

## DAILY BINGEING ON SUGAR REPEATEDLY RELEASES DOPAMINE IN THE ACCUMBENS SHELL

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**Abstract**—Most drugs of abuse increase dopamine (DA) in the nucleus accumbens (NAc), and do so every time as a pharmacological response. Palatable food also releases accumbens-shell DA, but in naïve rats the effect can wane during a long meal and disappears with repetition. Under select dietary circumstances, sugar can have effects similar to a drug of abuse. Rats show signs of DA sensitization and opioid dependence when given intermittent access to sucrose, such as alterations in DA and mu-opioid receptors, cross-sensitization with amphetamine and alcohol, and behavioral and neurochemical signs of naloxone-precipitated withdrawal. The present experiment asks whether sucrose-dependent rats release DA each time they binge. We also predict that acetylcholine (ACh), which rises as the end of a meal, will be delayed in rats with intermittent access to sucrose. To create dependency, the experimental group (Daily Intermittent Sucrose) was maintained on a diet of 12-h food deprivation that extended 4 h into the dark, followed by 12-h access to a 10% sucrose solution and chow, daily, for 21 days. As the main result, these rats gradually increased their sucrose intake from 37 to 112 ml per day (from 13 to 20 ml in the first hour of access), and repeatedly increased extracellular DA to 130% of baseline as measured in the NAc shell by microdialysis during the first hour of sucrose access on day 1, day 2 and day 21. Three control groups failed to show a significant increase in extracellular DA on day 21: Sucrose only for 1 h on days 1 and 21 (Sucrose Twice), *ad libitum* access to sucrose and chow (Daily *Ad libitum* Sucrose), and intermittent chow instead of sucrose (Daily Intermittent Chow). Acetylcholine measured at the same time as DA, increased significantly toward the end and after each test meal in all groups. In the Daily Intermittent Sucrose group, the highest ACh levels (133%) occurred during the first sample after the sucrose meal ended. In summary, sucrose-dependent animals have a delayed ACh satiation response, drink more sucrose, and release more DA than sucrose- or binge-experienced, but non-dependent animals. These results suggest another neurochemical similarity between intermittent bingeing on sucrose and drugs of abuse: both can repeatedly increase extracellular DA in the NAc shell. © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** acetylcholine, microdialysis, drug of abuse, feeding, sucrose.

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**Abbreviations:** ACh, acetylcholine; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC-EC, high performance liquid chromatography with electrochemical detection; HVA, homovanillic acid; NAc, nucleus accumbens.

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Drugs of abuse and palatable foods have similarities that suggest they share certain neural substrates (Wise, 1987; Hernandez and Hoebel, 1988). Both produce their behavioral reinforcement, in part, by releasing opioids (Capendijk et al., 1999; Nieto et al., 2002) and dopamine (DA) in the limbic system (Mark et al., 1991; Tanda and Di Chiara, 1998; Hoebel et al., 1999; Hajnal et al., 2004). But one major difference is that drugs of abuse can increase extracellular DA every time (Pothos et al., 1991; Wise et al., 1995), while during a meal the release of DA wanes and disappears with repeated access (Di Chiara and Tanda, 1997; Bassareo and Di Chiara, 1999). Suppose, however, that the animals were to eat in a manner that produced signs of substance abuse with a very palatable food. Would that be manifest in unfailing release of DA, such as seen with drugs of abuse?

Under select dietary circumstances some foods can elicit behavioral signs of dependence (Le Magnen, 1987; Colantuoni et al., 2001, 2002; Avena and Hoebel, 2003; Avena et al., 2004, 2005). Rats maintained on a diet of daily, intermittent access to sugar and chow (Daily Intermittent Sucrose) show both behavioral and neurochemical changes similar to rats that are dependent on addictive drugs. They will binge in the first hour of daily access and progressively escalate their daily sugar intake (Colantuoni et al., 2001). When food deprived for 24 h, they show somatic signs of withdrawal. Naloxone precipitates opioid withdrawal with immediate behavioral and neurochemical signs of anxiety and DA/acetylcholine (ACh) imbalance in the nucleus accumbens (NAc) (Colantuoni et al., 2002). They also show locomotor cross-sensitization with amphetamine and augmented intake of alcohol (Avena and Hoebel, 2003; Avena et al., 2004), increases in mu-opioid and D<sub>1</sub> receptor binding (Colantuoni et al., 2001), and increases in D<sub>3</sub> mRNA in the NAc (Spangler et al., 2004). This suggests that under select dietary circumstances palatable food can cause dependency on endogenous opioids and lasting sensitization to dopaminergic stimulants. If bingeing on sugar is, in fact, like a substance of abuse, such bingeing should release DA consistently.

Previous results suggest ACh in the NAc is involved in satiation when extracellular DA is high and aversion when DA is low (Mark et al., 1992b; Hoebel et al., 1999; Helm et al., 2003). An opiate, such as morphine, not only increases DA, it simultaneously lowers ACh (Pothos et al., 1991; Rada et al., 1991a,b, 1996; Fiserova et al., 1999). This raises a question as to whether sugar also acts like morphine by lowering ACh in the NAc. However, unlike morphine, which can stimulate feeding, sugar causes satiety, which might involve ACh release (Mark et al., 1992a,b). If

accumbens ACh does inhibit eating, the ACh satiety response should be delayed in rats that take very large meals of sucrose due to bingeing.

In summary, two hypotheses are posed: 1.) Daily intermittent sucrose bingeing can act like a drug of abuse and should release DA even after 3 weeks without waning of the DA response. 2.) Such sucrose bingeing should delay the ACh response that accompanies the satiation process.

## EXPERIMENTAL PROCEDURES

### Subjects

Male Sprague–Dawley rats were obtained from Taconic Farms (Germantown, NY, USA) or from the Princeton University (Princeton, NJ, USA) vivarium from a stock originating from Taconic Farms. All rats weighed 300–350 g at the onset of the experiment and were housed individually on a reversed 12-h light/dark schedule with Lab Diet rodent chow pellets (PMI Nutrition International, Brentwood, MO, USA) and water available *ad libitum*.

### Surgery

For surgery, animals were anesthetized with a combination of ketamine (80 mg/kg i.p.) and xylazine (10 mg/kg i.p.). All rats were stereotaxically implanted with bilateral 21 gauge stainless-steel guide shafts aimed at the posterior medial accumbens shell (Anterior/posterior: +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). Microdialysis probes, inserted 1 week later, protruded 5 mm from the guide shaft to reach the intended site in the accumbens shell. Efforts were made to use the minimum number of rats and allow them to recover comfortably in the home cage for at least 1 week before experiments began. All procedures were carried out in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals and the Princeton University Institutional Animal Care and Use Committee.

### Microdialysis procedures

Microdialysis probes were constructed of silica glass tubing (37  $\mu\text{m}$  inner diameter, Polymicro Technologies Inc., Phoenix, AZ, USA) 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, USA, 6000 molecular weight, 0.2 mm outer diameter  $\times$  2 mm long) (Hernandez et al., 1986). Probes were inserted and fixed in place with acrylic cement 14–16 h before each experiment to allow neurotransmitter recovery to stabilize. Probes were perfused with buffered Ringer's solution (142 mM NaCl, 3.9 mM KCl, 1.2 mM CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, 1.35 Na<sub>2</sub>HPO<sub>4</sub>, 0.3 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.35) at a flow rate of 0.5  $\mu\text{l}/\text{min}$  overnight and at 1.3  $\mu\text{l}/\text{min}$  2 h before starting and throughout the experiment. Neostigmine (0.3  $\mu\text{M}$ ) was added to the perfusion fluid to improve basal recovery of ACh by hindering its enzymatic degradation. Samples were collected every 30 min during the experiment and then each was split, half for DA analysis and half for ACh.

### DA and ACh assays

DA and its metabolites 3,4-dihydroxyphenylacetic 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- $\mu\text{l}$  sample loop leading to a 10-cm column with 3.2 mm-bore and 3  $\mu\text{m}$ , C18 packing (Brownlee Co. Model 6213, San Jose, CA, USA). The

mobile phase contained 60 mM sodium phosphate, 100  $\mu\text{M}$  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, USA) 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- $\mu\text{l}$  sample loop with a 10-cm C18 analytical column (Chrompack Inc., Palo Alto, CA, USA). ACh was converted to betaine and hydrogen peroxide by an immobilized enzyme reactor (acetylcholinesterase and choline oxidase from Sigma Chemicals, St Louis, MO, USA, and column from Chrompack Inc.). The mobile phase was 200 mM potassium phosphate at pH 8.0. An amperometric detector was used (EG&G Princeton Applied Research, Lawrenceville, NJ, USA). The hydrogen peroxide was oxidized on a platinum electrode (BAS, West Lafayette, IN, USA) set at 500 mV with respect to an Ag-AgCl reference electrode (EG&G Princeton Applied Research).

### Experimental design

Rats were divided into four groups for microdialysis. Except for days in which microdialysis samples were collected, rats in the experimental group (Daily Intermittent Sucrose;  $n=6$ ) rats were maintained on a diet of daily 12-h deprivation followed by 12-h access to food, starting 4 h into the dark phase. This feeding schedule is similar to our previous reports (Colantuoni et al., 2001, 2002; Avena and Hoebel, 2003; Spangler et al., 2004; Avena et al., 2004, 2005). For microdialysis, rats were brought from the home cage to the dialysis cage the night before experimentation, at which time a microdialysis probe was implanted. The testing cages had overhead fluid swivel joints and a counterbalanced arm to collect samples. Food and water were removed from the dialysis cage during the experiment. After a stable baseline was reached (variation between samples no more than 10% in three continuous samples), DA and ACh were monitored simultaneously in 30-min samples on days 1 and 2 as follows: three baseline samples, two samples during access to 10% sucrose (no chow available) and two samples after the sucrose was removed (no sucrose or chow available). Rats then remained in the dialysis cage on their usual diet of intermittent sucrose and chow until next day. After day-2 samples were collected, the rats were then moved back to the home cages where they remained on their intermittent sucrose and chow diet until day 20. On day 20, each rat was moved to the dialysis cage again, and a probe was implanted on the contralateral side (this was done counterbalancing left and right sides). On day 21, the same procedure as day 1 was performed, with samples collected before, during and after drinking sucrose.

Three control groups were also tested in a similar manner. One group (Sucrose Twice,  $n=6$ ) was maintained on *ad libitum* chow, with access to the sucrose solution for 1 h after 12-h of food deprivation only on days 1 and 21, with microdialysis samples collected only on these days. The procedure was similar to that of the Daily Intermittent Sucrose group: three baseline samples, two samples during access to 10% sucrose (no chow available) and two samples after the sucrose was removed (no sucrose or chow available). Samples were not taken and sucrose was not given on day 2, although the rats remained connected to the dialysis apparatus. Another control group (Daily Intermittent Chow,  $n=9$ ) was maintained on a 12-h food deprivation followed by 12 h access to chow starting 4 h into the dark cycle with microdialysis samples collected on days 1, 2 and 21. For this group, instead of sucrose, 1-h access to chow was given. A final group (Daily *Ad libitum* Sucrose,  $n=6$ ) had *ad libitum* 10% sucrose and chow with microdialysis samples taken on days 1, 2 and 21. For this group, food was not removed from the chambers until baseline microdialysis samples were collected. Access to sucrose was for 1 h during

sample collection, and no sucrose or chow was available during the three post-meal samples.

In between microdialysis sessions (days 2–20), all rats were moved back to the home cages where they remained on their respective diets until day 20: Daily Intermittent Sucrose rats with daily 12-h access to chow and sucrose, Daily Intermittent Chow rats with 12-h access to chow, Daily *Ad libitum* Sucrose rats with 24 h access to sucrose and chow and Sucrose Twice rats with *ad libitum* chow. The Daily Intermittent Sucrose and the Sugar Twice groups were tested first, followed by the other groups.

### Statistics and histology

Sucrose intake was recorded to the nearest ml and analyzed by Student's *t*-test, one-way ANOVA, or two-way repeated measures ANOVA with post hoc Student's *t*-test when justified. Microdialysis data were normalized to percent of baseline and analyzed by repeated measures one- or two-way ANOVA followed by Newman-Keuls post hoc comparisons when justified. The null hypothesis was rejected at  $P < 0.05$ .

Histology was performed to verify probe location. Rats received an overdose of sodium pentobarbital and were intracardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and frozen for sectioning. Sections, 40  $\mu$ m thick, were taken from the anterior lobe caudally until probe tracks were identified.

## RESULTS

### Sucrose-dependent rats escalate first-hour and daily intake of sucrose

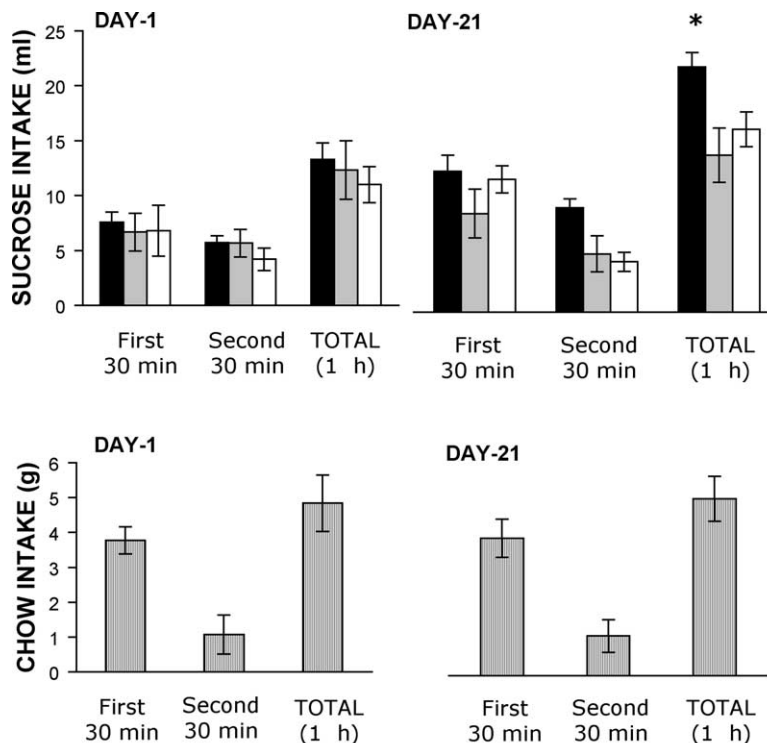
As seen in Fig. 1, during the first hour of access on day 1, when microdialysis was first performed, the Daily Intermittent

Sucrose rats drank  $13.3 \pm 1$  ml, the Sucrose Twice rats drank  $12.3 \pm 3$  ml, and the Daily *Ad libitum* Sucrose rats drank  $11 \pm 2$  ml of sucrose. A one-way ANOVA revealed no significant difference in sucrose intake between these groups. Rats in the Daily Intermittent Chow group ate  $4.8 \pm 0.8$  g during this time. On subsequent days, Daily Intermittent Sucrose rats progressively increased their first-hour sucrose intake to  $20 \pm 1$  ml by day 21 ( $t(4) = 4.52$ ,  $P < 0.01$ ; Fig. 1). There was no difference between day 1 and day 21 intake during the first hour for the control groups. The Intermittent Chow rats ate  $4.95 \pm 0.6$  g in 1 h on day 21, which was no different than their day-1 intake.

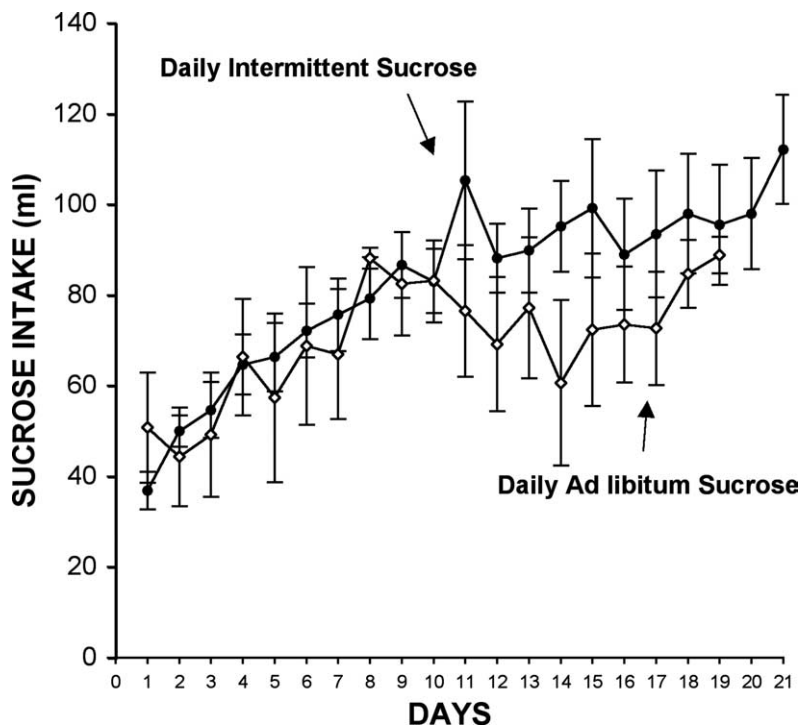
Total 12-h daily intake of sucrose significantly increased for the Daily Intermittent Sucrose group from  $37 \pm 4$  ml on day 1 to  $112 \pm 15$  ml on day 21, reaching an asymptote after 11 days ( $F(20,80) = 9.08$ ,  $P < 0.01$ ; Fig. 2). The Daily *Ad libitum* Sucrose rats also increased their total daily sugar intake over the 21 days, but this result was non-significant (Fig. 2). There was no significant difference in daily sucrose intake between the Daily Intermittent Sucrose and the Daily *Ad libitum* Sucrose groups over the 21 days.

### Sucrose-dependent rats release DA repeatedly in the NAc upon sucrose access

Basal levels of DA were not different for groups prior to eating (mean of group means =  $11.5 \pm 4.5$  fmol/sample). Animals on the daily, intermittent sucrose regimen main-



**Fig. 1.** Sugar or chow intake during microdialysis sessions on day 1 and day 21. For the sugar drinking groups (top panel), there was no significant difference in sugar intake on day 1. By day 21, only the Daily Intermittent Sugar group showed a difference in intake relative to day 1. Rats in the Daily Intermittent Chow group ate the same amount on day 21 as on day 1 (bottom panel). Top panel: ■ = Daily Intermittent Sucrose; □ = Sucrose Twice; □ = Daily *Ad libitum* Sucrose; bottom panel: Daily Intermittent Chow; \*  $P < 0.05$ .



**Fig. 2.** Gradual increase in total daily intake of 10% sucrose for the Daily Intermittent Sucrose and Daily *Ad libitum* Sucrose groups. The Daily Intermittent Sucrose group significantly increased their total daily sucrose intake over the 21 days. There was a non-significant increase for the Daily *Ad libitum* Sucrose group.

tained high DA release after 21 days of access. As shown in Fig. 3, on day 1, when the rats had access to a 10% sucrose solution for the first time, DA levels increased to  $131 \pm 9\%$  of baseline ( $F(6,42)=5.97$ ,  $P<0.01$ ; Fig. 3) for the Daily Intermittent Sucrose rats. For this group, an increase was also observed on day 2 ( $135 \pm 7\%$ ,  $F(6,18)=3.65$ ,  $P<0.02$ ; Fig. 3) and again on day 21 ( $130 \pm 7\%$ ,  $F(6,30)=3.17$ ,  $P<0.02$ ; Fig. 3).

Results for the control groups were markedly different than those of the experimental group. Rats with access to sucrose for 1 h only on days 1 and 21 showed a blunting of the DA response during the second access, on day 21. After tasting sucrose for the first time on day 1, these rats increased DA release to  $133 \pm 9\%$  of baseline ( $F(6,54)=3.65$ ;  $P<0.01$ ; Fig. 3). However, on day 21, which was the second time they drank sucrose, there was a non-significant increase to  $109 \pm 5\%$ , even though they drank about the same amount on both days ( $12.3 \pm 3$  and  $13 \pm 2$  ml, on day 1 and day 21, respectively). Similar blunting in the DA response was observed with the Daily *Ad libitum* Sucrose rats on day 21. On day 1, DA levels reached  $118 \pm 7\%$  ( $F(6,54)=5.88$ ,  $P<0.001$ ) in response to tasting sucrose for the first time. By day 2 and day 21, there was no increase in DA levels in response to drinking sucrose ( $104 \pm 2$  and  $97 \pm 1\%$ , respectively). For the Daily Intermittent Chow group, DA increased significantly to  $131 \pm 4\%$  ( $F(6,42)=4.06$ ,  $P<0.01$ ) on day 1, and this increase was less evident, but nonetheless significant, on day 2 reaching  $122 \pm 11\%$  of baseline levels ( $F(6,42)=2.58$ ,  $P<0.05$ ) and only a mild non-significant increase on day 21,  $109 \pm 2\%$ . There was a significant difference in the DA response

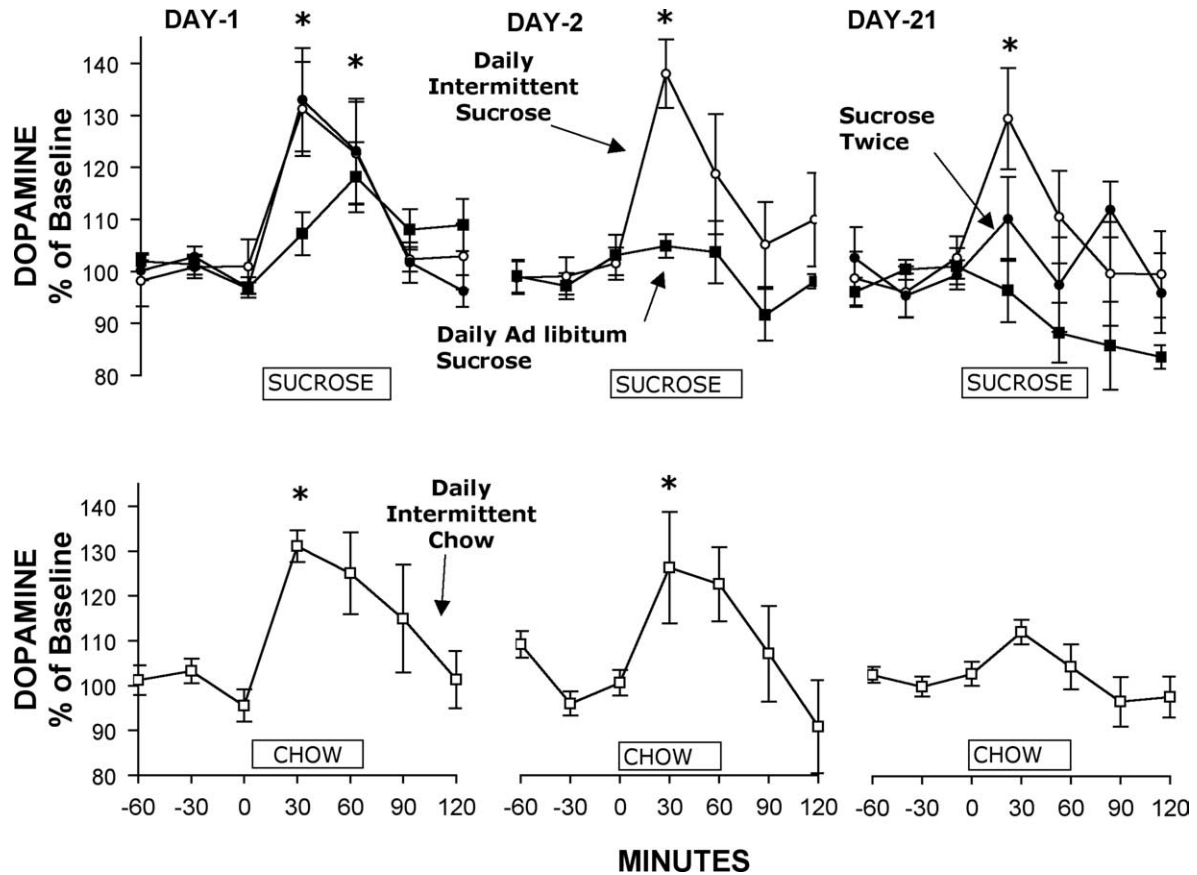
during 1-h sucrose access on day 21 comparing all three groups with access to sucrose ( $F(12,90)=2.081$ ,  $P<0.03$ ) and the difference is due to the significant increase in DA levels in the Daily Intermittent Sucrose rats. Comparing day 1 to day 21, DA levels significantly decreased for the control groups (Daily Intermittent Chow: ( $F(6,78)=3.11$ ,  $P<0.01$ ), Sucrose Twice: ( $F(6,54)=3.65$ ,  $P<0.005$ ), Daily *Ad libitum* Sucrose: ( $F(6,54)=5.88$ ,  $P<0.0001$ )) but not for the Daily Intermittent Sucrose group ( $F(6,54)=0.28$ ,  $P=n.s.$ ).

For all rats, DOPAC levels increased to approximately the same levels (109–113% of baseline) on day 1. However, by day 21, DOPAC increased to  $124 \pm 2\%$  for the Daily Intermittent Sucrose rats compared with  $108 \pm 7\%$  for the Sucrose Twice group, or  $108 \pm 4\%$  for the Daily *Ad libitum* Sucrose group (data not shown). DOPAC levels were maintained at initial levels on day 21 for the Daily Intermittent Chow group.

HVA levels changed similar to DOPAC levels with no significant difference between groups on day 1, but a significant increase on day 21 for the Daily Intermittent Sucrose rats (data not shown).

#### **Accumbal ACh rises at the end of a sucrose meal, and is delayed in sucrose-dependent rats**

There were no overall differences in the basal ACh levels (mean of group means =  $1.0 \pm 0.2$  pmol/sample). Extracellular ACh reached the same levels in all groups, but the time courses were different. In the Daily Intermittent Sucrose group, during the first access to the sucrose solution



**Fig. 3.** Animals on the Daily Intermittent Sucrose regimen maintained high DA release for 3 weeks. Microdialysis samples were collected on days 1, 2 and 21 of access. DA levels increased for the Daily Intermittent Sucrose rats (open circles) on days 1 and 2 and again on day 21. DA levels also increased significantly for the Sucrose Twice (filled circles), Daily *Ad libitum* Sucrose (filled squares), and Daily Intermittent Chow (open squares) control groups on day 1, but there was a blunting of this effect by day 21. The ordinate indicates the hour (0–60 min) of sucrose or chow availability for this test. \*  $P < 0.05$ .

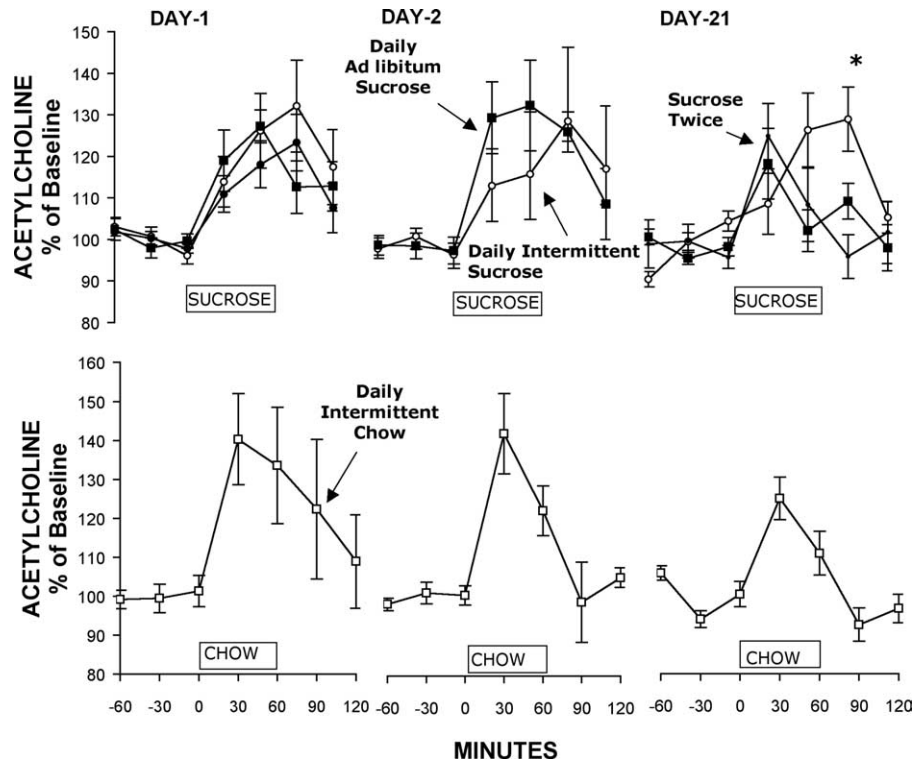
on day 1, ACh levels started to increase in the initial 30 min of feeding, reaching a maximum of  $132 \pm 11\%$  during the first post-meal sample ( $F(10,6) = 8.763$ ,  $P < 0.01$ ; Fig. 4). This same increase occurred after the meal of sucrose during day 2 and day 21 ( $133 \pm 14\%$ ,  $F(5,6) = 3.452$ ,  $P < 0.01$  and  $130 \pm 7\%$ ,  $F(5,6) = 7.158$ ,  $P < 0.01$ , respectively, Fig. 4). However, in the Sucrose Twice and Daily *Ad libitum* Sucrose groups, on day 21, the maximum ACh levels of  $125 \pm 7\%$  and  $119 \pm 7\%$ , respectively, were reached sooner, during the first half hour access to sucrose, and then fell to the original level during the first post-meal interval ( $F(5,6) = 2.83$ ,  $P < 0.03$ ; Fig. 4). Note, by comparing Figs. 3 and 4 that this Day-21 ACh peak in the control groups occurs at the same time they released less DA. For the Daily Intermittent Chow group, ACh increased to  $140 \pm 11\%$  during the first day ( $F(6,6) = 3.64$ ,  $P < 0.01$ ). A similar increase to  $141 \pm 10\%$  occurred the second day ( $F(6,6) = 7.75$ ,  $P < 0.001$ ) and a milder although significant increase to  $125 \pm 5\%$  on day 21 ( $F(5,6) = 8.10$ ,  $P < 0.001$ ). Significant changes were observed on day 21 when all three groups that drank sucrose were compared ( $F(12,90) = 2.688$ ,  $P < 0.01$ ) and it was due to the higher increase in ACh during the first post-meal sample in the Daily Intermittent Sucrose group ( $P < 0.05$ ).

Histology showed that probes were localized mainly in the shell of the NAc (Fig. 5).

## DISCUSSION

Rats on an intermittent 12-h sucrose-feeding regimen (Daily Intermittent Sucrose group) escalated intake of sucrose and consumed a large amount in the first hour of access, referred to as a “binge.” They released significant amounts of DA in the first hour of sucrose access when bingeing on day 1, day 2 and presumably every day through to the test on day 21. Control rats that were similarly deprived for 12 h but had sucrose only for 1 h on 2 days, 21 days apart (Sucrose Twice group), showed blunting of the DA response the second time they drank. This was also the case for rats that had *ad libitum* access to sugar (Daily *Ad libitum* Sugar group) or those rats with intermittent chow (Daily Intermittent Chow group).

Extracellular ACh levels increased every time animals drank a meal of 10% sucrose, but this response was delayed in the Daily Intermittent Sucrose rats when tested after 21 days of access. To the extent that intermittent intake of very palatable food, such as sugar, causes escalation of intake and large meals, the resultant release of



**Fig. 4.** ACh, measured at the same time as DA, increases toward the end of every 1-h meal, but the time courses were different. For the Daily Intermittent Sucrose group (open diamonds), day 21 ACh levels peaked later than they did for the Sucrose Twice (filled diamonds), Daily *Ad libitum* Sucrose (filled squares), and Daily Intermittent Chow (open squares) control groups. The ordinate indicates the hour (0–60 min) of sucrose or chow availability for this test. \*  $P < 0.05$ .

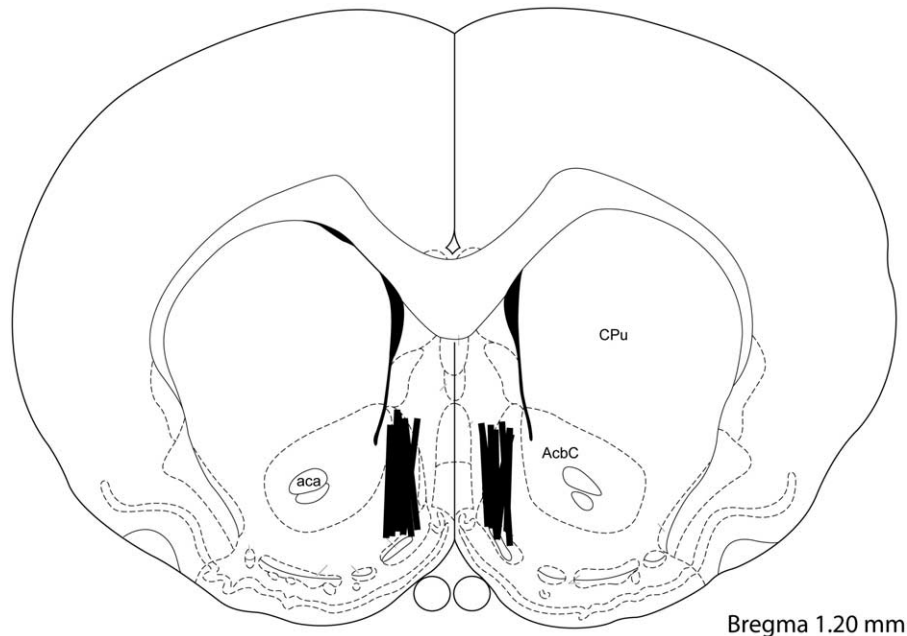
DA may cause dependence in partly the same manner as repeated administration of a drug of abuse.

DA in the NAc has been suggested to play a role in reinforced behaviors (Di Chiara and Imperato, 1988; Wise and Rompre, 1989; Hoebel et al., 1999; Koob and Le Moal, 1997). The mesoaccumbens DA system is activated by drugs of abuse and by natural stimuli such as food (Di Chiara and Imperato, 1988; Hernandez and Hoebel, 1988; Pothos et al., 1991; Wise et al., 1995; Acquas et al., 2002; Hajnal and Norgren, 2002). However, if natural stimuli are given repeatedly, a blunting of the DA increase may be observed. A single presentation of a palatable food can be sufficient to decrease subsequent DA release in the NAc shell and has been related to the familiarity of the taste (Di Chiara and Tanda, 1997; Bassareo and Di Chiara, 1999; Di Chiara, 2002). Bassareo and Di Chiara (1999) found that 24-h food deprivation was sufficient to restore DA release after a repeated palatable meal, suggesting that DA responsiveness is influenced by the physiological state. Therefore, in the present experiment, daily 12-h food deprivation may help to prevent blunting of DA release in rats in the Daily Intermittent Sucrose group. However, the Daily Intermittent Chow, and Sugar Twice, control groups were also deprived for 12 h on the days of testing and nevertheless did show the blunting. This suggests that the observed increase in DA release with daily intermittent access to sucrose is due not only to the deprivation, but also to the repeated intermittent sucrose diet, which

causes gradual escalation of intake, as well as other signs of dependence (Colantuoni et al., 2001, 2002; Avena and Hoebel, 2003; Spangler et al., 2004; Avena et al., 2004, 2005).

Taste without intermittent access was not sufficient to repeatedly increase DA in the NAc shell. Rats that drank sucrose *ad libitum* showed no increase in DA release when they drank sucrose on day 21. This would suggest that intermittency in the feeding schedule is a determinant of the persistent increase in DA release; however, rats maintained in an intermittent chow schedule also show blunting of the DA response suggesting that intermittent access may not be sufficient. A combination of a very palatable taste and the intermittent feeding schedule appears to be responsible for the phenomenon.

Rats progressively increased their sucrose intake when given intermittent access, reaching an asymptote 11 days after starting the restricted diet. This slow escalation suggests that this effect cannot be solely attributed to overcoming neophobia (Buresova and Bures, 1980). Similar gradual escalation in glucose intake was observed in previous reports from this laboratory (Colantuoni et al., 2001, 2002) and mimics the initial stages of dependency seen for drugs of abuse with escalated intake (Koob and Le Moal, 1997). The total sucrose intake in 24 h is similar between rats kept in the intermittent schedule or *ad libitum* schedule. However, the first hour intake is significantly different, with an increase in sugar intake in those rats kept



**Fig. 5.** Probe track placement indicates that samples were drawn predominantly from the NAc shell. CPu, caudate putamen; aca, anterior commissure; AcbC, accumbens core.

on intermittent sugar compared with *ad libitum* access or access to sugar twice. The control group on Daily Intermittent Chow ate the same amount during the first hour intake on day 1 and day 21. This suggests that the increase in sucrose intake during the first hour is not only dependent on the feeding schedule but also on the tastiness of the food. Additionally, the sucrose intake during the 1-h access was significantly higher in the intermittent group, and this was associated with a persistent increase in DA release in the NAc, while the other control groups showed blunting of this effect.

DA is taken into the cell by the dopamine transporter (DAT) and converted to DOPAC that diffuses to the synaptic cleft. On day 21 there was a significant increase in DOPAC levels only in the rats with 12-h daily access to sucrose. An increase in DA turnover and DAT has been reported in rats given a restricted sucrose diet for 6 days (Hajnal and Norgren, 2002), and it could explain the DOPAC increase observed in the present study.

Release of accumbens ACh appears to be related to the satiation process. Whereas DA peaks early in the meal, ACh peaks near the end (Mark et al., 1992b; Hoebel et al., 1999). In the present experiment, ACh levels reached a maximum during the first post-meal sample in all rats during their first access to sucrose. Similar increases occurred in the Daily Intermittent Sucrose group during day 2 and also during day 21 of access. Rats in the Sugar Twice group also showed a significant increase in ACh on day 21, but the peak occurred sooner. The delayed release of ACh in animals that drink excessive amounts of sugar may allow the animals to binge.

These findings support past studies implicating ACh in satiety signaling. Rats eating chow show a significant increase in ACh levels as eating slows down, suggesting

ACh might inhibit eating (Mark et al., 1992b; Hoebel et al., 1999). Accumbens ACh is released in part via neural circuits controlled by satiety-inducing neurotransmitters in the hypothalamus (Helm et al., 2003). The present experiment shows that ACh is released at the end of a meal of sucrose. This palatable food produces a delayed, longer lasting increase in ACh levels compared with plain chow.

In conclusion, an increase in extracellular DA following repeated intake of palatable solutions resembles the persistent increase in DA seen after administration of most drugs of abuse and suggests that intermittent, moderate food deprivation, combined with recurrent DA release due to sucrose intake might be responsible for modifying the mesoaccumbens DA system in a fashion similar to some aspects of drug abuse.

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