Br. J. Pharmac. (1981), **72**, 621–633

THE EFFECT OF FENFLURAMINE ON THE MICROSTRUCTURE OF FEEDING AND DRINKING IN THE RAT

M.J. BURTON, S.J. COOPER* & D.A. POPPLEWELL

Laboratory of Experimental Psychology, University of Sussex, Brighton BN1 9QG and
*Department of Psychology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT

1 The effects of three doses of fenfluramine on feeding and drinking in the rat were examined.

2 Feeding and drinking were subdivided into meals and bouts, and the changes in feeding/drinking were expressed in terms of meal/bout frequency, meal/bout size, meal/bout length, and eating/drinking rate.

3 The changes in these parameters were examined over different time periods after the injection.

4 Significant changes in the distribution of inter-response intervals within meals were found in time period 1 with 5 mg/kg and 10 mg/kg doses of fenfluramine. Videotape and computer analysis showed that the changes in inter-response interval histograms differed significantly from those seen in normal animals approaching satiety. Drinking parameters also changed.

5 Compensatory increases in feeding were observed in time period 4 with the 10 mg/kg dose.

6 The difficulties in designing and interpreting experiments in feeding are discussed, and the action of fenfluramine as an anorectic drug is considered.

Introduction

Fenfluramine is used clinically as an anti-obesity agent, but the pharmacological and behavioural bases of its anorectic action remain unclear, although a considerable literature exists concerning its mode of action (see Pinder, Brogden, Sawyer, Speight & Avery, 1975; Reuter, 1975 for reviews).

One approach to the analysis of feeding behaviour has recently been utilized by Blundell and his coworkers (Blundell, Latham & Leshem, 1976; Blundell, 1977; Blundell & Latham, 1978). They have examined the action of a variety of anorectic agents on rats feeding in both *ad libitum* and deprived conditions. Their analysis consisted of subdividing total food intake into meals and then examining the changes in meal frequency, meal size, meal length and eating rate following drug administration. Their findings suggest that anorectic drugs affect parameters of feeding selectively and differentially. For example, at anorexigenic doses, fenfluramine decreased food consumption by selectively reducing meal size and eating rate whereas amphetamine reduced food consumption by altering meal frequency.

These behavioural differences are useful in the classification of anorectic agents. Further, if feeding parameters are differentially sensitive to changes in the underlying regulatory physiology of energy balance (see Le Magnen, 1971; Panksepp, 1973), then these differences may serve as a guide to the drugs' mode of action. However, there are problems with the criteria used to generate meal analysis in that they are usually arbitrary and describe not simply feeding behaviour but also interpolated behaviours (e.g. grooming, exploration). It is possible to use statistical and observational techniques to produce and justify a subdivision of meals into shorter bouts of sustained feeding. These bouts may provide a further tool in the analysis of drug action and more reliable estimates of feeding rate.

A further problem in determining the overall action of anorectic agents stems from the demonstration that the duration of the test period is crucial (Blundell *et al.*, 1973). Thus the time course and nature of the changes that occur after the administration of anorectic drugs needs careful description. However, only one recent paper does this (Grinker, Drewnowski, Enns & Kissileff, 1980). It may well be that earlier findings based upon results averaged across extended periods concealed the true nature of changes in meal parameters.

The present experiments analysed the effects of three doses of fenfluramine on feeding behaviour: first, to replicate previous findings that fenfluramine alters only meal size and feeding rate; second, to obtain accurate measures of its time course of action, both to provide more substantial data than currently exist, and to test the adequacy of analyses based on total intake; third, to discover whether meal and bout

0007–1188/81/040621–013 $01.00

analyses can provide information about the cause of the observed anorexia and its relationship with natural processes of satiety. Drinking was also measured.

Method

Eight male hooded rats, (232 to 328 g) were used from the Laboratory of Experimental Psychology, University of Sussex. Initially they were individually housed in standard laboratory cages (North Kent Plastics), within the experimental room. Standard laboratory chow (Spratts rodent breeding diet 1) and tap water were continuously available. Lighting operated on a 12 h light/dark (L/D) cycle, with lights off at 19 h 00 min. The rats were allowed 7 days to habituate to these conditions and their body weights were recorded at 17 h 00 min daily.

Apparatus

The apparatus used to record feeding and drinking was designed and built by ourselves at Sussex and based upon a Motorola 6800 microprocessor. The cages consisted of 8 aluminium boxes, (450 mm × 300 mm × 300 mm). A food hopper and drinking bottle, (spout bore size 3 mm), were fixed adjacently on one wall of each box. Removal of a 45 mg food pellet, (Noyes formula 'A'), from the food hopper activated an infra-red photo-beam system, (see Kissileff, 1970), and a further food pellet was delivered. A delay of 1.75 s occurred before further activation of the food dispenser was possible. Licking the water spout activated a CMOS sensing circuit; a delay of 1.5 s followed before further sensing could occur.

Both the removal of a food pellet and the activation of the drinking sensor were recorded by the microprocessor. Each event was coded by box (i.e 1–8), event (i.e. feeding or drinking) and the time since the beginning of the experiment (to 0.1 s resolution). This information was stored on mini-floppy discs and later transferred to a PDP 11–40 minicomputer for long-term storage and analysis. A continuous record of feeding and drinking events was therefore available.

Procedure

Each animal was individually housed in one of the boxes. They were allowed 7 days to habituate to the pelleted food. Their feeding and drinking were monitored for 24 h periods and by day 5, total food intake was stable. Mean intake day 5 = 527.8 (s.d.=71.6) pellets, mean intake day 6 = 507.5 (s.d.=107.1) pellets, $t = 0.8683$, d.f. = 7, $P > 0.4$ for related t test (two-tail). They were handled and weighed between 17 h 00 min–17 h 30 min daily. The experiment began on day 8.

All injections were given between 17 h 20 min and 17 h 30 min. Following injection the animals were replaced in their experimental boxes with free access to food and water, and their feeding and drinking were recorded from 18 h 00 min on the same day to 17 h 00 min on the following day (i.e. 23 h). There were 4 injection conditions: (1) 2.5 mg/kg fenfluramine, (2) 5.0 mg/kg fenfluramine, (3) 10.0 mg/kg fenfluramine (4) 0.9% w/v NaCl solution (saline). Doses are expressed in terms of (±)-fenfluramine hydrochloride; solutions were made up in saline and injected intraperitoneally in a concentration of 5.0 mg/ml. Each animal received all conditions, 72 h separated injections, and the orders of injections were counterbalanced.

Analysis

Feeding criteria Two methods are available for analysing feeding behaviour; the first is to divide the feeding data into meals, and then to analyse feeding parameters associated with meals; the second, is to subdivide the data into bouts (see below), and analyse feeding parameters according to bouts.

Criterion of a meal When the feeding data were expressed in the form of frequency of inter-response intervals (times between removal of successive food pellets), no objective evidence to support a particular

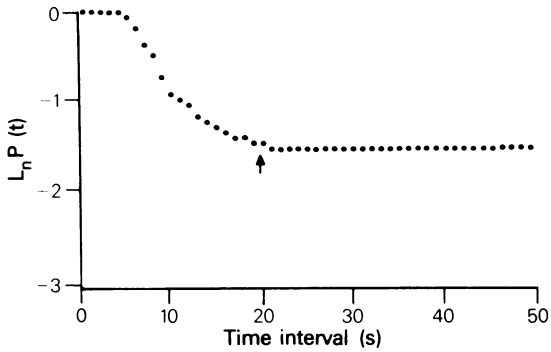

Figure 1 An example of the inter-response intervals over one day (derived from one animal) plotted as a log-survivor curve. LnP(t) is the natural logarithm of the proportion of intervals longer than time t, (where 0 <= t <= the maximum inter-response interval). The gradient of the line represents the rate of occurrence of responses. When responses occur in bouts (a sequence of short inter-response intervals followed by a much longer interval) a fairly sudden change in the gradient of the log-survivor curve occurs, indicating a change from a relatively high to low rate of responding (see Slater, 1974). The interval length at this gradient change gives a good estimate of the length of interval to use when distinguishing between intra- and inter-bout intervals. Hence it can be used to define the bout.

meal criterion could be found. A criterion of 10 min was adopted as the minimum inter-response interval separating two meals. This criterion value is representative of the literature, (Kissileff, 1970). For detailed analysis, the following 4 parameters were analysed: meal size (g), meal length (s), meal frequency, eating rate within meals (g/60 s).

A drinking response was taken as a single activation of a spout sensing device. A drinking meal criterion of 600 s was adopted.

Criterion of a bout The histograms of inter-response intervals were transformed into log-survivor curves (see Figure 1). These revealed that feeding occurred in groups of responses (bouts) separated from each other by periods of non-feeding (e.g. grooming, exploration). One or more bouts constituted a meal. Bout criteria were based on the analysis of log-survivor curves for each animal. Typically a bout consisted of a series of responses separated by less than 25 s. The behaviour which generated these bouts was examined by videotaping 3 animals (from a different experiment) over 3 days. Using these films the animals were then scored for different behavioural categories of ambulation, rearing, drinking, orientation to the food hopper, sleeping, feeding and grooming. Bout taking was defined as orientation to the food hopper and feeding with no other behaviours interpolated. These video-generated bouts were then compared to those obtained from the computer analysis, Pearson's cross-correlation giving a coefficient of $r = 0.90$. Thus, a bout as defined here corresponds to a period of feeding uninterrupted by other behaviours such as grooming or drinking. A meal consists of one or more of these bouts separated by grooming, drinking or ambulation.

The use of bouts derived from log-survivor curves closely parallels clearly definable behavioural sequences. It provides an objective criterion for each animal and avoids the confusions inherent in calculating eating rate from meals by eliminating most non-feeding behaviour. The consumption of small amounts of food in discrete clusters of responses appears to be a fundamental component of the feeding behaviour of the rat. A drinking bout criterion was also assessed for each animal by the log-survivor curve method.

Meal analysis In addition to total food intake within meals, four parameters of feeding were analysed: meal frequency; meal size; meal length and eating rate within meals.

Total session Meal frequency was taken as the number of meals in the total session; meal size was the total number of pellets for a given meal; meal length was the total time a meal lasted; and eating rate the number of pellets eaten per unit time within a given meal (i.e. meal size/meal length). Similar parameters were computed for drinking with number of drinking responses (see above) treated analogously to food pellets.

To analyse the effects of fenfluramine over the full session the mean of each parameter for the total session (i.e. 23 h) was calculated for each animal for each day.

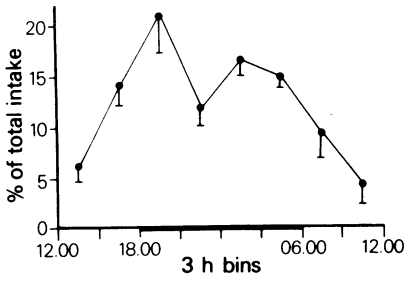

Figure 2 Three hour averages of food intake across the day, expressed as the means (and s.e.means—vertical lines) of the percentages of 24 h intake. The results represent the average of 6 animals measured over a 72 h period. Note the bimodal distribution of food intake across the dark period.

Time periods To analyse the time course of effect of fenfluramine the session was divided into four time periods. Normal animals consume most of their food in the dark part of the L/D cycle. Furthermore, as shown in Figure 2 this food intake occurs maximally at the beginning and end of the dark cycle. The time periods were therefore arranged to reflect these differences in food intake, (see Figure 2). Hence, time period 1 included all data from the start of the experiment (18 h 00 min) to 23 h 00 min; time period 2 from 23 h 00 min to 03 h 00 min; time period 3 from 03 h 00 min to 07 h 00 min; and time period 4 from 07 h 00 min to 17 h 00 min. The mean for each meal parameter of feeding and drinking was then calculated; for each time period; for each animal; for each day. Meals were assigned to the time period in which they began.

Bout analysis This was identical to the analysis of meals except that feeding and drinking were subdivided into bouts according to the bout criterion.

Statistical analysis One-way analyses of variance (ANOVAs) were used to analyse overall session effects, and effects within a given time period; data being grouped according to injection condition.

Two-way ANOVAs were used to analyse the time course of effect of fenfluramine; data were grouped according to injection condition and time period.

Table 1 Effects of fenfluramine on feeding and drinking over 23 h, (i.e. full session), expressed as means and s.e.mean (parentheses) of meal parameters

Feeding parameters

	Saline	*Fenfluramine condition* 2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	*Oneway-ANOVA* (*F*)
Total intake (g)	29.42 (1.77)	27.46 (1.76)	25.54 (1.54)	20.03*** (1.99)	5.212**
Meal freq. (no.)	15.29 (0.089)	17.86 (1.37)	20.57 (1.53)	15.53 (1.84)	2.957
Meal size (g)	1.96 (0.17)	1.59 (0.16)	1.24*** (0.05)	1.35** (0.13)	5.569**
Meal length (min)	7.58 (0.56)	6.89 (0.84)	6.19 (0.70)	6.64 (0.66)	0.685
Eating rate (g/min)	0.264 (0.024)	0.242 (0.010)	0.216 (0.010)	0.211 (0.008)	1.116

Drinking parameters

	Saline	*Fenfluramine condition* 2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	*Oneway-ANOVA* (*F*)
Total events (no. evnts)	1025.4 (73.02)	1075.7 (80.19)	1208.4 (133.3)	1274.7 (106.7)	1.298
Meal freq. (no.)	22.14 (1.35)	23.00 (0.84)	22.57 (1.60)	25.86 (2.14)	1.163
Meal size (no. evnts)	47.83 (5.45)	45.17 (6.03)	54.06 (5.78)	50.12 (3.89)	0.495
Meal length (min)	4.20 (0.60)	4.65 (0.58)	6.04 (0.68)	4.99 (0.52)	1.724
Drink rate (no./min)	12.01 (1.07)	10.49 (1.49)	9.62 (1.35)	10.54 (0.99)	0.630

One-way ANOVAs were carried out for each parameter; data being grouped according to injection condition, (d.f.=3 and 24. The *F*-values for these are given in the table, (**indicates a significant *F*-value at the 0.01 level). Differences between saline and specific injection conditions were assessed using *t* tests for simple effects, (** $P<0.01$; *** $P<0.001$).

Table 2 Summary of the results of two-way analyses of variance (ANOVAs) carried out to examine the time course of effect of fenfluramine (see method section)

Meal parameters

Feeding parameters			*Drinking parameters*		
Parameter	*F*	*P*	Parameter	*F*	*P*
Total intake	33.31	0.001	Total events	13.48	0.001
Meal freq.	6.94	0.001	Meal freq.	7.83	0.001
Meal size	5.32	0.001	Meal size	1.80	NS
Meal lnth.	0.71	NS	Meal lnth.	2.89	0.01
Eating rate	4.67	0.001	Drink. rate	1.75	NS

Bout parameters

Feeding parameters			*Drinking parameters*		
Parameters	*F*	*P*	Parameters	*F*	*P*
Bout freq.	4.88	0.001	Bout freq.	9.43	0.001
Bout size	5.88	0.001	Bout size	3.28	0.01
Bout lnth.	2.60	0.05	Bout lnth.	3.13	0.01
Eating rate	7.75	0.001	Drink. rate	0.75	NS

The *F*-value for the interaction injection condition × time period and associated significance levels are presented for each parameter of feeding and drinking examined, (d.f. = 9,54).

Differences between injection conditions were analysed using *t* tests for simple effects (Bruning & Kintz, 1968). This *a priori* test uses the results of one-way ANOVAs. Hence the degrees of freedom are computed from both the total number of conditions and the number of data points per condition.

All levels of significance were assessed using non-directional (two-tailed) tests.

Results

Due to a feeder fault, animal 8 was deprived of food on an injection day. All data from this animal were therefore excluded from the analysis, (i.e. $n = 7$ for all analyses). All the results and discussion will be assumed to be significant unless stated otherwise. Probability values for *t* tests and degrees of freedom can be obtained by consulting Tables 1 to 6.

Total session effects (18 h 00 min–17 h 00 min)

The analysis of meals (Table 1) revealed that there was a dose-dependent reduction in food intake which was significant at 10.0 mg/kg. Meal size was the only feeding parameter to be significantly different (i.e. reduced) from control in any drug condition.

Analysis of bouts revealed no significant effects. Table 1 summarizes the mean value for all parameters of meals over the total session.

Time period effects

The analysis of the time course of effect of fenfluramine gave strikingly different results. Table 2 shows that the interaction of injection condition × time period was significant for all parameters of feeding obtained from meal analyses with the exception of meal length. For drinking, number of responses, bout frequency and bout length showed significant interactions.

In order to understand the implications of these interactions it was necessary to examine the parameter in question for each of the four time periods. Complete data can be found in Tables 3 to 6.

Time period 1 (18 h 00 min–23 h 00 min)

Table 3 shows the dose-dependent reduction in food intake in the initial 4 h of the dark cycle when the first nocturnal peak of feeding occurs in normal animals (c.f. Figure 2). This reduction resulted from a decrease in feeding bout size at all doses in the bout analysis (Table 4) and at the two higher doses in the meal analysis (Table 3).

Time period 2 (23 h 00 min–03 h 00 min)

Tables 3 and 4 show that the second time period was characterized by a more complex pattern of changes.

Table 3 Summary of the time course of effect of fenfluramine on feeding, expressed as means and s.e.means (parentheses) of feeding meal parameters in 4 time periods (see methods section)

	Feeding meal parameters				
		Fenfluramine condition			*Oneway-ANOVA*
	Saline	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	(F)
Tot. intake (g)					
Period 1	9.681	6.332**	3.690***	1.684***	16.946***
	(0.84)	(1.02)	(0.91)	(0.49)	
Period 2	3.772	3.844	3.806	1.074***	7.666***
	(0.48)	(0.48)	(0.65)	(0.31)	
Period 3	9.624	10.749*	9.257	4.609***	23.074***
	(0.39)	(0.44)	(0.78)	(0.55)	
Period 4	6.242	6.519	8.460	12.644***	11.800***
	(0.96)	(0.70)	(0.65)	(1.07)	
Freq. (no.)					
Period 1	5.00	4.71	5.00	2.71	1.563
	(0.38)	(1.02)	(1.00)	(1.00)	
Period 2	2.00	2.71	3.71*	1.43	4.719**
	(0.38)	(0.18)	(0.64)	(0.42)	
Period 3	4.43	6.14**	6.43**	3.57	5.841**
	(0.53)	(0.26)	(0.88)	(0.37)	
Period 4	3.86	4.29	5.43	7.41***	8.163***
	(0.46)	(0.64)	(0.51)	(0.71)	
Size (g)					
Period 1	1.973	1.606	0.8398***	0.6910***	9.945***
	(0.17)	(0.25)	(0.17)	(0.18)	
Period 2	2.097	1.436*	1.103***	0.675***	12.185***
	(0.24)	(0.16)	(0.11)	(0.16)	
Period 3	2.341	1.778	1.543*	1.394**	3.859*
	(0.29)	(0.15)	(0.08)	(0.13)	
Period 4	1.637	1.706	1.559	1.672	0.072
	(0.18)	(0.26)	(0.09)	(0.11)	
Lnth. (min)					
Period 1	8.43	10.60	7.20	6.56	0.797
	(0.466)	(3.016)	(1.035)	(1.741)	
Period 2	8.20	4.83	4.54	5.67	2.282
	(1.318)	(0.628)	(0.546)	(1.313)	
Period 3	7.93	8.32	6.89	6.93	0.356
	(1.203)	(1.525)	(1.178)	(1.273)	
Period 4	6.34	6.41	6.56	6.48	0.005
	(1.233)	(1.666)	(1.226)	(0.618)	
Rate (g/min)					
Period 1	0.237	0.179	0.128*	0.074***	8.646**
	(0.020)	(0.029)	(0.026)	(0.018)	
Period 2	0.279	0.338	0.256	0.130**	5.477
	(0.039)	(0.043)	(0.032)	(0.038)	
Period 3	0.304	0.271	0.251	0.227	0.726
	(0.042)	(0.038)	(0.029)	(0.044)	
Period 4	0.289	0.308	0.291	0.275	0.140
	(0.031)	(0.037)	(0.044)	(0.032)	

Significant injection condition × time period interactions, (see Table 2), were analysed using oneway-ANOVAs; data being grouped according to injection condition, (d.f.=3, 24). The *F*-values for these are given in the table. Differences between saline and specific injection conditions were assessed using *t* tests for simple effects, d.f.=24. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 4 Summary of the time course of effect of fenfluramine on feeding, expressed as means and s.e.mean (parentheses) of feeding bout parameters in 4 time periods, (see methods section)

	Feeding bout parameters				
		Fenfluramine condition			*Oneway-ANOVA*
	Saline	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	(F)
Freq. (no.)					
Period 1	9.14	10.14	8.86	5.71	1.387
	(0.74)	(1.71)	(1.93)	(1.83)	
Period 2	2.86	3.29	6.00*	2.86	3.402*
	(0.40)	(0.29)	(1.21)	(0.99)	
Period 3	7.57	10.86*	9.86	6.00	2.309
	(1.55)	(1.70)	(1.45)	(0.97)	
Period 4	6.00	5.71	8.14	11.14***	7.086**
	(0.95)	(0.68)	(0.83)	(1.22)	
Size (g)					
Period 1	1.06	0.70*	0.40***	0.44***	6.747**
	(0.08)	(0.14)	(0.08)	(0.15)	
Period 2	1.49	1.22	0.75**	0.45***	9.860***
	(0.14)	(0.18)	(0.12)	(0.15)	
Period 3	1.61	1.11	0.99	1.01	2.504
	(0.29)	(0.15)	(0.08)	(0.13)	
Period 4	1.11	1.20	1.12	1.20	0.131
	(0.15)	(0.17)	(0.11)	(0.11)	
Lnth. (min)					
Period 1	2.92	2.16	1.60	2.13	1.564
	(0.187)	(0.313)	(0.272)	(0.740)	
Period 2	4.36	3.34*	2.49*	2.03**	3.812*
	(0.499)	(0.486)	(0.410)	(0.666)	
Period 3	4.41	2.94	2.60	3.45	2.873
	(0.688)	(0.342)	(0.293)	(0.454)	
Period 4	3.11	3.31	2.94	3.33	0.278
	(0.355)	(0.395)	(0.241)	(0.364)	
Rate (g/min)					
Period 1	0.413	0.312*	0.247**	0.190***	5.520**
	(0.027)	(0.033)	(0.024)	(0.043)	
Period 2	0.349	0.384	0.307	0.208**	4.089*
	(0.026)	(0.042)	(0.030)	(0.048)	
Period 3	0.358	0.384	0.391	0.300	1.490
	(0.025)	(0.033)	(0.039)	(0.036)	
Period 4	0.354	0.367	0.381	0.368	0.198
	(0.020)	(0.024)	(0.019)	(0.033)	

Significant injection condition × time period interactions, (see Table 2), were analysed using oneway-ANOVAs; data being grouped according to injection condition, (d.f.=3, 24). The *F*-values for these are given in the table. Differences between saline and specific injection conditions were assessed using *t* tests for simple effects, d.f.=24. $*P<0.05$; $**P<0.01$; $***P<001$.

Table 5 Summary of the time course of effect of fenfluramine on drinking, expressed as means and s.e.mean (parentheses) of drinking meal parameters in 4 time periods, (see methods section)

	Drinking meal parameters				
		Fenfluramine condition			*Oneway-ANOVA*
	Saline	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	(*F*)
Tot. evnts (no.)					
Period 1	285.00	245.86	191.57	152.86**	3.115*
	(27.21)	(16.60)	(36.63)	(44.54)	
Period 2	164.14	201.00	213.57	140.86	1.315
	(32.03)	(23.41)	(29.43)	(30.79)	
Period 3	356.29	422.29	528.14	437.57	1.902
	(32.09)	(39.97)	(77.04)	(44.21)	
Period 4	220.00	206.14	275.14	533.43***	13.100***
	(38.03)	(14.22)	(47.22)	(57.00)	
Freq. (no.)					
Period 1	6.14	5.14	5.00	4.71	0.366
	(0.86)	(0.51)	(1.13)	(1.39)	
Period 2	3.43	3.86	4.57	2.29	2.579
	(0.68)	(0.26)	(0.72)	(0.61)	
Period 3	7.00	8.71*	7.71	7.00	3.365*
	(0.44)	(0.18)	(0.67)	(0.31)	
Period 4	5.43	5.29	5.29	11.14***	13.71***
	(0.97)	(0.52)	(0.87)	(0.70)	
Size (no. evnts)					
Period 1	51.76	53.43	42.25	27.95	2.592
	(7.86)	(7.67)	(7.59)	(5.66)	
Period 2	51.61	54.00	50.66	61.86	1.263
	(8.46)	(8.35)	(7.55)	(13.74)	
Period 3	51.30	49.08	67.89	62.28	2.617
	(4.34)	(5.24)	(7.30)	(4.71)	
Period 4	40.80	56.77	53.10	46.53	0.648
	(5.31)	(15.34)	(4.26)	(5.11)	
Lnth. (min)					
Period 1	4.92	6.67	5.12	1.78	2.462
	(1.35)	(1.04)	(1.91)	(1.52)	
Period 2	5.22	5.59	3.26	5.64	0.534
	(1.99)	(1.35)	(0.49)	(1.89)	
Period 3	4.65	4.90	9.64***	6.10	7.192**
	(0.43)	(0.69)	(1.47)	(0.38)	
Period 4	2.83	2.22	6.81	4.67	2.657
	(0.62)	(0.39)	(2.33)	(0.67)	
Rate (evnts/min)					
Period 1	13.38	8.71	14.02	16.61	1.416
	(2.00)	(1.21)	(2.59)	(3.80)	
Period 2	19.20	14.38	18.31	12.95	0.372
	(5.17)	(4.92)	(3.62)	(3.69)	
Period 3	11.43	10.91	8.01	9.00	2.030
	(1.07)	(1.37)	(1.27)	(0.65)	
Period 4	17.50	23.92	14.95	11.14	2.003
	(2.63)	(5.08)	(4.66)	(1.70)	

Significant injection condition × time period interactions, (see Table 2), were analysed using oneway-ANOVAs; data being grouped according to injection condition, (d.f.=3, 24). The *F*-values for these are given in the table. Differences between saline and specific injection conditions were assessed using *t* tests for simple effects, d.f.=24. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 6 Summary of the time course of effect of fenfluramine on drinking, expressed as means and s.e.means (parentheses) of drinking bout parameters in 4 time periods, (see methods section)

	Drinking bout parameters				
		Fenfluramine condition			*Oneway-ANOVA*
	Saline	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg	(*F*)
Freq. (no.)					
Period 1	11.29	13.71	11.57	10.86	0.301
	(2.31)	(1.55)	(1.92)	(3.16)	
Period 2	8.86	8.86	13.14	8.29	1.447
	(1.72)	(0.80)	(2.56)	(1.88)	
Period 3	16.57	17.86	26.71**	22.57	4.523*
	(1.70)	(1.30)	(3.10)	(2.19)	
Period 4	11.71	7.86	14.43	32.86***	9.554***
	(2.89)	(0.96)	(2.57)	(5.96)	
Size (no. evnts)					
Period 1	29.91	19.04*	15.43**	12.04***	5.347**
	(5.44)	(1.91)	(2.31)	(2.49)	
Period 2	19.41	23.37	18.33	15.89	1.282
	(1.58)	(3.42)	(1.79)	(3.58)	
Period 3	22.33	23.92	19.82	19.35	1.074
	(2.36)	(1.74)	(2.15)	(1.99)	
Period 4	20.98	33.00	21.41	18.76	2.066
	(3.17)	(7.07)	(3.61)	(2.52)	
Lnth. (min)					
Period 1	0.70	0.44*	0.37*	0.28**	4.170*
	(0.149)	(0.047)	(0.060)	(0.058)	
Period 2	0.45	0.53	0.44	0.40	0.600
	(0.051)	(0.085)	(0.058)	(0.089)	
Period 3	0.51	0.54	0.46	0.46	0.421
	(0.069)	(0.053)	(0.063)	(0.060)	
Period 4	0.49	0.73	0.51	0.43	1.260
	(0.091)	(0.182)	(0.09)	(0.069)	
Rate (evnts/min)					
Period 1	44.18	43.96	41.88	36.50	0.804
	(2.95)	(2.92)	(2.02)	(6.48)	
Period 2	44.63	44.54	43.38	35.50	1.459
	(2.96)	(3.32)	(3.38)	(5.81)	
Period 3	44.77	45.50	44.20	43.42	0.095
	(2.70)	(2.99)	(2.79)	(2.93)	
Period 4	44.54	44.38	43.68	44.51	0.021
	(2.98)	(2.75)	(2.61)	(2.90)	

Significant injection conditions × time period interactions, (see Table 2), were analysed using oneway-ANOVAs; data being grouped according to injection condition (d.f.=3, 24). The *F*-values for these are given in the table. Differences between saline and specific injection conditions were assessed using *t* tests for simple effects, d.f.=24. *$P<0.05$; **$P<0.01$; ***$P<001$.

Table 7 The characteristics of inter-pellet intervals belonging to the 1st, 2nd, 3rd and 4th quarters of meals, in the first time period (i.e. 18 h 00 min to 23 h 00 min), for each injection condition

Condition	*Quarter*			
	1st	*2nd*	*3rd*	*4th*
Saline				
mean	10.4	12.4	13.2	11.9
	(1.6)	(1.8)	(2.4)	(1.6)
medn	7.0	7.0	8.0	8.0
	(6–9)	(6–9)	(6–10)	(6–10)
2.5 mg/kg				
mean	13.9	17.1	20.7	17.9
	(1.7)	(3.4)	(4.0)	(3.6)
medn	8.0	8.0	8.0	8.0
	(6–12)	(6–12)	(6–12)	(6–12)
5.0 mg/kg				
mean	21.1	22.1	27.7	33.6
	(3.2)	(4.9)	(6.6)	(6.0)
medn	13.0	12.0	12.0	14.0
	(9–21)	(9–15)	(10–18)	(10–21)
10.0 mg/kg				
mean	32.9	30.9	33.1	25.6
	(5.7)	(7.7)	(7.6)	(2.1)
medn	24.5	20.0	23.0	21.0
	(17–36)	(14–24)	(16–28)	(13–33)

Condition	D	nl	n4	*P*
Saline	0.0772	343	343	NS
2.5 mg/kg	0.0583	223	215	NS
5.0 mg/kg	0.0879	133	121	NS
10.0 mg/kg	0.1395	58	54	NS

The data from all animals ($n=7$) were combined. The intervals are expressed as the means and s.e.mean (parentheses) and the medians and interquartile ranges (parentheses), in seconds. Additionally the results of Kolmogorov-Smirnov two-tailed tests are presented for the comparison between the 1st and 4th quarters for each injection condition, (D = largest difference, nx = number of intervals in quarter x, NS = non significant difference).

Only at 10.0 mg/kg was total intake reduced although meal size was reduced at all doses. The anorectic effect at 10.0 mg/kg was characterized by a combination of reduced eating rate and meal size. The 2.5 and 5.0 mg/kg doses produced no anorectic effect because the reduction in meal size was compensated for by an increase in meal frequency which reached significance at the 5 mg/kg dose. This increased frequency was also seen in the bout analyses. The other changes in bouts were similar to those for meals; bout size was reduced at 5.0 and 10.0 mg/kg doses and bout rate was depressed at 5.0 mg/kg. The major dissociation between bouts and meals was the significant reduction in bout length at 5.0 and 10.0 mg/kg, for which there was no comparable change in meal length, as shown in Table 3.

Time period 3 (03 h 00 min–07 h 00 min)

In time period 3, Figure 2 shows that normal animals' intake rises to the second nocturnal peak before falling during the daylight hours. The bout analyses (Table 4) revealed no significant changes at any dose. Meal taking however continued to be disturbed. Thus meal frequency was significantly raised at 2.5 and 5.0 mg/kg, as shown in Table 3, and this resulted in an increase in total intake at 2.5 mg/kg. The anorectic effect remained significant at 10.0 mg/kg. At both 5.0 and 10.0 mg/kg meal sizes were still significantly smaller than control although the meal eating rate was no longer depressed.

Time period 4 (07 h 00 min–17 h 00 min)

Food-intake was measured during the entire 12 h of the light period (c.f. Figure 2). There was a dose-dependent increase in intake by the drug which was significant at 10.0 mg/kg. The primary cause of these increases were rises in meal and bout frequency, although again these effects were significant only at 10.0 mg/kg.

Drinking

Time periods 1 and 2 showed a reduction in total intake for 10.0 mg/kg and this was produced by a reduction in bout size and bout length. The increases in feeding were accompanied by similar increases in drinking.

Rate of feeding

In order to examine further the bout size and bout rate changes which characterize the anorexia seen for all animals in time period 1(2.5, 5.0 and 10.0 mg/kg) frequency histograms of inter-pellet intervals for meals in time period one were plotted (see Figure 3). There was a significant dose-dependent shift in inter-pellet intervals towards slower rates as compared with controls, (Kolmogorov—Smirnov two-tailed test, $P < 0.001$ for all cases).

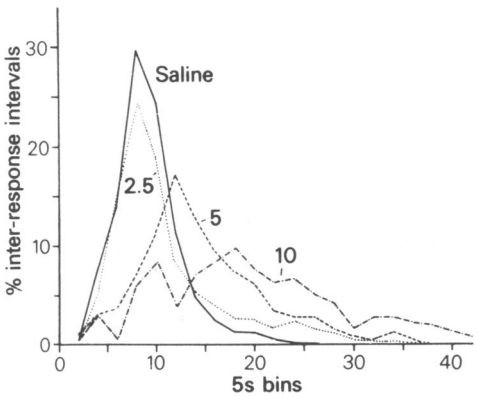

Figure 3 Frequency distributions of inter-pellet intervals for each injection condition (combined data for the 7 animals). Note the shift of distribution to the right, i.e. toward a slower eating rate, as the dose of fenfluramine (indicated in mg/kg) increases.

To assess whether the slower rate occurred differentially across the meal, meals for the time period of greatest anorexia (i.e. period 1) were divided into quarters on the basis of their size, as shown in Table 7. All intervals were consecutive and quarter 1 received the first group of intervals, quarter 2 the second etc. If the number of intervals within a given meal was not exactly divisible by 4 then quarter 1 was the first group to receive an extra interval, followed by quarter 2, and then 3 (thus the numbers of intervals in each quarter were not always equal). There was no evidence that control animals slow down their rate of eating towards the end of meals and the reduction in rate found with fenfluramine occurred equally in all four quarters of the meals for all doses.

Discussion

The results show that fenfluramine produces significant reductions in food intake at 2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg. However, the time course of the anorectic action is dose-dependent and averages taken over the total session compound the early reduction in food intake with the increase in feeding observed after recovery from the drug. This explains why total session averages only achieve significance in the 10 mg/kg condition: when the anorectic effects were sustained over three time periods. The reduced intake appears to derive from reductions in meal size and meal rate thus replicating the findings of Blundell & Latham (1978).

The rate changes observed in meals occur due to reduced intra-bout responding and increased inter-bout intervals (see Figure 3). This shift in inter-response times, both within and between bouts, is clearly crucial to the inderstanding of the anorectic actions of fenfluramine. An explanation offered by Blundell *et al.* (1976) is that the shift occurs because fenfluramine enhances the normal processes that contribute to the cessation of feeding, thus causing meal size to reduce. However, as Table 7 shows there is no evidence that normal animals slow their rate of feeding towards the end of meals.

Blundell *et al.* (1976) showed that fenfluramine-treated deprived rats initially ate at the same rate as control animals; only when some food had been consumed were the anorectic effects of fenfluramine observed. However, the analysis based upon dividing the meals into quarters (see Table 7) revealed that the feeding rates were reduced by fenfluramine by the same amount during all four quarters of meals, i.e. under free feeding conditions the anorectic effect of fenfluramine can be seen across the entire meal.

The shift in response rates is sufficient to explain most of the observed anorectic changes. However, observations by Blundell & Latham (1978) that similar reductions in rate produced by neuroleptics are associated with an increase in meal length suggest that fenfluramine may have some further action which prevents an increase in meal length. Although a direct effect on feeding motivation cannot be precluded several alternative explanations should be considered.

Fenfluramine is believed to produce anorexia by increasing 5-hydroxytryptamine (5-HT) availability at central synapses, by inhibiting the presynaptic reuptake pump (Garattini & Samanin, 1977). There are a number of reports of changes in responses other than feeding after treatments which raise available levels of 5-HT at central synapses. These changes include hyperthermia, resting tremor, rigidity, reciprocal forepaw treading, hindlimb abduction, lateral head weaving and Straub tail (Jacobs, 1976; Marsden & Curzon, 1979). Animals receiving doses of 10.0

mg/kg fenfluramine in the present experiments showed clear evidence of abnormal lateral head movements, resting tremor and reciprocal forepaw treading. Although there were no overt changes in behaviour of this kind at 2.5 mg/kg or 5.0 mg/kg, disruption by some component of these processes must at least be considered as an explanation for the reduced meal size.

A further possibility is that the behavioural actions of fenfluramine do not derive solely from its action as a reuptake inhibitor but from some alternative mechanism. This possibility is raised by the observation that 5-HT reuptake inhibition alone may be insufficient to induce substantial anorexia (see Samanin, Caccia, Bendotti, Borsini, Boronni, Invernzzi, Pataccini & Mennini, 1980), or may do so by changing meal frequency rather than meal size (e.g. the 5-HT reuptake inhibitor femoxitine: unpublished observation). It may be that fenfluramine's ability to release 5-HT from presynaptic terminals is important or that its actions on other monoamine systems may contribute to its rate slowing actions.

The other major change occurring after fenfluramine is the increase in feeding in the later time periods. This increase results from a rise in frequency of both bouts and meals even whilst meal size and rate remain suppressed. The observation that normal animals respond to deprivation by increasing meal size rather than frequency (Levitsky, 1970) suggests that this may not simply be a response to drug-induced deprivation but may reflect the continued action of the drug on non-5-HT-ergic systems or alterations in synaptic sensitivity. These hypotheses are the subjects of current investigations.

The evidence from these experiments support the idea that fenfluramine's anorectic actions are expressed behaviourally by reduction in meal size (Blundell & Latham, 1978; Grinker *et al.*, 1980). This reduction can be seen to result from the shift in inter-response intervals that might parsimoniously be ascribed to the animal's inability to generate high response rates. Although such disruption is clearly anorectic in that it reduces feeding it may not do so through changes in motivation. It is interesting to speculate whether the changes which cause reduced feeding in acute experiments are causative in the weight loss observed during chronic clinical treatment. Preliminary data from this laboratory and from other workers (Levitsky, personal communication) suggest that the reductions in food intake observed acutely with fenfluramine disappear after daily chronic dosing. Body weight however fails to show a similar recovery. This raises the possibility that one of the metabolic effects of fenfluramine, such as increased glucose transport into muscle or enhanced thermogenesis, might assist in the observed weight loss (see Pinder *et al.*, 1975 for review).

This work was supported by an M.R.C. project grant. D.A.P. is the recipient of an M.R.C. research scholarship. Servier laboratories kindly donated the fenfluramine. We would like to thank Dr R.A. Boakes for his helpful criticism of the early versions of this paper. All correspondence to D.A.P. please.

References

BLUNDELL, J.E. (1977). Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Internat. J. Obesity*, **1**, 15–42.

BLUNDELL, J.E. & LATHAM, C.J. (1978). Pharmacological manipulation of feeding behaviour: Possible influences of serotonin and dopamine on food intake. In *Central Mechanisms of Anorectic Drugs*. ed. Garattini, S. & Samanin, R., pp. 83–109. New York: Raven Press.

BLUNDELL, J.E., LATHAM, C.J. & LESHEM M.B. (1973). Biphasic action of 5-hydroxytryptamine inhibitor on fenfluramine-induced anorexia. *J. Pharm. Pharmac.*, **75**, 492–494.

BLUNDELL, J.E., LATHAM, C.J. & LESHEM, M.B. (1976). Differences between the anorexic actions of amphetamine and fenfluramine. Possible effects on hunger and satiety. *J. Pharm. Pharmac.*, **28**, 471–477.

BRUNING, J.L. & KINTZ, B.L. (1968). *Computational Handbook of Statistics*. Glenview (Illionois): Scott, Foresman & Co.

GARATTINI, S. & SAMANIN, R. (1977). Drugs Affecting Serotonin: A Survey. In *Serotonin in Health and Disease:* Volume II. ed. Essmann, W.B. New York: Spectrum Publication Inc.

GRINKER, J.A., DREWNOWSKI, A., ENNS, M. & KISSILEFF, H. (1980). Effects of d-amphetamine and fenfluramine on feeding patterns and activity of obese and lean Zucker rats. *Pharmac. Biochem. Behav.*, **12**, 265–275.

JACOBS, B.L. (1976). Minireview: An animal behavior model for studying central serotonergic synapses. *Life Sci.*, **19**, 777–784.

KISSILEFF, H.R. (1970). Free feeding in normal and "recovered lateral" rats monitored by a pellet-detecting eatometer. *Physiol. Behav.*, **5**, 163–173.

LE MAGNEN, J. (1971). Advances in studies on the physiological control and regulation of food intake. *Progr. Physiol. Phychol.*, **4**, 203–261.

LEVITSKY, D.A. (1970). Feeding patterns of rats in response to fasts and changes in environmental conditions. *Physiol. Behav.*, **5**, 291–300.

MARSDEN, C.A. & CURZON, G. (1979). The role of tryptamine in the behavioural effects of tranylcypromine + l-tryptophan. *Neuropharmac.*, **18**, 159–164.

PANKSEPP, J. (1973). Reanalysis of feeding patterns in the rat. *J. comp. Physiol. Phychol.*, **82**, 78–94.

PINDER, R.M., BROGDEN, R.N., SAWYER, P.R., SPEIGHT, T.M. & AVERY, G.S. (1975). Fenfluramine: a review of

its pharmacological properties and therapeutic efficacy in obesity. *Drugs*, **10**, 241–323.

REUTER, C.J. (1975). A review of the C.N.S. effects of fenfluramine, 780SE and norfenfluramine on animals and man. *Postgrad Med. J.*, **51**, 18–27.

SAMANIN, R., CACCIA, S., BENDOTTI, C., BORSININ, F., BORRONI, E., INVERNIZZI, R., PATACCINI, R. & MENNINI, T. (1980). Further studies on the mechanism of serotonin-dependent anorexia in the rat. *Phychopharmac.*, **68**, 99–104.

SLATER, P.J.B. (1974). The temporal pattern of feeding in the zebra finch. *Anim. Behav.*, **22**, 506–515.

(Received March 10, 1980.
Revised October 2, 1980.)