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Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats

Paul M Johnson & Paul J Kenny

We found that development of obesity was coupled with emergence of a progressively worsening deficit in neural reward responses. Similar changes in reward homeostasis induced by cocaine or heroin are considered to be crucial in triggering the transition from casual to compulsive drug-taking. Accordingly, we detected compulsive-like feeding behavior in obese but not lean rats, measured as palatable food consumption that was resistant to disruption by an aversive conditioned stimulus. Striatal dopamine D2 receptors (D2Rs) were downregulated in obese rats, as has been reported in humans addicted to drugs. Moreover, lentivirus-mediated knockdown of striatal D2Rs rapidly accelerated the development of addiction-like reward deficits and the onset of compulsive-like food seeking in rats with extended access to palatable high-fat food. These data demonstrate that overconsumption of palatable food triggers addiction-like neuroadaptive responses in brain reward circuits and drives the development of compulsive eating. Common hedonic mechanisms may therefore underlie obesity and drug addiction.

Feeding is influenced by pleasure and reward, and obtaining food reward can powerfully motivate consumption[1,2]. Nevertheless, the hedonic mechanisms contributing to obesity remain poorly understood. In hyperphagic humans with congenital leptin deficiency, activity in the dorsal and ventral striatum, which are core components of brain reward circuits increases markedly in response to images of food[3], and leptin replacement therapy attenuates both striatal activity and self-reported 'liking' of food[3]. This suggests that the striatum is important in hedonic aspects of feeding behavior. It was shown recently that activation of the striatum in response to highly palatable food is blunted in obese individuals when compared with lean controls[4]. Moreover, hypofunction of the dorsal striatum and long-term weight gain are most pronounced in individuals with the TaqIA allele of the *DRD2–ANKK1* gene locus, which results in decreased striatal D2R expression and has been shown to predispose individuals to substance dependence disorders[4,5]. These and similar observations have led to the proposal that deficits in reward processing may be an important risk factor for the development of obesity, and that obese individuals may compulsively consume palatable food to compensate for reward hyposensitivity[6]. Notably, it is unclear whether deficits in reward processing are constitutive and precede obesity, or whether excessive consumption of palatable food can drive reward dysfunction and thereby contribute to diet-induced obesity.

A defining characteristic of overweight and obese individuals is that they continue to overeat despite the well known negative health and social consequences. Indeed, many overweight individuals express a desire to limit their food consumption, yet struggle to control their intake and repeatedly consume beyond their energy requirements[7,8]. Development of feeding behavior that is insensitive to negative outcome is analogous to the compulsive drug-taking behavior seen in human drug addicts, which is similarly impervious to negative consequences[9]. Here we investigated the effects of extended access to a palatable high-fat diet on the sensitivity of brain reward systems in rats. We also examined the link between diet-induced hedonic dysregulation and the emergence of compulsive food seeking. Finally, we investigated the role for striatal D2Rs in these addiction-like behavioral responses.

RESULTS

Addiction-like reward deficits in obese rats

To test the effects of restricted or extended access to a palatable high-fat diet, we prepared male Wistar rats (300–350 g) with a bipolar stimulating electrode in the lateral hypothalamus and trained them for 10–14 d in a discrete-trial current-threshold brain stimulation reward (BSR) procedure until stable reward thresholds were established[4]. In the BSR procedure, rats respond vigorously to obtain rewarding electrical self-stimulation through the indwelling stimulating electrode, with the minimal stimulation intensity that maintains self-stimulation behavior termed the reward threshold[10]. Because reward thresholds remain stable and unaltered over prolonged times under baseline conditions, this procedure provides a sensitive measure of the responsiveness of brain reward systems. After establishment of stable BSR thresholds (defined as <10% variation in thresholds across three consecutive sessions), we allocated rats to three groups that showed no differences in mean body weights or reward thresholds between groups. The three groups were given differential access to a 'cafeteria-style' diet consisting of palatable energy-dense food readily available for human consumption (see Online Methods). Rats had 0 h (chow-only rats; $n = 9$), 1 h (restricted-access rats; $n = 11$), or 18–23 h (extended-access rats; $n = 11$) access to the diet per day for

Laboratory of Behavioral and Molecular Neuroscience, Department of Molecular Therapeutics, The Scripps Research Institute-Scripps Florida, Jupiter, Florida, USA. Correspondence should be addressed to P.J.K. (pjkenny@scripps.edu).

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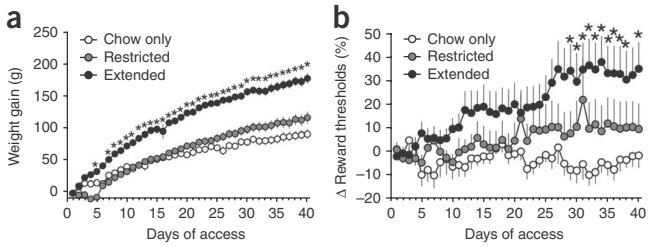


Figure 1 Weight gain and reward dysfunction in rats with extended access to a cafeteria diet. (**a**) Mean (± s.e.m.) weight gain in chow-only, restricted-access and extended-access rats (access × day interaction: $F_{39,702} = 7.9$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**b**) Mean (± s.e.m.) percentage change from baseline reward thresholds (access × time interaction: $F_{78,1092} = 1.7$, $P < 0.0005$; $^*P < 0.05$ compared with chow-only group, *post hoc* test).

40 consecutive days. Cafeteria diets are known to result in diet-induced obesity in rats[11]. All rats also had *ad libitum* access to standard laboratory chow, with reward thresholds, weight gain and caloric intake recorded throughout.

Weight increased markedly in rats with extended access to the cafeteria diet compared to the chow-only or restricted-access groups (**Fig. 1a**). Weight also tended to increase in the restricted-access rats compared to chow-only rats, but this effect did not reach statistical significance. The development of obesity in extended-access rats was closely associated with a worsening deficit in brain reward function, reflected in progressively elevated BSR thresholds (**Fig. 1b**). Because no differences in response latencies for BSR were observed among the three groups (**Supplementary Fig. 1**), deficits in behavioral performance cannot account for this observation. Similar deficits in brain reward function have been reported in rats with extended but not restricted access to intravenous cocaine or heroin self-administrations[12–14]. Thus, extended access to palatable high-fat food can induce addiction-like deficits in brain reward function, considered an important source of motivation that may drive overeating and contribute to the development of obesity[1,6].

When we examined feeding behavior in detail (**Fig. 2**), we found that total daily caloric intake was similar between chow-only and restricted-access rats (**Fig. 2a**,**d**). By contrast, total caloric intake in rats with extended access was almost twice that of the restricted-access and chow-only rats (**Fig. 2a**,**d**). Although the restricted-access and chow-only rats maintained approximately the same daily caloric intake (**Fig. 2a**,**d**), restricted-access rats obtained only ~33% of their daily calories from chow (**Fig. 2b**,**d**), indicating that they developed binge-like feeding behavior and consumed ~66% of their daily caloric intake during their 1 h access session to the cafeteria diet[15] (**Fig. 2d**). Extended-access rats obtained only a small fraction (~5%) of their total caloric intake from chow (**Fig. 2b**); they consumed the cafeteria diet almost exclusively (**Fig. 2d**). The shift in dietary preference in the restricted- and extended-access groups was also reflected in a marked increase in fat intake compared with chow-only rats (**Fig. 2c** and **Supplementary Fig. 2**). Consistent with previous reports[16], there was a tendency for consumption of the cafeteria diet to decrease over time in the extended-access rats. This may reflect the development of tolerance to the palatability of the food items provided as part of the cafeteria diet over time. Nevertheless, the preference for the cafeteria diet versus standard chow remained consistently high in these rats (**Supplementary Fig. 3**). These data demonstrate that extended but not restricted access to a palatable high-fat diet induces addiction-like reward deficits, overeating and loss of homeostatic energy balance. By contrast, restricted access to palatable food gives rise to binge-like patterns of consumption, but does not disrupt homeostatic energy balance nor brain reward function. However, it is possible that restricted access to the cafeteria diet for more than 40 consecutive days would induce significant weight gain and disruption of brain reward function.

After 40 d, rats were no longer permitted access to the palatable diet but continued to have *ad libitum* access to standard laboratory chow. We assessed reward thresholds and chow consumption daily during this enforced 'abstinence' period. The elevations in reward thresholds persisted for at least 2 weeks in the extended-access rats when they no longer had access to the palatable diet (**Fig. 3a**). This contrasts with the relatively transient (~48 h) deficits in reward function reported in rats undergoing abstinence from self-administered cocaine[13]. There was also a marked decrease in caloric intake (**Fig. 3b**) and a gradual decrease in body weight (**Fig. 3c**) in extended-access rats, and to a lesser extent in restricted-access rats, during this abstinence period, consistent with previous reports[11,15]. After 14 d of abstinence, rats were killed and electrode placements were determined by cresyl violet staining (**Fig. 3d**).

Striatal D2Rs in obese rats: role in reward deficits

We next tested the hypothesis that overconsumption of a palatable cafeteria diet might reduce striatal D2R density, contributing to the development of addiction-like reward hyposensitivity. A new cohort of

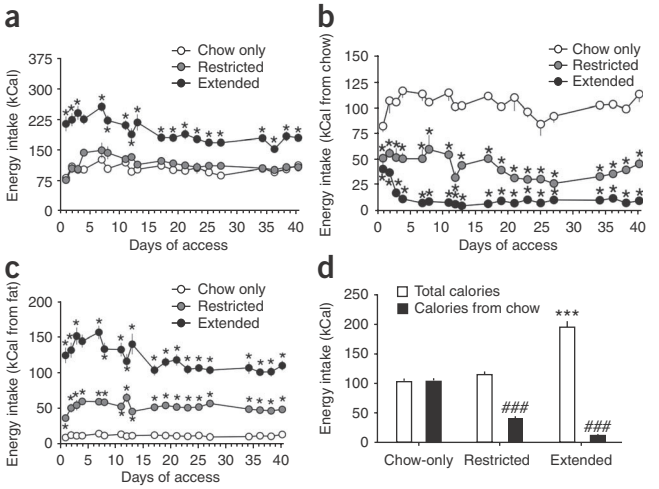


Figure 2 Patterns of consumption in rats with extended access to a cafeteria diet. (**a**) Mean (± s.e.m.) daily caloric intake in chow-only, restricted-access and extended-access rats (access: $F_{1,324} = 100.6$, $P < 0.0001$; time: $F_{18,324} = 7.8$, $P < 0.0001$; access × time interaction: $F_{18,324} = 4.6$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**b**) Mean daily caloric intake (± s.e.m.) from chow (access: $F_{2,504} = 349.1$, $P < 0.0001$; time: $F_{18,504} = 5.9$, $P < 0.0001$; access × time interaction: $F_{36,504} = 3.52$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**c**) Mean daily caloric intake (± s.e.m.) from fat (access: $F_{2,486} = 118.7$, $P < 0.0001$; time: $F_{18,486} = 8.8$, $P < 0.0001$; access × time interaction: $F_{36,486} = 6.2$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**d**) Comparison of mean (± s.e.m.) total caloric intake, and calories consumed exclusively from chow, during the entire 40-day period of access (access: $F_{2,54} = 25.0$, $P < 0.0001$; calorie source: $F_{2,54} = 1235.2$, $P < 0.0001$; access × calorie source interaction: $F_{2,54} = 485.7$, $P < 0.0001$; $^{***}P < 0.001$ compared with total calories in chow-only group, $^{###}P < 0.001$ compared with total calories in the same group of rats, *post hoc* test).

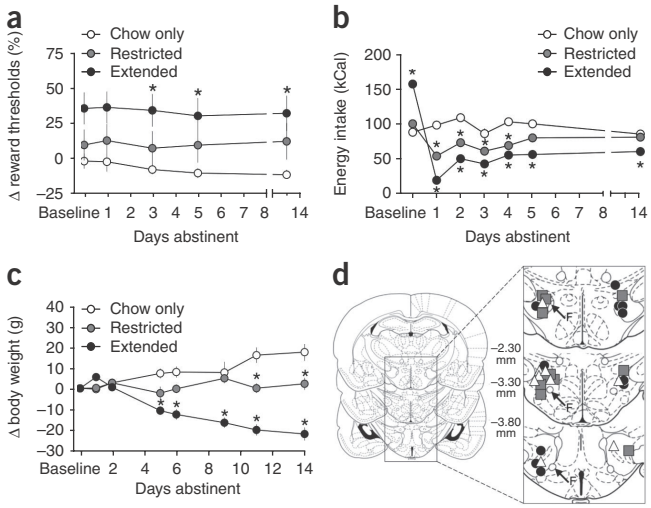


Figure 3 Persistent reward dysfunction and hypophagia during abstinence in rats with extended access to a cafeteria diet. (**a**) Mean percentage change from baseline reward thresholds (± s.e.m.) during abstinence from a palatable high-fat diet (access: $F_{2,112} = 3.7$, $P < 0.05$; time: $F_{4,112} = 2.3$, $P > 0.05$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**b**) Mean caloric intake (± s.e.m.) on the last day of access to the high-fat diet (baseline) and during the 14 d of abstinence when only standard chow was available (access: $F_{2,168} = 41.7$, $P < 0.0001$; time: $F_{6,168} = 65.6$, $P < 0.0001$; access × time interaction: $F_{12,168} = 38.3$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**c**) Change in mean body weight (± s.e.m.) compared with body weight on the last day of access to the high-fat diet (baseline) and during the 14 d of abstinence when only standard chow was available (access: $F_{1,126} = 37.2$, $P < 0.0001$; time: $F_{7,126} = 3.1$, $P < 0.01$; access × time interaction: $F_{7,126} = 40.9$, $P < 0.0001$; $^*P < 0.05$ compared with chow-only group, *post hoc* test). (**d**) Histological reconstruction of the location of BSR stimulating electrodes in the lateral hypothalamus of chow-only (triangles), restricted-access (squares) and extended-access (circles) rats.

chow-only, restricted-access and extended-access rats were permitted access to the cafeteria diet until there was a statistically significant increase in body weight in the extended-access rats compared to the chow-only group ($P < 0.05$; **Fig. 4a**). Striatal expression of the reportedly heavily glycosylated (~70 kDa) membrane-bound form of the D2R was lower in the extended-access rats than in the restricted-access or chow-only rats (**Fig. 4b**; see Online Methods). When we divided the rats in each access group into two subgroups on the basis of a median split of body weights (light or heavy), we found a clear inverse relationship between body weight and striatal D2R expression (**Fig. 4a**,**c**). We detected no statistically significant decreases in expression of the unglycosylated immature (~39 kDa) and intermediately glycosylated cytoplasmic (~51 kDa) forms of the D2R (**Supplementary Fig. 4**)[17], indicating that striatal D2R expression in extended-access rats is probably regulated through post-transcriptional mechanisms.

Next, to test the functional relevance of diet-induced reductions in striatal D2R to brain reward function, we designed and validated a lentiviral vector to deliver a short hairpin interfering RNA (shRNA) in order to knock down D2R (Lenti-D2Rsh; **Fig. 5** and **Supplementary Fig. 5**). Reward thresholds began to increase in rats treated with Lenti-D2Rsh almost immediately upon being permitted extended access to the cafeteria diet, whereas reward thresholds remained unaltered in extended-access rats treated with an empty lentivirus vector (Lenti-control) over the relatively short period of access to the cafeteria diet (14 d; **Fig. 6a**). Response latencies were unaltered in both groups of rats, showing that this effect was not secondary to deficits in task performance (**Supplementary Fig. 6**). Reward thresholds were also unaltered in rats treated with Lenti-D2Rsh or Lenti-control that had access to chow only over the same period (**Fig. 6b**). Thresholds remained persistently elevated during an extra 15 d of abstinence when all rats had access only to standard chow (**Supplementary Fig. 7**).

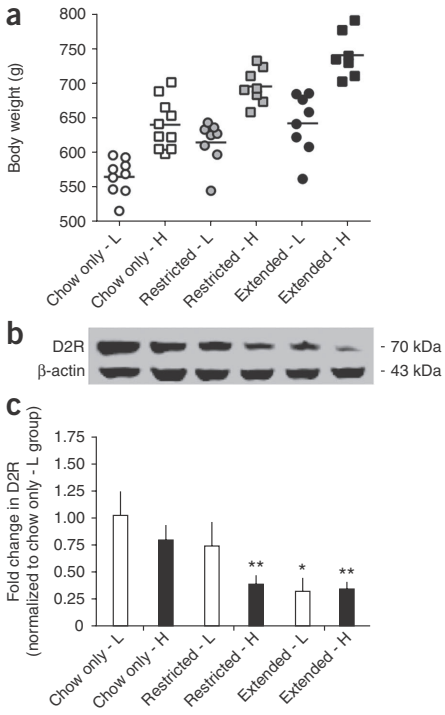
Figure 4 Weight gain is inversely related to striatal D2R levels. (**a**) Chow-only, restricted-access and extended-access rats were subdivided into two groups per access condition based on a median split of body weights: light (L) or heavy (H). (**b**) The entire striatal complex was collected from all rats and D2R levels in each group measured by western blotting. The membrane-associated D2R band was resolved at 70 kDa, and the protein-loading control is displayed below (β-actin, 43 kDa). Full-length immunoblots are shown in **Supplementary Figure 12**. (**c**) Relative amounts of D2R in the striatum of chow-only, restricted-access and extended-access rats were quantified by densitometry ($F_{2,6} = 5.2$, $P < 0.05$, main effect of access; $^*P < 0.05$ and $^{**}P < 0.01$ compared with chow-only-L group).

Knockdown of striatal D2R therefore increased vulnerability to diet-induced reward hypofunction but did not alter the baseline activity of brain reward systems.

We found that caloric intake (**Fig. 6c**) and weight gain (**Fig. 6d**) were similar in the Lenti-D2Rsh and corresponding Lenti-control groups under chow-only or extended-access conditions (**Supplementary Figs. 8** and **9**). Thus, striatal D2R knockdown altered neither preference for the cafeteria diet nor total caloric intake when the palatable food was freely available for consumption.

Compulsive eating in obese rats: role for striatal D2R

We next tested the hypothesis that compulsive-like eating might emerge in rats with extended access to the cafeteria diet and that deficits in striatal D2R signaling might contribute to this effect. A new cohort of chow-only, restricted-access and extended-access rats were permitted access to the cafeteria diet for >40 d until statistically significant weight increases occurred in the extended rats ($P < 0.05$ compared with chow-only rats; data not shown). All three groups of rats were



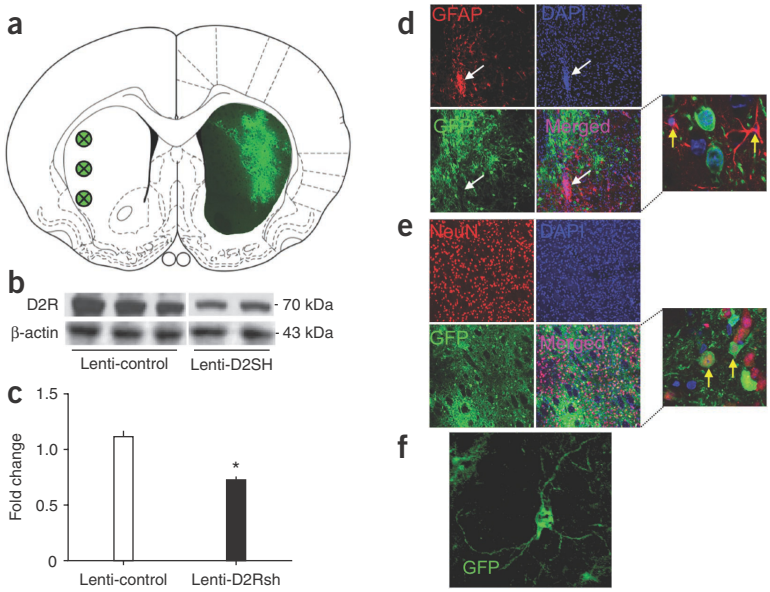


Figure 5 Lentivirus-mediated knockdown of striatal D2R expression. (**a**) Graphical representation of the striatal areas in which Lenti-D2Rsh was overexpressed. Green circles in the left striatal hemisphere represent the locations at which viral infusions were targeted. Green staining in the right striatal hemisphere is a representative immunochemistry staining for green fluorescent protein (GFP) from the brain of a Lenti-D2Rsh rat. (**b**) Representative immunoblot of the decreased D2R expression in the striatum of Lenti-D2Rsh rats. Full-length immunoblots are shown in **Supplementary Figure 13**. (**c**) Relative amounts of D2R in the striatum of Lenti-control and Lenti-D2Rsh rats, quantified by densitometry (*$P < 0.05$ compared with the Lenti-control group, *post hoc* test). (**d**) Infection of glial cells in the striatum by the Lenti-D2Rsh vector was not detected. Green staining is GFP from virus; red is the astrocyte marker glial fibrillary acidic protein (GFAP); cell nuclei are highlighted by DAPI staining in blue. White arrows indicate a localized area of gliosis found only at the site of virus injection in the striatum and not in the surrounding tissues into which the virus has diffused. Even in this area, none of the astrocytes are GFP-positive. The yellow arrows in the magnified image highlight the typical GFP-negative astrocytes that were detected. (**e**) High levels of neuronal infection in the striatum by the Lenti-D2Rsh vector. Green staining is GFP from virus; red is the neuronal nuclear marker NeuN; cell nuclei are highlighted by DAPI staining in blue. The yellow arrows in the magnified image highlight GFP-positive and NeuN-positive neurons in the striatum. (**f**) A higher-magnification image of a virally infected (GFP-positive) neuron in the striatum of Lenti-D2Rsh rats that shows the typical morphological features of medium spiny neurons.

then permitted only 30 min access per day to the cafeteria diet for 5–7 d in an operant chamber until stable intake was achieved (defined as <10% variation in daily intake). Half the rats in each access condition then were exposed to a light (conditioned stimulus) paired with delivery of foot shocks (punished group), whereas the remaining rats in each group were exposed to the cue light in the absence of foot shock (unpunished group). On the test day, we examined the effects of cue light exposure alone on palatable food consumption (**Fig. 7**; see Online Methods). We found that mean caloric intake during the 30-min baseline sessions was higher in the chow-only and restricted-access rats than in the extended-access rats (**Fig. 7a**,**b**). This suggests that the chow-only and restricted-access rats binged on the palatable food during the intermittent 30-min access sessions, reflected in the fact that these rats consumed ~40–50% of their daily caloric intake, typically ~100 kCal, during these sessions (**Fig. 7a**,**b**). By contrast, extended-access rats seem resistant to developing this binge-like feeding behavior, perhaps because their history of almost unlimited access to the palatable food for >40 consecutive days established patterns of eating that were relatively inflexible to change. On the test day, we observed no statistically significant effects of cue light replay on food consumption in the unpunished rats from the chow-only, restricted-access or extended-access groups when compared with intake during the baseline period (**Fig. 7a**). The cue light alone therefore had no motivational salience. In punished rats, the shock-paired cue light significantly decreased palatable food intake in the chow-only and restricted-access rats. However, the cue light had no effect on palatable food intake in the extended-access rats, showing that their consumption was insensitive to aversive environmental cues predicting adversity. Baseline energy intake in the extended-access rats was lower than that in the other groups. However, because chow intake during similar time-periods was far lower (**Fig. 7d**), it is unlikely that this represents a 'floor effect' that confounds our findings. Together, our data support the idea that compulsive-like eating behavior can emerge in extended-access rats in a manner analogous to the compulsive cocaine-taking seen in rats with a history of extended access to the drug[18].

Finally, we examined the effects of the punishment-paired conditioned stimulus on food intake in the Lenti-control and Lenti-D2Rsh

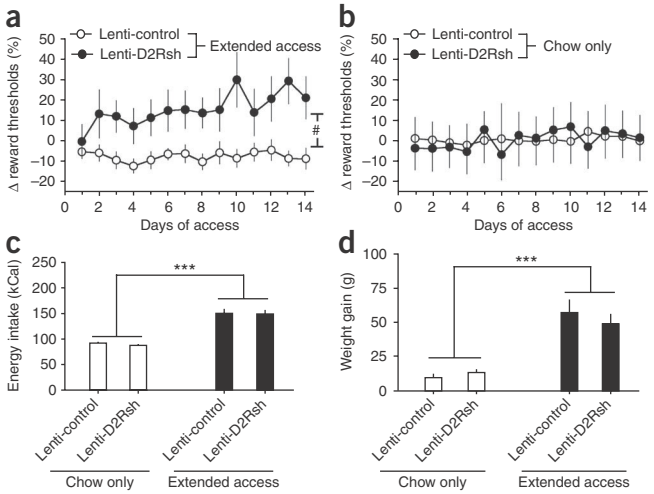


Figure 6 Knockdown of striatal D2R increases vulnerability to reward dysfunction in rats with extended access to a cafeteria diet. (**a**) Mean (± s.e.m.) percentage change from baseline reward thresholds in Lenti-control and Lenti-D2Rsh rats that had extended access to the cafeteria diet for 14 consecutive days (virus: $F_{1,156} = 5.9$, $P < 0.05$; time: $F_{13,156} = 2.2$, $P < 0.05$; virus × time interaction: $F_{13,156} = 2.2$, $P < 0.05$; #$P < 0.05$, interaction effect). (**b**) Mean (± s.e.m.) percentage change from baseline reward thresholds in Lenti-control and Lenti-D2Rsh rats that had chow-only access. (**c**) Mean (± s.e.m.) caloric intake of rats during 14 d of chow only or extended access (access: $F_{2,28} = 135.6$, ***$P < 0.0001$). (**d**) Mean (± s.e.m.) weight gain during 14 d of chow only or extended access (access: $F_{2,28} = 96.4$, $P < 0.0001$; ***$P < 0.001$, main effect of access).

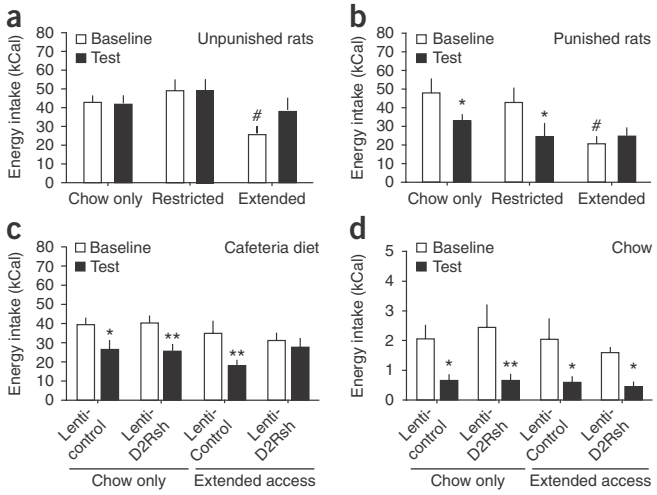


Figure 7 Compulsive-like responding for palatable food. (**a**) Mean (± s.e.m.) palatable diet consumption in unpunished rats during the 30-min baseline sessions and on the test day when rats were exposed to a neutral conditioned stimulus that was not previously paired with noxious foot shock (access: $F_{2,20} = 5.2$, $P < 0.05$; #$P < 0.05$ compared with chow-only rats). (**b**) Mean (± s.e.m.) palatable diet consumption in punished rats during the 30-min baseline sessions and on the test day when rats were exposed to a conditioned stimulus that was previously paired with noxious foot shock (access: $F_{2,21} = 3.9$, $P < 0.05$; cue: $F_{1,21} = 8.6$, $P < 0.01$; access × cue interaction: $F_{2,21} = 4.7$, $P < 0.05$; *$P < 0.05$ compared with intake during the baseline session, #$P < 0.05$ compared with chow-only rats). (**c**) Mean (± s.e.m.) palatable diet consumption during the 30-min baseline sessions and on the test day in Lenti-control and Lenti-D2Rsh rats that previously had chow-only or extended access to a cafeteria diet (cue: $F_{1,26} = 29.7$, $P < 0.0001$; *$P < 0.05$, **$P < 0.01$ compared with intake during the baseline sessions, *post hoc* test). (**d**) Mean (± s.e.m.) chow consumption during the 30-min baseline sessions and on the test day in Lenti-control and Lenti-D2Rsh rats that previously had chow only or extended access to a cafeteria diet (cue: $F_{1,26} = 44.9$, $P < 0.0001$; *$P < 0.05$, **$P < 0.01$ compared with intake during the baseline sessions, *post hoc* test).

rats that had previously had access to chow only or extended access to the cafeteria diet (rats from **Fig. 6**). We found that baseline palatable food intake during the 30-min baseline sessions was similarly high (~40 kCal) in all four groups (**Fig. 7c**). In addition, total daily chow consumption (in the home cage) was similar between all four groups of rats during the conditioning sessions and on the test day (**Supplementary Fig. 10**). The 14 d of prior access to the cafeteria diet was therefore not sufficient to block binge-like eating behavior in a manner similar to that seen in rats that had >40 d extended access to the cafeteria diet (**Fig. 7a**,**b**). The aversive cue light stimulus disrupted palatable food intake in Lenti-control and Lenti-D2Rsh rats that previously had chow-only access (**Fig. 7c**). Similarly, the aversive conditioned stimulus disrupted palatable food intake in the Lenti-control rats that previously had 14 d extended access to the cafeteria diet. By contrast, the aversive conditioned stimulus had no impact on palatable food consumption in the Lenti-D2Rsh rats that previously had 14 d extended access to the cafeteria diet (**Fig. 7c**). BSR thresholds remained significantly elevated in these rats when recorded 48 h after the test session, whereas thresholds remained stable and unaltered in the other three groups of rats (**Supplementary Fig. 11**). To verify that resistance to conditioned stimulus–induced suppression of palatable food intake in the Lenti-D2Rsh extended-access rats was not secondary to impairments in classical conditioning processes, we tested the effects of the aversive conditioned stimulus on consumption of less palatable standard chow in all four groups of rats. In contrast to the binge-like consumption of palatable food, we found that all four groups of rats consumed little chow (~2 kCal) during the 30 min baseline sessions (**Fig. 7d**) and that chow intake was disrupted in all four groups by a similar magnitude upon exposure to the aversive conditioned stimulus (**Fig. 7d**). These data demonstrate that knockdown of striatal D2Rs markedly accelerated the emergence of compulsive-like eating of palatable food, but only in rats with a history of extended access. Moreover, because compulsive eating was detected only in Lenti-D2Rsh rats that had elevated BSR thresholds, diet-induced reward hypofunction may be a necessary antecedent to the emergence of compulsive food seeking.

DISCUSSION

Ease of access to palatable high-fat food is considered to be an important environmental risk factor for obesity[19]. We found that extended access to a highly palatable cafeteria-style diet resulted in overeating and weight gain coupled with progressively elevating BSR thresholds in rats. This effect on BSR thresholds can be explained by gradually decreasing responsiveness of brain reward circuits, an interpretation consistent with the fact that food restriction and weight loss can increase[20], whereas acute overfeeding can transiently decrease[21], responding for BSR in rats. This finding represents an extension of work showing that acute overfeeding of rats through an intragastric feeding tube[21], and gastric distention or intravenous glucagon infusion that mimics postprandial satiety[22–24], decreases responding for rewarding lateral hypothalamic BSR and increases aversion-like responses to the stimulation[25]. Previous work has also shown that repeatedly force-feeding rats through intragastric tubes until their weight has increased by ~200 g similarly decreases rates of responding for BSR, an effect that persists until body weight has normalized[23]. As in these findings in rats, responding in cats for lateral hypothalamic BSR was inhibited by previous feeding to satiation[26], showing that interactions between brain reward function and metabolic state are conserved and thus are likely to occur in humans as well. Ease of access and consequent overeating of cafeteria-style diets in humans is considered an important environmental contributor to the current obesity epidemic in Western societies[19]. Our data show that reward hypofunction arises in rats that volitionally overeat a palatable cafeteria diet similar to that consumed by humans and that this effect becomes progressively worse as they gain more weight. Notably, all rats with reward threshold elevations ≥20% had BSR electrodes located within ~500 μm of the fornix dorsolaterally. The sensitivity of reward-related neurons in this area is increased by food restriction in a manner that is sensitive to the fat-derived hormone leptin, and this brain region is considered an important substrate for food reward[27]. The brain circuits that regulate food hedonics are therefore inhibited by postingestive signals that predict satiety, consistent with recent human imaging studies showing that gastric distention[28] and the gut-derived postprandial factor peptide YY3-36 (PYY)[29] modulate the activity of regions of the brain involved in reward processing. In addition, reward systems are also inhibited by excessive weight gain. Recent reports indicate that circulating leptin, a key regulator of energy balance, can penetrate into brain tissues and inhibit the activity of reward circuits[3,27,30,31].

Reward deficits in overweight rats may reflect counteradaptive decreases in the baseline sensitivity of brain reward circuits to oppose their overstimulation by palatable food. Such diet-induced reward hypofunction may contribute to the development of obesity by increasing

the motivation to consume high-reward 'obesogenic' diets to avoid or alleviate this state of negative reward[6,32]. This might account for the hypophagia we observed in extended-access rats and to a lesser degree in restricted-access rats when the palatable food was withdrawn and only the less palatable chow was available. Such a scenario is also consistent with data from human brain imaging studies in which blunted activation of the striatum in response to highly palatable food, particularly in individuals with genetic polymorphisms thought to decrease striatal D2R expression, is associated with long-term weight gain[4]. It has been unclear whether such reward hyposensitivity in obese individuals is manifested before the development of obesity and related solely to genetic factors ('reward deficiency syndrome') or whether overeating can cause disruption in reward processing. Our data demonstrate that extended access to palatable high-energy food and subsequent overeating blunts reward sensitivity and may therefore represent an important hedonic mechanism that promotes the development of obesity. Similar reward dysfunction to that reported here in obese rats is also detected in rats with a history of extended access to intravenous cocaine or heroin self-administration, but not in those with a history of restricted access[12–14]. Moreover, the transition from casual to compulsive drug seeking has been proposed to result from an attempt to alleviate the persistent state of diminished reward induced by this drug-induced reward dysfunction[12,32,33]. Thus, our data indicate that obesity and drug addiction may share underlying hedonic mechanisms.

The downregulation of striatal D2R expression is a notable neuroadaptive response to overconsumption of palatable food. Indeed, reductions in striatal D2R density are seen in overweight individuals[4,34] and rodents[35,36]. Conversely, individuals with anorexia nervosa have elevated striatal D2R[37], and weight loss in obese individuals after bariatric (gastric bypass) surgery is associated with elevated striatal D2R density[28]. The gene polymorphism referred to as the TaqIA A1 allele results in decreased striatal D2R density, and individuals harboring this allele are over-represented in obese populations[4]. The TaqIA allele also increases vulnerability to alcohol, opioid and psychomotor stimulant addiction[38]. Reductions in striatal D2R density occurring either through constitutive genetic factors or as a consequence of overeating may therefore contribute to the neurobiological mechanisms of obesity. We found that striatal levels of the 70 kDa D2R isoform, thought to reflect the membrane-associated D2R, were inversely related to body weight in rats from the chow-only, restricted-access and extended-access groups (**Fig. 4**). Knockdown of striatal D2R expression, most prominently in the dorsolateral striatum (**Fig. 5**), caused BSR thresholds to increase almost immediately upon exposure to the cafeteria diet. Decreases in striatal D2R expression therefore rapidly accelerated the emergence of reward hypofunction in rats with extended access to highly palatable food, a finding consistent with human brain imaging data that indicate that deficits in striatal D2R density contribute to reward hypofunction in obese individuals[4].

Three features of the Lenti-D2Rsh rats are also noteworthy. First, although striatal D2R knockdown combined with extended access to the palatable diet resulted in elevating BSR thresholds, there were no differences in caloric intake or weight gain in these rats compared to control rats. This might reflect that fact that rats only had 14 d of access to the cafeteria diet; longer periods of access might have resulted in higher weight gain over time, similarly to the higher susceptibility to weight gain seen in humans with deficits in striatal D2R signaling[4]. However, the advantage of limiting access to the cafeteria diet to only 14 d is that the knockdown rats with extended access were the only group to show elevated BSR thresholds, and this permitted us to assess the potential role of reward hypofunction in the development of compulsive eating (see below). Second, BSR thresholds remained stable and unaltered in the knockdown rats that had access to chow only. This indicates that reduced striatal D2R expression alone was not sufficient to induce reward hyposensitivity; instead, it seemed to interact with overconsumption of palatable food to accelerate the emergence of this state of reduced reward sensitivity. Other adaptive responses in brain reward circuits might therefore trigger reward hyposensitivity in rats with extended access to the cafeteria diet. With this in mind, we note that the D2R agonist bromocriptine reduces circulating levels of leptin[39], and leptin inhibits feeding at least in part by inhibiting the striatal regions that control hedonic responses to food[3,30,31]. Thus, it is possible that downregulation of striatal D2R in response to increasing body weight increases leptin signaling and thereby boosts the inhibitory effects of this adipokine on brain reward systems. Finally, we note that we targeted our lentivirus vectors towards the dorsolateral striatum. This was primarily for technical reasons, as lateral placement of cannulae for virus delivery into the striatum enabled us also to accommodate the indwelling hypothalamic stimulating electrode for BSR threshold determination. Thus, it is possible that targeting of D2Rs for knockdown in other areas of the striatum, particularly the dorsomedial and ventral areas (nucleus accumbens core and shell), might have elevated BSR thresholds even in the absence of the palatable diet.

The dorsolateral striatum has been heavily implicated in stimulus-response habit type learning, as reflected in the development of consummatory behavior that is insensitive to devaluation by prior feeding to satiation or by pairing with noxious stimuli[40]. By targeting predominately the dorsolateral striatum, we might have knocked down populations of D2Rs that regulate the rat's vulnerability to the development of compulsive-like eating. In keeping with a role for striatal D2Rs in compulsive behaviors, the TaqIA allele of the human *DRD2–ANKK1* gene locus—which results in low striatal D2R density[5], blunts striatal activation in response to palatable food[4] and elevates vulnerability to obesity[4]—is also associated with deficits in learning to avoid actions with negative consequences[41]. Loss of inhibitory control over behavior that can have a negative outcome is a characteristic feature of both obesity and drug addiction, in which consummatory behaviors persist despite negative social, health or financial consequences. Cocaine-taking behavior in rats with a history of extensive drug intake can become inflexible and resistant to disruption by an aversive conditioned stimulus that predicts a negative outcome (foot shock)[18]. Similarly, mice that previously had access to a palatable high-fat diet will spend more time in an aversive environment (brightly lit) to obtain the palatable food than mice that had no experience with the diet[42]. We found that palatable food consumption in rats with extended access to the cafeteria diet was similarly insensitive to an aversive conditioned stimulus. Consistent with a role for striatal D2Rs in this effect, compulsive-like eating was found in the striatal D2R knockdown rats that previously had 14 d extended access to the cafeteria diet but not in the control groups. From a neurocircuitry perspective, extended access to palatable food might trigger plasticity in corticostriatal pathways, thereby rendering animals more vulnerable to the development of compulsive-like behaviors, with deficits in striatal D2R signaling enhancing this process. Indeed, reduced striatal D2R density in obese individuals is correlated with reduced metabolism in prefrontal and orbitofrontal cortical areas[43] that exert inhibitory control over behavior[44].

Notably, compulsive-like consumption of palatable food was detected only in the knockdown rats that previously had extended access to the cafeteria diet, not in control rats that had extended access to the cafeteria diet for the same time period, nor in knockdown rats that had chow-only access. The main difference between the knockdown rats with prior extended access and to the other groups

was their persistently elevated BSR thresholds. This might reflect common neurobiological origins of reward hypofunction and the emergence of compulsive-like eating, which are temporally coincident yet independent phenomena. Alternatively, diet-induced reward hypofunction might serve as a substrate for negative reinforcement that facilitates the development of compulsive-like eating[14,32,33]. Whatever the underlying mechanisms, our findings demonstrate that addiction-like compulsive responding for palatable food can emerge in obese rats, and indicate that deficits in striatal D2R signaling increase vulnerability to the development of this behavior.

In summary, we found that over-stimulation of brain reward systems through excessive consumption of palatable, energy-dense food induces a profound state of reward hyposensitivity and the development of compulsive-like eating. These maladaptive behavioral responses in obese rats probably arise from diet-induced deficits in striatal D2R signaling. Overconsumption of drugs of abuse similarly decreases striatal D2R density, induces a profound state of reward hypofunction and triggers the emergence of compulsive-like drug-taking behaviors. Our findings therefore support previous work[4,19,42,45–47] in indicating that obesity and drug addiction may arise from similar neuroadaptive responses in brain reward circuits.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

P.M.J. conducted all experiments. P.M.J. and P.J.K. designed the experiments, analyzed the data and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Rats. Male Wistar rats weighing 300–350 g at the start of the experiments were obtained from Charles River. Upon arrival, rats were housed individually at constant temperature on a 12-h light–dark cycle (lights on at 2200 h). Rats were permitted *ad libitum* access to standard laboratory chow and water for the duration of the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of Scripps Florida, and rats were treated in accordance with the guidelines set forth by the National Institutes of Health regarding the principles of animal care.

Surgical procedures. Rats prepared with BSR stimulating electrodes were first anaesthetized by inhalation of 1–3% isoflurane in oxygen and positioned in a stereotaxic frame (Kopf). Bipolar BSR electrodes (11 mm long) were implanted into the posterior lateral hypothalamus (anteroposterior, −0.5 mm from bregma; mediolateral, ±1.7 mm from midline; dorsoventral, 8.3 mm from dura; incisor bar was adjusted to 5 mm above the interaural line)[47]. Rats receiving virus injections were also prepared with bilateral guide cannulae (23 gauge, 14 mm long) positioned above the striatum (anteroposterior, 2.8 mm from bregma; mediolateral, ±3.1 mm from midline; dorsoventral, −2.4 mm from dura)[48] and filled with 14-mm stylets. Four stainless steel skull screws and dental acrylic held the electrode and cannulae in place. The surgical wound was treated with topical antibiotic once every 12 h for 5 d after the surgery. Rats were allowed 7–10 d to recover from surgery and were then trained in the BSR threshold procedure.

BSR procedure. Rats were trained to respond for BSR stimulation according to a discrete-trial current-threshold procedure similar to that described elsewhere[10,14]. Briefly, BSR current levels were varied in alternating descending and ascending series in 5-μA steps. In each testing session, four alternating descending/ascending series were presented. The threshold for each series was defined as the midpoint between two consecutive current intensities for which rats responded in at least three of the five trials, and two consecutive current intensities for which rats did not respond in three or more of the five trials. The overall threshold of the session was defined as the mean of the thresholds for the four individual series. Each testing session was approximately 30 min in duration. Stable BSR thresholds were defined as ≤10% variation in thresholds over 5 consecutive days, usually established after 10–14 d of training. The response latency for each test session was defined as the mean response latency of all trials during which a positive response occurred.

Viral packaging and delivery. Short hairpin RNA was delivered and constitutively expressed using the pRNAT-U6.2/Lenti vector system (GenScript). Viral particles were prepared according to the manufacturer's protocol. Briefly, HEK 293FT cells were transfected with vector containing the shRNA insert (5′-GGATCCCGCGCAGCAGTCGAGCTTTCTTCAAGAGAGAAAGCTCGACTGCTGCGCTTTTTTCCAACTCGAG-3′) or the empty vector, plus ViraPower Packaging Mix (Invitrogen) for 72 h (medium replaced after 24 h). Supernatant was then collected and concentrated by ultracentrifugation (76,755g, Beckman Coulter SW 32 TI rotor., 90 min, 4 °C) and viral titer was determined by fluorescence-activated cell sorting according to the manufacturer's instructions. Virus was aliquoted and stored in light-protected boxes at –80 °C until use.

Rats with stable BSR thresholds received bilateral viral injections at three sites in the striatum of each brain hemisphere (2 μl per injection, 1 μl min^{-1}, 1 min between injections, a total of six injections per rat). Rats were allowed at least 2–3 d recovery from intrastriatal injections before BSR threshold assessment was resumed. Daily BSR threshold assessment continued for 33 d after virus injections to ensure maximal striatal D2R knockdown was achieved before permitting rats access to the cafeteria diet. There were no differences in BSR thresholds between the Lenti-control and Lenti-D2Rsh rats during these 33 d (data not shown).

Immunoblotting. Rats were killed approximately 1 h after their regularly scheduled access to the cafeteria diet, and brains were rapidly removed. Brain sections of ~1–2 mm thickness were prepared using a coronal brain matrix (1-mm slice interval; Plastics One) on an ice block, and tissue punches of dorsal striatum (bregma: ~2.2 to −0.26 mm) were taken. Striatal tissue punches were rapidly collected, snap frozen and stored at −80 °C until use. Individual samples were thawed on ice and equal amounts of striatal tissue were pooled on the basis of a weight-dependent median split of access groups (7–10 rats per pool). Tissue was resuspended in 500 μl ice-cold RIPA buffer (Thermo Scientific) containing sodium orthovanadate, phosphatase cocktail inhibitors 1 and 2 (Sigma-Aldrich), leupeptin and pepstatin before homogenization. Tissue lysates were boiled for 10 min in sample buffer and loaded onto 4%–20% or 10% Tris-glycine SDS gels (Invitrogen). Protein was transferred to nitrocellulose membranes, blocked for 1 h at ~23–25 °C (5% non-fat dry milk and 0.2% Tween-20 in PBS, pH 7.4), and incubated in primary antibody overnight at 4 °C. The following primary antibodies were diluted in block solution: D2R mouse monoclonal (Santa Cruz, 1:100) or β-actin mouse monoclonal (Santa Cruz, 1:200). Chemiluminescent ECL reagent was added after incubation with horseradish peroxidase–conjugated secondary antibodies (Amersham, 1:2,000). The mature membrane-associated form of D2DR (~70 kDa)[17,49] was normalized to a protein-loading control (β-actin; 43 kDa) and quantified by densitometry using NIH Image J software.

Immunochemical analysis. Rats were anesthetized and transcardially perfused with 4% paraformaldehyde in PBS (pH 7.6). Brains were removed, postfixed overnight and stored in sucrose (30% solution in PBS, pH 7.4) for at least 72 h. Frozen tissue sections (30 μm thickness) were collected from a microtome and blocked (3% BSA, 5% normal goat serum and 0.3% Triton X-100 in PBS) for 1 h at ~23–25 °C. The following primary antibodies were added to the block solution and incubated overnight at 4 °C: chicken polyclonal to GFP (Abcam, 1:1,000); rabbit monoclonal to GFAP (Millipore, 1:1,000); mouse monoclonal to NeuN (Millipore, 1:1,000). Sections were incubated with fluorescent-dye-conjugated secondary antibodies at ~23–25 °C: anti-chicken–488-nm dye (Jackson ImmunoResearch, 1:1,000), anti-rabbit–594-nm dye (Invitrogen, 1:1,000), and anti-mouse–594-nm dye (Invitrogen, 1:1000). Sections were mounted with Vectashield mounting media containing DAPI (Vector Labs) and coverslipped. Images were taken using an Olympus BX61 fluorescence microscope (×2 objective) or an Olympus confocal microscope (×10 and ×100 objectives).

Feeding procedure. Rats were housed individually on paper bedding (alpha pads; Shepherd Specialty Papers) to prevent food products from being soiled with loose bedding materials. The cafeteria diet consisted of bacon, sausage, cheesecake, pound cake, frosting and chocolate, which were individually weighed before being made available to the rats. The cafeteria diet food items were delivered in small metal receptacles. All food items, including standard laboratory chow, were weighed again on completion of the feeding session. Caloric intake from the various macronutrients was calculated using the nutritional information provided by the manufacturer.

Cue-induced suppression of feeding behavior. Feeding procedures took place in sound-attenuated operant chambers identical in dimensions to those used in the BSR experiments. Rats were placed into an operant chamber and had access to the cafeteria diet or chow for 30 min. The food products were delivered in small metal receptacles. All food items were weighed before and after feeding sessions, which were carried out during the rats' normal feeding period. Chow consumption was assessed by consumption of 45-mg chow pellets identical in composition to chow provided in the rats' home cages. Rats were then permitted 30 min access per day to the cafeteria diet until stable intake was achieved (defined as <10% variation in daily intake), requiring 5–7 d. After stabilization of palatable food intake during this baseline period, rats in each access condition were allocated to two groups: punished (those receiving foot shock) and unpunished (not receiving foot shock). Rats were then subjected to four conditioning sessions on consecutive days in the same operant chamber in which they previously had had access to the palatable food. During the 30-min conditioning sessions a cue light (conditioned stimulus) was activated for 10 min, turned off for 10 min, and then turned back on for 10 min. Punished rats received foot shock only during presentation of the cue light (0.5 mA for 1.0 s; 10 stimulations with ~1-min intervals). The unpunished rats were presented with the cue light in the same manner, but without the delivery of foot shock. On the test day, the day after the final conditioning session, rats in the punished groups received intermittent foot shock (five stimulations in total) paired with activation of the cue light for 5 min. The unpunished rats were again exposed to the cue light in the absence of foot shock. After the 5-min punishment period, all rats were permitted access to the palatable food for a 30-min session with the conditioned stimulus activated intermittently (10 min cue light on, 10 min cue light off, 10 min cue light on).

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Statistical analyses. Baseline reward thresholds were defined as the average threshold value for the 5 d before access to the cafeteria diet for each subject. Reward thresholds were expressed as the percentage change from the baseline threshold value. Data on percentage of baseline reward threshold values, weight gain, caloric consumption, and caloric consumption from fat were analyzed by two-factor, repeated-measures analysis of variance, with access (chow only, restricted access or extended access), calorie source (standard chow or cafeteria diet), virus (Lenti-control or Lenti-D2Rsh) and cue (paired or unpaired with punishment) as the between-subjects factors and time as the within-subjects factor. When appropriate, main effects in the analyses of variance were further analyzed by Bonferroni *post hoc* tests. All statistical analyses were performed using GraphPad Prism software.

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