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Behavioral consequences of exposure to a high fat diet during the post-weaning period in rats

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ABSTRACT

We explored the impact of exposure to an obesogenic diet (High Fat–High Sucrose; HFS) during the post-weaning period on sweet preference and behaviors linked to reward and anxiety. All rats were fed chow. In addition a HFS-transient group had access to this diet for 10 days from post-natal (PN) day 22 and a HFS-continuous group continued access until adult. Behavioral tests were conducted immediately after PN 32 (adolescence) or after PN 60 (adult) and included: the condition place preference (CPP) test for chocolate, sugar and saccharin preference (anhedonia), the elevated plus maze (anxiety-like behavior) and the locomotor response to quinpirole in the open field. Behavior was unaltered in adult rats in the HFS-transient group, suggesting that a short exposure to this obesogenic food does not induce long-term effects in food preferences, reward perception and value of palatable food, anxiety or locomotor activity. Nevertheless, rats that continued to have access to HFS ate less chocolate during CPP training and consumed less saccharin and sucrose when tested in adolescence, effects that were attenuated when these rats became adult. Moreover, behavioral effects linked to transient HFS exposure in adolescence were not sustained if the rats did not remain on that diet until adult. Collectively our data demonstrate that exposure to fat and sucrose in adolescence can induce immediate reward hypofunction after only 10 days on the diet. Moreover, this effect is attenuated when the diet is extended until the adult period, and completely reversed when the HFS diet is removed.

1. Introduction

It has become increasingly evident that the early life environment, including nutrition, plays a pivotal role in determining the subsequent body weight and metabolic phenotype when adult. It is known, for example, that under- or over-nutrition in utero predisposes individuals to overweight and obesity, including increased risk of cardiometabolic disease. The impact of different diets in the early life period has been widely studied, especially during the gestation and lactation periods. In rodents, the offspring of mothers consuming a high fat diet have an increased risk of obesity and insulin resistance in adulthood (Ainge et al., 2011). What has been much less studied is the impact of the composition of the food environment during that critical post-weaning period, when individual food preferences are first expressed by the offspring and when many of the key pathways important for subsequent dietary choice and associated behaviors are established. Although we do not yet have a clear view of the brain pathways critical for food choice, we can follow food-linked (including homeostatic and reward-driven) behaviors that would seem critical for individuals to make favourable decisions for one food over another.

By the time weaning takes place, which usually occurs around post-natal days (PN) 21–22 in rats and mice, neurogenesis of many of the key pathways important for energy balance and non-homeostatic feeding will already have occurred, although the fine tuning of their connections continues for some time afterwards. Thus, neurogenesis in the hypothalamus is estimated to occur between embryonic days 13–15 (Markakis, 2002), with the circuitry of the arcuate nucleus, a pivotal site for energy balance integration, estimated to happen between PN 7–18 (Bouret et al., 2004). Of the non-homeostatic networks, the midbrain dopamine system that confers reward from natural and artificial reinforcers, including food, is a candidate target for programming of early life appetitive behavior. The neurogenesis of the dopamine circuitry in the striatum and projections to cortical regions are mostly developed between PN 7–21 in rats (Van den Heuvel and Pasterkamp, 2008), although during the peri-adolescent period (PN 21–42), a lot of rearrangements in dopaminergic systems occur (Andersen, 2003; Bernheim et al., 2013). This rearrangement of the dopamine system has been linked to risk-taking behaviors during the peri-adolescent period (Bernheim et al., 2013). Therefore, the stimulation of these structures when eating palatable food will have different effects depending on which period in development this occurs. Moreover, there is

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evidence in humans that exposure to different types of food and the habits acquired early in life can influence our decisions and preferences for different diets (Beauchamp and Mennella, 2009; Schwartz et al., 2011).

Adult rats that are offspring to mothers consuming cafeteria diet (high in fat, sugar and salt) display altered reward-based behaviors due to an altered development of the mesolimbic reward pathway (Ong and Muhlhausler, 2011). They also have an increased preference for foods high in fat, sugar and salt (Bayol et al., 2007), indicative of metabolic imprinting (i.e. epigenetic modulation of metabolic and neurologic responses to food in the adult offspring). Mice fed high fat diet from weaning (PN 21) that continue on this diet for at least 15 weeks, displayed decreased expression of a number of dopamine-related genes in the mesolimbic reward circuit and increased expression of these genes in the hypothalamus (Vucetic et al., 2012), and decreased μ-opioid receptor expression in the mesolimbic reward circuits together with a decreased preference for saccharin (Vucetic et al., 2011), suggesting a state of reward hypofunction. Supportively, in pathological obese human subjects, a reduction in striatal dopamine D2-receptors, similar to that seen in individuals with substance abuse, has been reported (Volkow et al., 2013), a finding interpreted to indicate that the reward circuits are under-stimulated, predisposing these individuals to compensate for this "reward-deficiency" by overeating.

However, after the intrauterine and nursing period, very little is known about potential long-term effects of a short-transient exposure to obesogenic diets, during the post-weaning and adolescent period, on behaviors linked to appetite control, such as food motivation and anxiety-like behavior. Teegarden and colleagues demonstrated that mice given access to a high fat diet for 1 week after the weaning, subsequently showed higher preference for it (when adult) and this was accompanied by epigenetic changes in the ventral striatum (Teegarden et al., 2009). This supports the idea that exposure to an obesogenic diet in the post-weaning period can impact on adult food preferences and on the reward system.

In the present study, we sought to determine whether exposure to an obesogenic palatable diet high in fat and sugar during the post-weaning period (either transiently for 10 days or continued for >40 days into adult life) can alter behaviors linked to feeding control, including reward-linked and anxiety-like behaviors in rats. We also explored whether such behaviors can be altered immediately after transient exposure to the high fat diet in early life, or indeed, whether it is necessary to sustain the animals on the high fat diet in order to see behavioral changes. Finally, we also sought to discover whether there is an impact of the diet in the post-weaning period (transient or continued until adult life) on expression of candidate genes in key brain areas.

2. Methods

2.1. Animals

Pregnant Sprague-Dawley rats (Charles-River, Germany) delivered their pups by 8–10 days after their arrival to the animal facility. The litters were reduced to eight pups each, with a balance kept between males and females where possible. After weaning, on postnatal day (PN 22), the rats were regrouped with 4–5 per cage and in a way that ensured the animals in the same group were not all from one dam. Only the males were included in the experiments described here.

The animal room was maintained on a 12/12 hour light/dark cycle (lights on at 6 am), at 20 °C and 50% humidity. Rats always had ad libitum access to food and water. All procedures took place at the Laboratory for Experimental Biomedicine, University of Gothenburg. All animal procedures were carried out with ethical permission and in accordance with the University of Gothenburg Institutional Animal Care and Use Committee guidelines.

2.2. General procedure

After weaning (PN 22), the rats were divided in 2 groups balanced in body weight: (1) Chow rats: fed normal chow only (Teklad Global 16% Protein Rodent Diet, Harlan; 3 kcal/g) and (2) HFS rats: fed an obesogenic diet high in fat and sucrose (Western diet, TD88137, Harlan: 4.5 kcal/g; 15.2% kcal from protein, 42.7% kcal from carbohydrates and 42% kcal from fat. Sucrose represents 341.4 g/kg.) in addition to normal chow. The HFS was never offered alone but always in combination with chow in all experiments. After 10 days, on PN 32, half of the rats in the HFS group were returned to normal chow feeding (HFS-transient, HFS-T), while the other half continued to have access to HFS until the end of the experiments (HFS-continuous, HFS-C). Food and water were available ad libitum throughout the experiments, except during the behavioral tests. Rats were never food or water restricted. Three different cohorts of rats were tested immediately after PN 32 (puberty) in several reward-linked behavioral tests (see below), while another two cohorts of rats were evaluated in the same tests after PN 58 (adulthood) to study the long-term effects of the HFS diet (Fig. 1). The rats were handled at least three times prior to starting the behavioral tests. They were also habituated to the common procedures in the experiments like body weight measurements and intraperitoneal (i.p.) injections in order to reduce the stress associated with these procedures. All the experimental procedures were done during the light phase. The behavioral apparatus was cleaned with 5% ethyl alcohol between each individual test.

2.3. Behavioral tests

2.3.1. Sucrose and saccharin preference test

Rats were allowed to drink a saccharin solution (0.1% w/v) or sucrose (1% w/v) (Sigma, Dorset, England) ad libitum for 3 h/day on two consecutive days, and 1 h/day on the third day. The reduction to 1 h instead of 3 h on the third day aimed to increase the sensitivity of the test, as those with high preference for the solutions would be expected to drink a large volume in a very short time period. The rats were placed in individual cages for 1 h before starting the test. Saccharin and water were stored in special bottles to prevent leakage. The two bottles were refilled and side-switched each day. To habituate the rats to the procedure, we introduced the two bottles filled with water for 24 h in the home-cage before the test. Rats exposed to saccharin were never exposed to sucrose. The measures taken were saccharin, sucrose and water consumption per body weight. Preference for the sweet solutions over water was calculated as follows: (ml of sucrose or saccharin/ml of sucrose or saccharin + ml of water) * 100. The first day of exposure to sucrose or saccharin is not shown in the results since it is considered a day for adaptation to the solutions.

2.3.2. Conditioned place preference (CPP) for assessing food reward

The CPP test was performed in an apparatus comprised of two connected chambers with distinct visual and tactile qualities (Med Associates Inc., St Albans, Vermont, USA). Initial preference for one chamber was assessed on two consecutive days (15 min/day). The second day was used to determine the preference (Pre-test). The least preferred compartment was subsequently paired with rewarding/palatable food (chocolate pellets; Ms, Marabou, Kraft Foods, Väsby, Sweden). The preferred chamber was paired with normal chow. The pre-test was followed by 20 conditioning sessions (two sessions per day, 20 min each). One day following the last conditioning session, rats were tested again for their preference in the CPP apparatus for 15 min. During the CPP test, rats did not have access to food, enabling dissociation of the intake of palatable food from the reward evaluation process. The behavior of the animals was recorded and time spent in each compartment was determined automatically.

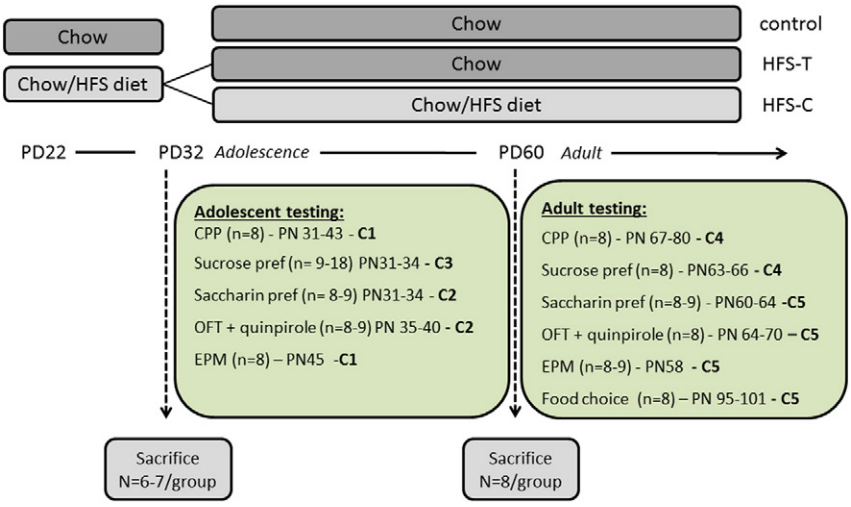

Fig. 1. Experimental design including all cohorts (C1–C5). CPP: conditioned place preference; OFT: open field test and EPM: elevated plus maze. The "n" size refers to the number of rats in each group.

2.3.3. Elevated plus maze (EPM) for assessing anxiety-like behavior

The EPM apparatus, adapted from Pellow and File (1986), consists of two open arms and two closed arms (50 cm long and 10 cm wide), arranged as a plus/cross, elevated 70 cm from the floor, the closed arms are sheltered by black walls, 40 cm high (Med Associates Inc., St. Albans, Vermont, USA). In between the arms there is a square area, 10 × 10 cm. Parameters measured were entries into each zone, total time in each zone and percentage of time spent in open arms as a measure of anxiety-like behavior. Rats were allowed to explore the maze during a 5 min test. To initiate the test, each rat was placed in the centre of the maze facing a closed arm.

2.3.4. Quinpirole induction of hyper-locomotor activity in the open field test (OFT)

The OFT can be used for a number of behavioral analysis that include general activity, exploration and anxiety-like behavior (Sousa et al., 2006). Rats were exposed to an open field arena (90 × 90 × 30 cm; Med Associates Inc.). A monitor registered locomotor activity with infrared beams in X-, Y- and Z-plane. Activities measured were: distance travelled (locomotion) and the central part of the arena versus the periphery to study the effect of diet on anxiety-like behavior (Time central / total time) ∗ 100.

It has been demonstrated that dopamine agonists increase locomotor activity in the OFT (Millan et al., 2004; Naef et al., 2008). To explore whether early-life access to a HFS diet alters dopamine-linked locomotor activity, we examined the locomotor response after the rats were injected with the selective dopamine (D-2/3-receptor) agonist, quinpirole in the OFT. A crossover design was used, such that each rat received both vehicle and quinpirole, with at least 72 h between injections. The elimination half-life of plasma quinpirole in the rat is about 9.5 h (Whitaker and Lindstrom, 1987). After 15 min of habituation in the open field arena, occurring 24 h before start of the OFT, the rats were injected IP with either vehicle (0.9 mg/ml saline) or quinpirole (0.5 mg/kg) and were exposed to the open field 1 h later, based on previous studies (Horvitz et al., 2001). The test was initiated by placing the rat in a corner facing the wall (the same corner was used for all rats). The rats remained in the open field for 1 h.

2.3.5. Food preference

To study whether short- or long-term access to a HFS diet changes subsequent food preference, in the adult period, all rats were placed in individual cages, and 2 weeks later (habituation period) they were given ad libitum amount of chow and HFS diet. The food intake was monitored daily for 8 days.

2.4. Sacrifice and tissue harvesting

One group of rats was sacrificed on PN 32 in studies investigating the effects of 10 days of HFS diet access on gene expression in specific brain areas. A second group of rats was sacrificed on PN 62–63 to evaluate the long-term effects of the diet. Rats were anaesthetized with isoflourane (Attane vet, Strömsholm, Sweden) and sacrificed by decapitation. The brains were rapidly removed and the following areas dissected with the help of a brain matrix: prefrontal cortex (PFC), nucleus accumbens (NAcc) and striatum. The borders of each region were determined based on a rat brain atlas (Paxinos and Watson, 2007). Tissue was collected and frozen in liquid nitrogen and stored at −80 °C for later determination of mRNA expression. The gonadal adipose tissue was removed and weighed immediately after dissection.

2.5. RNA isolation and mRNA expression

Total RNA from brain samples was extracted with the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) or RNeasy Micro Kit (Qiagen) according to the guidelines of the manufacturer. The RNA quantity and quality of the samples were checked with the NanoDrop. First strand cDNA synthesis was prepared with 500 ng total RNA and the iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, CA, USA). Gene expression profiling was performed using Custom TaqMan Assays (Life Technologies, Stockholm, Sweden) and the 7900HT system (Life Technologies). Data were normalized according to Livak and Schmittgen (2001), where the mean values of *Actb* (β-*actin*) and *Hmbs* expression were used as endogenous controls.

2.6. Statistical analysis

The 'Statistical Package for Social Science' (SPSS) (version 22 for Windows) was used for statistical analyses. Data were analysed using the generalized linear model (GzLM) with group as a between-subject variable in the analysis of food intake, body weight changes, gene expression, sucrose and saccharin intake and preference, EPM and food preferences. The generalized linear model with repeated measures (generalized estimating equations, GEE) was used to analyse the changes in the CPP and in the open field. In the CPP, the analysis included a between subject variable: group (chow, HFS-T and HFS-C) and one within-subjects variable (test: baseline and test). In the open field, the

analysis included a between subject variable: group (chow, HFS-T and HFS-C) and one within-subjects variable (drug: vehicle and Quinpirole). The generalized linear model was chosen because it is a more flexible tool than the standard general linear model as it does not require samples to be homogenous but offers the opportunity to choose several types of distribution (Hardin and Hilbe, 2013). Normal distribution with identity as a link function was chosen in most cases to be the one that best fit the data. When normal distribution was not fitting, inverse Gaussian or Gamma were chosen depending on the AIC index in SPSS. As a method of estimation, the maximum likelihood (ML) was used in all cases. The significance of the effects was determined by the Wald chi-square statistic and for multiple comparisons the least significant difference was used. Statistical significance was accepted if $p < 0.05$. Effect sizes of the pairwise comparisons were calculated as Cohen's d.

3. Results

3.1. Adolescent cohorts

3.1.1. Food intake and body weight gain from PN 22–32: cohorts 1 to 3

Although between PN 22 and 32, the rats from different cohorts were treated in the same way, significant differences in the body weight on PN 22 and PN 32 were observed, and, for that reason, the analysis was performed separately for the different cohorts. Differences in body weight gain depending on the diet were observed in all cohorts. Rats in the HFS group (with access to the HFS diet as well as chow) increased their body weight significantly more than those fed only chow (Cohort 1: Wald χ^2 (1) = 3.91, $p < 0.05$, d = 0.85; Cohort 2: Wald χ^2 (1) = 25.3, $p < 0.001$, d = 2.25; Cohort 3: Wald χ^2 (1) = 6.75, $p < 0.01$, d = 0.83). The total amount of Kcal consumed was also higher in the HFS group in all cohorts (Cohort 1: Wald χ^2 (1) = 11.5; $p < 0.001$, d = 1.34; Cohort 2: Wald χ^2 (1) = 70.4, $p < 0.001$, d = 3.13; Cohort 3: Wald χ^2 (1) = 51.3, $p < 0.001$, d = 2.25), with the preference for the HFS diet in these rats being above 90% (see Table 1 for the descriptive data). The subgroup of rats sacrificed on PN 32 did not differ in body weight at this time (Table 2), but showed differences in the absolute and relative white adipose tissue (WAT) weight (Wald χ^2 (1) = 16.62, $p < 0.001$, d = 2.29 and Wald χ^2 (1) = 29.75, $p < 0.001$, d = 3.09, respectively), demonstrating that the intake of HFS diet increases the amount of WAT significantly.

3.1.2. Gene expression results at PN 32

We hypothesized that transient and continuous exposure to HFS-chow diet would impact on the expression of neurotransmitters in brain areas critically involved in reward. For this reason, we explored the expression of genes linked to the dopaminergic and opioid systems (Table 1 in Supplementary material). We chose genes that are up or downregulated after long periods of HF diet exposure (i.e. Vucetic et al., 2011; Vucetic et al., 2012) with the aim to see whether we could detect differences in expression patterns in response to transient and continuous access to the HFS-chow diet. We decided to analyse genes related to the degradation of dopamine: Catechol-O-Methyltransferase (COMT) in prefrontal cortex (PFC), and genes involved in the modulation and transmission of the dopamine signaling: dopamine receptor D1 (D1R), dopamine receptor D2 (D2R), and dopamine- and cAMP-regulated phosphoprotein (DARPP-32) in Nucleus Accumbens (NAcc) and PFC (only DARPP-32). We also studied Mu and Kappa opioid receptors (MOR and KOR respectively) in the Dorsal Striatum (Str) and NAcc.

Although many genes were analysed, at PN 32, only an increase in COMT gene expression was observed in the PFC of HFS-T rats relative to those fed chow (Wald χ^2 (1) = 3.8, p = 0.05, d = 1.08, Table 3).

Table 1
Body weight gain, food intake and food preference between PN 22 to PN 32 in the different cohorts of rats.

	Diet	BW gain	Total kcal	% chow	% HFS diet
Cohort 1	Chow	64.1 ± 3.6	45.1 ± 1.6		
(n = 24)	HFS	73.3 ± 2.9*	55.5 ± 2.3***	7 ± 1.3	93 ± 1.3
Cohort 2	Chow	59.2 ± 1.5	43.3 ± 1.9		
(n = 26)	HFS	71.2 ± 1.4***	59.8 ± 1.3***	6.3 ± 0.5	93.7 ± 0.5
Cohort 3	Chow	61.8 ± 3.5	44.5 ± 0.7		
(n = 48)	HFS	74.3 ± 2.8**	58.7 ± 1.3***	8.4 ± 1.3	91.5 ± 1.3
Cohorts 4–5	Chow	45 ± 1.6	35.2 ± 1.1		
(n = 50)	HFS	56.8 ± 1.6***	51.2 ± 2.6***	5.8 ± 0.7	94.2 ± 0.7

Results expressed as mean ± SEM. * Significantly different from Control (Chow). Always: 1 symbol $p < 0.05$; 2 symbols $p < 0.01$ and 3 symbols $p < 0.001$. BW: body weight.

3.1.3. Behavioral effects during the adolescent period (PN 32–PN 40)

3.1.3.1. Sucrose preference: PN 34–35. A significant reduction in sucrose intake was observed in the rats fed HFS diet. A difference between groups in sucrose consumption was observed on day 2 and there was trend to significance on day 3 (Day 2: Wald χ^2 (2) = 9.1, $p < 0.01$ and Day 3: Wald χ^2 (2) = 5.9, p = 0.051). HFS-T and HFS-C rats consumed less sucrose than chow fed rats on day 2 ($p < 0.05$, d = −1.08; $p < 0.01$, d = −1.13, respectively), and the effect was similar on day 3 for HFS-C ($p < 0.05$, d = −0.98) (Fig. 2). There were no differences in the intake of water. The preference for sucrose over water was only statistically significant on day 2 (Wald χ^2 (2) = 5.9, $p < 0.05$). Thus, rats with access to the HFS diet showed reduced consumption and preference for sucrose compared to the Chow rats (HFS-T: $p < 0.05$, d = −1.12 and HFS-C: $p < 0.05$, d = −0.98). This difference might be explained by compensation for the amount of sucrose in the HFS diet. However, rats in HFS-T group did not have access to HFS diet during the days of the saccharin test.

3.1.3.2. Saccharin preference: PN 34–35. On testing days 2 and 3, there were differences between groups in the consumption of saccharin (Wald χ^2 (2) = 6.9, $p < 0.05$ and Wald χ^2 (2) = 6.7, $p < 0.05$ respectively), without differences in the water consumed. On day 2, the pairwise comparisons showed that HFS-C rats consumed less saccharin than HFS-T rats, and the same tendency was observed versus the chow fed rats ($p < 0.05$, d = −1.11 and p = 0.086, d = −1.85 respectively). The tendency became significant on day 3, when rats in the HFS-C group consumed less saccharin than the control-chow animals ($p < 0.05$, d = −1.66, see Fig. 3). The study exploring the preference of saccharin over water showed a significant group effect (Day 2: Wald χ^2 (2) = 13.74, $p < 0.001$ and Day 3: Wald χ^2 (2) = 14.0, $p < 0.001$). The group comparison demonstrated that HFS-C rats had a lower preference for the saccharin compared to chow fed rats on days 2 and 3 (day 2: $p < 0.001$, d = −2.26; day 3: $p < 0.001$, d = −2.14), while the HFS-T rats showed a significant difference only on day 3 ($p < 0.01$, d = −1.2). The results suggest that rats with access to a HFS diet find saccharin less rewarding than rats consuming normal chow. Due to the fact that saccharin does not have caloric value, the results would suggest that there is a reduction in the rewarding properties of the sweet solution in HFS exposed rats (Fig. 3).

3.1.3.3. Conditioned place preference (CPP): PN 33–45. The pre-test showed that the rats had a strong preference for one of the compartments (Wald χ^2 (1) = 49.8, $p < 0.001$), and a "biased design" was chosen. The time spent in the non-preferred compartment during habituation was significantly increased in the CPP-test (Wald χ^2 (1) = 36.2, $p < 0.001$), without differences between groups, suggesting that all the animals found the chocolate rewarding (Fig. 4A). The analysis of chocolate consumption (average intake of chocolate days 1–10 of conditioning, Fig. 4C) showed that the HFS-C group consumed less chocolate than the HFS-T and control chow-fed rats (Wald χ^2 (2) = 7.1, $p < 0.05$; d = −0.98 HFS-C compared to chow-fed rats).

3.1.3.4. Locomotor activity – Quinpirole: PN 36–41. All the rats injected with quinpirole showed an increase in the distance travelled (Wald χ^2

Table 2
Body weight and white adipose tissue at PN 32 and PN 62–63.

	PN 32			PN 62–63		
Group	BW	aWAT	rWAT	BW	aWAT	rWAT
Chow	132 ± 6.1	0.65 ± 0.05	0.49 ± 0.03	343 ± 12	3.7 ± 0.5	1.1 ± 0.2
HFS-T	136 ± 5.1	1.10 ± 0.10***	0.80 ± 0.05***	390 ± 20*	5.4 ± 0.9	1.3 ± 0.2
HFS-C				408 ± 14**	7.8 ± 0.7***/+	1.9 ± 0.2***/++

Results expressed as mean ± SEM. The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until adult. * Significantly different from Control (Chow), + significantly different from HFS-T. Always: 1 symbol $p < 0.05$; 2 symbols $p < 0.01$ and 3 symbols $p < 0.001$. PN 32: chow (n = 6) and HFS (n = 7). PN 62–63: chow (n = 7), HFS-T (n = 8) and HFS-C (n = 8). BW: body weight; aWAT: absolute gonadal adipose tissue weight (g); rWAT: aWAT/BW.

(1) = 15.09, $p < 0.001$) and in the time spent in the central part of the arena (Wald χ^2 (1) = 21.3, $p < 0.001$) compared to the vehicle condition. However, no differences dependent on the diet were observed (Fig. 4B and D).

3.1.3.5. EPM: PN 45. There were no differences between groups in any of the measurements. % time in open arms: chow: 35.4 ± 8.8; HFS-T: 40.2 ± 4.1; HFS-C: 34.5 ± 5.0 and % time in closed arms: chow: 38.0 ± 8; HFS-T: 37.6 ± 2.6; HFS-C: 34.9 ± 3.9.

3.2. Adult cohorts

3.2.1. Food intake and body weight gain from PN 22–32: cohorts 4 and 5

The rats from cohorts 4 and 5 were originally from the same litters and hence rats from these cohorts were siblings. For this reason, the body weight changes and food intake from PN 22 to PN 32 were analysed as if all the rats were in the same cohort. As observed in cohorts 1–3, those rats fed HFS diet in addition to chow, showed a higher increase in body weight (Wald χ^2 (1) = 20.7, $p < 0.001$; d = 1.4) (see Table 1 for the descriptive data). The total amount of kcal consumed was higher in the HFS group (Wald χ^2 (1) = 20.6, all $p < 0.001$; d = 1.24 Table 1), with a 94% of preference for HFS diet over chow.

The subgroup of rats sacrificed at PN 62–63 showed differences in the absolute body weight (Wald χ^2 (2) = 10.0, $p < 0.01$). Rats with access to HFS diet showed a higher body weight than Chow fed rats (HFS-T: $p < 0.05$, d = 1.07; HFS-C: $p < 0.01$, d = 1.89, see Table 2). Furthermore, a significant increase in absolute and relative WAT (Wald χ^2 (2) = 18.0, $p < 0.001$ and Wald χ^2 (2) = 15.7, $p < 0.001$, respectively) was observed in rats with access to HFS diet until PN 62–63 compared to chow fed and HFS-T rats (Table 2). Absolute WAT: HFS-C compared to control chow fed rats ($p < 0.001$, d = 2.5), and HFS-C compared to HFS-T ($p < 0.05$, d = 0.87). Relative WAT: HFS-C compared to control chow-fed rats ($p < 0.001$, d = 1.56), and compared to HFS-T ($p < 0.01$, d = 1.13).

3.2.2. Gene expression results at PN 62–63

In rats sacrificed at PN 62 and 63, there was a group effect regarding gene expression of DARPP-32 and D1R in the PFC (Wald χ^2 (2) = 9.65, $p < 0.01$ and Wald χ^2 (2) = 7.26, $p < 0.05$, respectively). We found that rats in the HFS-C group had an increased expression of DARPP-32 compared to the HFS-T group ($p < 0.01$, d = 1.41), and a decreased expression of D1R, also compared to HFS-T group ($p < 0.01$, d = −1.45). In the NAcc, a reduced gene expression of KOR was observed in the HFS-T and HFS-C rats compared to the chow fed rats (Wald χ^2 (2) = 6.05, $p < 0.05$; HFS-T versus chow: $p < 0.05$, d = −1.02 and HFS-C versus chow: $p < 0.05$, d = −1.47) (Table 3).

3.2.3. Behavioral effects during the adult period (PN 58–101)

3.2.3.1. Sucrose preference: PN 63–65. The rats tested during the adult period did not show any difference in their sucrose preference. This suggests that after removing (HFS-T) or extending (HFS-C) the HFS diets, the rats recover their preference for sucrose. % of sucrose preference day 2: chow: 76.8 ± 7.1; HFS-T: 77.7 ± 5.0 and HFS-C: 79.7 ± 3.9. % sucrose preference day 3: chow: 82.2 ± 3.4; HFS-T: 75.5 ± 3.8 and HFS-C: 81.6 ± 4.4.

3.2.3.2. Saccharin preference: PN 60–63. The group of rats tested in the adult period showed differences in saccharin consumption on day 3 and a trend to significance on day 2 (Day 2: Wald χ^2 (2) = 4.6, p = 0.095 and Day 3: Wald χ^2 (2) = 11.09, $p < 0.01$). Similar to the adolescent results, rats in HFS-C group drank less saccharin than chow fed rats on day 3 ($p < 0.001$, d = −1.2). A group effect in water consumption was observed on day 3 (Wald χ^2 (2) = 9.6, $p < 0.01$) and Chow fed

Table 3
Impact of HFS diet on gene expression in brain areas relevant to reward.

Area	Gene	PN 32			PN 62–63			
		Chow	HFS		Chow	HFS-T	HFS-C	
PFC	*Comt*	1.00 ± 0.03	1.11 ± 0.05	**P = 0.05**	1.00 ± 0.04	0.95 ± 0.06	1.09 ± 0.09	NS
	D1r	1.01 ± 0.09	0.85 ± 0.06	P = 0.093	1.02 ± 0.08	1.22 ± 0.11	0.89 ± 0.05+	**P = 0.027**
	D2r	1.04 ± 0.13	0.97 ± 0.04	NS	1.01 ± 0.07	1.18 ± 0.13	0.93 ± 0.05	NS
	Darpp32	1.02 ± 0.90	0.98 ± 0.05	NS	1.00 ± 0.03	0.81 ± 0.10	1.15 ± 0.08 +	**P = 0.008**
NAcc	*D1r*	1.01 ± 0.06	0.84 ± 0.13	NS	1.00 ± 0.04	1.07 ± 0.07	1.03 ± 0.04	NS
	D2r	1.02 ± 0.09	0.81 ± 0.15	NS	1.00 ± 0.04	1.07 ± 0.07	1.03 ± 0.04	NS
	Mor	1.01 ± 0.07	1.11 ± 0.25	NS	1.00 ± 0.06	0.93 ± 0.07	0.85 ± 0.04	NS
	Kor	1.00 ± 0.05	0.94 ± 0.11	NS	1.01 ± 0.06	0.88 ± 0.04*	0.84 ± 0.03*	**P = 0.048**
	Darpp32	1.01 ± 0.08	0.86 ± 0.13	NS	1.01 ± 0.06	0.88 ± 0.06	0.80 ± 0.09	NS
Striatum	*Mor*	1.06 ± 0.15	0.76 ± 0.07	P = 0.052	1.08 ± 0.17	1.05 ± 0.13	1.24 ± 0.20	NS
	Kor	1.01 ± 0.06	0.96 ± 0.06	NS	1.00 ± 0.03	1.04 ± 0.06	1.04 ± 0.05	NS

Results are expressed as relative fold change in mRNA expression, means ± SEM. The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until adult. + Significantly different vs HFS-T; * Significantly different vs controls (chow). PN 32: chow (n = 6) and HFS (n = 7). PN 62–63: chow (n = 7), HFS-T (n = 8) and HFS-C (n = 8). The statistically significant p values (= or < to 0.05) are shown in bold characters.

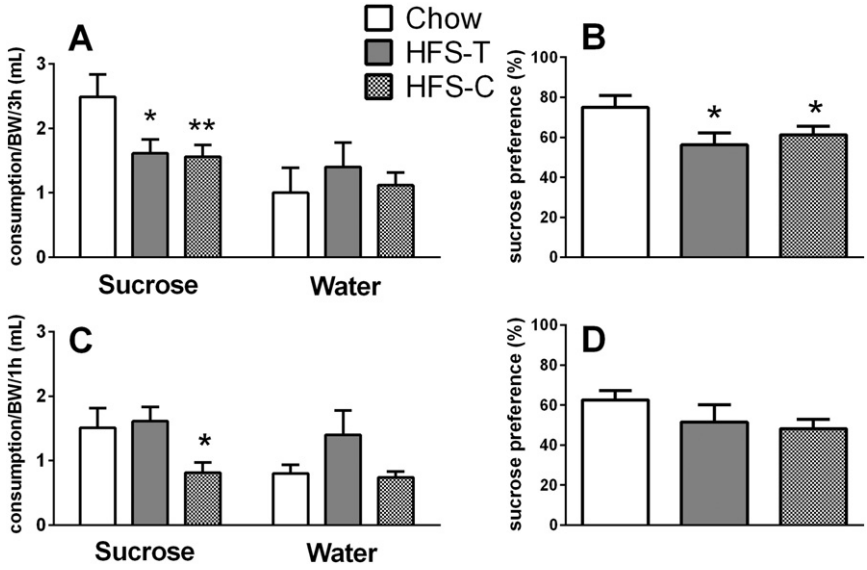

Fig. 2. Sucrose (1%) consumption and preference on PN 34–35. The panels on the top show the data on the second testing day (3 h/access to sucrose) and the bottom panels represent the third day of test (1 h/access to sucrose). The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until the end of the experiment. Consumption of sucrose and water (A, C), and preference for sucrose over water (B, C) are shown. Mean and SEM are represented (n = 9–18/group, cohort 3). *Significantly different from Chow (control). 1 symbol always $p < 0.05$; 2 symbols always $p < 0.01$.

rats drank more water than HFS-C rats ($p < 0.01$. $d = -1.7$). On the other hand, no differences in the preference for saccharin over water were observed in the adult period. Surprisingly, the diet effect on saccharin consumption differed from that on sucrose consumption in the adult rats. The results demonstrate that HFS-C rats express symptoms of a long term anhedonia, since they are less interested in saccharin than HFS-T and chow rats (Fig. 5).

3.2.3.3. Conditioned place preference (CPP): PN 67–80. As it was the case in the early period, the pre-test showed that the rats had a strong significant preference for one of the compartments (Wald χ^2 (1) = 22.19, $p < 0.001$), and a "biased design" was chosen. Time spent in the non-preferred compartment during habituation was significantly increased in the CPP-test (Wald χ^2 (1) = 46.43, $p < 0.001$), suggesting that all the animals found the chocolate rewarding (Fig. 6A). Also, a significant

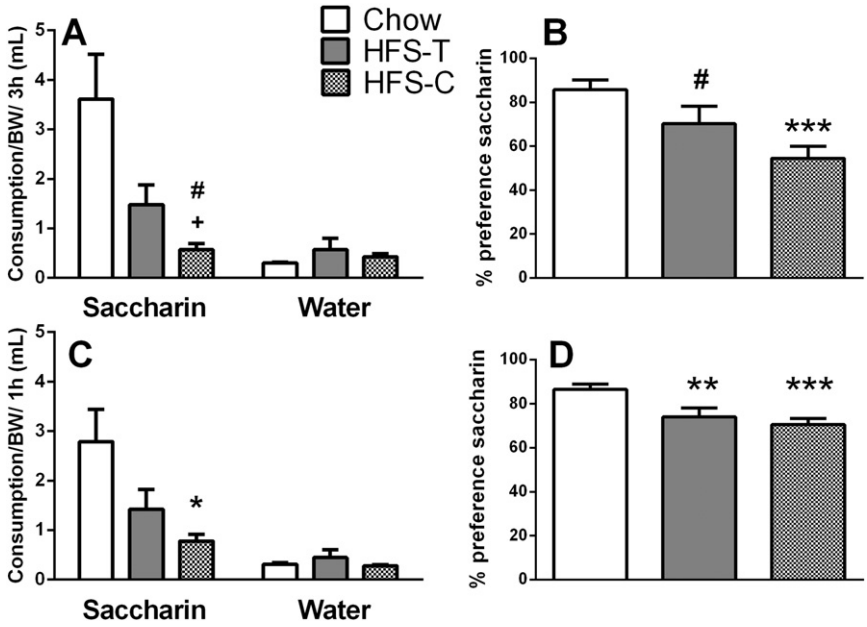

Fig. 3. Saccharin (0.1%) consumption and preference on PN 34–35. The panels on the top show the data on the second testing day (3 h/access to saccharin) and the bottom panels represent the third day of test (1 h/access to saccharin). The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until the end of the experiment. Consumption of saccharin and water (A, C), and preference for saccharin over water (B, C) are shown. Mean and SEM are represented (n = 8–9/group, cohort 2). *Significantly different from Chow (control), + significantly different from HFS-T and # trend to significance from Chow. 1 symbol always $p < 0.05$; 2 symbols always $p < 0.01$ and 3 symbols always $p < 0.001$.

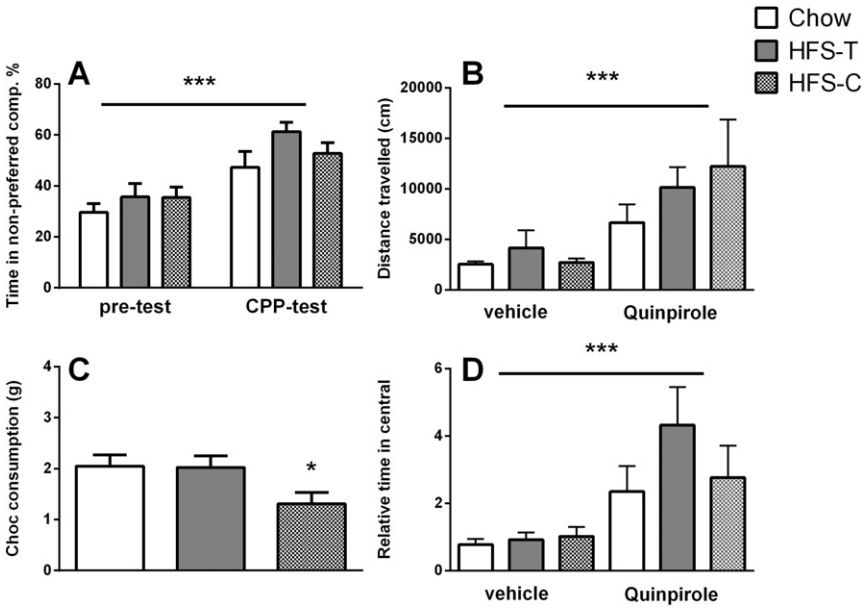

Fig. 4. CPP for chocolate on PN 33–45 (A, C) and locomotor activity in the open field after quinpirole injection on PN 36–41 (B, D). Independent groups of rats were exposed to each test. The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until the end of the experiment. Mean and SEM are represented (CPP: n = 8 rats/group, cohort 1; locomotor activity: n = 8–9 rats/group, cohort 2). CPP test: All rats increase the time spent in the compartment where they received chocolate in the CPP-test independently on the diet (Panel A) ***$p < 0.001$ between pre-test (baseline) and CPP-test. The average of chocolate/day ate by HFS-C group was significantly lower than chow and HF-T rats (Panel C) *$p < 0.05$ between HFS-C and Chow (control). Locomotor activity: quinpirole (0.5 mg/kg) injection increased the distance travelled (Panel B) and the time spent in the central part of the open field (Panel D) independently on the diet (***$p < 0.001$, quinpirole versus vehicle).

difference dependent on the diet was observed (Wald χ^2 (2) = 7.46, $p = 0.024$). Further comparisons demonstrated that HFS-C rats always spent less time in the non-preferred compartment, but the change after the training was not different from the other two groups. No differences were observed in chocolate consumption in the adult rats during the training (Fig. 6C).

3.2.3.4. Locomotor activity-quinpirole: PN 64–70. As for the cohort tested in adolescence, all rats injected with quinpirole showed an increase in the distance travelled (Wald χ^2 (1) = 9.5, $p < 0.01$) and in the time spent in the central part of the arena (Wald χ^2 (1) = 5.4, $p < 0.05$) compared to the vehicle condition (Fig. 6B and D). However, no differences dependent on the diet were observed.

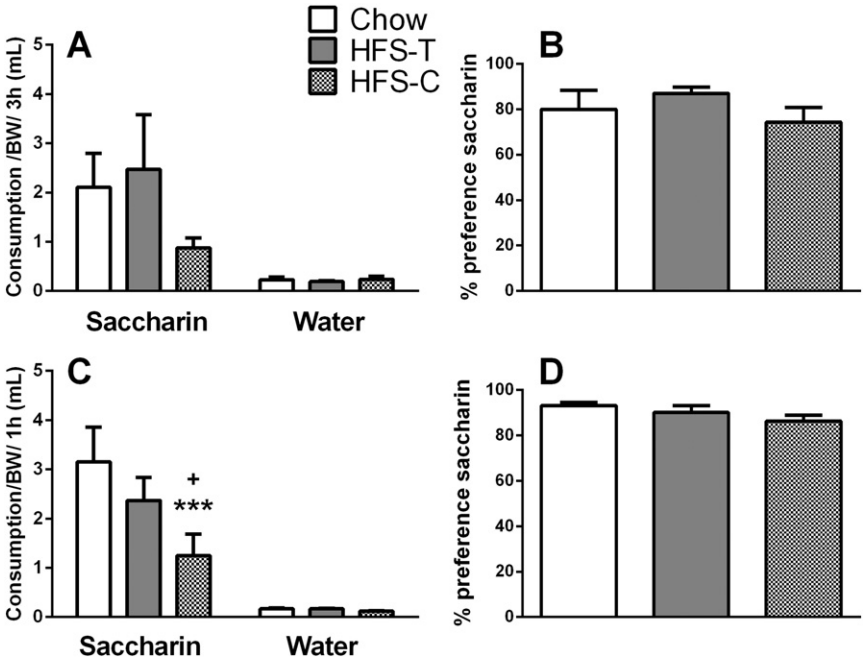

Fig. 5. Saccharin (0.1%) consumption and preference on PN 62–63. The panels on the top show the data on the second testing day (3 h/access to saccharin) and the bottom panels represent the third day of test (1 h/access to saccharin). The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until the end of the experiment. Consumption of saccharin and water (A, C), and preference for saccharin over water (B, C) are shown. Mean and SEM are represented (n = 8–9/group, cohort 5). *Significantly different from Chow (control), + significantly different from HFS-T. 1 symbol always $p < 0.05$; 2 symbols always $p < 0.01$ and 3 symbols always $p < 0.001$.

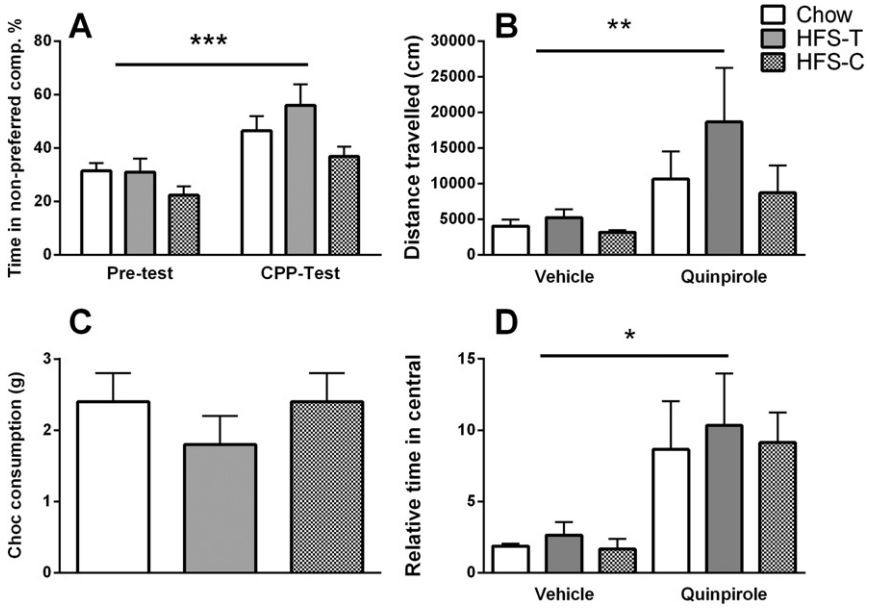

Fig. 6. CPP for chocolate on PN 67–80 (A, C) and locomotor activity in the open field after quinpirole injection on PN 64–70 (B, D). The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until adult. Independent groups of rats were exposed to each test. Data are expressed as Mean ± SEM (CPP: n = 8 rats/group, cohort 4; locomotor activity: n = 8 rats/group, cohort 5). CPP test: All rats increase the time spent in the compartment where they received chocolate in the CPP-test independently on the diet (Panel A) ***p < 0.001 between pre-test (baseline) and CPP-test. No differences were observed in the daily average of chocolate eaten by different groups (Panel C). Locomotor activity: quinpirole (0.5 mg/kg) injection increased the distance travelled (Panel B) and the time spent in the central part of the open field (Panel D) independently on the diet (**p < 0.01 and *p < 0.05 respectively, quinpirole versus vehicle).

3.2.3.5. EPM: PN 58. There were no differences between groups in any of the measurements. % time in open arms: chow: 44.6 ± 3.1; HF-T: 43.3 ± 3.4; HF-C: 36.3 ± 4.8 and % time in closed arms: chow: 39.5 ± 3.2; HF-T: 39.4 ± 2.6; HF-C: 49.3 ± 5.1.

3.2.3.6. Food preference: PN 95–102. Differences between groups were observed in the total amount of kcal eaten during the 8 days of ad libitum access to both diets (Wald χ^2 (2) = 6.5, p < 0.05). Rats in HFS-T group consumed more kcal than HFS-C rats but not more than control chow-fed rats (HFS-T versus HFS-C: p < 0.05, d = 1.2) (Fig. 7). Also, the choice between diets differed depending on the group. Control chow and HFS-T groups consumed significantly less chow (group effect: Wald χ^2 (2) = 10.3, p < 0.01) and more HFS diet than the HFS-C rats (group effect: Wald χ^2 (2) = 19.8, p < 0.001). Chow intake: HFS-C versus Chow: p < 0.01; d = 1.5 and HFS-C versus HFS-T: p < 0.01, d = 1.6. HFS intake: HFS-C versus Chow: p < 0.001; d = −1.6 and HFS-C versus HFS-T: p < 0.001, p = −2.2. The percentage of preference for HFS diet during the 8 days of testing also differed between groups (Wald χ^2 (2) = 20.7, p < 0.001). The percentages of HFS preference over chow were as follows: control-chow: 92.2 ± 1.6; HFS-T: 93.7 ± 1.6 and HFS-C: 81.6 ± 2.8. The HFS-C group had a lower preference than Chow fed (p < 0.001, d = −1.75) and HFS-T (p < 0.001, d = −2.0).

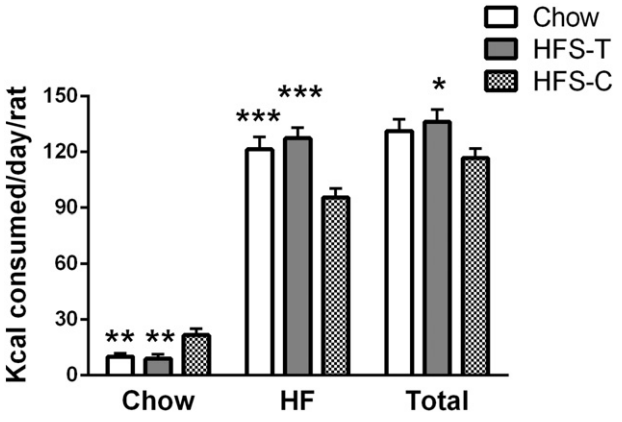

Fig. 7. Average of food intake in rats fed chow and HFS diet for 8 days (PN 95–102). The experimental groups are chow fed rats and rats offered access to a high fat and high sucrose diet superimposed on chow, offered either transiently (HFS-T, for 10 days after weaning) or continuously (HFS-C) until adult. Mean and SEM are represented (n = 8 rats/group, cohort 5). Chow and HFS-T groups ate less chow: **p < 0.01; and more HFS diet: ***p < 0.001 than HFS-C. The total amount of kcal was only increased in the HFS-T rats versus HFS-C, *p < 0.05.

4. Discussion

We sought to determine whether offering rats a palatable HFS diet during the post- weaning period, either transiently for 10 days or continuously until adult life, has any short- or long-term consequences for reward behaviors linked to dopaminergic function or on anxiety-like behaviors. The HFS diet was offered in an ad libitum choice situation as a supplement to regular chow from post-natal day 22. This day was chosen because it corresponds to a time point when pups are first exposed to solid foods and stop being dependent on their mother for nutrition. Furthermore neurogenesis of dopaminergic and hypothalamic pathways has largely taken place by then (Andersen, 2003; Bernheim et al., 2013; Bouret et al., 2004; Markakis, 2002; Van den Heuvel and Pasterkamp, 2008). Our data support the hypothesis that exposure to diets rich in fat and sucrose, whether transient during the adolescent period or continued into adult life, induces immediate changes in the reward function, evident in the behavioral tests. We did not, however, observe any long-term reprogramming of reward behavior in rats transiently exposed to this diet during the post-weaning period.

Obesogenic effects of short term (10 day) exposure to the HFS diet during the post-weaning period were evident in the adolescent rats, as shown previously in adult rats (la Fleur et al., 2007). In comparison to control chow-fed rats, on PN 32, the group of rats with access to HFS diet consumed more energy, gained more body weight and had more gonadal fat mass. These differences in adiposity and body weight between diets were even more evident in the subgroup of rats that

continued on the diets until adult life and were sacrificed on PN 62–63. Interestingly, the body weight phenotype of rats with transient (10 day adolescent) HFS access that were sacrificed when adult (at PN 62–63) was intermediate between that of the chow control rats and those with long-term access to the HFS diet. This effect is unlikely to be explained by differences in energy intake, since feeding was monitored the week before the sacrifice and there were no differences between the HFS-transient group and chow controls. Thus, the rats transiently fed HFS during the post-weaning period may sustain an elevated body weight that was already evident by 10 days of exposure to the HFS diet. We hesitate to draw strong conclusions about the effect of transient HFS feeding on long-term body weight, however, as such effects have not been consistently observed in all of our cohorts.

Sweet solutions are highly rewarding for rats, verified here by a 60–90% preference for sucrose or saccharin solution over water in control groups. Whereas both preference tests can be used to assess anhedonia, sucrose preference can also be linked to diet. In general, the preferences seen on 2 consecutive days were rather reproducible, with the exception of the HFS-transient group, whose preference appeared to be marginally influenced by the fact HFS diet access had just newly been terminated (two days earlier). Thus, rats with access to the HFS diet continuously from PN 22 and tested on PN 33–35 (while still on this diet) showed reduced intake and preference for sucrose and saccharin, compared to control chow-fed rats. The HFS-transient group, without access to the HFS diet from PN 32 onwards, showed a reduced intake and preference for sucrose on the first test day (PN 34), while their preference for saccharin was reduced on both testing days (PN 34–35). Therefore, 10 days access to the HFS diet was sufficient to reduce preference for sweet solutions, consistent with an increased anhedonia (a core reward symptom of major depression that reflects the inability to experience pleasure with things that were pleasant before). Thus, our results might indicate a state of reward hypofunction already after a very short dietary intervention in early adolescence.

Interestingly, the effects of diet on sweet preference tested in adolescence were observed for both sucrose (nutritive) and saccharine (non-nutritive), suggesting that this is not a compensatory reduction in energy intake linked to the high sugar intake associated with the HFS diet. Our results are similar to those reported previously for two bottle-drinking paradigms for sucrose or saccharin, but after longer periods of high fat diet exposure (>6 weeks) (Carlin et al., 2013; Chen et al., 2010; Vucetic et al., 2011). In line with this, a reduced motivation to obtain sucrose pellets has been observed in rats and mice fed a high fat diet for prolonged periods (Davis et al., 2008; Finger et al., 2012; Íbias et al., 2015; Tracy et al., 2015). In our CPP experiment, the data support the idea that exposure to fat, in this case the HFS diet, impacts on the consumption of sweet treats as chocolate consumption was lower in the HFS group during CPP training in the adolescent cohort. However, to our knowledge, this is the first dataset demonstrating that reduced preference for palatable solutions and food can already be observed after only 10 days of HFS diet exposure during early adolescence.

We also explored preference for sweet flavours in the adult rats. The intake and preference for sucrose were not affected by the diet when the groups were tested on PN 62–63, while the preference for saccharin was reduced only in the HFS-continuous group. The lack of effects in the HFS-transient group was not completely unexpected, since mice that have been on a high fat diet recover their sweet preference within 4 weeks of chow feeding (Carlin et al., 2013). The reason why saccharin but not sucrose was sensitive to long term exposure to the HFS diet is not entirely clear. Pre-exposure to sucrose in rats fed a high fat diet has been shown to prevent their lack of motivation to obtain sucrose in a progressive ratio paradigm (Tracy et al., 2015). The HFS diet used in our experiment contains very high amounts of sucrose (34% of the weight). Thus, in our study, the HFS rats are, in fact, exposed to sucrose (but not saccharin) throughout and this would be expected to impact upon their subsequent sucrose preference. Notably, even though HFS rats reduced their sucrose and saccharin preference compared to chow controls, they preferred the sweet solutions over water, indicating that these solutions were still rewarding for the rats. Nevertheless, the main conclusion from these preference studies in adult rats is that access to HFS diet induces some degree of anhedonia. Supportively, other studies have shown an increase in immobility in the forced swim test in mice after 12 weeks of high fat diet exposure (Kaczmarczyk et al., 2013; Sharma and Fulton, 2013), strengthening the idea of depressive-like behavior induced by HFS diets.

In the different dietary groups, condition place preference (CPP) tests were used to explore food reward behavior for chocolate. Exposure to the HFS diet did not modify the CPP acquisition during adolescence or in adulthood, and all groups of rats found the chocolate compartment more rewarding than the one paired with normal chow after training. In line with our results, Morales et al. (2012) observed that adult mice fed a high fat diet, showed preference for the compartment linked to a palatable treat in a CPP procedure similar to ours. However, the preference for this compartment was lower in high fat fed mice than in mice fed normal chow diet. Although differences between groups were not observed in our experiment, small differences in the procedures could explain these discrepancies. Collectively our CPP results , taken together with the saccharin and sucrose preference data, suggest that the ability of the rats to experience reward from sweet treats is not abolished by HFS exposure but may be slightly reduced.

The mesolimbic dopamine system is critically involved in locomotor activity induced by dopaminergic agonists. In the present study, dopamine-linked locomotor activity induced by quinpirole (Horvitz et al., 2001), a D2/D3 receptor agonist, was explored in the open field test. It has been reported that rats fed a high fat diet are more sensitive than chow fed rats to the yawning induced by low doses (0.01–0.03 mg/kg) of quinpirole (Baladi and France, 2009). We observed a quinpirole-induced hyperlocomotor response in all rats but there was no difference between groups, suggesting that dopaminergic function in the ventral striatum is preserved independently of the diet. Quinpirole also induced anxiolytic-like behavior, increasing the time spent in the central zone of the open field, but again, the effect was not diet-related. In line with this, there were no changes in anxiety-like behavior measured in the elevated plus maze in adolescence or in the adult period.

Hyperlocomotor activity induced by D1 and D2 receptor agonists engages different striatal areas including the NAcc (Gong et al., 1999; Ikemoto, 2002). In the present study, we explored changes in gene expression, including for dopamine-related genes, in these areas. In line with the results observed in the open field, there were no changes in the expression of dopamine-related genes in the NAcc after 10 or 40 days of access to the HFS diet. In fact, HFS diet exposure induced very few changes in gene expression, when compared to the chow diet. These results are surprising, since it has been demonstrated that feeding high fat diets can induce many changes in dopaminergic and opioid signaling in brain areas relevant to reward and locomotor activity (South and Huang, 2008; Teegarden et al., 2009; Vucetic et al., 2012, 2011). However, these differences may be linked to the length of the dietary intervention and the specific techniques to study the effects of the diet (Carlin et al., 2013; Vucetic et al., 2012).

The overarching collective result of the gene analysis (in PFC, Striatum and NAcc) is that very few genes showed changes in expression between the dietary groups and for those that did, the changes were very small, even if significant. We highlight one finding, that KOR expression in the NAcc was slightly decreased by HFS exposure irrespective of whether it was offered only transiently during the adolescent period or continuously into adulthood. The stimulation of KOR decreases dopamine release in the NAcc (Di Chiara and Imperato, 1988) and so our results might be a compensatory reaction to other changes in dopaminergic function.

One other gene dataset worthy of mention is the small increase in COMT in the PFC, which appeared to be linked to HFS exposure in adolescent rats (i.e. those sacrificed immediately after the 10 days of HFS

choice diet). COMT participates in the inactivation of cathecolamines, including dopamine. However, the changes in COMT did not persist in the adult period in any of the HFS groups (transient or continuous), demonstrating that the changes in COMT are short in duration. On the other hand, in the groups of rats sacrificed in the adult period (PN 62–63), some differences between HFS transient and HFS continuous were observed in the PFC. DARPP32 protein is highly expressed in neurons containing D1R (Rajput et al., 2009), and is phosphorylated in response to dopamine through the stimulation of D1R (Nishi et al., 1997). As reported previously (Carlin et al., 2013; Sharma and Fulton, 2013), PFC expression of these genes was inversely related in rats exposed to high fat and high sucrose diets. However, they found large differences in expression of these genes in high fat diet fed rats relative to the control diets, which was not the case in our study.

We also hypothesized that early life exposure to HFS diet could impact on subsequent food choice when they become adult. Using a similar design, Teegarden et al. (2009) observed an increase in preference for high fat diet in adult mice exposed to this diet for one week after weaning. Surprisingly, we did not find a similar effect, as HFS diet consumption in adult rats exposed to this diet in adolescence was not different from chow controls. On the other hand, the rats with long-term access were less interested in the HFS than the other groups, likely due to lack of novelty of the diet.

In conclusion, we show that exposure of rats to an obesogenic Western diet, rich in fat and sugar, for only 10 days during the post-weaning period, can have detrimental effects on energy balance, and on some behaviors important for reward and emotional reactivity. Exposure to an obesogenic diet reduced reward from sweet treats, which is consistent with reward hypofunction hypothesis of obesity and increased anhedonia. The ability of rats to experience reward from chocolate was not influenced by dietary manipulation, in adolescence or in adult life. Also, we found little evidence to support the idea that anxiety-like behavior is influenced by exposure to obesogenic diets on the adolescent period. Interestingly, the anhedonia was already evident after 10 days of HFS diet, and this effect were not strengthened but attenuated (i.e. sucrose preference and chocolate intake) when the HFS diet was extended until the adult period. The fact that the short-term effects of HFS diet on these reward-linked behaviors disappear if the animals remain on the diet until adulthood, suggests a possible effect of the diet to cause neurobiological adaptations over time. However, the expression of reward- related genes in brain areas critically involved in reward did not show large differences between groups and we do not know the extent to which these contribute to the behavioral changes. Thus, short term exposure to an obesogenic diet in adolescence may impact on reward-linked behaviors. The effects on these behaviors are not worsened but attenuated when rats continue on that diet until the adult period, and the same effects are completely reversed when the HFS diet is removed, demonstrating the non-permanent effects of feeding HFS diets in adolescence.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2016.07.008.

Disclosure statement

The authors have nothing to disclose.

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