0022-3565/93/2673-1256$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 267, No. 3
Printed in U.S.A.

Fluoxetine Prevents the Disruptive Effects of Fenfluramine on Differential-Reinforcement-of-Low-Rate 72-Second Schedule Performance[1]

JERRY B. RICHARDS, KAREN E. SABOL and LEWIS S. SEIDEN
The University of Chicago, Department of Pharmacological and Physiological Sciences, Chicago Illinois
Accepted for publication August 23, 1993

ABSTRACT

This study compared the effects of fenfluramine and fluoxetine on the differential-reinforcement-of-low-rate 72-s schedule of reinforcement. Fluoxetine, a clinically effective antidepressant, increases extracellular serotonin (5-HT) by blocking the uptake of 5-HT after release. Fenfluramine increases extracellular 5-HT through transporter-mediated release (although it also blocks 5-HT uptake). The following characteristics were identified. First, fenfluramine and fluoxetine had two different effects on the differential-reinforcement-of-low-rate 72-s schedule. Fluoxetine had an antidepressant-like effect by increasing reinforcement rate without disrupting the interresponse time distribution. Fenfluramine's effect on the differential-reinforcement-of-low-rate 72-s schedule was not antidepressant-like: it did not increase the reinforcement rate, whereas it did disrupt the interresponse time distribution. Second, when fluoxetine and fenfluramine were given in combination, fluoxetine prevented the disruptive effects of fenfluramine. This result is consistent with fluoxetine's ability to block fenfluramine-induced 5-HT release, and supports the argument that the uptake transporter mediates fenfluramine's effects on both 5-HT release and behavior. Putative behavioral mechanisms (waiting capacity and temporal discrimination) which may mediate the acute effects of fluoxetine are discussed.

A variety of antidepressant compounds have been shown to have distinctive effects on the DRL 72-s schedule of reinforcement (Seiden *et al.*, 1985). The DRL 72-s schedule requires rats to wait at least 72 s between bar press responses in order to earn a reinforcer. The IRT distributions of rats trained on the DRL 72-s schedule have modes (or peaks) at IRT durations less than the 72-s criterion for reinforcement. These peaks indicate that rats trained on the DRL 72-s schedule systematically wait between responses but that they do not wait long enough. Because the rats systematically respond too soon they typically obtain only 10 to 12 reinforcers on average in a 1-hr test session. Antidepressant compounds shift the peak of the IRT distribution toward longer IRT durations in a coherent fashion (*i.e.*, without disrupting the profile of the IRT distribution). The shift in the IRT distribution toward longer durations results in an increased reinforcement rate (O'Donnell and Seiden, 1982, 1983; Richards *et al.*, 1993; Richards and Seiden, 1991).

Manipulation of the 5-HT system has resulted in a variety of effects on the DRL 72-s schedule. Selective serotonergic lesions (induced by the neurotoxin 5,7-DHT) cause a decrease in earned reinforcers, which is accompanied by a complete disruption of the IRT distribution (Jolly *et al.*, 1991). 5-HTP (the precursor to 5-HT) increases reinforcers earned, without disrupting the IRT distribution (Marek *et al.*, 1989; Richards et al., in press). The gepirone-like 5-HT_{1A} agonists also increase reinforcement rate, however, this increase is accompanied by a disruption of the IRT distribution (Richards *et al.*, in press; Richards and Seiden, 1991). The serotonergic agonist fluoxetine (a 5-HT uptake inhibitor with antidepressant efficacy in humans [see Rudorfer and Potter, (1989)] also increases reinforcement rate (Marek *et al.*, 1989; Seiden *et al.*, 1985), but its effects on the distribution of IRTs have not previously been characterized.

The antidepressant fluoxetine is an indirect serotonergic agonist which facilitates serotonergic transmission by blocking the uptake of 5-HT back into the nerve terminal after release (Fuller *et al.*, 1991; Wong *et al.*, 1975). Another indirect 5-HT agonist is fenfluramine, a 5-HT releasing agent with uptake inhibition capability (Fuxe *et al.*, 1975; Garattini *et al.*, 1975; see Sabol *et al.*, 1992). Fenfluramine's efficacy as an antidepressant agent in humans has not been systematically tested,

Received for publication October 8, 1992.
[1] This work was supported by United States Public Health Service Grant MH-11191 and by Research Scientist Award RSA MH-10562 to L.S.S.

Abbreviations: DRL 72-s, differential-reinforcement-of-low-rate 72-s; IRT, interresponse time; 5-HT, 5-hydroxytryptamine (serotonin); 5,7-DHT, 5,7-dihydroxytryptamine; 5-HTP, 5-hydroxytryptophan; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; V, vehicle; PkA, peak area; PkL, peak location; BR, burst ratio.

but it has been described as both potentially effective (O'Rourke *et al.*, 1989), and ineffective (Price *et al.*, 1990) for the treatment of depression.

Both fenfluramine and fluoxetine are similar in that their effects are thought to be mediated primarily by an increase in extracellular levels of 5-HT. The two compounds are dissimilar, however, in their mechanism of action at the cellular level. Fenfluramine increases extracellular concentrations of 5-HT predominantly by transporter-mediated release. That is, fenfluramine is thought to enter the 5-HT nerve terminal through the uptake carrier and cause 5-HT to be released, also through the uptake carrier (Garattini *et al.*, 1975; see also Fuller, 1980). Fenfluramine-induced 5-HT release is independent of nerve cell firing *in vivo* (Carboni and Di Chiara, 1989). Fluoxetine, on the other hand, blocks the uptake of 5-HT after it is released into the synaptic cleft (Wong *et al.*, 1975). The increase in extracellular 5-HT concentrations with fluoxetine (Perry and Fuller, 1992), as well as with other uptake inhibitors (Carboni and Di Chiara, 1989; Matos *et al.*, 1990) is dependent on nerve cell firing *in vivo*. The amount of 5-HT overflow that occurs will depend on the amount of impulse-dependent release. Fluoxetine's effects are therefore dependent upon 5-HT cell firing.

Even though both fenfluramine and fluoxetine increase extracellular concentrations of 5-HT, these drug-induced increases in 5-HT are not additive. As described above, both fenfluramine and fluoxetine depend upon the 5-HT uptake transporter to increase extracellular 5-HT. Fluoxetine not only blocks the uptake of 5-HT, it also blocks the fenfluramine-induced release of 5-HT *in vivo* (Sabol *et al.*, 1992) and *in vitro* (Hekmatpanah and Peroutka, 1990).

Previous research has demonstrated that fluoxetine can prevent some but not all of the functional consequences of fenfluramine administration. Fluoxetine attenuates fenfluramine-induced hyperthermia (Sugrue, 1984), as well as the stimulation of prolactin and corticosterone secretion (McElroy *et al.*, 1984; Van de Kar *et al.*, 1985) by fenfluramine. Fluoxetine has been reported to either attenuate (Clineschmidt and McGuffin, 1978) or have no significant effect on (Fornal and Radulovacki, 1982) fenfluramine-induced head twitches. Fluoxetine also failed to prevent the sleep suppressing effects of fenfluramine (Fornal and Radulovacki, 1982). In other cases the prevention of fenfluramine's effects by fluoxetine is made unlikely by the fact that both compounds have similar effects. For example both fluoxetine and fenfluramine reduce food intake (Fuller and Wong, 1989; Fuxe *et al.*, 1975; Garattini *et al.*, 1975; Goudie *et al.*, 1976; Wurtman and Wurtman, 1977).

The first objective of this study was to determine the effects of fluoxetine and fenfluramine on DRL 72-s schedule performance. Because both fluoxetine and fenfluramine increase extracellular 5-HT (although through different mechanisms) the effects of these two compounds on DRL 72-s performance would be expected to be similar. However, in terms of antidepressant activity, the effects of fenfluramine and fluoxetine on the DRL 72-s schedule performance would be predicted to be different. The second objective of the study was to determine the ability of fluoxetine to prevent the effects of fenfluramine on DRL 72-s schedule performance. If fenfluramine's effects on DRL 72-s performance depend upon the transporter-mediated 5-HT release, then fluoxetine should attenuate fenfluramine's effects. The results showed that the antidepressant fluoxetine had antidepressant-like effects while fenfluramine did not. When given together fluoxetine attenuated the effects of fenfluramine in a dose-dependent fashion.

Methods

Animals. Nineteen male Sprague-Dawley rats (Holtzman, Madison, WI) weighing between 350 and 500 g were used. The rats were 11 months old at the beginning of the experiment reported below. The rats were housed two per cage in hanging stainless steel wire cages. Lights were on in the colony room from 7 A.M. to 7 P.M. Food (4% Teklad rat chow) was available *ad libitum*. Access to water was restricted to 20 min per day. On the training days the rats received 20-min access to water at the end of their training session. On nontraining days (weekends), the rats were given 20-min access to water between 10 A.M. and 2 P.M. Before the experiment, the rats had previously received dose-response determinations of various 5-HT_{1A} agonists. Six of the rats received previous injections of zalospirone (WY-47,846), 8-OH-DPAT and gepirone. Seven of the rats received previous injections of WY-48,723, buspirone and gepirone. Six of the rats received previous injections of WY-50,324, ipsapirone and gepirone.

Apparatus. Nineteen operant chambers were used. Each operant chamber was 20.5 cm wide, 20.5 cm deep and 23.5 cm long. The operant chambers had grid floors, aluminum front and back walls and Plexiglas sides. A lever was mounted 3 cm above the grid floor 4.5 cm from the nearest side. A downward force of approximately 0.15 N was required for a lever press to be detected. A solenoid-operated dipper was located 10 cm to the left of the lever. Access to the dipper was through a round 4.5-cm diameter hole in the front panel. Reinforcement consisted of lifting the dipper (0.025 ml) from a water trough to within reach of the rat's tongue for a period of 4 s. A stimulus light mounted 15 cm above the floor on the back wall of the chamber provided the only illumination within the chamber. The stimulus light was turned on when a training session began and off when the training session ended. The operant chambers were enclosed in 80-quart Coleman ice chests to attenuate external stimuli. Fans mounted on the ice chests provided ventilation and masking noise. The operant chambers were connected to a PDP-11/73 microcomputer *via* a Coulbourn Lablinc interface. The schedule contingencies were programmed using the SKED-11 software system (Snapper *et al.*, 1976). The timing resolution of the system was 0.01 s.

Training. Upon arrival in the colony the rats were adapted to the 20-min per day access to water regimen for 1 week. The rats were then trained to bar press in overnight training sessions using an alternative FR1, FT 1-min schedule. Rats which did not acquire the lever press response after five overnight training sessions were hand shaped. The rats were then shifted to a DRL 72-s training regimen. DRL 72-s overnight training consisted of six 1-hr sessions with a 30-min timeout (house light off) between each session. The rats were trained overnight on the DRL 72-s schedule for 10 nights. Finally, the rats were trained during daily (5 days a week) 1-hr sessions on the DRL 72-s schedule. At the beginning of the present experiment the rats had been trained on the DRL 72-s schedule for approximately 7 months (5 days a week) in 1-hr sessions.

Drug administration. Fenfluramine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and fluoxetine hydrochloride (a gift from Lilly, Indianapolis, IN) was dissolved in distilled water to form an injectable solution of 1 ml/kg. Both fenfluramine and fluoxetine were in their racemic forms. Fenfluramine was injected intraperitoneally 60 min before the start of the 1-hr session and fluoxetine was injected intraperitoneally 80 min before the start of the session. The animals received drugs in the following sequence: first, a dose response determination for fenfluramine (V, 0.5, 1.0, 2.0, 4.0 mg/kg); second, a dose response determination for fluoxetine (V, 2.5, 5.0, 10.0, 20.0 mg/kg); third, a single dose of fenfluramine (4.0 mg/kg) combined with a dose-response determination of fluoxetine (V, 2.5, 5.0, 10.0 mg/kg of fluoxetine). In the combined fenfluramine-fluoxetine experiment, fluoxetine was administered 20 min before fenfluramine. All doses were given in ascending order, with the exception of 4.0 mg/kg of fenfluramine + fluoxetine V, which was given last. All drug doses

were given as the salt. Drugs were administered on Tuesdays and Fridays. Control performance was the average of the Thursdays which occurred during each drug's dose response determination.

IRT analysis. The IRT distributions generated by DRL 72-s schedule performance were quantitatively characterized using peak deviation analysis. Peak deviation analysis includes three measures for the characterization of the profile of DRL IRT distributions: PkA, PkL and BR. The PkA metric indicates the proportion of responses in the peak of the IRT distribution which lie above a random prediction. The PkL metric indicates the central location of the peak. The BR metric indicates the propensity to burst. Each of these measures is briefly described below (see Richards *et al.*, 1993 for a detailed description).

An example DRL 72-s IRT distribution is shown in figure 1. The IRT distribution of most rats trained on the DRL 72-s schedule is bimodal, with one mode occurring at short IRT durations and a second mode occurring at longer IRT durations. Because of the bimodal nature of DRL 72-s IRT distributions, peak deviation analysis divides the obtained IRT distribution into separate burst (IRTs <6 s) and pause (IRTs ≥6 s) components.

The basis for peak deviation analysis is the comparison of each rat's obtained IRT distribution with a theoretical distribution that predicts the appearance of the obtained IRT distribution had the rat emitted responses at the same overall rate, but randomly in time with respect to the preceding response. This expected curve is called the corresponding negative exponential. The corresponding negative exponential was computed based on the mean of the obtained pause IRT durations with bursts (IRTs <6 s) excluded. [For computational details see Richards *et al.*, 1993, and Richards and Seiden (1991)]. Because the corresponding negative exponential is determined by the mean IRT duration of the obtained distribution the random prediction of the corresponding negative exponential adjusts as the mean of the obtained IRT distribution changes. Adjustment of the corresponding negative exponential insures that deviations from the corresponding negative exponential are equivalent for obtained distributions with different mean IRT durations. For example, drugs which change the obtained response rate (and its reciprocal, mean IRT duration) also change the corresponding negative exponential prediction.

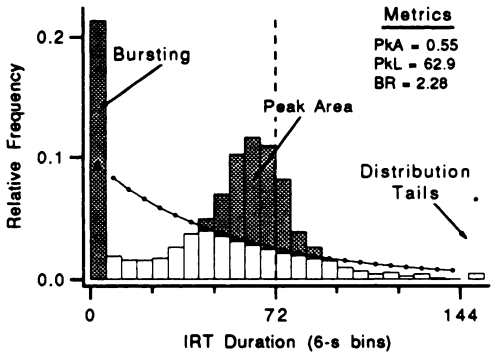

Fig. 1. Relative frequency histogram of the IRTs of rats trained on a DRL 72-s schedule of reinforcement illustrating the PkA, PkL and BR metrics. The single shaded histogram bar on the left indicates the burst component of the IRT distribution (IRTs <6 s). The bars to the right of the burst component indicate the pause component of the IRT distribution (IRTs ≥6 s). The connected dots indicate the expected appearance of the pause component of the IRT distribution if the rats emitted the same number of responses, but randomly in time with respect to the preceding response. This expected curve is called the corresponding negative exponential. The PkA is indicated by the shaded region of the obtained IRT histogram above the corresponding negative exponential. The PkL is the median IRT duration which bisects the shaded region above the corresponding negative exponential. The triangle in the burst category indicates the relative frequency of burst responses predicted by extrapolation of the corresponding negative exponential into the burst component. The ratio of the obtained to the predicted burst responses is designated the BR measure. The single dot at the far right indicates the relative frequency of IRT >144 s predicted to occur in the tail of the corresponding negative exponential. Similarly, the single histogram bar at the far right indicates the relative frequency of IRTs >144 s in the tail of the obtained IRT distribution. The dashed vertical line indicates the 72-s IRT duration requirement for reinforcement.

In figure 1 the single shaded histogram bar on the left indicates the burst component of the obtained IRT distribution (IRTs <6 s). The histogram bars to the right of the burst component indicate the pause component of the obtained IRT distribution. The single histogram bar at the far right of figure 1 indicates the relative frequency of IRTs >144 s in the tail of the obtained IRT distribution. Connected dots show the shape of the pause distribution (IRTs ≥6 s) if the rats had responded randomly (*i.e.*, the prediction made by the corresponding negative exponential curve). The triangle in the burst category indicates the relative frequency of burst responses predicted by the corresponding negative exponential. The single dot at the far right of figure 1 indicates the relative frequency of IRTs >144 s predicted to occur in the tail of the corresponding negative exponential.

The PkA and PkL metrics characterize the pause component (IRTs >6 s) of the IRT distribution. The PkA measure is the area of the obtained IRT distribution above the corresponding negative exponential (sum of the relative frequencies in the shaded area of the pause component of the distribution in fig. 1). The areas under the obtained pause distribution and corresponding negative exponential pause IRT distribution are equal. The largest possible PkA value (1.0) would occur only if all of the obtained IRT durations have exactly the same value. The smallest possible PkA value (0.0) would indicate that the obtained and corresponding negative exponential distributions are identical. Thus, decreases in PkA indicate that the rat's IRT distribution has become more similar to random performance. The PkL measure is the central IRT duration (median) of the shaded area in of the pause component in figure 1. The PkL does not always correspond to the modal value of the obtained IRT distribution because its computation does not include the area below the corresponding negative exponential.

The corresponding negative exponential shown in figure 1 is extrapolated into the burst category to provide a prediction for the number of IRT durations expected to occur in the burst category if the rat emitted responses randomly at a constant overall rate with no difference between burst and pause responding. This prediction (indicated by the triangle in the shaded burst category of the histogram in fig. 1) is used to calculate the BR metric. The BR is the number of obtained IRT durations in the burst category divided by the number of IRT durations predicted to occur in the burst category by the corresponding negative exponential. Measuring only the absolute or relative frequency of IRTs in the burst category ignores the fact that as the mean of the pause IRT distribution becomes smaller the chance probability of an IRT occurring in the burst category increases.

A computer-based method for computation of the PkA, PkL and BR metrics is fully described in Richards *et al.*, 1993. Disruption of the pause component of the IRT distribution by drug treatments frequently causes multiple peaks to occur. The above method uses a peak search algorithm which locates the largest deviation (peak) above the corresponding negative exponential and calculates its area and location. The algorithm computes PkA and PkL without sorting the IRTs into class intervals. A PASCAL routine which implements this algorithm is available from the authors.

Data Analysis. Response and reinforcement rate measures are valid even if the subjects make zero responses. However, in the case of IRT analysis, a minimum number of IRTs are required in order for there to be a distribution to measure. Reliable estimates of the peak deviation analysis metrics require at least 25 responses in the pause component (IRTs ≥6 s) of the IRT distribution. Under base-line conditions the rats trained on the DRL 72-s schedule in this study always made more than 25 pause responses. However, when given drugs, some rats failed to make 25 or more responses, particularly at higher doses. The PkA, PkL and BR metrics of individual rats were not included in the data analysis at doses where they failed to make at least 25 pause responses. To ensure that the IRT analysis corresponded to the response and reinforcement rate analysis, response and reinforcement rate measures were not included in the data analysis for individual rats which made

fewer than 25 pause responses at a given dose. Occasions when rats failed to make at least 25 pause responses are clearly indicated in the results and figures.

Response rate, reinforcement rate, PkA, PkL and BR measures were taken for each rat at each dose of the drug (except as noted above). Control values for each measure were compared to each drug dose (including vehicle) using *t* tests. A Bonferroni correction (Neter and Wasserman, 1974) was used to guard against a type 1 error due to multiple comparisons. The overall level of significance was set at $P < .05$, two-tailed.

Results

Fenfluramine. Response and reinforcement rates were not significantly affected by fenfluramine during the initial dose response determination (fig. 2, left panel). Two rats failed to make at least 25 responses at the 4.0-mg/kg dose of fenfluramine.

In contrast to the absence of a systematic effect on overall response and reinforcement rate fenfluramine dose-dependently decreased PkA (fig. 2, left panel). This decrease in PkA indicates that fenfluramine disrupted the distribution of IRTs. At the highest dose of fenfluramine the PkL was significantly shifted toward shorter IRT durations. The BR was not significantly affected at any dose of fenfluramine.

The disruptive effects of fenfluramine on the IRT distribution profile are shown graphically in figure 3 (left panel). As the dose of fenfluramine increased, the obtained IRT distribution became increasingly similar to the corresponding negative exponential distribution. The disruption of the IRT distribution is reflected in the significant decrease in PkA and indicates a decrease in temporal stimulus control.

Fluoxetine. Reinforcement rate was increased and response rate was decreased in a dose-dependent fashion by fluoxetine (fig. 2, left panel). Five rats failed to make 25 or more pause responses at the 20-mg/kg dose.

Fluoxetine had very different effects on the profile of the IRT distribution than fenfluramine (fig. 2, left panel). In strong contrast to the effects of fenfluramine, the PkA of the IRT distribution was not affected by any dose of fluoxetine. Also in contrast to fenfluramine the PkL of the IRT distribution was significantly shifted toward longer IRT durations. The BR was significantly decreased at the 20.0-mg/kg dose.

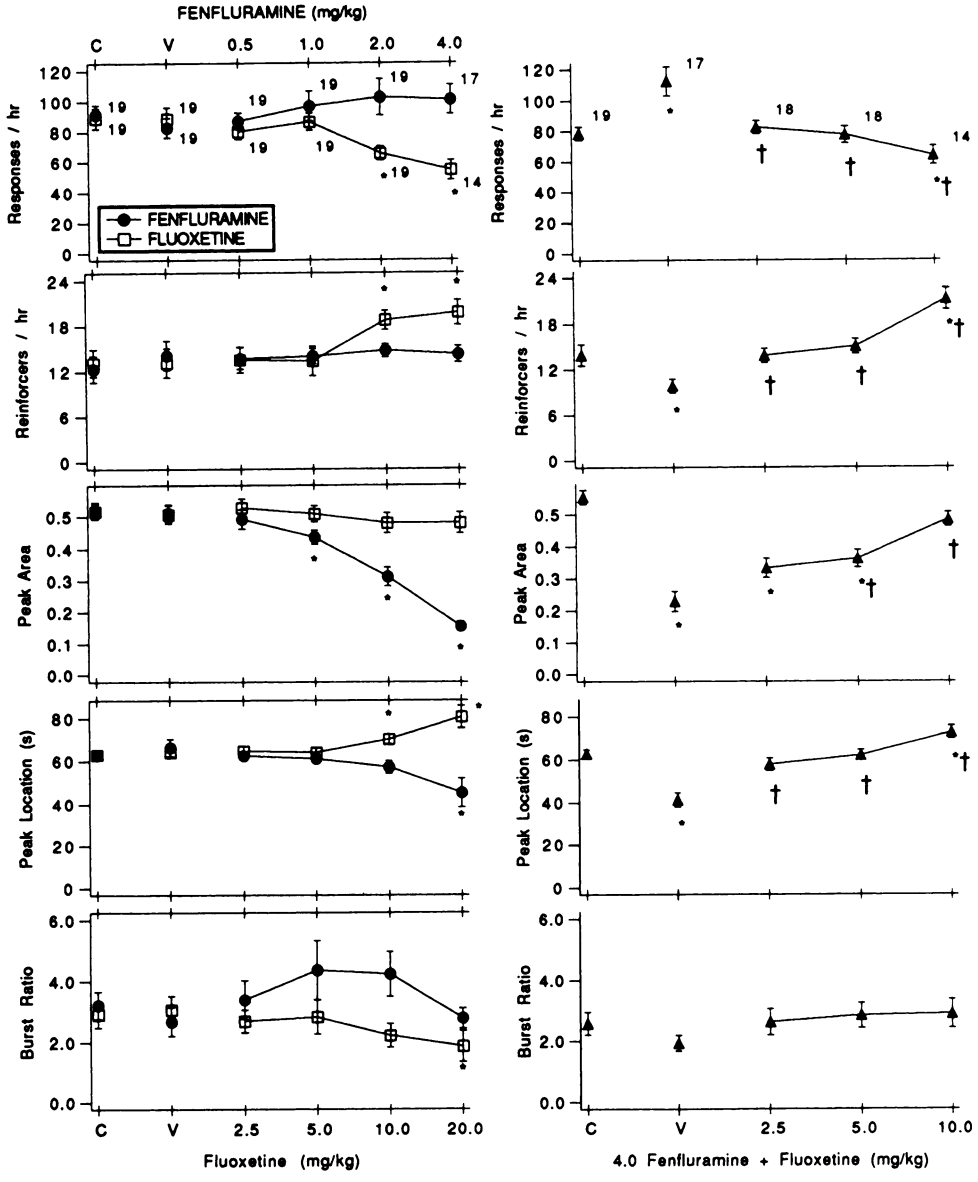

Fig. 2. Effects of fenfluramine and fluoxetine on response rate, reinforcement rate, PkA, PkL and BR measures of DRL 72-s schedule performance. The PkA, PkL and BR metrics quantitatively characterize the shape of the IRT distribution which resulted from training on the DRL 72-s schedule (see text and fig. 1 for explanation). The plots in the left panel show the effects of fenfluramine and fluoxetine when given alone. The doses of fenfluramine are shown on the top abscissa and the doses of fluoxetine are shown on the bottom abscissa. The plots in the right panel show the effects of 4.0 mg/kg of fenfluramine when administered after various doses of fluoxetine, including V. The data points indicate means, and the error bars indicate S.E.M. The "C" on the *x* axis indicates nondrug control days and the "V" indicates vehicle injection. Rats which made fewer than 25 responses were not included in the analysis. Numbers next to data points on response rate graphs indicate sample size. * $P < .05$ compared to nondrug control. † $P < .05$ compared to 4.0 mg/kg of fenfluramine + V.

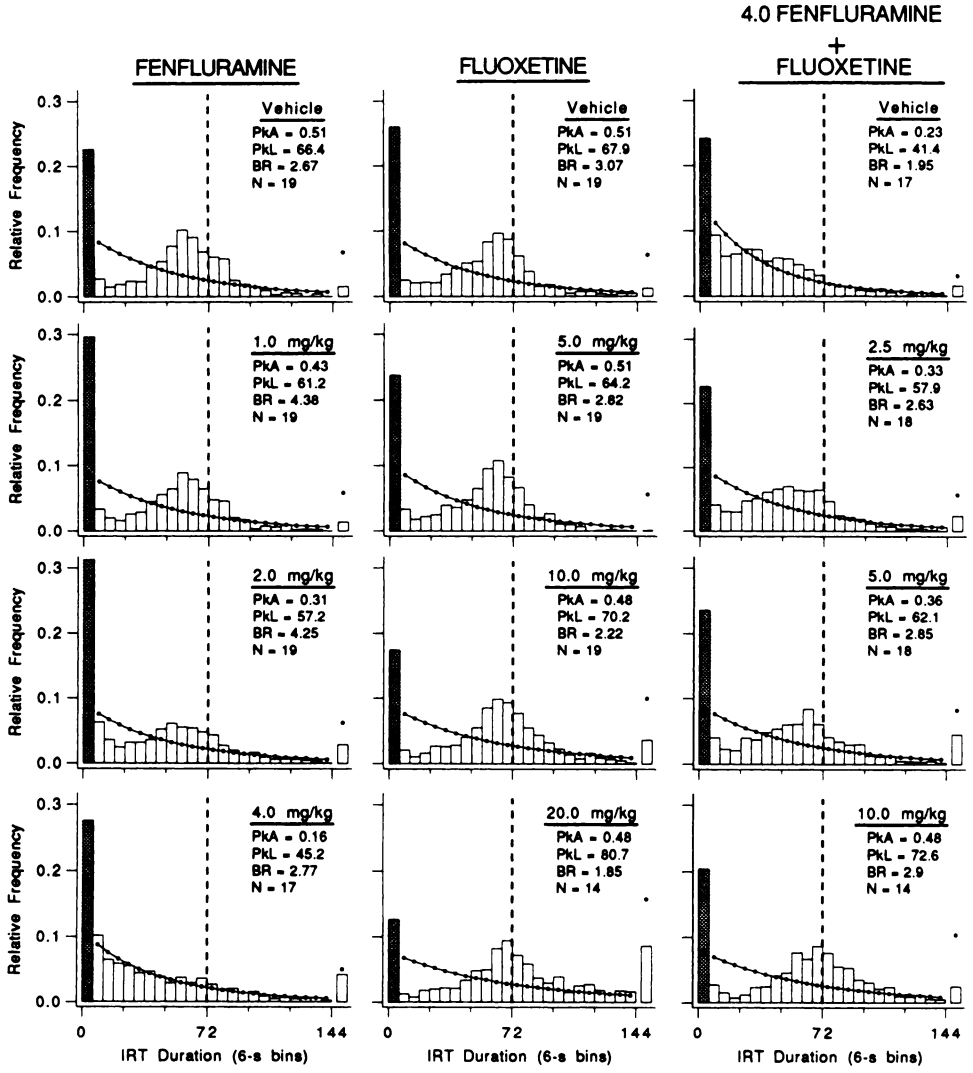

Fig. 3. Effects of fenfluramine and fluoxetine on DRL 72-s IRT distributions. The four histograms in the left panel show the effects of fenfluramine alone, the histograms in the center panel show the effects of fluoxetine alone and the histograms in the right panel show the effects of 4.0 mg/kg of fenfluramine when administered after various doses of fluoxetine, including V. Each histogram represents averaged relative frequencies. The average PkA, PkL and BR measures are shown for each dose of the drug. Rats which made fewer than 25 pause responses were not used in the analysis. The value of *n* indicates the number of rats that were used to determine the relative frequency plots. See text and figure 1 for a more detailed description.

Visual inspection of the IRT plots (fig. 3, middle panel) confirms the quantitative analysis provided by the PkA, PkL and BR measures. Fluoxetine did not cause the IRT distribution to become more similar to the corresponding negative exponential as did fenfluramine. This indicates that temporal stimulus control was not disrupted by fluoxetine, even at the highest dose at which five rats failed to make at least 25 responses.

Fluoxetine + 4.0 mg/kg of fenfluramine. Fluoxetine given in conjunction with fenfluramine, decreased response rate and increased reinforcement rate (fig. 2, right panel). When 4.0 mg/kg of fenfluramine was given after the fluoxetine V (water) an increase in response rate above nondrug control levels was observed. Two rats failed to emit at least 25 pause responses, and were not included in the analysis. The increase in response rate was not observed when 4.0 mg/kg of fenfluramine was given alone in the first dose-response determination (see above). Response rate was decreased below levels recorded for vehicle + 4.0 mg/kg of fenfluramine at the 2.5-, 5.0- and 10.0-mg/kg doses of fluoxetine plus 4.0 mg/kg of fenfluramine. Response rate was decreased below nondrug control performance only at the 10.0 mg/kg dose of fluoxetine plus 4.0 mg/kg of fenfluramine. One rat emitted less than 25 responses at the 2.5 and 5.0 mg/kg doses of fluoxetine + 4.0 mg/kg dose of fenfluramine. Five rats emitted less than 25 responses at the 10.0 mg/kg dose of fluoxetine + 4.0 mg/kg dose of fenfluramine.

A decrease in reinforcement rate below nondrug control levels was observed for V + 4.0 mg/kg of fenfluramine (fig. 2, right panel). Increasing doses of fluoxetine increased reinforcement rate above both the V + 4.0 mg/kg dose of fenfluramine and the nondrug control performance levels at the 10.0 mg/kg dose of fluoxetine + 4.0 mg/kg of fenfluramine.

The disruptive effects of 4.0 mg/kg of fenfluramine on the profile of the IRT distribution were reversed by fluoxetine. The PkA of the IRT distribution was decreased by V + 4.0 fenfluramine (fig. 2, right panel). This decrease was similar to the decrease in PkA observed in the first administration of 4.0 mg/kg of fenfluramine. This decrease was dose-dependently reversed by increasing doses of fluoxetine. The PkL of the IRT distribution was shifted to the left, toward shorter IRT durations by vehicle + 4.0 mg/kg of fenfluramine. Increasing doses of fluoxetine + 4.0 mg/kg of fenfluramine dose-dependently shifted the IRT distribution back to the right, toward longer IRT durations. The BR was not significantly affected.

Fluoxetine reversed the effects of fenfluramine on the IRT distribution profile (fig. 3). Vehicle + 4.0 mg/kg of fenfluramine disrupted the IRT distribution profile, indicating a decrease in temporal stimulus control. Increasing doses of fluoxetine in

combination with 4.0 mg/kg of fenfluramine reversed the disruption of the IRT distribution profile by fenfluramine and restored temporal stimulus control.

Discussion

The results demonstrate that fluoxetine and fenfluramine have very different effects on DRL 72-s schedule performance. Fluoxetine showed an increase in reinforcement rate, a decrease in response rate, a shift in the peak of the IRT distribution toward longer IRT durations, no change in PkA and a decrease in BR at the highest dose. The increase in reinforcement rate, without a disruption of the IRT distribution (as indicated by no decrease in PkA), shows that fluoxetine has an antidepressant-like effect on the DRL 72-s schedule. Despite fluoxetine's effects on response and reinforcement rate, it did not disrupt the profile of the IRT distribution. This is demonstrated by the fact that the PkA of the IRT distribution was not significantly affected at any dose of fluoxetine, including the high dose (20 mg/kg) at which five rats failed to make at least 25 responses.

Fenfluramine did not have consistent effects on response and reinforcement rate. In the initial dose-response determination for fenfluramine, there were no changes in response or reinforcement rate; with the second administration of 4.0 mg/kg of fenfluramine (given with the fluoxetine V) there was a small but significant increase in response rate, and a decrease in reinforcement rate. Fenfluramine's effect on PkA were, however, consistent. There was a dose-dependent decrease in PkA; the effects of 4.0 mg/kg of fenfluramine on PkA were replicated with the second administration of that dose. This result indicates that fenfluramine caused a profound disruption of the IRT distribution, an effect not observed with fluoxetine. The fenfluramine-induced decrease in PkA indicates that the IRT distribution became similar to the "random" prediction of the corresponding negative exponential indicating a loss of control by the DRL schedule.

The difference between fenfluramine alone and fenfluramine with water pretreatment is difficult to interpret. One explanation is that the rats changed their response to fenfluramine with repeated administration. (The V + 4.0 mg/kg dose of fenfluramine was the last treatment given in this experiment.) Because the effects of fenfluramine on response and reinforcement rate were not considered between the first and second administration of the 4.0-mg/kg dose, it could be argued that 4.0 mg/kg of fenfluramine was not a sufficiently high dose. However, at this dose, two rats stopped responding (for both administrations).

The differential effects of fenfluramine and fluoxetine on DRL 72-s schedule performance reported here are consistent with results reported by Willner *et al.* (1990). These authors found that fenfluramine disrupted a postprandial behavioral sequence of grooming and resting, whereas fluoxetine did not. Similarly, McElroy and Feldman (1984) found that whereas other 5-HT-releasing agents (such as *para*-chloroamphetamine) substituted for fenfluramine in a drug discrimination paradigm, fluoxetine did not.

Fluoxetine, given in combination with fenfluramine, caused a dose-dependent reversal of the effects of fenfluramine on PkA. That is, fluoxetine in combination with fenfluramine dose-dependently increased the PkA of the IRT distribution. At the 10.0 mg/kg dose of fluoxetine plus 4.0 mg/kg of fenfluramine, the PkA was not different from control. The IRT distributions of figure 3 graphically demonstrate this result. In terms of response and reinforcement rate, the combination of fenfluramine and fluoxetine had effects similar to fluoxetine alone. Fluoxetine plus fenfluramine increased reinforcement rate and decreased response rate.

The prevention of fenfluramine's disruptive effects on DRL 72-s performance by fluoxetine is consistent with previous work indicating that 5-HT uptake blockers inhibit fenfluramine's effects on 5-HT release (Hekmatpanah and Peroutka, 1990; Sabol *et al.*, 1992), 5-HT depletions (Fuller *et al.*, 1978; Steranka and Sanders-Bush, 1979), hyperthermia (Sugrue, 1984), hormone secretion (McElroy *et al.*, 1984; Van de Kar *et al.*, 1985) and drug discrimination (McElroy and Feldman, 1984). These results support the interpretation that fenfluramine's effects on DRL 72-s schedule performance are due to transporter-mediated 5-HT release.

Antidepressant-like effects on the DRL 72-s screen. The antidepressant fluoxetine caused a shift of the IRT distribution toward longer intervals without changing PkA, and without causing disruption. Similar observations have been made for the antidepressants desipramine, and 5-HTP using the same quantitative IRT analysis (Richards *et al.*, in press; Richards et al., 1993; Richards and Seiden, 1991). These quantitative observations for fluoxetine, desipramine and 5-HTP are consistent with previous qualitative reports indicating that antidepressants cause a coherent shift of the DRL 72-s IRT distribution toward longer intervals (O'Donnell and Seiden, 1982; O'Donnell and Seiden, 1983).

As described above, fenfluramine had very different effects from fluoxetine on DRL schedule performance. The large effect of fenfluramine on the PkA in the absence of a systematic effect on response and reinforcement rates shows that IRT analysis adds important information to the assessment of drugs on DRL performance. By using the PkA measure, in addition to response and reinforcement rate, we were able to detect changes caused by fenfluramine that would not have been detected by response and reinforcement rate alone. This result indicates that peak deviation analysis, by providing three new measures, allows for a more complete description of the effects of drugs on DRL schedule performance.

Chemical-induced release *vs.* impulse-dependent release. Because both fenfluramine and fluoxetine cause increases in extracellular 5-HT *in vivo* (Sabol *et al.*, 1992), it is not clear why they should have different effects on DRL schedule performance. One explanation for the differences observed between fenfluramine and fluoxetine on DRL 72-s schedule performance is that they induced unequal increases in extracellular 5-HT. However, the dose response determinations for fenfluramine and fluoxetine make this seem unlikely. At no dose was the effect of fenfluramine similar to the effect of fluoxetine on DRL 72-s schedule performance.

A second explanation for the differences between fenfluramine and fluoxetine on the DRL 72-s schedule is that fenfluramine may not be as selective a 5-HT agent as is fluoxetine. It has been reported that L-fenfluramine (but not D-fenfluramine) has effects on *in vivo* dopamine release (Bettini *et al.*, 1987), and amphetamine-induced stereotypies [see Invernizzi *et al.*, 1989)] similar to neuroleptic agents. If one were to take into account L-fenfluramine in DL-fenfluramine's effects on the DRL 72-s schedule, neuroleptic-like effects would be predicted. This is not the case however. DL-Fenfluramine did not decrease response rate, whereas the neuroleptics haloperidol and chlo-

ropromazine both decreased response rate on the DRL 72-s schedule (Britton and Koob, 1989; O'Donnell and Seiden, 1983; Pollard and Howard, 1986; Seiden *et al.*, 1985).

A third explanation of the differential effects of fenfluramine and fluoxetine on DRL 72-s schedule performance may be the different mechanisms through which fluoxetine and fenfluramine cause increases in extracellular 5-HT. The increases in 5-HT caused by fenfluramine are due to the transporter-mediated release and uptake inhibition of 5-HT (Fuxe *et al.*, 1975; Garattini *et al.*, 1975). The fenfluramine-induced release of 5-HT *in vivo* does not rely upon normal cell firing (Carboni and Di Chiara, 1989). Fluoxetine, on the other hand, increases extracellular 5-HT by blocking the uptake of 5-HT that is released into the synapse by nerve impulses (Perry and Fuller, 1992; Wong *et al.*, 1975). The distinction between impulse-dependent release and chemically-induced release may be important for determining the behavioral consequences of increases in extracellular 5-HT. The disruption of the IRT distribution after fenfluramine may reflect the fact that the chemically induced release caused by fenfluramine occurs independently of inputs to 5-HT neurons which mediate impulse-dependent 5-HT release. These mediating inputs could determine not only the amount but also the sequence and anatomical locus of impulse-dependent 5-HT release. Fenfluramine may cause an inappropriate global release of 5-HT which in effect "short circuits" the effects of normal cell firing. In this manner fenfluramine may disrupt behavioral output which requires selective release of 5-HT rather than an enhancement of release. In contrast, fluoxetine, by blocking uptake and not inducing 5-HT release, may serve to increase the effects of impulse-dependent release. Consistent with these results, the 5-HT precursor, 5-HTP, has effects on the DRL schedule similar to fluoxetine (Richards *et al.*, in press). 5-HTP-induced 5-HT release has been suggested to be exocytotic; it is blocked by 8-OH-DPAT (Gartside *et al.*, 1992).

The effects of fenfluramine on DRL 72-s performance were similar to the effects of 5-HT depletions induced by intracerebral injections of 5,7-DHT (Jolly *et al.*, 1991). Although an apparent contradiction, both chemically induced 5-HT release and the 5-HT depletions may act to interfere with normal serotonergic neuronal functioning, and result in disruption of DRL performance.

Behavioral mechanisms underlying the effects of the antidepressant fluoxetine on DRL 72-s schedule performance. Rats responding on DRL schedules of reinforcement have peaks in their IRT distributions near the temporal requirement of the particular DRL schedule in use [for example see (Malott and Cumming, 1964)]. Because of the functional relationship between the peak of the IRT distribution and the IRT requirement, DRL performance has often been characterized as reflecting the animal's ability to make a temporal discrimination (Kramer and Rilling, 1970; Malott and Cumming, 1964; Platt, 1979; Zeiler, 1986). Because of the timing requirement, it is tempting to link changes in DRL performance induced by drugs, to changes in the discrimination of time intervals. However, the observed changes in performance on the DRL schedule may be only indirectly related to the temporal requirement of the DRL schedule.

Performance of the DRL task potentially involves many behavioral processes other than time perception. Recently, it has been suggested that the coherent shift to the right of the IRT distribution induced by antidepressants such as fluoxetine, on the DRL 72-s schedule, may be due to an enhanced capacity to wait (Soubrie and Bizot, 1990; Thiebot *et al.*, 1991). These authors have presented data which indicates that a variety of antidepressant compounds increase waiting capacity (Bizot *et al.*, 1988). In these studies rats are given a choice between an immediate small magnitude reinforcer and a delayed large magnitude reinforcer in a T maze (*i.e.*, two food pellets given immediately *vs.* 10 food pellets given after a delay of 25 s). Antidepressant compounds increased the frequency with which the rats chose the delayed large magnitude reinforcer. This increase in the choice of the delayed large magnitude reinforcer was interpreted as indicating an increase in waiting capacity. These authors have also reported that compounds that decrease 5-HT transmission, such as benzodiazepine anxiolytics, decreased the frequency with which the rats chose the delayed large magnitude reinforcer (Thiebot *et al.*, 1985; Thiebot, 1986). Conversely, the increase in waiting capacity induced by antidepressant compounds was hypothesized to be associated with increased serotonergic transmission (Soubrie and Bizot, 1990; Thiebot *et al.*, 1991).

As was pointed out in the introduction, rats performing on the DRL 72-s schedule have peaks in their IRT distributions which occur before the criterion IRT duration for reinforcement. The observation that rats have peaks at all indicates that the rats are systematically waiting, however, the observation that the peaks occur before 72 s indicates that they do not wait long enough. Increases in the ability to wait or inhibit responding induced by the antidepressants on the DRL 72-s schedule could cause a peak shift toward longer IRT durations resulting in an increase in reinforcement rate and a decrease in response rate. A reasonable alternative to inaccurate timing for the early peaks in DRL 72-s IRT distributions, is an inability to wait long enough between responses.

However, both the temporal discrimination and capacity to wait explanations make similar predictions in both the DRL 72-s schedule and T maze tasks described above. If antidepressants such as fluoxetine altered the perception of time so that the delay before the larger reward was perceived as shorter, the rats may choose the delayed large reward more frequently. This same alteration in time perception could also cause rats to wait longer between presses on the DRL 72-s schedule. Therefore, the effects of fluoxetine on DRL 72-s performance can be attributed to either changes in temporal discrimination or the capacity to wait.

Acknowledgments

We wish to thank Matthew Baggott and Kelly Layton for technical assistance.

References

BETTINI, E., CECI, A., SPINELLI, R. AND SAMANIN, R.: Neuroleptic-like effects of the l-isomer of fenfluramine on striatal dopamine release in freely moving rats. Biochem. Pharmacol. **36:** 2387–2391, 1987.

BIZOT, J. C., THIEBOT, M. H., LE BIHAN, C., SOUBRIE, P. AND SIMON, P.: Effects of imipramine-like drugs and serotonin uptake blockers on delay of reward in rats. Possible implication in the behavioral mechanism of action of antidepressants. J. Pharmacol. Exp. Ther. **246:** 1144–1151, 1988.

BRITTON, K. T. AND KOOB, G. F.: Effects of corticotropin releasing factor, desipramine and haloperidol on a DRL schedule of reinforcement. Pharmacol. Biochem. Behav. **32:** 967–970, 1989.

CARBONI, E. AND DI CHIARA, G.: Serotonin release estimated by transcortical dialysis in freely-moving rats. Neuroscience **32:** 637–645, 1989.

CLINESCHMIDT, B. V. AND MCGUFFIN, J. C.: Pharmacological differentiation of the central 5-hydroxytryptamine-like actions of MK-212 (6-chloro-2-[1-piperazinyl]-pyrazine), p-methoxyamphetamine and fenfluramine in an in vivo model system. Eur. J. Pharmacol. **50:** 369–375, 1978.

FORNAL, C. AND RADULOVACKI, M.: Sleep suppressant action of fenfluramine in rats. II. Evidence against the involvement of presynaptic serotonergic mechanism. J. Pharmacol. Exp. Ther. **225:** 675–681, 1982.

FULLER, R. W.: Mechanism by which uptake inhibitors antagonize p-chloroamphetamine-induced depletion of brain serotonin. Neurochem. Res. **5:** 241–245, 1980.

FULLER, R. W., SNODDY, H. D. AND HEMRICK, S. K.: Effects of fenfluramine and norfenfluramine on brain serotonin metabolism in rats (40021). Proc. Soc. Exp. Biol. Med. **157:** 202–205, 1978.

FULLER, R. W. AND WONG, D. T.: Fluoxetine: A serotonergic appetite suppressant drug. Drug Dev. Res. **17:** 1–15, 1989.

FULLER, R. W., WONG, D. T. AND ROBERTSON, D. W.: Fluoxetine, a selective inhibitor of serotonin uptake. Med. Res. Rev. **11:** 17–34, 1991.

FUXE, K., FARNEBO, L. O., HAMBERGER, B. AND OGREN, S. O.: On the *in vivo* and *in vitro* actions of fenfluramine and its derivatives on central monoamine neurons, especially 5-hydroxytryptamine neurons, and their relation to the anorectic activity of fenfluramine. Postgrad. Med. J. **2:** suppl. 1: 35–45, 1975.

GARATTINI, S., BUCZKO, W., JORI, A. AND SAMANIN, R.: The mechanism of action of fenfluramine. Postgrad. Med. J. **51:** suppl. 1: 27–35, 1975.

GARTSIDE, S. E., COWEN, P. J. AND SHARP, T.: Effect of 5-hydroxy-L-tryptophan on the release of 5-HT in rat hypothalamus in vivo as measured by microdialysis. Neuropharmacology **31:** 9–14, 1992.

GOUDIE, A. J., THORNTON, E. V. AND WHEELER, T. J.: Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotonergic inhibition of feeding. J. Pharm. Pharmacol. **28:** 318–320, 1976.

HEKMATPANAH, C. R. AND PEROUTKA, S. J.: 5-Hydroxytryptamine uptake blockers attenuate the 5-hydroxytryptamine-releasing effect of 3,4-methylenedioxymethamphetamine and related agents. Eur. J. Pharmacol. **177:** 95–98, 1990.

INVERNIZZI, R., BERTORELLI, R., CONSOLO, S., GARATTINI, S. AND SAMANIN, R.: Effects of the 1 isomer of fenfluramine on dopamine mechanisms in rat brain: Further studies. Eur. J. Pharmacol. **164:** 241–248, 1989.

JOLLY, D. C., RICHARDS, J. B. AND SEIDEN, L. S.: Depletion of brain serotonin with 5,7-dihydroxytryptamine is associated with a persistent behavioral deficit in rats performing on the differential reinforcement of low rate-72 second operant schedule of water reinforcement. Soc. Neurosci. Abstr. **17:** 148, 1991.

KRAMER, T. J. AND RILLING, M.: Differential reinforcement of low rates: A selective critique. Psychol. Bull. **74:** 225–254, 1970.

MALOTT, R. W. AND CUMMING, W. W.: Schedules of interresponse time reinforcement. Psychol. Rec. **14:** 211–252, 1964.

MAREK, G. J., LI, A. A. AND SEIDEN, L. S.: Evidence for involvement of 5-hydroxytryptamine-1 receptors in antidepressant-like drug effects on differential reinforcement-of-low-rate 72-second behavior. J. Pharmacol. Exp. Ther. **250:** 60–70, 1989.

MATOS, F. F., ROLLEMA, H. AND BASBAUM, A. I.: Characterization of monoamine release in the lateral hypothalamus of awake, freely moving rats using in vivo microdialysis. Brain Res. **528:** 39–47, 1990.

MCELROY, J. F. AND FELDMAN, R. S.: Discriminative stimulus properties of fenfluramine: Evidence for serotonergic involvement. Psychopharmacology **83:** 172–178, 1984.

MCELROY, J. F., MILLER, J. M. AND MEYER, J. S.: Fenfluramine, p-chloroamphetamine and p-fluoroamphetamine stimulation of pituitary-adrenocortical activity in rat: Evidence for differences in site and mechanism of action. J. Pharmacol. Exp. Ther. **228:** 593–599, 1984.

NETER, J. AND WASSERMAN, W.: Applied Linear Statistical Models, Richard D. Irwin, Inc., Homewood, IL, 1974.

O'DONNELL, J. M. AND SEIDEN, L. S.: Effects of monoamine oxidase inhibitors on performance during differential reinforcement of low response rate. Psychopharmacology **78:** 214–218, 1982.

O'DONNELL, J. M. AND SEIDEN, L. S.: Differential-reinforcement-of-low-rate 72-second schedule: Selective effects of antidepressant drugs. J. Pharmacol. Exp. Ther. **224:** 80–88, 1983.

O'ROURKE, D., WURTMAN, J. J., WURTMAN, R. J., CHEBLI, R. AND GLEASON, R.: Treatment of seasonal depression with d-fenfluramine. J. Clin. Psychiatry **50:** 343–347, 1989.

PERRY, K. W. AND FULLER, R. W.: Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. Life Sci. **50:** 1683–1690, 1992.

PLATT, J. R.: Temporal differentiation and the psychophysics of time. *In* Advances in Analysis of Behaviour. Reinforcement and the Organization of Behaviour, ed. by M. D. Zeiler and P. Harzem, Wiley, Chichester, pp. 1–29, 1979.

POLLARD, G. T. AND HOWARD, J. L.: Similar effects of antidepressant and nonantidepressant drugs on behavior under an interresponse-time >72-s schedule. Psychopharmacology **89:** 253–258, 1986.

PRICE, L. H., CHARNEY, D. S., DELGADO, P. L. AND HENINGER, G. R.: Fenfluramine augmentation in tricyclic-refractory depression. J. Clin. Psychopharmacol. **10:** 312–317, 1990.

RICHARDS, J. B., SABOL, K. E., HAND, T. H., JOLLY, D. C., MAREK, G. J. AND SEIDEN, L. S.: Buspirone, gepirone, ipsapirone, and zalospirone have unique effects on the differential-reinforcement-of-low-rate 72-second schedule when compared to 5-HTP and diazepam. Psychopharmacology, in press, 1993.

RICHARDS, J. B., SABOL, K. E. AND SEIDEN, L. S.: DRL interresponse time distributions: Quantification by peak deviation analysis. J. Exp. Anal. Behav. **60:** 361–365, 1993.

RICHARDS, J. B. AND SEIDEN, L. S.: A quantitative interresponse-time analysis of DRL performance differentiates similar effects of the antidepressant desipramine and the novel anxiolytic gepirone. J. Exp. Anal. Behav. **56:** 173–192, 1991.

RUDORFER, M. V. AND POTTER, W. Z.: Antidepressants. A comparative review of the clinical pharmacology and therapeutic use of the "newer" versus the "older" drugs. Drugs **37:** 713–738, 1989.

SABOL, K. E., RICHARDS, J. B. AND SEIDEN, L. S.: Fluoxetine attenuates the DL-fenfluramine-induced increase in extracellular serotonin as measured by *in vivo* dialysis. Brain Res. **585:** 421–424, 1992.

SEIDEN, L. S., DAHMS, J. L. AND SHAUGHNESSY, R. A.: Behavioral screen for antidepressants: The effects of drugs and electroconvulsive shock on performance under a differential-reinforcement-of-low-rate schedule. Psychopharmacology **86:** 55–60, 1985.

SNAPPER, A. G., STEPHENS, K. R., COBEZ, R. I. AND VAN HAAREN, F.: The SKED Software system: OS8 and time Share SKED, State Systems, Kalamazoo, MI, 1976.

SOUBRIE, P. AND BIZOT, J. C.: Monoaminergic control of waiting capacity (impulsivity) in animals. *In* Violence and Suicidality: Perspectives in Clinical and Psychobiological Research, ed. by H. M. van Praag, R. Plutchik and A. Apter, Brunner/Mazel, Inc. NY, pp. 257–272, 1990.

STERANKA, L. R. AND SANDERS-BUSH, E.: Long-term effects of fenfluramine on central serotonergic mechanisms. Neuropharmacology **18:** 895–903, 1979.

SUGRUE, M. F.: Antagonism of fenfluramine-induced hyperthermia in rats by some, but not all, selective inhibitors of 5-hydroxytryptamine uptake. Br. J. Pharmacol. **81:** 651–657, 1984.

THIEBOT, M. H.: Are serotonergic neurons involved in the control of anxiety and in the anxiolytic activity of benzodiazepines? Pharmacol. Biochem. Behav. **24:** 1471–1477, 1986.

THIEBOT, M., BIZOT, J. AND SOUBRIE, P.: Waiting capacity in animals: A behavioral component crossing nosologic boundaries of anxiety and depression? *In* Anxiety, Depression, and Mania, ed. by P. Soubrie, S. Karger, Basel, pp. 48–67, 1991.

THIEBOT, M., LEBIHAN, C., SOUBRIE, P. AND SIMON, P.: Benzodiazepines reduce the tolerance to reward delay in rats. Psychopharmacology **86:** 147–152, 1985.

VAN DE KAR, L. D., URBAN, J. H., RICHARDSON, K. D. AND BETHEA, C. L.: Pharmacological studies on the serotonergic and nonserotonin-mediated stimulation of prolactin and corticosterone secretion by fenfluramine. Neuroendocrinology **41:** 283–288, 1985.

WILLNER, P., MCGUIRK, J., PHILLIPS, G. AND MUSCAT, R.: Behavioural analysis of the anoretic effects of fluoxetine and fenfluramine. Psychopharmacology **102:** 273–277, 1990.

WONG, D. T., BYMASTER, F. P., HORNG, J. S. AND MOLLOY, B. B.: A new selective inhibitor for uptake of serotonin into synaptosomes of rat brain: 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. J. Pharmacol. Exp. Ther. **193:** 804–811, 1975.

WURTMAN, J. J. AND WURTMAN, R. J.: Fenfluramine and fluoxetine spare protein consumption while suppressing caloric intake by rats. Science (Wash. DC) **198:** 1178–1180, 1977.

ZEILER, M. D.: Behavior units and optimality. *In* Analysis and Integration of Behavioral Units, ed. by T. Thompson and M. D. Zeiler, Erlbaum, Hillsdale, NJ, pp. 81–116, 1986.

Send reprint requests to: Jerry B. Richards, The University of Chicago, Department of Pharmacological and Physiological Sciences, 947 East 58th St., Chicago, IL 60637.