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A novel procedure for assessing the effects of drugs on satiation in baboons: effects of memantine and dexfenfluramine

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Abstract

Rationale—Procedures for studying the effects of medications on satiation will assist the development of obesity medications.

Objectives—Develop a procedure for measuring satiation during consumption of bland and highly palatable food, and determine the effect of acute intramuscular administration of dexfenfluramine (DFEN), which increases serotonin levels, and memantine (MEM), which blocks N-methyl-D-aspartate receptors.

Methods—A modified progressive ratio (PR) procedure was used to track changes in reinforcing strength when a food was consumed. The response requirement increased after each reinforcement, and reinforcing strength was estimated using the breakpoint (BP), which was the last completed response cost. There was one preferred food (sweet candy) and one chow pellet PR session per week. During each session, 4 male and 4 female adult baboons experienced three 1-hr PR trials, separated by 30 min. Chow pellets were available at all other times. We examined the BP for 1 to 20 candies or chow pellets. Drug effects were examined when baboons had access to 1 and 10 candies/pellets.

Results—Breakpoints for candy were greater than for pellets. Varying the pellet/candy pieces per delivery, produced an inverted U-shaped function on the first trial, i.e., maximal BP was observed for 3 items, and the BP for multiple items, but not a single item, decreased across trials, i.e., BP decreased with food intake and satiation. DFEN and MEM decreased responding with the greatest effects at 10 deliveries suggesting that DFEN and MEM enhanced satiation.

Conclusion—Drugs that enhance satiation for several types of food may be particularly effective for decreasing food intake.

Keywords

Food Intake; Baboon; Motivation; Satiation; Memantine; Dexfenfluramine; NMDA

Introduction

In the early 1960s, Hodos introduced a procedure that he hypothesized allowed for determining the reinforcing strength of a commodity (Hodos 1961). The response schedule requires that an incremental number of operant responses be made for each commodity delivery or reinforcer, i.e., a progressive ratio (PR). Responding was recorded during a long-single session, in which

the animals were given ample time to complete the ever-increasing response requirement, with the session ending when no responses had been made for a long period of time, e.g., 15 min (Hodos and Kalman 1963). The value of the completed response requirement prior to the cessation of responding provided the PR breakpoint (BP). The greater the BP the greater the reinforcing strength.

PR procedures have both face and predictive validity. For example, non-human primates had greater BPs for a sweet chocolate (M & M®) or jelly (Skittles®) candy than either a sweetened fruit drink (Foltin and Evans 2001), or the standard food pellet (Foltin 2006b), and the greater BPs were predictive of greater single-meal intake and choice behavior. In addition to comparing commodities, BPs have been shown to vary systematically as a function of the stimulus qualities of the commodity or magnitude of the commodity. In general, increasing the amount of commodity delivered per reinforcement increases the BP for that commodity (e.g., Dantzer 1976; Hodos 1961; Hodos and Kalman 1963; Sclafani and Ackroff 2003; Reilly 1999), but Brennan et al. (2001) reported an inverted-U function with BP increasing and then decreasing at the highest concentration of a sucrose solution. Given the greater BP for highly palatable food (candy) in monkeys and the role that such food plays in the development of obesity (e.g., Blundell and Finlayson 2004) it was of interest to develop procedures for measuring satiation on both palatable and standard food intake.

Acute food deprivation or food deprivation associated with reduced body weight generally increased BP for food in rats (Ferguson and Paule 1997; Thorpe et al. 2005; but see Ferguson and Paule 1995, for exception) and small pigs (Dantzer 1976; Lawrence and Illius 1989). Caloric pre-feeding before a session decreased food BP in small pigs (Dantzer 1976), and induction of physical dependence on ethanol increased the BP for self-administered ethanol in rats (Walker and Koob 2007). These studies demonstrate that PR procedures provide a dynamic measure of reinforcing strength that is sensitive to acute and long-term environmental manipulations. Given its sensitivity to environmental manipulations, BP may provide a useful behavioral measure of differential sensitivity to environmental factors related to overeating and obesity.

Vaughan et al. (2006) used PR procedures to define meal size and frequency in wildtype (WT) and melanocortin-r receptor knockout (KO) mice. The hyperphagic obese KO mice had larger BPs and larger meal sizes than the WT mice suggesting a deficit in within-meal satiety in the KO mice. Sclafani et al. (2007) also described a type of satiety deficit in oxytocin KO mice, who had more meals than WT mice. Of note was the finding that the BP for sucrose did not differ between WT and KO mice, indicating that sucrose *per se* was not more reinforcing, or more palatable, in KO mice. As a final example, beta-endorphin deficient mice, who are obese, had BPs for a variety of foods that did not differ from WT mice when the animals were food deprived. In contrast, when food was available *ad libitum*, KO mice had smaller BPs than WT mice suggesting that endorphins modulated food reinforcement, and that modulation was in turn regulated by deprivation state. Thus, food BPs are dynamic and can be used as tools to understand normal and disordered eating behavior.

Hodos and Kalman (1963) cautioned that PR schedules with too small an increment in response requirement or too large a reinforcer magnitude could result in satiation thus confounding the estimate of reinforcer strength. Because it takes longer to complete the response requirement as the PR value gets larger, the interval between reinforcer deliveries necessarily gets larger, and the satiating effects of the food already eaten decreases. In this way PR schedules do limit the confounding effects of satiation. For example, Sclafani and Ackroff (2003) demonstrated that increasing sucrose concentration when responding was reinforced using a fixed (unchanging) ratio resulted in an inverted-U shape intake curve, while responding during PR sessions increased linearly with concentration. Studies of drug-reinforced responding also

show inverted-U shaped dose-response functions using fixed ratio schedules and linear dose-response function using PR schedules (see review by Stafford et al. 1998). In the case of drug-reinforced responding the PR schedules limit the effects of both drug satiation and non-specific response disruptions.

While PR schedules evolved, in part, to provide a measure of the direct reinforcing effects of a stimulus without satiation, the first purpose of the present study was to develop a PR procedure that could be used to provide a measure of satiation. Measurement of satiation requires recording the effects of a commodity at multiple time points during consumption, or comparing the effects of varying amounts of a commodity consumed in a similar timeframe. We hypothesized that satiation would be accompanied by a decrease in the reinforcing strength of the consumed food, i.e., the greater the satiating strength of a commodity the greater the decrease in its BP following consumption. This was tested by increasing the number of 1-g foods delivered upon completion of each ratio, and by having 3 consecutive PR trials per assessment. We hypothesized that changing reinforcer magnitude would result in an inverted-U response function, and that BP for the multiple items would decrease across the 3 trials, i.e., satiation.

The second purpose of the study was to determine if satiation would vary as a function of palatability of the food. We compared BPs for the maintenance diet banana-flavored pellet to that of a high-sugar palatable preferred food (Skittles candy). In a previous study, when access to the palatable food was limited to a single meal in the morning 3 days a week, baboons consumed a quantity of candy in one meal equivalent to the quantity of food pellets eaten in multiple meals over an entire day (Foltin 2006b). We hypothesized that BP's for the highly palatable food items would be greater than BP's for the standard diet items reflecting greater reinforcing strength and smaller satiating strength.

The third purpose of the study was to determine how pharmacological manipulations affected satiation. PR schedules have been used to evaluate the effects of pharmacological manipulations on the reinforcing strength of food. For example, orexin-A (Thorpe et al. 2005) and diazepam (Dantzer 1976) increased food BP, while haloperidol (Reilly 1999), and amphetamine (Foltin and Evans 2001) decreased food BP. In the present study, we evaluated the effects of two pharmacological manipulations. Although no longer used clinically, dexfenfluramine (DFEN), which increases serotonin, and to a lesser extent dopamine levels (Foltin 2004) is commonly used as a prototypic anorectic agent. DFEN has been reported to decrease the BP for standard food in rats (LeSage et al. 2004). A further advantage of using DFEN is that it has been hypothesized to decrease food intake by enhancing satiation (Clifton et al. 2000; Halford et al. 1998; Kirkham and Blundell 1986). We hypothesized that dexfenfluramine would decrease the BP measure of satiating strength for the standard and preferred food item. Glutamatergic systems have also received attention for their effects on eating behavior (e.g., Parsons et al. 2005). For example MPEP, a metabotropic glutamate receptor 5 antagonist, has been shown to decrease the BPs for standard food, and intravenously self-administered cocaine or nicotine (Paterson and Markou 2005). Recently, we have shown that memantine (MEM), which blocks glutamatergic N-methyl-D-aspartate (NMDA) receptors, also decreased intake of both highly palatable food and standard food pellets by baboons, but only increased the latency to the first meal when the standard diet was available (Bisaga et al. submitted). A similar pattern was observed for DFEN. In the present study we further examine mechanism of memantine's effects of food intake. The final purpose of this study was to evaluate possible effects of sex on response to drugs by testing male and female baboons.

Methods and materials

Animals

Four adult male baboons (*Papio cynocephalus anubis*), weighing 21.3 to 25.4 kg, and four adult female baboons, weighing 12.1 to 17.6 kg, were individually housed in standard non-human primate cages ($0.94 \times 1.21 \times 1.52$ m high) at The New York State Psychiatric Institute. The room was illuminated with fluorescent lighting from 7:00 AM to 7:00 PM daily. In addition to food and candy earned during experimental sessions, two chewable vitamins, two pieces of fresh fruit, and a dog biscuit were also given daily. Water was available *ad libitum* from a spout located at the back of each cage. All baboons had experience responding for both chow pellets and candy and experience responding for a single candy or pellet under PR schedules of reinforcement (Foltin 2006b), and had received acute doses of drugs (Foltin, 2006b; Foltin & Haney, 2007). All aspects of animal maintenance and experimental procedures complied with the U.S. National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the New York State Psychiatric Institute Animal Care and Use Committee.

Progressive Ratio Sessions

As shown in the top part of Table 1, baboons were run as 2 cohorts (2 males and 2 females each) and PR sessions were accomplished on Monday and Thursday for Cohort A and Tuesday and Friday for Cohort B. Food pellets were available in morning before the first PR session, and again after the third PR session, and all day on Sundays, Wednesdays and Saturdays using the procedures described in other studies (e.g., Foltin 2006b, Foltin and Haney 2007), i.e., multiple meals available without any limits on pellet intake.

When working during PR or regular sessions for chow pellets, baboons received banana-flavored 1-g food pellets containing 3.3 kcal: 0.55 g carbohydrate, 0.03g fat, 0.2 g protein (Bio-Serv, Frenchtown, NJ). Pellet delivery was accompanied by the illumination of all 4 stimulus lights above the 2 manipulanda for 8 s. When working during PR sessions for highly palatable candy, baboons received Skittles® containing 4.3 Kcal: 0.9 g carbohydrate, 0.04 g fat, 0 g protein (Mars Corp., Hackettstown, NJ). There are 5 flavors of Skittles®. Candy delivery was accompanied by the flashing (1 s on: 1 s off) of 2 white stimulus lights located above the food hopper for 8 s.

In a previous study with these same baboons, the BP for a single chow food pellet was about 80 and the BP for a single Skittle candy was about 200 (Foltin 2006a); BP was defined as the last response requirement completed before the baboon stopped responding. The greater BP for candy meant that the baboons consumed about 50 percent more candy than chow pellets before they stopped responding. Thus, the greater number of candies consumed may have affected the BP. In an attempt to have baboons eat a similar number of items before reaching a BP, in the present study, the initial response cost was 10 for chow pellets and 60 for candies.

As shown in the bottom part of Table 1, baboons worked for candy or food using PR schedules of reinforcement 3 times a test session with separate trials beginning at 9:00 AM, 10:30 AM, 12:00 PM. Sessions lasted for 1 hour. During the PR sessions, the ratio value increased by 20% after each reinforcer delivery. Based on previous work, we had hypothesized that responding would stop after a similar number of reinforcer deliveries for candy and pellets, but the candy BP would be larger. At the start of each trial, all baboons received the number and type of food that they would be working for in their food hopper without having to make any responses, i.e., responding was “primed” by free food. This indicated to the baboons what items are available that trial (always 3 trials for same item during PR test sessions).

Using 1 candy as an example, completion of the first 60 responses resulted in the delivery of a single piece of candy. Another piece of candy was available after a 5-s timeout, but that piece

of candy required 20% more responses, and so on (e.g., 60, 72, 86, 103, 123, 147, 176...). The 1-hr session length was sufficient for all baboons to reach a breakpoint during all PR trials. Thus, each trial consisted of the opportunity to work for candy or pellets using progressively larger response costs. For example, if an animal had a breakpoint of 211 for 1 candy then she or he had received 8 candy deliveries during that trial at costs of 60, 72, 86, 103, 123, 147, 176, and 211 then she or he did not complete the next larger ratio (253). If an animal had been working for 10 candies and had the same breakpoint, she or he would have received 80 candies (8 deliveries $\times$ 10 candies each) during that trial.

Procedure and Drugs

Initially the effect of reinforcer magnitude on PR breakpoints for candy or chow pellets was determined by varying the number of candies (1, 3, 10, or 20) then pellets (1, 3, or 10) delivered upon completion of each ratio. The effect of DFEN then MEM on PR breakpoints for 1 and 10 candies, and 1 and 10 pellets was then determined. The multiple-item conditions were tested before the single-item condition for MEM, and the order of testing conditions was varied across baboons for DFEN. Two doses of each drug were tested: 0.50, and 1.0 mg/kg under each condition, e.g., 1.0 mg/kg - 1 food pellet, 1.0 mg/kg - 1 candy, 1.0 mg/kg - 10 food pellets, 1.0 mg/kg - 10 candies. Placebo was also tested under each condition. Drug dose levels were chosen that were in the middle of the previously tested dose range based on a study examining the effects of these drugs on intake of candy and normal food pellet consumption in these same baboons (Bisaga et al. submitted).

Memantine HCl (0.5 – 1.0 mg/kg; Merz Pharmaceuticals, Frankfurt/Main, Germany) was dissolved in sterile saline at a concentration of 25 or 40 mg/ml. Dexfenfluramine HCl (0.5 – 1.0 mg/kg; Sigma Chemical Corp., St. Louis, MO, USA) was dissolved in sterile saline at a concentration of 25 mg/ml. Drug doses are expressed as total weight of the salt. Drugs were given intramuscularly (i.m.) in a thigh muscle (location varying among sessions) at 08:50 AM before PR sessions at 09:00. Dose was counterbalanced such that 2 females and 2 males received the smallest dose of each dose pair first, and 2 females and 2 males received the largest dose of each dose pair first.

Data Analysis

In traditional PR procedures, a specific length of time in the absence of responding is used to define that a breakpoint has been reached. In the current procedure, responding was limited to 60 – min PR trials such that animals might still have been responding at the end of a session. This was not the case, however, as all baboons stopped responding before the end of the PR trials. Progressive ratio breakpoints (the largest response requirement ratio completed before baboons stopped responding during a trial) for candy and pellets were calculated for each trial during each PR session. The effects of reinforcer magnitude on PR breakpoint for candy and pellets were analyzed separately using repeated-measures ANOVA with Sex as a between-groups factor. The within-groups factors were Number of Reinforcers (1, 3, 10 food pellets; 1, 3, 10, 20 candies), and Trial (there were 3 trials each session). Two sets of planned comparisons were also calculated based on the Number of Reinforcers $\times$ Trial interaction. In one set, each of the larger reinforcer magnitudes was compared to the single reinforcer on the first trial. In the second set, each of the larger reinforcer magnitudes was compared to the single reinforcer on the third trial.

Progressive ratio breakpoint was analyzed for each drug and reinforcer value (1 vs 10) separately using repeated-measures ANOVA with Sex as a between-groups factor. The within-groups factors were Food Type (candy vs. pellet), Drug dose (0, 0.5, 1.0 mg/kg), and Trial. Two sets of planned comparisons were also calculated based on the Drug $\times$ Food $\times$ Trial interaction. In one set, each dose of drug was compared to placebo for the candy and food pellet

condition, collapsed across trial. In the second set, each dose of drug was compared to placebo for the candy and food pellet condition for the first trial only. Data were considered significantly different at $P < 0.05$, using Huynh-Feldt corrections where appropriate.

Results

Because there was only one significant interaction based on Sex, data will be presented averaged across sex of the baboons. There was a significant effect of number of pellets on PR breakpoint [$F(2, 12) = 19.7, P < 0.0002$]. As shown in the right panel of Figure 1, The pellet-magnitude function had an inverted-U shape on the first trial with the breakpoint being the greatest for 3 pellets [$F(1, 24) = 3.7, P < 0.066$], and similar for 1 and 10 pellets. Although there was no main effect of Trial on pellet breakpoint, the breakpoint for 10 pellets was significantly smaller than the breakpoint for 1 pellet during the third trial [$F(1, 24) = 7.8, P < 0.01$]. Finally, the breakpoint for 10 pellets across all 3 trials was significantly smaller than the breakpoint for either 1 pellet [$F(1, 24) = 36.2, P < 0.0001$] or 3 pellets [$F(1, 24) = 20.9, P < 0.0006$] across all 3 trials.

There was a significant effect of number of candies on PR breakpoint [$F(3, 18) = 12.5, P < 0.0001$]. As shown in the left panel of Figure 1, the candy-magnitude function had an inverted-U shape on the first trial with the breakpoint being the greatest for 3 candies [$F(1, 36) = 35.7, P < 0.0001$], and greater for 10 candies compared to 1 candy [$F(1, 36) = 4.9, P < 0.041$]. There was a main effect of Trial on candy breakpoint [$F(2, 12) = 25.6, P < 0.0001$], and a significant Trial $\times$ Number of Reinforcers interaction [$F(6, 36) = 4.1, P < 0.006$]. Inspection of the graph clearly shows that candy breakpoint decreased across trials when baboons received multiple candies, but remained stable when baboons received 1 candy, although only the breakpoint for 20 candies was significantly smaller than the breakpoint for 1 candy during the third trial [$F(1, 36) = 11.6, P < 0.003$]. The attempt to have baboons eat a similar number of pellets and candies at the BP for each item by using different initial response costs failed; baboons ate on average 50 percent more chow pellets than candies under each reinforcer magnitude.

As shown in the left panel of Figure 2, breakpoints were greater for 1 candy (171 ± 10 ; mean $\pm$ SEM) than 1 pellet (77 ± 5) collapsed across all DFEN conditions [$F(1, 6) = 33.9, P < 0.001$]. Although there was no main effect of DFEN Dose ($P < 0.06$), the planned comparisons indicated that 1.0 mg/kg DFEN decreased the breakpoint for 1 candy [$F(1, 24) = 7.8, P < 0.01$] and 1 pellet [$F(1, 24) = 4.4, P < 0.05$] on the first trial, and decreased the breakpoint for 1 pellet across all 3 trials [$F(1, 24) = 58.6, P < 0.07$].

As shown in the right panel of Figure 2, breakpoints were greater for 10 candies (168 ± 11) than 10 pellets (36 ± 4) collapsed across all DFEN conditions [$F(1, 6) = 98.6, P < 0.0001$]. There was a significant effect of DFEN Dose [$F(2, 12) = 10.8, P < 0.002$] and a significant Food $\times$ Dose $\times$ Session interaction [$F(4, 24) = 2.8, P < 0.05$]. The planned comparisons indicated that 1.0 mg/kg DFEN decreased the breakpoint for 10 candies [$F(1, 24) = 24.0, P < 0.0001$] and 10 pellets [$F(1, 24) = 6.0, P < 0.02$] on the first trial, and decreased the breakpoint for 10 candies across all 3 trials [$F(1, 24) = 6.7, P < 0.02$], but this effect appears only due to the significant decrease on the first trial. Finally, in contrast to when 1 pellet or candy was delivered, the breakpoint for 10 candies or pellets significantly decreased as a function of Trial [$F(2, 12) = 22.0, P < 0.0001$].

As shown in the left panel of Figure 3, breakpoints were greater for 1 candy (196 ± 11 ; mean $\pm$ SEM) than 1 pellet (63 ± 5) collapsed across all MEM conditions [$F(1, 6) = 50.4, P < 0.0004$]. Although there was a significant effect of MEM Dose [$F(2, 12) = 4.1, P < 0.045$], the planned comparisons indicated that 1.0 mg/kg MEM only decreased the breakpoint for 1 candy [$F(1, 24) = 4.7, P < 0.04$]. There was a significant Food $\times$ MEM Dose $\times$ Trial $\times$ Sex interaction [F

(4, 24) = 4.1, $P < 0.01$]. Inspection of the data indicates that when females received the 0.5 mg/kg MEM dose their breakpoint for 1 candy increased on the third trial; an effect not observed in males or under other dose, session and food type conditions.

As shown in the right panel of Figure 3, breakpoints were greater for 10 candies (170 ± 12 ; mean $\pm$ SEM) than 10 pellets (26 ± 4) collapsed across all MEM conditions [$F(1, 6) = 46.4$, $P < 0.0005$]. There was a significant effect of MEM Dose [$F(2, 12) = 7.1$, $P < 0.009$] and a significant Food $\times$ Dose interaction [$F(4, 24) = 4.6$, $P < 0.03$]. The planned comparisons indicated that 0.5 mg/kg MEM decreased the breakpoint for 10 candies [$F(1, 24) = 19.4$, $P < 0.0009$] and 10 pellets [$F(1, 24) = 5.9$, $P < 0.03$] on the first trial, 1.0 mg/kg MEM also decreased the breakpoint for 10 candies [$F(1, 24) = 17.3$, $P < 0.001$] and 10 pellets [$F(1, 24) = 7.7$, $P < 0.02$] on the first trial, and decreased the breakpoint for 10 candies [$F(1, 24) = 16.1$, $P < 0.002$] and 10 pellets [$F(1, 24) = 8.1$, $P < 0.02$] across all 3 trials. Finally, as observed when DFEN was tested, the breakpoint for 10 candies or pellets significantly decreased as a function of Trial [$F(2, 12) = 30.0$, $P < 0.0001$].

Discussion

Breakpoints were greater for highly palatable food than for the maintenance diet (Foltin 2006b; Foltin and Evans 2001) in non food-deprived baboons. Increasing the number of candies or pellets per delivery initially increased the BP, but the curves had an inverted-U shape such that the BP for larger numbers of reinforcers did not differ from the BP for a single reinforcer. The subtle increase in BP for the chow diet in comparison to the dramatic tripling in BP for the highly palatable food in non-food deprived baboons highlights the role that palatability and variety can play in food consumption, and overeating (e.g., Hetherington et al. 2006).

In contrast, nearly all the previous studies using food-deprived laboratory rodents and small pigs examining reinforcer magnitude have reported that increasing either the concentration of a palatable fluid or the duration of access to the food increased BP (e.g., Dantzer 1976; Ferguson and Paule 1997; Lawrence and Illius 1989; Sclafani and Ackroff 2003). Because increasing food deprivation is known to increase food BP (e.g., Thorpe et al. 2005), it is likely that animals in the previous studies had greater motivation to eat and a greater satiety threshold.

Non food-deprived animals and larger amounts of food were tested in order to develop a model for assessing satiation. In addition to using amounts of food (10 or 20 pieces) that should result in consumption of a normal size “meal” of the item before or at the BP value, we conducted 3 PR trials during each session to test the assumption that BP for the larger reinforcer magnitudes would decrease across trials, while BP for a single reinforcer would remain stable. Indeed, BPs for multiple items significantly decreased across the trials. We suggest that the decrease in BP across trials provides a measure of satiation.

Although PR procedures were used, the methods differed from previous studies: 1) the trials, though long enough for responding to end, set a time limit on responding; 2) the ratio increment, i.e., “step size,” varied across commodities; 3) animals had the pellet diet available *ad libitum*, and 4) 3 trials, rather than 1 trial, were conducted during each PR session. Because baboons always ate a pellet meal in the morning after the room lights were illuminated, the first PR trial consistently began after a brief interval without food, but intervals without food varied greatly among baboons between the first and later trials. The consistent BPs with single food items and decreasing BPs with multiple items across the 3 trials support the hypothesis that multiple sessions in non-food deprived animals would be needed to develop a PR measure of satiating strength.

While we hypothesize that the changes in BP across trials and across multiple items reflect satiation strength, it is of course possible that we are measuring some other change in the

motivation to eat. For example, Lawrence and Illius (1989) pointed out that the motivation to perform an operant for food in pigs is not only due to hunger, but also due to the pig's innate motivation to "root" or forage. Similarly, we have shown that some baboons will respond for a sugar-coated chocolate candy, but then not eat them when only 10 responses were required for 1 candy (Foltin 2006a).

MEM decreased the BP for a single candy on the first trial without affecting BP for a single food pellet or BP across all 3 trials for either food. In contrast to the specificity shown by MEM for highly-palatable food or multiple food items, DFEN decreased the BP for both a single candy and food pellet on the first trial and the large dose decreased BP for a single food pellet across all 3 trials. Both doses of MEM, but only the larger DFEN dose significantly decreased the BP for 10 candies and 10 food pellets on the first trial. Finally, only the larger MEM dose decreased BP for 10 candies and 10 food pellets across all 3 trials. These findings argue that both drugs were increasing the satiating effects of multiple items. Alternatively, as cautioned above, both drugs decreased the reinforcing strength of multiple items for a reason other than satiation. However, it should be stressed that these drugs have completely different mechanisms of action, dexfenfluramine affecting primarily serotonin release whilst MEM blocks NMDA receptors. While it may be tempting to conclude that MEM was more efficacious than DFEN, the absence of a complete dose-response function prevents this conclusion.

It should be noted that weight loss has not been described either in clinical practice or in experimental animals after MEM treatment (Danzysz unpublished observations). Drugs such as MEM may be clinically effective in modulating abnormal or dysregulated behavior, i.e., cognitive benefit in patients with Alzheimer's disease, compared to healthy behavior (Parsons et al. 2007).

The present findings argue that drugs that increase serotonin may be clinically effective in obesity because they reduce meal size (also see Clifton et al. 2000; Halford et al. 1998). Recently, much attention has focused on the consumption of large "binge" meals as a factor in obesity (Yanovski 2003), and medications that decrease binge-meal size may have therapeutic relevance for the obese and obese binge eaters (Aronne and Thornton-Jones 2007). Indeed, Stunkard et al. (1996) reported that obese patients who reported binge eating decreased the rate of binge eating during a 8-week DFEN treatment trial faster than individuals receiving placebo. Subsequent studies confirmed that medications that increase serotonin levels were effective in decreasing binge eating in obese patients (e.g., Hudson et al. 1998; McElroy et al. 2000; Ricca et al. 2001). A medication that reduced abnormally large meals should be effective for the treatment of obesity.

Although serotonergic systems have been hypothesized to play a role in meal size and satiation, glutamatergic systems have received less attention in this regard (e.g., Parsons et al. 2005). A large series of studies demonstrated a role for glutamate in meal size using localized micro-infusions in brain areas associated with feeding or reinforcement (e.g., Zeni et al. 2000). Infusions of glutamate in the Nucleus Accumbens decreased food intake, while infusions of glutamate antagonists in the Nucleus Accumbens increased food intake by increasing meal size rodents (Maldonado-Irizarry et al. 1995; Stratford et al. 1998; Zeni et al. 2000), and central infusions of glutamate agonists and antagonists in the pigeon produced a similar pattern of results (Da Silva et al. 2006; Gillespie et al. 2005). Several studies have also shown that peripheral administration of the NMDA non-competitive antagonist (+)MK-801 (dizocilpine) also increased meal size in the rat (Treece et al. 2000; Jahng and Houpt 2001), perhaps due to an increase in the rate of gastric emptying (Covasa et al. 2000).

The above findings conflict with the current report of NMDA-antagonism reducing meal size, presumably by enhancing satiation. On the other hand, administration of glutamate agonists

directly into the lateral hypothalamus induces binge-type eating in rats, and this effect is blocked by the administration of NMDA receptor antagonists (Khan et al. 2004). Peripheral administration of the mGlu5 antagonists MPEP or MTEP reduced food intake in rodents (Bradbury et al. 2005; Semenova and Markou 2007), and food BP in rats (Paterson and Markou 2005). And, finally, NMDA receptor antagonists also suppress feeding induced by food deprivation (Stanley et al. 1996) and by the infusion of neuropeptide Y (Lee and Stanley 2005).

Clearly the effects of glutamatergic manipulations on feeding behavior are complex and dependent upon site and type of injection, deprivation state of the animal (e.g., Zeni et al. 2000), specificity of the receptor subtype affected by the drug (e.g., Semenova and Markou 2007), and perhaps species.

In addition to decreasing glutamate, MEM has also increases dopamine and acetylcholine levels in the rat brain (Shearman et al. 2006), with the magnitude of effects varying as a function of MEM dose and brain region. Hoebel and colleagues have reported that acetylcholine increases as a meal progresses, and have hypothesized that acetylcholine functions as a satiety signal (Mark et al. 1992; Rada et al. 2000). It is tempting to surmise that the greater decrease in BP with multiple items and trials is related to an increase in acetylcholine produced by MEM. If this were the case then MEM might have increased satiation by increasing acetylcholine and decreasing glutamate levels, while DFEN might have increased satiation by increasing serotonin levels.

The final purpose of the study was to look for possible sex differences in either BP or response to the drugs. While the sample size of each sex was small, there was no evidence for any effects of sex, other than greater food pellet intake for males than females on some occasions. One study reported that the anorectic effect of DFEN varied subtly across the estrous cycle in female rats (Eckel et al. 2005). The pattern of results, however, highlights a weakness of the present study in that, although the female baboons had menstrual cycles, dose-response functions were obtained irrespective of cycle phase.

The present procedures are time consuming and result in varying amounts of food consumed between PR trials. An alternative approach would be to determine dose-response functions after animals consumed varying levels of a caloric preload. In fact, we (Foltin and Schuster 1983) have shown that intragastric caloric preloads shifted the dose-response function for DFEN to the left in rhesus monkeys, i.e., DFEN enhanced satiation. A limitation to using similar procedures in non-cannulated animals is that the animals must voluntarily consume the preload. Because non-food deprived non-human primates will only consistently consume palatable foods, the ability to compare across food types, as was accomplished here, would be lost.

Developing medications that increase satiation and decrease normal meal size, or binge meal size may be an effective approach for treating overeating and obesity. Furthermore, the current novel procedure for assessing the satiating strength and reinforcing effects of foods during consumption of that food may provide a preclinical tool for the assessment of such medications.

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Figure 1.

Progressive ratio breakpoint as a function of number of candies or food pellets delivered and PR trial ($n = 8$). Error bars represent 1 *SEM*. An § indicates that the first trial for that number of items differed significantly ($P < 0.05$) from the first trial when 1 item was delivered, and an §§ indicates that the third trial for that number of items differed significantly ($P < 0.05$) from the third trial when 1 item was delivered.

Figure 2.

Progressive ratio breakpoint as a function of number of candies or food pellets delivered, PR trial and DFEN dose ($n = 8$). Error bars represent ± 1 SEM. A data point contained in a grey circle indicates that that data point differed significantly ($P < 0.05$) from placebo on that trial.

Figure 3.

Progressive ratio breakpoint as a function of number of candies or food pellets delivered, PR trial and MEM dose ($n = 8$). Error bars represent ± 1 SEM. A data point contained in a grey circle indicates that that data point differed significantly ($P < 0.05$) from placebo on that trial.

Table 1
Weekly Schedule of Activities and Daily Schedule on Progressive Ratio Test Days

	Weekly Schedule						
	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Cohort A	Food	Candy PR	Food	Food	Pellet PR	Food	Food
Cohort B	Food	Food	Pellet PR	Food	Food	Candy PR	Food
Daily Schedule on PR Test Days							
		8:50 AM	Drug Injection				
		9:00 AM	Give Free Candy or Pellet(s)				
			Start PR Session #1				
		10:00 AM	End PR Session #1				
		10:30 AM	Give Free Candy or Pellet(s)				
			Start PR Session #2				
		11:30 AM	End PR Session #2				
		12:00 PM	Give Free Candy or Pellet(s)				
			Start PR Session #3				
		1:00 PM	End PR Session #3				
			Food Pellets Available Until Next Day at 8:45 AM				