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Evidence for a compulsive-like behavior in rats exposed to alternate access to highly preferred palatable food

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ABSTRACT

Converging evidence suggests that recurrent excessive calorie restriction causes binge eating by promoting behavioral disinhibition and overeating. This interpretation suggests that cognitive adaptations may surpass physiological regulations of metabolic needs after recurrent cycles of dieting and binging. Intermittent access to palatable food has long been studied in rats, but the consequences of such diet cycling procedures on the cognitive control of food seeking remain unclear. Female Wistar rats were divided in two groups matched for food intake and body weight. One group received standard chow pellets 7 days/week, whereas the second group was given chow pellets for 5 days and palatable food for 2 days over seven consecutive weeks. Rats were also trained for operant conditioning. Intermittent access to palatable food elicited binging behavior and reduced intake of normal food. Rats with intermittent access to palatable food failed to exhibit anxiety-like behaviors in the elevated plus maze, but displayed reduced locomotor activity in the open field and developed a blunted corticosterone response following an acute stress across the diet procedure. Trained under a progressive ratio schedule, both groups exhibited the same motivation for sweetened food pellets. However, in contrast to controls, rats with a history of dieting and binging exhibited a persistent compulsive-like behavior when access to preferred pellets was paired with mild electrical foot shock punishments. These results highlight the intricate development of anxiety-like disorders and cognitive deficits leading to a loss of control over preferred food intake after repetitive cycles of intermittent access to palatable food.

Keywords Anxiety, compulsive-like behavior, diet, palatable food, stress.

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INTRODUCTION

In developed countries, where nutrition and life-style have dramatically changed in the last decades, more than 1.5 billion adults are overweight. The World Health Organization reports that the increased body weight in the general population correlates with consumption of larger amounts of calorie-dense food and reduced physical activity (WHO 2009). A large body of evidence suggests that overweight and obese people have higher risks of developing diabetes, heart diseases and cancer (Allison *et al.* 1999). According to the current epidemiologic data, overweight/obesity, high blood glucose and high cholesterol have become the leading causes of preventable death behind high blood pressure and tobacco use worldwide (WHO 2009). In parallel, patients suffering from eating disorders, particularly anorexia nervosa, frequently experience mood disorders, including negative emotionality, neuroticism, generalized anxiety and depression (Kaye 2009; Luppino *et al.* 2010).

Current considerations on feeding regulation take into account two overlapping brain mechanisms, the *homeostatic* and the *hedonic* control of food intake (Berthoud 2007; Lutter & Nestler 2009). Clinical researchers have proposed that the hedonic control of food intake surpassed the homeostatic metabolic needs in overeaters, especially in compulsive overeaters (Zheng *et al.* 2009; Parylak, Koob & Zorrilla 2011). Concomitantly, recent evidence collected in pre-clinical models has suggested that compulsive overeating might share some neurobiological adaptations reminiscent of drug addiction (Avena, Rada & Hoebel 2008; Davis & Carter 2009; Kenny

2011b). Even if the debate remains open, and thoroughly discussed (Benton 2010; Wilson 2010; Ziauddeen, Farooqi & Fletcher 2012), most of recent clinical and pre-clinical data support the view that compulsive overeating and addiction share common neural substrates (Barry, Clarke & Petry 2009; Corsica & Pelchat 2010; Grosshans, Loeber & Kiefer 2011; Volkow *et al.* 2013). Of particular interest, sugar and sweet fat diets have been shown to stimulate the brain reward system (Hernandez & Hoebel 1988; Avena *et al.* 2008; Berridge *et al.* 2010) and it appears that hypersensitivity of reward circuitries may predispose an individual to overeating and weight gain (Kenny 2011b). In particular, the key role of dopamine receptor 2 has been demonstrated in both clinical (Stice *et al.* 2010, 2011) and pre-clinical studies (Johnson & Kenny 2010), emphasizing the plausibility of common cellular and molecular mechanisms in obesity and drug addiction (Volkow *et al.* 2013). Further, it has also been proposed that acute physical or emotional stress increased preference for fat and/or sugar contents even in absence of hungriness, thus defining the notion of comfort food (Dallman *et al.* 2003; Dallman 2010). Consumption of comfort food would alleviate signs of distress and anxiety by reducing the hypothalamic-pituitary-adrenal (HPA) axis activity (Dallman *et al.* 2003; Pecoraro *et al.* 2004; Gibson 2012). Such a relief, together with the direct activation of brain reward systems, would promote sweetened food seeking and exacerbate craving for calorie-dense food. This effect could be mediated by insulin and glucocorticoids (Dallman 2010). However, recent evidence demonstrated that the caloric load is not as critical as the hedonic aspect of palatable food consumption for reducing stress since saccharin-fed (or sexually active) rats had attenuated HPA axis responses to acute stress, like sucrose-fed animals (Ulrich-Lai *et al.* 2010). This observation is of significant importance since it suggests that the subjective feeling of pleasantness is fundamental for promoting palatable food consumption. This assumption may also explain why it is not the palatable food *per se* that promotes compulsive overeating, but most likely its pattern of consumption (Corwin & Grigson 2009; Corwin 2011). Polivy & Herman (1985) already reported that dieting often preceded binge eating chronologically. They proposed that recurrent excessive calorie restriction, in order to prevent weight gain, might cause binging by promoting behavioral disinhibition and overeating (Polivy & Herman 1985). Interestingly, these authors have also suggested that, with cognitive controls supplanting physiological regulatory processes, eating and weight begin to dominate the dieter's thought. Ultimately, dieting makes the dieter vulnerable to break the restrictive rules and lose control over incentive 'forbidden' calorie-dense food (Polivy & Herman 1985). In line with this interpretation, recent findings point out that alterations in cognitive processes, such as the appreciation of the rewarding value of palatable food and the capacity to inhibit hedonic feeding, may represent vulnerability factors promoting bingeing and overeating (Appelhans *et al.* 2011).

In parallel to these clinical observations, sugar binging models have been established in laboratory animals. However, escalation in sucrose consumption often required recurrent cycles of food restriction followed by periods of full access to sucrose or palatable food intake (Hagan *et al.* 2003; Corwin 2006, 2011; Avena *et al.* 2008), which raises the possibility of confounding stress-like effects due to chronic starvation. Noteworthy, recent pre-clinical evidence confirmed that alternate access to sweet fat food elicited binging behavior in non-food-deprived laboratory animals (Berner, Avena & Hoebel 2008; Cottone *et al.* 2008a,b, 2009a,b). Rats with alternate access to a palatable food exhibited binging behavior, metabolic alterations and anxiety-like behaviors (Cottone *et al.* 2009a,b). This model appears to be the most relevant for studying long-term consequences of 'yo-yo dieting', as defined as recurrent cycles of dieting followed by disinhibited hyperphagia. Indeed, intermittent access to rewards (palatable food or drugs of abuse) leads to negative emotional states when the rewarding substance is no longer available, and it is considered that such negative emotional states may be sufficient for motivating substance use and abuse via negative reinforcement mechanisms.

In summary, the present study investigated whether rats with a history of alternating access to highly preferred food (but only containing 5% more calories than regular chow pellets) would exhibit anxiety-like traits and cognitive alterations defined as a loss of control over palatable food intake.

MATERIALS AND METHODS

Animals

Thirty adolescent female Wistar rats (140–200 g, 45–60 days old at the beginning of the experiment) were individually housed and kept under reversed light-dark cycle conditions (12 hours light–dark cycle, lights off at 8 am) where humidity (50–60%) and temperature (22 ± 1°C) were controlled. Food and water were available *at libitum* during the procedure. All behavioral experiments were carried out during the dark phase of the cycle. The procedures were conducted in conformity with the Swiss National Institutional Guidelines on Animal Experimentation, and approved by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation (authorization 1999 to B.B.)

Table 1 Diet composition and energy density.

Diet	*Energy density (kcal/g)*	*Macronutrient composition (kcal%)*		
		Carbohydrate	*Protein*	*Fat*
3436 Kliba (standard food)	3.129	77.0	18.5	4.5
5TCY Test Diets (preferred food)	3.290	63.6	22.5	13.9
Precision pellets BioServ (sweet pellets)	3.600	67.2	20.5	12.3

Alternate feeding protocol

After 1 week of habituation, animals matched for food intake and body weight were divided in two groups. One group (named C/C) received standard chow 7 days a week, whereas the second group (named C/P) was given 5 days of standard chow (defined as the C phase, for access to chow food) followed by 2 days of a more palatable food (defined as the P phase, for access to preferred food) for a total of seven consecutive weeks. Standard diet (3436 Kliba Nafag, Provimini Kliba AG, Kaiseraugst, Switzerland) and palatable diet (5TCY Test Diets, IPS Ltd, London, UK) had comparable energy density and macronutrient composition (see Table 1), and palatable diet was chocolate flavored. Daily caloric food intake and body weight were measured every day, around 1 hour after the onset of the dark phase.

Food operant conditioning

Apparatus

Food conditioning was conducted in six operant chambers (Med Associates Inc., St. Albans, VT, USA) placed in sound-attenuated cubicles equipped with ventilation fans. Inside cages, a food receptacle was located between two retractable levers and connected to a food dispenser. Presses on the 'active lever' resulted in the release of a 45 mg food pellet whereas presses on the 'inactive lever' had no consequence. A cue light illuminated for 1 second above the active lever signaled food delivery. The grid floor of the cage was connected to a shocker apparatus for administration of electrical foot shocks. Custom-made schedule programming and data acquisition were driven by a Med-PC software package (Med Associates Inc.).

Training for food reinforcement

During the 'C phase' of the third cycle of alternate feeding, rats were trained in the operant cages to acquire the lever pressing behavior and get food pellet rewards under a fixed ratio 1 time out 1 second (Dustless precision pellet 45 mg, rodent purified diet, BioServ, Frenchtown, NJ, USA). Training sessions lasted until the rat collected 60 rewards or terminated after a maximum of 30 minutes. Rats were trained twice a day and after five sessions, all rats succeeded to collect 60 pellets in less than 30 minutes.

Progressive ratio measurements

One week after, all animals were submitted to a progressive ratio (PR) schedule of reinforcement (meaning during the 'C phase' of the fourth cycle). Rats had to increase progressively the number of lever presses to receive one pellet reward. The progression of responses required was set to 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, etc. Both groups of rats underwent two PR sessions a day, one session with sweet pellets (45 mg Dustless precision pellets, BioServ) and the other session with chocolate pellets (45 mg 5TCY pellets, Test Diets). Rats were randomly assigned to sessions so that half of them received chocolate pellets in the morning and the others in the afternoon. Each session ended when no response was emitted for 20 minutes or after a total duration of 90 minutes. The number of lever presses was measured as an index of motivation. The test was performed twice, on the first and fifth day after home cage palatable food withdrawal.

Compulsive food seeking

Perseverance to get food pellets despite shock punishments was assessed in rats pressing a lever paired with the delivery of a 45-mg food pellet immediately followed by a moderate foot shock punishment (0.22 mA, 0.5 second) during a 30-minute test session, on the first and fifth day after preferred food withdrawal. Since repeated exposure to foot shocks may have induced confounding effects in rats, C/P and C/C groups were randomly divided in two subgroups. Rats were exposed to foot shocks only twice, on day 1 and day 5 as mentioned above, and thus were tested with one type of food reward only (half rats tested on chocolate pellets and half rats tested on standard pellets).

Anxiety-related behaviors

Rats were tested in the open field–novel object (during the fifth cycle) and in the elevated plus maze (during the sixth cycle) paradigms on the first day of palatable food withdrawal for evaluating anxiety-like behaviors. Both

experiments were conducted under a dim light (10–15 lx). Animal tracks were recorded by a digital video camera mounted above the maze and connected to a computer running a tracking software (Ethovision v.3.1—Noldus Technology, Wageningen, the Netherlands).

Open field–novel object

Rats were placed in a round arena (140 cm of diameter, 30 cm of depth) and their motor and exploratory activities were monitored for 10 minutes, after which a novel object (a white plastic bottle) was introduced in the center of the maze. The distance travelled in the arena, the time spent in the central part of the arena and the time spent touching the novel object were assessed for additional 5 minutes.

Elevated plus maze

The elevated plus maze consisted of two opposite open arms (50 cm L × 10 cm W × 42.5 cm H) and two opposite closed arms (50 cm L × 10 cm W) arranged in a cross and elevated 50 cm above the floor. In the center, a small platform (10 cm × 10 cm) gave access to all arms. Rats were gently placed in the center of the maze face to a close arm and their behavior was monitored for 5 minutes. The time spent on open arms was used as an index of anxiety.

Plasma corticosterone levels in response to acute stress

Plasma corticosterone levels were measured in response to an acute stress at the beginning (third cycle) and at the end (seventh cycle) of the entire protocol. The acute stress (rats were placed on an elevated platform 12 × 12 × 101 cm above the floor) was applied for 30 minutes on the first day of preferred food withdrawal. Corticosterone levels were assessed at three different timepoints, always in the middle of the dark (active) period: 24 hours before the acute stress (basal), 5 minutes and 60 minutes (t5 and t60) after the stress procedure. A blood sample (200–300 µl) was systematically collected using heparin Microvette CB 300 (Sarstedt AG, Sevelen, Switzerland) after tail vein incision. Blood samples were then centrifuged at 4°C for 20 minutes at 4500 rpm. Plasma samples were analyzed using a commercial enzymatic-immuno assay kit (Corticosterone EIA kit, Enzo Life Sciences, Lausen, Switzerland).

Statistical analysis

Data are expressed as mean ± standard error (SE). Food intake, body weight gain, operant conditionings and corticosterone measures were evaluated by using a two-way analysis of variance (ANOVA) with 'group' as between-subject factor and sampling 'time' as repeated-measures within-subject factor, followed by Fischer's PLSD *post-hoc* tests for multiple comparisons. Student's *t*-test was used to compare the distance moved in the open field and the time spent on the open arms of the elevated plus maze. For the novel object touching observation, a Shapiro–Wilks test revealed that the data were not normally distributed ($W = 0.8798$, $P = 0.0259$). Hence, a Mann–Whitney *U*-test was used to compare the duration of contact with the novel object. All statistical analyses were conducted using the statistical software STATISTICA 10 (Stat Soft, Tulsa, OK, USA). The level of significance was set at 0.05.

RESULTS

Alternate palatable food access changes food-taking behavior

During the last 3 days of habituation in individual cages, rats assigned to the standard laboratory chow pellets (C/C group) and rats exposed to alternating access to standard chow and preferred food (C/P group) exhibited similar body weight (180.7 ± 7.35 g and 182.8 ± 5.73 g, respectively). Their daily caloric consumption was also identical (49.9 ± 1.85 kcal/day and 47.2 ± 0.78 kcal/day, respectively).

Afterwards, as shown on Fig. 1, chow/preferred rats overate during the P phases and underate during the C phases, as compared to chow/chow control rats. Consumption of palatable food was particularly elevated on the first day of each 'P phase' [$F_{1.28}$ (group) = 43.55, $P < 0.001$]. Monitoring of food intake revealed that C/P rats displayed a binge eating behavior for the first 8 hours of access to preferred food compared to C/C rats [$F_{1,14}$ (group) = 12.20, $P < 0.01$; $F_{2,28}$ (time) = 217.57, $P < 0.001$; $F_{2,28}$ (group × time) = 3.84, $P < 0.05$; Fig. 1b]. When C/P rats returned to standard food, they displayed a significant reduction of food taking, again during the first day after preferred food withdrawal [$F_{1.28}$ (group) = 80.93, $P < 0.001$]. However, C/P rats ate progressively more food during the next days and, on the fifth day, their food intake was similar to controls. Overall, the mean caloric intake of C/P rats during 'P phases' (Fig. 1c) was significantly higher compared to C/C rats [$F_{1,28}$ (group) = 21.85, $P < 0.01$] and this effect was stable across the procedure [$F_{6,168}$ (group × phase) = 1.14, $P = 0.338$]. Meanwhile, when exposed to regular chow pellets ('C phases'), the C/P group displayed a drastic reduction of mean caloric intake [$F_{1,28}$ (group) = 20.43, $P < 0.01$; $F_{6,168}$ (group × phase) = 1.08, $P = 0.376$] compared to controls. As expected, body weight gain in C/P rats changed accordingly with palatable food availability. When rats had access to palatable food, their body weight gain was significantly increased compared to that of control rats [$F_{1,28}$ (group) = 7.72, $P < 0.01$]. Conversely, when exposed to laboratory chow

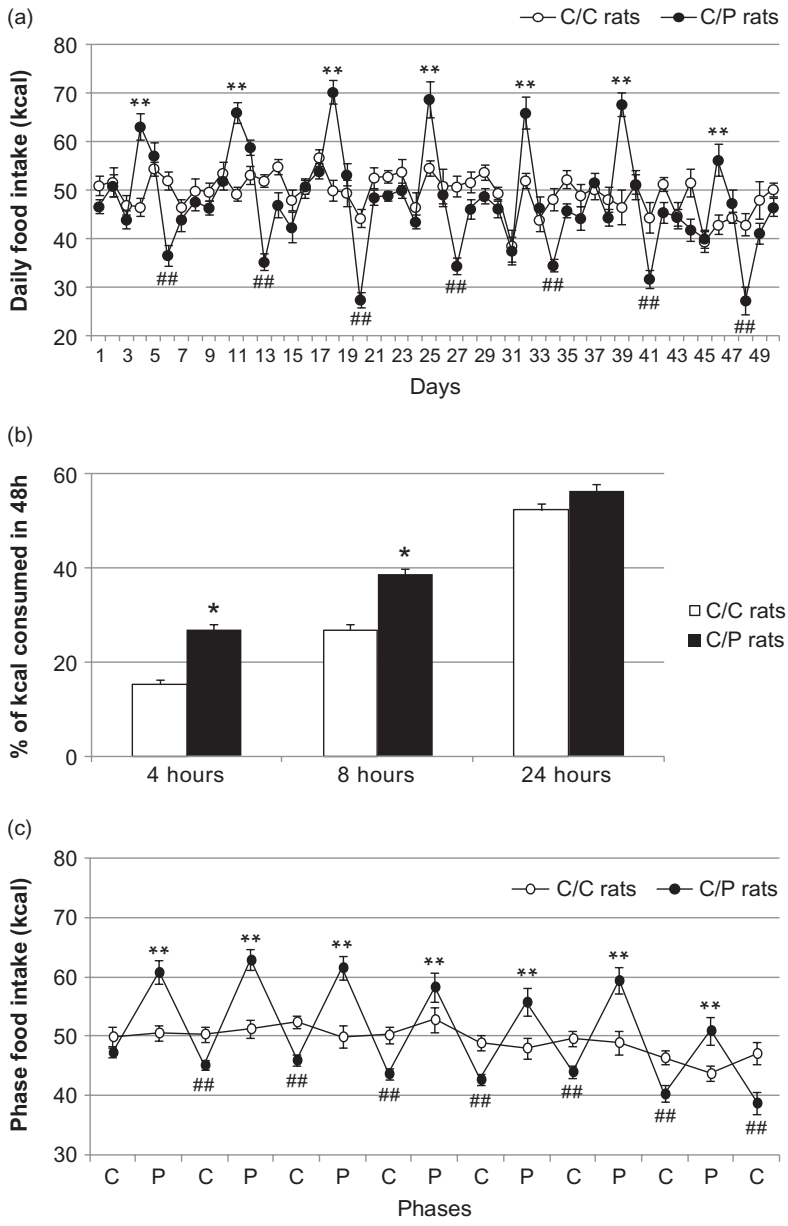

Figure 1 Intermittent access to preferred palatable food induced recurrent periods of food binging and restriction. (a) Data represent mean daily food intake (±SE) expressed in kcal. C/P rats ($n = 16$) had access to palatable food for 2 days followed by 5 days of standard chow pellet while C/C (control) rats had a continuous access to standard chow pellet (white circles, $n = 14$). (b) Data represent the mean cumulative food intake (±SE) expressed in percent of the total intake over the 48 hours of access to preferred food in C/P rats ($n = 8$) compared to standard food intake in C/C animals ($n = 8$) at the end of the procedure. (c) Data represent the average daily food intake (±SE) during the C (5 days of standard food) and P (2 days of preferred food) phases in C/P rats ($n = 16$) compared to controls ($n = 14$). Symbols (** and ##) indicate significant differences ($P < 0.01$) from controls (two-way repeated-measure ANOVA followed by Fisher's PLSD *post hoc* tests)

pellets, the body weight gain of C/P rats tended to be reduced [$F_{1,28}$ (group) = 3.92, $P = 0.057$].

As a result of these fluctuations in body weight gain, the cumulative body weight gain was similar in both groups of rats at the end of experimental procedure (Fig. 2).

Alternate palatable food access does not change motivation for preferred food in a PR schedule

All rats exhibited similar performances for collecting sweetened (BioServ) food pellets in a fixed ratio 1 schedule of reinforcement (C/C rats: 64.79 ± 1.20 and C/P rats: 64.31 ± 1.28 lever presses; *t*-test, $P = 0.791$). After acquisition of the operant conditioning procedure, C/C and C/P rats were tested on a PR schedule with both sweetened (BioServ) and chocolate (Test Diets) pellets. The experiment was repeated twice, on the first (test 1) and fifth day (test 2) of the 'C phase', meaning on the first and fifth day after preferred food withdrawal (Fig. 3a & b).

All rats exhibited similar performance during test 1 and test 2, suggesting similar motivation for the sweetened (BioServ) food pellets [$F_{1,56}$ (group) = 0.020, $P = 0.888$; $F_{1,56}$ (test) = 1.428, $P = 0.237$; Fig. 3a]. Interestingly, when exposed to the chocolate (Test Diets) food pellets, all rats again manifested similar performances, either during test 1 or test 2 [$F_{1,56}$ (group) = 0.433, $P = 0.512$; $F_{1,56}$ (test) = 0.374, $P = 0.543$; Fig. 3b], suggesting that C/P rats did not exhibit a higher motivation

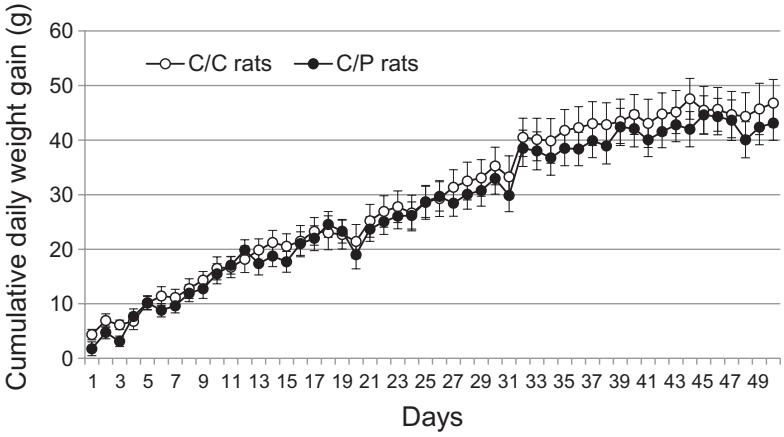

Figure 2 C/P ($n = 16$) and C/C (control) rats ($n = 14$) displayed similar cumulative weight gain curves during the seven weeks of the experimental procedure. Data represent mean weight (±SE) expressed in grams

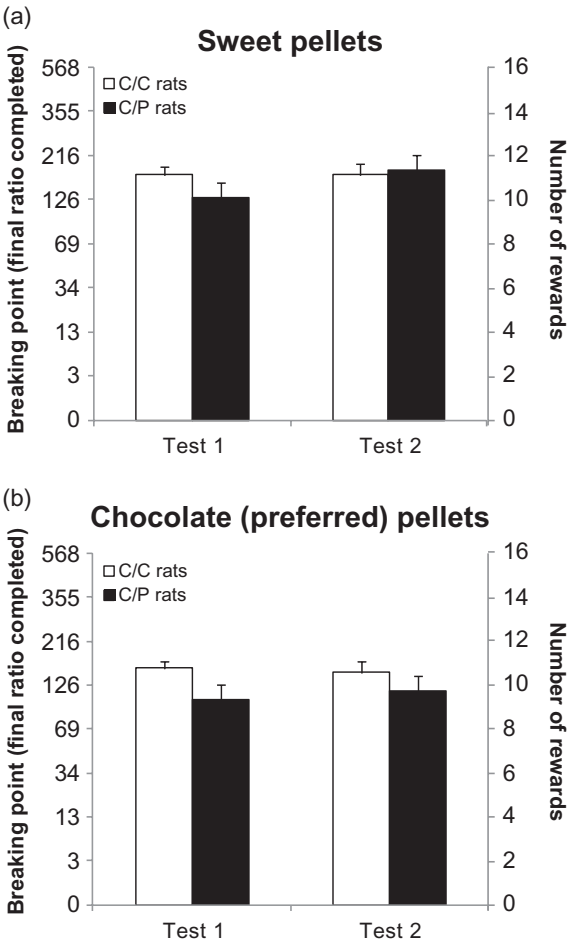

Figure 3 C/P ($n = 16$) and C/C (control) rats ($n = 14$) displayed similar motivation for sweetened and chocolate (preferred) pellets. Data represent mean breaking points (±SE) and concomitant mean rewards (±SE)

for preferred food compared to control animals. All rats, including controls for which access to palatable food was unusual, displayed a robust effort to collect sweetened food rewards, which reflects the high reinforcing properties of these pellets.

Noteworthy, standard laboratory chow pellets (3436 Kliba Nafag) were not available in 45 mg size. Consequently, we could not assess motivation for standard food using the PR schedule of reinforcement. Nonetheless, sated animals may not have worked thoroughly for non-rewarding/non-palatable food.

Alternate palatable food access promotes compulsive-like behavior

Perseverance to get food pellets despite shock punishment is considered to reflect compulsive-like behavior. Hence, rats were exposed to foot shocks twice, on day 1 and day 5 after preferred food withdrawal. They were tested with one type of food reward only, half rats were tested with Test Diet pellets, and the other half was tested with BioServ pellets.

All rats exposed for the first time to the foot shock procedure (test 1) accepted a few number of aversive stimuli for collecting sweet (BioServ) food pellets. However, when re-exposed to the experimental paradigm (test 2), all rats drastically reduced their number of food rewards and concomitant foot shocks. A two-way repeated-measure ANOVA revealed a significant main effect for test ($F_{(1,18)} = 16.87$, $P < 0.001$) but not for group ($F_{(1,18)} = 2.17$, $P = 0.157$) suggesting that the aversion for foot shocks surpassed motivation for BioServ food pellets in both C/C and C/P rats (Fig. 4a).

Strikingly, all animals exposed for the first time to the foot shock procedure (test 1) paired with preferred (Test Diets) food pellets exhibited an enhanced perseverance for reward-associated punishments compared to rats exposed to sweet (BioServ) pellets ($F_{(1,24)} = 6.95$, $P = 0.014$; Fig. 4a & b), whatever their group ($F_{(1,24)} = 0.854$, $P = 0.364$). However, only rats with a history of intermittent access to preferred food persisted to lever press during test 2. A two-way repeated-measure ANOVA revealed a significant group × test interaction ($F_{(1,18)} = 5.14$, $P < 0.05$), and Fisher's PLSD *post-hoc* tests among means demonstrated a significant decrease in reward

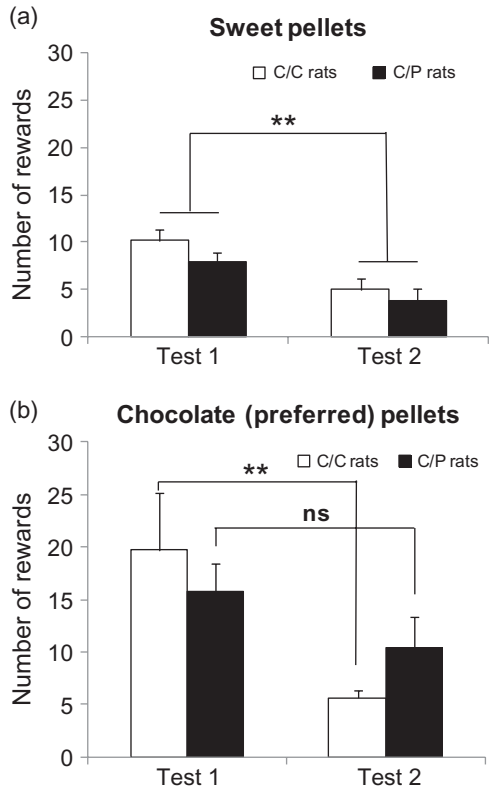

Figure 4 C/P rats persisted in taking preferred food pellets despite shock punishment compared to C/C (control) rats. Data represent the mean number of reward/punishments (±SE). (a) Both groups of rat (C/C, $n = 6$; C/P, $n = 5$) significantly reduced their lever pressing behavior on the second test for sweet pellets. (b) C/C rats ($n = 7$) significantly reduced their lever pressing behavior on the second test for preferred food pellets whereas C/P ($n = 10$) rats persisted in seeking palatable food despite aversive consequences. Symbols ** indicate a significant difference ($P < 0.01$) from test 1 (two-way repeated-measure ANOVA followed by Fisher's PLSD *post hoc* tests)

taking between test 1 and test 2 in C/C rats, whereas C/P rats displayed unchanged reward/punishment taking. The incentive for preferred (Test Diets) food pellets surpassed the aversion for foot shocks in C/P rats only, thus demonstrating that intermittent access to highly rewarding food promotes persistent palatable food-seeking behavior despite aversive consequences.

Alternate palatable food accesses moderately affect anxiety-like behaviors

Previous reports established that intermittent access to palatable food elicits signs of anxiety in rats. Hence, we evaluated anxiety-like behaviors in C/C and C/P rats at the end of the experimental procedure (fifth and sixth food cycles).

Open field–novel object

Although both groups spent similar amount of time in the central zone of the arena, C/P rats exhibited a reduced exploratory behavior assessed by the decreased distance travelled in the entire arena compared to C/C rats (5921.47 ± 162.91 cm versus 6473.81 ± 189.76 cm, respectively; *t*-test, $P = 0.0413$; Fig. 5a). Further, both groups displayed similar approaching behavior after introduction of the novel object in the central zone of the open field (10.00 ± 3.25 and 18.80 ± 4.53 seconds contacting the object, respectively; Mann–Whitney test, $P = 0.1097$; Fig. 5b).

Elevated plus maze

Both groups of rats spent an average of 20 to 25% of the total time on the open arms of the elevated plus maze suggesting similar emotional states while performing this test (C/C rats: 61.73 ± 19.47 and C/P rats: 68 ± 21.16 seconds; *t*-test, $P = 0.8117$; Fig. 5c).

Alternate palatable food access alters corticosterone response after an acute stress

We measured corticosterone levels in all rats at the beginning (third cycle) and at the end (seventh cycle) of the experimental procedure. At the beginning of the feeding protocol (Fig. 6a), both C/C and C/P rats displayed similar basal corticosterone levels (85.6 ± 13.7 versus 62.0 ± 10.3 ng/ml, respectively). Plasma levels strongly increased after the acute stress in both groups (321.3 ± 49.0 ng/ml and 276.3 ± 58.7 ng/ml, respectively) and returned to basal values later on (105.0 ± 26.5 ng/ml and 120.5 ± 50.3 ng/ml, respectively). A two-way repeated-measure ANOVA showed a main significant effect for time ($F_{2,40} = 28.75$, $P < 0.001$) but no difference between groups ($F_{1,20} = 0.175$, $P = 0.679$) suggesting that all rats responded similarly to the acute stress.

When rats were again tested at the end of the feeding protocol (Fig. 6b), C/C and C/P rats still exhibited similar basal levels of corticosterone (41.94 ± 10.6 versus 58.41 ± 8.11 ng/ml, respectively). However, whereas C/C rats still exhibited a drastic response the acute stress procedure, C/P rats displayed blunted corticosterone levels (298.4 ± 48.7 versus 186.8 ± 21.0 ng/ml, respectively; Fig. 6b). A two-way repeated-measure ANOVA revealed a main significant effect for time ($F_{2,40} = 47.52$, $P < 0.001$) and a significant interaction time × group ($F_{2,40} = 4.35$, $P = 0.019$), and Fisher's PLSD *post-hoc* tests among means demonstrated a significant decrease in corticosterone levels after acute stress in C/P rats compared to C/C controls. Taken together these results revealed an adaptation of the HPA axis in animals with a history of intermittent access to preferred palatable food.

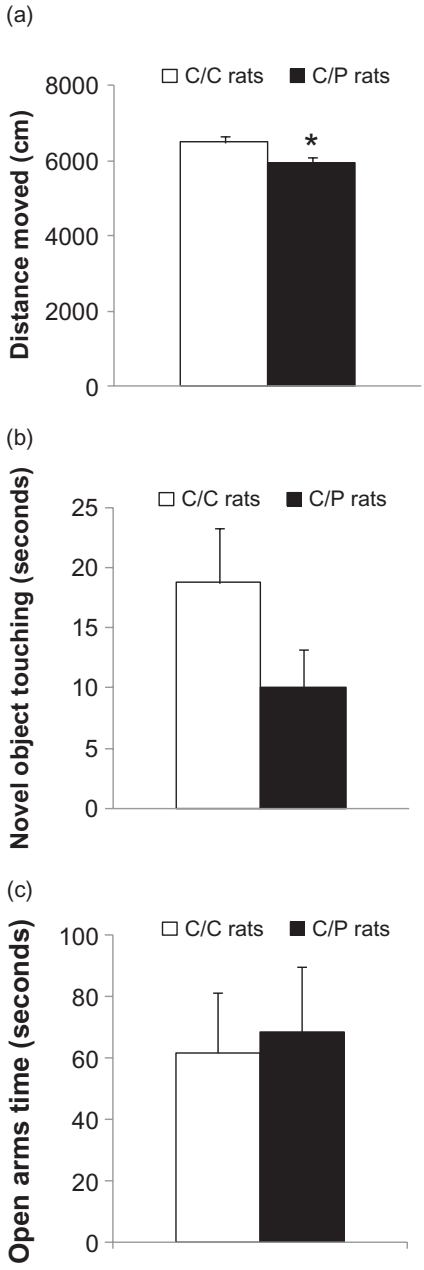

Figure 5 Measures of anxiety like-behaviors in C/C ($n = 14$) and C/P ($n = 16$) rats. (a) Data represent the mean distance (±SE) travelled in cm. C/P rats exhibited a reduced exploratory behavior compared to C/C rats in the open field (*t*-test, $^{*}P < 0.05$). (b) Data represent the mean duration (±SE) of contact with the novel object expressed in second. C/P rats showed a reduced, but not significant, interaction with the novel object compared to C/C rats (Mann–Whitney test, $P = 0.1097$). (c) Data represent the mean duration (±SE) expressed in second of the time spent on the open arms of the elevated plus maze. Both groups of rats spent similar amount of time on the open arms (*t*-test, $P = 0.8117$)

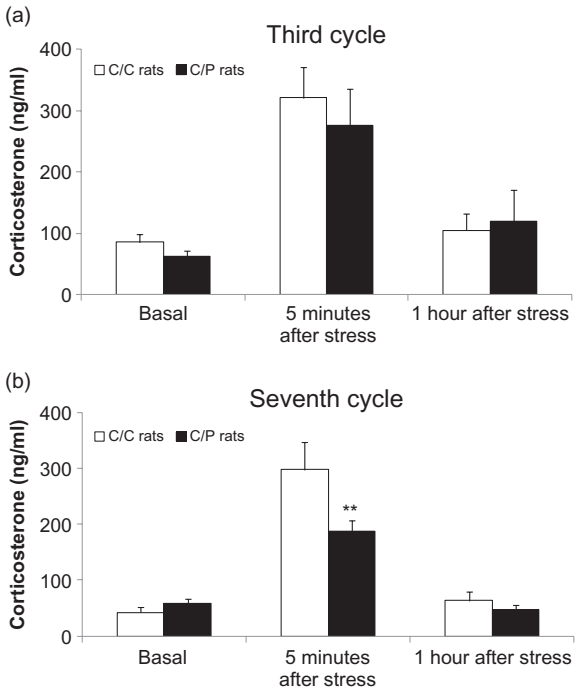

Figure 6 C/P ($n = 16$) rats exhibited blunted corticosterone levels after an acute stress compared to C/C (control) rats ($n = 14$). Data represent mean levels of corticosterone (±SE) expressed in ng/ml. (a) At the beginning of the procedure, C/P and C/C rats responded to the acute stress with increased levels of plasma corticosterone compared to basal measures. (b) At the end of the procedure, C/P rats displayed blunted corticosterone levels following the acute stress compared to C/C (control) rats. Fisher's PLSD *post hoc* tests, $^{**}P < 0.01$ versus C/C rats

DISCUSSION

Rats with a history of intermittent access to highly preferred food developed a 'yo-yo dieting', defined as recurrent cycles of reduced feeding behavior followed by disinhibited hyperphagia. We confirmed that alternate access to sweet fat food elicited binging behavior in non-food-deprived laboratory animals (Cottone *et al.* 2008a,b, 2009a,b). Furthermore, we also confirmed that not only did this diet cycling procedure elicit binge-like intake of preferred food, but it also caused a reduced intake of less preferred food between palatable food accesses (Cottone *et al.* 2008a,b, 2009a,b). It could be argued that this 'yo-yo dieting' is no more than a homeostatic control of feeding since both groups exhibited similar weight gain across the procedure. This assumption is most likely correct, at least at the beginning of the experiment, but our demonstration emphasizes the long-term consequences of this diet cycling on the stress response. Indeed, the blood corticosterone release following an acute stress was normal in rats exposed to a 3-week period of alternate diet, but was significantly reduced after 7 weeks. This apparent stress hyporesponsiveness, considered as a hallmark of persistent disruption of HPA function, is in line with a recent report demonstrating that intermittent access to palatable diets induces an allostatic shift in brain stress systems (Cottone *et al.* 2009a). In our hands

though, rats with intermittent access to palatable food did not exhibit any increased anxiety-like behaviors assessed with the elevated plus maze (both groups of rats spent around 20% of the total time on the open arms). This observation differs from that of Cottone *et al.* (2009b) in which control rats spent twice as much time on the open arms. Therefore, we may have missed the diet cycling-induced anxiety effect due to a possible floor effect. Nonetheless, in line with the idea that intermittent access to preferred food leads to negative emotional states, we report a compulsive-like behavior in C/P rats since they persisted to accept punishments when access to preferred food pellets was paired with mild electrical foot shocks. Overall, our results highlight the intricate development of disrupted HPA function and cognitive deficits leading to a loss of control over preferred food intake after repetitive cycles of intermittent access to palatable food.

Consumption of sugar and high-fat contents is considered to lower the stress response (Dallman 2010). According to this assumption, occasional binge eating may reflect a coping strategy aimed at alleviating signs of anxiety. This behavior could trigger the development of 'bad' habits leading to obesity due to recurrent and exaggerated accesses to high caloric foods (Dallman 2010). Meanwhile, recurrent withdrawal from calorie-dense contents could precipitate an increased stress state further promoting calorie-dense food craving (Teegarden & Bale 2007). However, the present findings support that food inclination, and not caloric load, is sufficient for promoting emotional changes upon cessation of preferred food access (Cottone *et al.* 2009b; Ulrich-Lai *et al.* 2010). Indeed, as reported in Table 1, preferred food content is quite similar to that of standard laboratory chow pellet, and its caloric load is unlikely to explain our behavioral observations. A more plausible explanation is that carbohydrate-rich foods may directly activate brain reward systems that produce a concomitant sensation of wellbeing, inherently reducing subjective feelings of anger and tension. Repeated overconsumption of palatable foods may also produce long-term neuroadaptations in brain reward and stress pathways that ultimately may promote depressive or anxious responses when those foods are no longer available or consumed (Parylak *et al.* 2011). Repeated overconsumption of highly palatable foods may initiate a downregulation of dopaminergic reward circuitry via mechanisms that mirror those commonly observed in drug addiction: reduced striatal dopamine D2 receptor availability and blunted dopamine release (Kenny 2011b; Volkow *et al.* 2013). Such a reduced dopaminergic tone in the striatum may lead to impaired inhibitory control over food intake and thereby increase risk of overeating (Johnson & Kenny 2010).

In parallel, a concurrent amplification of brain stress or 'antireward' systems may contribute to the shift from a positive to a negative reinforcement of palatable food, thus exaggerating the vulnerability to lose control over food intake (Parylak *et al.* 2011). Indeed, converging evidence revealed a key role for corticotropin releasing factor (CRF) in promoting food seeking. Infusion of CRF into the nucleus accumbens shell enhanced the incentive salience of a contextual cue previously paired with palatable food availability (Pecina, Schulkin & Berridge 2006; Berridge *et al.* 2010). Further, mice with extended access to palatable high-fat diet exhibited decreased expression of CRF in the central amygdala (CeA), whereas mice undergoing a palatable food withdrawal had increased CRF expression in this brain area (Teegarden & Bale 2007). Mice with increased CRF expression in the CeA also spent significantly more time in an aversive environment to obtain palatable food compared to control animals fed with regular chow pellets (Teegarden & Bale 2007).

In our hands, C/C and C/P rats exhibited similar breaking points in the PR schedule. This is not surprising considering that C/C rats had been briefly exposed to sweetened food during acquisition of the operant conditioning. They logically manifested an elevated motivation for both types of sweetened food pellets when given access to, since enhanced motivation does not correspond to loss of control. This high motivation was again observed on the first exposure to foot shocks; however, the key observation is that all rats drastically decreased their food-seeking behavior on the second exposure to punishments at the notable exception of C/P rats working for chocolate (Test Diets) pellets. This persistent food-seeking behavior despite adverse consequences is strikingly reminiscent of that observed in cocaine addict rats (Koob & Le Moal 2001, 2008; Deroche-Gamonet, Belin & Piazza 2004; Parylak *et al.* 2011).

Interestingly, a recent study established that food seeking in spite of harmful consequences depended on noradrenergic neurotransmission in the prefrontal cortex (Latagliata *et al.* 2010). Noteworthy, the nucleus of the tractus solitarius (NTS) contains neurons producing catecholamine neurotransmitters (including noradrenaline) and is involved in the regulation of feeding behavior. The NTS relays visceral signals to homeostatic feeding centers in the hypothalamus, and also projects to brain areas involved in the regulation of stress and reward processing, including the nucleus accumbens, the amygdala and the prefrontal cortex. Hence, neuroadaptations within the NTS could contribute to an altered perception of food reward (Kenny 2011a) and could play a key role in the intricate networks of brain nuclei involved in the stress-induced loss of control over palatable food intake after chronic episodes of binging and dieting.

With regards to the blunted corticosterone levels displayed by rats with a history of dieting and binging, it is

important to mention that apart from two brief exposures on elevated platforms, all rats remained in a quiet environment for the duration of the entire procedure. Thus, these blunted corticosterone levels cannot be attributed to external factors or stressful situations. Strikingly, blunted cortisol, and concomitant blunted cardiac reactions to acute psychological stress, have been frequently reported in patients with eating disorders (Pirke *et al*. 1992; Koo-Loeb *et al*. 2000; Ginty *et al*. 2012), but also in abstinent alcoholic and polysubstance-abusing patients (Lovallo *et al*. 2000; Lovallo 2006). It seems therefore that a common spiraling distress (defined as a progressive dysregulation of the brain reward function and concomitant development of counter adaptive processes within the brain stress system) may grow with repeated cycles of binging (or drug abuse) and dieting (or protracted abstinence), producing an allostatic state that drives further consumption, and ultimately compulsive intake (Koob & Le Moal 2001, 2008). Blunted cortisol in humans (and corticosterone in rats) may represent a biological marker of this allostatic state that may increase the vulnerability to develop negative affect and depressive-like behaviors. Indeed, blunted cardiac reactions to acute psychological stress have been correlated with an increased vulnerability to depression in humans (Phillips *et al*. 2011). And it has been recently demonstrated that rats with a history of intermittent access to palatable food exhibited signs of depression-like behaviors (Iemolo *et al*. 2012).

Overall, the present study is a novel contribution to the concept of dark side of food addiction (Parylak *et al*. 2011), in which a downregulation of brain reward systems that subserve appetitive responses to rewards and a concomitant amplification of brain stress or 'antireward' systems concur to elicit anxiety-like disorders and cognitive deficits potentially responsible for the loss of control over palatable food intake after repetitive cycles of dieting and binging.

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Authors Contribution

CR, OH and BB were responsible for the study concept and design. CR, GS and BB acquired the data and performed the analysis. CR and BB drafted the manuscript. All authors critically reviewed the content and approved the final version for publication.

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