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d-Fenfluramine Anorexia: Dissociation of Ingestion Rate, Meal Duration, and Meal Size Effects

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KAPLAN, J. M., J. DONAHEY, J.-P. BAIRD, K. SIMANSKY AND H. J. GRILL. d-*Fenfluramine anorexia; Dissociation of ingestion rate, meal duration, and meal size effects.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 223–229, 1997.—In the present study, we ask whether the suppressive effect of *d*-fenfluramine (*d*-FEN) on short-term intake can be better explained in terms of a primary action on particular behavioral parameters (e.g., ingestion rate or meal duration), as proposed by several investigators, or in terms of a primary effect on an intake "target" that can be achieved via diverse behavioral strategies. We applied two specialized intake testing paradigms that constrain the behavioral structure of the rat's meal in different ways, and determined whether or not the meal-size result varied in turn. (1) In the intraoral intake test, the rate of ingestion was clamped by the rate (1.0 ml/min) at which the test stimulus (12.5 % glucose) was intraorally delivered. A *d*-FEN (3 mg/kg) suppression of intraoral intake was obtained demonstrating that ingestion rate adjustment is not necessary for the anorexic effect. In addition, for both *d*-FEN and vehicle conditions, comparable amounts were consumed when the intraoral intake test was either continuous or interrupted for 10 min beginning 6 min after test onset. For *d*-FEN, the increase in meal duration (mean = 11.98 min) required to compensate for the imposed interruption indicates that the drug does not specify an absolute limit for meal duration. (2) In the drop size-controlled spout-licking test, the volume of 12.5% glucose delivered for each lick was fixed at either 8 or 4 μl. There was an overall reduction in intake with *d*-FEN (0.75 mg/kg), but as under vehicle injection conditions, the number of licks emitted approximately doubled when lick volume was halved. As a result, meal size was unaffected by the drop size manipulation. The drop size manipulation affected several other behavioral parameters under respective *d*-FEN and vehicle injection conditions, including: average rate of ingestion (ml/min), initial ingestion rate, and ingestion duration (meal duration minus pause time). The two experiments together demonstrate that the anorexic effect of *d*-FEN does not depend on adjustment of any particular behavioral parameter. The results suggest, rather, that given doses of *d*-FEN establish a particular degree of intake suppression that the rat defends via diverse behavioral strategies. © 1997 Elsevier Science Inc.

d-Fenfluramine Ingestion Ingestion rate Meal Serotonin Licking behavior

d-FENFLURAMINE (*d*-FEN), the serotonin releaser and reuptake inhibitor, has been a prototypical agent for probing the serotonergic contribution to food intake control (for reviews, see 6,12,25,28). In recent years, researchers have turned to the measurement of meal-taking parameters, particularly of ingestion rate and meal duration, to gain insight into the mechanism by which *d*-FEN (or *dl*-fenfluramine, the less serotonin-selective form used in earlier studies) reduces meal size. In one study, the reduction in meal size following *d*-FEN was accounted for in part or entirely by significant reductions in meal duration (13). More commonly, and under various testing conditions in rats, humans and nonhuman primates, the reduction of meal size following treatment with *d*-FEN (or *dl*-fenfluramine) was mediated in largest part, or exclusively, by a reduction in the rate of ingestion (1,4,5,7,8,19,11,14,18,23,24). The effect of fenfluramine on the short-term control of food intake has been attributed to such factors as an enhancement in the satiating value of food (3,8), a decrease in the hedonic value of ingestate (1), and a disruption of oral motor competence (2,7). Although interpretations differ (see reviews in

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6,28), common to most discussions is some notion about how the overall intake effect of *d*-FEN (or *dl*-fenfluramine) is a consequence of the drug's primary action on behavioral parameters.

In the present study, we question whether changes in either ingestion rate or meal duration are necessary or sufficient for *d*-FEN's effect on overall intake. We begin by distinguishing, using the language of control theory, two contrary views of the meal size result. In one, meal size is seen as a "controlled" parameter. Here, direct influences on behavioral parameters such as average ingestion rate and meal duration, determine the magnitude of the resulting change in meal size. The notion that reduced intake is secondary to a direct action of *d*-FEN on ingestion rate and/or the duration of feeding is consistent with this view. The opposing idea holds that the primary effect of *d*-FEN is on meal size itself. From this perspective, changes in ingestion rate (or in meal duration) are seen not as the cause of the intake effect, but as one behavioral strategy, however typical, by which the targeted intake is achieved. Support for this view of meal size as a "regulated" parameter would be provided if a given dose of *d*-FEN reduces intake by the same magnitude despite constraints placed on either the ingestion rate or the temporal distribution of feeding responses. Evidence to favor one or the other interpretation of FEN's influence on meal size is not available.

Before we ask whether, under *d*-FEN, meal size is a controlled or a regulated parameter, it is appropriate to ask the same question of the untreated, intact rat. Recent experiments in our laboratory lead us to conclude that meal size, in fact, is a regulated parameter. Rats return stable meal sizes despite experimenter-imposed constraints on meal-taking behavior. Two test meal paradigms were developed for our analyses.

Interruption of Intraoral Intake

In the intraoral intake test, the test fluid is infused directly into the oral cavity at a fixed rate. The rat actively ingests (transports and swallows [19]; infusion rate = ingestion rate) the fluid until a satiety criterion (fluid rejection) is met. Seeley et al., (27) measured intraoral intake of 12.5% glucose across tests in which the infusions were either continuous or interrupted for variable durations. Rats demonstrated behavioral flexibility in order to defend meal size against the "interrupt" challenge. They dramatically prolonged their test meal as a direct function of the duration of the imposed pause in ingestion (other examples of behavioral flexibility in support of a stable intraoral intake are provided in refs 21 and 22).

Spout-licking With Drop Size Manipulation

We (Kaplan, Baird and Grill, in preparation) trained rats to take 12.5% glucose meals from a drinking spout that delivered a calibrated amount of fluid (drop size) for each lick. Intake stabilized after a set of habituation sessions run with drop size held constant at 5 μl/lick. We then varied drop size across tests and found that meal size was affected only slightly. When drop size was reduced from 7.5 to 2.5 μl/lick, rats almost tripled the number of licks emitted during the meal. Meal duration and feeding duration (= meal duration minus pause time between licking bursts) were substantially higher, and average ingestion rate was substantially lower, under the small versus large drop size conditions. We concluded, at least in relation to glucose intake, that rats demonstrate a remarkable degree of behavioral flexibility in defending what may be reasonably regarded as a meal size "goal."

We apply these two paradigms (interruption of intraoral intake, spout-licking with drop size manipulation) in a behavioral analysis of the intake suppression following IP *d*-FEN administration. It is possible that suppression of intake by *d*-FEN can be best explained in terms of a primary effect on the expression of ingestive behavior or on motor behavior more generally. Such effects may be expressed in the rat's inability to increase the number of licks to defend intake against a reduction of the lick drop size (Exp. 2), or to prolong their intraorally delivered meals to compensate for the interrupt challenge (Exp. 1). If, on the other hand, rats treated with *d*-FEN defend meal size via necessarily broad behavioral adjustments to the respective constraints, we would suggest that the drug acts primarily in relation to the meal size parameter itself. The intake "goal" would be lower with than without *d*-FEN, but achieved, nevertheless, via a behavioral flexibility typical of normal meal size control.

METHOD: EXPERIMENT 1

Subjects

Male Sprague–Dawley rats (Charles River) weighing 350–450 g were housed individually in hanging cages and maintained on a 12L:12D schedule. Individual rats were tested at the same time each day between 5 and 8 h after lights on. Food and water were available ad lib.

Surgery

Rats were anesthetized with ketamine (9 mg/kg) and xylazine (1.5 mg/kg), IM, and implanted with two intraoral cannulas. The PE-100 cannula tubing was led from just lateral to the first maxillary molar to emerge at the top of the head. Stainless steel tubing (19 G) was press fitted into the distal end of the tube and secured to the skull with screws and dental acrylic (see 17 for details.) At least 1 week was allowed for recovery before habituation training and testing began.

Apparatus

Rats were run in groups of 3 to 6 in individual hanging cages (18 × 25 × 36 cm). The infusion line for each cage was led to the rat from an infusion pump (Harvard Apparatus, Pump 44) through a miniature 3-way solenoid valve (Lee). A PC-AT personal computer with custom software and interface controlled infusion delivery to the rat by solenoid activation and tracked cumulative intake.

Procedure

Intraoral intake test. A 12.5% glucose solution was infused at 1.0 ml/min for each intraoral intake test. The infusion was initiated 5 min after the rat was placed into the test cage. Satiety criterion: When the solution was first seen to drip from the rat's mouth, the infusion was halted for 30 s. The test was terminated if the rat rejected the fluid a second time within 60 s after the infusion was resumed. Otherwise, the test continued until this criterion (2 rejections within a 90 s period) was met.

Habituation training. Beginning at least 1 week after surgery, rats received a series of 10 daily habituation training sessions during each of which, one intraoral intake test was delivered. Rats then entered the experimental phase.

Experimental design. Rats ingested 12.5% glucose infused at 1.0 ml/min in a series of four intraoral intake tests run on consecutive days. *d*-FEN (S(+)-fenfluramine HCl; RBI,

Natick, MA), or saline vehicle (0.5 ml), was injected IP 30 min prior to each test.

Dose selection. The *d*-FEN dose tested (3 mg/kg) was chosen on the expectation, based on pilot testing, that an intermediate degree of intraoral intake suppression (i.e., in the 30–50% range), on average, would be obtained.

For drug and vehicle injection conditions, one test was run in which the intraoral infusion was delivered continuously, and a second test was interrupted for 10 min, beginning 6 min after infusion onset. The same satiety criterion (see above) was applied in all cases. Testing order for the 4 conditions was counterbalanced across rats.

Tests were run on 14 rats. Seven were naive and 7 had been previously exposed to *d*-FEN in a preliminary dose-response study (data not shown). A comparable degree of intake suppression to *d*-FEN was obtained in both groups, permitting a pooling of their data for statistical analysis. However, results from only those rats ($n = 10$) that ingested for at least 6 min (= 6 ml) under both *d*-FEN conditions were included in the analysis. (Note that the removal of rats that were most sensitive to the drug results in an underestimate the magnitude of the drug's overall group effect on intraoral intake.) Amount consumed (ml) was used as the dependent measure for a 2-way (drug × infusion condition) ANOVA.

METHOD: EXPERIMENT 2

Subjects

Ten naive rats were maintained as described in Experiment 1. No surgery was performed.

Apparatus

Up to 6 rats at a time were tested in individual hanging wire test chambers. A drinking spout (Girton Inc.) was mounted on the front panel of each chamber, 4 cm above the chamber floor. Fluid was delivered by a PE-100 tube with its flared end fitted tightly in the opening at the tip of the drinking spout. Fluid (12.5% glucose), maintained under constant pressure (4.0 psi) was delivered to each chamber from individual reservoirs. The fluid line from reservoir to spout was interrupted by a 2-way solenoid valve. The duration of solenoid activation was calibrated to deliver a preset volume (± 5%) of glucose to the drinking spout.

The system registered the time of occurrence of each lick event via a lickometer circuit that passed less than 50 μA through the rat. The system also included a custom interface, and an 80286 processor running a program of our own design. With each lick registration, the corresponding solenoid was activated for the appropriate precalibrated duration. Predicted intake (number of licks × lick volume) was confirmed by pre- and post-test weight measurements of the fluid reservoir's content.

Intake Test

Rats were placed into the testing chamber where they remained for 1 h. The test meal began with the first spout-lick.

Satiety Criterion

Meal's end was defined as the time of occurrence of the first of two consecutive licks separated by at least 10 min.

Procedure

Habituation training. Rats received a series of 18 daily habituation training sessions. During each 1 h session, rats had access to a drinking spout that delivered 12.5% glucose at 6 μl/lick, a drop size halfway between the two (4.0 and 8.0 μl/lick) sizes tested in the experiment.

Experimental design. Half of the rats received, over 3 consecutive days, 12.5% glucose in intake tests with drop size set at 4.0 μl/lick, followed by 3 tests with an 8.0 μl/lick drop size. Remaining rats received drop size test blocks in reverse order. Saline vehicle (0.5 ml) was injected IP 30 min before the first and third test of each block. *d*-FEN (S(+)-fenfluramine HCl) was injected IP 30 min prior to the second test of each block.

Dose selection. The *d*-FEN dose selected for testing (0.75 mg/kg) was shown in pilot tests to yield, on average, an intermediate degree (30–50% range) of intake suppression. [Note that the dose tested is 4 times lower than that selected (by a similar intake suppression criterion) for testing in Exp. 1. The different sensitivities of intraoral- and bottle-intakes to *d*-FEN has been described previously (30) and is treated in Discussion.]

Dependent measures selected for statistical analysis were: meal size (ml), number of licks in meal, meal duration, average ingestion rate (ml/min) for meal, initial (60 s) ingestion rate (ml/min), and number of licks emitted in first min. In addition, within-burst lick frequency (licks/s) was taken as the reciprocal of the principal (first) peak of the whole-meal inter-lick interval distribution. (Inter-lick intervals falling beyond the principal mode of the distribution, typically used to define lick burst durations and inter-burst intervals, were not treated in the present analysis.) Effects of drop size and day-within-3-session block were evaluated for each dependent measure via 2-way repeated-measures ANOVA. When significant main effects of day-within- block were obtained, planned contrasts were obtained comparing (1) the pre- and post-drug vehicle conditions, and (2) *d*-FEN versus both vehicle conditions.

RESULTS

Experiment 1

Figure 1 shows mean intraoral intakes for vehicle (left pair of bars) and *d*-FEN injection (3 mg/kg; right pair of bars) tests under continuous (filled bars) and 10 min interrupted meal (open bars) conditions. Analysis of variance revealed a significant main effect of drug ($F[1, 9] = 35.64$, $p < 0.001$) and of meal interruption ($F[1, 9] = 6.473$, $p = 0.031$). Post-hoc analysis showed that the interrupt manipulation significantly increased intake for the vehicle tests ($t = 3.14$, $p = 0.012$), but not for the *d*-FEN tests ($t = 1.11$, NS). Importantly, however, the overall ANOVA yielded no significant two-factor [drug × interrupt] interaction ($F[1, 9] = 0.80$, NS).

Meal duration (not graphed) was lengthened substantially by the interrupt manipulation ($F[1, 9] = 129.28$, $p < 0.001$). The main drug effect was significant ($F[1, 9] = 35.64$, $p < 0.001$) and there was no 2-factor interaction ($F[1, 9] = 0.39$, NS) for this parameter.

RESULTS

Experiment 2

Post-hoc analyses showed that all significant day-within-3-session-block (sequence: vehicle - *d*-FEN - vehicle) effects were clearly attributable to the difference between the *d*-FEN

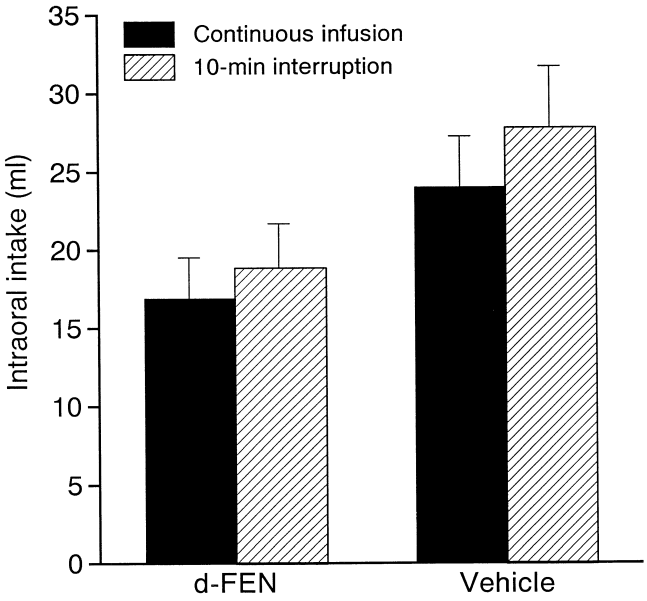

FIG. 1. Mean (± SEM, $n = 10$) intraoral intakes (ml) for *d*-fenfluramine (3.0 mg/kg; left bars) and vehicle control (right bars) conditions for tests in which the intraoral infusion of 12.5% glucose was either continuous (filled bars) or interrupted for 10 min beginning 6 min after infusion onset (diagonal bars).

test and the two (pre- and post-drug) vehicle tests. For no parameter did the pre- and post- drug vehicle test values significantly differ. We therefore chose to present day-within-block results from the overall ANOVA for a concise representation of drug effects.

Meal size (Figure 2). *d*-FEN administration reduced meal size from control levels by an average 43% ($F[2, 18] = 22.01$, $p < 0.001$). Drop size, however, was without effect either for the vehicle or for the *d*-FEN conditions, as indicated by the absence of a main effect of drop size ($F[1, 9] = 2.45$, NS) and the absence of an interaction between drug and drop-size factors in the overall ANOVA ($F[2, 18] = 0.17$, NS).

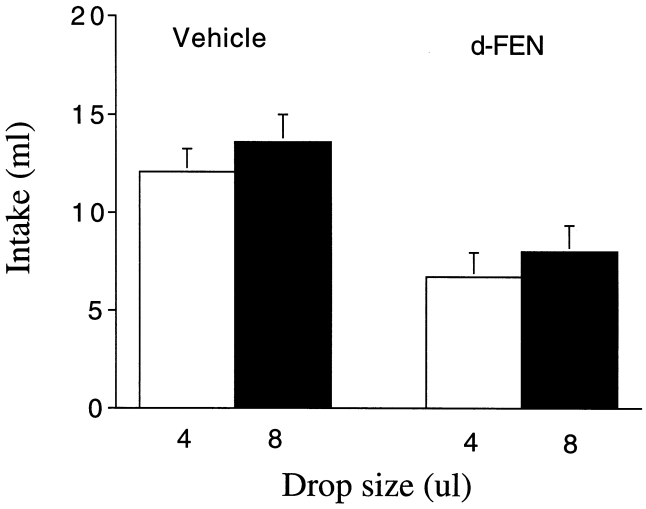

FIG. 2. Mean (± SEM, $n = 10$) meal size (ml) for *d*-fenfluramine (0.75 mg/kg; right bars) and vehicle control conditions (left bars) for tests in which volume of 12.5% delivered for each spout-lick was either 4 μl (open bars) or 8 μl (filled bars).

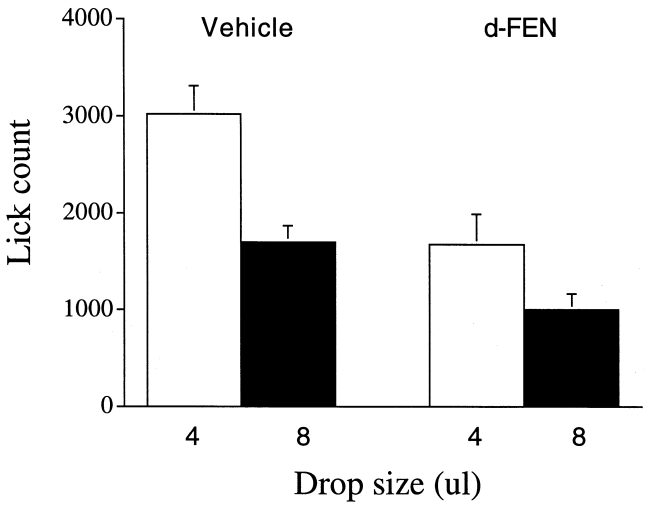

FIG. 3. Mean (± SEM, $n = 10$) number of licks per meal for *d*-fenfluramine (0.75 mg/kg; right bars) and vehicle control conditions (left bars) for tests in which volume of 12.5% delivered for each spout-lick was either 4 μl (open bars) or 8 μl (filled bars).

Licks. The reader can deduce that with no effect of drop size on meal size, the number of licks per meal would have approximately doubled when lick drop size was halved (from 8 μl to 4 μl). This result is shown in Fig. 3, and is expressed in a significant overall effect of drop size on lick count ($F[1, 9] = 43.43$; $p < 0.001$). A main drug effect, as expected given the results for meal size, was obtained ($F[2, 18] = 18.03$, $p < 0.001$). As was true for the meal size ANOVA, there was no interaction between drug and drop-size factors ($F[2, 18] = 2.64$, NS). The dramatic increase in number of licks emitted under *d*-FEN when drop size was reduced argues against viewing the drug's intake effect as secondary to a general action on behavioral performance.

Lick frequency and ingestion duration. There was no hint of either a drop size ($F[1, 9] = 0.08$, NS) or a drug treatment effect ($F[2, 18) = 0.003$, NS) on the within-burst lick frequency (Table 1), the overall average of which was 6.26 licks/s. It is clear then, that the dramatic increase in the number of licks as drop size was decreased—both for the *d*-FEN tests and for the vehicle tests—entailed a commensurate increase in time spent licking during the meal (i.e., in ingestion duration).

Average ingestion rate and meal duration. There was no main effect of *d*-FEN on the average ingestion rate for the meal ($F[2, 18] = 2.49$, NS; Table 1). Thus, *d*-FEN's suppression of meal size was due in largest part to an action on meal duration (Table 1), for which a main effect of drug condition was indeed obtained ($F[2, 18] = 8.65$, $p < 0.005$). Average ingestion rate, however, did vary with drop size ($F[1, 9] = 15.25$, $p < 0.001$); Average ingestion rate under *d*-FEN was reduced by 34% when drop size decreased from 8 to 4 μl (Table 1). For the vehicle sessions, the reduction in mean average ingestion rate with decreased drop size was somewhat less than that for *d*-FEN, although the 2-factor (drug × drop size) interaction was not significant ($F[2, 18] = 1.14$, NS). For meal duration, there was no significant effect of drop size

TABLE 1

MEAN (± SEM) VALUES FOR FIVE BEHAVIORAL MEASURES FROM THE LICKING TESTS (EXPERIMENT 2) RUN AT THE 4 AND 8 μl DROP SIZES UNDER VEHICLE AND *d*-FEN INJECTION CONDITIONS

	Vehicle		*d*-FEN	
	4 μl	8 μl	4 μl	8 μl
Within-burst lick frequency (licks/sec)	6.33 (± 0.16)	6.18 (± 0.31)	6.12 (± 0.15)	6.35 (± 0.35)
Injestion rate (ml/min)	0.752 (± 0.077)	0.878 (± 0.095)	0.602 (± 0.093)	0.918 (± 0.119)
Meal duration (sec)	1073 (± 149)	1071 (± 183)	720 (± 125)	648 (± 152)
First min ingestion rate (ml/min)	1.06 (± 0.12)	1.62 (± 0.19)	0.77 (± 0.17)	0.94 (± 0.10)
First min licks	264 (± 30)	203 (± 24)	193 (± 42)	117 (± 12)

See text for description and statistical comparisons.

($F[1, 9] = 0.05$, NS) and no 2 factor interaction ($F[2, 18] = 0.04$, NS).

Ingestion rate and licks during first minute of meal. As described above, *d*-FEN did not significantly affect the average rate of ingestion or the within-burst lick frequency. These whole-meal parameters belied a clear effect of *d*-FEN on ingestive microstructure that was apparent at the beginning of the meal. A significant main drug effect was obtained on the ingestion rate ($F[2, 18] = 10.38$, $p < 0.002$) and on the number of licks emitted ($F[2, 18] = 9.36$, $p < 0.005$) during the first 60 s of the meal (Table 1). Drop size also affected initial ingestion rate ($F[1, 9] = 11.60$, $p < 0.01$) and first-min licks ($F[1, 9] = 6.11$, $p < 0.05$). There were no significant 2-factor interactions for either parameter.

DISCUSSION

In the present study we ask whether the intake suppressive effect of *d*-FEN can be better explained in terms of a primary action on the behavioral structure of the meal or in terms of a primary effect on intake. Two different intake testing paradigms, intraoral intake and spout- licking, were used. In each case, a dose of *d*-FEN was chosen to produce an intermediate suppression of intake relative to baseline values. A behavioral challenge was then imposed: the intraorally delivered meal was either continuous or interrupted (Exp. 1), and the lick drop size of the spout-delivered meal was varied (Exp. 2). The respective manipulations altered the behavioral structure of the meals taken under *d*-FEN, but not the amount consumed. The relative stability of meal size was not a simple no-effect. The flexibility of the meal structure appeared, rather, to serve an active defense of the amount consumed. We infer that *d*-FEN does not act on particular behavioral parameters (ingestion rate, meal duration, number of ingestive movements), but rather acts at the level at which an overall intake result is specified.

Intraoral Intake

Under the special conditions of the intraoral intake test, changes in ingestion rate were not necessary for intake suppression following *d*-FEN treatment. Indeed, ingestion rate adjustments were not possible as infusion (= ingestion) rate was held constant during each test. For the uninterrupted infusions, intake reduction under *d*-FEN was entirely (and unavoidably) mediated by an adjustment in meal duration. Our result is in agreement with that of Wolgin et al. (30) who demonstrated a dose-related *d*-FEN suppression of intraoral intake of milk diet.

Perhaps intraoral intake suppression by *d*-FEN is secondary to a direct effect of a given magnitude on meal duration. The interrupt conditions of Experiment 1 show that this is not the case. Under *d*-FEN, the interrupt manipulation produced a large and significant (70.1%) increase in meal duration relative to that of continuous tests. Moreover, despite the effect of *d*-FEN on intake, and the small but significant overall effect of interruption, the analysis of variance revealed no interaction between drug and interrupt treatment factors. Thus, the flexibility of meal duration and the relative inflexibility of the meal size result seen under *d*-FEN, was mirrored in the vehicle condition results (Fig. 1). [We note here a minor contrast between the interrupt effect for the vehicle tests and the results of our previous study (27). In both studies a small increase in intraoral intake as a function of interruption was reported, but only in the present study was this trend statistically significant.]

We have developed the intraoral intake paradigm as a model for the study of normal ingestion controls. A number of treatments (e.g., deprivation, gastric loading, sugar concentration) yield similar effects on intraoral intake and on intake under standard testing conditions (10,15,18,21,26). The paradigm offers a special advantage for pharmacological analyses. A drug may affect intake by an action on physiological mechanisms of intake regulation and/or on general behavioral processes, such as arousal or motor control, that support the acquisition of food. Insofar as the appetitive phase of ingestion is discounted in intraoral intake, a drug effect is less likely, as compared to standard tests, to reflect an action on behavioral processes not specific to ingestion control.

The arbitrary control of the parameters of intraoral fluid delivery enabled the interrupt manipulation of the present experiment and offers other unique advantages (e.g., 20,22). As a matter of course, however, this arbitrariness should prompt a critical evaluation of the validity of the intraoral intake paradigm for the analysis of normal intake control. Indeed, particular concerns arise in relation to the analysis of intake suppression by *d*-FEN. First, higher doses were required for suppression of intraoral intake than of intake tested under standard conditions [see also 30]. This disparity may

reflect an appetitive action of *d*-FEN on normal intake that is absent here (30; see above), but a common effect on consummatory mechanisms of both intraoral and normal intake. On the other hand, the controls of consummatory behavior under the two paradigms may be affected differently by *d*-FEN.

As noted in Introduction, the typical behavioral adjustment in response to *d*-FEN under normal conditions is a reduction in ingestion rate. It is possible, then, that the character of *d*-FEN's action is altered when ingestion rate is constrained and all adjustment must be in terms of meal duration. A question arises more generally, with respect to the nature of the meal duration adjustments of intraoral intake. In standard tests, the rat is free to vary meal duration, (i.e., the time from meal onset to meal's end) and/or "ingestion duration," (i.e., the actual time spent feeding = meal duration minus pauses during the meal). The two parameters, by contrast, covary across all continuous intraoral intake tests. Here, the significance of the arbitrarily altered relation between these parameters by the interrupt manipulation (meal duration increased; ingestion duration little changed) is not clear. These concerns, as they relate to the interpretation of *d*-FEN anorexia, were addressed in Experiment 2.

Lick Analysis (Drop Size)

The behavioral form of the test meals under *d*-FEN varied as a function of drop size (4, 8 μl/lick). The average and initial (60 s) ingestion rates were higher for the meal ingested at 8 μl/lick. Interestingly, overall meal duration did not differ for the two drop size conditions. Ingestion duration, however, was greatly increased at the smaller drop size. Given no change in within-burst lick frequency, this increase in ingestion duration (time spent ingesting during the meal) was in direct proportion to the increase (almost a doubling) in the number of licks. This dramatic variation in number of licks in the meal (Fig. 3) was the most salient behavioral effect of the drop size manipulation.

Meal size under *d*-FEN did not vary as a function of drop size (Fig. 2). The doubling of the number of licks when drop size was halved indicates that meal size was actively defended. The behavioral differences for the two drop size conditions were apparently organized in service of a stable intake result.

The effects of the drop size manipulation under vehicle control conditions were comparable to those just described for *d*-FEN conditions. Average and initial ingestion rates increased with drop size, as did ingestion duration. Number of licks approximately doubled when drop size was halved, resulting in a stable intake across drop size conditions. These results are comparable to those obtained in our recent study (Kaplan, Baird and Grill, in preparation) in which intake varied only slightly across meals taken with drop sizes ranging from 2.5 to 7.5 μl/lick. Thus, for both *d*-FEN and control conditions, the behavioral structure of the meal was altered in compensation for the drop size challenge.

The two experiments taken together lead us to infer that *d*-FEN reduces intake not by a direct action on any particular behavioral parameter, but by an action on meal size itself. Rats prolonged their intraoral intake in response to the interrupt challenge, apparently in order to return a stable meal size. For spout-licking, rats returned stable intakes via great flexibility in ingestion duration, average ingestion rate, and, especially, the number of licks emitted. The dramatic increase in number of licks under *d*-FEN when drop size was halved shows definitively that *d*-FEN does not specify a reduced amount of ingestive behavior, and, moreover, that the intake effect does not depend on the drug's action on general motor performance. We do not argue that *d*-FEN has no primary actions on ingestive or more general behavioral parameters. The reduction in the initial rate of ingestion under *d*-FEN in Experiment 2 may indeed reflect such an action. The point here is that such influences cannot explain the intake suppression observed.

If the primary action of *d*-FEN is to specify a lower intake, should we be surprised that intake suppression by *d*-FEN (or *dl*-fenfluramine) is most commonly associated with adjustment of one behavioral parameter: i.e., a reduction in the rate of ingestion (1,4,5,7,8,19,11,14,18,23,24)? Such a result was obtained by Asin et al. (1) in rats ingesting sugar solution from a drinking spout. A counterexample, however, is provided in Experiment 2. Here, average ingestion rate was not significantly affected by *d*-FEN; the intake effect was carried by the meal duration parameter. Grignaschi et al. (13), in one experiment, obtained both types of behavioral mediation of *d*-FEN's intake suppression. In their study, rats received *d*-FEN before each of seven consecutive daily intake tests. On the first day, intake reduction relative to baseline was mediated largely by a reduction in ingestion rate. Thereafter, ingestion rate increased as meal duration shortened. Importantly for our perspective, the degree of intake suppression by *d*-FEN relative to control values remained stable throughout that experiment. Across studies, therefore, we see the same kind of flexibility reported here under the respective drug and control conditions of Experiment 2.

Recent experiments in the untreated, intact rat, consistently demonstrate relatively stable meal sizes in the face of a variety of challenges to the behavioral structure of the meal (21,22,27; see ref. 29 for a related study in humans). If defense of amount consumed is indeed a hallmark of normal short-term intake control, then short-term intake under *d*-FEN can be regarded as normal-like, at least in this respect. Support for this suggestion derives from both experiments presented, where no significant interaction between drug and behavioral (interrupt, drop size) treatments was obtained. The intake testing paradigms applied here to *d*-FEN anorexia may prove useful for the interpretation of the intake effects of other pharmacological agents.

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