particles (*18*). The sections on grids were stained with uranyl acetate followed by lead citrate, and were then examined with an AEI transmission electron microscope at 50 kv. The experiment was repeated with surface-sterilized seeds grown on glass beads partially immersed in a sterile nutrient solution (*19*) and enclosed in sealed, aerated glass containers.

A diffuse network of distinctive, curved, electron-opaque fibrils (and loosely assembled tufts of them) was found at the rhizoplane of the tip of all roots examined. These rhizoplane fibrils at the root-soil interface, between the root hair zone and the root cap, are described here for the first time. They are curved ribbons whose diameter usually varies from 3 to 10 nm. Their form is similar to that of fibrils of a recently described, complex material composed mainly of polygalacturonic acid (*10*) and found at the surface of some plant cells grown axenically. Figure 1A shows the distinctive appearance of some fibrils which form a loose tuft projecting from the rhizoplane into the rhizosphere. Rhizosphere bacteria were often well preserved, and the fixation image of root cells internal to the epidermal layer was similar in quality to that of previous work (*13*) done with similar combinations of fixatives and "electron stains." Substances in the soil solution had no obvious deleterious effect on the primary fixation, but mineral particles interfered with the quality of sectioning. The distribution of fibrils in the rhizoplane of root tips was patchy, as was the distribution of fibril tufts projecting into the rhizosphere volume. Penetration of mineral particles by the fibrils could not be studied in sections approximately 50 nm thick. Sections of the root tips grown under sterile conditions (Fig. 1B) revealed rhizoplane fibrils (including a patchy distribution of tufts of fibrils) and a total absence of microbes. Scanning electron microscopy confirmed the absence of surface microbes. Thus, the fibrils were derived from plant cells.

The rhizoplane fibrils were revealed by a combination of electron stains previously used to reveal fibrillar polygalacturonic acid. The fibrils in each case have a similar form (*13*), polymers containing galacturonic acid do exist at some root surfaces (*20*), and the enzyme polygalacturonase is important in the invasion of some roots by microbes (*21*). These facts are consistent with the assumption that the rhizoplane fibrils may be composed in part of galacturonic acid polymers. Detailed knowledge of their chemistry is required, particularly in view of recent suggestions by Ramamoorthy and Manning (*22*) concerning nutrient and heavy metal uptake by roots.

Structures considered to be composed partially of uronic acid polymers have been described previously for root surfaces (*3, 8*). These structures were not resolved as fibril aggregates, but perhaps reinvestigation would show them to be fibrillar. It is interesting to note that rhizoplane fibrils do not normally appear in electron micrographs in the literature on roots. A major reason for this anomaly appears to be inadequate enhancement of contrast by most electron-staining procedures. That the ruthenium-osmium stain enhances contrast of existing fibrils (as opposed to creating artifactual fibrils) has been shown previously (*13*).

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Fenfluramine: Amphetamine Congener That Fails to Maintain Drug-Taking Behavior in the Rhesus Monkey

Abstract. *Fenfluramine, over a dose range from 0.003 to 3 milligrams per kilogram of body weight, failed to maintain self-injection behavior in rhesus monkeys that had initiated and maintained responding for cocaine or methohexital. This absence of a positive reinforcing effect could not be attributed to a slow onset of drug effect or to the use of behaviorally inactive doses. Fenfluramine, because of its distinctive properties, may produce fewer problems of human abuse than do amphetamine-type agents.*

Fenfluramine [*N*-ethyl-*m*-(trifluoromethyl)amphetamine] manifests some, but not all, of the pharmacological actions of amphetamine and related phenethylamines. Like the latter compounds, fenfluramine decreases food intake in animals (*1*) and is clinically efficacious in the initiation of treatment for obesity in man (*2*). In contrast to amphetamine, however, fenfluramine has much less pressor activity in animals and little, if any, pressor activity in man, it does not induce amphetamine-like response stereotypy, and it lacks psychomotor stimulant actions in animals or humans (*1, 3*). Furthermore, the electroencephalographic effects of fenfluramine in man resemble those of amobarbital rather than those of amphetamine (*4*). The possibility that fenfluramine lacks an amphetamine-like subjective effect in man is suggested by the results of a study in which amphetamine users were asked to compare fenfluramine's effects to those of amphetamine. They judged fenfluramine to be no more similar to amphetamine than was the placebo (*5*).

Thus, while fenfluramine retains a capacity to reduce appetite, it differs markedly from amphetamine-like agents in several pharmacological aspects.

Amphetamine and a large number of substituted phenethylamines also strengthen (reinforce) responding when drug injections are made contingent upon the occurrence of a response or a sequence of responses. In this behavioral paradigm, rats and monkeys demonstrate a pattern of amphetamine-reinforced responding similar to that of amphetamine-dependent humans, that is, periods of sustained intoxication alternating with abstinence from amphetamine (*6–8*).

The purpose of the present experiments was to determine whether fenfluramine would function similarly to other amphetamine-like agents in maintaining lever-press responding in monkeys. Since amphetamine and other phenethylamines function as reinforcers in animals, and in view of the similarity of the temporal pattern of self-administration by animals and humans, demonstration that fenfluramine functions as a reinforcer in animals would suggest that it might also be abused by man; the inability to obtain such a relationship would suggest the converse.

The procedure used in these experiments takes advantage of the observation that amphetamine maintains high rates of self-injection responding in the rhesus monkey when the drug is substituted for cocaine or for a barbiturate. On the other hand, after replacement of these latter compounds by saline, rates of responding decrease markedly (*9*). In our protocol, monkeys were initially trained to press a lever that led to the intravenous injection of either cocaine or a barbiturate (methohexital). Subsequently, fenfluramine was injected after a lever-press response. Therefore, if fenfluramine is like amphetamine in this regard, it should maintain high self-injection rates.

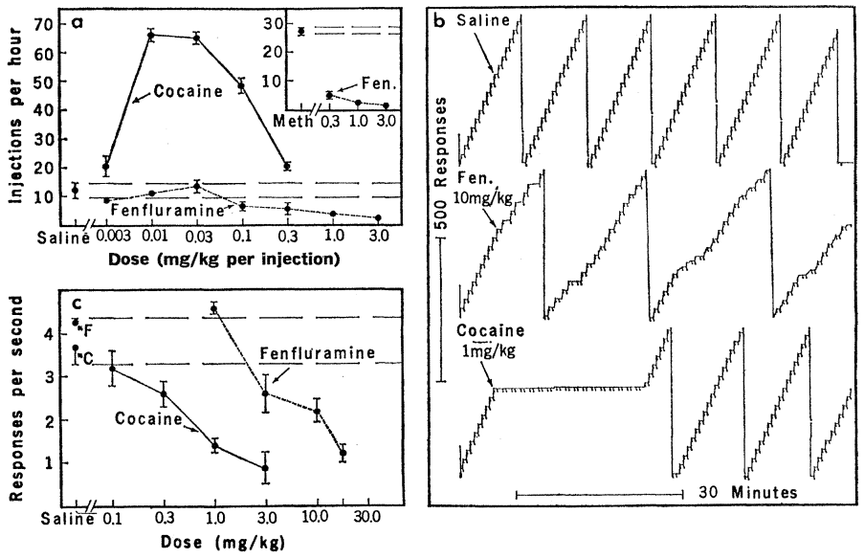

Fig. 1. (a) Response-produced injection rates of saline, cocaine, methohexital, or fenfluramine. Lever-press responding was maintained by cocaine (0.1 mg/kg per injection) in two groups of monkeys and by methohexital (0.3 mg/kg per injection) (inset) in a third group. In the methohexital group and one of the cocaine groups, saline or one of a variety of fenfluramine doses was substituted for ten 1-hour sessions. In the second cocaine group, one of several doses of cocaine was substituted for the same number of sessions. Saline and the substituted doses are indicated on the abcissa. After each substitution, either cocaine (0.1 mg/kg per injection) or methohexital (0.3 mg/kg per injection) was made available until responding returned to the presubstitution rate for at least five 1-hour sessions. Data points are the average of at least 30 observations (ten 1-hour sessions for each of three monkeys). Brackets indicate ± 1 standard error of the mean. (b) Cumulative records for a single rhesus monkey illustrating the effects of saline (upper record), fenfluramine, 10.0 mg/kg (middle record), and cocaine, 1.0 mg/kg (bottom record) on food-reinforced responding. Thirty lever-press responses in the presence of a red light resulted in delivery of a 300-mg food pellet. A 30-second green-light period followed either food presentation or failure to complete the response requirement within 1 minute. Upward steps of the recorder pen indicate lever-press responses; downward strokes of the pen denote changes of light condition. The recorder reset automatically after approximately 550 responses. Drugs were administered after the tenth food delivery. Saline had no effect on responding whereas fenfluramine produced an immediate and long-lasting decrease in response rate; cocaine produced an immediate cessation of responding for approximately 30 minutes. (c) Dose-effect relation of cocaine and fenfluramine on the rates of food-reinforced responding. The procedure is summarized in (b). The data points represent the mean rates of responding (brackets, ± 1 standard error of the mean) and are based on at least six observations per dose, three observations in each of two monkeys. Control rates of responding for the two pairs of monkeys are shown at left. Both cocaine and fenfluramine had dose-related rate-decreasing effects on responding; fenfluramine was approximately ten times less potent than cocaine.

Subjects were nine rhesus monkeys, prepared with intravenous catheters (*10*). Six monkeys were given access to cocaine at 0.1 mg per kilogram of body weight per injection and three monkeys were given access to methohexital at 0.3 mg/kg per injection; initially, drug was delivered after every lever press. When the rate of responding increased to approximately 20 to 30 lever-presses per hour, drug access was limited to two equally spaced, 1-hour sessions (10 a.m. and 10 p.m.) every 24 hours. Access to drug was signaled by the illumination of a red light above the lever. When the red light was not illuminated, lever responses had no programmed consequence. Within 20 sessions, drug-reinforced responding was occurring regularly during each 1-hour session. A series of cocaine doses was substituted for the maintenance dose (0.1 mg/kg per injection) in three monkeys receiving cocaine. For the other three monkeys in which responding was maintained by cocaine and for those in which responding was maintained by methohexital, a series of fenfluramine doses was substituted for the maintenance drug. Each new dose of cocaine or fenfluramine remained available during the illumination of the red light for ten sessions, whereupon the maintenance dose of cocaine or methohexital was reinstated until drug-reinforced responding was occurring at presubstitution rates for five or more sessions. In this manner, observations for a range of cocaine or fenfluramine doses and for an equal volume of saline were obtained in an unsystematic sequence.

Fenfluramine (0.003 to 3.0 mg/kg per injection) failed to maintain rates of lever-press responding above those maintained by saline in monkeys that had previously initiated and maintained responding for cocaine (0.1 mg/kg) (Fig. 1a). In addition, when monkeys were given access to fenfluramine (0.3 to 3.0 mg/kg per injection) after having initiated and maintained responding for methohexital (0.3 mg/kg per injection), responding was negligible (inset in Fig. 1a). Low doses of fenfluramine produced response rates ap-

proximately equal to those produced by saline, whereas high doses depressed rates.

Response rates for the various doses of cocaine were an inverted U-shaped function of dose, with peak rates at 0.01 and 0.03 mg/kg per injection. The shape of the cocaine dose function and the doses that produce maximal injection rates are consistent with previous research (*11, 12*). The lowest dose of cocaine that served a reinforcing function under these conditions was 0.003 mg/kg per injection. Presumably, under these conditions this dose was simply too weak to support greater responding. As the dose increased, rates of drug-reinforced responding increased; further increments in dose led to decreased responding. These results suggest that larger doses of drug impede the expression of the rate-increasing or reinforcing effects by the induction of other behavioral effects (*12, 13*).

By analogy to the situation with cocaine, the identification of a drug as a reinforcer in this behavioral procedure might lead to an incorrect conclusion if the dose used were either too small or too large. It thus appeared appropriate to use some other behavioral effect of fenfluramine to assure that a behaviorally significant range of doses was being examined. Furthermore, a relatively slow onset of behavioral effect could also have been a contributing factor to the failure of fenfluramine to serve as a reinforcer. To investigate these considerations, the effects of intravenously administered fenfluramine and cocaine were determined and compared in food-deprived rhesus monkeys for which lever-press responding was maintained by presentations of food pellets. Thirty lever-press responses in the presence of a red light were required for the delivery of a 300-mg banana-flavored food pellet. A green light was turned on for 30 seconds after each food delivery or when the monkey failed to complete the 30-response requirement within 1 minute. During the green-light period, food pellets were not delivered and each lever-press response delayed the next red-light period by 30 seconds.

Figure 1b illustrates the pattern of responding for a single monkey during portions of three experimental sessions in which saline, fenfluramine (10.0 mg/kg), or cocaine (1.0 mg/kg) was intravenously administered after the tenth food delivery. During the saline control session, the monkey responded at a rate of approximately four responses per second in the presence of the red light but almost never responded in the presence of the green light. Intravenous administration of saline had no discernible effect upon responding. Fenfluramine had an almost immediate rate-decreasing effect. Cocaine also had a rapid effect; the rate decrease was more marked and of shorter duration.

As noted earlier, cocaine in large doses may impede responding that leads to further drug delivery. The behavioral rate-decreasing effects of cocaine upon food-reinforced responding may provide an approximate guide toward establishing a dose regimen that might impede self-injection responding. As seen in Fig. 1b, responding for food ceased for 30 minutes after administration of cocaine (1 mg/kg), while a single dose of 0.3 mg/kg produced a pause of approximately 10 minutes. Successive rapid cocaine injections of 0.1 or 0.3 mg/kg per injection are thus in the dose range that suppresses food-reinforced responding and, by analogy, might suppress responding that leads to cocaine delivery.

In Fig. 1c, the dose-effect relations of cocaine and fenfluramine on the rate of food-reinforced responding are summarized. Fenfluramine was approximately ten times less potent than cocaine in suppressing responding (*14*). By comparing the data from Figs. 1a and 1c, it can be concluded that cocaine serves as a reinforcer in a dose approximately one-hundredth that necessary to produce a reliable decrease in food-reinforced responding. If fenfluramine were similar to cocaine with respect to relative potency in these two procedures, fenfluramine should have had its maximal rate-increasing (reinforcing) effects in the dose range of 0.03 to 0.1 mg/kg per injection. Yet these and smaller doses maintain responding at rates comparable to those of saline. Fenfluramine doses of 3 mg/kg or greater suppress food-reinforced responding when given as a single injection, and cumulative self-injected doses of this or slightly smaller magnitudes appear as well to suppress responding that leads to their injection. Together with the rapid onset of the effect on food-reinforced responding, these data confirm that the failure of fenfluramine to reinforce self-injection responding was due neither to the use of an inappropriate dose range nor to the lack of immediacy of behavioral effect.

We conclude that fenfluramine does not reinforce responding that leads to fenfluramine administration in monkeys that had initiated and maintained cocaine- or methohexital-reinforced responding. Since amphetamine serves as a reinforcer in the rhesus monkey, in a protocol similar to one used in the present experiment (*9, 12, 15*), the structural congeners of fenfluramine deserve further behavior and pharmacological study. The data also lend credence to the notion that fenfluramine may not possess some of the pharmacological and behavioral attributes associated with human abuse of amphetamines. In addition, amphetamine-dependent individuals often exchange drugs with similar pharmacological action (*8, 16*); these data strongly suggest that fenfluramine would not act as an amphetamine-like substitute in these individuals.

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