

Decreased rates of operant food self-administration are associated with reward deficits in high-fat feeding mice

Javier Íbias² · Miguel Miguéns² · Danila del Río¹ · Ismael Valladolid-Acebes^{1,5} · Paula Stucchi^{1,4} · Emilio Ambrosio³ · Miriam Martín¹ · Lidia Morales¹ · Mariano Ruiz-Gayo¹ · Nuria Del Olmo¹

Received: 19 December 2014 / Accepted: 29 June 2015 / Published online: 7 August 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Purpose Highly palatable foods behave as appetitive reinforcers and tend to be consumed compulsively. Nevertheless, the motivation for this kind of diets in experimental diet-induced obesity models has not been well established. Our hypothesis is that obesity caused by a regular consumption of high-fat diet (HFD) occurs concomitantly with the inhibition of food reward. The ultimate goal of our study was to further analyze the extent to which the perception of food as an appetitive reinforcer is a necessary condition for obesity.

Methods We have evaluated the influence of HFD on operant food self-administration (FSA) during a whole light–dark (12–12-h) cycle in mice that consumed HFD either during 1, 4 or 8 weeks. The study has been complemented by a two-bottle free-choice assay between tap water and sweetened drinks.

Results These data show that both 4- and 8-week HFD treatments induced a significant decrease in operant FSA rate. Moreover, HFD impaired the sweetened-conditioned flavor preference in the two-bottle choice assay.

Conclusion Our results, showing a reduction in how hard an animal is willing to work for food reinforcers, provide evidence that chronic consumption of HFD negatively contributes to the incentive motivation to acquire food/drink reinforcers. We demonstrate that energy homeostasis imbalance triggered by HFD is associated with the inhibition of hedonic feeding.

Keywords Food self-administration · High-fat diets · Reward · Two-bottle choice · Motivation · Obesity

Introduction

Obesity is a complex pathological condition that deals with both energy metabolism and feeding behavior disorders. It is widely assumed that obesity is a matter of excessive caloric intake and less attention has been paid to other aspects such as circadian distribution of meals and motivational components of food intake. Physiological mechanisms regulating feeding behavior are integral to the gut–adipose tissue–brain axis, and therefore, levels of adiposity and hormones such as leptin, cholecystokinin, ghrelin or insulin are involved in regulating both energy metabolism and appetite/satiety by acting on brainstem and hypothalamic nuclei as well as on mesocorticolimbic areas [1, 2].

Easy accessibility to palatable energy-dense food is considered a major environmental risk factor contributing to the recent surge in obesity [3–6]. Growing evidence suggests that the hedonic properties of food stimulate food consumption, even after energy requirements are met,

✉ Nuria Del Olmo
nolmo@ceu.es

¹ Laboratorio de Farmacología, Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad CEU–San Pablo, Campus de Montepríncipe, Boadilla del Monte, 28668 Madrid, Spain

² Departamento de Psicología Básica I, Facultad de Psicología, Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain

³ Departamento de Psicobiología, Facultad de Psicología, Universidad Nacional de Educación a Distancia (UNED), Madrid, Spain

⁴ Present Address: Departamento de Biología Celular, Instituto de Biología, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

⁵ Present Address: Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

leading to compulsive eating behavior, progressive body weight (BW) gain and obesity that some authors consider as “food addiction” [7–9]. A number of studies have evidenced that food and drugs of abuse share reward neural circuits that involve mesocorticolimbic nuclei [10–14].

Substance abuse is associated with the disruption in the circadian oscillation of physiological functions such as sleep, body temperature and hormone levels [15–17]. More recent studies have evidenced that food, considered both as a *zeitgeber* and as an element of potential abuse, simultaneously influences all circadian feeding behavior, energy homeostasis and reward processes, suggesting that motivation for food might be analyzed in a chronobiological context [18–22]. Other studies have shown that high-fat diets (HFD) enhance BW gain in animals with food availability restricted to the light phase and also trigger dramatic changes in the circadian pattern of clock gene expression [18, 23–25]. Closely related to that, we have reported that the inhibition of conditioned place preference (CPP) to cocaine/food triggered by HFD is linked to the disorganization of scheduled meals [26].

Our hypothesis is that exposition to HFD triggers a loss of the rewarding properties of food, at least during periods of feeding activity. Therefore, we have evaluated the influence of HFD on operant food self-administration during a whole light–dark (12–12-h) cycle, in order to detect eventual variations of circadian activity. The study was carried out in mice that consumed HFD either during 1, 4 or 8 weeks in order to identify the turning point from which animals start to perceive food as a non-rewarding stimulus. This study was complemented by a two-bottle free-choice assay between tap water and sweetened drinks. Our final goal was to further analyze the extent to which the perception of food as an appetitive reinforcer is a necessary condition for obesity.

Materials and methods

Animals and dietary treatment

Five-week-old C57BL/6 J male mice (CRIFA, Barcelona, Spain) weighing 16–18 g were housed under 12-h light/12-h dark cycle (lights on at 8:00 a.m., defined as *zeitgeber* 0, ZT0), in a temperature-controlled room (22 °C). Three cohorts of 18 mice for operant food self-administration (1-, 4- and 8-week on dietary treatment) and one additional group of 20 animals for the two-bottle choice drinking test were used. Every cohort was divided in two groups with similar average BW. Animals were housed three *per* cage and assigned (free access) either to a standard chow (control diet, CD; 18 % kcal from fat, 58 % kcal from carbohydrates and 24 % kcal from protein; 3.3 kcal/g, (Teklad

Table 1 Composition of control diet, high-fat diet and food-self administration (FSA) pellets

	Control diet	High-fat diet	FSA pellets
Energy density kcal/g	3.1	4.73	3.3
Calories from protein	24 %	20 %	24.1 %
Calories from fat	18 %	45 %	10.4 %
Calories from carbohydrate	58 %	35 %	65.5 %
Crude protein	18.6 %	20 %	19.9 %
Carbohydrate (available)	44.2 %	46.8 %	54.1 %
Fat (ether extract)	6.2 %	22.3 %	3.8 %
Crude fiber	3.5 %	5.8 %	4.4 %
Sucrose	No	20 %	3 %

Global 18 % Protein Rodent Diet) or to a HFD (D12451, 45 % kcal from fat, 35 % kcal from carbohydrates and 20 % kcal protein; 4.73 kcal/g; Research Diets, EEU). Table 1 shows the composition of diets used for this study. BW was monitored twice a week during dietary treatment. All experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (2010/63/EU). Protocols were approved by the local ethics committee of the Universidad CEU-San Pablo. All efforts were made to minimize the number of animals used and their suffering.

Operant food self-administration

Food self-administration (FSA) studies were carried out in six operant conditioning chambers (Med Associated Inc., Saint Albans, VT, USA) equipped with a lever connected to a food reservoir. Active lever presses resulted in a pellet delivery, while inactive presses had no programmed consequences. All lever presses were recorded. In response to an active press, a pellet (Test Diet 1811143; 5TUM MLab Rodent Tablet 20 mg; 10.4 % kcal from fat, 65.5 % kcal from carbohydrates and 24.1 % kcal protein; 4.73 kcal/g; Test Diet Limited BCM IPS Ltd, UK) was delivered from food reservoir and was available for the animal.

After 1-, 4- or 8-week dietary treatment, mice were introduced in self-administration cages. Autoshaping procedure (training) consisted of (1) initial 48-h food restriction (daily food intake was adjusted to 85 % basal food consumption), and (2) four consecutive daily sessions of autoshaping, previous to the differentiated diet offering. During 1-h sessions, animals had the option to press the left lever to obtain a pellet. After this period, animals were retired to their home cages, and the procedure was repeated in the following day. A minimum of 30 responses in the active lever was set as lever-press acquisition criterion. A mean of 52 ± 9 responses was recorded during the last session, and all animals reached the criterion on

the third session. After training, animals were shifted to the FSA protocol consisting in a single 23-h session during which animals were maintained under the same lighting program scheduled in the animal facility. Mice spent 23 h (ZT1–ZT24) in operant conditioning chambers and were allowed to press the retractable active lever on a fixed ratio 1 (FR-1). Responses on the active lever throughout the five first presses after each pellet delivery were recorded but not reinforced. The number of lever presses and the number of delivered and split pellets were counted. At the end of each session, mice were removed from the chamber and returned to their cages. As pellets delivered after lever presses were not immediately consumed, food intake was determined by calculating the difference between delivered food and food spillage. Food intake was normalized by BW as previously described [27, 28]. No external cues were applied. BW and food intake were daily monitored during both autoshaping and testing. Two days before re-testing, after 4- and 8-week dietary treatment, mice were submitted to 30-min autoshaping tests. The animals showed 15 ± 7 and 20 ± 10 responses, respectively, without differences between CD and HFD mice. Animals used for FSA experiments had water available ad libitum both during the training and the actual FSA protocol.

Two-bottle choice test

Mice ($n = 10$ per group) were given ad libitum access to water and CD/HFD rations during 3 weeks. After this period, animals were transferred to individual chambers for two-bottle test sessions during five additional weeks. During testing, mice had the same dietary regime, but they had the option between regular tap water and either sucrose (1 %) or saccharine (0.05 %) solutions, presented in graduated 100-mL glass bottles with a drinking well that extended 4 cm into the cage. Concentrations of sucrose and saccharin were chosen following the ones used by other authors [29–32]. Bottles were placed symmetrically on the food container, and they were switched every day to avoid position preference. BW and liquid consumption were monitored daily during 5 weeks at ZT4. Sucrose/saccharine preference ratio was calculated based on the equation: % preference = (volume sweetened drink/total volume consumed) \times 100.

Statistics

Data are reported as mean \pm SEM. Effects were evaluated using two- and three-factor ANOVA tests. Factors of variation were (1) *dietary treatment* (HFD or CD), (2) *zeitgeber time* (ZT) and (3) *weeks on treatment* (1, 4 or 8 weeks). Factors 2 and 3 were repeated-measures factors. *Post hoc* comparisons were carried out by using Bonferroni's

adjusts. Data from the two-bottle choice test were analyzed using a two-factor ANOVA with repeated measures on the second factor (5-week two-bottle choice protocol). All statistical analyses were carried out using the SPSS 17.0 statistical package (IBM Corporation, Armonk, NY, USA). Differences were considered significant if $p < 0.05$.

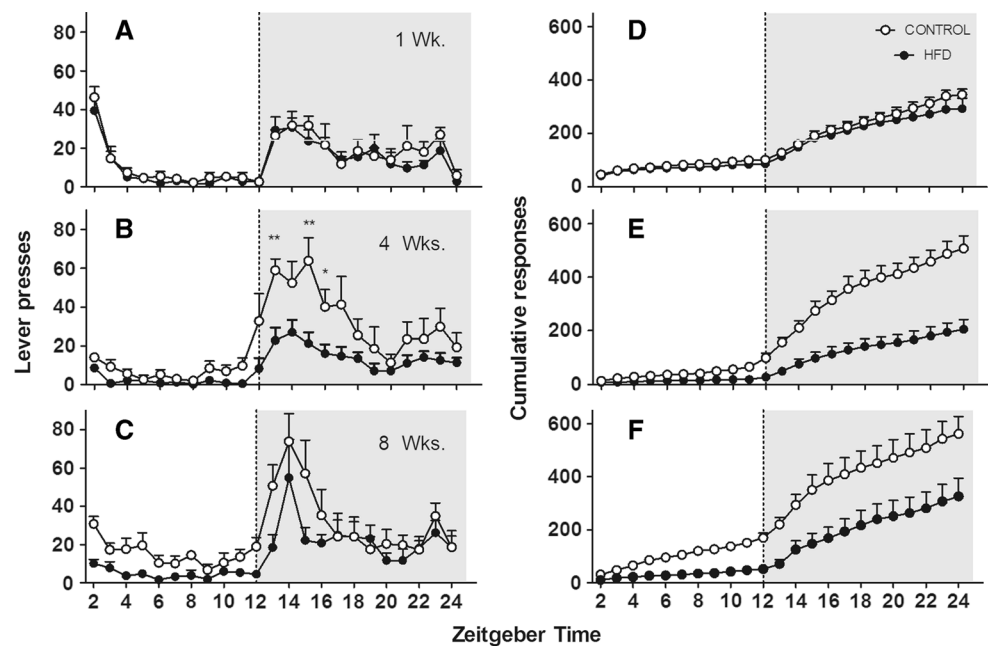
Results

High-fat diet decreases food self-administration

Figure 1 illustrates the number of lever presses per session operated by HFD and CD mice on a FR-1 schedule of reinforcement. After 1-week dietary treatment, both CD and HFD mice displayed a similar behavior, characterized by low activity during the light phase of the circadian photoperiod and an abrupt increase from the time at which lights were off (8:00 p.m., ZT12) (Fig. 1a). In contrast, 4-week HFD treatment triggered the inhibition of the operant behavior that was more evident during the dark period. In this case, a significant effect for *dietary treatment* ($F_{1,12} = 143.504$, $p < 0.001$) as well as a significant interaction *dietary treatment* \times *ZT* ($F_{22,264} = 1.861$, $p < 0.05$) was detected. Statistical differences were found during the dark period (Fig. 1b) at ZT13 ($p < 0.001$), ZT15 ($p < 0.01$) and ZT16 ($p < 0.05$). Eight-week HFD triggered a similar behavior (Fig. 1c). In this case, repeated-measures ANOVA revealed a significant *dietary treatment* effect ($F_{1,12} = 6.104$, $p < 0.05$), although no interaction *dietary treatment* \times *ZT* was observed. This might be due to the attenuation of the inhibition of the operant behavior observed during the dark period. Right panels (Fig. 1d–f) illustrate the influence of HFD on cumulate lever presses. HFD significantly decreased this parameter after 4- ($F_{1,12} = 143.504$, $p < 0.001$; Fig. 1e) and 8-week treatment ($F_{1,12} = 5.620$, $p < 0.05$; Fig. 1d). Regarding the whole procedure, a significant interaction *dietary treatment* \times *weeks on treatment* ($F_{2,264} = 4.005$, $p < 0.05$) was detected. CD mice made more lever pressings during the 4- and 8-week tests compared with HFD ($p < 0.05$). On the other hand, CD mice increased the number of lever presses since the 4-week test ($p < 0.05$), and this increase was not observed in HFD mice.

Figure 2a shows the evolution of BW through the FSA procedure. A significant interaction *dietary treatment* \times *weeks on treatment* ($F_{2,24} = 7.112$, $p < 0.003$) showed that HFD animals were heavier than their paired controls after 8-week treatment ($p < 0.01$). Figure 2b shows food consumption (g) normalized to BW (interaction *dietary treatment* \times *weeks on treatment* $F_{2,24} = 6.680$, $p < 0.005$). In this case, HFD mice consumed less food than their control mates after 4- and 8-week treatment

Fig. 1 Time course of changes in operant responses during a 23-h (ZT1–ZT23) food self-administration (FSA) session performed after 1, 4 and 8 weeks of ad libitum HFD treatment. *Left panels (A, B and C)* illustrate the number of lever presses per h under a FR-1 schedule of reinforcement. *Right panels (D, E and F)* represent cumulative responses during the experimental process. Data are expressed as mean \pm SEM for HFD ($n = 9$) and control ($n = 9$) groups. * $p < 0.05$; ** $p < 0.01$. Shaded areas correspond to the night-time schedule in the self-administration boxes



($p < 0.01$). In addition, CD animals exhibited increased food intake, compared with the HFD group, from the eighth week of treatment ($p < 0.01$).

Figure 3A shows that HFD animals presented less caloric intake and consumed less pellets than CD mice during the FSA protocol (interaction *dietary treatment* \times *weeks on treatment* $F_{2,24} = 5.831$, $p < 0.001$), independently of the duration of the dietary treatment ($p < 0.01$ after 1-, 4- and 8-week treatment). In this case, CD mice consumed more calories after 8-week treatment than they did previously after 1- and 4-week treatment ($p < 0.01$ in both cases). As illustrated in Fig. 3b, the FSA procedure evoked BW loss (interaction *dietary treatment* \times *weeks on treatment*, $F_{2,24} = 3.434$, $p < 0.05$) that was significantly higher in HFD compared with CD mice after 8-week dietary treatment ($p < 0.05$). Although normalization of food intake data to BW during FSA (Fig. 3b) shows that HFD mice were hypophagic compared with control animals, relative BW lost was similar between HFD and controls (Fig. 3b), suggesting that energy balance is not differently affected by FSA. In any case, BW loss was more intense in mice after 8-week treatment, independently of the dietary treatment they adhered ($p < 0.01$ in both cases).

High-fat diet decreases sweetened-drink preference in the two-bottle choice test

To assess the apparent lack of motivation displayed by HFD mice, we tested the influence of the dietary treatment in the two-bottle choice test by analyzing the preference between tap water and water sweetened either with sucrose or saccharine. Volumes of both tap water and sweetened

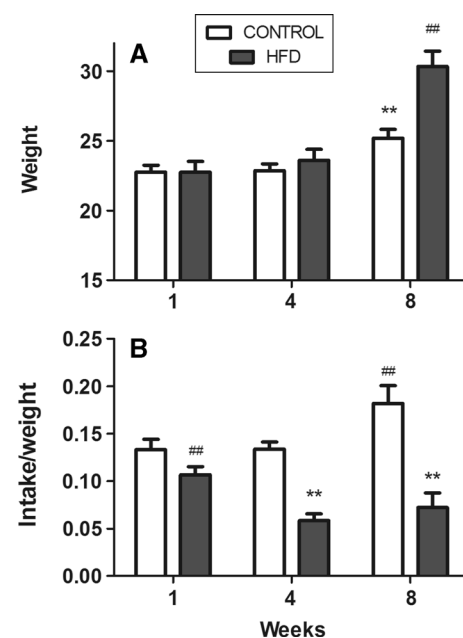


Fig. 2 Body weight (BW) and normalized food intake before each food self-administration (FSA) session. **a** Body weight. **b** (BW) Food intake (g) normalized by BW (g) after 1-, 4- and 8-week dietary treatment. Data are expressed as mean \pm SEM for HFD ($n = 9$) and control ($n = 9$) groups. ** $p < 0.01$ for between groups comparisons. ## $p < 0.01$ for within group comparisons

beverages consumed by CD and HFD mice were measured twice a week, during the five last weeks of an 8-week HFD protocol. Results appear illustrated in Fig. 4; ANOVA revealed that HFD mice displayed less preference than controls for both saccharine ($F_{1,90} = 75.630$, $p < 0.001$;

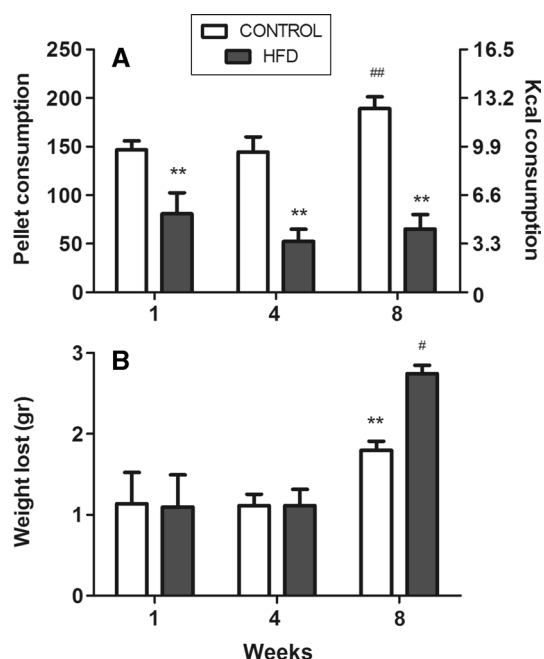


Fig. 3 Pellet consumption and loss of BW during food self-administration (FSA) sessions. **a** Number of pellets consumed during FSA sessions in animals that ate either HFD or control diet (CD) during 1, 4 or 8 weeks. **b** Animals on FSA displayed a decrease in BW during testing that is similar between HFD and CD receiving the dietary treatment during either 1 or 4 weeks. In contrast, 8-week HFD mice lost more weight than their respective controls. Data are expressed as mean \pm SEM for HFD ($n = 9$) and CD ($n = 9$) groups. ** $p < 0.01$ for between groups comparisons. # $p < 0.5$; ## $p < 0.01$ for within group comparisons

Fig. 4 Preference for saccharin and sucrose sweetened drinks during the last 5 weeks of an 8-week HFD treatment. **Panels A and B** show the mean \pm SEM of preference ratios (%) for saccharin (A) or sucrose (B) in control diet- (saccharin, $n = 10$; sucrose, $n = 9$) and HFD-treated animals (saccharin, $n = 10$; sucrose, $n = 11$). **Panels C and D** show the animal's weight during the 5 weeks of free-choice paradigm for saccharin (C) and sucrose (D) in both groups of animals. * $p < 0.05$; ** $p < 0.01$

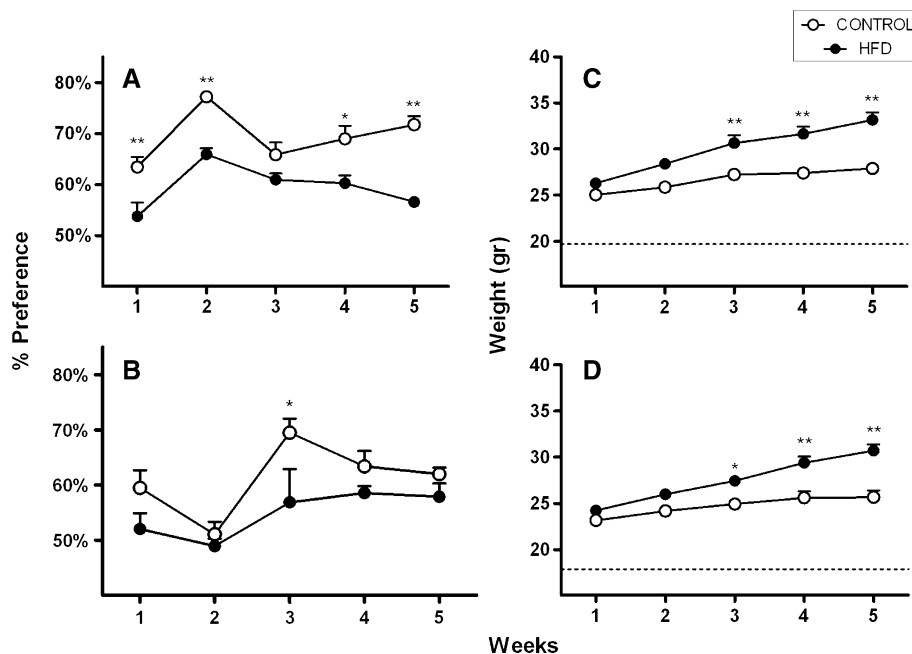


Fig. 4a) and sucrose ($F_{1,90} = 10.740$, $p < 0.001$; Fig. 4b). These data show that the effect of HFD on sweetened-drink preference is independent of the caloric density of the beverage presented as alternative to tap water.

Mice that had access to sweetened drinks exhibited an increase in BW ($F_{1,90} = 68.390$, $p < 0.001$ and $F_{1,90} = 61.450$, $p < 0.001$, for saccharin and sucrose, respectively) that was similar to that of CD animals growing under HFD/tap water (Fig. 4c, d).

Discussion

In a previous study [26], we reported that HFD decreases conditioned place preference (CPP) to cocaine and food by a mechanism apparently linked to the circadian desynchronization of food intake triggered by this kind of diets. We present here data showing that HFD also inhibits food-motivated operant behavior as well as motivation for sweetened beverages.

In FSA studies, we observed that although HFD mice maintained a similar FSA pattern, independently of the length of exposure to HFD, CD mice showed increased operant responding that could be attributable to the normal maturation of the mesocorticolimbic system, as treatment started during adolescence. Thus, our data suggest that HFD treatment has an impact on mesocorticolimbic function. In this sense, we have previously shown that chronic HFD produce deleterious effects in other parts of the brain

such as hippocampus and hippocampal-dependent memory in adolescent mice [33–35]. It can be argued that anhedonia experimented by HFD mice might be partially due to differences in fat/sucrose content between the diet consumed during the dietary treatment and that delivered during the FSA protocol, especially considering that the HFD used also contains a really considerable quantity of sucrose (20 %). Nevertheless, the results yielded by the two-bottle choice test point to an anhedonic state. This experimental paradigm is conceptually different from FSA since animals have access to the reinforcer during a long period (5 weeks) and thus allows discarding the eventual influence of novelty in behavioral inhibition. Otherwise, this test shows that the effect observed was not only a matter of specific demotivation toward caloric dense aliments, as HFD similarly decreased motivation for sucrose and saccharine sweetened beverages. In any case, it has to be considered that the low consumption of sucrose/saccharine by HFD mice might be due to an eventual misperception of sweet reward as an appetitive reinforcer compared with HFD reward. Nevertheless, other authors have shown that obese rats under HFD also increase the motivation to work for sucrose [36].

Because food intake pattern follows a circadian variation, we explored FSA behavior during a full dark/light cycle in animals that were not previously fasted. Our goal was to characterize the influence of HFD during both resting and activity periods. This protocol is pertinent because limiting the study to a shorter period would yield results of difficult interpretation. For instance, an eventual inhibitory effect of HFD on FSA would be hardly detected during the light phase since mice mostly fast during this period of the day. In fact, our current data show that the FSA inhibition observed in HFD mice was more evident during darkness and already perceptible after 4-week dietary treatment. In contrast, the effect was only observed after 8-week treatment during the light phase. At this point, it has to be stated that HFD triggers relevant changes in distribution of meals characterized by an increase in food intake during the light period and slight hypophagia during the night [37], thus suggesting that demotivation for food rather affects nocturnal feeding. Our current data give support to the inhibitory effect of HFD [26] as they show that this kind of diets reduces motivation for food also during the light period and point to the inhibition of food reward as a main trigger of changes in feeding behavior leading to obesity.

It is possible to consider that an increase in the reinforcer potency could theoretically account for a decrease in the self-administration ratio, as it has been demonstrated for cocaine or heroin, in which case a decrease in self-administration rates has been shown to inversely correlate with unit dose [38]. Although we cannot discard this possibility, the unit-dose effectiveness has not been established for food and shifting the fixed ratio to a higher ratio schedule should

be performed to verify this hypothesis. Alternatively, a consummatory negative contrast (cSNC) might account for a reduced palatability of pellets used in the operant chambers [39]. Nevertheless, such an effect should have been already observed after 1-week HFD and maintained during all the experimental phases. Besides, cSNC should be accompanied by an increase in other behaviors such as rearing, nose-down locomotion, ambulation, sampling new sources and grooming [40], which is not the case in our study (data not shown).

A pivotal question that emerges from the current study concerns the relationship between consumption of palatable food, reward dysfunction and obesity. This is a relevant issue because the role of palatable food as a motivating force able to override homeostatic signals still remains controversial [41–43]. Indeed, a profound state of reward hyposensitivity accompanied by compulsive-like eating, as a consequence of over-stimulation of brain reward systems triggered by cafeteria diet, has been reported [44]. In a similar context, Wang et al. [45] have suggested that obese humans compulsively consume palatable food to compensate reward hyposensitivity [46]. Nevertheless, our current data rather suggest that HFD reduces compulsive behavior toward comfort food.

The poor motivation for food displayed by HFD animals is coherent with BW loss observed during the last FSA session. Until we know, BW loss during FSA has not been previously reported. We hypothesize that our experimental conditions could facilitate BW loss due to the increase in physical activity together with a lower food intake accounting for a decrease in energy efficiency, as suggested in previous studies [47]. Other possibility with respect to this point is related to stress component of being inside the Skinner box. The connection between reward deficits and obesity exceeds the goal of the current study, but we have already proposed that circadian desynchronization of meals triggered by HFD may be a pivotal trigger event. Indeed, we have observed that forced synchronization of food intake prevents obesity and allows recover reward performance [26, 37, 48]. Otherwise, mismatch between feeding and light/dark cycle has been shown to disrupt energy metabolism in skeletal muscle and has significant consequences for whole-body energy homeostasis [49].

Our data, taken together with all these antecedents, suggest a situation of HFD-induced anhedonia and are coherent with other findings showing sucrose-induced anhedonia, anxiety-like behavior and hypersensitivity to stress after HFD treatment [50, 51].

In conclusion, our results show that the inhibition of operant behavior triggered by HFD might be integral to behavioral impairment in obesity. These data further support the connection between behavior and metabolic alterations typical of obesity.

Acknowledgments This work was supported by grants from Ministerio de Economía y Competitividad (SAF2011-25300, BFU2012-35353), Fundación Universitaria San Pablo–CEU (Spain) and UNED. P.S. is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brasil). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest The authors declare no conflict of interest.

References

- Abizaid A, Liu Z-W, Andrews ZB et al (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 116(12):3229–3239. doi:10.1172/JCI29867
- Gao Q, Horvath TL (2007) Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci* 30:367–398. doi:10.1146/annurev.neuro.30.051606.094324
- Finkelstein EA, Ruhm CJ, Kosa KM (2005) Economic causes and consequences of obesity. *Annu Rev Public Health* 26:239–257. doi:10.1146/annurev.publhealth.26.021304.144628
- Hill JO, Astrup A (2003) What diets should we be recommending for obesity? *Obes Rev* 4(2):77–78. doi:10.1046/j.1467-789X.2003.00103.x
- Swinburn BA, Sacks G, Lo SK et al (2009) Estimating the changes in energy flux that characterize the rise in obesity prevalence. *Am J Clin Nutr* 89(6):1723–1728. doi:10.3945/ajcn.2008.27061
- Volkow ND, Wise RA (2005) How can drug addiction help us understand obesity? *Nat Neurosci* 8(5):555–560. doi:10.1038/nn1452
- Kenny PJ (2011) Reward mechanisms in obesity: new insights and future directions. *Neuron* 69(4):664–679. doi:10.1016/j.neuron.2011.02.016
- Lutter M, Nestler EJ (2009) Homeostatic and hedonic signals interact in the regulation of food intake. *J Nutr* 139(3):629–632. doi:10.3945/jn.108.097618
- Saper CB, Chou TC, Elmquist JK (2002) The need to feed: homeostatic and hedonic control of eating. *Neuron* 36(2):199–211. Available at <http://www.ncbi.nlm.nih.gov/pubmed/12383777>. Accessed 16 Dec 2014
- Del Parigi A, Chen K, Reiman EM (2007) Is the brain representation of hunger normal in the Prader-Willi syndrome? *Int J Obes (Lond)* 31(2):390; author reply 390–1. doi:10.1038/sj.ijo.0803411
- Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008) Dorsomedial prefrontal cortex contribution to behavioral and nucleus accumbens neuronal responses to incentive cues. *J Neurosci* 28(19):5088–5098. doi:10.1523/JNEUROSCI.0253-08.2008
- Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* 162(8):1403–1413. doi:10.1176/appi.ajp.162.8.1403
- Ventura R, Morrone C, Puglisi-Allegra S (2007) Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. *Proc Natl Acad Sci USA* 104(12):5181–5186. doi:10.1073/pnas.0610178104
- Del Rio D, Cano V, Martín-Ramos M et al (2015) Involvement of the dorsomedial prefrontal cortex in high-fat food conditioning in adolescent mice. *Behav Brain Res* 283:227–232. doi:10.1016/j.bbr.2015.01.039
- Mukai M, Uchimura N, Hirano T, Ohshima H, Ohshima M, Nakamura J (1998) Circadian rhythms of hormone concentrations in alcohol withdrawal. *Psychiatry Clin Neurosci* 52(2):238–240. doi:10.1111/j.1440-1819.1998.tb01051.x
- Spanagel R, Pendyala G, Abarca C et al (2005) The clock gene *Per2* influences the glutamatergic system and modulates alcohol consumption. *Nat Med* 11(1):35–42. doi:10.1038/nm1163
- Wasielowski JA, Holloway FA (2001) Alcohol's interactions with circadian rhythms. A focus on body temperature. *Alcohol Res Health* 25(2):94–100. Available at <http://www.ncbi.nlm.nih.gov/pubmed/11584555>. Accessed 16 Dec 2014
- Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW (2009) Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring)* 17(11):2100–2102. doi:10.1038/oby.2009.264
- Escobar C, Salgado R, Rodríguez K, Blancas Vázquez AS, Angeles-Castellanos M, Buijs RM (2011) Scheduled meals and scheduled palatable snacks synchronize circadian rhythms: consequences for ingestive behavior. *Physiol Behav* 104(4):555–561. doi:10.1016/j.physbeh.2011.05.001
- Falcón E, McClung CA (2009) A role for the circadian genes in drug addiction. *Neuropharmacology* 56(Suppl 1):91–96. doi:10.1016/j.neuropharm.2008.06.054
- Froy O, Miskin R (2010) Effect of feeding regimens on circadian rhythms: implications for aging and longevity. *Aging (Albany NY)* 2(1):7–27. Available at <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2837202&tool=pmcentrez&rendertype=abstract>. Accessed 16 Dec 2014
- Turek FW, Joshu C, Kohsaka A et al (2005) Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 308(5724):1043–1045. doi:10.1126/science.1108750
- Barnea M, Madar Z, Froy O (2010) High-fat diet followed by fasting disrupts circadian expression of adiponectin signaling pathway in muscle and adipose tissue. *Obesity (Silver Spring)* 18(2):230–238. doi:10.1038/oby.2009.276
- Cano P, Jiménez-Ortega V, Larrad A, Reyes Toso CF, Cardinali DP, Esquifino AI (2008) Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. *Endocrine* 33(2):118–125. doi:10.1007/s12020-008-9066-x
- Kohsaka A, Laposky AD, Ramsey KM et al (2007) High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* 6(5):414–421. doi:10.1016/j.cmet.2007.09.006
- Morales L, Del Olmo N, Valladolid-Acebes I et al (2012) Shift of circadian feeding pattern by high-fat diets is coincident with reward deficits in obese mice. *PLoS One* 7(5):e36139. doi:10.1371/journal.pone.0036139
- Tschöp MH, Speakman JR, Arch JRS et al (2012) A guide to analysis of mouse energy metabolism. *Nat Methods* 9:57–63. doi:10.1038/nmeth.1806
- Butler AA, Kozak LP (2010) A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. *Diabetes* 59:323–329. doi:10.2337/db09-1471
- Strekalova T, Spanagel R, Bartsch D et al (2004) Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* 29:2007–2017. doi:10.1038/sj.npp.1300532
- Briones TL, Woods J (2013) Chronic binge-like alcohol consumption in adolescence causes depression-like symptoms possibly mediated by the effects of BDNF on neurogenesis. *Neuroscience* 254:324–334. doi:10.1016/j.neuroscience.2013.09.031
- Dym CT, Kraft TT, Bae VS et al (2012) Double-dissociation of D1 and opioid receptor antagonism effects on the acquisition of sucrose-conditioned flavor preferences in BALB/c and SWR mice. *Pharmacol Biochem Behav* 103:26–32. doi:10.1016/j.pbb.2012.07.018
- Novati A, Hulshof HJ, Koolhaas JM et al (2011) Chronic sleep restriction causes a decrease in hippocampal volume in adolescent rats, which is not explained by changes in

- glucocorticoid levels or neurogenesis. *Neuroscience* 190:145–155. doi:[10.1016/j.neuroscience.2011.06.027](https://doi.org/10.1016/j.neuroscience.2011.06.027)
33. Valladolid-Acebes I, Stucchi P, Cano V et al (2011) High-fat diets impair spatial learning in the radial-arm maze in mice. *Neurobiol Learn Mem* 95(1):80–85. doi:[10.1016/j.nlm.2010.11.007](https://doi.org/10.1016/j.nlm.2010.11.007)
 34. Valladolid-Acebes I, Merino B, Principato A et al (2012) High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. *Am J Physiol Endocrinol Metab* 302:E396–E402. doi:[10.1152/ajpendo.00343.2011](https://doi.org/10.1152/ajpendo.00343.2011)
 35. Valladolid-Acebes I, Fole A, Martín M et al (2013) Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? *Neurobiol Learn Mem* 106:18–25. doi:[10.1016/j.nlm.2013.06.012](https://doi.org/10.1016/j.nlm.2013.06.012)
 36. La Fleur SE, Vanderschuren LJM, Luijendijk MC, Kloeze BM, Tiesjema B, Adan RAH (2007) A reciprocal interaction between food-motivated behavior and diet-induced obesity. *Int J Obes (Lond)* 31(8):1286–1294. doi:[10.1038/sj.ijo.0803570](https://doi.org/10.1038/sj.ijo.0803570)
 37. Stucchi P, Gil-Ortega M, Merino B et al (2012) Circadian feeding drive of metabolic activity in adipose tissue and not hyperphagia triggers overweight in mice: is there a role of the pentose-phosphate pathway? *Endocrinology* 153(2):690–699. doi:[10.1210/en.2011-1023](https://doi.org/10.1210/en.2011-1023)
 38. Koob GF, Le Moal M (2006) *Neurobiology of addiction*. Academic Press, London
 39. Becker HC, Jarvis MF, Wagner GC, Flaherty CF (1984) Medial and lateral amygdectomy differentially influences consummatory negative contrast. *Physiol Behav* 33(5):707–712. doi:[10.1016/0031-9384\(84\)90035-0](https://doi.org/10.1016/0031-9384(84)90035-0)
 40. Lopez Seal MF, Cuenya L, Suarez AB, Mustaca AE (2013) Consummatory suppression due to incentive downshift is not a consequence of enhanced search behavior. *Behav Processes* 98:69–71. doi:[10.1016/j.beproc.2013.05.004](https://doi.org/10.1016/j.beproc.2013.05.004)
 41. Lenoir M, Serre F, Cantin L, Ahmed SH (2007) Intense sweetness surpasses cocaine reward. *PLoS One* 2(8):e698. doi:[10.1371/journal.pone.0000698](https://doi.org/10.1371/journal.pone.0000698)
 42. Shomaker LB, Tanofsky-Kraff M, Zocca JM et al (2010) Eating in the absence of hunger in adolescents: intake after a large-array meal compared with that after a standardized meal. *Am J Clin Nutr* 92(4):697–703. doi:[10.3945/ajcn.2010.29812](https://doi.org/10.3945/ajcn.2010.29812)
 43. Wang G-J, Volkow ND, Telang F et al (2004) Exposure to appetitive food stimuli markedly activates the human brain. *Neuroimage* 21(4):1790–1797. doi:[10.1016/j.neuroimage.2003.11.026](https://doi.org/10.1016/j.neuroimage.2003.11.026)
 44. Johnson PM, Kenny PJ (2010) Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci* 13(5):635–641. doi:[10.1038/nn.2519](https://doi.org/10.1038/nn.2519)
 45. Wang G-J, Volkow ND, Fowler JS (2002) The role of dopamine in motivation for food in humans: implications for obesity. *Expert Opin Ther Targets* 6(5):601–609. doi:[10.1517/14728222.6.5.601](https://doi.org/10.1517/14728222.6.5.601)
 46. Figee M, Vink M, de Geus F et al (2011) Dysfunctional reward circuitry in obsessive-compulsive disorder. *Biol Psychiatry* 69(9):867–874. doi:[10.1016/j.biopsych.2010.12.003](https://doi.org/10.1016/j.biopsych.2010.12.003)
 47. Garland T, Schutz H, Chappell MA et al (2011) The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. *J Exp Biol* 214:206–229. doi:[10.1242/jeb.048397](https://doi.org/10.1242/jeb.048397)
 48. Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O (2012) Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB J* 26(8):3493–3502. doi:[10.1096/fj.12-208868](https://doi.org/10.1096/fj.12-208868)
 49. Reznick J, Preston E, Wilks DL, Beale SM, Turner N, Cooney GJ (2013) Altered feeding differentially regulates circadian rhythms and energy metabolism in liver and muscle of rats. *Biochim Biophys Acta* 1832(1):228–238. doi:[10.1016/j.bbadis.2012.08.010](https://doi.org/10.1016/j.bbadis.2012.08.010)
 50. Sharma S, Fernandes MF, Fulton S (2013) Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int J Obes (Lond)* 37(9):1183–1191. doi:[10.1038/ijo.2012.197](https://doi.org/10.1038/ijo.2012.197)
 51. Isingrini E, Camus V, Le Guisquet A-M, Pingaud M, Devers S, Belzung C (2010) Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. *PLoS One* 5(4):e10404. doi:[10.1371/journal.pone.0010404](https://doi.org/10.1371/journal.pone.0010404)