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Baclofen, raclopride, and naltrexone differentially reduce solid fat emulsion intake under limited access conditions

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Abstract

Previous work in rats has demonstrated that an Intermittent (Monday, Wednesday, Friday) schedule of access promotes binge-type consumption of 100% vegetable shortening during a 1-h period of availability. The present study used novel shortening-derived stable solid emulsions of various fat concentrations. These emulsions were the consistency of pudding and did not demonstrate oil and water phase separation previously reported with oil-based liquid emulsions. Male Sprague–Dawley rats were grouped according to schedule of access (Daily or Intermittent) to one of three concentrations (18%, 32%, 56%) of solid fat emulsion. There were no significant Intermittent vs. Daily differences in amount consumed, due to high intakes in all groups. This indicated the acceptability of the emulsions. Baclofen ($GABA_B$ agonist) and raclopride (D2-like antagonist) both significantly reduced emulsion intake in all Daily groups, but only in the 56% fat Intermittent group. Naltrexone (opioid antagonist), in contrast, significantly reduced 32% and 56% fat emulsion intake in the Intermittent, as well as the Daily groups. These results indicate that the fat intake-reducing effects of $GABA_B$ activation and D_2 blockade depend upon fat concentration and schedule of fat access, while the fat intake-reducing effects of opioid blockade depend upon fat concentration but not schedule of access.

Keywords: Binge-type behavior; Binge eating; Dopamine; D2 receptors; Fat emulsions; Fat intake; $GABA_B$; Opioid

1. Introduction

A behavioral model of binge-type eating in rats has been developed over the past few years in which an Intermittent (Monday, Wednesday, Friday or every third day) schedule of access to 100% vegetable shortening provided during a brief (1–2 h) period of availability promotes excessive intakes relative to a Daily schedule of access (Corwin, 2004; Corwin and Buda-Levin, 2004; Corwin et al., 1998; Davis et al., 2007; Dimitriou et al., 2000; Thomas et al., 2002). It has also been shown that under an Intermittent schedule of access rats consume as much shortening during the brief period of availability as is consumed in a 24-h period when rats have continuous shortening access (24-h per day — 7 days/week) (Dimitriou et al., 2000).

While the use of 100% vegetable shortening provides a way to model bouts of excessive fat consumption, foods upon which people binge typically contain lower amounts of fat, ranging from ~18% (cookies, some ice creams) to ~32% (chocolate candy) of the weight of the food. Developing the means to use solid fats of different concentrations, therefore, would provide a new and clinically relevant tool for use in the study of binge-type behavior. Recent research has shown that when liquid sucrose is provided on an Intermittent or Daily schedule of access, the occurrence of binge-type behavior depends upon the sucrose concentration (Wojnicki et al., 2007). In addition, intermittent brief access to a high-fat (~38%) chow did not promote binge-type consumption, relative to intakes induced by daily brief access to the same chow in otherwise non-food-deprived rats (Davis et al., 2007). Taken together, these reports suggest that the concentration of fat in the binge food may be critical to the expression of binge-type eating in rat behavioral

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models of binge eating. Therefore, one goal of the present study was to assess the effects of different concentrations of solid shortening on binge-type eating.

Previous work has shown that both the form and type of fat can affect intake (Lucas et al., 1989). While gelatinized emulsions are one way to create solid shortening emulsions, the texture of gels is different from that of shortening itself. The present study developed and utilized stable solid emulsions of three different shortening concentrations (18%, 32% and 56%) that had the consistency of moist whipped shortening (like icing or pudding) across all concentrations.

Limited progress has been made in understanding the neurological controls that distinguish binge consumption from that of normal consumption of palatable foods. Previous work has shown that baclofen, a gamma-aminobutyric $acid_B$ ($GABA_B$) agonist, reduces intake of 100% vegetable shortening under limited access conditions. While baclofen was equally effective in "binge" and "non-binge" groups, the same study showed no effect of baclofen on intake of a continuously available fat-matched chow (~15% fat by weight, or ~30% fat by energy), suggesting the importance of the manner in which the fat is consumed and/or its concentration (Buda-Levin et al., 2005).

The food intake-reducing effects of baclofen appear to be specific to fat, as baclofen had no effect on sucrose intake under limited access conditions (Corwin and Wojnicki, 2006). In addition, another study showed that baclofen reduced fat-maintained lever pressing at a lower dosage than was necessary for reductions in chow-maintained lever pressing (Wojnicki et al., 2006). Previous research, therefore, suggests that $GABA_B$ receptor activation can selectively reduce intake of and responding for fat under limited access conditions. The use of solid emulsions of different fat concentrations in the present study made it possible to determine the importance of fat concentration to the observed effects of baclofen.

In addition to GABAergic effects, dopamine signaling has also been implicated in fat intake in binge as well as non-binge protocols. One study, which made use of the sham-feeding binge protocol, showed a dose-related inhibition of 100% corn oil sham-feeding with peripheral administration of the D_2 antagonist raclopride, but not the D_1 antagonist SCH23390 (Weatherford et al., 1990). More recent work using real-feeding has shown that higher dosages of peripherally administered raclopride decrease intake of powdered chow mixed with shortening (33% shortening by weight), while lower dosages stimulate intake (Baker et al., 2001). In that same study, the D_1 antagonist SCH23390 had no effect on consumption of the high-fat chow. Finally, recent work using our limited access binge protocol indicates that SCH23390 only reduces intake of 100% shortening at dosages that also suppress intake of chow (Corwin and Wojnicki, 2006). This indicates that the intake reductions were due to non-specific motor impairment. In contrast, raclopride had more fat-specific effects (Corwin and Wojnicki, 2006). Taken together, the available evidence indicates that fat intake can be modulated by peripheral injections of D_2, but not D_1, receptor antagonists, and that these effects are not explained by generalized motor disruption.

Opioid signaling also can modulate high-fat feeding. Studies have shown mu-opioid antagonism reduces short-term intake of high-fat food as well as corn oil (Islam and Bodnar, 1990; Mizushige et al., 2006). In a binge protocol in which combination foot-shock stress and dietary restriction induce binge eating, mu-opioid antagonism suppressed binge consumption of a high-fat food (Boggiano et al., 2005). In addition, at least one study has shown a greater potency for the fat intake-reducing effects of mu-opioid blockade relative to carbohydrate intake-reducing effects, when the foods being compared were both highly palatable (chocolate vs. sugar-coated cereal) and equally preferred (Hagan et al., 1997). It has been suggested that the mu-opioid modulation of high-fat feeding operates via a signaling pathway that is independent of dopamine (Will et al., 2006). Systematic comparisons of mu-opioid effects on consumption of different concentrations of solid fat have not been reported.

The present study was designed to assess: 1) intake of various concentrations of solid fat emulsions under conditions of limited availability, and 2) the effects of $GABA_B$, D_2, and opioid ligands on this intake.

2. Methods

2.1. Preparation of solid fat emulsions

All solid fat emulsions consisted of tap water, Ticaloid 103-S Mayo (Tic Gums, Belcamp, MD), bovine sodium caseinate solution (Sigma Aldrich, St. Louis, MO; 3.88 kcal/g) and solid vegetable shortening (Crisco® All-Vegetable shortening, J.M Smucker Co., Orrville, OH; 9.17 kcal/g). The Ticaloid 103-S Mayo [2.48/kcal/g (metabolizable energy)], a non-commercially available developmental product, contained starch, microcrystalline cellulose, agar and xanthan gum. According to the manufacturer's specifications it contained (per 100 g) 270 mg of sodium, 45 mg of potassium, 19 mg of calcium, and 87 g of total carbohydrate (11 g soluble dietary fiber, 14 g insoluble dietary fiber, 0 g simple carbohydrates, and 62 g complex carbohydrates).

Each solid fat emulsion was created by combining two sets of products. The first product was prepared by mixing tap water with the Ticaloid 103-S Mayo and heating to 85 °C for 2 h to allow it to fully hydrate and form a thick paste. The second product was prepared by dissolving the sodium caseinate in warm water, adding melted vegetable shortening to the dissolved sodium caseinate and homogenizing for 2 min using a high speed mixer. Note that at this temperature, vegetable shortening is fully liquid and can therefore be readily homogenized to form a stable oil-in-water emulsion. This second product was a turbid liquid with a texture similar to milk or cream. Finally, the hydrated Ticaloid 103-S Mayo and oil-in-water emulsion were combined, blended and then placed in a drying oven for 2 h at 85 °C. The product was then stored overnight at 4 °C to allow it to set, resulting in a pudding-like consistency. The appearance and texture of the product did not appear to depend on the amount of fat and was stable over the course of the experiment. See Table 1 for the contribution

Table 1
The energy derived from shortening, sodium casein, and gum represent fat, protein, and carbohydrate, respectively

Emulsion	Energy density (kcal/g emulsion)	Shortening (wt.%, energy)	Sodium casein (wt.%, energy)	Gum (wt.%, energy)
18%	1.8	18, 89.8	0.6, 1.3	6.6, 8.9
32%	3.1	32, 94.7	0.94, 1.2	5.2, 4.2
56%	5.2	56, 97.9	1.86, 1.4	1.6, 0.8

of each ingredient to the mass and energy of the different emulsions.

All emulsions were refrigerated when not in use, and prior to each daily session each emulsion was brought to room temperature. Fat emulsions were presented in glass jars (6.5 cm diameter) placed in stainless steel clips attached to the front of the home cage to prevent tipping and spillage. After each session, the emulsions were collected and refrigerated for the next day's use. Fresh emulsion was made each week.

2.2. Animals

Sixty male Sprague–Dawley rats, 60 days of age and weighing 266–300 g (277.68 +/− 0.9 g) (Harlan, Indianapolis, IN) at the start of the study, were housed in individual hanging stainless steel wire cages in a temperature- and humidity-controlled facility under a 12:12 light:dark cycle. Throughout the study, all rats were provided free access to a nutritionally complete, pelleted, commercial laboratory rodent chow (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN; percent of calories as protein: 28.05%, as fat: 12.14%, as carbohydrate: 59.81%; 3.3 kcal/g) placed in hanging stainless steel feeding hoppers at the front of the cage. All rats were allowed a two-week adaptation period to the animal colony before the start of the study. Chow and tap water were available ad libitum throughout the study, i.e., the rats were never food-deprived. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

During the first two weeks of adaptation to the vivarium, chow intake was measured on a daily basis, and body weights were determined three times per week. Prior to the start of the experiment, six groups of 10 rats each were matched by body weight and average amount of daily chow consumed during the last 3 consecutive 24-h periods [$F(5,54) < 1$, NS for all].

2.3. Procedure

After matching of the groups, all rats were given 4-h access to their designated fat emulsion to prevent the effects of neophobia on subsequent emulsion intake. Two groups of rats were provided an 18% solid fat emulsion, two groups a 32% solid fat emulsion, and two groups a 56% solid fat emulsion. Within each of these concentrations two groups of 10 rats each were again matched for emulsion intake, average chow intake and body weight [p NS for all] and assigned to either a Daily or Intermittent schedule of access for the remainder of the study. Rats on the Daily schedule of access (D-18%, D-32%, and D-56%, $N = 10$ each) were provided 1-h of availability to their assigned solid fat emulsion every day of the week. Rats on the Intermittent schedule of access were provided 1-h of availability to their assigned solid fat emulsion on Mondays, Wednesdays, and Fridays only (I-18%, I-32%, and I-56%, $N = 10$ each). These schedules were chosen based upon studies in which intermittent access induced binge-type consumption of 100% solid vegetable shortening (Corwin, 2004; Corwin et al., 1998; Dimitriou et al., 2000; Thomas et al., 2002; Wojnicki et al., 2006).

Rats were then placed on their assigned access schedules for a period of five weeks to establish a baseline before drug dose–effect curves were determined. Previous research has shown binge-type behavior to develop and become stable within this time frame (Corwin, 2004; Corwin et al., 1998; Dimitriou et al., 2000; Thomas et al., 2002). Chow consumption was monitored in addition to the grams of emulsion consumed during the 1-h period of availability. Chow intake during the 1-h period of emulsion availability served as a control for the behavioral specificity of the drugs tested. Rats were weighed on Thursdays and Sundays throughout the study.

2.4. Drugs

All rats received all drug dosages; dosages were assigned to each rat using a uniform Latin square. All rats received injections

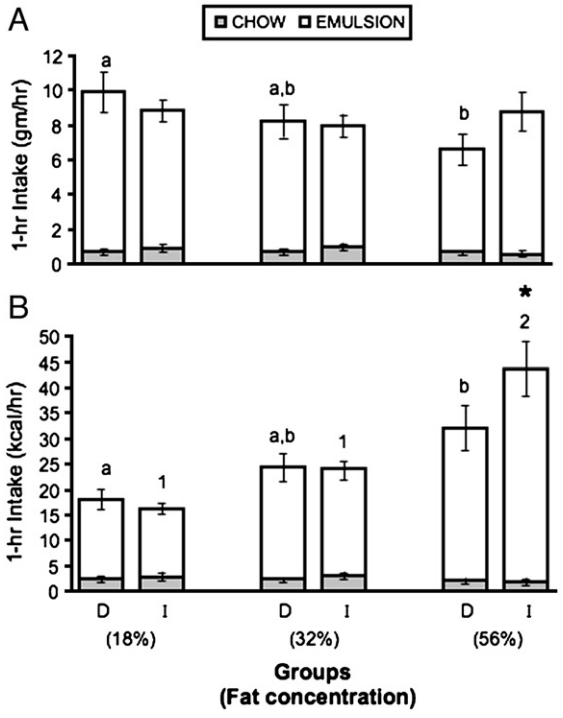

Fig. 1. 1-h intake in grams (A, top) and energy (B, bottom) for all three solid fat emulsions and simultaneously available chow. Different letters indicate significant differences in emulsion intakes among the Daily groups while different numbers indicate significant differences in emulsion intakes among the Intermittent groups. Vertical lines represent Standard Error of the Mean (SEM). The asterisk indicates a significant difference in emulsion intake between the D-56% and I-56% group. D = Daily, I = Intermittent; 18%, 32%, and 56% fat concentrations.

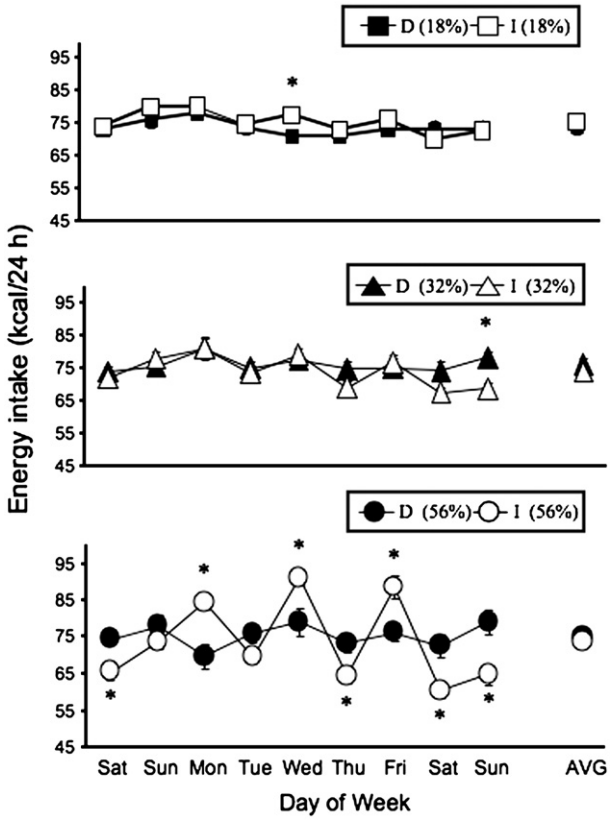

Fig. 2. 24-h total daily energy (fat emulsion plus chow) intake over the course of week 5. Significant Intermittent vs. Daily differences indicated with an asterisk.

on Mondays and Fridays with Wednesdays serving as non-drug baseline days to assess intake stability. The following drugs (Tocris, Ellisville, MO) were dissolved in saline and administered intraperitoneally (IP) in a volume of 1 ml/kg: the $GABA_B$ agonist (*R*-*S*)-baclofen (0.0 (vehicle), 0.6, 1.0, and 1.8 mg/kg, 30-min pre-treatment), the D_2-like antagonist raclopride (0.0 (vehicle), 0.03, 0.1, and 0.3 mg/kg, 20-min pre-treatment), and the opioid antagonist naltrexone (0.0 (vehicle), 0.03, 0.1, and 0.3 mg/kg, 20-min pre-treatment). These drugs and dosages were selected based upon previous reports of their effects on palatable food intake (Baker et al., 2001; Buda- Levin et al., 2005; Stein et al., 2000), as well as a recent report describing effects on binge-type consumption of 100% vegetable shortening (Corwin and Wojnicki, 2006).

2.5. Statistics

SAS v.9.1 (SAS Institute, Cary, NC) was used to analyze all data. Body weight change during the pre-drug intake period (week 5 body weight − week 1 body weight), as well as average 1-h gram and energy intakes during the emulsion access period in the week prior to the initiation of drug testing (week 5; see Fig. 1) was analyzed using a 2-way ANOVA (concentration × access) followed by pre-planned LS mean comparisons with a Bonferroni correction applied; for the LS mean comparisons, alpha was set at 0.0167 ($p = 0.05/3$ comparisons per mean). Within each concentration "bingeing" was operationally-defined as having occurred when 1-h emulsion intakes of the Intermittent groups significantly exceeded that of the respective Daily groups. Daily 24-h intakes during the week prior to initiation of drug testing (week 5; see Fig. 2) were compared within each concentration using independent *t*-tests to determine differences between the Intermittent and Daily groups. Baclofen, raclopride, and naltrexone data (Figs. 3–5) were analyzed using a 3-way ANOVA (access schedule × dosage × fat concentration), followed by one-way within group ANOVA and Tukey's HSD to determine the effect of dosage on intake within each group. For Tukey's HSD, alpha was set at 0.05. Stability of emulsion intake was analyzed using a 1-way repeated measures ANOVA and Tukey's HSD to determine differences across baseline days with intake expressed as kcal/body weight$^{0.67}$

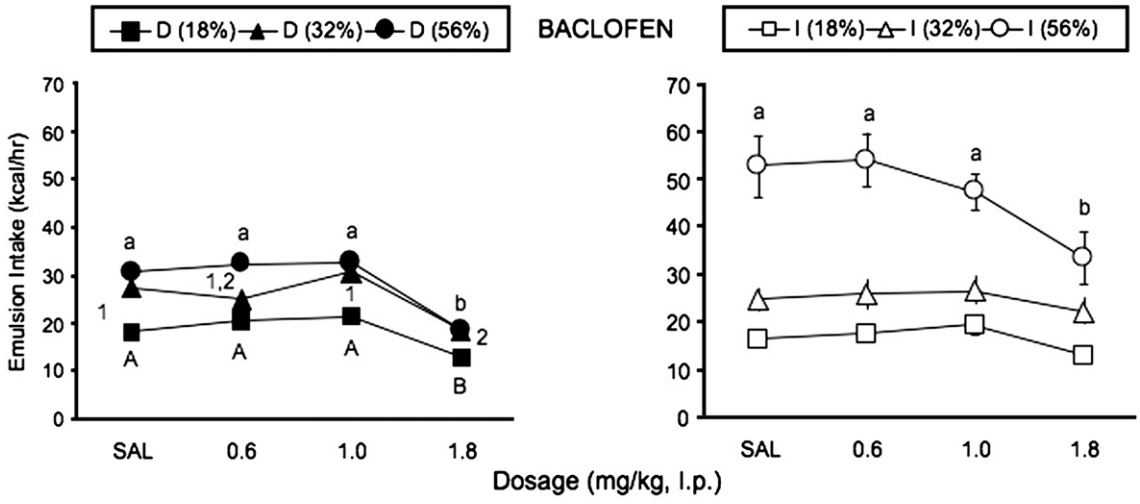

Fig. 3. Effects of baclofen on 1-h solid fat emulsion intake. 18% fat concentration groups are marked with capital letters; 32% fat concentration groups are marked with numbers; and 56% fat concentration groups are marked with lower-case letters. Different letters or numbers represent significant differences among dosages. Vertical lines represent Standard Error of the Mean (SEM).

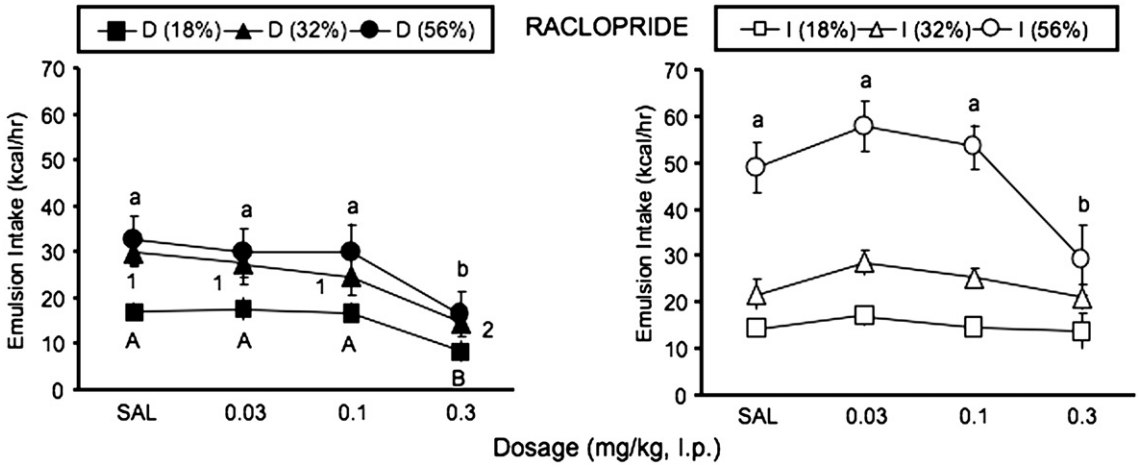

Fig. 4. Effects of raclopride on 1-h solid fat emulsion intake. Symbols are as described for Fig. 3.

(Heusner, 1985). If F was <1, results were not significant and p-values are not reported.

3. Results

3.1. Pre-drug data

The average 1-h emulsion and chow intakes expressed in grams for each of the groups during week 5 of exposure are depicted in Fig. 1, panel A. There were no main effects of access schedule or of fat concentration. Regardless of the fat concentration of the emulsion, binge-type emulsion intake was not observed within each concentration, i.e. there were no significant differences between the Daily and Intermittent groups within each of the fat concentrations, although at the 56% concentration the difference between Intermittent and Daily groups was marginally non-significant ($p<0.08$). Although the ANOVA showed no main effect of fat concentration, pre-planned LS mean comparisons revealed that the D-56% group consumed significantly less emulsion than did the D-18% group ($p<0.0167$, LS means). Emulsion intakes among concentrations did not differ in the Intermittent groups. 1-h chow intake was not statistically different among the groups. The total gram intake (emulsion plus chow) reflected the emulsion intake, i.e., the D-56% group consumed significantly less than did the D-18% group ($p<0.0167$, LS mean comparisons).

The average 1-h energy intake for week 5 from emulsion and chow are depicted in Fig. 1, panel B. With respect to 1-h emulsion intake alone there was a main effect of fat concentration [$F(2,54)=22.29$; $p<0.0001$] due to the elevated intake of emulsion in the I-56% and D-56% groups relative to the I-18% and D-18% groups, respectively ($p<0.0167$, LS means). In addition, the I-56% group consumed significantly more energy from the emulsion than did the D-56% group ($p<0.0167$, LS means). With respect to 1-h energy intake from

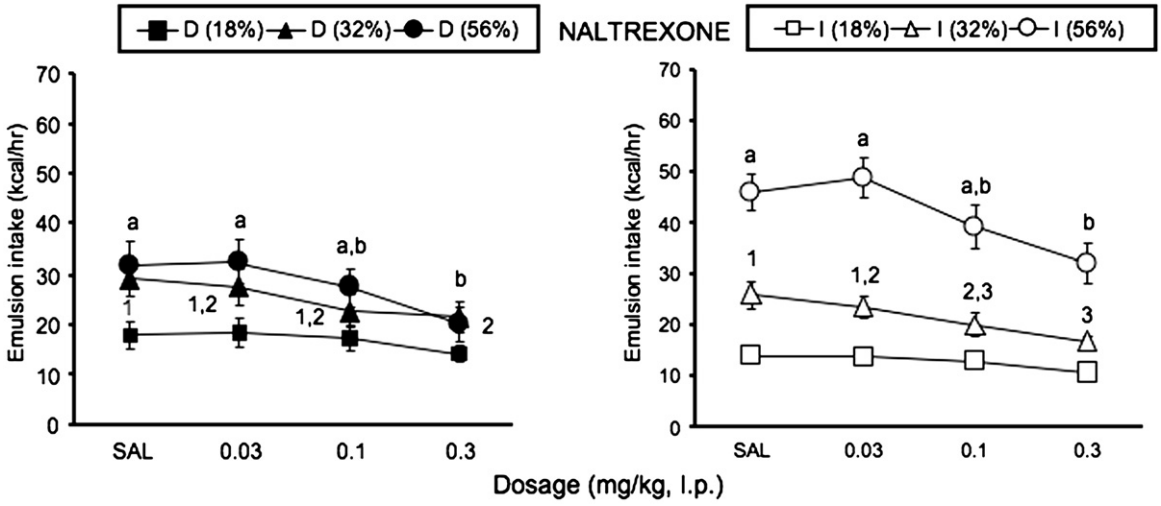

Fig. 5. Effects of naltrexone on 1-h solid fat emulsion intake. Symbols are as described for Fig. 3.

chow alone there were no statistically significant differences among groups. Total 1-h energy intake (chow plus emulsion) reflected the emulsion intake. That is, total 1-h energy intake was significantly affected by emulsion concentration [main effect of concentration: $F(2,54)=24.82$; 22.83; $p<0.0001$], due to the significantly elevated intakes of the 56% fat groups ($p<0.0167$, LS mean comparisons). The only differences between Intermittent and Daily intakes occurred in the 56% fat concentration groups; the I-56% group ate significantly more total 1-h energy than did the D-56% group ($p<0.0167$, LS means).

There were no significant differences in the average total weekly energy intakes among the groups (Fig. 2, avg symbol). However, the daily energy intake patterns differed across fat concentrations and schedules of access. While I-18% and I-32% groups showed no statistically reliable over-eat/under-eat pattern relative to their respective Daily groups, the I-56% group over-ate on the days they received emulsion, and under-ate on the non-emulsion days, relative to the D-56% group. Significant differences between the I-56% and D-56% groups (independent t-test, $p<0.05$) occurred on seven of the nine measurement days. A significant difference between Intermittent and Daily groups occurred only on one day (independent t-test, $p<0.05$) in the 18% and 32% fat concentration groups.

There were no significant differences in body weight or in body weight change among the groups.

3.2. Baclofen

Baclofen had differential effects in the Intermittent and Daily groups. Overall, there was a main effect of group [$F(1,54)=5.45$, p 0.02], fat concentration [$F(2,54)=28.41$ p 0.0001] and a group by concentration interaction [$F(2,54)=8.41$ p 0.0007]. There was also a main effect of baclofen dosage [$F(3,162)=24.90$, p 0.0001], and a dose by concentration interaction [$F(6,132)=3.60$ p 0.0022]. More specifically, baclofen significantly decreased emulsion intake in each of the Daily groups, at the 1.8-mg/kg dosage ($p<0.05$, Tukey's HSD, Fig. 3). However, in the Intermittent groups, a significant decrease was observed at the 1.8-mg/kg dosage only for the I-56% group ($p<0.05$, Tukey's HSD). There was no effect of baclofen on emulsion intake in the I-18% or I-32% groups.

In contrast to its effects on emulsion intake, baclofen significantly stimulated intake of the simultaneously available chow (main effect of dosage [$F(3,162)=16.96$, p 0.0001]) in some of the groups, while having no effect on chow intake in other groups. Specifically, the 1.8 mg/kg dosage stimulated chow intake in the D-32%, D-56%, and I-18% groups (Tukey's HSD p 0.05), but had no effect in the remaining groups. Thus, baclofen reduced emulsion intake at dosages that either stimulated or had no effect on chow intake, i.e. effects were not due to generalized behavioral suppression.

3.3. Raclopride

The effects of raclopride on emulsion intake were similar to those of baclofen (Fig. 4). Overall, there was a main effect of raclopride dosage [$F(3,162)=21.14$, p 0.0001] as well as a significant group by concentration interaction [$F(2,54)=3.22$, p 0.0477]. Specifically, raclopride significantly decreased shortening intake in each of the Daily groups, at the 0.3 mg/kg dosage ($p<0.05$, Tukey's HSD). In the Intermittent groups, however, a significant decrease was observed at the 0.3-mg/kg dosage only for the I-56% group ($p<0.05$, Tukey's HSD). There was no effect of raclopride in the I-18% or I-32% groups. Thus, raclopride had differential effects in the Intermittent and Daily groups consuming the 18% and 32% fat emulsions, a finding identical to the effects of baclofen.

Raclopride had no significant effect on simultaneously available chow intake in five of the groups. However, at the 0.3 mg/kg dosage chow was significantly decreased in the D-32% group relative to saline (Tukey's HSD, p 0.05). This indicates that, in all but the D-32% group, the ability of raclopride to reduce emulsion intake was not due to generalized behavioral suppression.

3.4. Naltrexone

The effects of naltrexone on emulsion intake were related to fat concentration, but not access schedule. Overall, there was a main effect of concentration [$F(2,54)=28.22$, $p=0.0153$] and a group by concentration interaction [$F(2,54)=7.2$, p 0.017]. There was also a main effect of naltrexone dosage [$F(3,162)=26.47$, $p<0.0001$], as well as a significant dose by concentration [$F(2,54)=4.10$, $p=0007$] interaction. More specifically, naltrexone significantly decreased emulsion intake in both the Daily and Intermittent 56% fat groups at the 0.3 mg/kg dosage and in both the Daily and Intermittent 32% fat groups at the 0.1 and 0.3 mg/kg dosages (Tukey's HSD p 0.05). In contrast, naltrexone had no effect on emulsion intake in either the Daily or Intermittent 18% fat groups (Tukey's NS, Fig. 5). Naltrexone had no significant effect on intake of the simultaneously available chow in any of the groups, again indicating no generalized behavioral suppression.

3.5. Effects across test periods

In comparing emulsion intakes throughout the drug testing period, there were no differences across the Wednesday control days within each of the groups, i.e., there were no shifts in the Wednesday control baseline emulsion intakes as a function of drug testing, thus indicating no carry-over effects of the drugs. Furthermore, in comparing emulsion intakes on the Wednesday control days for each of the drugs to the respective saline intakes only two differences emerged. For the D-32% group emulsion intakes during the Wednesday control days during baclofen testing were significantly greater than emulsion intakes during the saline days, indicating no suppressive carry-over effect. A similar result was obtained for the D-56% group during naltrexone testing.

Throughout the study, there were no differences in body weights among the groups.

4. Discussion

A number of new findings are reported: (1) stable solid fat emulsions of various concentrations (18%, 32% and 56%) were developed that were acceptable to rats without phase-dependent confounds (e.g. separation) or the need to gelatinize the product. (2) No operationally-defined binge-type gram intake (Intermittent > Daily) occurred with any of the solid fat emulsions. (3) The $GABA_B$ agonist baclofen as well as the D_2-like antagonist raclopride had differential effects on emulsion intake between the two schedules of access. Both drugs reduced emulsion intake in all three Daily groups, but only in the Intermittent 56% group. (4) Naltrexone, a non-specific mu-opioid receptor antagonist, had differential effects on emulsion intake among the different fat concentrations. Specifically, naltrexone reduced intake of the 32% and 56% fat emulsions regardless of access schedule, but had no effect on intake of the 18% emulsion in either group.

4.1. Pre-drug solid fat emulsion and chow intake

In the present study solid fat emulsions engendered reliable consumption and were physically stable. The development of a relatively simple procedure for the preparation of solid fat emulsions allows for the determination of solid fat concentration-effect functions for emulsion intake and will facilitate the study of how dietary fats influence ingestive behavior.

The emulsions were clearly acceptable to the rats, with intakes approaching 10 g at the lowest fat concentration. Average 1-h gram intakes for all groups (~7–10 g) were greater than intakes of 100% shortening reported in previous studies (~5 g in Intermittent access groups) using similar protocols with male Sprague–Dawley rats. In contrast, only the energy intake of the I-56% group (~40 kcal) approached energy intakes reported in previous studies of 100% shortening in Intermittent access groups (~45–50 kcal) (Corwin, 2004; Corwin et al., 1998). The higher gram intakes, therefore, are at least partially accounted for by the reduced energy density of the solid fat emulsions. Although the 1-h energy intakes were lower relative to 100% shortening, they were comparable to the 24-h intake of 35% shortening or oil gels in a previous report (Lucas et al., 1989). Thus, the emulsions used in the present study were acceptable to the rats and relatively large amounts were consumed during the brief period of emulsion access.

Although the emulsions were clearly acceptable to the rats, there were no differences in 1-h gram intakes between Intermittent and Daily groups at any concentration. That is, binge-type eating as operationally-defined by Intermittent intakes > Daily, did not occur, likely because of the relatively high intakes in all groups. The large intakes of the 18% and 32% fat emulsions may have been close to the maximum capacity of the rats, thus eliminating the possibility of differential daily and intermittent intakes. This is in contrast to prior studies using a similar rat model of binge-type eating in which rats on an intermittent schedule of access consumed significantly more solid 100% vegetable shortening ("binge") during their brief access period than did rats given daily brief access to the shortening (Corwin, 2004; Corwin et al., 1998; Davis et al., 2007; Dimitriou et al., 2000; Thomas et al., 2002). The large intakes of the Intermittent groups in the present study would typically be perceived as binge-type eating if not for the concurrently elevated intake in the Daily groups. These results emphasize the importance of including an appropriate control group against which binge behavior can be operationally-defined.

The I-56% group showed a significant over-eat/under-eat pattern of daily total energy intake, i.e. they ate less of their chow diet on days following emulsion intake, an effect consistent with prior work with 100% shortening (Corwin, 2004; Corwin et al., 1998; Dimitriou et al., 2000). Average 24-h energy intake and body weight were the same across all groups, suggesting compensation for emulsion intake in all groups.

4.2. Baclofen: $GABA_B$ agonist

Baclofen (1.8 mg/kg) significantly reduced emulsion intake in all of the Daily groups and in the 56% Intermittent group. Baclofen had no effect on emulsion intake in the I-18% and I-32% groups. These results demonstrate a difference in the actions of the $GABA_B$ agonist baclofen between the two schedules of access despite similar (Intermittent vs. Daily) baseline intakes. Intakes after vehicle were comparable among groups that were (Daily groups) and were not (I-18%, I-32%) affected by baclofen, indicating that baseline intakes cannot account for the results obtained. The differential Daily and Intermittent results demonstrate that the effects of baclofen cannot be explained by generalized behavioral disruption. In addition, reductions in emulsion intake occurred at dosages that stimulated chow intake, again indicating that the rats did not suffer from any loss of motor function as a result of the drug administration. Baclofen's effect on simultaneously available chow in the present study (either no effect or a significant increase) is consistent with previous reports of baclofen's effects on chow intake (e.g., Brebner et al., 2000; Buda-Levin et al., 2005; Ebenezer, 1995; Ebenezer and Pringle, 1992; Ebenezer and Patel, 2004; Higgs and Barber, 2004; Wojnicki et al., 2006).

The issue of fat-specificity in the effects of baclofen raises some important questions relating to the amount of fat consumed and the manner of fat intake. One possible explanation for the lack of baclofen-induced reductions in intake of the I-18% and I-32% groups is that the total amount of fat consumed was much lower in these than in the other groups. The average one-week total intake of fat from the emulsion in the I-56% group was ~15 g and for the D-18% group ~11 g. Both of these groups showed a reduction of intake with the highest dosage of baclofen. The two groups that didn't show a reduction in emulsion intake were the I-18% and I-32% groups. These groups had average weekly emulsion-derived fat intakes of only about 4 and 7 g, respectively. It is possible that $GABA_B$ actions potentially affected by fat consumption (e.g. altered association with lipid rafts; Becker et al., 2001; Koyrakh et al., 2005; Stillwell et al., 2005) require a certain threshold level of weekly fat intake. In addition, the present study involved consumption

of a large quantity of emulsion in a relatively short period of time (1 h). Previous studies have shown the fat intake-reducing effects of baclofen occur under both the Daily and Intermittent access protocols, but not when fat is included in a fat-matched chow available 24 h a day (Buda-Levin et al., 2005). Consequently, it is important to consider the quantity of fat consumed, as well as the manner in which it is consumed, when looking at the fat-specific effects of baclofen.

4.3. Raclopride: dopamine D_2-like antagonist

Raclopride produced an effect on emulsion intake that was similar to that of baclofen. That is, raclopride reduced fat emulsion intake in all of the Daily groups at the 0.3-mg/kg dosage, but reduced emulsion intake only in the I-56% fat group. These differential effects occurred even though intakes after vehicle were comparable in groups that were (D-18%) and were not (I-18%, I-32%) affected by raclopride. Like baclofen, the differential Daily and Intermittent results demonstrate that the effects of raclopride cannot be explained by generalized behavioral disruption. Raclopride generally had no effect on chow intake, again suggesting that the reduction in emulsion intake was not the result of motor impairment. The failure of raclopride to reduce chow intake in the present study is consistent with other reports in which chow intake was either unaffected or stimulated by peripheral raclopride at dosages that had other behavioral effects (e.g. Lutz et al., 2001; Salamone et al., 2002).

Peripherally administered D_2 antagonists have been reported to reduce consumption of high-fat foods in some studies, but not others. For instance, raclopride failed to reduce consumption of a chow diet made with 18% fat at a dosage (0.1 mg/kg) that reversed amylin-induced satiety (Lutz et al., 2001). The lack of effect on the 18% fat diet may have been due to the relatively low dosage used. Higher dosages of raclopride (0.2–0.4-mg/kg) produced a dose-related inhibition of corn oil sham-feeding (Weatherford et al., 1990, 1988), indicating the involvement of D_2 receptors in the positive reinforcing effects of orosensory stimulation by fats. In a real-feeding study, raclopride (~0.3 mg/kg) reduced consumption of a shortening-based high-fat diet (33% shortening) when rats were given daily 2-h access to the diet (Baker et al., 2001). Rats in that study had access to a standard chow diet at all other times. In contrast to the present results, lower dosages (~0.07, 0.15-mg/kg) of raclopride significantly increased intake of the high-fat diet in that same study. Baker, et al. (2001), suggested that one explanation for these results could be differential actions of low and high raclopride dosages at pre- and post-synaptic D_2 sites. In the present study, no stimulation of intake occurred at the lower dosages. This may have been due to a ceiling effect on intake, i.e. the animals were already eating to their maximum capacity, and thus a further stimulation may not have been possible.

Given the similar profile of effects that resulted from the administration of baclofen and raclopride, it is tempting to speculate that similar mechanisms may be operating. GABA is the main inhibitory neurotransmitter in the central nervous system, and GABAergic projections to the VTA are thought to synapse on dopamine neurons at $GABA_B$ receptor sites (Johnson and North, 1992; Sugita et al., 1992). One way that baclofen might act, therefore, would be to reduce the rewarding properties of fatty foods by inhibiting DA neurons in the VTA. Higher dosages of raclopride may, likewise, inhibit DA actions via blockade of post-synaptic D_2 receptors within dopaminergic terminal fields. The fact that effects were only seen at the highest fat concentration in the intermittent groups suggests that both the manner and amount of fat consumed may influence actions within regions of the brain relevant to reward. For instance, fat intake, either total amount over time or large bolus amounts, may alter D_2 actions via changes in arachidonic acid-dependent signal transduction and/or the formation of lipid rafts (Bhattacharjee et al., 2005, 2006; Jacobowitz and Kallarakal, 2004).

4.4. Naltrexone: opioid antagonist

Naltrexone significantly reduced consumption of the 32% and the 56% fat emulsion at the 0.3 mg/kg dosage in the Intermittent and Daily groups, but had no effect on consumption of the 18% fat emulsion in either group. Unlike the results reported above with baclofen and raclopride, there was no effect of access schedule on naltrexone-induced reductions of emulsion intake. In addition, there was no effect of naltrexone on chow intake, once again indicating that no motor impairment was realized at any of the dosages administered. Naltrexone's lack of effect on chow intake accords with other reports (e.g. Kanarek et al., 1997). Baselines were comparable among groups whose intakes were and were not reduced by naltrexone, indicating that baseline intake cannot account for the results obtained.

These results support previous research suggesting that opioid signaling plays a prominent role in the consumption of foods rich in fat and/or sugar. Naltrexone has been reported to significantly reduce the percentage of time spent on both a solid high-calorie sucrose and a high-calorie fat-based food-conditioned side in conditioned place preference tests (Jarosz et al., 2006). While preference clearly can influence the effects of mu-opioid blockade on food intake (Levine et al., 2003), greater effects on consumption of a fatty food, relative to a sugary food, have been reported when the foods being compared were equally preferred (Hagan et al., 1997). Previous research has implicated mu_2- as well as mu_1-opioid receptors in the consumption of dietary fats (Islam and Bodnar, 1990; Mizushige et al., 2006). Taken together, the available data suggest mu-opioid receptors, whether mu_1 or mu_2, play some role in the intake of fats; since naltrexone is a non-specific mu-antagonist, the respective contributions of the mu_1 and mu_2 receptors to the present results are not known.

4.5. Conclusions

A major outcome of this study was the successful creation of stable solid fat emulsions, allowing the study of fat concentration without having to rely on liquid oils. This is an

important advance, as others have shown that rats will consume more solid fat than liquid oil (Lucas et al., 1989). Solid forms of fat, therefore, are optimal for studying the contribution of fat to dietary excess. All rats in this study consumed relatively large quantities of their respective fat emulsions, demonstrating the palatability and acceptability of the emulsions. Additionally, differential effects of compounds known to modulate consumption of palatable foods are described. These results suggest that the schedule of access promotes differential involvement of $GABA_B$ and D_2 receptors, while fat concentration promotes differential involvement of mu-opioid receptors. Together, these receptors may serve to promote the development and/or maintenance of large bouts of fat intake during brief periods of time.

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References

Baker RW, Osman JJ, Bodnar RJ. Differential actions of dopamine receptor antagonism in rats upon food intake elicited by either mercaptoacetate or exposure to a palatable high-fat diet. Pharm Biochem Behav 2001;69: 201–8.

Becker A, White JH, McIlhinney RA. The gamma-aminobutyric acid receptor B, but not the metabotropic glutamate receptor type-1, associates with lipid rafts in the rat cerebellum. J Neurochem 2001;79:787–95.

Bhattacharjee AK, Chang L, Lee HJ, Bazinet RP, Seemann R, Rapoport SI. D2 but not D1 dopamine receptor stimulation augments brain signaling involving arachidonic acid in unanesthetized rats. Psychopharmacology (Berl) 2005;180:735–42.

Bhattacharjee AK, Chang L, White L, Bazinet RP, Rapoport SI. D-Amphetamine stimulates D_2 dopamine receptor-mediated brain signaling involving arachidonic acid in unanesthetized rats. J Cereb Blood Flow Metab 2006;26:1378–88.

Boggiano MM, Chandler PC, Viana JB, Oswald KD, Maldonado CR, Wauford PK. Combined dieting and stress evoke exaggerated responses to opioids in binge-eating rats. Behav Neurosci 2005;119:1207–14.

Brebner K, Phelan R, Roberts DCS. Effect of baclofen on cocaine self administration in rats reinforced under fixed-ratio 1 and progressive ratio schedules. Psychopharmacology 2000;148:314–21.

Buda-Levin A, Wojnicki FHE, Corwin RLW. Baclofen reduces fat intake under binge-type conditions. Physiol Behav 2005;86:176–84.

Corwin RL. Binge-type eating induced by limited access in rats does not require energy restriction on previous day. Appetite 2004;42:139–42.

Corwin RL, Buda-Levin A. Behavioral models of binge-type eating. Physiol Behav 2004;82:123–30.

Corwin RL, Wojnicki FHW, Fisher JO, Dimitriou SG, Rice HB, Young MA. Limited access to a dietary fat option affects ingestive behavior but no body composition in male rats. Physiol Behav 1998;65:545–53.

Corwin RL, Wojnicki FHE. Differential effects of D_1, D_2, opioid, and GABA-B receptor ligands in rats with intermittent access to fat and sucrose. Program No. 457.8. 2006.Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience; 2006. Online.

Davis JF, Melhorn SJ, Shurdan JD, Heiman JU, Tschop MH, Clegg DJ, et al. Comparison of hydrogenated vegetable shortening and nutritionally complete high-fat diet on limited access-binge behavior in rats. Physiol Behav 2007;92:924–30.

Dimitriou SG, Rice HB, Corwin RL. Effects of limited access to a fat option on food intake and body composition in female rats. Int J Eat Disorders 2000;28: 436–45.

Ebenezer IS. Intraperitoneal administration of baclofen increases consumption of both solid and liquid diets in rats. Euro J Pharmacol 1995;273: 183–5.

Ebenezer IS, Pringle AK. The effect of systemic administration of baclofen on food intake in rats. Neuropharmacology 1992;31:39–42.

Ebenezer IS, Patel SM. Effects of the GABAB receptor agonists baclofen and 3-aminopropylphosphinic acid (3-APA) on food intake in rats. Methods Find Exp Clin Pharmacol 2004;26:627–30.

Hagan MM, Holguin FD, Cabello CE, Hanscom DR, Moss DE. Combined naloxone and fluoxetine on deprivation-induced binge eating of palatable foods in rats. Pharm Biochem Behav 1997;58:1103–7.

Heusner AA. Body size and energy metabolism. Ann Rev Nutr 1985;5: 267–93.

Higgs S, Barber DJ. Effects of baclofen on feeding behavior examined in the runway. Prog Neuropsychopharmacol Biol Psychiatry 2004;28: 405–8.

Islam AK, Bodnar RJ. Selective opioid receptor antagonist effects upon intake of a high-fat diet in rats. Brain Res 1990;508:293–6.

Jacobowitz DM, Kallarakal AT. Flotillin-1 in the substantia nigra of the Parkinson brain and a predominant localization in catecholaminergic nerves in the rat brain. Neurotox Res 2004;6:245–57.

Jarosz PA, Sekhon P, Coscina DV. Effect of opioid antagonism on conditioned place preferences to snack foods. Pharm Biochem Behav 2006;83: 257–64.

Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol 1992;450:455–68.

Kanarek RB, Mathes WF, Heisler LK, Lima RP, Monfared LS. Prior exposure to palatable solution enhances the effects of naltrexone on food intake in rats. Pharm Biochem Behav. 1997;57:377–81.

Koyrakh L, Lujan R, Colon J, Karschin C, Kurachi Y, Karschin A, et al. Molecular and cellular diversity of neuronal G-protein-gated potassium channels. J Neurosci 2005;25:11468–78.

Levine AS, Kotz CM, Gosnell BA. Sugars and fats: the neurobiology of preference. J Nutr 2003;133:831S–4S.

Lucas FF, Ackroff K, Sclafani AA. Dietary fat-induced hyperphagia in rats as a function of fat type and physical form. Physiol Behav 1989;45: 937–46.

Lutz TA, Tschudy S, Mollet A, Geary N, Scharrerr E. Dopamine D_2 receptors mediate amylin's acute satiety effect. Am J Physiol Regulatory Integrative Comp Physiol 2001;280:R1697–703.

Mizushige T, Matsumura S, Yoneda T, Tsuzuki S, Inoue K, Fushiki T. Daily increase of fat ingestion mediated via mu-opioid receptor signaling pathway. Biomed Res 2006;27:259–63.

Salamone JD, Arizzi MN, Sandoval MD, Cervone KM, Aberman JE. Dopamine antagonists alter response allocation but do no suppress appetite for food in rats: contrast between the effects of SKF 83566, raclopride, and fenfluramine on a concurrent choice task. Psychopharmacology 2002;160: 371–80.

Stein JA, Znamensky V, Baumer F, Rossi GC, Pasternak GW, Bodnar RJ. Mercaptoacetate induces feeding through central opioid-mediated mechanisms in rats. Brain Res 2000;864:240–51.

Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassall SR. Docosahexaenoic acid affects cell signaling by altering lipid rafts. Reprod Nutr Dev 2005;45: 559–79.

Sugita S, Johnson SW, North RA. Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. Neurosci Lett 1992;134(2): 207–11 Jan 6.

Thomas MA, Rice HB, Weinstock D, Corwin RL. Effects of aging on food intake and body composition in rats. Physiol Behav 2002;76:487–500.

Weatherford SC, Smith GP, Melville LD. D-1 and D-2 receptor antagonists decrease corn oil sham feeding in rats. Physiol Behav 1988;44:569–72.

Weatherford SC, Greenberg DD, Gibbs JJ, Smith GP. The potency of D-1 and D-2 receptor antagonists is inversely related to the reward value of sham-fed corn oil and sucrose in rats. Pharm Biochem Behav 1990;37: 317–23.

Will MJ, Pratt WE, Kelley AE. Pharmacological characterization of high-fat feeding induced by opioid stimulation of the ventral striatum. Physiol Behav 2006;89:226–34.

Wojnicki FHE, Roberts DCS, Corwin RLW. Effects of Baclofen on operant performance for food pellets and vegetable shortening after a history of binge-type behavior in non-food deprived rats. Pharm Biochem Behav 2006;84:197–206.

Wojnicki FHE, Stine JG, Corwin RLW. Liquid sucrose bingeing in rats depends on the access schedule, concentration and delivery system. Physiol Behav 2007;92:566–74.