

Evidence That Intermittent, Excessive Sugar Intake Causes Endogenous Opioid Dependence

Carlo Colantuoni,* Pedro Rada,*† Joseph McCarthy,* Caroline Patten,* Nicole M. Avena,* Andrew Chadeayne,* and Bartley G. Hoebel*

Abstract

COLANTUONI, CARLO, PEDRO RADA, JOSEPH MCCARTHY, CAROLINE PATTEN, NICOLE M. AVENA, ANDREW CHADEAYNE, AND BARTLEY G. HOEBEL. Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obes Res.* 2002;10:478–488.

Objective: The goal was to determine whether withdrawal from sugar can cause signs of opioid dependence. Because palatable food stimulates neural systems that are implicated in drug addiction, it was hypothesized that intermittent, excessive sugar intake might create dependency, as indicated by withdrawal signs.

Research Methods and Procedures: Male rats were food-deprived for 12 hours daily, including 4 hours in the early dark, and then offered highly palatable 25% glucose in addition to chow for the next 12 hours. Withdrawal was induced by naloxone or food deprivation. Withdrawal signs were measured by observation, ultrasonic recordings, elevated plus maze tests, and in vivo microdialysis.

Results: Naloxone (20 mg/kg intraperitoneally) caused somatic signs, such as teeth chattering, forepaw tremor, and head shakes. Food deprivation for 24 hours caused spontaneous withdrawal signs, such as teeth chattering. Naloxone (3 mg/kg subcutaneously) caused reduced time on the exposed arm of an elevated plus maze, where again significant teeth chattering was recorded. The plus maze anxiety effect was replicated with four control groups for comparison. Accumbens microdialysis revealed that naloxone (10 and 20 mg/kg intraperitoneally) decreased extracellular dopamine (DA), while dose-dependently increasing acetylcholine (ACh). The naloxone-induced

DA/ACh imbalance was replicated with 10% sucrose and 3 mg/kg naloxone subcutaneously.

Discussion: Repeated, excessive intake of sugar created a state in which an opioid antagonist caused behavioral and neurochemical signs of opioid withdrawal. The indices of anxiety and DA/ACh imbalance were qualitatively similar to withdrawal from morphine or nicotine, suggesting that the rats had become sugar-dependent.

Key words: glucose, addiction, dopamine, plus maze, acetylcholine

Introduction

Palatable foods release brain opioids and dopamine in the limbic system (1–3). For example, a tasty food can activate opioid receptors in the ventral tegmental area (VTA) and thereby stimulate cells that release dopamine in the nucleus accumbens (NAc) (4). Conversely, opiates or dopamine injected into the NAc can stimulate animals to eat palatable food (5–7). Injections of this type can reinforce behavior, as evidenced by rats that self-inject opiates in the VTA and self-inject dopaminergic drugs or opiates in the NAc (8–12). Thus, opioid and dopamine synapses in the mesolimbic system may play important roles in the reinforcement of behavior by natural rewards, such as food (13–15). Because evidence suggests that drugs of abuse can create dependency via this system (15,16), it was hypothesized that palatable foods might also create dependency under appropriate conditions. Specifically, bingeing on a very palatable food, such as sugar, was predicted to cause dependency as defined by naloxone-induced withdrawal.

The first stage of dependency is expressed when animals escalate their intake of a substance and show underlying neurochemical changes indicative of sensitization or tolerance. In the eating disorder model of Hagan and Moss (17), rats that were raised from a young age with periodic food restriction and refeeding, became sensitized to opiate-induced food intake. Such animals were also prone to self-administer cocaine (18). To develop a

Submitted for publication August 27, 2001.

Accepted for publication in final form March 8, 2002.

*Department of Psychology, Princeton University, Princeton, New Jersey; and the †Department of Physiology, University of Los Andes, Merida, Venezuela.

Address correspondence to Dr. Bartley G. Hoebel, Department of Psychology, Green Hall, Princeton University, Princeton NJ 08544.

E-mail: hoebel@princeton.edu

Copyright © 2002 NAASO

more rapid model, we deprived adult rats of food for 12 hours daily, including the early-dark period, and then gave them access to 25% glucose and chow for the next 12 hours for 1 to 4 weeks. Their glucose intake gradually doubled, and they learned to drink large amounts of glucose in the first hour of daily access (19,20). After a month on this schedule, autoradiography performed on brain sections of female rats showed dopamine D-1 and opioid μ receptors were altered in several regions, including significantly increased binding in the NAc (19,20). In a similar model of binge eating, rats on a schedule of intermittent deprivation and a palatable high-fat diet learned to eat excessive amounts at the onset of daily food access (21). Thus, it seems that either a palatable sugar or fat-rich diet can effectively induce bingeing under the appropriate access schedule.

The second stage of dependency is the emergence of withdrawal symptoms. In the case of opiate dependency, withdrawal can be precipitated with an opioid receptor antagonist such as naloxone. Morphine withdrawal is characterized by autonomic nervous system abnormalities and physical signs such as changes in body temperature, tremors, and shakes (22). Behavioral manifestations that include ultrasonic, distress vocalizations (23), and expressions of anxiety (22,24) also have been observed. Withdrawal from food was first reported by Le Magnen (25) who found that rats on a palatable, cafeteria diet displayed body shakes when administered naloxone. Food withdrawal was discovered independently as part of this study using rats on a diet of intermittent glucose and chow.

There are also neurochemical changes associated with withdrawal. For example, in naive rats, an initial injection of morphine releases dopamine (DA) and lowers extracellular acetylcholine (ACh) in the NAc (26,27). After repeated morphine administration, the ACh effect diminishes, suggesting tolerance (28,29). This can be overcome by escalating doses of morphine (30). Subsequent administration of naloxone, locally or directly in the NAc, reveals opiate dependence by triggering the opposite state, which is the release of ACh and a decrease in extracellular DA (28–30). For this study, it was hypothesized that bingeing on sugar would lead to both behavioral and neurochemical signs of withdrawal in response to naloxone. Preliminary reports of the results have been published (31–36).

Research Methods and Procedures

Experiment 1

Male Sprague–Dawley rats from the Princeton University vivarium were housed on a 12-hour light, 12-hour dark cycle in individual cages. Water was available ad libitum. The rats in the main experimental group were maintained on a daily cycle of laboratory chow and 25% aqueous glucose

available for 12 hours each day beginning 4 hours after the onset of the dark period. For the other 12 hours each day, they were deprived of food. Control groups are specified in experiments below. All procedures were approved by the Institutional Animal Care and Use Committee.

Experiment 1A: Feeding Behavior of Males on Cyclic-Glucose/Chow. As a preliminary test to determine how much and when males would consume glucose and chow on the experimental schedule, males ($n = 6$) weighing 300 to 475 g were maintained on cyclic glucose and chow for 8 days, as described above. Glucose and chow intakes were measured after 1, 2, 3, and 12 hours of access on days 1, 4, and 8. Glucose intakes were analyzed by two-way ANOVA (hour \times day) and chow intake by one-way ANOVA. Dunnett Multiple Comparison post hoc tests were used for both analyses.

Experiment 1B: Somatic Signs During Precipitated Withdrawal. Male rats ($n = 16$) weighing 300 to 475 g were maintained on cyclic glucose and chow for 8 days, and control rats ($n = 16$) received ad libitum chow. On day 9, after the usual 12-hour deprivation period, instead of receiving glucose and chow, the rats were placed in individual tilt cages for 20 minutes, and observers blind to the experimental condition counted baseline frequency of rearing, grooming, cage crossing, teeth chattering, head shaking, forepaw tremors, and wet-dog shakes. Next the rats received an injection of naloxone [20 mg/kg intraperitoneally (IP), $n = 8$] or saline vehicle ($n = 8$) and were observed for another 20 minutes. Control rats in the ad libitum chow group were food-deprived for 12 hours, put in the tilt cages, observed in the same way, and given the same injections (naloxone, $n = 8$; saline, $n = 8$). The 12-hour period of food deprivation was included for the control group to exclude the possibility that acute food deprivation caused the observed effects. Statistical analysis was performed by two-way ANOVA (drug treatment \times somatic sign) followed by post hoc Bonferroni test. For a replication of the teeth-chattering measurement with a lower dose of naloxone under different conditions see Experiment 2B.

Experiment 1C: Somatic Signs during Spontaneous Withdrawal. The 16 saline-injected rats from Experiment 1B, 8 experimental and 8 control, were tested for the emergence of spontaneous withdrawal during 20-minute observations. Measurements were taken after the usual 12-hour food deprivation when they received the saline injection, and then again after 24 hours and 36 hours of food deprivation. Statistical analysis was performed by two-way ANOVA (time \times somatic sign) followed by post hoc Bonferroni test to determine differences from the 12-hour deprivation condition.

Experiment 2

Experiment 2A: Ultrasonic Vocalization. Males ($n = 60$) were housed three per large cage for 28 days while given four different dietary schedules for comparison: cyclic glucose and chow ($n = 24$), cyclic chow ($n = 12$), ad libitum

glucose and chow ($n = 12$), and ad libitum chow ($n = 12$). One-half of the cyclic-glucose/chow group received an injection of naloxone [3 mg/kg subcutaneously (SC) $n = 12$] and the other one-half received a saline injection ($n = 12$). The other three groups received naloxone (3 mg/kg SC). After the injection, each rat was placed in a clear plastic cylinder (8 × 20 cm). An air puff (10 ± 5 psi) was directed through a hole in the cylinder at the scruff of the rat's neck once per minute for 5 minutes of gentle confinement. Then a computerized ultrasonic detector (Noldus Information Technology, Leesburg, VA) recorded and analyzed the number of 20 kHz vocalizations lasting at least 0.075 seconds separated by 0.05 seconds for the next 4 minutes. Significance was judged by Wilcoxon nonparametric test for unpaired replicates. This procedure and the statistical analysis are similar to that used by others to detect ultrasonic vocalizations during withdrawal from drugs of abuse (22).

Experiment 2B: Elevated Plus Maze. Each rat from the ultrasonic-vocalization test was transferred 5 minutes later to an elevated plus-shaped maze. The apparatus had four arms 10 cm wide × 50 cm long and 2 ft above the floor. Two opposite arms were enclosed by high opaque walls, and the other two arms had no walls. The rats were placed in the center facing an open arm. During a 5-minute test, the time spent with all four feet on an open arm was recorded with a stopwatch by an observer blind to the experimental condition. Data from rats receiving naloxone were analyzed by one-way ANOVA with Dunnett Multiple Comparison post hoc test. The two cyclic-glucose/chow groups, one with saline and one with naloxone, were compared by Student's *t* test.

Experiment 2C: Somatic Signs. While each rat was in the plus maze, instances of audible teeth chatter were recorded by the condition-blind observer. Data were analyzed as in Experiment 2B.

Experiment 3: Elevated Plus Maze, Replication

Single-housed male rats ($n = 12$ /group) were given one of the following five diets for 32 to 36 days. 1) Cyclic glucose and chow: animals in this main experimental group were maintained on a daily cycle of intermittent access to 25% aqueous glucose and laboratory chow for 12 hours each day beginning 4 hours after the onset of the dark period, as usual; 2) ad libitum glucose and chow, i.e., 24-hour access to both foods; 3) cyclic glucose: daily 12-hour glucose with 4-hour delay in the dark and ad libitum chow; 4) cyclic chow: daily 12-hour chow starting 4 hours into the dark period with no glucose; and 5) ad libitum chow: the basic control group. Glucose intake was measured daily and body weight recorded every third day.

Between days 33 and 36, in the dark when the food-cycled groups usually would be fed, each rat received naloxone (3 mg/kg SC) and was placed 15 minutes later in the

center of the maze facing in a different direction on a random basis. The time a rat spent with four feet in an open arm, not facing into a closed arm, was recorded under red light by an observer blind to the experimental condition. If a rat fell off the maze, it was picked up and put back in the same spot on the maze. The maze was wiped with dilute alcohol after each rat. The procedure is similar to that used by other investigators as an index of withdrawal-induced anxiety (23). Statistics were performed by contrast ANOVA.

Experiment 4: Accumbens DA and ACh during Glucose Withdrawal

Surgery. Animals for microdialysis in Experiments 4 and 5 were anesthetized with xylazine (10 mg/kg IP) supplemented by ketamine (40 mg/kg IP). Bilateral 21-gauge stainless steel guide shafts aimed at the posterior medial NAc were stereotaxically implanted as follows: NAc: B + 1.2, L 1.2, V 4.0 with reference to bregma, midsagittal sinus, and the surface of the level skull, respectively. Guide shafts were kept patent with 26-gauge stylets. Microdialysis probes, which were inserted later, extended 5 mm beyond the guide shafts to reach the NAc, on the border of the shell and core, but largely in the shell. The rats recovered at least 1 week before being put on a feeding schedule.

Microdialysis Probes and Perfusate. Probes were constructed of silica glass tubing (37 μm inner diameter) inside a 26-gauge stainless steel tube with a microdialysis tip of cellulose tubing (Spectrum Medical Co., Rancho Dominguez, CA) sealed at the end with epoxy cement (6000 MW cutoff, 0.2 mm outer diameter × 2 mm long). Probes were inserted and fixed in place 14 to 16 hours before the experiment and perfused at 0.5 μL/min overnight to allow neurotransmitter recovery to stabilize. During the experiment, probes were perfused with buffered Ringer's solution (142 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, 1.35 mM Na₂HPO₄, and 0.3 mM NaH₂PO₄, pH 7.3) at a flow rate of 2.0 μL/min. Neostigmine at a low concentration (0.3 μM; Sigma, St. Louis, MO) was added to the perfusion fluid to improve recovery of ACh by hindering its enzymatic degradation while still allowing the extracellular concentration of ACh to vary up or down as a result of opioid withdrawal.

DA Assay. DA content in microdialysates was analyzed by reverse phase, high performance liquid chromatography coupled to electrochemical detection (HPLC-EC). Samples were injected immediately into an HPLC system that used a 20-μL sample loop leading to a 10-cm column with 3.2 mm-bore and 3 μm, C-18 packing (Brownlee Co. Model 6213; San Jose, CA). The mobile phase contained 60 mM sodium phosphate, 100 μM EDTA, 1.24 mM heptanesulfonic acid, and 5% to 6% vol/vol methanol. DA was measured with a coulometric detector (ESA Co. Model 5100A; Chelmsford, MA) with the conditioning cell potential set at

Table 1. Changes in body weight and intake of 25% glucose and chow

| Day | Glucose intake per hour (mL \pm SEM) | | | Total glucose (mL) | Chow (g) | Weight (g) |
|-----|--|--------------|--------------|-----------------------|-----------------|---------------|
| | Hour 1 | Hour 2 | Hour 3 | Hour 12 | 1st 3 Hours | |
| 1 | 5 \pm 2 | 2 \pm 0.6 | 1 \pm 0.4 | 30 \pm 9 | 2.7 \pm 0.6 | 387 |
| 4 | 12 \pm 2 | 2 \pm 0.5 | 2 \pm 0.5 | 46 \pm 9 | 6.9 \pm 0.7 | 378 |
| 8 | 16 \pm 1* | 8 \pm 0.2* | 6 \pm 0.4* | 79 \pm 1* | 10.5 \pm 0.8* | 395 |

* $p < 0.05$.

+500 mV, and working cell potential at -400 mV.

ACh Assay. ACh was measured by a reverse phase HPLC system using a 20- μ L sample loop with a 10-cm C18 analytical column (Chrompack Inc., Palo Alto, CA). ACh was converted to betaine and hydrogen peroxide by an immobilized enzyme reactor (acetylcholinesterase and choline oxidase from Sigma; columns from Chrompack Inc.). The mobile phase was 200 mM potassium phosphate at pH 8.0. An amperometric detector (EG&G Princeton Applied Research, Lawrenceville, NJ) was used. The hydrogen peroxide was oxidized on a platinum electrode (BAS, West Lafayette, IN) set at 0.5 V with respect to a Ag-AgCl reference electrode (EG&G Princeton Applied Research).

Microdialysis Procedure. In Experiment 4, three groups of male rats were maintained on a 12-hour cycle of glucose and chow for 28 days, and a control group was given free access to chow ($n = 5$ to 8/group). The microdialysis procedure was performed 4 hours into the dark period at the time when the cycled rats would normally have received glucose and chow. The rats had no access to food during dialysis sessions, but water was available. Microdialysis samples (40 μ L) were divided in half to conduct both DA and ACh assays with the 20-minute samples. After establishing consistent baseline levels of DA and ACh for at least 60 minutes, groups received naloxone (10 or 20 mg/kg IP) or saline (equal volume). Microdialysis samples were collected for 80 minutes after injection. Microdialysis data were converted to the percentage of the mean of the three successive baseline samples and analyzed by ANOVA for repeated measures (condition \times time) with Newman-Keuls post hoc tests when justified.

Experiment 5: Accumbens DA and ACh during Sucrose Withdrawal

This was a replication of Experiment 4 using 10% sucrose, instead of 25% glucose, and a lower dose of naloxone (3 mg/kg SC) to match the dose in Experiments 2 and 3. The experimental group ($n = 6$) received cyclic sucrose and chow and the control group ($n = 6$) was fed ad libitum chow for 21 days. Data for DA and ACh in split 20-minute

samples were converted to the percentage of the mean of three successive baseline samples and analyzed by ANOVA with Bonferroni's post hoc tests.

Subjects received an overdose of sodium pentobarbital before perfusion with 0.9% saline solution followed by formalin. Brains were removed, frozen, and sectioned at 40 microns to identify probe tracks.

Results

Experiment 1A: Glucose and Chow Intake

Table 1 shows glucose intake during the first, second, and third hours of access. Two features of the data stand out. The rats consumed a large amount of glucose in the first hour of access after being deprived for 12 hours, and the amount increased from 5 mL on day 1 to 16 mL on day 8 ($F_{(2,17)} = 6.64, p < 0.01$). Total 12-hour glucose intake increased from 30 to 79 mL ($F_{(2,17)} = 11.5, p < 0.01$). Daily chow intake also escalated significantly during the three measurement periods ($F_{(2,17)} = 30.69, p < 0.01$, Table 1). Body weight decreased during the first 4 days and then returned to normal by the end of the 8 days.

Experiment 1B: Somatic Signs during Precipitated Withdrawal

Male rats ($n = 8$ /group) were given glucose and chow for 12 hours each day and then received naloxone (20 mg/kg IP) or saline on day 9 at the end of the 12-hour deprivation. They showed an increase in the incidences of teeth chattering, forepaw tremor, and head shaking. This was significant ($F_{(1,56)} = 40.90, p < 0.01$) in the cyclic glucose/chow group compared with a similar group given saline injections (Figure 1A, left bar graph). Naloxone was not different than saline in the control rats that had been fed chow ad libitum (Figure 1A, right hand bar graph).

Experiment 1C: Somatic Signs during Spontaneous Withdrawal

The saline-injected rats in Figure 1A, left ($n = 8$ /group) were food-deprived for an additional 12 hours (24 hours

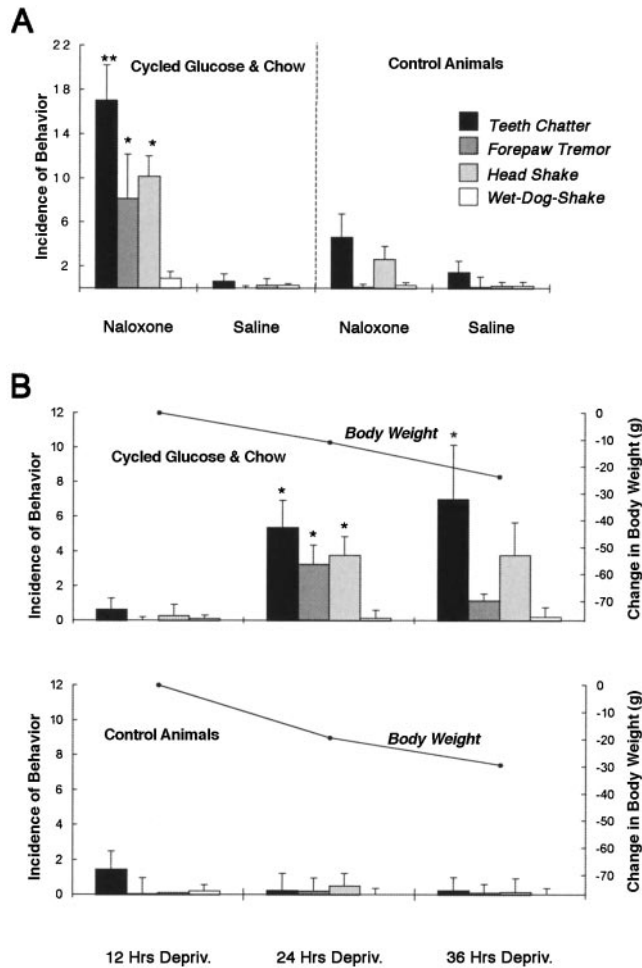


Figure 1: (A) Somatic signs of naloxone-induced opioid withdrawal. On the left, naloxone (20 mg/kg intraperitoneally) precipitated significantly more withdrawal signs than saline when administered to rats with a history of daily 12-hour food deprivation and 12-hour access to glucose and chow (* $p < 0.05$, ** $p < 0.01$). At the right, the same dose of naloxone or saline had no significant effect when administered to a control group maintained on ad libitum chow. (B) Spontaneous withdrawal. Rats that had been on the cyclic-glucose/chow diet showed a significant increase in spontaneous withdrawal signs after 24 and 36 hours of food deprivation, compared with the same rats at 12 hours, or compared with the control group that had free access to chow (* $p < 0.05$). Body weight decreased similarly in both groups as indicated by the scale at the right.

total), at which time (Figure 1B), teeth chatter, forepaw tremor, and head shaking emerged spontaneously. These signs of withdrawal were observed again after 36 hours of deprivation (Figure 1B; $F_{(2,84)} = 7.3, p < 0.001$). There was a lack of these effects in the chow-fed control group that was food-deprived for an equal length of time and that lost body weight at an equal rate (Figure 1B, lower graph).

Experiment 2A: Ultrasonic Vocalization

This experiment was designed to replicate Experiment 1A with a lower dose of naloxone and to look for other signs

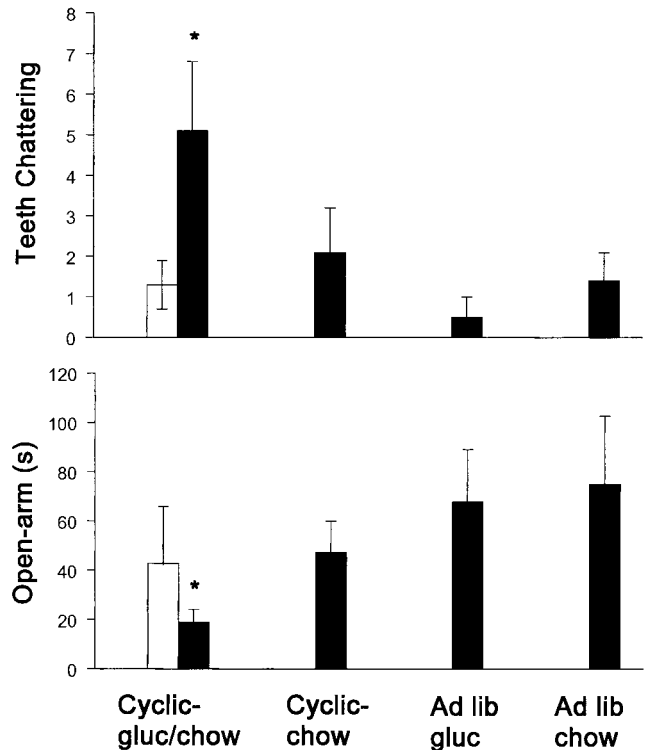


Figure 2: Lower graph: time spent on the open arms of an elevated plus maze. Four groups of rats received naloxone (20 mg/kg intraperitoneally, black bars) and one group received saline (white bars) following airpuffs to heighten anxiety and induce ultrasonic calls. The cyclic-glucose/chow group spent less time on the open arms of the maze (* $p < 0.05$ compared with other naloxone groups). Upper graph: audible teeth chattering while in the plus maze. The cyclic-glucose/chow group teeth chattered the most (* $p < 0.05$ compared with other naloxone groups and the saline group). White bars, saline group; black bars, naloxone groups; gluc, glucose; lib, libitum.

of withdrawal. In rats ($n = 12$ /group) that had been group housed on the 12-hour schedule of cyclic glucose and chow, naloxone administered (3 mg/kg SC) when the rats had been without food for 12 to 14 hours, caused three signs of anxiety characteristic of withdrawal. In Experiment 2A, ultrasonic vocalizations at 20 kHz were detected in each group that received naloxone. This occurred in 50% of the cyclic glucose-chow rats, compared with 44% of the cyclic chow rats, 33% of the ad lib glucose rats and 37% of the ad lib chow group. Although significant ($p < 0.05$) based on nonparametric statistics, the effect was minimal.

Experiment 2B: Anxiety in an Elevated Plus Maze

When the same rats were transferred to the plus maze, the cyclic-glucose/chow rats given naloxone spent significantly less time venturing onto the exposed arms than any of the other three naloxone-treated groups ($F_{(3,37)} = 2.95, p < 0.05$, black bars in Figure 2, lower graph). However, the

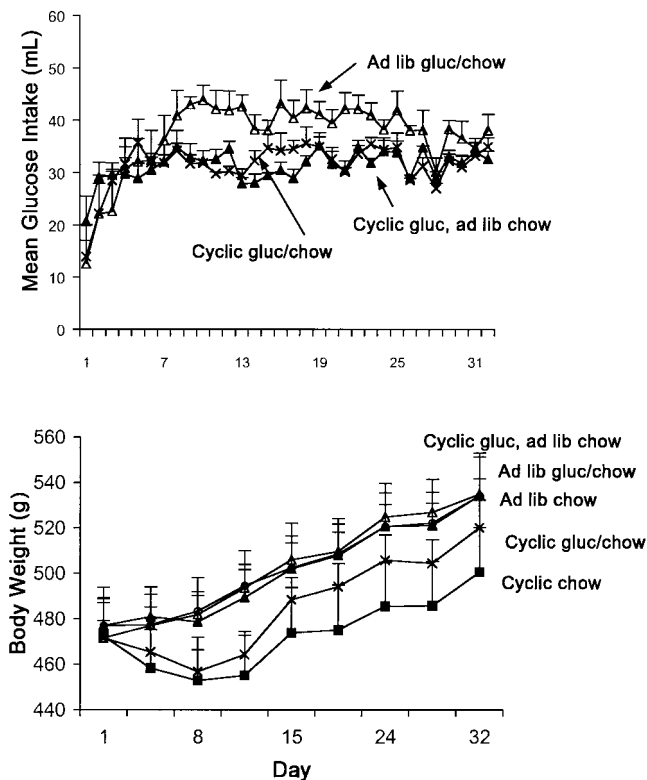


Figure 3: Lower graph: body weight of rats in Experiment 3. The two groups that were deprived of food 12 hours each day lost weight during the first week. Upper graph: glucose intake per day. All three groups with access to glucose, regardless of the schedule, increased their intake over the course of the first week.

difference between the cyclic-glucose/chow group given naloxone and the similar group given saline did not reach significance (black vs. white bar in Figure 2, lower left).

Experiment 2C: Teeth Chattering

While the above rats were in the plus maze, the cyclic-glucose/chow rats given naloxone exhibited significantly more bouts of audible teeth chattering than the other three groups ($F_{(3,37)} = 3.95, p < 0.05$) and more teeth chattering than those given saline (black vs. white bar in Figure 2, upper left; $t_{(10)} = 2.49, p < 0.05$).

Experiment 3: Sugar Intake, Body Weight, and Anxiety, Replication

This experiment replicated the plus-maze test, but without the preliminary procedure for inducing ultrasonic calls. In this case, rats ($n = 12$ /group) were housed singly on one of five different feeding regimens for 1 month. Figure 3 (upper graph) shows that the mean glucose intake increased gradually for all groups that had glucose and reached an asymptote after 8 to 10 days as reported earlier for females (20). Body weight decreased during the first 8 days for the

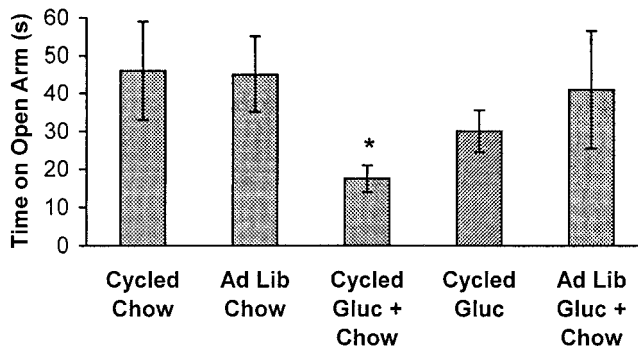


Figure 4: Time spent on the open arm of the maze. The cyclic-glucose/chow group spent the least amount of time venturing onto the exposed arm of the elevated maze (* $p < 0.05$).

two groups on the 12-hour cyclic schedules and then weight resumed its normal rate of gain (Figure 3, lower graph).

After 1 month, rats in all groups received 3 mg/kg naloxone SC at the time of day when the intermittent-fed rats had been deprived for the usual 12 hours. As shown in Figure 4, the cyclic-glucose group spent significantly less time on the open arm of the elevated plus maze than did the ad libitum chow group ($F_{(1,49)} = 2.19, p < 0.05$).

Experiment 4: Accumbens DA/ACh Imbalance

Microdialysis showed that naloxone injection in cyclic-glucose/chow rats caused marked changes in the balance of DA and ACh in the NAc (Figure 5). Naloxone at two doses (10 and 20 mg/kg; $n = 8$ and 6/group, respectively) caused extracellular ACh to increase significantly to 118% and 134% of baseline, respectively ($F_{(18,126)} = 4.388; p < 0.001$). At the same time, DA decreased significantly to 80% and 73% of baseline ($F_{(18,126)} = 3.738; p < 0.001$). The effect of naloxone on ACh was dose related. As seen in Figure 4, there was no effect of naloxone in rats fed ordinary chow.

Experiment 5: Accumbens DA/ACh Imbalance, Replication

The microdialysis study was repeated in new groups of rats with 3 mg/kg of naloxone. Male rats were maintained on the 12-hour experimental feeding schedule for 21 days, using 10% sucrose instead of 25% glucose. Daily intake of sucrose nearly tripled, from 37 mL on day 1 to 112 mL on day 21. When given naloxone, extracellular DA decreased to 82% of baseline ($F_{(1,56)} = 7.35, p < 0.01$) and ACh increased to 157% of baseline ($F_{(1,70)} = 16.23, p < 0.01$) in the cyclic-sucrose/chow rats, but not in the ad libitum chow group (Figure 6).

Histology showed that the microdialysis probes were primarily in the NAc shell (Figure 7).

Discussion

In brief, the results demonstrate that daily exposure to food deprivation followed by excessive glucose intake can

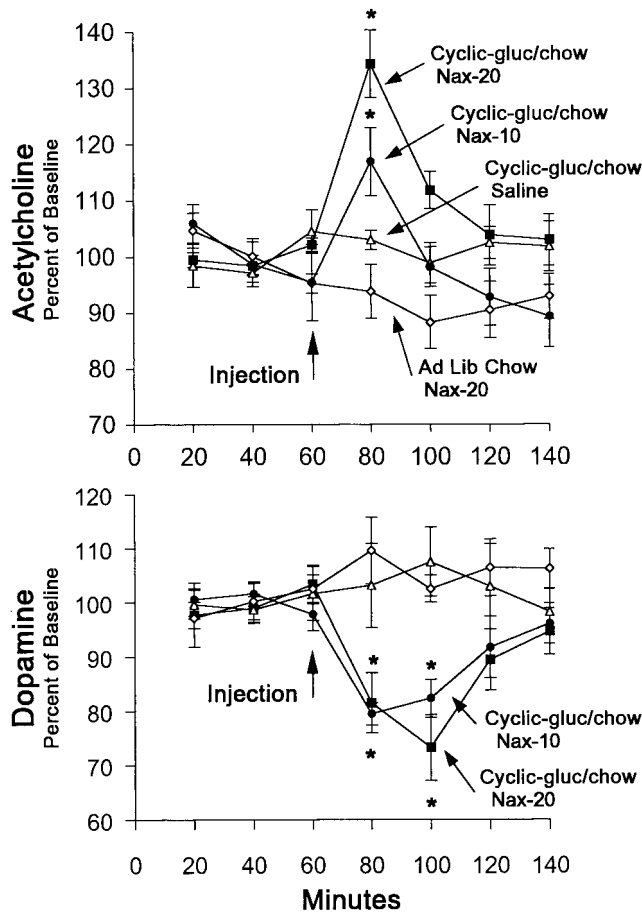


Figure 5: Microdialysis measurements of dopamine/ acetylcholine (DA/ACh) balance in the NAc before and after naloxone injection in Experiment 4. Extracellular DA decreased (bottom graph) and ACh increased (top graph) after naloxone injection in rats with a history of daily deprivation and refeeding glucose and chow. The arrow indicates the time of naloxone injection [20 mg/kg intraperitoneally (IP), ■ and 10 mg/kg IP, ●]. No effect was seen in rats that underwent this same dietary treatment followed by saline injection (△) or in a control group with free access to chow followed by naloxone injection (20 mg/kg IP, ◇; **p* < 0.05 compared with baseline).

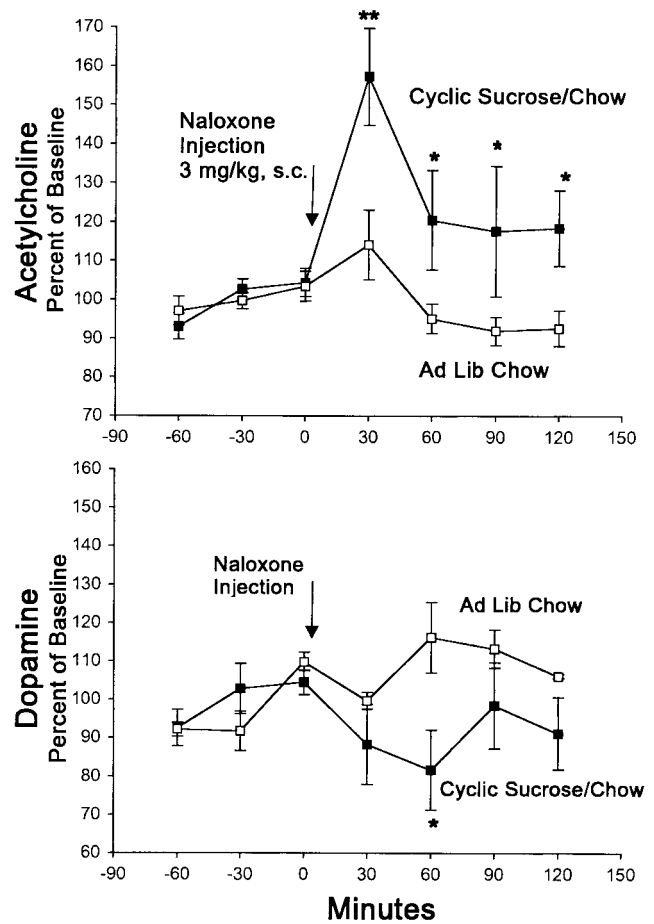


Figure 6: Replication of dopamine/ acetylcholine (DA/ACh) measurements in Experiment 5. Rats with a history of cyclic glucose/ chow and given naloxone (3 mg/kg subcutaneously) show the same DA/ACh withdrawal effect. DA release decreases and ACh increases (**p* < 0.05, ***p* < 0.01 compared with baseline).

alter the central nervous system. Spontaneous withdrawal caused by a 24-hour fast led to minor withdrawal symptoms in the form of teeth chatter, tremor, and shakes. When withdrawal was precipitated with naloxone, signs of anxiety were also observed, and there was a reversal of DA/ACh balance in the NAc that was similar to morphine withdrawal.

The doses of naloxone used in this study will precipitate withdrawal in morphine-treated rats (28), although much lower doses are also effective with morphine (37,38). The effective doses in the cyclic-glucose/chow rats were 10 mg/kg IP and 3 mg/kg SC. Classic, somatic signs of opiate withdrawal include teeth chattering, piloerection, diarrhea,

head shaking, and wet-dog shakes. Some of these may require a drug such as morphine that acts on opiate receptors in the intestines and elsewhere throughout the body. Other symptoms are generated in the hindbrain centers for autonomic control (39). However, in terms of a psychological addiction, the most relevant withdrawal symptoms would be signs such as anxiety, aversion, depression, or dysphoria as predicted by the opponent process theory (40,41). Anxiety is a sign of withdrawal from most drugs of abuse (21) and may be reflected in the 20-kHz ultrasonic call of rats (22) and in their avoidance of dangerous locations in an elevated plus maze (23). Both somatic signs and the anxiety were observed during withdrawal in this study after exposure to the deprivation/refeeding regimen.

Further research will be needed to establish the most potent diet and eating schedule for producing these dependency effects. As shown in Figure 3 for males and in our previous article for females (20), rats will gradually escalate

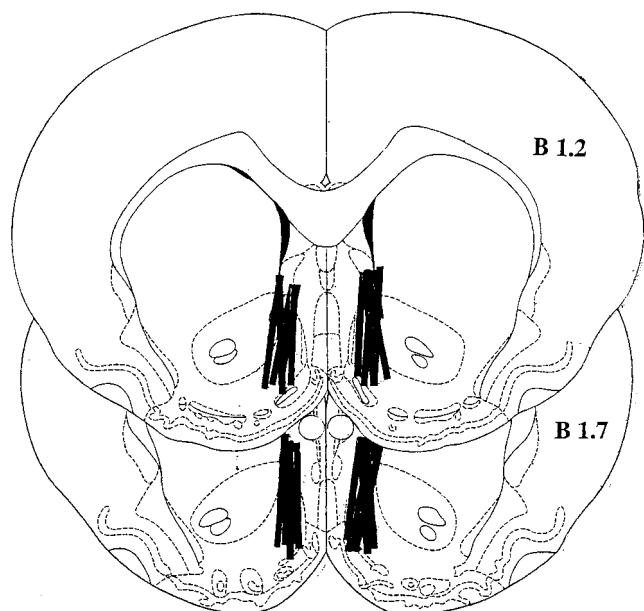


Figure 7: Histology: microdialysis probes (2 mm long and 0.02 mm in diameter) were located at stereotaxic planes B 1.2 and B 1.7 in Experiment 5. Probes were bilaterally placed as shown, with one-half from each side used to collect dopamine and acetylcholine samples. Probe tips are primarily in the nucleus accumbens shell.

their intake of glucose regardless whether it is given ad libitum or for 12 h/d. However, there seems to be more to the phenomenon than just consuming large amounts of sugar. Intermittency may be an important factor. Food deprivation can release opioids (42), and deprivation promotes eating in a large binge when the food becomes available (20,30). This was evident in these data (Table 1), showing that males on the intermittent cycle consumed 16 mL of 25% glucose in the first hour of access.

Underlying withdrawal, there must be neurochemical changes in the brain's emotion or motivation systems. We reported that rats bingeing on sugar have up-regulation of both D-1 and μ receptors in the accumbens shell. This is one of several forebrain sites in which naloxone will precipitate signs of withdrawal (28,43,44). This study shows that during naloxone-precipitated withdrawal, sugar-treated rats display a rise in extracellular ACh, whereas DA decreases. This DA/ACh imbalance is characteristic of intermittent morphine followed by naloxone (28) and was also found with nicotine followed by a nicotine receptor antagonist mecamylamine (45). This suggests that withdrawal from sugar shares features with withdrawal from morphine and nicotine, and in this respect, they all have a common basis. Dependence on naturally activated endogenous opioids is a clear possibility based on these results and earlier evidence (29,46).

Palatable foods and addictive drugs bear many interesting similarities. For example, food restriction enhances the re-

inforcing effect of both food and drugs, such as cocaine, alcohol, or opiates (47–49). Animals may take less of these drugs if there is saccharin to drink (50). A sweet flavor can suppress pain (51,52), or it can potentiate opiate-induced analgesia (53), and opiate analgesics can potentiate ingestion of sweet flavors (54). Logically, a drug such as naloxone, which blocks brain opioid receptors, will reduce sugar intake (51,54–56). The importance of endogenous opioids for palatability is shown by the finding that naloxone can reduce intake of a preferred food without affecting intake of ordinary chow (57–60).

The behavioral paradigm used in this study shares some aspects with a pattern of ingestive behavior self-imposed by people diagnosed with binge-eating disorder or bulimia nervosa. Bulimics often restrict intake early in the day and then binge later in the afternoon or evening. Having achieved the effects of this behavior, some bulimics then purge the food, and others do not. The rats in this model were deprived of food during their period of sleep and the early hours normally devoted to the most vigorous caloric intake (onset of darkness). After this delay, they were allowed access to highly palatable food. Based on the previous article (20) showing up-regulation of accumbens dopamine D-1 and μ opioid receptors, it is probable that the rats were sensitized to food stimuli that act via these receptors. Such neurochemical changes, particularly in the NAc, could reflect underlying molecular changes, which have been proposed to cause addiction (16). These same processes could underlie the increased frequency of comorbid drug abuse and food abuse observed in bulimic individuals (61,62).

Marrazzi and Luby (42) put forward a hypothesis of “auto-addiction” in anorexia in which endogenous opioid peptides mediate an addiction to starvation. In contrast, these results suggest that intense opioid and DA activation during intermittent overingestion of palatable food sensitizes reinforcement systems on a background of lowered DA activity. This may lead to dependency on the palatable food to release opioids and DA. These theories are not mutually exclusive. It is likely that food deprivation activates opioid systems in the hindbrain parabrachial region (63), and palatable food activates μ -opioid systems in the nucleus tractus solitarius (63), midbrain VTA (64), amygdala (65), and NAc (5), with any or all of these sites contributing to food dependency. The hypothalamus is also involved in opioid control of food intake (66–68), so there is no reason to believe one brain region accounts for all aspects of addictive eating disorders, and one cannot assume that all eating disorders involve aspects of addiction.

Everyone depends on food. Under normal circumstances, this may not involve the severe conditions of alternating deprivation and feasting that contribute to an abnormal state. Steady access to a palatable food results in diminished DA release in the NAc (69), which would lessen the chances of abnormal dependency. In contrast, the neuro-

chemical adaptations observed in the present model, when fasting and bingeing alternate, may overcome this normal habituation process, sensitizing the individual to the DA- and opioid-activating properties of palatable foods. This could be followed by the withdrawal symptoms, which promote self-medication by bingeing, and hence, rendering the behavior an eating disorder.

In conclusion, behavioral and neurochemical signs of opioid withdrawal in rats consuming sugar suggest the emergence of substance dependence. This rat model seems to apply to some aspects of human eating disorders. It also has relevance to the survival value of opioid-based motivation and reinforcement that is activated by highly caloric food encoded as being palatable in an environment where food is scarce (70). Some of the neurochemical adaptations that accompany this eating pattern (20) seem to parallel changes observed in stimulant sensitization (71,72) and may be causally linked to the behavioral changes observed during withdrawal. In summary, an opioid-mediated dependence on sugar has been demonstrated at both the behavioral and neurochemical level. The withdrawal signs observed in this study suggest that dependence on endogenous opioids can develop during the ingestion of very palatable food on some eating schedules.

Acknowledgments

The research was supported by USPHS Grant NS-30697 followed by DA-10608. We thank M. Rada for her contribution to this research.

References

1. **Hernandez L, Hoebel BG.** Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci.* 1988;42:705–12.
2. **Radhakishun FS, Van Ree JM, Westerink BHC.** Scheduled eating increases dopamine release in the nucleus accumbens of food-deprived rats as assessed with on-line brain dialysis. *Neurosci Lett.* 1988;85:351–6.
3. **Salamone JD, Cousins MS, McCullough LD, Carriero DL, Berkowitz RJ.** Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. *Pharmacol Biochem Behav.* 1994;49:25–31.
4. **Tanda G, Di Chiara G.** A dopamine-mu1 opioid link in the rat ventral tegmentum shared by palatable food (Fonzies) and non-psychostimulant drugs of abuse. *Eur J Neurosci.* 1998;10:1179–87.
5. **Kelley AE, Bakshi VP, Fleming S, Holahan MR.** A pharmacological analysis of the substrates underlying conditioned feeding induced by repeated opioid stimulation of the nucleus accumbens. *Neuropsychopharmacology.* 2000;23:455–67.
6. **Pal GK, Thombre DP.** Modulation of feeding and drinking by dopamine in caudate and accumbens nuclei in rats. *Ind J Exp Biol.* 1993;31:750–4.
7. **Ragnauth A, Moroz M, Bodnar RJ.** Multiple opioid receptors mediate feeding elicited by μ - and Δ -opioid receptor subtype agonists in the nucleus accumbens shell in rats. *Brain Res.* 2000;876:76–87.
8. **Guerin B, Goeders NE, Dworkin SI, Smith JE.** Intracranial self-administration of dopamine into the nucleus accumbens. *Soc Neurosci Abstr.* 1984;10:1072.
9. **Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L.** Self-injection of amphetamine directly into the brain. *Psychopharmacology.* 1983;81:158–63.
10. **McBride WJ, Murphy JM, Ikemoto S.** Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behav Brain Res.* 1999;101:129–52.
11. **Olds ME.** Reinforcing effects of morphine in the nucleus accumbens. *Brain Res.* 1982;237:429–40.
12. **Wise RA.** Opiate reward: sites and substrates. *Neurosci Biobehav Rev.* 1989;13:129–33.
13. **Di Chiara G, Tanda G, Bassareo V, et al.** Drug addiction as a disorder of associative learning: role of nucleus accumbens shell/extended amygdala dopamine. In: McGinty JF, ed. *Advancing from the Ventral Striatum to the Extended Amygdala.* *Ann N Y Acad Sci.* 1999;877:461–85.
14. **Hoebel BG.** Brain neurotransmitters in food and drug reward. *Am J Clin Nutr.* 1985;42:1133–50.
15. **Wise RA.** Common neural basis of brain stimulation reward, drug reward, and food reward. In: Hoebel BG, Novin D, eds. *The Neural Basis of Feeding and Reward.* Brunswick, ME: Haer Institute; 1982, pp. 445–54.
16. **Nestler EJ, Aghajanian GK.** Molecular and cellular basis of addiction. *Science.* 1997;278:58–63.
17. **Hagan MM, Moss DE.** An animal model of bulimia nervosa: opioid sensitivity to fasting episodes. *Pharmacol Biochem Behav.* 1991;39:421–2.
18. **Specker SM, Lac ST, Carroll ME.** Food deprivation history and cocaine self-administration: an animal model of binge eating. *Pharmacol Biochem Behav.* 1994;48:1025–9.
19. **Colantuoni C, Schwenker J, Landenheim B, et al.** Circadian cycle of dietary restriction and overeating induces dopamine receptor and transporter changes. *Soc Neurosci Abstr.* 1998;24:193.
20. **Colantuoni C, Schwenker J, McCarthy J, et al.** Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *NeuroReport.* 2001;12:3549–52.
21. **Dimitriou SG, Rice HB, Corwin RL.** Effects of limited access to a fat option on food intake and body composition in female rats. *Int J Eat Disord.* 2000;28:436–45.
22. **Koob GF.** Drug reward and addiction. In: Zigmond MJ, Bloom FE, Landis SC, Roberts JL, Squire LR, eds. *Fundamental Neuroscience.* San Diego, CA: Academic Press; 1999, pp. 1245–79.
23. **Mutschler NH, Miczek KA.** Withdrawal from a self-administered or non-contingent cocaine binge: differences in ultrasonic distress vocalizations in rats. *Psychopharmacology.* 1998;136:402–8.
24. **File SE, Andrews N, Al-Farhan M.** Anxiogenic responses of rats on withdrawal from chronic ethanol treatment: effects of tianeptine. *Alcohol.* 1993;28:281–6.

25. **Le Magnen J.** A role for opiates in food reward and food addiction. In: Capaldi ED, Powley TL, eds. *Taste, Experience, and Feeding*. Washington, DC: American Psychological Association; 1990, pp. 241–54.
26. **Pothos E, Rada P, Mark GP, Hoebel BG.** Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. *Brain Res.* 1991;566:348–50.
27. **Rada P, Mark GP, Pothos E, Hoebel BG.** Systemic morphine simultaneously decreases extracellular acetylcholine and increases dopamine in the nucleus accumbens of freely moving rats. *Neuropharmacology.* 1991;30:1133–6.
28. **Rada P, Pothos E, Mark GP, Hoebel BG.** Microdialysis evidence that acetylcholine in the nucleus accumbens is involved in morphine withdrawal and its treatment with clonidine. *Brain Res.* 1991;561:354–6.
29. **Fiserova M, Consolo S, Krasiak M.** Chronic morphine induces long-lasting changes in acetylcholine release in the nucleus accumbens core and shell: an in vivo microdialysis study. *Psychopharmacology.* 1999;142:85–94.
30. **Rada PV, Mark GP, Taylor KM, Hoebel BG.** Morphine and naloxone, i.p. or locally, affect extracellular acetylcholine in the accumbens and prefrontal cortex. *Pharmacol Biochem Behav.* 1996;53:809–16.
31. **Colantuoni C, McCarthy J, Gibbs G, Searls E, Alisharan S, Hoebel BG.** Repeatedly restricted food access combined with highly palatable diet leads to opiate-like withdrawal symptoms during food deprivation in rats. *Soc Neurosci Abstr.* 1997;23:517.
32. **Hoebel BG, Colantuoni C, McCarthy J, et al.** Sugar addiction: neural and behavioral symptoms of sensitization and withdrawal. *Obes Res.* 1999;7:41S.
33. **Hoebel B G, Colantuoni C, McCarthy J, et al.** Evidence for sugar addiction in rats. *Appetite.* 2000;35:292.
34. **Hoebel BG, Chau D, Kosloff RA, Taylor JL, Rada P.** Possible role of accumbens dopamine and acetylcholine in sugar withdrawal and behavioral depression. *Appetite.* 2001;37:142.
35. **Hoebel BG, Rada PV, Mark GP, Pothos EN.** Neural systems for reinforcement and inhibition of behavior: relevance to eating, addiction and depression. In: Kahneman D, Diener E, Schwarz N, eds. *Well Being: The Foundations of Hedonic Psychology*. New York, NY: Russel Sage Foundation; 1999, pp. 560–74.
36. **Hoebel BG, Patten CS, Colantuoni C, Rada, PV.** Sugar withdrawal causes symptoms of anxiety and acetylcholine release in the nucleus accumbens. *Soc Neurosci Abstr.* 2000; 501.12:257.
37. **Schulteis G, Markou A, Gold LH, Stinus L, Koob GF.** Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. *J Pharmacol Exp Ther.* 1994;271:1391–8.
38. **Bodnar RJ.** Opioid receptor subtype antagonists and ingestion. In: Cooper SJ, Clifton PG, eds. *Drug Receptor Subtypes and Ingestive Behaviour*. San Diego, CA: Academic Press; 1996, pp. 127–66.
39. **Maldonado R, Stinus L, Gold LH, Koob GF.** Role of different brain structures in the expression of the physical morphine withdrawal syndrome. *J Pharmacol Exp Ther.* 1992; 261:669–77.
40. **Koob GF, Stinus L, LeMoal M, Bloom FE.** Opponent process theory of motivation: neurobiological evidence from studies of opiate dependence. *Neurosci Biobehav Rev.* 1989; 13:135–40.
41. **Markou A, Kosten TR, Koob GF.** Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology.* 1998;18:135–74.
42. **Marrazzi MA, Luby ED.** The neurobiology of anorexia nervosa: an auto-addiction? In: Cohen M, Foa P, eds. *The Brain as an Endocrine Organ*. New York, NY: Springer-Verlag; 1990, pp. 46–95.
43. **Aston-Jones G, Hirata H, Akaoka H.** Local opiate withdrawal in locus coeruleus in vivo. *Brain Res.* 1997;765:331–6.
44. **Stinus L, Le Moal M, Koob GF.** Nucleus accumbens and amygdala are possible substrates for the aversive stimulus effects of opiate withdrawal. *Neuroscience.* 1990;37:767–73.
45. **Rada P, Jensen K, Hoebel BG.** Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens. *Psychopharmacology.* 2001;157:105–10.
46. **Mercer ME, Holder MD.** Food cravings, endogenous opioid peptides, and food intake: a review. *Appetite.* 1997;29:325–52.
47. **Carr KD, Kim GY, Cabeza de Vaca S.** Chronic food restriction in rats augments the central rewarding effect of cocaine and the $\Delta 1$ opioid agonist, DPDPE, but not the $\Delta 2$ agonist, deltorphin-II. *Psychopharmacology (Berlin).* 2000; 152:200–7.
48. **Carroll ME, France CP, Meisch RA.** Food deprivation increases oral and intravenous drug intake in rats. *Science.* 1979;205:319–21.
49. **Wolinsky TD, Carr KD.** Effects of chronic food restriction on μ and κ binding in rat forebrain: a quantitative autoradiographic study. *Brain Res.* 1994;656:274–80.
50. **Campbell UC, Carroll ME.** Reduction of drug self-administration by an alternative non-drug reinforcer in rhesus monkeys: magnitude and temporal effects. *Psychopharmacology (Berlin).* 2000;147:418–25.
51. **Le Magnen J.** Palatability: concept, terminology, and mechanisms. In: Boake RA, Popplewell DA, Burton MJ, eds. *Eating Habits: Food, Physiology and Learned Behaviour*. New York, NY: John Wiley & Sons; 1987, pp. 131–54.
52. **Blass EM, Hoffmeyer LB.** Sucrose as an analgesic for newborn infants. *Pediatrics.* 1991;87:215–8.
53. **Kanarek RB, White ES, Biegen MT, Marks-Kaufman R.** Dietary influences on morphine-induced analgesia in rats. *Pharmacol Biochem Behav.* 1991;38:681–4.
54. **Levine AS, Billington CJ.** Opioids: are they regulators of feeding? *Ann N Y Acad Sci.* 1989;575:209–20.
55. **Bellinger LL, Bernardis LL, Williams FE.** Naloxone suppression of food and water intake and cholecystokinin reduction of feeding is attenuated in weanling rats with dorsomedial hypothalamic lesions. *Physiol Behav.* 1983;31: 839–46.
56. **Drewnowski A, Krahn DD, Demitrack MA, Nairn K, Gosnell BA.** Naloxone, an opiate blocker, reduces the consumption of sweet high-fat foods in obese and lean female binge eaters. *Am J Clin Nutr.* 1995;61:1206–12.

57. **Levine AS, Weldon DT, Grace M, Cleary JP, Billington CJ.** Naloxone blocks that portion of feeding driven by sweet taste in food-restricted rats. *Am J Physiol.* 1995;268:R248–R52.
58. **Sclafani A, Aravich PF, Xenakis S.** Dopaminergic and endorphinergic mediation of a sweet reward. In: Hoebel BG, Novin D, eds. *The Neural Basis of Feeding and Reward.* Brunswick, ME: Haer Institute; 1982, pp. 507–15.
59. **Siviy SM, Calcagnetti DJ, Reid LD.** Opioids and Palatability. In: Hoebel BG, Novin D, eds. *The Neural Basis of Feeding and Reward.* Brunswick, ME: Haer Institute; 1982, pp. 517–24.
60. **Glass MJ, Grace M, Cleary JP, Billington CJ, Levine AS.** Potency of naloxone's anorectic effect in rats is dependent on diet preference. *Am J Physiol.* 1996;271:R271.
61. **Brewerton TD, Lydiard RB, Herzog DB, Brothman AW, O'Neil PM, Ballenger JC.** Comorbidity of axis I psychiatric disorders in bulimia nervosa. *J Clin Psychiatry.* 1995;56:77.
62. **Mitchell JE, Specker SM, De Zwaan M.** Comorbidity and medical complications of bulimia nervosa. *J Clin Psychiatry.* 1991;52:S13–S20.
63. **Carr KD, Papadouka V.** The role of multiple opioid receptors in the potentiation of reward by food restriction. *Brain Res.* 1994;639:253–60.
64. **Levine AS, Grace M, Billington CJ.** The effect of centrally administered naloxone on deprivation and drug-induced feeding. *Pharmacol Biochem Behav.* 1990;36:409–12.
65. **Glass MJ, Billington CJ, Levine AS.** Opioids, food reward, and macronutrient selection. In: Berthoud HR, Seeley RJ, eds. *Neural and Metabolic Control of Macronutrient Intake.* New York, NY: CRC Press; 2000, pp. 407–24.
66. **Grandison L, Guidotti A.** Stimulation of food intake by muscimol and beta-endorphin. *Neuropharmacology.* 1977;16:533.
67. **Leibowitz SF, Hor L.** Endorphinergic and α -noradrenergic systems in the paraventricular nucleus: effects on eating behavior. *Peptides.* 1982;3:421–8.
68. **McLean S, Hoebel BG.** Opiate and norepinephrine-induced feeding from the paraventricular nucleus of the hypothalamus are dissociable. *Life Sci.* 1982;31:2379–82.
69. **Di Chiara G, Tanda G.** Blunting of reactivity of dopamine transmission to palatable food: a biochemical marker of anhedonia in the CMS model? *Psychopharmacology (Berlin).* 1997;134:351–3,371–7.
70. **Scott TR, Mark GP.** The taste system encodes stimulus toxicity. *Brain Res.* 1987;414:197–203.
71. **Unterwald EM, Fillmore J, Kreek MJ.** Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction. *Eur J Pharmacol.* 1996;318:31–5.
72. **Vaderschuren LJ, Kalivas PW.** Alterations in dopaminergic and glutaminergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berlin).* 2000;151:99–120.