Extinction and reinstatement of an operant responding maintained by food in different models of obesity

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Category of the article: Regular research article

Number of references: 27

Words in Abstract: 250

Words in Article (excluding abstract, references and figure legends): 5560

Number of figures: 5

Running title: Reinstatement in obesity

Abstract

A major problem in treating obesity is the high rate of relapse to abnormal food-taking habits after maintaining an energy balanced diet. Alterations of eating behavior such as compulsive-like behavior and lack of self-control over food intake play a critical role in relapse. In this study, we used an operant paradigm of food-seeking behavior on two different diet-induced obesity models, a free-choice chocolate-mixture diet and a high-fat diet with face validity for a rapid development of obesity or to unhealthy food regularly consumed in our societies. A reduced operant performance and motivation for the hedonic value of palatable chocolate pellets was revealed in both obesity mouse models. However, only mice exposed to high-fat diet showed an increased compulsive-like behavior in the absence of the reinforcer further characterized by impaired operant learning, enhanced impulsivity and intensified inflexibility. We used principal component analysis to globally identify the specific behaviors responsible for the differences among diet groups. Learning impairment and inflexible behaviors contributed to a first principal component, explaining the largest proportion of the variance in the high-fat diet mice phenotype. Reinforcement, impulsion and compulsion were the main contributors to the second principal component explaining the differences in the chocolate-mixture mice behavioral phenotype. These behaviors were not exclusive of chocolate group since some high-fat individuals showed similar values on this component. These data indicate that extended access to hypercaloric diets differentially modifies operant behavior learning, behavioral flexibility, impulsive- and compulsive-like behavior and these effects were dependent on the exposure to each specific diet.

Keywords: Eating behavior, relapse, operant behavior, principal component analysis, learning, impulsivity, compulsivity.

Introduction

Overweight is now recognized as a worldwide public health problem with major socio-economic consequences in Europe and North America (WHO, 2015). It is associated with increased risk for mortality and several medical morbidities. Although lacking well-defined criteria in medical science, the concepts of relapse to anomalous intake habits and addictive behavior towards eating are receiving increased attention and contribute to the development of overweight and obesity (Volkow and O'Brien, 2007; Volkow et al., 2012). These addictive-like behaviors have been widely investigated and shares similarities with drug addiction (Corwin and Grigson, 2009; Burokas *et al.*, 2012). Indeed, parallels in the compulsion for the reinforcer and the loss of control over intake despite recognizing its deleterious effects exist in both drug addiction and addictive-like behavior towards eating (Volkow *et al.*, 2008; Mancino *et al.*, 2015).

Diet-induced obesity (DIO) models in rodents have increased our knowledge about the mechanisms underlying neurobiological, behavioral and metabolic alterations associated to energy imbalance disorders (Martin-Garcia et al., 2010). Traditional DIO models have used high-caloric food with an increase of fat-derived calories in the chow (Kennedy et al., 2010; Sampey et al., 2011). In contrast, the recent use of cafeteria diet has improved the validity to these models, since it directly uses the obesogenic unhealthy food regularly consumed by humans that includes high-salt, high-palatable and high-fat foods, such as chips, cookies or processed meats, and provide animals with free simultaneous *ad libitum* access to standard chow and this unhealthy food (Corwin and Buda-Levin, 2004). Several preclinical studies have investigated the possible development of addictive-like behavior after DIO exposure. Interestingly, rats with a schedule of intermittent access to a sugar solution and chow for several weeks showed opiate-like withdrawal symptoms such as anxiety and depression,

bingeing and craving (Avena et al., 2008). Other studies reported that rats fed with cafeteria diet gained weight and showed downregulation of striatal dopamine D2 receptor (Johnson and Kenny, 2010). These studies propose that obesogenic food may lead to addictive-like behavior and suggest that overlapping neural circuits are involved in the processing of food- and drug-related cues and the control of food and drug use.

In the present study, we have used prolonged exposure to a free-choice of chocolate-mixture diet and a forced exposition to high-fat food in mice to investigate the consequences of long-term obesogenic diet exposure in reinstatement of food seeking behavior. In the first model, mice had access to standard chow and chocolate-mixture palatable hypercaloric diet, which is highly preferred by animals producing compulsive food-intake and progressive weight gain. In the second model, diet was based on a forced exposition to high-fat food. Behavioral responses were evaluated in a recently validated model (Martin-Garcia et al., 2011) that allows to determine operant learning, reinforcement, motivation for the hedonic value of palatable food and reinstatement of food seeking behavior induced by associated cues. As operant reward, we used chocolate-flavored pellets with similar caloric content (3.48 kcal/g) and composition (21% protein, 13% fat and 67% carbohydrate) than regular standard chow to enhance the hedonic value of the pellet. Thus, chocolate-flavored pellets used in the operant boxes constitute a different reward from the chocolate mixture (17% protein, 24% fat and 52% carbohydrate with a caloric value of 4.92 kcal/g) or the high fat diet (19% protein, 60% fat and 21% carbohydrate with a caloric value of 5.21 kcal/g) used in DIO models and rather similar to the standard food (18% protein, 7% fat and 75% carbohydrate with a caloric value of 3.52 kcal/g). Principal component analysis (PCA) was used to globally assess the effects of each type of diet in order to identify which behavioral phenotypes assessed on the reinstatement model best define each experimental group. PCA allows to understanding the

combinations of variables that capture a major portion of the data variance (Catuara-Solarz et al., 2015). PCA identified that learning impairment and inflexible behaviors explained the largest proportion of the variance in the high-fat diet mice phenotype, while reinforcement, impulsion and compulsion were the main contributors to the differences in the chocolate-mixture mice.

Material and methods

Animals

Male C57BL/6 mice (Charles River, France), weighing 24-26 g at the beginning of the experiment were used in this study. Mice were housed individually in controlled laboratory conditions with the temperature maintained at 21 ± 1 °C and humidity at $55\pm10\%$. Mice were tested during the dark phase of a reverse light cycle (lights off at 8.00 a.m. and on at 20.00 a.m.). Food and water were available *ad libitum* except during 5 days before starting operant sessions and during the first 10 days of the operant behavior training. During this first period, the animals were food deprived to maintain their weights at 85% of initial values (study design in Fig. 1). All experimental protocols were performed in accordance with recommendations for the proper care and use of laboratory animals [local regulations (law 32/2007); European (EU directive n° 86/609, EU decree 2001-486) regulations, and the Standards for Use of Laboratory Animals n° A5388-01 (NIH)] and were approved by the local ethical committee (CEEA-PRBB). Body weight was registered twice a week during the obesity development period (Monday and Friday) and once daily (Monday) during the whole period of the operant conditioning experiment (Fig. 1).

Diet

Animals were randomly assigned to the different mouse obesity models. In the chocolate-mixture diet model, mice were fed *ad libitum* with chocolate-mixture diet having free-choice between standard chow diet containing 3.52 kcal/g (75% energy from carbohydrates, 18% from protein and 7% from fat) and chocolate-mixture hypercaloric palatable diet containing 4.92 kcal/g with equal amounts of Bounty®, Snickers®, Mars® and Milka® chocolate prepared as homogenous food pellets; 52% energy from carbohydrates, 17% from protein and

24% from fat. The proportion of sugars within this carbohydrate content was 44.4% of the total vs 8.3% in the standard chow diet (Heyne et al., 2009; Martin-Garcia et al., 2010). The corresponding control lean group received standard chow *ad libitum*.

In the high-fat diet model, animals assigned to obese group were fed *ad libitum* with a high-fat hypercaloric food (Test Diet®, USA) containing 5.21 kcal/g (21% energy from carbohydrates, 19% from protein and 60% from fat). The proportion of sugars within this carbohydrate content was 22.9% of the total vs 24.7% in the standard diet. Animals assigned to the control group (lean) were maintained *ad libitum* under standard diet (Test Diet®, USA) containing 3.87 kcal/g (70% energy from carbohydrates, 19% from protein and 12% from fat).

Glucose tolerance test

For the glucose tolerance test, animals exposed to 24 weeks of obesity development were fasted for 14 hours prior to the beginning of the blood sample collection. Blood was sampled from the tail using an Ascensia ELITE® glucose monitoring system (Bayer HealthCare). This method is based on a slip-in sampling test strip technology, which requires a tiny amount of blood (5 µl). The glucose detection range and the relative standard variation stated by manufacturer for the Ascensia ELITE® are 20-600 mg/dL and less than 3.1%, respectively. After measuring basal levels of glucose (0 time point) animals were injected glucose 2 g/kg intraperitoneally (i.p.), and blood glucose levels were measured at 15, 30, 45, 60 and 120 min.

Operant self-administration apparatus

Operant responding maintained by food was investigated in mouse operant chambers (Model ENV-307A-CT, Med Associates, Georgia, VT, USA) equipped with two retractable levers, one randomly selected as the active and the other as the inactive. Pressing on the active lever resulted in a pellet delivery with the associated cue, while pressing on the inactive had no consequences. A stimulus light (associated cue), located above the active lever was paired contingently with the delivery of the reinforcer (chocolate flavored pellet). The chambers were made of aluminum and acrylic, had grid floors connected to an electrical shocker (EVB-414, Med. Associates Inc., St Albans, USA), and were housed in sound- and light-attenuated boxes equipped with fans to provide ventilation and white noise. A food dispenser equidistant between the two levers permitted delivery of food pellets when required.

Acquisition of operant conditioning maintained by food

Sessions of operant responding maintained by palatable chocolate-flavored pellet were performed in accordance to protocols previously described (Martin-Garcia et al., 2011). Responding for all the animals was maintained by chocolate-flavored pellets (20 mg), containing 21% protein, 13% fat and 67% carbohydrate with a caloric value of 3.48 kcal/g, with similar caloric value than the maintenance diet provided for mice in their home cage (see Diet section above), and with some slight modifications in its composition: addition of chocolate flavour (2% pure unsweetened cocoa) and modification in the sucrose content. Indeed, although the carbohydrate content was similar in maintenance diet (75%) and highly palatable isocaloric pellets (67%), the sucrose content in standard chow was 8.3% of the total carbohydrates and 50.1% percent in highly palatable isocaloric pellets. These pellets were

presented only during the 1-hour daily operant session, and animals were maintained on their corresponding diet for their daily food intake.

1-hour daily operant responding sessions were conducted 7 days per week during 20 days. The animals were food deprived 5 days before starting sessions and during the first 10 sessions of the operant behavior training to maintain their weight at 85% of their *ad libitum* initial weight adjusted for growth. Animals were feed *ad libitum* during the last 10 days of operant conditioning maintained by chocolate. The house light was on at the beginning of the session for 3 sec and off during the remaining time of the session, this means that no extra house light was turned on during session. The side of active and inactive lever was counterbalanced between animals. Each daily session started with a priming delivery of one chocolate-flavored pellet. Mice were trained under a fixed ratio 1 (FR1) schedule of reinforcement. The stimulus light (associated cue) signaled delivery of the chocolate-flavored pellet. A time-out period of 10 sec was established after pellet delivery. During this period, the cue light was off and no reinforcer was provided after responding on the active lever. Responses on the inactive lever and all the responses elicited during time-out periods were also recorded. The session was terminated after 100 reinforcers were delivered or after 1 hour, whichever occurred first. As previously described (Martin-Garcia et al., 2011), the criteria for the achievement of the operant responding was acquired when all of the following conditions were met: 1) mice maintained a stable responding with less than 20% deviation from the mean of the total number of reinforcers earned in three consecutive sessions (80% of stability); 2) at least 75% responding on the active hole, and 3) a minimum of 10 reinforcers per session. After each session, mice were returned to their home-cages. Each chamber was cleaned at the end of each session to prevent the presence of odor of the previous mouse. After 20 days of operant responding, mice were moved to the progressive ratio phase.

Progressive ratio

Reinforcement schedule was changed during the progressive ratio (PR) session in which the response requirement to earn a chocolate-flavored pellet escalated according to the following series: 1-2-3-5-12-18-27-40-60-90-135-200-300-450-675-1000. The PR session lasted for 4 hours or until mice did not complete the ratio for delivery of one reinforcer within 1 hour, and was performed only once. The breaking point to extinguish self-administration behavior was determined in each animal. After PR session, mice were moved to the extinction phase (Fig. 1). The PR session was performed before extinction and the cue-induced reinstatement without introducing a retest baseline as it has been published before (Gutierrez-Cuesta et al., 2014; Charbogne et al., 2017).

Extinction of operant conditioning

Animals were maintained with the corresponding diet during extinction and cue-induced reinstatement to evaluate the food-seeking behaviour in the same conditions of acquisition of operant behavior. During the extinction phase, animals were feed *ad libitum* and the experimental conditions were similar to sessions of acquisition of operant conditioning maintained by food sessions except that chocolate-flavored pellets were not available and the stimulus light was not presented after responding in the active lever. Mice were given 1-hour daily sessions 7 days per week during 20 days. The criterion for extinction was achieved when mice made during 3 consecutive sessions a mean number of lever-pressing in the active lever of less than 30% of the responses obtained during the mean of the three days of achievement of the acquisition criteria. After 20 days of extinction sessions, mice that acquired the extinction criterion were tested by cue-induced reinstatement.

Cue-induced reinstatement

Test for cue-induced reinstatement was conducted under the same conditions used in the training phase except that pellets were not available (Martin-Garcia et al., 2011). Each pressing in the active lever led to the presentation of the cue-associated stimulus light for 2 sec. The reinstatement criterion for the experiments was achieved when responding in the active lever was double than responding in the active lever during the 3 consecutive days that mice acquired the extinction criterion.

Principal component analysis

We used principal component analysis (PCA) technique to interpret the multidimensional data obtained in our behavioral study. We first annotated variables according to the behavior they should measure by combining relevant experimental readouts (supplementary table 1). This resulted in a data matrix containing 28 individuals and 24 variables. PCA was used to learn which of these annotated behaviors best explain the variance present in the data set. Data were scaled to unit variance to allow the combined analysis of the annotated variables that were originally measured in different units. PCA yields a new coordinate space consisting of linear combinations of the original variables. These principal components are orthogonal to each other and explain a percentage of the phenotypic variance present in the data set. Dimensionality reduction is achieved by choosing the minimum number of components that maximize the phenotypic variance explained. To further characterize the behavioral phenotypes that explain the group separation in specific sessions, PCA was performed using only variables that belong to each specific operant training period:

acquisition, maintenance and extinction of operant conditioning. PCA was performed using R (R Core Team, 2015) and specifically R-package FactoMineR package (Lê et al., 2008).

Data analysis

Body weight values were analyzed in each experiment (chocolate-mixture and high-fat diet) using two-way ANOVA considering diet (lean/obese) as between-subjects factor and experimental phase (last two weeks of obesity development, food deprivation, acquisition of operant conditioning with food deprivation, operant conditioning maintenance *ad libitum*, extinction and reinstatement) as within-subject factor.

To evaluate the fasted glucose levels, two-way ANOVA was performed with model (chocolate-mixture/high-fat) and diet (lean/obese) as between-subject factors. For the glucose tolerance test, three-way ANOVA was performed with model and diet as between-subjects factors and time point as within-subject factor.

For each experiment (chocolate-mixture and high-fat diet) and experimental phase of operant conditioning (acquisition, maintenance and extinction), three-way ANOVA was conducted in operant responding with diet as between-subjects factor (obese/lean) and day (daily sessions) and lever (active/inactive) as within-subjects factor. For impulsivity, two-way ANOVA was done with diet as between-subjects factor and day as within-subject factor. For progressive ratio data, three-way ANOVA was performed with obesity model and diet as between-subjects factor (obese/lean) and lever (active/inactive) as within-subjects factor. Three-way ANOVA was conducted for cue-induced reinstatement in each experiment with diet as between-subjects factor (obese/lean) and phase (acquisition, extinction and reinstatement) and lever (active/inactive) as within-subjects factors. Post-hoc analyses were performed when required in all the cases (Newman-Keuls). Pearson's χ^2 test was used to

compare the percentage of animals that reach the acquisition of operant learning, extinction and reinstatement criteria in the different experimental groups. Differences were considered significant at $P < 0.05$. All results are expressed in mean $\pm$ S.E.M. The statistical analysis was performed using the Statistical Package for Social Science program SPSS® 19.0 (SPSS Inc, Chicago, USA).

Results

Body weight and glucose tolerance test

Body weight was progressively increased by the continued exposure to chocolate-mixture diet (Fig. 2A) or high-fat diet (Fig. 2B) (see supplementary information for detailed results).

Fasted glucose levels in blood were increased in mice exposed to high-fat diet. Two-way ANOVA revealed significant main effects of obesity model [$F_{(1,31)} = 22.77$; $P < 0.001$], diet [$F_{(1,31)} = 16.784$; $P < 0.001$] and interaction between both factors [$F_{(1,31)} = 12.223$; $P < 0.01$]. Subsequent Newman-Keuls analysis showed a significant increase of fasted glucose levels in mice exposed to high-fat diet ($P < 0.001$) compared to other groups (Fig. 2C). Glucose tolerance was impaired in mice exposed to both chocolate-mixture and high-fat diet (Fig. 2D) (see supplementary information for detailed results).

Acquisition and maintenance of operant conditioning maintained by food

Lean and obese mice from both experimental models were first food deprived in their home cages and trained in the operant chambers to acquire an operant responding maintained by chocolate-flavored pellets. After 10 sessions, mice were exposed to food *ad libitum* in their home cages and trained in the operant chambers for maintenance of operant responding (Fig. 3A,B). In both experimental conditions, lean and obese mice discriminated between active and inactive lever and differences with standard diets were mainly revealed during the food deprivation period. In this period, lean mice pressed more the active lever than obese mice, specifically during 8 over 10 days in the chocolate-mixture model and during 10 over 10 days in the high-fat diet model. During *ad libitum* period, lean mice also pressed more the active lever than obese mice but only during 2 over 10 days in the chocolate-mixture model

and during 7 over 10 in the high-fat diet model. In addition, obese mice from high-fat diet model showed low discrimination between levers during the first day of operant conditioning (57.8%). Then after, the levels of responding in the inactive lever were higher in this group compared to control group in 7 over 10 days. Accordingly, obese mice showed lower levels of discrimination between levers (64.1%) than control mice (83.1%). In contrast, obese mice from chocolate-mixture group showed similar levels of discrimination between levers (90.4%) than control mice (88.4%).

Impulsivity-like behavior was evaluated considering the number of active lever-presses performed during the 10 sec of time-out period established after each pellet delivery. During this period, the cue light was off and no reinforcer was provided after responding on the active lever. Obese mice exposed to high-fat diet showed higher levels of responding than corresponding control lean mice mainly during the acquisition and at the end of the maintenance period. In addition, obese mice exposed to chocolate-mixture diet showed higher levels of responding than corresponding control lean mice at the beginning of acquisition period (Fig. 3C,D). The stability, discrimination and reinforcing criteria of operant learning (see supplementary material and methods) was achieved by 62.5% of obese high-fat diet mice and by 100% of mice of the obese chocolate-mixture diet and control groups and this difference was statistically significant [$\chi^2 = 4.80$; $P < 0.05$]. Finally, obese mice exposed to high-fat diet, but not those exposed to chocolate-mixture diet, required more time than corresponding lean control to achieve the criteria of operant responding (Fig. 3E,G) (see supplementary information for detailed results). A pattern of responses performed on the active and inactive levers (days 1 and 10 of acquisition and maintenance) for a representative mouse is depicted in Fig. S1.

Progressive ratio

The motivation for chocolate-flavored pellets was lower in obese mice of both experimental models than the corresponding lean control groups (Fig. 3F,H) (see supplementary information for detailed results).

Extinction

After progressive ratio, lean and obese mice from both experimental models were trained to extinguish operant responding maintained by palatable pellets (Fig. 4A,B). In the chocolate-mixture diet experiment, the lean mice showed higher number of responses in the active lever in comparison to the responses of the obese group during the first two sessions of extinction ($P < 0.001$). In the high-fat diet experiment, the lean mice showed higher number of responses in the active lever than the obese group during the first session of extinction ($P < 0.01$). The extinction criterion (see materials and methods) was achieved by 88% of obese high-fat diet mice and by 100% of mice of the rest of the groups. This difference did not reach statistical significance [$\chi^2 = 1.14$; *n.s.*]. In agreement, one-way ANOVA for extinction acquisition day did not reveal significant main effect of diet in the chocolate-mixture experiment [$F_{(1,10)} = 0.50$; *n.s.*] or in the high-fat diet experiment [$F_{(1,14)} = 1.85$; *n.s.*] (Fig. 4C,D) (see supplementary information for detailed results). A pattern of responses performed on the active and inactive levers (days 1 and 20 of extinction) for a representative mouse is presented in Fig. S1.

Reinstatement

Cue-induced reinstatement was observed in lean and obese mice from both experimental models. However, obese mice exposed to high-fat diet did not discriminate between the

active and inactive lever after cue exposure in contrast to the significant discrimination in all of the other experimental groups. All control animals and 83% of obese animals having chocolate-mixture diet reached criteria of reinstatement of food-seeking behavior. Meanwhile, only 50% of obese mice exposed to high-fat diet acquired such criteria (Fig. 4E,F) (see supplementary information for detailed results). During cue-induced reinstatement, the level of active lever-presses of lean mice was 62.3% of maintenance active lever-presses and 35.2% of obese mice in chocolate-mixture diet, while in high-fat diet the level of active lever-presses of lean mice was 80.1% and 63% of obese mice (Fig. 4G,H). A pattern of responses performed on the active and the inactive levers for a representative mouse under cue-induced reinstatement is depicted in Fig. S1.

Multidimensional analysis of factors separating mice

PCA reduces the dimensionality of the multivariate operant behavior data by identifying those linear combinations of the annotated variables (table S1) that capture most of the phenotypic variance. In this way, combinations of those behaviors that are relevant for a characterization of each diet group can be found. At the same time, the individuals are also displayed, thus allowing visualization of the behavioral variation within the diet groups. Since this is an unsupervised technique, group information is not used to obtain the coordinates. Nevertheless, we found a neat separation between chocolate-mixture and high-fat diet groups along PC1 (which accounts for 35% of the variance). PC1 alone suffices to separate high-fat mice from the rest of individuals. PC2 (accounting for 14% of the variance) is needed to separate the chocolate-mixture group from their controls. Obese chocolate-mixture mice loaded towards negative values of PC2, while controls tend to positive values. In contrast, obese high-fat mice spread out along the entire PC2 axis, some animals of this

group having PC2 values similar to controls and others similar to the chocolate-mixture group (Fig. 5A).

The contributions of variables to the principal axes can best be visualized by plotting the variables in the PCA space, thus showing their directionality and interdependence and relating them to the diet groups (Fig. 5B). This representation reveals the biological meaning of the observed group separations and within-group variations. Most learning-related variables including primary reinforcement discrimination, inactive maintenance, extinction inflexibility, inactive cue and day of acquisition have a main contribution to PC1 (Fig. 5B,C). Hence, this axis represents a composite learning variable (with a notable exclusion of extinction learning) where high values correspond to learning impairment. Since the PC2 axis is orthogonal to PC1, it is uncorrelated with the composite variable described by PC1. PC2 receives a high contribution of variables such as reinforcement, impulsivity/compulsivity-related variables and extinction learning (Fig. 5B,D). The fact that extinction learning and impulsivity/compulsivity-related variables contribute to PC2 and not to PC1 suggests that extinction learning is more related to impulsivity and compulsivity than to the operant learning described by PC1.

A considerable amount of variation was revealed within the high-fat group. The main direction of this within-group variance follows a diagonal at 45 degrees to both principal axes, and cannot be explained by one of the principal axes alone (Fig. 5A). Interestingly, a variable that follows the direction of this diagonal in the two-dimensional representation and thus seems to represent well the individual variability is impulsivity/compulsivity during the acquisition of operant conditioning.

To understand the specific contribution of behavioral phenotypes within each session, we further performed three independent PCAs for acquisition/maintenance (Fig. S2), extinction

(Fig. S3) and cue-induced reinstatement (Fig S4) including only the variables belonging to the respective sessions. Specifically, PC1 can still be interpreted as the composite learning variable and receives contributions from the same variables given the session (Fig. S2A,B,C), consistently separating the high-fat group from the other groups. Variable contribution to PC2 is also maintained although the chocolate-group's separation from the control is only attained when combining all variables that contribute to PC2 in the global PCA.

Discussion

In the present study, we compared for the first time operant learning, reinforcement, motivation, extinction and cue-induced reinstatement for palatable food of two different mouse DIO models with face validity to mimic unhealthy food regularly consumed in our societies or to obtain a rapid development of obesity. The first model was a validated obesity paradigm of compulsive food-taking behavior (Heyne et al., 2009; Martin-Garcia et al., 2010), and consisted on a free-choice between standard chow and a high caloric palatable chocolate-containing diet. The second model was based on exposure to a high-fat hypercaloric diet. Both diets led to overweight and glucose intolerance, with more important alterations detected in high-fat mice. Importantly, our data reveal that a high fat diet produces deleterious effects on learning and flexibility, whereas both high fat and chocolate-mixture diet have an important influence on reinforcement, impulsivity and compulsivity.

In our experimental conditions, both diets induced significant weight gain, although the effects were more pronounced in high-fat than in chocolate-mixture diet exposed mice. Glucose tolerance was impaired in obese mice regardless the kind of diet, as revealed by an upward shifted glucose curve. However, obese chocolate-mixture mice showed lower values than obese high-fat animals at the last time-point suggesting better recovery. As expected, high-fat diet obese mice also showed the highest fasted glucose levels, which could be related to their highest increase of body weight. These results validate the obesity models used in the present study because raised fasting glucose levels have been demonstrated in diet-induced and genetic mouse models of obesity and metabolic syndrome similarly to humans (Kennedy et al., 2010).

Learning of operant conditioning maintained by chocolate-flavored pellets was mainly impaired in high-fat obese mice since these mice required more time to acquire operant

learning than obese chocolate-mixture and control mice. In agreement, variables related to learning separated high-fat obese mice from the other groups in the PCA analysis. Moreover, during food deprivation, 100% of chocolate-mixture and control mice acquired criteria of discrimination, stability and reinstatement, while only 62.5% of high-fat obese mice reached criteria and even those needed longer time than the other groups. In agreement, high-fat diet has been reported to impair learning and memory by reducing hippocampal levels of brain-derived neurotrophic factor, a crucial modulator of synaptic plasticity, and a predictor of learning efficacy (Molteni *et al.*, 2004; Kaczmarczyk *et al.*, 2013).

During acquisition and maintenance of operant conditioning obese mice showed increased impulsivity-like behavior as revealed by the rate of responding during the time-out period established after pellet delivery. Impulsivity is the inability to refrain an action once is initiated and shares similarities with compulsivity, such as the inhibitory control dysfunction. However, compulsivity is more related to persistence of a response that is inappropriate, repetitive and perseverative (Robbins et al, 2012).

Obese mice of both models showed lower levels of operant responding than control mice, suggesting that the primary reinforcing effects of chocolate-flavored pellets were diminished. This difference could be due to a satiety state produced by the excessive access to hypercaloric diets. Indeed, the caloric value of each obesogenic diet was higher than standard chow producing different satiety state in lean and obese mice. In our experimental conditions, mice were food deprived only during the first training phase to facilitate operant learning. Mice were fed *ad libitum* during the remaining experiment to return to the normal physiological situation and to dissociate between learning and the hedonic value of the primary reinforcement. When mice were fed *ad libitum*, high-fat and lean mice decreased active-lever presses compared to previous sessions during food deprivation suggesting that

the absence of hunger reduced the reinforcing effects of the palatable pellet. In contrast, mice fed with the chocolate-mixture did not show a reduction of the primary reinforcing effects of palatable pellets and maintained similar levels of response and discrimination during food deprivation and *ad libitum* training. Instead, high-fat mice increased the responses on the inactive lever when fed *ad libitum*, suggesting impaired discrimination between levers and presented increased impulsivity during the last training sessions. This operant inflexible behavior could be related to a loss of control over food intake that has been reported to play a key role in obesity (Volkow and O'Brien, 2007; Volkow et al., 2008).

Motivation to obtain palatable pellets in the progressive ratio session was lower in obese mice exposed to both diets than in lean control groups. The satiety state previously discussed in obese mice could also participate in this lower motivation for chocolate-flavored pellets. Extinction learning was similar among groups, but again, high-fat obese mice showed higher number of inactive lever-presses during nearly all sessions, suggesting an increased cognitive inflexibility and difficulty to refrain their response. Cue light used as conditioned stimulus was effective in reinstating chocolate-seeking behavior in obese mice from both models. However, high-fat mice could not discriminate between the active and inactive lever after cue exposure maintaining the inflexibility previously described in the extinction phase. All control animals and 83% of obese animals having chocolate-mixture diet reached criteria of reinstatement of food-seeking behavior, whereas only 50% of obese mice of high-fat diet reached these criteria. Mice reaching the criteria showed high levels of food seeking revealed by the enhanced number of active and inactive lever-presses and by the high percentage of responding relative to the maintenance phase. Therefore, the extended access to hypercaloric diets has an effect in operant behavior and reinstatement. In agreement, other studies have reported addictive-related behavior after hypercaloric food access. Thus, the scheduled cycle

of food access combined with daily food deprivation for 12 h with 12 h access to a sugar solution and chow provoked bingeing, an opiate-like withdrawal state and craving in rats (Colantuoni et al., 2001; Colantuoni et al., 2002; Avena et al., 2005; Avena et al., 2008). These manipulations were correlated with increases in dopamine extracellular levels in the nucleus accumbens due to repeated bingeing (Avena et al., 2006). In agreement, excessive consumption of palatable energy-dense food has been shown to over-stimulate the brain reward system leading to a downregulation of striatal dopamine receptor D2 and a state of reward hyposensitivity that develops compulsive-like eating behavior (Johnson and Kenny, 2010). Drug abuse exposure also decreases striatal dopamine receptor D2 density, induces a reward hypofunctionality state and activates the emergence of compulsive-like drug-taking behavior (Volkow et al., 2008). Thus, obesity and drug-addiction may be related to similar neuroadaptive alterations in the brain reward circuitries.

PCA can improve the interpretation of results where the high dimensionality of the data may hinder their global understanding (Catuara-Solarz et al., 2015; Keeley and McDonald, 2015). Here, PCA enabled to better understand in this study the behavioral characteristics of the diet groups. PCA reveals the directions of highest variability when combining all the variables into a joint analysis and it can be used to extract those combinations of variables that are most relevant for a description of the behaviors observed. These relevant behaviors also separate well in experimental conditions the diet groups, i.e. individuals from the same group cluster together in the new coordinates obtained. The behavioral variation within the groups which is usually smaller, can also be characterized by this way.

The major source of variability was related to learning impairment/inflexibility (PC1), setting the high-fat group apart from both controls and the chocolate group and also explaining a considerable amount of the variation among high-fat individuals.

Complementary to this finding, reinforcement, impulsivity/compulsivity-related variables and extinction learning as captured by PC2 provide another source of variability (less than half of the amount of variance compared with PC1), which is related to the separation of the chocolate group from controls. This separation is perpendicular to PC1 and less pronounced, indicating that the behavioral alterations experienced by the chocolate group are lesser than and uncorrelated with the main alterations experienced by the high-fat group. However, these behaviors are not exclusive of chocolate-mixture mice since they cannot be distinguished from high-fat diet mice using PC2.

In summary, our results suggest that a free-choice chocolate diet leads to more subtle, reinforcement, impulsivity/compulsivity-related changes and is not enough to trigger the deleterious effects on learning and flexibility that are observed when forcing a high fat diet. On the other hand, high-fat mice, although clearly characterized by deficient learning, do neither behave homogeneously in terms of learning impairment/inflexibility (PC1) nor reinforcement, impulsivity and compulsivity (PC2). Indeed, on the PC2 composite measure, some high-fat individuals showed values similar to controls and others values similar to the chocolate-mixture group, suggesting that some high-fat diet mice experience a behavioral disruption in terms of reinforcement, impulsivity and compulsion comparable to chocolate-diet mice.

Our results indicate that extended access to hypercaloric diets impairs operant behavior and produces differential effects on learning, reinforcement, motivation, extinction and food-seeking reinstatement depending on the exposure to the different high caloric and palatable diets. The most relevant impairment was revealed after forced high-fat diet exposure leading to important deleterious effects on learning and flexibility.

Acknowledgements

This work was supported by the DG Research of the European Commission FP7 (#HEALTH-F2 2013-602891 and PHECOMP, No. LHSM-CT-2007-037669), the Spanish ‘RETICS-Instituto de Salud Carlos III’ (#RD12/0028/0023), the Spanish ‘Ministerio de Ciencia e Innovación’ (#SAF2011-29864), the Spanish ‘Ministerio de Economía y Competitividad’ (#SAF-2014-59648P), the ‘Plan nacional sobre drogas’ (#PNSD-2013-5068), the Catalan Government ‘AGAUR-Generalitat de Catalunya’ (#2009SGR00731 and #2014-SGR-1547) and the Catalan foundation ‘La Marató de TV3’ (201620-30-31-32).

The laboratory of M.D. is supported by DIUE de la Generalitat de Catalunya (Grups consolidats SGR 2014/1125). This work was supported by Fondation Jérôme Lejeune (Paris, France), MINECO (SAF2013-49129-C2-1-R), CDTI (“Smartfoods”) and EU (Era Net Neuron PCIN-2013-060). The CRG is a Center of Excellence Severo Ochoa SEV-2012-0208.

The CIBER of Rare Diseases is an initiative of the ISCIII.

The laboratory of C.N. acknowledge the funding from the Spanish Ministry of Economy and Finance (MINECO), grant number BFU2011-28575. J.E. received the FI grant from Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) de la Generalitat de Catalunya.

Authors contribution

RM, MD and NC were responsible for the study concept and design. AB and JMc contributed to the acquisition of animal data. JEC and IE performed the PSA analysis. AB, RM and E.M-G participated in the interpretation, writing and revision of the manuscript. RM, MD, and CN provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

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Figure legends

Figure 1. Experimental design. GTT = Glucose tolerance test, PR = Progressive ratio, Cue R = Cue-induced reinstatement.

Figure 2. Body weight in (A) lean and obese mice exposed to chocolate-mixture diet (n = 6 per group) or (B) high-fat diet (n = 8 per group) during the different periods of the experiment: before obesity development, two last weeks of obesity development, food deprivation period (5 days), acquisition of operant responding (10 days), maintenance of operant responding (10 days), extinction training (20 days) and reinstatement session (one day). Fasted glucose levels after food deprivation (12 hours) in (C) animals exposed to chocolate-mixture diet (n = 6 per group) and high-fat diet (n = 8 per group) and glucose tolerance test (D) after glucose administration (2 mg/kg, i.p.) in chocolate-mixture diet (n=6 per group) and high-fat diet groups (n = 8 per group), and respective lean controls after food deprivation (12 hours). Data are expressed as mean $\pm$ SEM. ★ $P<0.05$; ★ ★ $P<0.01$; ★ ★ ★ $P<0.001$ differences between lean and obese group. ☆ ☆ ☆ $P<0.001$ differences between obese mice of chocolate-mixture diet and high-fat diet (Newman-Keuls).

Figure 3. Acquisition of operant responding maintained by chocolate-flavored pellets in food deprived mice and maintenance of operant conditioning to seek chocolate-flavored pellets in mice fed *ad libitum* in (A) chocolate-mixture diet and (B) high-fat diet mice. Mice were trained daily in 1 hour sessions to obtain chocolate-flavored pellets during 20 days under a fixed ratio 1 schedule of reinforcement. Impulsivity-like behavior during acquisition and maintenance of operant responding in (C) chocolate-mixture diet and (D) high-fat diet mice. Acquisition day of learning of operant conditioning in (E) chocolate-mixture diet and (G)

high-fat diet mice. Total number of lever-presses during the 4 hours of progressive ratio session of (F) chocolate-mixture diet (n = 6 per group) and (H) high-fat diet groups (n=8 per group), and respective lean controls. Data are expressed as mean $\pm$ SEM of number of presses on the active and the inactive lever. ★ $P<0.05$; ★ ★ $P<0.01$; ★ ★ ★ $P<0.001$ differences between lean and obese group in the active lever. ☆ $P<0.05$; ☆ ☆ $P<0.01$ differences between lean and obese group in the inactive lever. ## $P<0.01$; ### $P<0.001$ differences between levers in the same diet group (Newman-Keuls).

Figure 4. Extinction of operant responding to seek chocolate-flavored pellets (mice fed *ad libitum*). Mean number of presses on the active and the inactive lever in (A) chocolate-mixture diet (n = 6 per group) and (B) high-fat diet mice (n = 8 per group), and respective lean controls. Extinction day of learning of operant conditioning in (C) chocolate-mixture diet and (D) high-fat diet mice. Cue-induced reinstatement in (E) chocolate-mixture diet (n = 6 per group) and (F) high-fat diet mice (n= 8 per group), and respective lean controls: mean number of presses in the active and the inactive lever during the different experimental phases (mean of the three days of the acquisition criteria under food deprivation condition, mean of the three days of the maintenance criteria under food *ad libitum* condition, mean of three days of extinction criterion and reinstatement induced by cue). Percentage of cue-induced reinstatement versus maintenance (3 days criteria) in (G) chocolate-mixture diet and (H) high-fat diet mice. Data are expressed as mean $\pm$ SEM. ★ $P<0.05$; ★ ★ $P<0.01$; ★ ★ ★ $P<0.001$ significant differences when comparing lean to obese group. ☆ $P<0.05$; ☆ ☆ $P<0.01$; ☆ ☆ ☆ $P<0.001$ differences when comparing the responses in the different lever in the same diet group. ## $P<0.01$; ### $P<0.001$, differences between levers in the same

diet group and experimental phase. @@ $P<0.01$; @@@ $P<0.001$, differences between the distinct experimental phases in the response on the same lever. ### $P<0.001$, differences between levers in the same diet group and experimental phase. @ $P<0.05$; @@ $P<0.01$; @@@ $P<0.001$, differences between the distinct experimental phases in the response on the same lever, \$\$\$ $P<0.001$, significant differences when comparing lean to obese group during the same experimental phase in the response on the same lever (Newman-Keuls).

Figure 5. PCA of the annotated variables from operant conditioning sessions revealed the main behavioral elements that separated experimental groups. (A) Mice clustered by diet group on the space yielded by the two first components of the PCA that account for almost half of the original data variance. (B) Annotated variables colored by the behavioral phenotype they are informative of, represented on the PCA space. Variables reaching values close to 1 in a given axis represent high contribution of the variable to this principal component. (C) Percentage of the data variance explained by each variable to first and (D) second principal components. Variables are annotated as described in supplementary table 1 and correspond to: ACQD (Acquisition Day), CACQ (Compulsivity Acquisition), CM (Compulsivity Maintenance), CUERATIO (Relapse Inflexibility), EXTD (Extinction Day), EXTIN (Extinction Inflexibility), EXTLAUC (Extinction Learning Area Under the Curve), EXTLTD (Extinction Learning Delta), EXTR (Extinction Inflexibility Ratio), IACQ (Impulsivity Acquisition), ICM (Impulsivity/Compulsivity Maintenance), INACQ (Inactive Acquisition), INM (Inactive Maintenance), LAUC (Learning Area Under the Curve), LD (Learning Delta), LDIS (Learning Discrimination), PRBP (Progressive Ratio Breaking Point), PRIMR (Primary Reinforcement), PRIMRD (Primary Reinforcement Discrimination),

PRIMRH (Primary Reinforcement Habituation), IM (Impulsivity Maintenance), RFC (Relapse Fold Change), INCUE (Inactive Cue).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure Supp 1

Figure Supp 2

Figure Supp3

Figure Supp4

Supplementary information

Supplementary Results

Body weight

In chocolate-mixture diet, two-way ANOVA revealed significant main effects of diet [$F_{(1,10)} = 10.187$; $P < 0.01$], experimental phase [$F_{(5,50)} = 44.611$; $P < 0.001$] and significant between both factors [$F_{(5,50)} = 4.367$; $P < 0.01$]. Subsequent Newman-Keuls analysis showed a significant body weight increase in obese animals during all the experimental phases (from $P < 0.05$ to $P < 0.01$) (Fig. 2A). In high-fat diet, two-way ANOVA also revealed a significant main effect of diet [$F_{(1,46)} = 176.2$; $P < 0.001$], experimental phase [$F_{(5,230)} = 215.587$; $P < 0.001$] and interaction between both factors [$F_{(5,230)} = 18.810$; $P < 0.001$]. Subsequent Newman-Keuls analysis showed a significant body weight increase in obese animals during all the experimental phases ($P < 0.001$) (Fig. 2B).

Glucose tolerance test

Glucose tolerance was impaired in obese mice of both models. Three-way ANOVA revealed significant main effects of diet [$F_{(1,31)} = 84.96$; $P < 0.001$], time point [$F_{(5,155)} = 550.876$; $P < 0.001$] and interaction between both factors [$F_{(5,155)} = 44.739$; $P < 0.001$], as well as interactions between model (chocolate-mixture diet/high-fat diet) and time point [$F_{(5,155)} = 10.454$; $P < 0.001$] and among the three factors [$F_{(5,155)} = 7.261$; $P < 0.001$]. Subsequent Newman-Keuls analysis showed a significant increase of glucose levels in obese mice at the chocolate-mixture diet compared to corresponding lean controls in all the time points after glucose administration (from $P < 0.01$ to $P < 0.001$) and in obese mice of the high-fat diet compared to corresponding lean controls in all the time points

before and after glucose administration ($P<0.001$) (Fig. 2D). Finally, post hoc test revealed higher glucose levels in obese mice of high-fat diet than obese mice of chocolate-mixture diet at time point 0, 15 and 120 min ($P<0.001$; Newman-Keuls).

Acquisition and maintenance of operant conditioning maintained by food

During food deprivation, in chocolate-mixture diet three-way ANOVA revealed significant main effects of diet [$F_{(1,10)} = 5.686$; $P<0.05$], day [$F_{(9,90)} = 7.206$; $P<0.001$], lever [$F_{(1,10)} = 733.581$; $P<0.001$] and no significant interaction among these three factors [$F_{(9,90)} = 124.302$; $n.s$] (Fig. 3A). In high-fat diet three-way ANOVA also revealed significant main effects of diet [$F_{(1,14)} = 6.276$; $P<0.05$], day [$F_{(9,126)} = 5.311$; $P<0.001$], lever [$F_{(1,14)} = 123.96$; $P<0.001$] and no significant interaction among these three factors [$F_{(9,126)} = 0.699$; $n.s$] (Fig. 3B).

For maintenance of operant conditioning *ad libitum* in chocolate-mixture diet, three-way ANOVA revealed significant main effects of diet [$F_{(1,10)} = 18.478$; $P<0.05$], lever [$F_{(1,10)} = 620.8$; $P<0.001$], but not day [$F_{(9,90)} = 1.618$; $n.s$], nor interaction among these three factors [$F_{(9,90)} = 124.302$; $n.s$] (Fig. 3A). In high-fat diet, three-way ANOVA also revealed significant main effect of lever [$F_{(1,14)} = 32.875$; $P<0.001$] but no effect of diet [$F_{(1,14)} = 0.728$; $n.s$], day [$F_{(9,126)} = 1.83$; $n.s$], and no interaction among these three factors [$F_{(9,126)} = 0.956$; $n.s$] (Fig. 3B).

Impulsivity-like behavior was also evaluated considering the number of active lever-presses performed during the 10 s of time-out period established after pellet delivery (Fig. 3C,D). During this period, the cue light was off and no reinforcer was provided after responding on the active lever. During food deprivation, in chocolate-mixture diet two-way ANOVA revealed no significant main effect of diet [$F_{(1,10)} = 3.074$; $n.s$] nor day [$F_{(9,90)} = 1.056$; $n.s$], but a significant interaction between these two factors [$F_{(9,90)} =$

2.296; $P<0.05$] (Fig. 3C). Subsequent *post hoc* analysis (Newman-Keuls) showed significant differences between diet groups on day 3 ($P<0.001$) and 4 ($P<0.01$) of acquisition. In high-fat diet two-way ANOVA revealed no significant main effect of diet [$F_{(1,14)} = 0.042$; *n.s.*], but significant effect of day [$F_{(9,126)} = 4.143$; $P<0.001$] and interaction between diet and day [$F_{(9,126)} = 3.092$; $P<0.01$] (Fig. 3D). Subsequent *post hoc* analysis (Newman-Keuls) showed significant differences between diet groups on day 1 ($P<0.05$) and 8 ($P<0.05$) of acquisition.

Impulsivity was also measured during maintenance of operant conditioning *ad libitum* in chocolate-mixture diet, and two-way ANOVA revealed no significant main effect of diet [$F_{(1,10)} = 0.150$; *n.s.*], day [$F_{(9,90)} = 1.541$; *n.s.*] nor interaction between these two factors [$F_{(9,90)} = 0.637$; *n.s.*] (Fig. 3C). In high-fat diet two-way ANOVA revealed no significant main effect of diet [$F_{(1,14)} = 2.647$; *n.s.*], day [$F_{(9,126)} = 1.285$; *n.s.*], but significant interaction between these two factors [$F_{(9,126)} = 2.171$; $P<0.05$] (Fig. 3D). Subsequent *post hoc* analysis (Newman-Keuls) showed significant differences between diet groups on day 9 ($P<0.05$) and 10 ($P<0.05$) of maintenance.

The stability, discrimination and reinforcing criteria of operant learning (see supplementary material and methods), was achieved by 62.5% of obese high-fat diet mice and by 100% of mice of the rest of the groups and this difference was statistically significant [$\chi^2 = 4.80$; $P<0.05$]. In chocolate-mixture diet, one-way ANOVA of acquisition day did not reveal significant main effect of diet [$F_{(1,10)} = 0.48$; *n.s.*] meanwhile in high-fat diet, one-way ANOVA revealed a significant main effect of diet [$F_{(1,14)} = 35.44$; $P<0.001$] (Fig.s 3E and 3G). Thus, obese mice from high-fat food obesity model needed more time than corresponding lean control to achieve criteria (Fig.s 3E and 3G).

Progressive ratio

Two-way ANOVA revealed a significant main effect of lever [$F_{(1,24)} = 80.68$; $P < 0.001$], diet [$F_{(1,24)} = 9.63$; $P < 0.01$] and interaction between lever and diet [$F_{(1,24)} = 6.02$; $P < 0.05$]. Subsequent *post hoc* analysis (Newman-Keuls) showed significant differences between diet groups in chocolate-mixture ($P < 0.01$) and high-fat obesity model ($P < 0.01$) or between active and inactive levers (see figures 3F and 3H for details). In contrast, no effect of obesity model [$F_{(1,24)} = 0.66$; *n.s.*], interaction between diet and obesity model [$F_{(1,24)} = 0.03$; *n.s.*], lever and obesity model [$F_{(1,24)} = 0.58$; *n.s.*] or among diet, lever and obesity model [$F_{(1,24)} = 0.01$; *n.s.*] were revealed (Fig.s 3F and 3H). The breaking point in chocolate-mixture diet was 351.7 for lean mice and 204.2 for obese and in high-fat diet, 406.3 for lean and 221.9 for obese mice. In chocolate-mixture diet, one-way ANOVA of breaking point did not reveal a significant main effect of diet [$F_{(1,10)} = 2.58$; *n.s.*], whereas in high-fat diet revealed a significant main effect of diet [$F_{(1,14)} = 5.58$; $P < 0.05$.] (Fig. 3F and H).

Extinction

After progressive ratio, lean and obese mice from both experimental models were trained to extinguish operant conditioning maintained by chocolate-flavored pellets (Fig. 4A and 4B). In chocolate-mixture diet, three-way ANOVA revealed significant main effect of lever [$F_{(1,10)} = 46.434$; $P < 0.001$], day [$F_{(19,190)} = 14.425$; $P < 0.001$], but not diet [$F_{(1,10)} = 1.812$; *n.s.*], and a significant interaction among these three factors [$F_{(19,190)} = 2.349$; $P < 0.01$] (Fig. 4A). During the first two sessions of extinction, the lean mice showed higher number of responses in the active lever in comparison to the responses of the obese group ($P < 0.001$). Subsequently, the responses in the active lever went down across sessions in both genotypes. The discrimination between levers only

occurred in the first two days of extinction and on day 15th in the lean mice ($P<0.01$ and $P<0.05$). Obese mice did not discriminate during the majority of sessions of the extinction period. A significant enhancement in the inactive lever pressing responses was also observed in the first day of extinction in both genotypes compared to previous inactive lever-pressing responses during acquisition ($P<0.05$) that went down across sessions.

In high-fat diet groups, three-way ANOVA also revealed significant main effects of lever [$F_{(1,14)} = 8.891$; $P<0.01$], day [$F_{(7,498)} = 4.461$; $P<0.001$], but not diet [$F_{(1,14)} = 2.797$; *n.s.*], and a significant interaction among these three factors [$F_{(6,426)} = 1.741$; $P<0.05$] (Fig. 4B). During the first session of extinction, the lean mice showed higher number of responses in the active lever than the obese group ($P<0.01$). Subsequently, the responses in the active lever went down across sessions. The discrimination between levers was absent in lean and obese mice during the majority of days of extinction. A significant enhancement in the inactive lever-presses responses was also observed in the first day of extinction in both genotypes compared to previous inactive lever-pressing responses during acquisition ($P<0.05$). This enhancement was maintained in the obese group and went down across sessions in the lean mice.

The extinction criterion (see materials and methods) was achieved by 88% of obese high-fat diet mice and by 100% of mice of the rest of the groups. This difference did not reach statistical significance [$\chi^2 = 1.14$; *n.s.*]. In chocolate-mixture diet, one-way ANOVA of extinction acquisition day did not reveal significant main effect of diet [$F_{(1,10)} = 0.50$; *n.s.*]. Similarly in high-fat diet, one-way ANOVA did not reveal a significant main effect of diet [$F_{(1,14)} = 1.85$; *n.s.*] (Fig.s 4C and 4D).

In both diets, one-way ANOVA of percentage of cue-induced reinstatement versus maintenance (3 days criteria) did not reveal a significant main effect in chocolate-mixture diet [$F_{(1,10)} = 3.59$; *n.s.*] or in high-fat diet [$F_{(1,14)} = 1.13$; *n.s.*] (Fig.s 3F and 3H).

Reinstatement

In chocolate-mixture diet, three-way ANOVA revealed a significant main effect of diet [$F_{(1,10)} = 10.276$; $P < 0.01$], lever [$F_{(1,10)} = 550.001$; $P < 0.001$], phase [$F_{(3,30)} = 101.172$; $P < 0.001$] and interaction between lever and phase [$F_{(3,30)} = 114.793$; $P < 0.001$], lever and diet [$F_{(1,10)} = 11.259$; $P < 0.01$] and among all three factors [$F_{(3,30)} = 2.899$; $P < 0.05$], but not interaction between diet and phase [$F_{(3,30)} = 1.752$; *n.s.*]. Subsequent Newman-Keuls analysis showed significant differences in responding on active and inactive levers in acquisition, maintenance and reinstatement for obese and lean mice ($P < 0.001$), as well as between lean and obese on response on the same lever in cue-induced reinstatement ($P < 0.001$). Three-way ANOVA also showed significant differences in responding on active levers during the distinct experimental phases for lean mice (acquisition versus maintenance, $P < 0.01$, maintenance versus extinction or extinction versus cue-induced reinstatement, $P < 0.001$) and for obese mice (acquisition versus maintenance, $P < 0.05$, maintenance versus extinction, $P < 0.001$ and extinction versus cue-induced reinstatement, $P < 0.05$) (Fig. 4E).

In high-fat diet, three-way ANOVA revealed a significant main effect of lever [$F_{(1,14)} = 127.769$; $P < 0.001$], phase [$F_{(3,42)} = 35.119$; $P < 0.001$], as well as significant interaction between lever and phase [$F_{(3,42)} = 72.815$; $P < 0.001$], lever and diet [$F_{(1,14)} = 49.455$; $P < 0.001$] and among all three factors [$F_{(3,42)} = 7.129$; $P < 0.001$] but not effect of diet [$F_{(1,14)} = 0.121$; *n.s.*] nor interaction of diet and phase [$F_{(3,42)} = 1.981$; *n.s.*]. Subsequent Newman-Keuls analysis showed significant differences in responding on active and inactive levers for obese and lean mice during acquisition ($P < 0.001$) and for lean mice

during maintenance and reinstatement ($P<0.001$). Newman-Keuls analysis also showed significant differences in responding on active levers during the distinct experimental phases for lean mice (acquisition versus maintenance, $P<0.01$, maintenance versus extinction or extinction versus cue-induced reinstatement, $P<0.001$) and for obese mice (acquisition versus maintenance, $P<0.01$, maintenance versus extinction, $P<0.001$ and extinction versus cue-induced reinstatement, $P<0.01$) (Fig. 4F).

The reinstatement criterion (see materials and methods) was achieved by 75% of obese high-fat diet mice, 83% of obese chocolate mixture and by 100% of both lean groups. No significant differences between lean and obese in the high-fat [$\chi^2 = 2.67$; *n.s.*] and chocolate-mixture groups were revealed [$\chi^2 = 1.20$; *n.s.*].

Supplementary figure legends

Fig. S1. Representative patterns of active and inactive lever-presses on various sessions for (A) chocolate-mixture diet and (B) high-fat diet experiments. Each vertical line represents one active or inactive lever-press. The horizontal line represents the 1-h session. The upper pattern (line 1) corresponds to the active and the lower (line 2) to the inactive lever-pressing responses.

Fig. S2. PCA of annotated variables from acquisition and maintenance of operant conditioning. (A) High-fat diet group are separated of the rest of the diet groups by PC1 (44% of the variance), PC2 (25% of the variance) is not able to discriminate groups (B) Direction of the variables, coloured by its behavioral phenotype, on the PCA space formed by PC1 and PC2. (C) Bars represent the contribution of the variables to PC1 and (D) PC2. Variables are annotated as described in supplementary table 1 and correspond to: LAUC (Learning Area Under the Curve), LD (Learning Delta), LDIS (Learning Discrimination), IACQ (Impulsivity Acquisition), ICACQ (Impulsivity/Compulsivity Acquisition) and CACQ (Compulsivity Acquisition).

Fig. S3. PCA of annotated variables from extinction of operant conditioning. (A) High-fat diet group are separated of the rest of the diet groups by PC1 (44% of the variance), PC2 (25% of the variance) is not able to discriminate groups (B) Direction of the variables, coloured by its behavioral phenotype, on the PCA space formed by PC1 and PC2. (C) Bars represent the contribution of the variables to PC1 and (D) PC2. Variables are annotated as described in supplementary table 1 and correspond to: EXTLAUC (Extinction Learning Area Under the Curve), EXTLD (Extinction Learning

Delta), EXT_D (Extinction Day), EXT_{IN} (Extinction Inflexibility) and EXT_R (Extinction Inflexibility Ratio).

Fig. S4. PCA of annotated variables from cue-induced reinstatement. (A) High-fat diet group are separated of the rest of the diet groups by PC1 (68% of the variance), PC2 (28% of the variance) is not able to discriminate groups (B) Direction of the variables, coloured by its behavioral phenotype, on the PCA space formed by PC1 and PC2. (C) Bars represent the contribution of the variables to PC1 and (D) PC2. Variables are annotated as described in supplementary table 1 and correspond to: CUERATIO (Reinstatement inactive lever presses divided by active lever presses), INCUE (Reinstatement inactive lever presses) and RFC (Reinstatement active lever presses divided by mean of active lever presses during three last days of extinction minus 1).