Published in final edited form as:

Physiol Behav. 2008 June 9; 94(3): 309–315. doi:10.1016/j.physbeh.2008.01.008.

After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance

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Abstract

Bingeing on sugar may activate neural pathways in a manner similar to taking drugs of abuse, resulting in related signs of dependence. The present experiments test whether rats that have been bingeing on sucrose and then fasted demonstrate signs of opiate-like withdrawal. Rats were maintained on 12-h deprivation followed by 12-h access to a 10% sucrose solution and chow for 28 days, then fasted for 36 h. These animals spent less time on the exposed arm of an elevated plus-maze compared with a similarly deprived ad libitum chow group, suggesting anxiety. Microdialysis revealed a concomitant increase in extracellular acetylcholine and decrease in dopamine release in the nucleus accumbens shell. These results did not appear to be due to hypoglycemia. The findings suggest that a diet of bingeing on sucrose and chow followed by fasting creates a state that involves anxiety and altered accumbens dopamine and acetylcholine balance. This is similar to the effects of naloxone, suggesting opiate-like withdrawal. This may be a factor in some eating disorders.

Keywords

Bingeing; Dopamine; Acetylcholine; Microdialysis; Nucleus accumbens; Binge eating

Withdrawal is a factor in the etiology of drug addiction [1]. Le Magnen [2] found that the opioid antagonist naloxone produced opiate-like withdrawal signs in rats fed a palatable cafeteria-style diet. Similarly, rats maintained on a diet to induce daily sugar bingeing also show signs of opiate-like withdrawal in response to naloxone [3]. These rats show somatic signs of withdrawal, anxiety on the elevated plus-maze, and decreased extracellular dopamine (DA) with increased acetylcholine (ACh) in the nucleus accumbens (NAc). While the use of an opioid antagonist is important for understanding the underlying neural mechanisms of a behavior, it is unlike the natural situation. Abstinence or spontaneously emergent withdrawal is more realistic and reflective of animals in the wild or the human condition during famine or severe dieting.

Mere abstinence from a drug of abuse is sufficient to elicit behavioral and biochemical signs of withdrawal. Rats abstaining from morphine display withdrawal signs such as tremors and wet-dog shakes [4,5]. These behaviors are coupled with changes in the DA system, including a decrease in striatal D₁ and D₂ receptor mRNA [6], decreased extracellular DA in the NAc [7,8] and an increase in accumbens ACh [9].

Similarly, deprivation from palatable foods can result in behavioral signs of opiate-like withdrawal. Rats previously maintained on a diet with intermittent access to sugar show behaviors indicative of a withdrawal state when the food and/or sugar are removed for 24 or 36 h [3,10]. Furthermore, food deprivation has been shown to enhance drug reinforced behavior, suggesting a link between food abstinence and addictive behaviors [11,12].

It is unknown whether fasting after excessive sugar intake can alter extracellular levels of DA and ACh in the NAc. In the present experiment, these neurochemicals were monitored during fasting from sugar and chow on the theory that the lack of natural opioid stimulation would cause a disruption similar to the effects of naloxone-precipitated withdrawal, specifically a decrease in DA and increase in ACh release in the NAc shell. To further complement the findings of somatic signs of opiate-like withdrawal in our previous report [3], anxiety on the elevated plus-maze and blood glucose levels were measured during fasting following sugar bingeing.

1. Materials and methods

1.1. General methods

Male Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY) or bred at the Princeton University vivarium from a stock originating from Taconic Farms. Rats were housed individually on a reversed 12-h light: 12-h dark cycle. All procedures were approved by the Princeton University Institutional Animal Care and Use Committee.

1.2. Experiment 1: Is anxiety evident while fasting in sugar-bingeing rats?

Rats (300–450 g) in the main experimental group (intermittent sugar + chow; $n = 9$) were maintained on a diet of 12-h deprivation followed by 12-h access to a 10% (w/v) sucrose solution plus standard rodent chow (LabDiet #5001, PMI, St. Louis, MO, 3.02 kcal/g) starting 4 h into the dark phase each day for 28 days [13]. A control group (ad libitum chow; $n = 7$) was allowed ad libitum access to standard rodent chow. All animals had water available ad libitum. Other groups (intermittent chow and ad libitum sugar) used in Experiments 2 and 3 were not tested for anxiety because they failed to show behavioral signs of withdrawal following naloxone or fasting in a previous report [3].

On Day 28, after the usual 12-h deprivation, the rats in the experimental group were denied access to sugar and chow for an additional 24 h. The control group was also deprived for chow for 36 h. During this time, the animals continued to have ad libitum access to water. Then the animals were individually placed in the elevated plus-maze for 5 min using the technique of File, Lippa, Beer, and Lippa [14]. The apparatus had four arms, each 10 cm wide by 50 cm long, and was elevated 60 cm above the floor. Two opposite arms were enclosed with high opaque walls. The other two arms had no protective walls. The

experiment was conducted under red-light. The rats were placed in the center of the maze and alternated facing an open or closed arm. Each plus-maze trial was video taped and scored for the amount of time spent with head and forepaws on the open arm, closed arm or middle section of the maze by an observer blind to the treatment condition.

1.3. Experiment 2: Do sugar-bingeing rats have altered DA and ACh release in the accumbens while fasting?

A separate group of rats (350–450 g) underwent surgery to implant guide cannulas for microdialysis. Rats were anesthetized with 20 mg/kg xylazine and 100 mg/kg ketamine (i.p.) supplemented with ketamine as needed (100 mg/kg, i.p.). Bilateral 21 gauge stainless-steel guide cannulas were aimed at the posterior medial accumbens shell (anterior: + 1.2 mm, lateral: 0.8 mm and ventral: 4.0 mm, with reference to bregma, midsagittal sinus, and surface of the level skull, respectively) using a stereotaxic instrument.

Rats were allowed to recover from surgery for at least 1 week. Similar to the procedures in Experiment 1, an experimental group ($n = 6$) was maintained on daily 12-h deprivation followed by 12-h access to 10% sucrose and standard rodent chow, starting 4 h into the dark phase, for 28 days to induce bingeing (i.e., intermittent sugar + chow). One control group was maintained on the same schedule with no sucrose (intermittent chow, $n = 7$), while another group was maintained on daily ad libitum chow ($n = 6$). On Day 28, each rat was moved into the microdialysis chamber and a probe was inserted and fixed in place with acrylic cement 14–16 h before the experiment to allow neurotransmitter recovery to stabilize. Microdialysis probes were constructed of silica glass tubing (37 μm inner diameter, Polymicro Technologies Inc., Phoenix, AZ) inside a 26 gauge stainless steel tube with a microdialysis tip of cellulose tubing sealed at the end with epoxy (Spectrum Medical Co., Los Angeles, CA, 6000 MW, 0.2 mm outer diameter $\times$ 2.0 mm long) [15]. The probes protruded 5 mm from the guide cannula to reach the intended site in the accumbens shell. Probes were perfused with buffered Ringer's solution (142 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl_2 , 1.0 mM MgCl_2 , 1.35 mM Na_2HPO_4 , 0.3 mM NaH_2PO_4 , pH7.35) at a flow rate of 0.5 $\mu\text{L}/\text{min}$ for the stabilization period and at 1.3 $\mu\text{L}/\text{min}$ 2 h before and throughout the experiment. Neostigmine (0.3 μM) was added to the Ringer's solution to improve basal recovery of ACh by hindering its enzymatic degradation.

When the final 12-h sucrose access period was over on Day 28, chow, sucrose and water were removed from all of the rats. Water was removed for the 36 h of dialysis experiment because drinking water can alter baseline levels of DA and ACh [16], which would confound the results. Microdialysis samples were collected for 1 h (3 $\times$ 20-min samples) after 12, 24 and 36 h of fasting (no food, sugar or water available). Each sample was split, half for DA analysis and half for ACh.

1.4. Dopamine and acetylcholine assays

DA and its metabolites 3,4-dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA) were analyzed by reverse phase, high performance liquid chromatography with electrochemical detection (HPLC-EC). Samples were injected into a 20- μL sample loop leading to a 10-cm column with 3.2 mm-bore and 3 μm , C18 packing (Brownlee Co. Model

6213, San Jose, CA). The mobile phase contained 60 mM sodium phosphate, 100 μ M EDTA, 1.24 mM heptanosulfonic acid, and 5% vol/vol methanol. DA, DOPAC and HVA were measured with a coulometric detector (ESA Co. Model 5100A, Chelmsford, MA) with the conditioning potential set at +500 mV, and working cell potential at -400 mV.

ACh was measured by reverse phase HPLC-EC 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, St Louis, MO and column from Chrompack Inc., Palo Alto, CA). The mobile phase was 200 mM potassium phosphate at pH 8.0. An amperometric detector was used (EG&G Princeton Applied Research, Lawrenceville, NJ). The hydrogen peroxide was oxidized on a platinum electrode (BAS, West Lafayette, IN) set at 500 mV with respect to an Ag-AgCl reference electrode (EG&G Princeton Applied Research).

Three, 20-min samples were collected at 12, 24 and 36 h of fasting. For each hour, data for the three samples were averaged. Data for DA and ACh were converted to percent of the 12-h deprivation time point for each group, when the intermittent-fed rats would normally expect food.

1.5. Histology

At the end of the experiment histology was performed to verify microdialysis probe placement. Rats received an overdose of sodium pentobarbital and when deeply anesthetized were intracardially perfused with 0.9% saline followed by 10% formaldehyde. The brains were removed, frozen, and the experimenter inspected the sections as they were cut (40 μ m slices, starting anterior to the accumbens) until the sites of probe tips were located. Once probe tracks were visualized, they were plotted using the atlas of Paxinos and Watson [17].

1.6. Experiment 3: Are there changes in blood glucose levels due to chronic bingeing on sucrose?

Rats (300–350 g) in three groups were maintained for 28 days on (a) intermittent sugar + chow (12-h deprivation followed by 12-h access to a 10% sucrose solution and chow, starting 4 h into the dark phase; $n = 10$), (b) intermittent chow (12-h deprivation followed by 12-h access to standard rodent chow (no sucrose), starting 4 h into the dark phase; $n = 10$), or (c) ad libitum chow ($n = 9$). Chow and sugar were removed and tail-blood samples were collected following 12, 24 and 36 h of deprivation. Blood was collected from the tip of the tail by one experimenter gently holding the animal while another made a small incision about 5 mm from the tip of the tail with a sterile scalpel. Blood was collected in a capillary tube, centrifuged and serum was then analyzed for glucose levels with an Analox GM7 Fast Enzymatic Metabolizer (Analox, Lunenburg, MA). During the 28-day access period, sugar and chow intakes were measured daily, and body weights were measured weekly. Body weights were also measured at each time point during deprivation.

1.7. Statistics

Plus-maze data were analyzed with a one-tailed, unpaired Student's t -test. Cohen's d , which measures effect size [18], and p_{rep} , which provides the probability of replication [19], were

also calculated. Data for DA and ACh were analyzed as percent difference from normalized baseline as described above, using a two-way repeated measures ANOVA followed by post hoc Tukey tests. Blood glucose levels, body weight and intake data were analyzed by two-way repeated measures ANOVA.

2. Results

2.1. Body weight, sugar intake and chow intake

Data collected during the 28-day access period in Experiment 3 revealed that rats with binge-access to sucrose escalated their intake of sucrose over the 28-day exposure period ($F(27, 279) = 4.9, p < 0.001$; Fig. 1A), a finding similar to what has been shown in our previous reports with sucrose or glucose [3,20]. Chow intake data showed a significant difference among groups. The rats with intermittent sugar access ate less chow than the ad libitum and intermittent chow groups ($F(2,26) = 60.8, p < 0.001$; Fig. 1B). However, there was no difference among groups in total daily caloric intake (Fig. 1C).

There were no differences in body weight among groups during the 28-day access period; however, there was an effect of time, with all three groups gaining weight over the 28 days ($F(4,104) = 298.9, p < 0.001$). During the 36 h of deprivation, body weight decreased over time for all groups ($F(2,52) = 1957.8, p < 0.001$), with no difference among groups at any time point (12, 24 or 36 h).

2.2. Experiment 1: Behavioral indices of anxiety after fasting in sugar-bingeing rats

When placed in the elevated plus-maze for 5 min, after 36 h of food deprivation the rats that had previously been maintained on intermittent sugar + chow spent less time (18 ± 4 s, 6% of total time) on the open arm of the plus-maze compared with the equally deprived ad libitum-chow group that had no sucrose experience (34 ± 8 s, 11% of total time; $t(16) = 2.01, p < 0.05, d = 1.03$, where 0.8 or higher is considered a large effect size [18], and $p_{\text{rep}} = 0.87$; Fig. 2).

2.3. Experiment 2: Sugar-bingeing rats have reduced extracellular DA and increased ACh in the NAc shell while fasting

There was a significant interaction between group and time (12, 24 and 36 h deprivation) ($F(4,28) = 2.86, p < 0.05$; Fig. 3A). After 24 h of fasting, DA release decreased to $68 \pm 6\%$ for the group previously fed intermittent sugar + chow, and $72 \pm 5\%$ for the ad libitum chow group, while remaining unchanged for the intermittent chow group ($95 \pm 7\%$). After 36 h of fasting extracellular DA remained low for the intermittent sucrose + chow group ($61 \pm 14\%$), and at this time point it was significantly less than both the ad libitum chow group ($113 \pm 14\%, p < 0.05$) and intermittent chow group ($104 \pm 15, p < 0.05$).

There were no differences amongst groups after 12 h of deprivation for either DA or ACh (intermittent sugar + chow = 1.6 ± 0.3 pg and 0.4 ± 0.1 pmol/sample; intermittent chow = 1.5 ± 0.4 pg and 0.7 ± 0.3 pmol/sample; ad libitum chow = 1.4 ± 0.3 pg and 0.7 ± 0.3 pmol/sample; DA and ACh, respectively).

After 24 h of fasting, DOPAC levels were decreased for all groups ($F(2,34) = 33.8, p < 0.001$). A similar, although non-significant trend was observed at 36 h of fasting. There was also an effect of time on HVA release ($F(2,34) = 6.97, p < 0.001$). Like DOPAC and DA, HVA was decreased at 24 h of fasting for all groups (Table 1). However, by 36 h of fasting, HVA was higher for the intermittent sugar + chow group ($119 \pm 20\%$), but remained slightly decreased for the ad libitum chow and intermittent chow groups.

Extracellular ACh changed in the opposite direction of DA. There was a significant interaction between group and time ($F(4, 30) = 4.81, p < 0.005$; Fig. 3B). ACh increased after 24 h of fasting for the intermittent sucrose + chow group ($115 \pm 10\%$; $p < 0.05$), but not for the ad libitum chow group ($77 \pm 13\%$) or the intermittent chow group ($90 \pm 15\%$). This difference was enhanced after 36 h of fasting, with ACh rising for the intermittent sucrose + chow group ($164 \pm 14\%$) compared with the levels observed in the ad libitum chow ($97 \pm 17\%$; $p < 0.05$) and intermittent chow ($104 \pm 15\%$; $p < 0.05$) control groups.

Note that the baseline measures were taken after the first 12 h of fasting when the intermittent sucrose + chow and intermittent chow rats would normally get food. Thus, the 36-h fasting time-point was exactly 24 h after the 12-h measure. At this point in the circadian cycle the chow-fed control groups did not show changes in DA or ACh, while the sugar-bingeing group had significantly low DA and high ACh.

Histology verified that probe placements were primarily in the shell of the NAc (Fig. 4).

2.4. Experiment 3: Withdrawal signs following fasting in sugar-bingeing rats are not directly related to hypoglycemia

There were no significant differences in blood glucose levels among groups (range for 12 h = 5.1–7.8 mmol, range for 24 h = 4.6–6.9 mmol, range for 36 h = 4.2–6.4 mmol). There was, however, an effect of time, with blood glucose levels dropping for all groups over the course of the 36 h of deprivation ($F(2,52) = 52.8, p < 0.001$).

3. Discussion

3.1. Behavioral indications of anxiety during fasting in sugar-bingeing rats

The elevated plus-maze is one of the most commonly used animal tests of anxiety [14,21], and has been extensively validated for both general anxiety [22] and anxiety induced by drug withdrawal [23]. The results of Experiment 1 suggest that fasting following a diet of intermittent access to sugar can result in anxiety as measured by the elevated plus-maze. Rats that had previously been bingeing on sugar spent 6% of the time on the open arm of the maze, compared with 11% for the ad libitum chow group. These data are in the range of values obtained by others, and the results are similar to those typically found using this procedure [24,25]. This finding is similar to the decreased open-arm exploration that has been observed following spontaneous withdrawal from morphine [26]. In previous studies, animals that have been maintained on an ad libitum diet of sugar and chow showed no signs of anxiety when administered naloxone, while animals maintained on an intermittent sugar and chow diet did show anxiety when administered the same dose of naloxone [3]. Ad libitum access to sugar has also failed to produce other behavioral signs of dependence,

including cross sensitization to amphetamine [27] and a proclivity to consume alcohol [28]. Intermittent access to sugar does produce these behaviors. The importance of intermittent access in eliciting the observed effects is further suggested by findings in which abstinence from ad libitum saccharin did not result in depressive-like behaviors [29], which is another behavior that can be observed during withdrawal. Given these previous studies, ad libitum sugar was not tested in the present experiment.

Studies have also shown that it is not the administration of the sucrose diet, but prolonged abstinence from the diet that precipitates signs of anxiety in sucrose-bingeing rats. We have previously reported that sugar-bingeing rats with daily 12-h access, followed by 12-h deprivation, do not show somatic signs of anxiety, ultrasonic distress calls or anxiety on the elevated plus-maze following the usual 12-h, daily food deprivation period [3]. The present results confirm that 36-h deprivation does cause the anxiety phenomenon.

The finding of anxiety during fasting in Experiment 1 is similar to opiate-like withdrawal signs that can be precipitated with the opioid antagonist, naloxone [3]. Sensitivity to naloxone in sugar-bingeing rats suggests an alteration in endogenous opioid receptors as a result of the diet. This has been confirmed in reports showing that bingeing on palatable food alters enkephalin mRNA and μ -opioid receptor binding in the NAc [30–32]. It is likely that the withdrawal signs following deprivation that are observed in the present study are due to a lack of endogenous opioid stimulation in the sugar-bingeing animals.

These results are in agreement with other reports of opiate-like withdrawal signs that follow fasting, or that emerge spontaneously, in rats that have previously been bingeing on sugar. In addition to somatic signs of distress [3], aggressive behaviors and decreases in body temperature have been noted [10]. These changes in behavior and physiology are similar to those observed during withdrawal from opiates [33,34], and support the theory that a diet of intermittent access to a sugar solution can result in signs of opiate-like withdrawal.

3.2. Extracellular DA and ACh in the accumbens during fasting in sugar-bingeing rats

At 36 h of fasting, compared with both control groups, DA levels were significantly decreased for the intermittent sugar + chow group. This suggests that food and water deprivation can cause a loss of DA tone in rats with a history of bingeing on sugar. At the same time, extracellular ACh is elevated, suggestive of an opioid withdrawal-like state.

The control groups did not show this effect. At this 36-h time point, which is the same phase of the light/dark cycle as the 12-h time point, DA had returned to baseline levels for the ad libitum chow group (Fig. 3A). This suggests that accumbens DA release in the ad libitum chow group followed a diurnal rhythm, as suggested by Paulson and Robinson [35]. Others have suggested similar changes in the striatum [36,37]. This diurnal effect was not observed with the intermittent chow group, possibly because the cyclic feeding may alter the normal circadian rhythm of DA release.

The prolonged decrease in extracellular DA in the intermittent sugar + chow group is similar to what has been reported during spontaneous withdrawal from morphine [7], and may play a role in fostering reinstatement of sugar intake after abstinence [38]. The results obtained

with the intermittent chow group, which showed relatively little change in DA release at any time point, suggests that the combination of bingeing on sugar and chow, not just chow, is important in producing the observed effects.

Although DOPAC and HVA usually follow patterns similar to DA, this is not always the case. In the present experiment, DOPAC and HVA did not show a diurnal variation like that observed with DA, and instead remained suppressed over time. Although others have reported circadian fluctuations in these metabolites in the NAc [35], we are not aware of any papers that have measured these levels during fasting for 36 h. Thus, in the present experiment, fasting may have affected DA metabolism in the chow control group.

ACh levels showed a significant difference between groups after 36 h of fasting. ACh in the NAc has been implicated in ingestive behavior [39] and satiety in particular [40–44], and, when DA is low, ACh may foster aversion [40,45–48]. The significant increase in ACh observed in the intermittent sugar + chow rats during fasting in the present experiment may correspond to the negative aspects of being reward-deprived. Previous studies lend support to the theory that the findings reported here are the result of deprivation from the sucrose diet. Rats bingeing on sucrose release DA and show attenuation of ACh release in the NAc [20,49], which is the opposite of the present results seen during prolonged deprivation. The imbalance between accumbens DA and ACh in the intermittent sugar + chow group, but not in the control groups, may contribute to the anxiety observed in Experiment 1.

3.3. Blood glucose levels during fasting in sugar-bingeing rats

Hypoglycemia can lead to an aversive state from which an animal may attempt to escape by eating. Behaviors associated with this aversive state are similar to those observed during naloxone administration or fasting in sucrose-bingeing rats [50]. A multitude of factors can influence the brain reward system. However, because of the similarity between behaviors observed during hypoglycemia and those observed during anxiety, this study measured blood glucose levels to ensure that the observed effects were not simply due to an aberrant glycemic status. Blood glucose levels were similar in all groups and therefore do not appear to account for the behavioral differences or changes in DA and ACh release. It can be inferred that mean insulin levels remained consistent across groups, since alterations in blood glucose levels were not observed and body weights did not differ as a result of the feeding schedules. Thus, the present findings, as well as those in our previous report [30], suggest the behavioral and neurochemical changes are not the result of differences in blood glucose levels. Instead they may be due to a combination of alterations in the endogenous opioid and DA systems.

4. Conclusion

Long-term deprivation following sugar bingeing can result in behavioral and neurochemical adaptations similar to those observed when opioid-dependent animals are deprived of an abused substance, such as morphine. These indicators of opiate-like withdrawal are signs of dependence. This finding, combined with previous studies showing that sugar bingeing can result in other signs of dependence, including dopaminergic and opioid changes [30,32], naloxone-precipitated and spontaneous withdrawal [3], cross-sensitization with drugs of

abuse [27,51], increased intake of sugar after abstinence [38], a time-dependent increase in responding for cues previously associated with sugar [52], and a proclivity to consume alcohol [28], suggests that dependency is evident on several dimensions [53,54]. The present findings may be important for understanding the aversive components that could contribute to binge eating.

Acknowledgements

This research was supported by USPHS grant AA-12882 (to BGH) and DA-16458 and DK-79793 (fellowships to NMA).

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Fig. 1. Sugar and chow intake during the 28-day access period. A) Rats with intermittent sugar + chow escalated their total daily sugar intake over time. B) Rats with intermittent sugar + chow ate fewer grams of chow than the intermittent chow and ad libitum chow control groups; however, C) there was no difference between groups in total daily caloric intake.

Fig. 2. Percent of time spent on the open arm of the elevated plus-maze. Rats that had been previously fed intermittent sugar + chow spent significantly less time on the open arm following 36 h of fasting compared with an equally deprived ad libitum chow group. This suggests anxiety. * = $p < 0.05$.

Fig. 3.

Extracellular DA and ACh in the NAc following 24 and 36 h of fasting. A) After 36 h of fasting, DA release in the intermittent sugar + chow group (black bar) was significantly less than both the intermittent chow (grey bar) and ad libitum chow (white bar) control groups. B) Extracellular ACh was significantly increased in the intermittent sugar + chow group at the 36 h fasting point compared with both control groups. * = $p < 0.05$ compared with both intermittent chow and ad libitum chow.

Fig. 4.

Probe track placement indicates that microdialysis samples were drawn predominantly from the medial NAc shell at planes 1.2 and 1.7 anterior to bregma [17]. CPu = caudate putamen, AcbC = accumbens core, AcbSh = accumbens shell.

Table 1

Values for DOPAC and HVA levels in Experiment 2

	Intermittent sucrose + chow	Ad libitum chow	Intermittent chow
<i>DOPAC</i>			
24 h	73±3% *	62±9% *	78±8% *
36 h	66±15%	64±10%	60±8%
<i>HVA</i>			
24 h	78±5% *	63±8% *	84±11% *
36 h	119±20%	89±12%	94±10%

*
= $p < 0.05$.