Video Article

A Procedure to Study the Effect of Prolonged Food Restriction on Heroin Seeking in Abstinent Rats

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URL: <http://www.jove.com/video/50751>

DOI: [doi:10.3791/50751](https://doi.org/10.3791/50751)

Keywords: Behavior, Issue 81, Animal, Drug-Seeking Behavior, Fasting, Substance-Related Disorders, behavioral neuroscience, self-administration, intravenous, drugs, relapse, food restriction

Date Published: 11/11/2013

Citation: Sedki, F., D'Cunha, T., Shalev, U. A Procedure to Study the Effect of Prolonged Food Restriction on Heroin Seeking in Abstinent Rats. *J. Vis. Exp.* (81), e50751, doi:10.3791/50751 (2013).

Abstract

In human drug addicts, exposure to drug-associated cues or environments that were previously associated with drug taking can trigger relapse during abstinence. Moreover, various environmental challenges can exacerbate this effect, as well as increase ongoing drug intake.

The procedure we describe here highlights the impact of a common environmental challenge, food restriction, on drug craving that is expressed as an augmentation of drug seeking in abstinent rats.

Rats are implanted with chronic intravenous i.v. catheters, and then trained to press a lever for i.v. heroin over a period of 10-12 days. Following the heroin self-administration phase the rats are removed from the operant conditioning chambers and housed in the animal care facility for a period of at least 14 days. While one group is maintained under unrestricted access to food (sated group), a second group (FDR group) is exposed to a mild food restriction regimen that results in their body weights maintained at 90% of their nonrestricted body weight. On day 14 of food restriction the rats are transferred back to the drug-training environment, and a drug-seeking test is run under extinction conditions (*i.e.* lever presses do not result in heroin delivery).

The procedure presented here results in a highly robust augmentation of heroin seeking on test day in the food restricted rats. In addition, compared to the acute food deprivation manipulations we have used before, the current procedure is a more clinically relevant model for the impact of caloric restriction on drug seeking. Moreover, it might be closer to the human condition as the rats are not required to go through an extinction-training phase before the drug-seeking test, which is an integral component of the popular reinstatement procedure.

Video Link

The video component of this article can be found at <http://www.jove.com/video/50751/>

Introduction

One of the major difficulties in the treatment of drug addiction is the high rate of relapse, even after prolonged periods of abstinence^{1,2}. Studies in human addicts have identified a critical contribution of environmental challenges, such as the exposure to drug-associated discrete cues or environments³ and stressful life events⁴, to relapse. A common environmental challenge that was found to have an effect on drug taking and craving is chronic food restriction. For example, it has been reported that chronic caloric restriction is associated with increased cigarette smoking⁵. Furthermore, dieting severity (caloric restriction) is positively correlated with an increased prevalence of drug use in young women⁶. However, the underlying brain mechanisms are not fully understood.

Due to practical and ethical considerations related to human research, animal models have been developed to identify the relevant neural mechanisms. We have previously used the reinstatement procedure^{7,8} to demonstrate resumption of extinguished drug seeking in rats that are exposed to acute (24-48 hr) food deprivation^{9,10}. However, an integral part of the reinstatement procedure is an extinction period that is not a common feature of drug addiction treatments¹¹ (but see Epstein *et al.*¹² for a comprehensive discussion on the validity of the reinstatement procedure). Moreover, in the particular case of dietary manipulations in humans, only chronic food restriction, but not acute food deprivation, was shown to effectively increase drug-taking^{5,13}.

Consequently, we have suggested an "abstinence procedure"¹⁴ that allows the study of the effects of chronic food restriction on drug seeking. Using this procedure we have recently demonstrated a robust augmentation of heroin seeking in abstinent rats following exposure to 14 days of mild food restriction¹⁵.

Protocol

1. Animals

All rats are 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.

1. Obtain rats at 275-300 g (males) or 225-250 g (females). House rats in pairs until surgery in standard plastic cages containing corncob bedding and shredded paper.
2. Maintain rats on a reversed light-dark cycle (light off at 9:30 AM).
3. Make sure food and water are available *ad libitum*, except during the abstinence period. We use a regular rat chow (20.9% protein, 67.2% carbohydrates and 11.8% fat).

2. Catheters and Connectors

1. Make catheters in bulk and use as needed over a long period of time following autoclave sterilization.
2. Cut the Silastic tubing to 12 cm long pieces.
 1. Use a toothpick, to place a small drop of silicon around the catheter, 3 cm from the tip.
 2. Let the silicon dry for 24 hr and then autoclave and store the catheters.
3. Bend the long tube of the "5-up" cannula, that will serve as a connector, in a right angle to the plastic pedestal, then autoclave and store.
4. Assemble, aseptically, the catheters on the day of the surgery, by pushing the "long" side of the Silastic tube over the bent metal tube of the "5-up" cannula, about half way to the plastic pedestal.
 1. Connect the "5-up" tube via Tygon tubing and a blunted 22 G needle to a 1 ml syringe filled with sterile saline.
 2. Flush saline through the catheter to ensure unobstructed flow.

3. Intravenous Catheterization Surgery

1. Allow the rats one week of acclimatization in the animal care facility (ACF) before surgery. Do not perform the surgery on rats with body weights lower than 300-325 g (males) or 250-275 g (females).
2. Before surgery weigh and anesthetize the rats with a ketamine/xylazine mixture (90/13 mg/kg; i.p.).
 1. Shave, and then clean the head and the area on the right shoulder (close to base of the neck) with alcohol and surgical scrub.
 2. Inject penicillin (450,000 IU/rat, s.c.) to protect against infection, and 2 ml of saline (s.c.) for hydration. Apply tear gel to the rat's eyes to prevent dryness induced by anesthesia.
3. Place the rat on its abdomen and make an incision, using a scalpel, at the top of the head, between the eyes, and caudally towards the ears of the rat.
4. Place the rat on its back with the forearms taped to the surface. Lift the skin in the incision area, just rostral to the clavicle, with forceps and make a 1 cm longitudinal cut with small scissors.
5. Using two straight forceps, separate the fat and other tissue until the external jugular vein is exposed.
6. Push the tips of straight forceps under the vein to lift and separate the vein from surrounding tissue. Carefully clean the vein from any adhering tissue and then insert curved forceps under the vein to keep it lifted and slightly stretched.
7. Make a small, v-shaped, cut on the upper surface of the vein using small spring scissors.
 1. Hold the cut open using a vessel dilator, and advance the "short", 3 cm tip of the catheter into the vein towards the heart until the silicone drop is at the incision point.
 2. Tie the catheter to the vein with 3 sutures: one as far caudal as possible, one just rostral to the silicone drop and the third one as far rostral as possible.
 3. Pass saline through the catheter following each suture to ensure that it remains unobstructed and that the sutures are not too tight.
8. Remove the tape around the arms and place the rat ventral side down.
 1. Insert a hemostat through the caudal side of the head incision and push to separate the skin from the underlying tissue to create a subcutaneous "pocket".
 2. Guide the hemostat under the skin towards the opening on the shoulders where the catheter was inserted.
 3. Break through the connective tissue using the hemostat, and tightly clamp the catheter at the most rostral point, below the base of the "5-up."
9. Detach the "5-up" and thread the long end of the catheter under the skin and out the incision located at the top of the rat's head.
 1. Use scissors to cut the catheter below where the hemostat was clamped and reattach the long end of the "5-up."
 2. Pull the catheter along the metal shaft of the "5-up" all the way to the plastic pedestal. Pass saline through to ensure free flow.
10. Place the rat on its back, and suture only the incision near the clavicle. The head incision should remain open.
11. Place the rat in a stereotaxic apparatus. Pull the skin away at the incision at the top of the head using bulldog clamps and clean and dry the surface of the skull.
12. Use a manual drill bit, to drill four to five holes into the skull and insert machine screws into the holes to act as scaffolding for the head-cap.

1. Place the "5-up" connector between the screws and tuck the excess length of the catheter into the subcutaneous "pocket" previously made using the hemostat.
13. Use dental cement to affix the "5-up" to the top of the skull. Remove the rat from the stereotaxic apparatus after the cement is fully dried.
14. Give the rat post-operative care, which consists of ketoprofen (analgesic; 5 mg/kg, s.c.) and 2 ml of saline (s.c.). Administer ketoprofen for an additional 2 days following surgery to aid with pain management.
15. Beginning 48 hr following surgery, flush rats daily with a mixture of heparin and gentamicin in sterile saline (7.5 IU + 12 µg; 0.2 - 0.3 ml) throughout the recovery period of ~2 days and through the heroin self-administration phase.

4. Behavioral Procedure

The timeline for the procedure of food restriction-induced augmentation of heroin seeking is presented in **Figure 1**.

1. Operant conditioning chambers:
 1. House rats individually in operant conditioning chambers enclosed in sound attenuating wooden compartments equipped with a fan.
 2. Each chamber consists of a stainless steel metal grid floor, a front and back Plexiglas wall and two metal panel sidewalls. Mount two retractable levers 9 cm above the floor of the right sidewall, and locate a cue-light above each lever. Responding on the drug-paired (active) lever activates an infusion pump while responding on the nondrug-paired (inactive) lever has no programmable consequences.
 3. Locate a tone module (2.9 kHz) above the active lever, and position a red house-light at the top-center of the left sidewall.
 4. Connect the drug pump to the catheter via a liquid swivel and Tygon tubing protected with a metal spring. In addition, equip each chamber with a food hopper and a water bottle allowing unrestricted access to food and water.
2. Habituation day:
 1. Following recovery from surgery move the rats to the operant training chambers and allow for a 24 hr habituation period.
 2. Do not connect the rats to the metal spring and do not activate the cue-light/tone complex.
 3. Make sure that the active lever remains retracted, but ensure the inactive lever is extended to help with the discrimination between the two levers during training.
3. Heroin self-administration:
 1. Connect the 5-up connector to the Tygon tube and attach the metal spring.
 2. Allow the rats to self-administer heroin (0.1 mg/kg/infusion) for 10 days during three 3 hr sessions separated by 3 hr intervals.
 3. Begin each session following the onset of the dark phase (around 9:30 AM in our laboratory) with the extension of the active and inactive levers, as well as illumination of the house-light and activation of the cue-light/tone complex for 30 sec.
 4. Responses on the active lever result in activation of the drug pump (5 sec, 0.13 ml / infusion) and initiation of a 20 sec timeout period during which the house-light is turned off and the cue light/tone complex above the active lever is activated (FR-1, 20 sec timeout). During the timeout period, active lever responses are recorded but not reinforced. Inactive lever responses are recorded but have no programmable consequences.
 5. Following each 3 hr session, the active lever is retracted whereas the inactive lever remains extended until 1 hr before the first session of the following day.
4. Abstinence and food restriction:
 1. Following self-administration training, remove the rats from the operant conditioning chambers and individually house them in the ACF, and give unrestricted access to food and water for one drug-washout day.
 2. Divide the rats into two groups: food restricted (FDR) or sated, matched according to body weight, number of infusions and active lever responses across the last 5 days of training.
 3. Following the drug-washout day, remove the FDR rats' food, and feed them approximately 15 g of rat chow at 1:30 PM. Adjust the amount of food across 14 days of abstinence to maintain the FDR rats' body weight (BW) to approximately 90% of their own baseline BW.
5. Drug-seeking test: On the morning of abstinence day 14, return the rats to the operant conditioning chambers and attach them to the metal spring. Use a 1 hr or 3 hr session during which active and inactive lever responses have the same consequences as in self-administration training, excluding the availability of the drug (*i.e.* under extinction conditions).

Representative Results

Body weights over time: Typical results demonstrate that body weights remain stable throughout heroin self-administration training. During abstinence, rats with unrestricted access to chow increase their body weight, while those that are food restricted decrease their body weight to approximately 90% of their own original body weight or 75-80% of the sated rats' body weight (**Figure 2**).

Infusions, active and inactive lever responses over training: Infusions and active lever responding increase over training days, whereas inactive lever responding does not (**Figure 3**). Typically, active lever responding increases throughout training and stabilizes at a higher rate of responding. Inactive lever responding remains minimal throughout training, signifying that the rats have properly discriminated between both levers. Finally, the number infusions taken will increase throughout training and remain stable at a rate lower than those of the active lever responses. This dissociation is possible due to the 20 sec timeout following drug infusions.

Active and Inactive lever responses on test day: Active lever responding for the food restricted rats significantly increases compared to the sated rats (we often observed a 250% difference between the treatment groups, see¹⁵). Inactive lever responding is minimal and comparable between the food restricted and sated rats (**Figure 4**).

Abstinence procedure: timeline

Figure 1. A timeline of the experimental procedure. The procedure consists of three phases. Animals are first trained to self-administer a drug in the presence of a cue/tone complex (heroin self-administration phase), then moved to a different context and undergo a one-day drug washout period. Washout day is followed by a prolonged period of abstinence, where rats are given a regimen of food restriction or unlimited access to food. On the 14th day of abstinence, rats are returned to the self-administration environment for a drug-seeking test in the presence of drug-paired cues under extinction conditions (drug-seeking test phase). [Click here to view larger image.](#)

Figure 2. Body weights in the food restricted (FDR) and Sated groups, across experimental days. All rats undergo 10 days of heroin self-administration training in operant training chambers and a 1 day drug washout in the animal care facility. Rats are matched according to body weight, active lever responding and drug infusions before separation into the FDR or Sated group. [Click here to view larger image.](#)

Figure 3. The number of infusions, active, and inactive lever responses made during heroin self-administration training. Heroin (0.13 mg/kg/infusion) is self-administered in three 3 hr sessions, over 10 days, under a FR-1, 20 sec time out schedule of reinforcement. [Click here to view larger image.](#)

Figure 4. The effect of exposure to prolonged food restriction (FDR) on heroin seeking in abstinent rats. The data represents typical active and inactive lever responding on test day. Test day consists of one 1 hr (or 3 hr) test session under extinction conditions, following heroin self-administration training and 14 days of abstinence under FDR or sated conditions. [Click here to view larger image.](#)

Discussion

The procedure described here has strong face validity because 1) it allows for voluntary drug intake and control over drug administration, 2) it uses a route of administration that is common in human addicts, 3) it reflects (relatively) prolonged periods of drug exposure and abstinence, and 4) it avoids the extinction training before the drug-seeking test that is integral to the reinstatement procedure but is rarely observed in human addicts¹⁴. In addition, the procedure models a prolonged food restriction condition that is thought to be critical when considering the effects of caloric restriction on drug seeking⁵.

In the protocol we describe here, the rats are housed in the operant conditioning chambers through the drug self-administration period (24 hr/day). It might be possible to increase the efficiency of data collection by using a "squads" protocol in which the rats are transferred back to the ACF after a single daily training session, allowing for multiple groups to be trained every day. However, this approach might be more suitable for cocaine training, since heroin is preferably administered in the "home" environment¹⁶. We have not assessed the efficiency of the procedure using the squads option. Another way to increase the throughput of the procedure is by staggering the drug self-administration training for two groups of rats by 10-12 days. Using this design, rats in the second group are trained for self-administration while the first group goes through the abstinence period in the ACF. Drug self-administration training for the second group must be concluded at least a day before the planned test for the first group to allow for thorough cleaning of the chambers.

We have reported before¹⁵ that the length of the restriction period is critical, with 3-5 days of food restriction failing to augment heroin seeking at the end of the abstinence period. We have not observed an increase in the efficacy of the food restriction manipulation with increased exposure periods (~28 days; unpublished data).

It is also worth noting that the change in context between the self-administration phase and the abstinence period seems to be important for the augmentation of drug seeking. We have collected data that suggest a much-reduced effect for food restriction when the rats are maintained in the drug-training environment during the abstinence period (unpublished observation).

In conclusion, the food restriction-induced augmentation of heroin seeking is a robust, easily replicable effect. It therefore allows for efficient designs of studies on the neural mechanisms that underlie relapse following abstinence, using pharmacological pretreatments, online monitoring of neurochemical or neuronal activity using microdialysis or electrophysiological techniques, and post-test histological analyses.

Disclosures

Approval for all the experimental procedures was granted by the Concordia University Animal Research Ethics Committee, in accordance with the guidelines of the Canadian Council on Animal Care. The authors declare that they have no competing financial interests. FS and TD equally contributed to the development of the procedure presented here and to the writing of the manuscript.

Acknowledgements

This work was supported by the Natural Sciences & Engineering Council Discovery Program (US: 298915), the Fonds de Recherche du Quebec - Santé (CSBN), and the Canada Research Chairs Program (US).

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