The effects of chronic food restriction on cue-induced heroin seeking in abstinent male rats

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Abstract

Rationale and objectives: Previous research with an animal model of relapse has shown that acute food deprivation will reinstate extinguished drug seeking. Recent evidence with humans, however, suggests that chronic food restriction rather than acute food deprivation is related to increases in drug taking and relapse, emphasizing a need for an animal model to elucidate the neural mechanisms mediating the effects of chronic food restriction on drug seeking. Here we studied the effects of chronic food restriction during a period of abstinence on heroin seeking in rats. *Methods:* Rats were trained to self-administer heroin over 10 days (0.1 mg/kg/infusion; i.v.). Rats were then removed from the operant conditioning chambers and exposed to a mild food restriction (resulting in 10-15% decrease in body weight) or given unrestricted access to food for 14 days while abstinent. The abstinence period was followed by a drug-seeking test under extinction conditions. Subsequent experiments manipulated the length of restriction and test conditions. *Results:* Rats that were food restricted throughout the abstinence period demonstrated a robust increase in cue-induced heroin seeking compared to sated rats. Re-feeding prior to testing or decreasing the length of the food restriction period prevented the augmentation of drug seeking. *Conclusions:* A combination of chronic food restriction and a concurrent state of hunger appears to be necessary for an increase in cue-induced heroin seeking following abstinence. The procedure presented here may serve as a useful model to study the increased risk for relapse following dietary manipulations in abstinent subjects.

Keywords: heroin, self-administration, food restriction, relapse, drug seeking, abstinence

Introduction

Drug addiction is a chronic condition that is characterized by compulsive drug seeking, and involves interchanging periods of drug abstinence and relapse (O'Brien 1997; O'Brien and McLellan 1996). In abstinent addicts, drug craving and relapse can be triggered by exposure to the self-administered drug (de Wit 1996; Meyer and Mirin 1979), drug-associated cues (Carter and Tiffany 1999; Childress et al. 1992), or stressful life events (Sinha 2001).

In humans, a relatively common stressor, restricted food intake, leads to increased intake of coffee and tobacco products (Franklin et al. 1948), as well as coca leaf chewing (Hanna and Hornick 1977). More recently, the risk for relapse in abstinent smokers was shown to be higher in subjects under caloric intake restriction (Hall et al. 1992), and the level of dietary restriction has been positively associated with the prevalence of alcohol, cigarettes, and marijuana use in young women (Krahn et al. 1992). In laboratory animals, the effects of dietary manipulations on drug-associated behaviors have been unequivocally demonstrated. Carroll and colleagues have described an increase in self-administration of drugs in chronically food-restricted subjects across multiple pharmacological classes, animal species and routes of administration (Carroll and Meisch 1981; Carroll and Meisch 1984; Carroll et al. 1979; Rodefer and Carroll 1996). In addition, drug conditioned-reward was shown to increase in food-restricted animals using the conditioned place preference procedure (Stuber et al. 2002). Food restriction-induced enhancement in the rewarding effectiveness of drugs was also inferred through augmented drug-induced lowering of stimulation threshold in the lateral hypothalamic self-stimulation paradigm (LHSS) in rats (Carr 2007). Furthermore, using the reinstatement procedure, a commonly used model for drug relapse (de Wit and Stewart 1983; Epstein et al. 2006), we have shown that acute (24-48 h) food deprivation restores extinguished cocaine and heroin seeking in rats (Shalev et al. 2000; Shalev et al. 2003).

We also found that this effect of food deprivation is mediated by extra-hypothalamic corticotropin-releasing factor (CRF), and dopamine transmission (Shalev et al. 2006; Tobin et al. 2009).

Two types of dietary manipulations are often mentioned in the literature, acute food deprivation and chronic food restriction, although they are not always clearly defined. For the purpose of the following discussion, chronic food restriction is defined as a period of limited food availability, either by limiting the amount of food or the time subjects have access to the food, that extends from days to weeks. Acute food deprivation is defined as a period during which no food is available, ranging from one to four days.

There are clear indications that acute food deprivation and chronic food restriction manipulations differentially affect both drug and non-drug reward seeking. Thus, in humans, only prolonged food restriction, and not acute food deprivation, is related to increased drug taking (Cheskin et al. 2005; Zacny and de Wit 1991). This effect might be explained, at least in part, by the different pattern of feeding in rats and humans; while humans typically consume food in 3-5 day-time meals (Bellisle et al. 1997), rats show a consistent distribution of frequent feeding bouts over the dark period of the daily cycle, and in laboratory rats meals are also observed during the light phase of day (although at a much reduced frequency) (Glendinning and Smith 1994). Nevertheless, the unique effect of chronic food restriction on reward-related behavior is observed in rats as well, since only prolonged food restriction significantly augments the reinforcing properties of LHSS (Fulton et al. 2000).

It therefore seems that a more clinically relevant animal model for dietary manipulations-induced relapse to drug seeking should include a prolonged period of food restriction, rather than acute food deprivation. Moreover, relapse in humans usually occurs after a period of abstinence rather than explicit extinction of drug seeking behavior, which is an integral part of the

reinstatement procedure, thus somewhat diminishing the face validity of the reinstatement procedure (Fuchs et al. 2008). This difference involves more than a simple procedural dissociation, as different neural mechanisms underlie the two behavioral phenomena. For example, compared to cocaine withdrawal (a 1-week abstinence period), extinction of cocaine self-administration behavior over the same period resulted in increases in the GluA1 and GluA2/3 AMPA receptor subunits in the nucleus accumbens shell, a brain area that is critically involved in drug reward and reinstatement of drug seeking (Sutton et al. 2003). Further support for a mechanistic dissociation comes from Fuchs et al. (2006), who have demonstrated that different neural substrates mediate discrete drug-associated-cue-induced reinstatement of cocaine seeking following extinction training versus a similar length abstinence period (Fuchs et al. 2006).

Here we present a novel model for relapse to heroin seeking following abstinence and 14 days of mild food restriction in the rat. Following the demonstration of a robust augmenting effect of food restriction on heroin seeking after 14 days of abstinence, we investigated the importance of the "hunger" state during the test by re-feeding before the drug-seeking test. Finally, to determine if prolonged food restriction is a necessary condition for the augmentation of drug seeking, we manipulated the length of the food restriction period.

Materials and Methods

Subjects

A total of 100 male Long Evans rats (Charles River, St. Constant, Quebec, Canada) were used in five separate experiments. Rats were approximately 325-350 g at the beginning of each experiment and were allowed to acclimate to the animal care facility (ACF) for a week prior to surgery. Rats were housed in pairs until surgery (see below) in standard clear shoebox cages under a reversed 12 h light-dark cycle (lights OFF 9:30 AM), with temperature at approximately 21˚C.

Following recovery from intravenous (i.v.) catheter surgery rats were housed individually in operant conditioning training chambers with unrestricted access to food and water, unless otherwise indicated (see below).

After self-administration training, rats were transferred back to the animal facility and housed individually in clear shoebox cages during the food restriction phase (see below). Finally, following an abstinence period the rats were brought back to the operant conditioning chambers for the drug-seeking tests. All rats were treated in accordance with the guidelines of the Canadian Council on Animal Care and approval for all the experimental procedures was granted by the Concordia University Animal Research Ethics Committee.

Surgical procedures

Rats were implanted with i.v. Silastic catheters (Dow Corning, Midland, MI, USA) into the jugular vein under xylazine + ketamine (10 + 100 mg/kg, i.p.) anesthesia, as described previously (Shalev et al. 2000). Following surgery rats were given penicillin (450 000 IU/rat, s.c.) and the analgesic buprenorphine (10 µg/kg, s.c.). Throughout self-administration training catheters were flushed daily with heparin and gentamicin in sterile saline (7.5 IU + 12.0 µg per day per rat) to prevent catheter blockage.

Apparatus

The apparatus used here was similar to the one described previously (Maric et al. 2011). Briefly, experiments were conducted in operant conditioning chambers (Med Associates Inc., St. Albans, Vermont, USA; 32.0 cm × 24.0 cm × 25.0 cm), placed in individual sound-attenuation cubicles. Each box had 2 levers located 5 cm above the grid floor. Responses on the "active",

retractable, lever activated the infusion pump (Med Associates), whereas responses on the "inactive", non-retractable, lever were recorded but had no programmable consequences.

Procedure

Different cohorts of rats were used for each of the five experiments, which followed a similar general procedure. All experiments consisted of three phases: heroin self-administration training in operant conditioning chambers, an abstinence phase in the ACF during which some rats were food restricted, and a testing phase in the operant conditioning chambers. Timelines for the different experiments are presented in Fig. 1.

Training

Following a 24 h habituation period to the operant conditioning chambers, heroin self-administration training was conducted over a period of 10 days. Rats were given three 3-h training sessions per day separated by a 3 h period under a fixed ratio 1 (FR-1) schedule with a 20 s timeout. The initial session began shortly after the onset of the dark phase and was marked by the insertion of the active lever, the illumination of a houselight, the activation of a cue-light above the active lever, and sounding a tone (2.9 kHz; 10 dB above background level). The cue-light/tone complex remained on for 30 s or until the active lever was pressed. A response on the active lever resulted in the delivery of 0.1 mg/kg of heroin (diacetylmorphine HCL; provided by the National Institute for Drug Abuse, Research Triangle Park, NC, USA) in 0.13 ml infusion over 12 s and the initiation of a 20 s timeout, during which the houselight was turned off, the cue-light/tone complex remained on, and additional responses on the active lever were recorded but not reinforced. At the end of each 3 h session the active lever retracted and the houselight turned off.

Experiment 1: The effect of 14 days of food restriction on heroin seeking in abstinent rats

Following self-administration training, rats were housed individually in standard shoebox cages in the ACF with unrestricted access to food and water for a drug washout day. The next day, rats were divided into 2 groups: food restricted (FDR) and sated. Groups were matched for average number of infusions, active lever responses and body weight during the last 5 days of training. The FDR rats had their food removed, and were fed ~15 g of rat chow at 1:30 PM; this amount was adjusted daily to bring the FDR rats' body weight to approximately 75-80% of the sated rats'.

On food-restriction day 14 (FDR14; abstinence-day 15), rats were brought back to the operant conditioning chambers and attached to the liquid swivel. FDR rats had food hoppers in their operant conditioning chambers, however they contained no food. Testing took place under extinction conditions over a 3 h session. Active lever responses resulted in the same consequences as in training with the exception that no heroin infusions occurred.

Experiment 2: The effects of a 24 h re-feeding period (following 14-day food restriction) and 5-day re-food-restriction on heroin seeking in abstinent rats

Here we investigated the importance of the state of hunger during the drug-seeking test for the expression of the augmentation effect seen in Experiment 1. Rats were tested twice, once under sated conditions and a second time following a re-food-restriction (re-FDR) period.

As in Experiment 1, rats were given a washout day and separated into FDR and sated groups for the abstinence period in the animal facility. On abstinence-day 16, FDR rats were given unrestricted access to food. The additional day of food restriction was given to keep the number of restriction days equal to Experiment 1, including the test day (abstinence-day 15). On the next day (abstinence-day 17), rats were brought back to the operant conditioning chambers for testing. All

rats were given unrestricted access to food and water during the test. Testing was conducted under extinction conditions as in Experiment 1, except that the duration of the tests was shortened to 1 h to minimize extinction over repeated tests. Following Test 1, rats were removed from the operant conditioning chambers and returned to the animal facility, where the previously FDR rats were re-restricted on abstinence-day 18 and kept under food restriction through Test 2, which took place on abstinence-day 22 (re-FDR day 5) under extinction conditions.

Experiment 3: The effects of a 2 h re-feeding period on heroin seeking in abstinent rats

Over a 24 h re-feeding period, food digestion and metabolic changes could contribute to the results observed in Experiment 2. Therefore, to examine the effect of the hunger state *per se*, rats were exposed to a short, 2 h, re-feeding period that allowed for very little digestion to occur, although considerable amounts of food were consumed.

FDR and sated groups of rats were treated as described in Experiment 1. On abstinence-day 15, FDR rats were given unrestricted access to food for 2 h in the ACF prior to the test session. Testing was conducted under extinction conditions over a 1 h session. All rats were given unrestricted access to food and water during the test.

Experiment 4: The effects of a 24 h re-feeding period, and 5-day food restriction on heroin seeking in abstinent rats

The purpose of this experiment was to replicate the re-feeding effects reported in Experiment 2, and to explore the effects of a short-term food restriction on the augmentation of heroin seeking in abstinent rats.

As in Experiment 1 and 2, rats were removed from the operant conditioning chambers following training, brought to the animal facility for a washout day, separated into FDR and sated

groups, and food restricted for 14 days. On abstinence-day 16, FDR rats were re-fed and tested the following day (abstinence-day 17), over a 1 h session (similar to Experiment 2), under extinction conditions (Test 1). Following Test 1, rats were returned to the animal facility, and the previously FDR rats were allowed unrestricted access to food for 5 days whereas the previously sated rats were now food restricted for 5 days. On abstinence-day 22 rats were tested for the second time under extinction conditions (Test 2).

Experiment 5: The effect of a 3-day food restriction period on heroin seeking in abstinent rats

In order to avoid the possible confounding effects of the repeated tests in Experiment 4, here the rats were exposed to only one drug-seeking test following a short food restriction period.

After the washout day in the animal facility, rats had 11 additional days of unrestricted access to food and water before being separated into a sated group and an FDR group that was food restricted for 3 days. Testing took place on abstinence day 15 (FDR3 for food restricted rats) under extinction conditions over a 1 h session.

Statistical Analysis

For Experiments 1, 4 and 5 the numbers of responses made on the active and inactive levers during the test session were analyzed separately using independent samples, two-tailed *t*-test to compare the means of the FDR and sated groups. For Experiments 2 and 3 the numbers of responses made on the active and inactive levers during the test session were analyzed separately using repeated measures ANOVA with the between subjects factor of *food restriction* (FDR, Sated) and the within subjects factor of *test day* (Test 1, Test 2). Statistically significant interactions were followed by post hoc (Fisher's LSD) tests. Significant results are reported for $p \leq 0.05$.

Results

Rats in all experiments acquired reliable heroin self-administration behavior. Mean ± SEM number of infusions, and number of responses on the active and inactive levers made on the last day of heroin self-administration training, for each experiment, are shown in Table 1. Mean ± SEM body weights of the FDR and sated groups for each experiment are detailed in Table 2. In addition, in representative groups of rats, the average 24 h food intake for the sated rats over the abstinence period was 30.39 ± 0.89 g, while the FDR rats were fed, on average, 14.21 ± 0.13 g of chow per day over the same period.

Experiment 1: The effect of 14 days of food restriction on heroin seeking in abstinent rats

Exposure to 14 days of mild food restriction resulted in a robust augmentation of heroin seeking behavior in abstinent rats.

The average body weights for the rats throughout the experiment are presented in Fig. 2. On test day, a 14-day food restriction period resulted in a significant increase in responding on the active lever compared to sated rats, when the rats were returned to the drug-training environment, $t(16) = 2.30$, $p = 0.03$ (Fig. 3). There were no significant differences in the number of inactive lever responses between groups. The individual distribution of responses on the active and inactive levers during the test session in the FDR and sated rats is presented in Fig. 4.

Experiment 2: The effects of 24 h re-feeding (following 14-day food restriction) and 5-day re-food restriction on heroin seeking in abstinent rats

Re-feeding over 24 h prior to the test session eliminated the food-restriction effect on heroin seeking when animals were returned to the drug-training environment. When rats where food restricted again for 5 additional days before Test 2, however, the FDR rats had a higher rate of

responding on the active lever than sated rats (Fig. 5). Repeated measures ANOVA revealed significant effects for *food restriction* ($F_{1,18} = 5.07$, $p = 0.04$) and *food restriction* × *test day* ($F_{1,18} = 6.09$, $p = 0.02$), but the *test day* effect was not significant. Post hoc tests revealed a significant difference between the FDR and sated rats on Test 2 ($p < 0.05$). There were no significant differences between the groups in inactive lever responding.

Experiment 3: The effects of 2 h re-feeding on heroin seeking in abstinent rats

A short, 2-h, re-feeding period prior to the test session eliminated the food restriction effect on heroin seeking when animals were returned to the drug-training environment.

The mean number of active lever responses made by the FDR rats that were allowed 2 h of unrestricted access to food prior to the heroin-seeking test was not statistically different from the number of responses in the sated group (Fig. 6). There were no significant differences in inactive lever responses between the groups.

Experiment 4: The effects of 24 h re-feeding and 5-day food restriction on heroin seeking in abstinent rats

Re-feeding over the 24 h prior to the test session eliminated the food restriction effect on heroin seeking when animals were returned to the drug-training environment. In addition, when the previously sated rats were exposed to a 5-day food restriction period, no augmentation of heroin seeking was observed.

No significant differences between the groups were found in active lever responses when previously FDR rats had been re-fed for 24 h (Test 1) or when the previously sated rats had been food restricted for 5 days (Test 2). The number of responses on the active lever made during Test 2 was lower than in Test 1 in both groups, suggesting extinction of responding over repeated tests, but

the difference was not statistically significant ($F_{1,8} = 8.08$, $p < 0.07$). The effect of *food restriction* and the interaction effect for *food restriction* × *test day* were found to be non-significant (Fig. 7). The number of inactive lever responses was very low compared to active lever responses, and was lower on Test 2 than on Test 1. Repeated measures ANOVA performed on the inactive lever data revealed a significant effect for *test day* ($F_{1,8} = 8.08$, $p = 0.02$), but not for *food restriction* or *food restriction* × *test day* interaction.

Experiment 5: The effect of 3-day FDR on heroin seeking in abstinent rats

Following 11 days of abstinence in the animal colony with both groups sated, 3 days of food restriction were not sufficient to cause a statistically significant increase in active lever pressing during tests for heroin seeking under extinction conditions compared to sated rats (Fig. 8). There were no significant differences between the groups in inactive lever responding.

Discussion

As expected, all the rats that had experienced a period of abstinence showed robust heroin-seeking behavior when re-exposed to the contextual and discrete drug-associated cues. Responding maintained by stimuli previously paired with drug delivery following prolonged periods of abstinence has been previously demonstrated in cocaine- and heroin-trained rats (Fuchs et al. 2006; Neisewander et al. 1996; Shalev et al. 2001a). The major finding in this study, however, is that the chronically food restricted rats showed dramatically higher levels (>250%) of heroin seeking compared to the sated rats. Food restriction-induced augmentation of drug seeking proved to be a reliable effect, with at least 60% of the subjects in Experiment 1 producing more than 350 responses during the 3 h test, compared to only 12.5% of the sated rats.

Our current findings are consistent with reports from studies in humans that describe a positive correlation between the level of food restriction and drug-related behaviors (e.g., (Cheskin et al. 2005; Hall et al. 1992; Krahn et al. 1992), and with substantial evidence for an augmenting effect of dietary restriction on drug taking and seeking, and the reinforcing properties of drugs in animals (Carr 2007; Carroll and Meisch 1984; Stuber et al. 2002). Moreover, the present report extends our previous demonstration of an acute, 24 h food-deprivation-induced reinstatement of drug seeking (Shalev et al. 2000). The failure, in the current study, to observe an effect for short-term (3-5 days) food restriction emphasizes the importance of the chronic property of this mild dietary manipulation in augmenting the effects of drug-associated cues on drug seeking behavior.

Drug abusers frequently cite stressful experiences as a reason for continued drug use and relapse (Sinha et al. 1999). Food restriction acts as a stressor by inducing an aversive state of hunger, resulting, among other responses, in the activation of the stress-responsive hypothalamic-pituitary-adrenal (HPA) axis (Tomiyama et al. 2010). Thus, the augmented heroin seeking observed in the FDR group could be the result of exposure to prolonged stress. A role for the "stress aspect" of food restriction in the enhanced drug-seeking behavior is indicated by the finding that heroin seeking was attenuated following both brief (2 h) and prolonged (24 h) undisturbed access to food that eliminated the aversive state of hunger. These results are in agreement with the previously reported rapid decline in cocaine-reinforced behavior in FDR rats following a return to free feeding (Papasava and Singer 1985). Interestingly, our findings do not agree with a report by Carr (Carr 2002) describing a persistence of the food restriction-induced increase in the rewarding effects of amphetamine following re-feeding over several days, until body weights were restored to pre-restriction baseline. However, as we further discuss below, rats in Carr's report were exposed

to a considerably more severe food restriction regimen (20-25% weight loss compared to baseline over a few weeks), making a direct comparison difficult.

Since the rats' behavior, other than responding on the active and inactive levers, during the test sessions that followed re-feeding periods, was not observed, we cannot entirely rule out the possibility that food-directed behavior somewhat interfered with drug seeking. However, as the food-intake data described below suggest, the amount of food consumed during the re-feeding period makes such interference seem highly unlikely.

In a sentinel group of rats, with a history of heroin self-administration and a 14-day abstinence period under food restriction, we have recorded re-feeding food consumption of approximately 48 g (over 24 h) and 12 g (over 2 h), resulting in body weight gains of about 9% and 6%, respectively. Such rapid food intake (gorging) might result in general discomfort and behavioral inhibition or lethargy that could explain the attenuated drug seeking during tests. However, this seems unlikely; although the re-fed and sated rats showed no statistically significant differences in drug seeking, the mean response rate was over 100 lever presses per hour, suggesting that the re-fed rats were not lethargic. In addition, in a different group of rats that were exposed to a similar experimental procedure, 2 h or 24 h re-feeding did not result in any significant changes in general locomotor behavior in a novel environment compared to sated or food-restricted rats (data not shown).

It is important to note, however, that our data indicate that the state of hunger, by itself, is not sufficient to induce an increase in heroin seeking in abstinent rats. Thus, a short, 5-day, food restriction period failed to induce an augmentation of heroin seeking in rats that were sated through the 14-day abstinence period (Experiment 4, Test 2). In contrast, in rats that were food restricted over the abstinence period, re-fed and then re-food restricted for 5 days, a robust increase in heroin

seeking was observed (Experiment 2, Test 2). Importantly, these results and the lack of effect for short, 3-day, food restriction in Experiment 5 strongly suggest that the difference in the effectiveness of short food restriction period is not simply a result of the repeated tests procedure used in Experiment 4.

It could be argued that the rapid reversal of prolonged food-restriction-induced augmentation of heroin seeking by re-feeding indicates a lack of long-term plasticity in brain systems. Instead, metabolic signals related to food restriction and satiety conditions could acutely modulate heroin seeking. Obvious candidates for such signaling systems would be the peripheral adiposity signals that are involved in long-term body-weight regulation, e.g., insulin and leptin (Morton et al. 2006), and the orexigenic gastrointestinal peptide ghrelin, all are known to directly interact with midbrain reward system and modulate the hedonic and rewarding properties of natural rewards as well as drugs (Cummings et al. 2007; Figlewicz 2003). In support of the above argument, we have previously demonstrated that acute leptin administration can block acute food-deprivation-induced reinstatement of heroin seeking (Shalev et al. 2001b). However, the attenuation of the food restriction effect following an acute, 2 h, re-feeding period, which would have minimal impact on circulating levels of leptin (Schneider et al. 2000, but see Xu et al. 1999), suggests that leptin has no or only a minor role in the food restriction effect on heroin seeking in abstinent rats. In contrast, plasma levels of ghrelin increase during periods of food restriction, and drop sharply following a meal (Drazen et al. 2006; Tschop et al. 2000), a pattern that parallels our behavioral findings with food restriction and acute re-feeding. Ghrelin might, therefore, mediate the effect of food restriction on heroin seeking following prolonged abstinence.

Nevertheless, the acute reversal of the augmentation of heroin seeking by re-feeding in FDR rats does not necessarily rule out a role for adaptations in relevant brain areas that develop over the

restriction period. The robust augmentation of heroin seeking that was observed in the 5-day re-FDR rats (Experiment 2, Test 2), suggests that the expression of the behavioral sensitization to the effects of the drug context and cues was attenuated by re-feeding, but the critical adaptation have not been "erased". A rapid development, over the 5-day re-FDR period, of the conditions that are essential for the augmentation of drug seeking seems unlikely, since a similar food restriction period did not result in an increase in drug seeking in previously sated rats (Experiment 4). Future studies would explore the possibility that prolonged food-restriction-induced brain adaptations are involved in the augmentation of heroin seeking in abstinent rats.

There are two important methodological points worth noting. First, the food restriction regimen we have utilized here is mild compared to most other studies cited above. On test day the body weights of FDR rats in our study were 72-80% of the sated rats, or about 90% of their pre-restriction body weight. In contrast, in Carr's or Fulton et al.'s studies (Carr 2007; Fulton et al. 2000), rats were food restricted to 75-80% of their pre-restriction body weights. It is unusual to observe a weight loss of 20-25% in healthy humans. This further emphasizes the clinical relevance of the procedure used here. Second, in most of the previous studies that explored the effect of dietary manipulations on drug seeking behavior, the drug of choice was a psychostimulant, while the rats in the current study were trained with heroin. Drug associated behaviors, as well as brain adaptations that might underlie these behaviors, were shown to differ in animals exposed to psychostimulant and opiate drugs (Badiani et al. 2011). Therefore, at this point it is unclear how well previously suggested neuronal mechanisms can explain the behaviors described here.

In conclusion, we present here a procedure that may be highly valuable in elucidating the brain mechanisms that underlie the effects of environmental conditions, and more specifically, mild, clinically relevant dietary restriction, on relapse to drugs following a period of abstinence.

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Figure Captions

Fig. 1 Overview of timelines for Experiments 1 through 5.

Fig. 2 Mean (± SEM) body weights of all rats over the course of Experiment 1 in the food restricted (FDR, n=10) and sated (n=8) groups. * $p < 0.05$ compared to FDR group

Fig. 3 The effect of 14 days of food restriction and abstinence on heroin seeking (Experiment 1). Data are mean (+ SEM) number responses made on the active and inactive levers on the test day (FDR 14) in the food restricted (FDR, n=10) and sated groups (n=8). * $p < 0.05$ compared to the sated group

Fig. 4 The effect of 14 days of food restriction and abstinence on heroin seeking (Experiment 1). Data are the number responses made by each food restricted (FDR) and sated rat on the active and inactive levers on the test day (FDR 14)

Fig. 5 Heroin seeking following 14 days of food restriction and re-feeding (24 h) in abstinent rats (Experiment 2). Data are mean (+SEM) number responses made on the active and inactive levers on Test 1 (abstinence-day 17) following 14 days of food restriction and one day of unrestricted access to food (FDR → Re-fed 24 h; n=10), compared to continuously sated rats (n=10), and following 5 days of re-food restriction (Re-FDR; Test 2). * $p < 0.05$ compared to the sated group

Fig. 6 The effects of 2 h re-feeding on heroin seeking following a 14 days of food restriction in abstinent rats (Experiment 3). Data are the mean (+SEM) number responses made on the active and inactive levers on the test day in the food restricted (FDR)-re-fed (FDR → Re-fed 2 h, n=13) and sated groups (n=12)

Fig. 7 Heroin seeking following a 24 h re-feeding period, and after a short (5 days) food restriction period in abstinent rats (Experiment 4). Data are mean (+SEM) number responses made on the

active and inactive levers on Test 1 (abstinence-day 17) following 14 days of food restriction and one day of unrestricted access to food (FDR → Re-fed 24 h; n=9), compared to continuously sated rats (Sated, n=10), and following 5 additional days of unrestricted access to food (FDR → Re-fed 6 days) compared to 5 days food restriction in the previously sated rats (Sated → FDR 5 days) on Test 2. * $p < 0.05$ compared to Test 2

Fig. 8 The effect of a short (3 days) food restriction period on heroin seeking in abstinent rats (Experiment 5). Data are the mean (+SEM) number responses made on the active and inactive levers on the test day (abstinence day 15) in the food restricted (FDR 3 days, n=10) and sated (n=8) groups

Table 1. Mean ± SEM of the number of infusions taken, and the number of active and inactive lever responses made on the last training day (9 h) in each experiment.

	Infusions	Active lever	Inactive lever
Exp. 1	34.72 ± 3.66	89.11 ± 16.47	12.61 ± 4.12
Exp. 2	41.65 ± 6.23	145.05 ± 37.89	11.10 ± 3.19
Exp. 3	47.58 ± 4.74	150.42 ± 26.44	6.32 ± 1.99
Exp. 4	44.00 ± 4.56	145.72 ± 27.69	6.39 ± 1.63
Exp. 5	40.16 ± 6.81	173.48 ± 62.97	3.44 ± 0.75

Table 2. Mean ± SEM body weight (g) in the sated and food restricted (FDR) groups. Numbers in parentheses indicate percent body weight compared to the sated group, and compared to own body weight on the washout day, respectively.

	Group	FDR 14-day	Re-feeding 24 h	Re-feeding 2 h	FDR 5-day	FDR 3-day
Exp. 1	Sated n = 8	464.88 ± 16.60	-	-	-	-
	FDR n = 10	336.30 ± 9.77 (72%, 89%)	-	-	-	-
Exp. 2	Sated n = 10	514.7 ± 13.35	527.20 ± 14.35	-	-	-
	FDR n = 10	388.40 ± 6.54 (75%, 90%)	424.10 ± 7.61 (80%, 98%)	-	-	-
Exp. 3	Sated n = 12	454.25 ± 11.96	-	459.17 ± 12.07	-	-
	FDR n = 13	352.85 ± 5.27 (78%, 93%)	-	375.46 ± 5.03 (82%, 99%)	-	-
Exp. 4	Sated n = 10	462.30 ± 11.48	474.60 ± 10.08	-	456.30 ± 6.93* (101%, 118%)	-
	FDR n = 9	369.33 ± 13.10 (80%, 94%)	405.22 ± 9.93 (85%, 103%)	-	452.78 ± 4.09#	-
Exp. 5	Sated n = 8	-	-	-	-	482.63 ± 14.45
	FDR n = 10	-	-	-	-	451.00 ± 12.63 (93%, 112%)

* Following a 5-day food restriction period

Following a 6-day period of unrestricted access to food