GS 455534 selectively suppresses binge eating of palatable food and attenuates dopamine release in the accumbens of sugar-bingeing rats

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Binge eating palatable foods has been shown to have behavioral and neurochemical similarities to drug addiction. GS 455534 is a highly selective reversible aldehyde dehydrogenase 2 inhibitor that has been shown to reduce alcohol and cocaine intake in rats. Given the overlaps between binge eating and drug abuse, we examined the effects of GS 455534 on binge eating and subsequent dopamine release. Sprague–Dawley rats were maintained on a sugar (experiment 1) or fat (experiment 2) binge eating diet. After 25 days, GS 455534 was administered at 7.5 and 15 mg/kg by an intraperitoneal injection, and food intake was monitored. In experiment 3, rats with cannulae aimed at the nucleus accumbens shell were maintained on the binge sugar diet for 25 days. Microdialysis was performed, during which GS 455534 15 mg/kg was administered, and sugar was available. Dialysate samples were analyzed to determine extracellular levels of dopamine. In experiment 1, GS 455534 selectively decreased sugar intake food was made available in the Binge Sugar group but not the Ad libitum Sugar group, with no effect on chow intake. In experiment 2, GS 455534 decreased fat intake in the Binge Fat group, but not the Ad libitum Fat group, however, it also reduced chow intake. In experiment 3, GS 455534 attenuated accumbens dopamine release by almost 50% in binge eating rats compared with the vehicle injection. The findings suggest that selective reversible aldehyde dehydrogenase 2 inhibitors may have the therapeutic potential to reduce binge eating of palatable foods in clinical populations. *Behavioural Pharmacology* **25:147–157 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Binge eating is a symptom of bulimia nervosa (BN), and binge eating disorder (BED) is now recognized as an eating disorder in the DSM-V (American Psychiatric Association, 2013). This behavior has been associated with increased depression, stress, and Body Mass Index (BMI) (Striegel *et al.*, 2012). Similarly, binge eating behavior has been reported among a subset of individuals with obesity (Smith *et al.*, 1998; Darby *et al.*, 2007). Given its clinical relevance and the lack of effective pharmacological treatments (Berner *et al.*, 2011; Marazziti *et al.*, 2011), research targeting novel treatment strategies for this behavior is needed.

A growing body of laboratory animal and clinical research suggests that binge eating shares similarities to drug addiction, both behaviorally and neurochemically (Cassin and von Ranson, 2007; Avena *et al.*, 2008b; Gearhardt *et al.*, 2011, 2012; Oswald *et al.*, 2011; Broft *et al.*, 2012). Specifically, rats with limited daily access to sugar (10% sucrose) for 3 weeks develop a pattern of binge intake as well as a series of behaviors similar to those observed within the context of drug addiction, including tolerance, somatic and behavioral signs of opiate-like withdrawal, and increased intake after a period of abstinence (Colantuoni *et al.*, 2002; Rada *et al.*, 2005). Animals maintained on this or similar sugar feeding schedules also show cross-sensitization to other drugs of abuse (Gosnell, 2000; Avena and Hoebel, 2003; Avena *et al.*, 2004). Concomitant with these behaviors are several neurochemical changes similar to those observed in models of addiction. One of the strongest neurochemical commonalities between binge consumption of sugar and drug addiction is the effect on extracellular levels of dopamine (DA) in the nucleus accumbens (NAc). Binge eating rats show nonabating DA release with sugar intake, which is more similar to the DA response observed with drugs of abuse than with traditional food consumption (Rada *et al.*, 2005). Alterations in DA receptor binding and gene expression, as well as μ-opioid receptor binding and enkephalin mRNA levels in the NAc, are also found in binge eating rats (Colantuoni *et al.*, 2001; Spangler *et al.*, 2004). In humans, high-frequency binge eaters with BN have reduced cerebrospinal fluid DA metabolite levels (Kaye *et al.*, 1990; Jimerson *et al.*, 1992). Further, PET

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imaging has shown decreased DA release in the putamen of individuals with BN compared with controls in response to a methylphenidate challenge (Broft *et al.*, 2012), similar to that found among individuals with substance use disorders (Volkow *et al.*, 1997, 2007). In addition, striatal DA release was associated with binge frequency (Broft *et al.*, 2012). Interestingly, PET imaging has shown greater DA release in the caudate of individuals with both obesity and BED when presented with food stimuli along with methylphenidate, which inhibits the DA reuptake transporter, compared with obese individuals without BED. In this study, increased DA in the caudate was correlated with binge eating scores (Wang *et al.*, 2011). Collectively, these findings suggest a strong relationship between binge eating and DA.

Given the overlaps between binge eating and addiction, pharmaceutical interventions that have been shown to be effective in treating drug dependency might also be useful in the treatment of binge eating behavior. GS 455534 (formally CVT-10216), a highly selective and reversible aldehyde dehydrogenase 2 (ALDH-2) inhibitor, has been shown to decrease alcohol consumption and suppress relapse, and reduce cocaine seeking in rats (Arolfo *et al.*, 2009; Yao *et al.*, 2010). The aim of the current study was to test the effects of GS 455534 on binge-type consumption of food in rats.

During alcohol consumption, ALDH-2 oxidizes acetaldehyde to acetic acid in the liver (Fernandez *et al.*, 2006). Inhibition or decreased activity of ALDH-2 in the liver increases acetaldehyde levels, which in turn can cause adverse symptoms (Eriksson, 2001) that may deter drinking (Arolfo *et al.*, 2009). However, it has also been shown that ALDH-2 inhibitors reduce alcohol seeking in the presence or absence of acetaldehyde (Keung *et al.*, 1995; Arolfo *et al.*, 2009), indicating that they may be functioning through another mechanism to decrease alcohol and drug intake. This may be explained by an ALDH-2-mediated change in brain DA metabolism. DA is synthesized in the ventral tegmental area (VTA) and transported for release into the NAc (Koob *et al.*, 1998; Wise, 2002). ALDH-2 is highly expressed in dopaminergic neurons in the VTA (McCaffery and Drager, 1994), and has been suggested to be involved in DA metabolism, and, more specifically, the metabolism of 3,4-dihydroxyphenylacetaldehyde (DOPAL) to 3,4-dihydroxyphenylacetic acid (DOPAC), by some (Florang *et al.*, 2007), but not all (Fernandez *et al.*, 2006) studies. DA has a known role in drug abuse, with addictive drugs activating VTA neurons, leading to the release of DA in the NAc (Balter, 1996; Berke and Hyman, 2000). Furthermore, increased DA release in the NAc is related to increased drug seeking and consumption (Weiss *et al.*, 1993). Recent evidence suggests that inhibition of ALDH-2 in the rodent brain, through GS 455534, reduces this drug-stimulated increase in accumbal DA without affecting basal levels [see Arolfo *et al.* (2009) for a discussion of the precise mechanisms], providing a mechanism by which GS 455534 may be decreasing alcohol and cocaine intake as well as preventing reinstatement of drug-seeking behavior (Arolfo *et al.*, 2009; Yao *et al.*, 2010).

In light of such findings and the repeated release of DA observed in the NAc of sugar-bingeing rats (Rada *et al.*, 2005), GS 455534 has been identified as a potentially promising pharmaceutical candidate for the cessation or attenuation of binge eating behavior. In the current study, we tested the effects of GS 455534 on binge-type consumption in rats. In particular, we investigated whether GS 455534 would reduce binge consumption of palatable foods, such as sugar (experiment 1) and fat (experiment 2), without affecting *ad libitum* intake of these same foods. In addition, we determined whether GS 455534 would attenuate accumbens DA levels in sugar-bingeing rats, without affecting basal DA levels (experiment 3).

Methods

Subjects and housing

Male Sprague–Dawley rats were obtained from Taconic Farms (Germantown, New York, USA) and housed individually on a 12-h reversed light/dark cycle, with lights on at 18:00 h and off at 06:00 h. All rats were fed standard rodent chow (LabDiet #5001; PMI Nutrition International, Richmond, Indiana, USA; 10% fat, 20% protein, 70% carbohydrate, 3.02 kcal/g). All procedures were approved by the Princeton University Institutional Animal Care and Use Committee.

Experiment 1: effect of GS 455534 on sugar intake in bingeing and freely-fed rats

Diets

Animals were allowed to acclimate to the vivarium for 1 week, and were then weight matched and assigned to three groups (250–260 g, $n = 12$/group). The experimental group (Binge Sugar) was allowed 12-h access daily to a 10% sucrose solution (w/v, 0.4 kcal/ml) and standard rodent chow starting 4 h into the dark cycle, an access schedule that our laboratory has used previously to elicit binge sugar consumption (Avena *et al.*, 2006a). A second group was maintained on *ad libitum* sucrose and chow (*Ad libitum* Sugar). A third group was maintained on *ad libitum* chow (*Ad libitum* Chow). The sucrose solution was available in a graduated cylinder with a sipper tube attached to the animals' cages. During diet training, body weight and chow intake were recorded weekly. Sucrose intake was measured every 12 h. Water was freely available.

Injections

After 25 days of diet access, drug and vehicle administration commenced. On the first 3 days, rats were acclimated to handling and injection procedures with

daily vehicle injections. For days 4–8, either vehicle or drug (7.5 mg/kg) was administered alternatively, starting with drug. The drug concentration was then increased to 15 mg/kg and injections were continued on days 9–14, starting with vehicle and again alternating vehicle and drug (Fig. 1). An alternating drug–vehicle paradigm was implemented to allow for drug washout between dosing (GS 455534 has been shown to clear from most tissue within 24 h; Gilead Sciences, unpublished data). This facilitated the collection of multiple intake readings per animal, which is important in controlling for potential day-to-day intake variations.

Before injections, animals were weighed, and food and palatable diet were removed. All animals were allowed access to their assigned diets 30 min after injections, which was 4 h into the dark phase. Previous studies of GS 455534 have found that it takes ~30 min for a systemic injection of the drug to take effect (Arolfo *et al.*, 2009; Overstreet *et al.*, 2009; Yao *et al.*, 2010). Intake of sucrose and chow was measured 1, 2, 4, 12, and 24 h after food was made available.

Experiment 2: effect of GS 455534 on fat intake in bingeing and *ad libitum*-fed rats

Diets

As in experiment 1, rats were weight matched and assigned to three groups (250–260 g, $n = 12$/group). The experimental group (Binge Fat) was allowed 12-h access daily to solid vegetable shortening (Crisco; J.M. Smucker Company, Orrville, Ohio, USA; 9.17 kcal/g) and standard rodent chow starting 4 h into the dark cycle. A second group was maintained on *ad libitum* shortening and chow (*Ad libitum* Fat). A third group was maintained on *ad libitum* chow (*Ad libitum* Chow). Shortening was made available in a glass jar attached to the animals' cages. During diet training, body weight and chow intake were recorded weekly. Shortening intake was measured twice weekly. Water was freely available. Injection procedures were performed as described in experiment 1 (Fig. 1).

Experiment 3: effect of GS 455534 on extracellular levels of dopamine while sugar bingeing

Animals were allowed to acclimate to the vivarium for 1 week. Surgery to implant guide cannulae for microdialysis was then performed. Rats were anesthetized with 20 mg/kg xylazine and 100 mg/kg ketamine (intraperitoneally), supplemented with ketamine as needed. Using a stereotaxic instrument, 21 G stainless-steel guide cannulas (Plastics One, Roanoke, Virginia, USA) were implanted unilaterally and aimed at the posterior medial accumbens shell, using coordinates established previously in our laboratory (Rada *et al.*, 2005; Avena *et al.*, 2006b, 2008a, 2008c), and based on the atlas of Paxinos and Watson (2005): +1.2 mm anterior to the bregma, 0.8 mm lateral to the midsagittal sinus, and 4.0 mm ventral to the surface of the level skull.

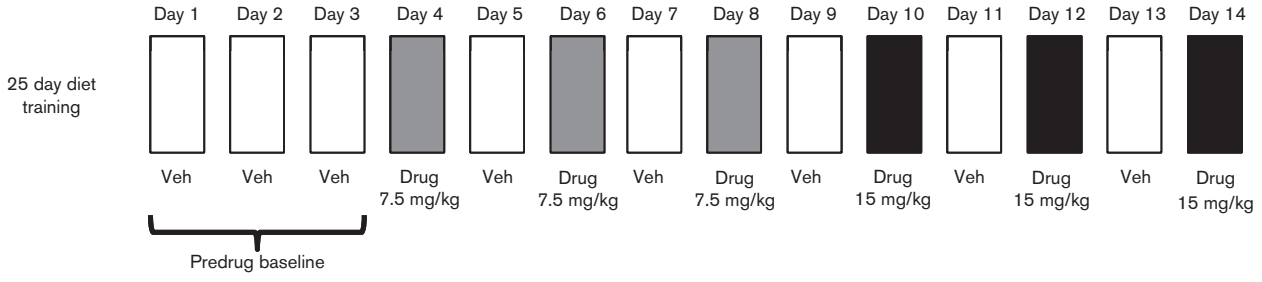
Diets

Animals were allowed 1 week to recover from surgery before diet access began. Rats were weight matched and then divided into two groups ($n = 17$–18/group). The rats were provided access to either Binge Sugar or *Ad libitum* Sugar, as described in experiment 1.

Microdialysis

After 25 days of diet access, a microdialysis probe (Plastics One, HMD1/CS, 13 KD with a 3 mm membrane) was inserted and cemented in place, protruding 5 mm from the guide cannula to reach the intended site in the accumbens shell (9 mm ventral to the skull surface). Probes were fixed in place and perfused (at a flow rate of 0.1 µl/min) with artificial cerebral spinal fluid (142 mmol/l NaCl, 3.9 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 1.0 mmol/l $MgCl_2$, 1.35 mmol/l Na_2HPO_4, 0.3 mmol/l NaH_2PO_4, pH 7.4) ~16 h before the microdialysis session to allow neurotransmitter recovery to stabilize. The flow rate was increased to 1.0 µl/min 2 h before the start of the microdialysis session and 2 µl of HCl 0.1 mol/l was added to the sample collection tubes before the collection of the samples to prevent DA degradation.

Fig. 1



Experimental paradigm. Veh, vehicle.

Experiment design

For microdialysis, each rat was brought from its home cage to the microdialysis cage the night before the study (16 h before experimentation), at which time the microdialysis probe was implanted. For the Binge Sugar group, sugar and chow were removed 4 h into the light cycle. The *Ad libitum* Sugar group was maintained on their diet through the night, but sugar and chow were removed 2 h into the dark cycle in preparation for obtaining baseline microdialysis readings. Microdialysis samples were collected starting 4 h into the dark cycle, at which time water was also removed from the cage. After baseline samples were collected, rats were administered an intraperitoneal injection of 15 mg/kg GS 455534 ($n = 8$–9 for each diet condition) or vehicle ($n = 9$ for each diet condition) in a between-groups design. Samples were collected for two time points over 40 min after the injection, and then sugar was returned to all rats. Sucrose intake was monitored and microdialysis samples were collected every 20 min for 2 h.

High-performance liquid chromatography

Microdialysis samples were frozen at −80°C until analysis with high-performance liquid chromatography (HPLC). Levels of DA were measured in dialysates using HPLC combined with an electrochemical detection system (HPLC-EC) with a microbore column (100 mm × 1 mm, 3 μm particle, ODS column; Bioanalytical Systems, West Lafayette, Indiana, USA). DA was eluted with a mobile phase consisting of 75 mmol/l sodium phosphate, 1.55 mmol/l octane sulfonic acid, 1 mmol/l EDTA, and 9% acetonitrile (v/v), and analyzed in a radial flow carbon working electrode and a Ag/AgCl reference electrode set at an oxidation voltage of 700 mV in a digital detector (Epsilon; Bioanalytical Systems). The range of detection was 2 nA and the average retention time for DA was 7 min. DA was quantified by comparing peak areas of the sample relative to the external standard.

Histology

At the end of the experiment, histology was performed to verify microdialysis probe placement. Rats were killed by rapid decapitation and brains were kept in formaldehyde (4% in PBS) for 24 h and then transferred to 30% sucrose in PBS. They were then sliced into 40 μm sections on a freezing microtome and slide-mounted for microscopic verification of probe placement. Once visualized, probe tracks were plotted using the atlas of Paxinos and Watson (2005). Rats with tracks outside the targeted area were removed from analysis, such that the final $n = 7$–8/group.

Drug

GS 455534 (in acid form at 0, 7.5, and 15 mg/kg; Gilead Pharmaceuticals, Foster City, California, USA) was administered intraperitoneally. Drug doses were chosen on the basis of previous studies using this compound (Arolfo *et al.*, 2009; Overstreet *et al.*, 2009). GS 455534 was suspended in 0.5% methylcellulose, which also served as a vehicle control. The drug solution was prepared daily.

Statistics

In experiments 1 and 2, repeated-measures analyses of variance (ANOVAs) were used to examine the amount of palatable food (sucrose or shortening) consumed over the 25-day training period. The amount of palatable food and chow consumed on day 25 of diet training, the day before injections, were also compared between groups using independent *t*-tests. Differences in body weight were assessed by one-way ANOVA or repeated-measures ANOVA with post-hoc Tukey's HSD (at the least significant level) when warranted by significance on the ANOVA. Palatable food and chow intake following injections were assessed using repeated-measures ANOVA comparing baseline intake and intakes at the lower and higher drug doses. Baseline intake was calculated by averaging intakes over the initial 3 days of vehicle injections. Intakes following drug injections were calculated by averaging intakes over the three drug injections for each dose.

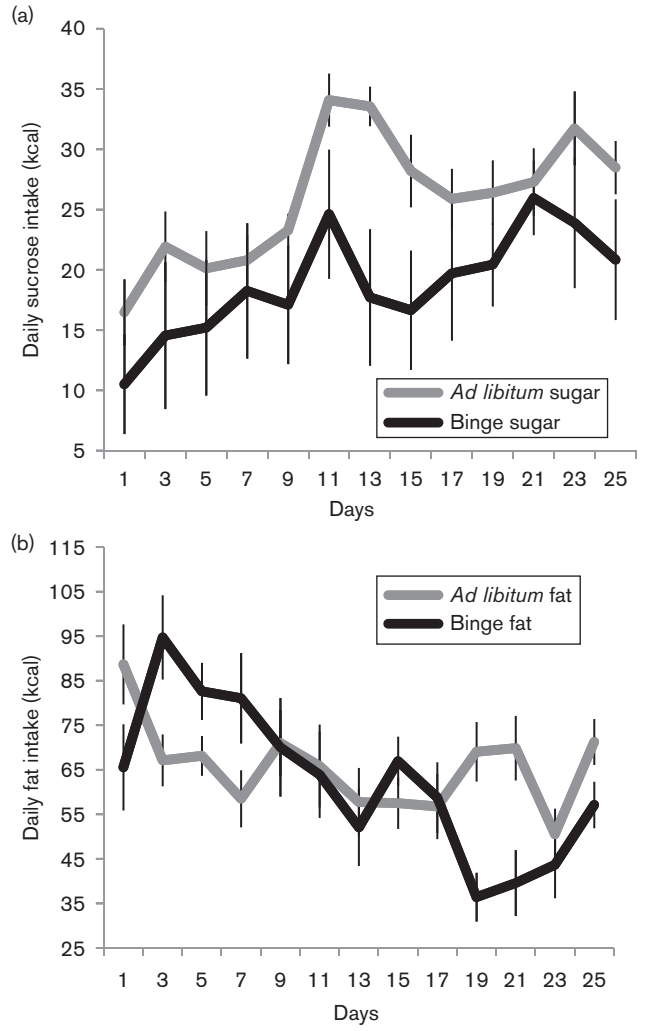
In experiment 3, microdialysis data were analyzed with a three-way repeated-measures ANOVA of dose (vehicle, drug), sucrose access schedule (binge, *ad libitum*), and time (−40, −20, 0, 20, 40, 6, 80, 100, 120 min). All four groups were included in the ANOVA. DA levels were normalized as percent of baseline. An additional one-way ANOVA was performed with raw data to compare groups' baseline DA values. In addition to the ANOVA, we calculated DA area under the curve and compared the binge group with vehicle and the binge group with drug using a Student's *t*-test.

Results

Preinjection palatable food and chow intake and body weight

Animals not consuming sucrose (<5 ml/daily) or shortening (<0.5 g/daily) were removed from analysis ($n = 1$ in the Binge Sugar group, $n = 2$ in the *Ad libitum* Sugar group, $n = 2$ in the Binge Fat group). Palatable food intake during the 25-day diet training period is shown in Fig. 2. For experiment 1, as described previously using this sugar binge model (Avena *et al.*, 2006a), rats increased intake over the access period, but intake leveled out in the final week (for the last week of training, there was no main effect of day [$F(6,96) = 0.68$, NS]). Similarly, over the last week of fat intake, before injections, intake was relatively stable, with no significant differences across days [$F(6,96) = 1.69$, NS]. On day 25 of the diet training, before injections, binge eating behavior, as defined by first-hour intake in the Binge group and intake during the equivalent time period in the *Ad libitum* group, was compared between groups. In experiment 1, the average sucrose intake during the first hour of access was

Fig. 2



Daily sugar (a) and fat (b) intake during the 25-day diet training period before injections. Error bars represent SEM.

significantly greater in the Binge group (13.3 ± 0.9 ml) than the *Ad libitum* group [8.8 ± 1.9 ml; $t(16) = 2.23$, $P < 0.05$; data not shown]. When sucrose consumption during the first hour was compared with overall daily intake, the Binge group consumed 22.7% of its daily intake during this time period, whereas the *Ad libitum* group consumed only 11.8%. There was no difference between groups in the total daily sugar intake on day 25 of diet training, the day before injections began [$t(17) = 1.36$, NS; Fig. 2]. In experiment 2, the average fat intake during the first hour was also significantly greater in the Binge group (2.14 ± 0.5 g) than the *Ad libitum* group [0.8 ± 0.3 g; $t(20) = 2.67$, $P < 0.02$; data not shown]. The Binge group consumed 32.9% of its daily fat intake during the first hour of access, whereas the *Ad libitum* group consumed only 16.9%. There was no difference between groups in the total daily fat intake the day before injections began [$t(20) = 1.53$, NS; data not shown].

In experiment 1, before injections, a difference in chow intake was observed at the first hour [$F(2,30) = 7.73$, $P < 0.01$; data not shown], with differences between the Binge Sugar and the *Ad libitum* Sugar groups ($P < 0.01$), as well as between the Binge Sugar and the *Ad libitum* Chow groups ($P < 0.05$), with the *Ad libitum*-fed groups consuming less chow than the Binge Sugar group. Differences in the total daily chow intake were also observed [$F(2,30) = 8.82$, $P < 0.002$; data not shown], with differences between the Binge Sugar and the *Ad libitum* Chow groups ($P < 0.01$), as well as between the *Ad libitum* Sugar and the *Ad libitum* Chow groups ($P < 0.005$), with the groups with sugar access consuming less chow than the *Ad libitum* Chow group. Before injections, no differences were found in the total daily energy intake [$F(2,30) = 0.35$, NS] or body weight between groups [$F(2,27) = 0.48$, NS; data not shown].

In experiment 2, before injections, differences in chow intake were also observed at the first hour [$F(2,32) = 11.23$, $P < 0.0001$; data not shown], with differences between the Binge Fat and the *Ad libitum* Fat groups ($P = 0.001$), as well as between the *Ad libitum* Chow and the *Ad libitum* Fat groups ($P = 0.001$). The *Ad libitum* Fat group consumed less chow in the first hour than the Binge Fat and *Ad libitum* Chow groups. There were also differences in the total daily chow intake [$F(2,32) = 54.10$, $P < 0.0001$; data not shown], with differences between the Binge Fat and the *Ad libitum* Chow groups ($P < 0.0001$), and between the *Ad libitum* Chow and the *Ad libitum* Fat groups ($P < 0.0001$). Both of the groups with access to fat consumed less chow than the *Ad libitum* Chow group. Before injections, no differences were found between groups in the total energy intake [$F(2,32) = 2.75$, NS] or body weight [$F(2,35) = 3.00$, NS; data not shown].

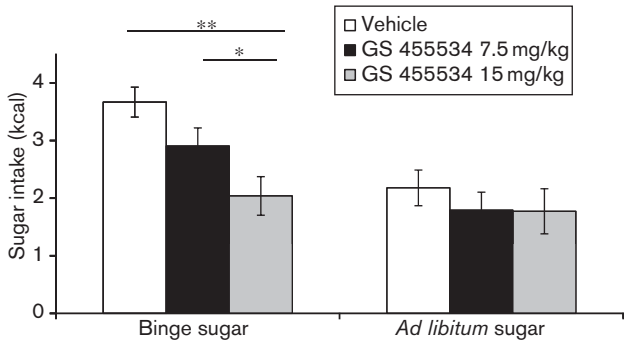
Experiment 1: GS 455534 selectively suppresses binge, not *ad libitum*, intake of sucrose

There was a decrease in sugar consumption during the first hour of intake in the Binge Sugar group following the administration of GS 455534 (Fig. 3). A follow-up ANOVA showed a significant reduction in the mean sugar intake in this group [$F(2,9) = 8.34$, $P < 0.005$]. Pair-wise comparisons showed a significant difference between vehicle and drug injection at 7.5 mg/kg ($P < 0.05$) and 15 mg/kg ($P < 0.002$). For the *Ad libitum* Sugar group, sugar intake was not significantly different at either dose compared with vehicle injection during this 1-h time period [$F(2,7) = 1.42$, NS]. Although there were no apparent effects of the drug at 2 h [$F(2,34) = 3.38$, $P = 0.05$; Table 1], at 4 h, there was an effect of the drug [$F(2,34) = 5.65$, $P < 0.01$], with pair-wise comparisons showing a decrease in sugar intake in the Binge Sugar group ($P < 0.05$) at the high dose compared with vehicle. No significant effect was observed in the *Ad libitum* Sugar group. Repeated-measures ANOVA showed a trend

similar to that observed during the first hour at 12 h. For the Binge Sugar group, GS 455534 significantly decreased sugar intake [$F(2,9) = 7.39$, $P < 0.005$], with no effect in the *Ad libitum* Sugar group [$F(2,7) = 2.11$, NS]. Post-hoc tests for the Binge Sugar group showed a significant difference between vehicle and the 7.5 mg/kg ($P < 0.05$) as well as between vehicle and the 15 mg/kg dose ($P < 0.01$) at 12 h. Post-hoc tests showed no effect of the drug at either dose on daily (24 h) sugar intake in the *Ad libitum* Sugar group [$F(2,16) = 1.30$, NS].

In the first hour, standard chow intake in the Binge Sugar group did not differ significantly between drug and vehicle treatments [$F(2,9) = 1.75$, NS; Table 2]. However, differences in chow intake were noted for the *Ad libitum* Sugar and *Ad libitum* Chow groups [$F(2,7) = 13.16$, $P < 0.001$ and $F(2,11) = 4.52$, $P < 0.02$, respectively].

Fig. 3



Sucrose intake during the first hour for each group in experiment 1. Both doses significantly reduced sucrose intake in the Binge Sugar group compared with vehicle. No effect was observed at either dose in the *Ad libitum* Sugar group. *$P<0.05$, **$P<0.01$. Error bars represent SEM.

Post-hoc Tukey's tests indicated a significant decrease in chow intake for the *Ad libitum* Sugar group (7.5 mg/kg, $P < 0.001$; 15 mg/kg, $P < 0.002$; both compared with vehicle). In the *Ad libitum* Chow group, a decrease was found in chow intake following treatment with the 15 mg/kg dose of GS 455534 compared with vehicle ($P = 0.014$). There was also a significant main effect of drug on the total daily chow intake [$F(2,58) = 17.03$, $P < 0.001$], interaction effect between drug and group [$F(4,58) = 7.24$, $P < 0.001$], and a main effect of group [$F(2,29) = 13.95$, $P < 0.001$]. Follow-up tests indicated a decrease in the total chow intake in the *Ad libitum* Sugar group [$F(2,18) = 19.71$, $P < 0.001$], as well as the *Ad libitum* Chow group [$F(2,22) = 12.02$, $P < 0.001$], but not the Binge Sugar group [$F(2,18) = 0.93$, NS, data not shown]. Repeated-measures ANOVA indicated an overall effect of the drug on the total daily energy intake [$F(2,56) = 26.09$, $P < 0.001$; Table 2], as well as an interaction effect between drug dose and group [$F(4,56) = 3.24$, $P < 0.02$]. Follow-up tests indicated a decrease in the total energy intake in the *Ad libitum* Sugar group [$F(2,16) = 13.12$,

Table 2 Average first-hour chow intake (kcal) and average daily energy intake (kcal) for experiment 1

	Vehicle	7.5 mg/kg	15 mg/kg
Average first-hour chow intake (kcal)			
Binge Sugar	16.3 ± 4.5	13.6 ± 3.9	12.7 ± 5.1
Ad libitum Sugar	7.9 ± 2.1	2.4 ± 2.4*	3.0 ± 2.4*
Ad libitum Chow	7.9 ± 4.2	6.0 ± 3.6	3.6 ± 2.7*
Average daily energy intake (kcal)			
Binge Sugar	80.9 ± 2.1	77.2 ± 2.2	77.4 ± 2.5
Ad libitum Sugar	90.0 ± 5.3	79.4 ± 4.5	75.8 ± 3.9*
Ad libitum Chow	79.0 ± 2.9	73.3 ± 2.5	65.6 ± 3.3*

First-hour chow intake and daily energy intake were significantly reduced by GS 455534 (15 mg/kg) compared with vehicle in all groups, except for the Binge Sugar group.
Error is SEM.
*$P<0.05$.

Table 1 Sugar and fat intake (kcal) at 1, 2, 4, 12, and 24 h after food was made available

	H1	H2	H4	H12	H24
Sugar intake					
Binge Sugar					
Vehicle	3.67 ± 0.26	4.95 ± 0.51	11.41 ± 1.08	22.79 ± 2.05	–
7.5 mg/kg	2.91 ± 0.31*	4.31 ± 0.53	9.56 ± 1.01	18.27 ± 1.52*	–
15 mg/kg	2.04 ± 0.33*	4.04 ± 0.57	8.53 ± 1.00*	16.24 ± 1.79*	–
Ad libitum Sugar					
Vehicle	2.18 ± 0.32	5.39 ± 0.88	10.41 ± 1.34	19.01 ± 2.44	27.96 ± 3.54
7.5 mg/kg	1.79 ± 0.31	3.88 ± 0.66	8.05 ± 0.98	16.55 ± 1.78	27.44 ± 3.07
15 mg/kg	1.77 ± 0.37	3.56 ± 0.77	7.55 ± 1.15	14.48 ± 1.99	26.30 ± 3.23
Fat intake					
Binge Fat					
Vehicle	19.56 ± 4.24	24.07 ± 5.07	31.09 ± 5.01	59.22 ± 5.88	–
7.5 mg/kg	18.83 ± 2.19	24.76 ± 3.96	34.95 ± 4.11	64.09 ± 4.26	–
15 mg/kg	9.45 ± 1.95*	16.34 ± 1.95	24.51 ± 2.13	**47.13 ± 2.56**	–
Ad libitum Fat					
Vehicle	7.45 ± 2.29	14.16 ± 2.29	19.10 ± 2.44	43.91 ± 4.14	71.25 ± 5.15
7.5 mg/kg	6.16 ± 1.50	10.88 ± 2.07	19.54 ± 3.46	**32.27 ± 4.12**	84.42 ± 6.01*
15 mg/kg	4.56 ± 1.14	8.03 ± 2.05*	14.59 ± 2.28	28.26 ± 3.35*	56.08 ± 3.32

Bold font indicates a significant difference ($P<0.05$) between the high and the low dose.
Error is SEM.
*Significant difference ($P<0.05$) compared with vehicle.

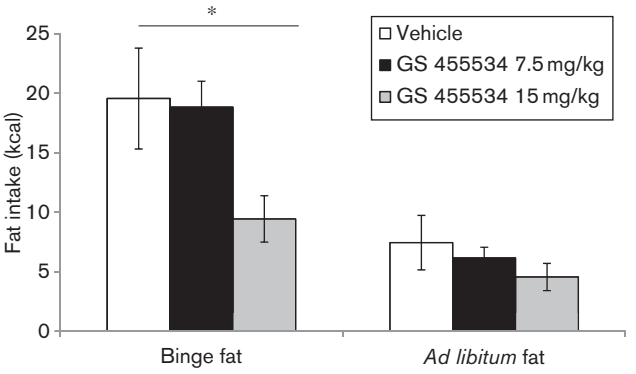
$P < 0.001$]. There was also a dose-dependent decrease in the total energy intake in the *Ad libitum* Chow group [$F(2,22) = 12.12$, $P < 0.001$]. No effect was observed on total energy intake in the Binge Sugar group [$F(2,18) = 2.89$, NS].

Experiment 2: GS 455534 suppresses binge intake of fat and reduces intake of chow

There was an effect of GS 455534 on fat intake in the Binge Fat group (Fig. 4). Repeated-measures ANOVA showed a within-subjects effect of dose on first hour fat intake [$F(2,40) = 6.08$, $P < 0.005$] as well as a between-subjects effect [$F(1,20) = 97.81$, $P < 0.001$]. Follow-up ANOVAs indicated statistically significant decreases in first-hour fat intake in the Binge Fat group [$F(2,29) = 3.66$, $P < 0.05$], but not in the *Ad libitum* Fat group [$F(2,35) = 0.84$, NS]. Pair-wise comparisons showed that for the Binge Fat group, differences were observed between the vehicle and the 15 mg/kg dose ($P < 0.05$). At 2 h, the *Ad libitum* Fat group showed a decrease in fat intake, compared with vehicle, when administered the high dose ($P < 0.05$), but no differences were noted at 4 h (Table 1). At 12 h, post-hoc tests showed an effect of drug on fat intake in the Binge Fat group [$F(2,18) = 5.46$, $P < 0.02$], with differences between the low and the high doses ($P < 0.005$). Post-hoc tests also found an effect of drug on fat intake in the *Ad libitum* Fat group at 12 h [$F(2,22) = 12.18$, $P < 0.001$], with significant differences between the high and the low doses ($P < 0.001$), as well as between vehicle and the high dose ($P < 0.005$). Follow-up ANOVA showed an effect of drug on 24 h fat intake in the *Ad libitum* Fat group [$F(2,33) = 9.80$, $P < 0.001$], with pair-wise comparisons indicating an increase in intake at the 7.5 mg/kg dose ($P < 0.05$), but no significant effect at the higher dose.

Repeated-measures ANOVA indicated dose-dependent effects of the drug on chow intake across all groups [$F(2,64) = 16.71$, $P < 0.001$; Table 3]. Follow-up ANOVA with pair-wise comparisons indicated that for the *Ad libitum* Chow group, chow intake was significantly decreased during the first hour when administered the 15 mg/kg dose compared with vehicle [$F(2,22) = 9.31$, $P < 0.001$]. Follow-up ANOVA with pair-wise comparisons further indicated that chow intake was decreased during the first hour in the *Ad libitum* Fat and Binge Fat groups when administered either the low or the high dose compared with vehicle [$F(2,18) = 8.80$, $P < 0.002$, and $F(2,18) = 44.24$, $P < 0.001$, respectively]. There was also a main effect of drug on the total daily chow intake [$F(2,64) = 192.12$, $P < 0.001$], a main effect of group [$F(2,32) = 105.95$, $P < 0.001$], and an interaction effect [$F(4,64) = 59.17$, $P < 0.001$]. Follow-up tests showed decreases in the total chow intake in the Binge Fat [$F(2,22) = 32.51$, $P < 0.001$], *Ad libitum* Fat [$F(2,20) = 19.24$, $P < 0.001$], and *Ad libitum* Chow groups [$F(2,22) = 14.27$, $P < 0.001$] (data not shown). Given the reductions in both fat and chow intake, a decrease in the total daily energy intake was also observed at the 15 mg/kg dose [$F(2,64) = 17.53$, $P < 0.001$; Table 3]. Although this effect was present among all groups ($P < 0.05$), it was most pronounced in the groups with access to fat.

Fig. 4



Fat intake during the first hour for each group in experiment 2. Fat intake was significantly decreased by the 15 mg/kg dose in the Binge Fat group compared with the vehicle. No effect was observed at either dose in the *Ad libitum* Fat group. *$P < 0.05$. Error bars represent SEM.

Table 3 Average first-hour chow intake (kcal) and average daily energy intake (kcal) for experiment 2

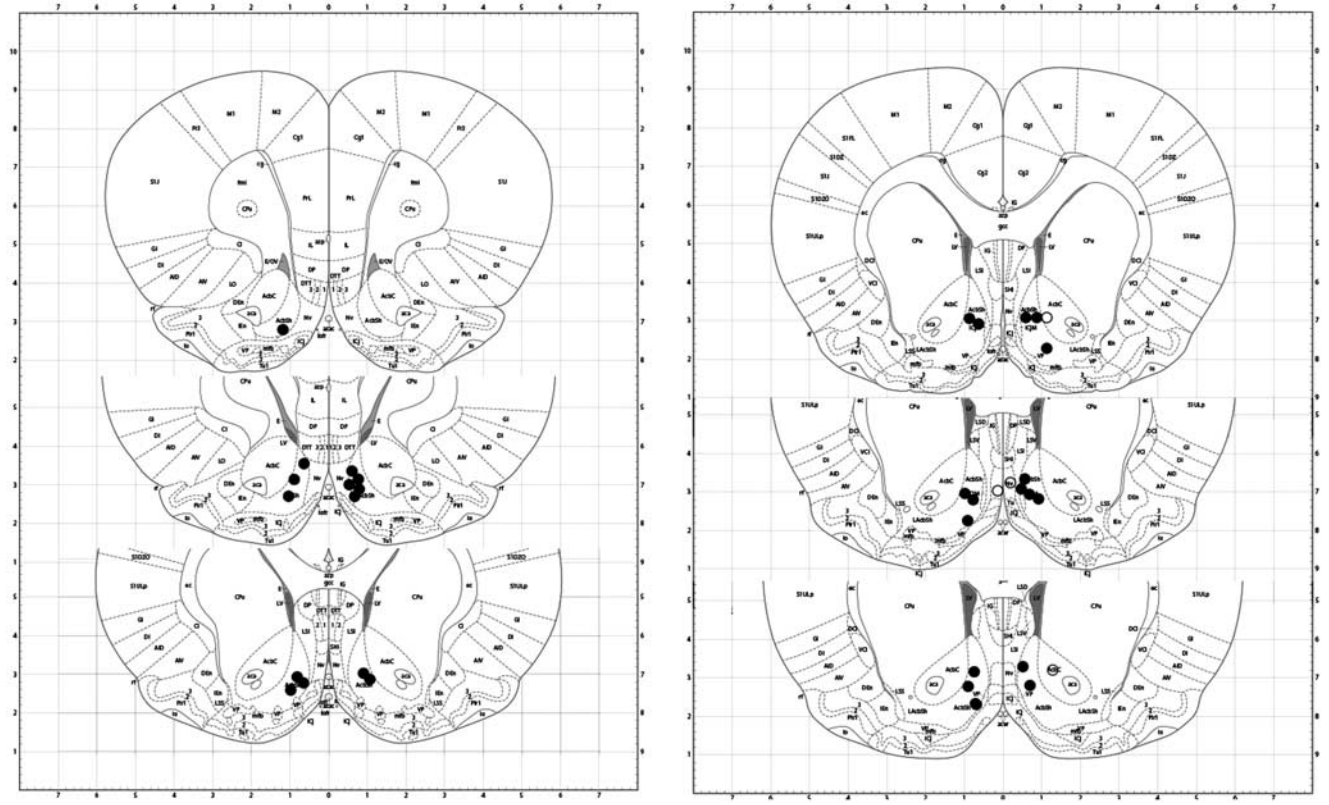
	Vehicle	7.5 mg/kg	15 mg/kg
Average first-hour chow intake (kcal)			
Binge Fat	11.5 ± 1.8	5.4 ± 0.9*	3 ± 0.9*
Ad libitum Fat	3.6 ± 1.2	0.9 ± 0.3*	2.1 ± 0.9*
Ad libitum Chow	12.1 ± 2.1	8.5 ± 2.7	4.2 ± 1.5*
Average daily energy intake (kcal)			
Binge Fat	90.8 ± 4.0	95.8 ± 3.8	69.6 ± 3.5*
Ad libitum Fat	109.7 ± 7.3	121.5 ± 6.4	83.4 ± 4.9*
Ad libitum Chow	108.9 ± 2.9	99.9 ± 4.0	90.3 ± 2.5*

First-hour chow intake and daily energy intake were significantly reduced by GS 455534 (15 mg/kg) compared with vehicle in all groups.
Error is SEM.
*$P < 0.05$.

Experiment 3: GS 455534 is associated with an attenuation of nucleus accumbens dopamine levels in sugar-bingeing rats

After histology, rats ($n = 4$) with microdialysis probe placement not in the NAc shell were removed from analysis (Fig. 5). A one-way ANOVA found no difference between baseline DA levels (mean ± SEM) of the *Ad libitum* vehicle (1.52 ± 0.12 nmol/l), *Ad libitum* drug (1.47 ± 0.10 nmol/l), Binge vehicle (1.51 ± 0.10 nmol/l), and Binge drug (1.46 ± 0.09 nmol/l) groups [$F(3,89) = 0.07$, NS]. A repeated-measures three-way ANOVA showed a significant three-way interaction between dose (vehicle, drug), sucrose access schedule (*ad libitum*, binge), and time (−40, −20, 0, 20, 40, 6, 80, 100,

Fig. 5



Histology. Open circles were removed from analysis ($n=4$).

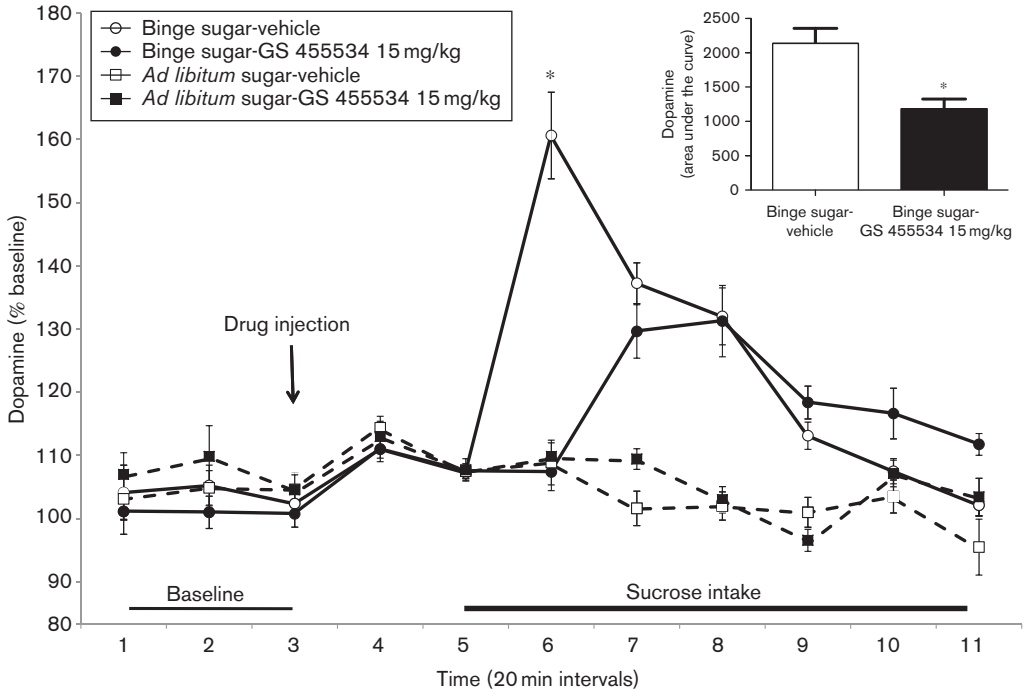
120 min) on DA levels (% baseline) in the NAc shell [$F(8,216) = 8.98$, $P < 0.001$]. In addition, there were significant interactions between sucrose access schedule and time [$F(8,216) = 18.49$, $P < 0.001$] and dose and time [$F(8,216) = 9.91$, $P < 0.001$], as well as significant effects of sucrose access schedule [$F(1,27) = 67.45$, $P < 0.001$] and time [$F(8,216) = 22.07$, $P < 0.001$] on extracellular DA levels in the NAc shell. The results showed significantly greater levels of extracellular DA in the NAc of the binge groups in response to drinking sucrose compared with the *Ad libitum* groups ($P < 0.05$; Fig. 6). GS 455534 significantly attenuated and delayed accumbal DA levels compared with those animals maintained on Binge Sugar and administered vehicle ($P < 0.05$; see inset of area under the curve). These effects on DA cannot be accounted for by differences in sucrose intake; on the microdialysis test day, no differences were observed in sucrose intake between drug and vehicle conditions [$F(3,32) = 1.31$, NS; Fig. 7]. Rats with *ad libitum* access to sucrose did not show a significant change in the extracellular levels of accumbal DA in response to drinking sucrose, and no differences in DA levels were observed between the *Ad libitum* Sucrose groups administered vehicle versus drug.

Discussion

In the current study, GS 455534 decreased the intake of both sugar and fat in binge eating rats at 1 h, but not in rats with *ad libitum* access to the same diets. Previous research from several laboratories has shown behavioral and neurochemical differences between binge eating and normal eating patterns [see the review by Corwin *et al.* (2011)]. Most notably, rats with a history of binge eating sugar show behavior and neurochemical states more often associated with drug addiction (Avena *et al.*, 2008b, 2011). Given the efficacy of GS 455534 in limiting the intake of some drugs of abuse (Arolfo *et al.*, 2009; Yao *et al.*, 2010), and the overlaps between binge eating and drug addiction, it is consistent that GS 455534 also decreases binge eating of palatable food.

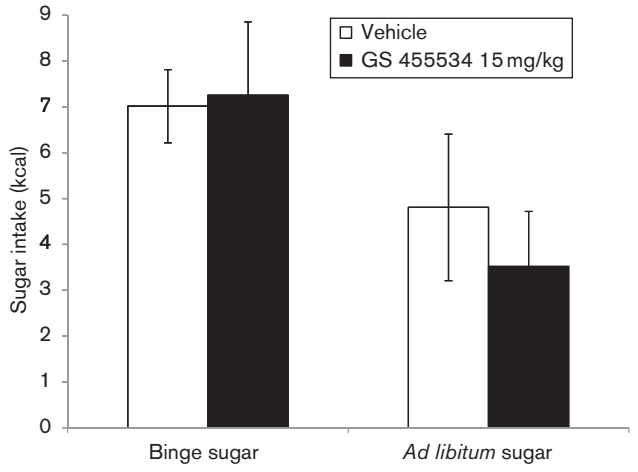
In experiment 1, both doses of GS 455534 resulted in decreased sugar intake at 1 h in the Binge Sugar group, an effect that was not observed in the *Ad libitum* Sugar group. This suppression of sugar intake was still apparent at 12 h in the Binge Sugar group, but, again, no significant effect was observed in the *Ad libitum* Sugar group. In addition, although the drug was associated with a decrease in both total daily chow and energy intake in both the *Ad libitum*

Fig. 6



Dopamine (DA) release, as % of baseline, at 2 h after injection and area under the curve during sucrose intake period (inset). Baseline DA levels (mean ± SEM) for each group were as follows: *ad libitum* vehicle (1.52 ± 0.12 nmol/l), *ad libitum* drug (1.47 ± 0.10 nmol/l), binge vehicle (1.51 ± 0.10 nmol/l), and binge drug (1.46 ± 0.09 nmol/l). GS 455534 significantly attenuated and delayed accumbal DA levels compared with those animals maintained on binge sugar and administered vehicle (see inset). *$P<0.05$. Error bars represent SEM.

Fig. 7



Sucrose intake during the 2 h access period after injection. No significant differences were found between drug and vehicle in the Binge Sugar or *Ad libitum* Sugar groups. Error bars represent SEM.

Sugar and Chow groups, this was not found in the Binge Sugar group. These findings indicate that GS 455534 selectively reduced sugar intake in the Binge Sugar group.

In experiment 2, the high dose of GS 455534 reduced fat intake at 1 h in the Binge Fat group, whereas no significant effect was found in the *Ad libitum* Fat group at this time. Daily fat intake was also reduced in the Binge Fat group at the high dose. Interestingly, daily fat intake was increased in the *Ad libitum* Fat group at the low dose, with no effect at the high dose. In contrast to the findings of experiment 1, the total daily chow and energy intake were reduced in all three groups. These results show that although fat intake was reduced in the Binge Fat group at 1 h, but not the *Ad libitum* Fat group, indicating a selective reduction of binge consumption of fat, the effect of GS 455534 was not selective for palatable food in this experiment. Rather, this compound resulted in a general suppression of food intake. The reason for this is unclear; however, it does not seem to be because of sickness in response to the drug as previous studies indicate that the drug leads to increased social interaction and has no effect on locomotor activity (Overstreet *et al.*, 2009) and decreases in either would indicate malaise. As our laboratory has suggested previously, it is possible that binge consumption of sugar affects the brain differently to not only normal eating, but also binge eating of fat (Berner *et al.*, 2009; Bocarsly *et al.*, 2011; Avena *et al.*, 2012). Future studies would benefit from examining the effects of GS 455534 on diets that

include both sugar and fat, which are more representative of the human diet. Given the marked overall decrease in the total energy intake observed in every group except the Binge Sugar group following drug administration, it is possible that GS 455534 might be an effective weight-control drug for certain individuals; however, this hypothesis requires further investigation.

It has been suggested that GS 455534 decreases drug intake by operating on the brain DA system. Binge eating sugar, similar to cocaine use, has been shown to increase extracellular DA in the NAc (Rada *et al.*, 2005). This was replicated in experiment 3, where binge, but not *ad libitum*, intake of sugar, resulted in an increase in extracellular DA levels in the NAc following vehicle injection. However, after the injection of GS 455534, the increase in DA in response to sugar intake in the binge eating rats was delayed and attenuated. It appears that GS 455534 only attenuated NAc DA release in sugar-bingeing rats 20 min following sugar access; however, this is normally when DA is most markedly increased (Rada *et al.*, 2005), making this time period an important target for intervention. It should be noted that sugar-bingeing rats show a less pronounced increase in NAc DA compared with rats administered drugs of abuse. For example, Pontieri *et al.* (1995) have found cocaine at 0.5 mg/kg (intravenous) to increase DA in the NAc shell approximately 200% of baseline, whereas in the current study, we observed an increase of approximately 160% in sugar-bingeing rats. These data indicate that although the two may share a number of similar effects, drugs of abuse appear to affect the DA system to a greater extent than sugar bingeing.

A mechanism has been suggested that may explain how GS 455534 acts to suppress DA levels (Yao *et al.*, 2010). Cocaine consumption results in increased extracellular DA. This activates DA D2 autoreceptors and leads to a downstream activation of tyrosine hydroxylase and increased DA synthesis. DA is transformed into DOPAL, which is a substrate for ALDH-2; however, by inhibiting ALDH-2, DOPAL can condense with DA and form tetrahydropapaveroline, which inhibits activated tyrosine hydroxylase, resulting in decreased DA production (Yao *et al.*, 2010). It has been suggested that GS 455534 is effective in limiting cocaine intake because of its ability to reduce cocaine-associated and craving-associated increases in DA release. Moreover, GS 455534 does not affect basal DA levels, suggesting specificity for addictive behaviors. Possibly the decrease in NAc DA release in sugar-bingeing rats observed in the current study is a result of this same mechanism. Future research may benefit from replicating the results found here as well as exploring other biological variables implicated in addiction or binge food consumption aside from, or in addition to, DA within the NAc to determine whether GS 455534 may also be acting by other mechanisms.

It is notable that GS 455534 attenuated binge sugar intake in experiment 1, but not in experiment 3, when microdialysis samples were simultaneously being collected. There are several reasons for the difference in results. First, the microdialysis testing may have been stressful for the animals (i.e. being tethered and not in their home cage), which may have influenced intake. Second, in experiment 1, rats had three days to acclimate to handling and injection procedures before drug injections, whereas in experiment 3, drug injections were administered without a period of acclimation, perhaps creating additional stress for the animals.

Conclusion

The results presented here extend the findings of previous studies showing that GS 455534 reduces consumption of drugs of abuse to binge eating. Given the observed effects of GS 455534 on binge consumption of palatable foods, this drug might be effective in treating binge eating behavior among individuals with BN and BED. The observed decreases in the daily energy intake in all of the *ad libitum*-fed groups (*Ad libitum* Sugar, Fat, and Chow) as well as the Binge Fat group suggest that GS 455534 might also be effective in certain cases of obesity.

Acknowledgements

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Conflicts of interest

Drs Maria P. Arolfo, Lina Yao, and Ivan Diamond are employed by Gilead Sciences, who funded the research. For the remaining authors there are no conflicts of interest.

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