

Fenfluramine and Fluoxetine Spare Protein Consumption While Suppressing Caloric Intake by Rats



Judith J. Wurtman; Richard J. Wurtman

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(Fig. 1B). Differing responses to particular periods of rainfall seem to be implicated. For instance, after a dry August in 1971, the flowering of *Heliconia* sp. 3 was retarded while that of *Costus malortieanus* was accelerated. The very wet period from December 1970 to January 1971 seemed to accelerate flowering of *Passiflora vitifolia* while retarding that of *Jacobinia aurea*, in comparison to other years. A severe drought between late February and April 1973 strongly affected the flowering of several species, producing a major flower shortage in May and June. This shortage was to some extent ameliorated by *Malvaviscus arborea*, which put on an unusually synchronized burst of flowering in June 1973; normally the species maintains a very low level of blooming year-round with little synchrony between individual plants, and is of only minor importance as a hermit food plant.

Two further conclusions are suggested by Fig. 1: first, unseasonably wet or dry periods affect flowering most strongly if they occur just before anthesis, perhaps interfering with differentiation of the flower bud itself (14). Second, the phenology of dry season flowerers may be more sensitive to unusually wet periods, while rainy season flowerers may be more affected by unusually dry periods and less by very wet ones. However, a sufficiently severe drought can retard or abolish flowering of even dry season bloomers. Only in such severe droughts will the advantages of flowering in sequence be overcome by physiological stress to the plant.

These data suggest that compensatory phenological reactions to periods of unusual rainfall exist among the hummingbird-pollinated plants of La Selva, and that these reactions may play a fundamental role in the year-to-year organization of the bird-flower community. Corresponding data from a California bird-flower community (15) and tropical bee-flower community with strong differentiation between wet and dry seasons (2, 16) suggest that in more seasonal climates displacement of the entire flowering sequence is the major mechanism for preserving staggered blooming sequences. Quantitative phenological studies of several years' duration, combined with ecologically based experimentation on physiological control mechanisms may prove essential to an understanding of the temporal organization of tropical plant communities.

F. GARY STILES

Escuela de Biología, Universidad de Costa Rica, Ciudad Universitaria Rodrigo Facio, Costa Rica

References and Notes

1. D. W. Snow, *Oikos* **15**, 274 (1965); B. Heinrich and P. H. Raven, *Science* **176**, 597 (1972); A. H. Gentry, *Biotropica* **6**, 64 (1974).
2. G. W. Frankie, "Tropical forest phenology and pollinator-plant coevolution," in *Co-Evolution of Animals and Plants*, L. E. Gilbert and P. H. Raven, Eds. (Univ. of Texas Press, Austin, 1975).
3. F. G. Stiles, *Ecology* **56**, 285 (1975); and unpublished.
4. D. A. Levin and W. W. Anderson, *Am. Nat.* **104**, 455 (1970); T. Mosquin, *Oikos* **22**, 398 (1971); E. R. Heithaus, *Ann. Mo. Bot. Gard.* **61**, 675 (1974).
5. V. Grant, *Origins of Adaptations* (Columbia Univ. Press, New York, 1963).
6. G. M. Allen, *Bats* (Harvard Univ. Press, Cambridge, Mass., 1939); H. G. Baker, *Q. Rev. Biol.* **36**, 64 (1961); *Science* **139**, 887 (1973).
7. H. H. Clayton and F. L. Clayton, *Smithson. Misc. Collect.* (1977), vol. 105; R. R. Ricklefs, *Ecology* (Chiron Press, Portland, Ore., 1973).
8. P. de T. Alvim, *Science* **132**, 354 (1960).
9. Climate and vegetation of Finca La Selva are described by L. R. Holdridge, W. C. Grenke, W. H. Hatheway, T. Liang, J. A. Tosi Jr., *Forest Environments in Tropical Life Zones: A Pilot Study* (Pergamon Press, Oxford, 1971); see also Stiles (3).
10. Quantitative data on the flowering of 42 species of hummingbird-pollinated plants were taken between January 1971 and March 1975; flower counts were used to define periods of "good" and "peak" bloom; for details of methods see Stiles (12).
11. L. L. Wolf, F. R. Hainsworth, F. G. Stiles, *Science* **176**, 1351 (1972).
12. F. G. Stiles, *Biotropica*, in press.
13. ——— and L. L. Wolf, *Am. Ornithol. Union Monogr.*, in press.
14. W. S. Hillman, *The Physiology of Flowering* (Holt, Rinehart and Winston, New York, 1962); N. Gopal, *Turrialba* **23**, 146 (1973).
15. F. G. Stiles, *Univ. Calif. Berkeley Publ. Zool.* **97**, 1 (1973).
16. P. A. Opler et al., *J. Biogeogr.*, in press.
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Fenfluramine and Fluoxetine Spare Protein Consumption While Suppressing Caloric Intake by Rats

Abstract. *The effects of fenfluramine and other anorectic drugs on the consumption of both protein and total calories by rats given simultaneous access to two isocaloric diets containing 5 or 45 percent casein were examined. Anorectic doses of fenfluramine failed to decrease protein intake but increased the proportion of total dietary calories represented by protein. In contrast, anorectic doses of d-amphetamine decreased protein and calorie consumption proportionately. Subanorectic doses of fenfluramine also increased the proportion of caloric intake represented by protein among animals given prior treatment with the serotonin precursor tryptophan. Fluoxetine, a drug that blocks reuptake of serotonin, similarly spared protein consumption while reducing caloric intake. These observations indicate that two distinct brain mechanisms, sensitive to different drugs, underlie the elective consumption of protein and calories.*

The effects of anorectic drugs on food consumption have traditionally been studied by giving a rat access to a single diet, usually rat chow, and then measuring its total food intake. This procedure allows the investigator to detect changes in the total number of calories that the animal consumes. It does not, however, allow detection of any changes that the drug might cause in food preference: the

test animal has no way of displaying its choice.

We now describe an experimental procedure that allows the investigator to distinguish the effects of a drug on the intake of calories, per se, from its effects on the consumption of a particular nutrient—in this case, protein. We treat animals with a drug and then allow them to select among two or more isocaloric

Table 1. Effects of fluoxetine on food intake and protein consumption. Groups of 8 rats (34- to 41-day-old males), which were trained to consume their daily food intake during an 8-hour dark period, received fluoxetine or saline intraperitoneally and were given access to two isocaloric diets (5 and 45 percent protein). C, control; F, fluoxetine.

Time (hours)	Dose (mg/kg)	Food consumed (g)		Protein as percent of total calories consumed*	
		C	F	C	F
1	5	11.6 ± 0.8	6.5 ± 1.0*	22 ± 2	28 ± 2
1 to 3	5	4.0 ± 0.4	4.7 ± 0.7	26 ± 2	34 ± 2†
3 to 5	5	5.6 ± 0.9	4.7 ± 1.1	25 ± 4	25 ± 4
5 to 8	5	5.3 ± 0.5	5.6 ± 1.1	23 ± 4	24 ± 5
1	10	11.7 ± 0.9	4.4 ± 0.9‡	17 ± 2	26 ± 3§
1 to 3	10	3.6 ± 0.7	4.6 ± 0.7	14 ± 4	28 ± 4*
3 to 5	10	3.2 ± 0.5	4.5 ± 0.8	24 ± 5	21 ± 5
5 to 8	10	5.5 ± 0.6	7.0 ± 1.0	30 ± 4	19 ± 5

*P < .005. †P < .025. ‡P < .001. §P < .05. All as compared to the control.

diets containing different amounts of protein. With this procedure, we found that fenfluramine (1) and fluoxetine (2), anorectic drugs that presumably increase the amounts of serotonin present within brain synapses, also cause animals to preserve normal protein intake while decreasing their consumption of calories. This test procedure may have general utility for exploring the effects of drugs on feeding behavior, as well as for examining the mechanisms that couple post-prandial changes in plasma composition to subsequent food choice (3).

Male rats 21 to 48 days old (4) were trained to consume all of their daily food during an 8-hour dark period and to select their food from two or three pans containing different diets. The diets were isocaloric and contained 5 or 45 percent protein (5) or, in one study, 24 or 48 percent sucrose (6). Animals were injected with either a drug or saline at the beginning of the dark period and were given

access to the food pans immediately thereafter. The pans were weighed before presentation and at intervals during the 8-hour feeding period. The number of grams of protein that each animal had consumed and the proportion of total caloric intake represented by this protein were calculated for each interval.

In the first set of experiments, rats received fenfluramine (2.5 mg per kilogram of body weight, intraperitoneally) (7) or its diluent and were given access to diets containing 5 or 45 percent casein. No changes in feeding behavior were observed during the next hour (Fig. 1). During the following 3 hours, however, fenfluramine-treated animals selectively decreased their consumption of the low-protein diet and increased their consumption of the high-protein food so that total caloric intake was depressed while protein consumption was spared (8). The proportion of the total caloric intake represented by protein thus increased significantly (Fig. 1). During the final 4 hours of food consumption, the feeding behaviors of fenfluramine-treated and control animals did not differ. A higher dose of fenfluramine (5 mg per kilogram of body weight) depressed total food intake so markedly during the first hour after its administration that it also significantly decreased protein consumption (from 2.0 ± 0.43 to 0.8 ± 0.15 g, $P < .05$). During the subsequent 3 hours, total caloric intake was no longer affected, but protein intake, and thus the percentage of calories represented by protein (Fig. 2), both increased significantly over control levels.

Like fenfluramine, fluoxetine (7) in doses of 5 or 10 mg per kilogram of body weight suppressed food intake but spared protein consumption, thereby causing the proportion of total calories represented by protein to rise (Table 1). The time course of this drug's action differed from that of fenfluramine: fluoxetine most effectively reduced food intake during the first hour after its administration but continued to facilitate protein consumption throughout the first 3 hours (Table 1).

The ability of fenfluramine to spare protein consumption was unrelated to either the number of food pans provided or the concentration of protein in the diets. When fenfluramine-treated animals (2.5 mg per kilogram) were allowed to choose among three isocaloric diets containing 5, 20, or 50 percent protein, they chose the same proportions of protein (as the percentage of calories) as animals given only the 5 and 45 percent protein foods (23 ± 2 vs. 25 ± 2 percent).

Fenfluramine failed to affect the elec-

tive consumption of sucrose, a sweet-tasting carbohydrate. When animals were allowed to choose between two isocaloric diets that contained equal amounts of protein but different concentrations of sucrose (24 as compared to 48 percent) (6), no differences were observed in diet choice after fenfluramine administration (2.5 mg per kilogram of body weight).

Anderson and his associates (3) have proposed that the likelihood that a rat, given the choice, will elect to eat a particular diet, containing a particular proportion of protein, varies inversely with that diet's effect on the plasma amino acid pattern—specifically, the change that its consumption produces in the ratio of plasma tryptophan to the sum of the other neutral amino acids that compete with tryptophan for uptake into the brain (9). Other studies have shown that this plasma ratio determines both the uptake of tryptophan into the brain and its subsequent conversion to the neurotransmitter serotonin (9, 10). To test the hypothesis that the differential effects of fenfluramine (and fluoxetine) on protein and calorie consumption involved their capacity to enhance transmission across serotonergic synapses, we examined the ability of preliminary treatment with tryptophan to potentiate fenfluramine's protein-sparing effect. (Presumably, by

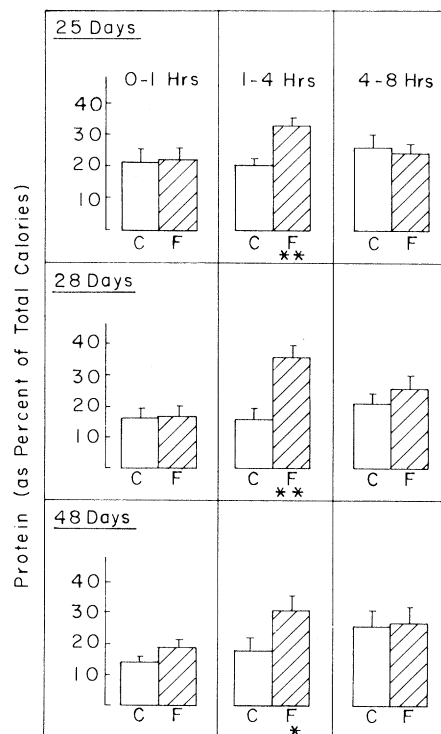


Fig. 1. Effect of fenfluramine on protein consumption. Groups of eight rats were given fenfluramine or its diluent and presented with two food pans containing 5 or 45 percent casein diets. The total amounts of each diet consumed during the next 0- to 1-, 1- to 4-, and 4- to 8-hour intervals were measured by weighing the pans. From these data, the grams of protein, total calories, and protein calories consumed were all calculated. The figure illustrates the percentage of total calories represented by protein that was consumed during each interval. In none of these studies did fenfluramine significantly alter the number of grams of protein consumed. Data are presented as the mean \pm the standard error of the mean. C, control; F, fenfluramine; *, $P < .01$; **, $P < .001$ (compared to controls).

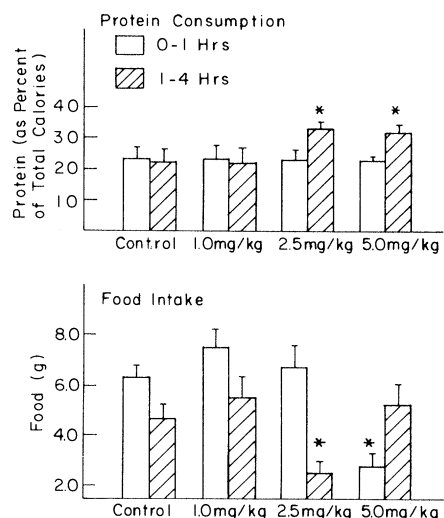


Fig. 2. Effect of fenfluramine on protein consumption and food intake. Groups of eight animals were treated as described in the legend to Fig. 1 and given the indicated fenfluramine doses. The 2.5 and 5.0 mg per kilogram doses significantly increased the percentages of total calories represented by protein 1 to 4 hours after fenfluramine administration. The dose at 2.5 mg per kilogram significantly decreased total food intake during this interval while the dose at 5.0 mg per kilogram decreased food intake during the first hour after its administration. Data are presented as means \pm standard error of the mean. *, $P < .01$, compared to control.

raising neuronal serotonin levels, tryptophan increases the amount of serotonin released by a given dose of fenfluramine.) Rats were treated with tryptophan (100 mg per kilogram of body weight) or its diluent 30 minutes before receiving a dose of fenfluramine that would not in itself modify food consumption (1 mg per kilogram) (Fig. 2). Those receiving both the amino acid and fenfluramine consumed no less food during the next 4 hours than control animals (5.25 as compared to 5.20 g); however, they ate significantly more protein [as the percentage of total calories (29 ± 3 as compared to 21 ± 3 , $P < .05$)]. Our data thus support a relation between serotonergic transmission and protein consumption but suggest that, under the conditions of our experiment, this relation differs from that proposed earlier (3)—that is, serotonin release by anorectic drugs preserves, and somehow stimulates, protein intake. Additional support for the view that brain serotonin mediates fenfluramine's differential effects on protein and calorie consumption was obtained from studies with another anorectic drug, *d*-amphetamine (2.5 mg per kilogram of body weight, intraperitoneally), whose actions in the central nervous system are thought to depend on the release of catecholamines, not serotonin (11). Amphetamine doses that consistently reduced food consumption never altered the proportion of total calories consumed as protein.

These observations suggest the following: (i) Anorectic drugs can differentially affect protein consumption regardless of calorie consumption. Drugs that sustain protein intake while reducing the consumption of calories may have special value in the long-term management of obesity. (ii) Protein consumption apparently is facilitated by anorectic drugs that increase intrasynaptic serotonin, but is not spared by anorectic agents that act primarily at nonserotonergic synapses. (iii) Different neuronal pathways thus underlie the mechanisms controlling the elective consumption of proteins and total calories (12).

JUDITH J. WURTMAN
RICHARD J. WURTMAN

Laboratory of Neuroendocrine
Regulation, Department of Nutrition
and Food Science, Massachusetts
Institute of Technology, Cambridge

References and Notes

1. J. E. Blundell, C. J. Latham, M. B. Leshem, *J. Pharm. Pharmacol.* **25**, 492 (1973); J. E. Blundell and M. B. Leshem, *ibid.* **27**, 31 (1975); W. Buczko, G. de Gaetano, S. Garattini, *Br. J. Pharmacol.* **53**, 563 (1975).
2. A. Goudie, E. W. Thornton, T. Wheeler, *J. Pharm. Pharmacol.* **28**, 318 (1976).
3. D. Ashley and G. H. Anderson, *J. Nutr.* **105**,

- 1412 (1975); T. Woodger, A. Sirek, G. H. Anderson, *Fed. Proc.* **36**, 1153 (1977).
4. Rats (Charles River Breeding Laboratories, Wilmington, Mass.) were housed singly in suspended cages and allowed free access to water. Light from cool-white fluorescent bulbs was provided between 9 p.m. and 9 a.m., and room temperature was kept at 22°C.
5. Both experimental diets contained (per kilogram): 150 g of corn oil, 22 g of vitamin mix (ICN Pharmaceuticals, Cleveland, Ohio), 40 g of Harper-Rogers Mineral Mix (Tekland Test Diets, Madison, Wis.), 35 g of agar, and 1000 ml of water. The 5 percent protein diet contained, in addition, 50 g of casein and 800 g of dextrin per kilogram; the 45 percent protein diet contained 450 g of casein and 400 g of dextrin per kilogram. Diets were put in individual feeding dishes; the locations of these pans within the cages were shifted daily.
6. The sucrose diets contained 23 percent casein, 5 percent corn oil, and agar, vitamin mix, and mineral salts as described in (4). The 24 percent sucrose diet contained 24 percent sucrose, 24 percent dextrin, and 24 percent dextrose; the 48 percent sucrose diet contained 48 percent sucrose, 12 percent dextrin, and 12 percent dextrose.
7. Fenfluramine was provided by the A. H. Robins Co., Richmond, Va.; fluoxetine was provided by the Eli Lilly Research Laboratories, Indianapolis, Ind.

8. In a typical experiment with 28-day-old rats, fenfluramine administration decreased consumption of the 5 percent casein diet from 3.1 to 0.5 g during this interval and increased consumption of the 45 percent casein diet from 3.0 to 4.0 g. Caloric intake thus fell from 29.0 to 21.3, whereas protein consumption rose from 1.50 to 1.82 g among rats receiving the drug.
9. J. D. Fernstrom and R. J. Wurtman, *Science* **178**, 414 (1972); *Sci. Am.* **230** (No. 12), 84 (1974); W. M. Partridge, in *Nutrition and the Brain*, R. J. Wurtman and J. J. Wurtman, Eds. (Raven, New York, 1977), vol. 1, p. 141.
10. J. L. Colmenares, R. J. Wurtman, J. D. Fernstrom, *J. Neurochem.* **25**, 825 (1975).
11. S. G. Holtzman and R. E. Jewett, *Psychopharmacologia* **22**, 151 (1971); R. Schulz and H. H. Frey, *Acta Pharmacol. Toxicol.* **31**, 12 (1972); S. F. Leibowitz, in *Hunger: Basic Mechanisms and Clinical Implications*, D. Novin, W. Wyrwicka, G. Bray, Eds. (Raven, New York, 1976), p. 1.
12. A brief description of some of these findings has appeared [J. J. Wurtman and R. J. Wurtman, *Fed. Proc.* **36**, 1132 (1977)].
13. These studies were supported in part by grants from the Kroc Foundation, the U.S. Public Health Service (AM-14228), and the National Aeronautics and Space Administration (NGR-22-009-627).

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“Bound Water” in Barnacle Muscle as Indicated in Nuclear Magnetic Resonance Studies

In their report on transverse nuclear magnetic resonance (NMR) relaxation in barnacle muscle, Foster *et al.* (1) described useful experiments. However, we think that their conclusion that “one water molecule per thousand, which is briefly and irrotationally bound, will produce the observed relaxation properties” of muscle water is misleading and that there are problems with the approach used.

1) To explain the relaxation time T_2 of muscle water, Foster *et al.* assumed a two-fraction exchange model in which the “bound” water molecule is “irrotationally bound.” This assumption is not supported by other experimental evidence. In fact, it has been shown that such a simplified assumption is incorrect for biological systems: on the basis of proton, deuterium, and oxygen-17 relaxation data, Koenig *et al.* (2) found that the two-fraction exchange model with irrotationally bound water failed to explain the relaxation times observed in protein solutions. Held *et al.* (3) studied the frequency dependence of the relaxation time T_1 in skeletal muscle, and their experimental results were also incompatible with the model of irrotationally bound water. Woessner and Snowden (4) found evidence for anisotropy of the rotational motion of bound water in agar solutions, which would not be obtained if the water molecules were irrotationally bound. The assumption of Foster *et al.* is therefore apparently incorrect.

2) It has been estimated from freezing

experiments (5, 6) that the fraction of bound water in skeletal muscle is of the order of 10 to 20 percent. Foster *et al.* reported that “this nonfreezing water [which is ascribed to bound water] is equivalent to about 0.07 g of water per gram of wet tissue.” (Taking the dry weight of barnacle muscle as 30 percent, their figure is equivalent to 10 percent bound water.) They also reported that this water exchanges with D_2O . We think that their approach may not adequately take into account the contribution of this water to the relaxation of the major portion of the cell water.

3) If Foster *et al.* draw a distinction between their observed nonfreezing bound water and their theoretical irrotationally bound water, they are, in effect, proposing two kinds of bound water. Since they argued that the nonfreezing bound water exchanged with the rest of tissue water, the relaxation effect of the nonfreezing water cannot be ignored. According to figure 2 in (1), the nonfreezing water has a T_2 of about 100 μ sec. For temperatures below 243°K, Belton *et al.* (6) gave an activation energy of 5.2 kcal/mole for the temperature dependence of T_2 of the unfrozen water in frog muscle. Using this activation energy, we extrapolate 100 μ sec at -34°C to 878 μ sec at 298°K. If there is rapid exchange between the nonfreezing bound water and the muscle water, as Foster *et al.* implied, the T_2 of the whole tissue water at 298°K would be approximately 8.8 msec. The relaxation effect of this nonfreezing water alone is more than enough