exposure to HFD disrupts the tropism and the motivational drive associated with food-reward events. Adiposity signals (i.e. leptin) are known to contribute to food-reward events [46–48]. However, our findings indicated that dysregulations of the reward system can occur regardless of body weight

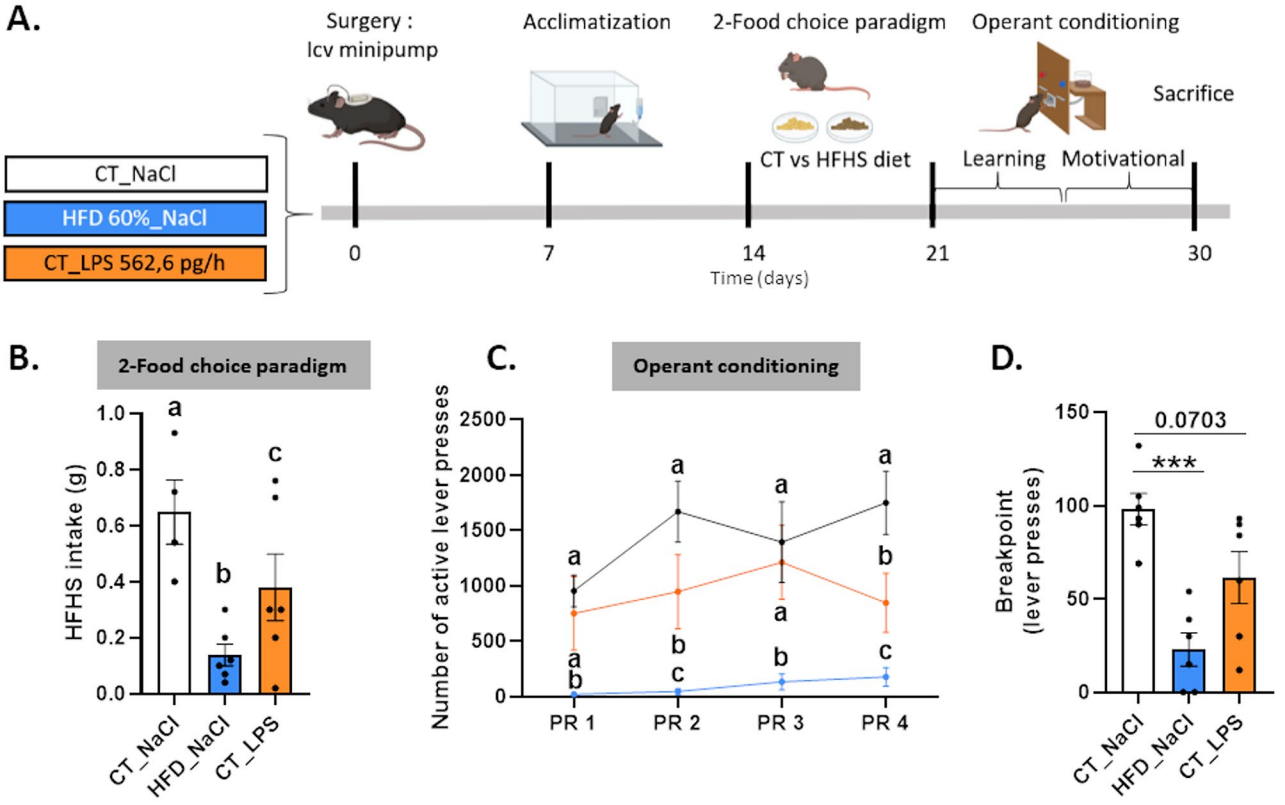


Fig. 5 Chronic ventricular diffusion of LPS at low-dose induces partial dysregulation of food-reward behaviours. The mice were monitored for 5 weeks on the CT or HFD diet after stereotaxic cannulation and mini-pumps filled with saline solution (NaCl) or LPS implantation. (**A**) Experimental plan for Experiment 5 ($n = 8$–9/group). (**B**) 2-Food choice paradigm: HFHS diet-based food intake in grams by CT_NaCl, CT_LPS and HFD_NaCl mice ($n = 6$/group). (**C**) Operant conditioning test showing the number of active lever presses during the four progressive ratio (PR) sessions and (**D**) the breakpoint during the PR4 session by CT_NaCl, CT_LPS and HFD_NaCl mice ($n = 6$/group). The data are shown as the means ± SEMs. P values were obtained after one-way ANOVA followed by the Holm–Sidak test (**B**, **D**) and after two-way repeated-measures ANOVA followed by Bonferroni post hoc correction (**C**). Different letters indicate significant differences at p values < 0.05 between CT_NaCl, CT_LPS and HFD_NaCl. ***: p value < 0,001 between CT_NaCl and HFD_NaCl

gain, thereby suggesting an involvement of other factors and mediators.

Using pharmacological approaches to challenge the integrity of DA signalling, we observed impaired functions of the dopaminergic system in HFD-fed mice as previously reported [7–11]. However, the causal role of this impairment in obesity-related food-reward behaviours remains uncertain [49]. We found a tendency towards reduced expression of DA receptor transcripts (*Drd1*, *Drd2*) as well as a reduced expression of DA synthesis enzyme transcript (*Th*) in HFD-fed mice compared to control mice, mainly in the striatum. However, since the analysis of gene expression related to dopaminergic markers does not fully reflect the functional activity of the system, we cannot exclude the possibility that other variables, such as receptor availability, could also influence the observed effect of HFD feeding on dopamine signalling. Moreover, we focused on DA, as it is the major driver of food-reward events within the mesocorticolimbic pathway [3, 4]. Other neurotransmitters/modulators, such as opioids [50], endocannabinoids [43, 51], serotonin [52] and a variety of hormones [53], are also involved in the regulation of food-reward behaviours. Exploring these pathways could offer valuable insights, but this is beyond the scope of our study.

Exposure to HFD leads to both cytokine and inflammatory-like responses in the brain together with DA signalling dysfunctions [16, 17]. Here we showed that short-term or chronic exposure to HFD induced dysregulation in food-reward behaviours associated with inflammation in the NAc and DS, consistent with published observations [22]. In the past decade, some studies also reported that obese rodents present inflammation in the NAc [24, 25, 54, 55]. Décarie-Spain et al. reported that 12 weeks of a HFHS induced the activation of astrocytes and microglia and that inhibition of nuclear factor kappa B (NFκB) in the NAc protected against accumbal inflammation and blunted compulsive sucrose-seeking behaviour [56]. Finally, Soto et al. demonstrated that HFD-fed mice showed increased proinflammatory cytokine expression in the NAc [23]. In line with our results, these studies indicate a significant association between

neuroinflammation and food-reward dysregulations in obese individuals. Neuroinflammation may arise from local inflammatory processes and/or from increased BBB permeability, thus facilitating the diffusion of pro-inflammatory mediators from the periphery to the brain. During obesity, the BBB continually faces challenges from proinflammatory stimuli [57, 58]. In this study, we observed that HFD-fed mice showed decreased expression of tight junction proteins (*Cldn1* and *Ocln*). Therefore, alterations of the BBB in the NAc could contribute to the development of local neuroinflammation.

To investigate the link between gut microbes and local neuroinflammation, we explored PAMPs receptor expression and detected increased *Tlr4* in the NAc and DS of HFD-fed mice. The role of TLR4 in alcohol and drug addiction has been extensively studied, and inhibiting TLR4 appears to reduce seeking-related behaviours [59–61]. Therefore, we examined whether TLR4 was involved in HFD-induced disrupted food-reward behaviours in TLR4-deleted mice. We selected C57BL/6 TLR4-deleted mice as they do not exhibit resistance to HFD-induced body weight gain, eliminating potential confounding factors (i.e. fat mass) observed in other strains [62, 63]. In this study, we revealed that TLR4 KO mice showed partial protection against HFD-induced dysregulations of food-reward tropism and motivational drive. The partial restoration of tropism for palatable food in TLR4 KO HFD-fed mice occurred despite *ad libitum* access to HFD between tests. Furthermore, TLR4 deletion suppressed some HFD-induced neuroinflammatory markers in the NAc and DS, including those of astrocytes. Therefore, our study highlights the role of TLR4 in neuroinflammation and food-reward dysregulations during HFD exposure. We used total TLR4 KO model since the role of peripheral versus central TLR4 in inflammation is still not well understood. Indeed, in an activity-based anorexia model, Belmonte et al. reported that *Tlr4* expression increased in the periphery without changes in the hypothalamus [64]. However, administration of LPS did not cause rapid-onset anorexic effects in TLR4 null mice re-expressing TLR4 specifically in peripheral afferents [65]. Interestingly, we also found a positive trend in the expression of TLRs for peptidoglycan and flagellin in the NAc and DS of WT HFD-fed mice as compared to WT lean mice (Fig. 2C and E). Since these pathways may also be involved in inflammation, exploring their involvement in changes to food-reward mechanisms might also be worthwhile [66].

In this study we mainly focused on the NAc and DS which represent the main mesolimbic dopaminoceptive regions. However, we cannot exclude that VTA DA-projecting neurons as well as midbrain neuroinflammatory processes may also contribute to our phenotypes. Further studies will be required to fully dissect the mechanistic and anatomo-functional features underlying food-reward dysfunctions under obesogenic conditions.

Evidence from humans and rodents strongly suggests that inflammation contributes in altering DA system [67]. First, inflammation reduces the availability of tetrahydrobiopterin (BH4), the cofactor necessary for the activity of tyrosine hydroxylase (TH), which is the limiting enzyme for DA synthesis [68–75]. Second, proinflammatory cytokines decrease the expression of vesicular monoamine transporter-2 (VMAT2), reducing the release of DA from vesicles via exocytosis and therefore DA availability [76]. Finally, proinflammatory cytokines decrease the binding of DA to DRD2 [68]. Interestingly, in the frontal cortex, the levels and activity of DA-regulated phosphoprotein 32 (DARPP-32), which is a key factor in DA signal transduction pathways, are affected in TLR4 KO mice [77]. Moreover, Li et al. reported that TLR4 deletion specifically in VTA DA-neurons decreased the amount of released accumbal dopamine. These mice presented dysregulated food-reward behaviours, which were rescued by reexpressing TLR4 [78]. However, the authors did not explore the effects of TLR4 deletion in the context of overeating and obesity. In this work, by using pharmacological tools, we shed light on the role of TLR4 in altering DRD2 during HFD consumption. Indeed, we observed protection against HFD-induced dysregulations of DRD2 signalling in TLR4-deleted mice. However, more studies are needed to better understand the precise interaction between TLR4 and DRD2. Moreover, elucidating the function of TLR4 across various brain cell types, despite its primary expression in microglia, holds significant research value [79].

In the context of obesity, small but chronic increase in circulating LPS and fatty acids are observed, both of which can activate TLR4. We aimed to elucidate the specific role of LPS in food-reward dysregulation through TLR4. High doses of LPS decrease food tropism and motivational drive in response to palatable food [80–83], but their relevance to obesity is questionable, as they do not replicate the chronic low concentrations observed in obesity. Therefore, we studied whether and how metabolic endotoxaemia affects inflammation and associated food-reward dysfunctions. We found that chronic low concentrations of LPS diffused into the brain induced inflammation similar to that in HFD-fed mice and altered food-reward behaviours, suggesting that chronic exposure to low-dose LPS, which mimics HFD-induced inflammation, may contribute to the dysregulation of food-reward processes. Interestingly, LPS can directly impact the DA signalling by promoting the degradation of central monoamines (norepinephrine, serotonin and DA) as well as DAT activity in the NAc [84]. To confirm our hypothesis of a causal role of TLR4-mediated inflammation induced by LPS in food-reward dysregulation

associated to obesity, we showed that TLR4 deletion provided protection against LPS-induced dysregulated food-reward behaviours.

Limitation of the study

Our study used only male mice, and sex-based variations could affect food-reward behaviours [53]. Second, while the 60% HFD model is commonly used to simulate human obesity, each nutritional model has inherent limitations [85–87]. The HFD is more calorically dense than the HFHS diet used in the 2-food choice paradigm, which could impact tropism, although our findings suggest that caloric intake is not the main factor driving food tropism [88]. Additionally, distinguishing between sensory signalling and caloric content in food-reward behaviours is challenging. These limitations highlight the complexity of studying food-reward behaviours and the need for cautious interpretation of the results.

Conclusion

Our study, by shedding light on the role of the LPS-TLR4 pathway, provides a new potential mechanism underlying food-reward dysregulation in an obesogenic context. These findings indicate that neuroinflammation triggered by the gut microbiota components LPS may contribute to food-reward dysregulation, thus paving the way for future treatment approaches.

Abbreviations

BBB	Blood–brain barrier
CLDN1	Claudin-1
CLDN5	Claudin-5
CD45	Cluster of differentiation 45
CT	Control diet
DA	Dopamine
DAT	Dopamine transporter
DARPP-32	Dopamine-regulated phosphoprotein 32
DRD1	Dopamine receptor 1
DRD2	Dopamine receptor 2
DS	Dorsal striatum
FR	Fixed ratio
GFAP	Glial fibrillary acidic protein
HFD	High-Fat diet
HFHS	High-fat high-sugar
IBA1	Ionized calcium-binding adaptor protein-1
IL1b	Interleukin–1β
KO	Knock-out
LPS	Lipopolysaccharide
NAc	Nucleus accumbens
OCLN	Occludin
PAMPS	Pathogen-associated molecular patterns
PR	Progressive ratio
TH	Tyrosine hydroxylase
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TLR5	Toll-like receptor 5
TNFa	Tumour necrosis factorα
VMAT2	Vesicular monoamine transporter-2
VTA	Ventral tegmental area
WT	Wild-type
ZO1	Zonula occludens

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12974-024-03297-z.

Supplementary Material 1

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Author contributions

Conceptualization, A.E; methodology, A.E., S.J.P.H., G.G., S.L. and P.D.C.; investigation S.J.P.H., A.E. and C.F.; writing - original draft S.J.P.H. and A.E.; writing - review & editing, A.E., S.J.P.H., C.F., G.G., S.L. and P.D.C; Visualization, S.J.P.H. and A.E; supervision, A.E.; funding acquisition; A.E. and P.D.C.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethical approval

The mouse experiments were approved by the UCLouvain Health Sector's ethical committee (approval numbers: 2022/UCL/MD/05, 2023/UCL/MD/A5, and 2023/UCL/MD/A12), conducted in accordance with the local ethics committee guidelines and compliant with the Belgian Law of May 29, 2013, concerning the protection of laboratory animals (agreement numbers: LA1230314 and LA2230641).

Competing interests

A.E. and P.D.C. are inventors on patent applications dealing with the use of A. muciniphila and its components in the treatment of metabolic disorders. A. E., SJPH and P.D.C. are inventors on patent applications dealing with gut microbes in food reward dysregulations. P.D.C. was cofounder of The Akkermansia company SA and Enterosys. All other authors declare they have no competing interests. The funders had no role: in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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