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# Amphetamine analogs have differential effects on DRL 36-s schedule performance

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**Abstract** Amphetamine and related compounds have previously been shown to differentially release dopamine (DA) and serotonin (5HT) in vivo and in vitro. The purpose of this report is directly to compare five amphetamine analogs on differential reinforcement of low rate 36-s (DRL 36-s) schedule performance, and to determine whether the reported increases in dopamine and/or serotonin release induced by these drugs can be related to observed behavioral differences. Amphetamine (AMPH) and methamphetamine (METH) induced large increases in response rate, methylenedioxymethamphetamine (MDMA) and para-chloroamphetamine (PCA) caused small increases in response rate, while fenfluramine (FEN) had no effect on response rate. AMPH, METH, PCA and MDMA caused a dose-dependent decrease in reinforcement rate, and FEN had no effect on reinforcement rate. AMPH, METH, and PCA but not FEN, shifted the peak of the inter-response time (IRT) distribution toward shorter intervals. MDMA decreased peak location only at the highest dose. All five drugs caused a dose-dependent decrease in peak area, indicating a loss of schedule control on the DRL 36-s schedule. Consistent with in vitro and in vivo release studies, the differential results of these five drugs on DRL 36-s schedule performance suggest a predominant dopamine role for AMPH and METH, a predominant serotonin role for FEN, and different degrees of combined dopaminergic and serotonergic roles for MDMA and PCA in the mediation of the task.

**Key words** Operant behavior · Amphetamine · Methamphetamine · Methylenedioxymethamphetamine · Fenfluramine · Para-chloroamphetamine · Dopamine · Serotonin · Rat

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## Introduction

Amphetamine (AMPH), methamphetamine (METH), methlyenedioxymethamphetamine (MDMA), fenfluramine (FEN), and para-chloroamphetamine (PCA) are psychoactive substituted amphetamines which release dopamine and serotonin. Each compound affects dopamine and serotonin release to different degrees, however. AMPH is primarily a dopamine releasing agent (Kannengiesser et al. 1976; Kuczenski and Segal 1989), FEN is primarily a serotonin releasing agent (Kannengiesser et al. 1976) and METH, MDMA, and PCA fall in between these two compounds in their ability to release dopamine and serotonin (Johnson et al. 1986; Sharp et al. 1986; Schmidt et al. 1987, 1991; McKenna et al. 1991).

In Table 1 we summarize the literature regarding the ability of different AMPH analogs to release DA versus 5HT. This table combines results from studies using a variety of techniques including in vitro synaptosomal release, in vitro slice release, and in vivo dialysis. In order to integrate the information from these studies, we have developed a release ratio (see Table 1). From these release ratios, it can be seen that the order of a drug's DA releasing ability relative to its serotonin releasing ability is the following: AMPH > METH > MDMA > PCA > FEN.

Amphetamine derivatives have differential effects on the behavior of rats (see Paulus and Geyer 1992). For example, METH and MDMA both induce locomotor behavior, but the pattern of locomotion induced by the drugs is qualitatively different. When tested in an activity monitoring apparatus, MDMA-treated rats walk in straight lines along the walls of the chamber. In contrast, METH treated rats move throughout the space of the chamber, exhibiting long straight-line movements as well as short movements (Gold et al. 1988; Paulus and Geyer 1992). In addition, MDMA and PCA both cause increases in tactile and acoustic startle reflexes, while FEN does not (Kehne et al. 1992).

In order to explore further the behavioral differences of amphetamine-like compounds, we studied the effects of AMPH, METH, MDMA, PCA and FEN on DRL 36-s schedule performance.

Amphetamine (Schuster et al. 1966; Campbell and Seiden 1973; Richards et al. 1993a) and MDMA (Li et al. 1989) increase response rate and disrupt performance on DRL schedules in rats. FEN, on the other hand, disrupts DRL performance without increasing response rate (Richards et al. 1993b). We now compare five amphetamine analogs on DRL 36-s schedule performance, and evaluate whether the reported increases in dopamine and/or serotonin release induced by these compounds (Kannengiesser et al. 1976; Liang and Rutledge 1982; Sharp et al. 1986; Kuczenski and Segal 1989; McKenna et al. 1991; Schmidt et al. 1991) can be related to behavioral differences on DRL performance.

Using peak deviation analysis (see Richards and Seiden 1991; Richards et al. 1993a), we evaluated changes in response rate, reinforcement rate, and the shape of the inter-response time (IRT) distribution that is characteristic of DRL schedules. We found that METH and AMPH caused large increases in response rate, MDMA and PCA induced small increases, while FEN did not increase response rate. AMPH, METH, PCA and MDMA caused a dose-dependent decrease in reinforcement rate; FEN did not affect reinforcement rate. All five drugs changed the shape of the IRT distribution, indicating disruption of schedule control over DRL performance.

## Material and methods

### Animals

Fifteen male Sprague-Dawley rats (Holtzman, Madison, Wis.) weighing between 350 and 500 g were used. Rats were housed two per cage in hanging stainless steel wire cages. Lights were on in the colony room from 0700 to 1900 hours. Food (4% Teklad rat chow) was available ad lib. Access to water was restricted to 20 min per day. On training days the rats received 20 min access to water at the end of their training session. On non-training days (weekends), the rats were given 20 min access to water between 1000 and 1400 hours. The rats had no prior history of drug treatment.

### Apparatus

Operant test chambers, as well as the hardware and software to control the schedule contingencies, were described in detail in Richards et al. (1994).

### Training

The rats were adapted to the 20 min per day access to water regimen for 1 week. The rats were then trained to respond (bar press) in overnight training sessions using an alternative FR1, FT 1-min schedule. Each overnight training session was terminated after 10 h or 100 responses, whichever occurred first. The rats acquired the bar press response within five training sessions. When the rats made 100 responses in two overnight training sessions, they were trained during 3-h sessions on a DRL 36-s schedule of reinforcement for 10 days. On the DRL 36-s schedule, responses were followed by water presentation only if the rats had waited 36 s since the last response before pressing the lever. Finally, the rats were trained during daily (5 days a week) 1-h sessions on the DRL 36-s schedule. At the beginning of the present experiment the rats had been trained on the DRL 36-s schedule for approximately 12 weeks (5 days a week) in 1-h sessions.

### Drug administration

*d*-Amphetamine sulfate (Sigma), *d*-methamphetamine hydrochloride (Sigma), *dl*-methylenedioxymethamphetamine hydrochloride (NIDA), *dl*-*p*-chloroamphetamine hydrochloride (Sigma) and *dl*-fenfluramine hydrochloride (Sigma) were dissolved in saline to form an injectable solution of 1 ml/kg (doses were calculated as the salt). Drugs were injected intraperitoneally 20 min before the start of the 1-h session. For eight of the rats the dose-response determinations were made in the following order: AMPH, METH, MDMA, FEN and PCA. In the remaining seven rats the dose-response determinations were made in the opposite order: FEN, MDMA, METH, AMPH and PCA. PCA was given last to both groups because of its potential for neurotoxic effects at the doses tested. The doses tested were: AMPH (Vehicle (V), 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg); METH (V, 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg); MDMA (V, 0.5, 1.0, 2.0, 4.0, 8.0 mg/kg); FEN (V, 0.5, 1.0, 2.0, 4.0 mg/kg); and PCA (V, 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg). Doses were given in ascending order. 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 effects of drug treatment on the IRT distributions generated by the DRL 36-s schedule were quantitatively characterized. The quantitative IRT analysis used, peak deviation analysis, includes three measures for the characterization of DRL IRT distributions: peak area (PkA), peak location (PkL) and burst ratio (BR). A PASCAL routine which implements peak deviation analysis is available from the authors.

The IRT distributions of most rats trained on the DRL 36-s schedule are 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 36-s IRT distributions, peak deviation analysis divides the obtained IRT distribution into separate burst (IRTs $< 3$ s) and pause (IRTs $\geq 3$ s) components.

Peak deviation analysis compares each rat's obtained IRT distribution to a theoretical distribution which 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 $< 3$ s) excluded (see Richards and Seiden 1991 and Richards et al. 1993a).

The peak area (PkA) and peak location (PkL) metrics were used to characterize the pause component (IRTs $> 3$ s) of the IRT distribution. The PkA measure was the area of the obtained IRT distribution above the corresponding negative exponential. The largest possible PkA value (1.0) would occur only if all of the obtained IRT durations had exactly the same value. The smallest possible PkA value (0.0) would indicate that the obtained and corresponding negative exponential distributions were identical. Thus, decreases in PkA indicate that the rat's IRT distribution had become more similar to random performance indicating disruption or loss of schedule control. The PkL measure was the median of the area of the obtained IRT distribution above the corresponding negative exponential.

The corresponding negative exponential was 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. The burst ratio (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. The BR metric indicates the propensity to burst.

### Data analysis

Response and reinforcement rate measures are valid even if the subjects make no 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. Estimates of the peak deviation analysis metrics require at least 25 responses in the pause component (IRTs ≥ 3 s) of the IRT distribution. Without drug the rats trained on the DRL 36-s schedule always made more than 25 pause responses. However, at high drug doses, some rats failed to make 25 or more responses. 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. When eight or more rats (out of 15) failed to make at least 25 pause responses the data for that dose were not analyzed. Occasions when rats failed to make at least 25 pause responses are clearly indicated in the Results and Fig. 1.

Response rate, reinforcement rate, PkA, PkL and BR were measured 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 I error due to multiple comparisons. The *P* value (two tailed) required for significance was set at $P < 0.05$

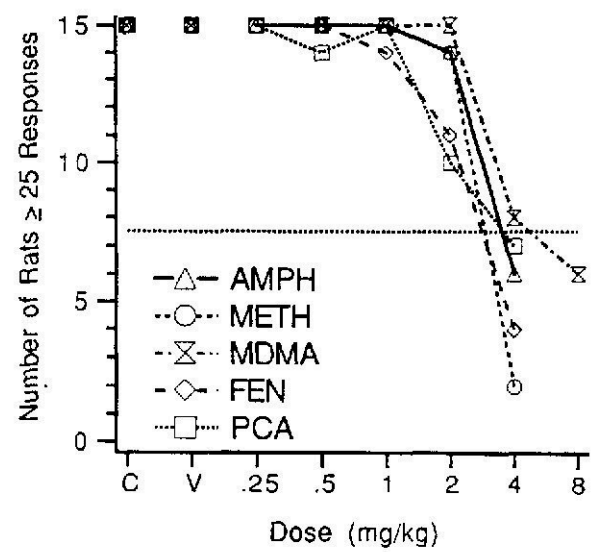


**Fig. 1** Five substituted amphetamines were tested, amphetamine (*AMPH*), methamphetamine (*METH*), methylenedioxymethamphetamine (*MDMA*), para-chloroamphetamine (*PCA*), and fenfluramine (*FEN*). In order to determine an equivalent range of behaviorally effective doses for each of the five substituted amphetamines, the rats were given increasing doses of each compound until over half of the rats failed to make at least 25 responses. Points below the horizontal dashed line indicate when fewer than half of the rats made at least 25 responses. For AMPH, METH, MDMA and PCA fewer than half of the rats responded at the 4.0 mg/kg dose. For MDMA fewer than half of the rats responded at the 8.0 mg/kg dose. Only those doses of the compounds at which greater than half of the rats responded were analyzed

## Results

### Failure to make ≥ 25 pause responses

The number of rats which made at least 25 pause responses at each dose of the five compounds is shown in Fig. 1. The drugs were given in increasing doses until over half of the rats failed to make at least 25 pause responses. The doses at which over half of the rats failed to make at least 25 responses were: AMPH = 4.0 mg/kg, METH = 4.0 mg/kg, MDMA = 8.0 mg/kg, FEN = 4.0 mg/kg, and PCA =4.0 mg/kg (see Fig. 2). Only doses of each test drug at which at least half of the rats made > 25 pause responses were used in the analysis reported below.

### *d*-Amphetamine

AMPH increased response rate and decreased reinforcement rate in a dose-dependent fashion. In addition, PkA and PkL were dose-dependently decreased by AMPH (Fig. 2). Note that in Fig. 2, the filled symbols indicate significantly different from control ($P < 0.05$). The BR metric was not significantly changed by AMPH. The disruption of the IRT distribution by AMPH is shown graphically in Fig. 3B.

### *d*-Methamphetamine

The effects of METH on DRL 36-s schedule performance were very similar to those of AMPH. METH increased response rate and decreased reinforcement rate in a dose-dependent fashion. PkA and PkL were decreased by METH (Fig. 2). The BR metric was not significantly changed by METH. The disruption of the IRT distribution by METH is shown graphically in Fig. 3C.

### *dl*-Para-chloroamphetamine

PCA administration resulted in a significant increase in response rate at a single dose (1.0 mg/kg). Reinforcement rate was dose-dependently decreased after PCA administration. PkA and PkL were also dose-dependently decreased following PCA administration (Fig. 2).The BR metric was not significantly changed by PCA. Disruption of the IRT distribution by PCA is shown graphically in Fig. 3D.

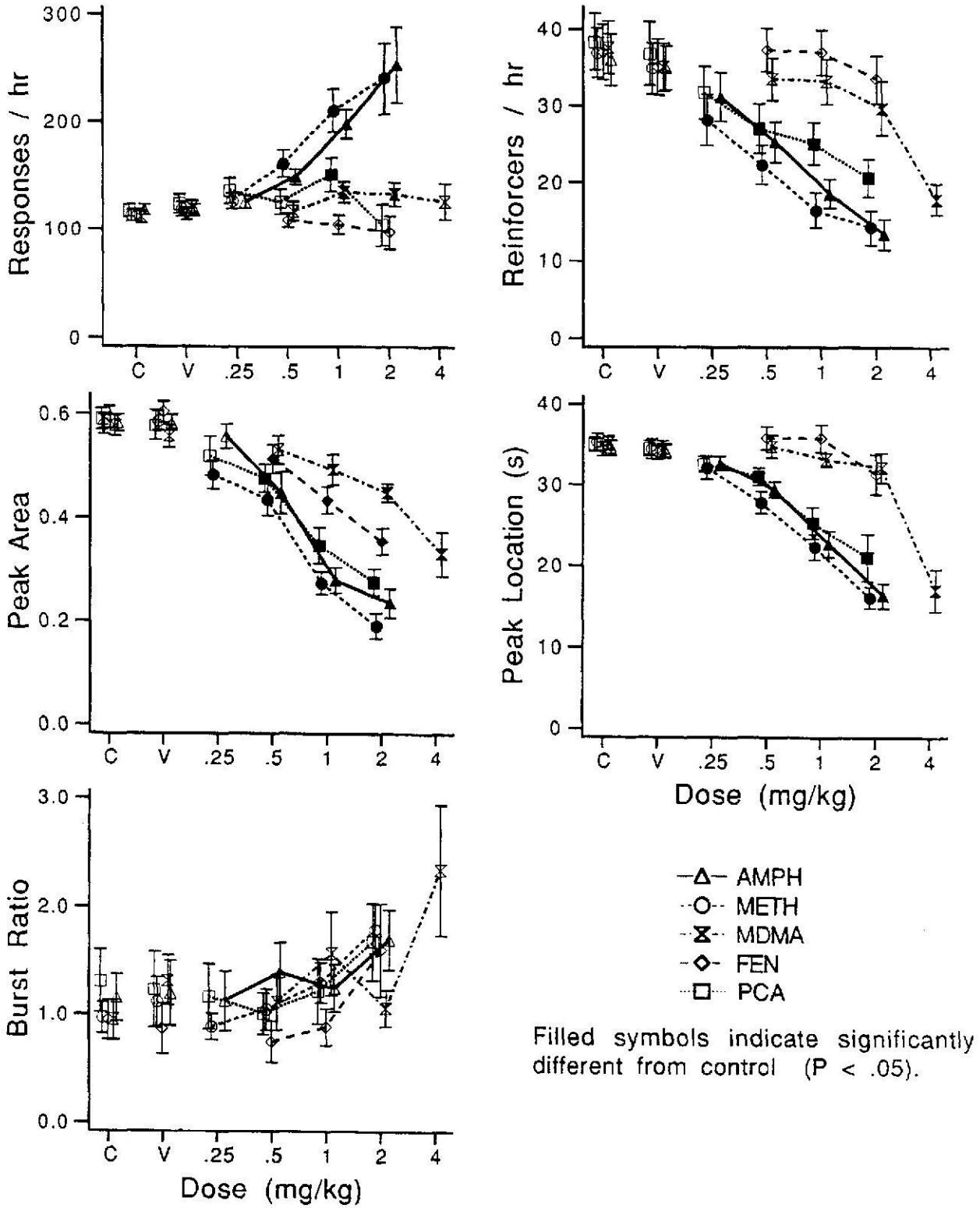


**Fig. 2** Effects of five substituted amphetamines on response rate, reinforcement rate, peak area, peak location and burst ratio. Peak area, peak location and burst ratio are metrics designed to measure the IRT distribution profile. AMPH, METH, MDMA and PCA increased response rate and decreased reinforcement rate. FEN did not significantly change either response or reinforcement rate. All five amphetamine analogs decreased peak area. Four of the amphetamine analogs (AMPH, METH, MDMA, and PCA shifted the peak location toward shorter IRT durations. FEN did not significantly change the peak location. The burst ratio measure was not significantly affected by any compound. The *filled symbols* indicate significantly different from control ($P < 0.05$). Abbreviations: *AMPH* amphetamine, *METH* methamphetamine, *MDMA* methylenedioxymethamphetamine, *PCA* para-chloroamphetamine, *FEN* fenfluramine

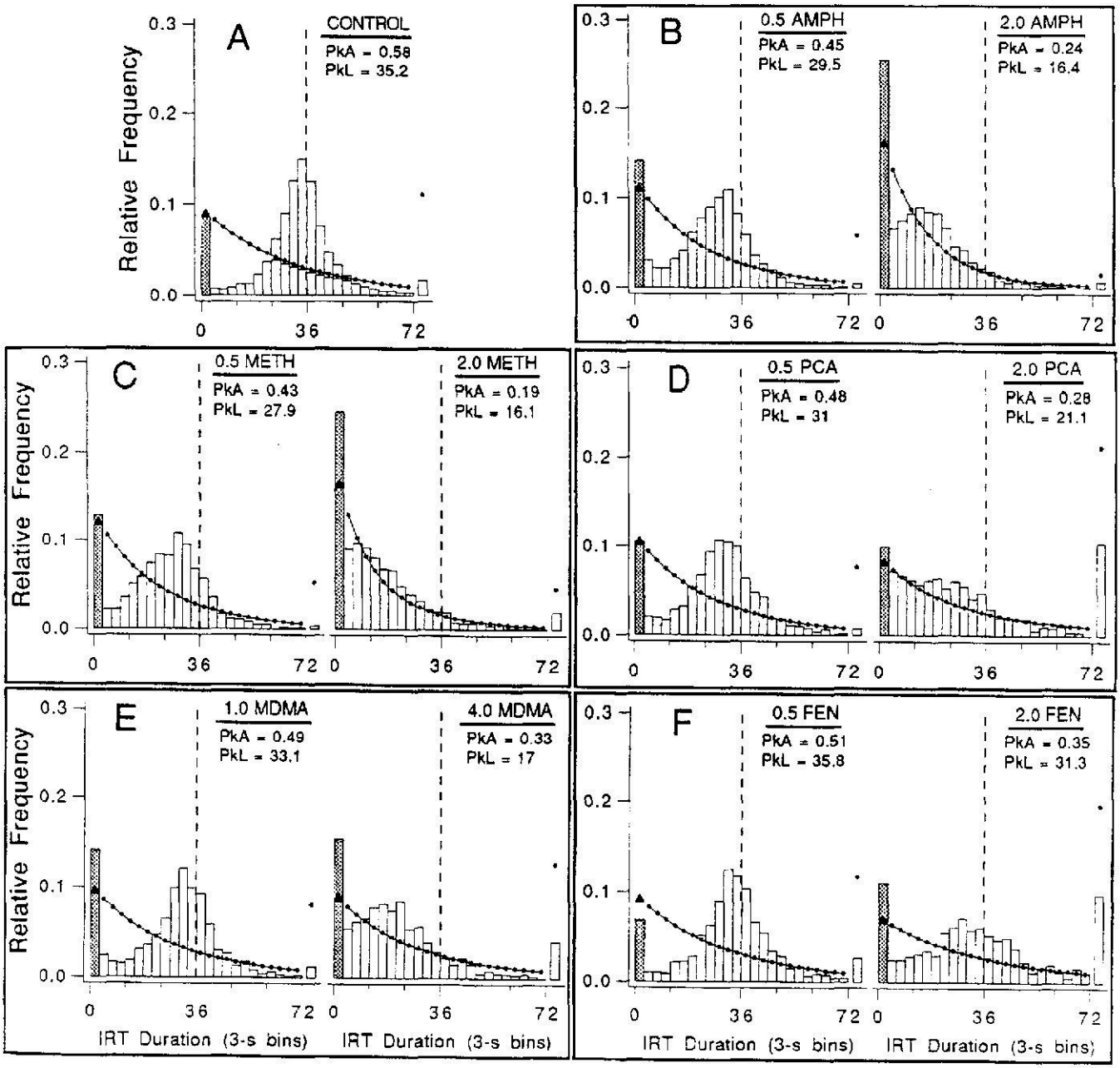


**Fig. 3A–F** The inter-response time (*IRT*) histograms displayed above visualize how the five substituted amphetamines disrupted performance on the DRL 36-s schedule. The control distribution in panel **A** shows that the DRL 36-s IRT distribution is bimodal with one mode at short IRT durations (IRT<3 s, darkened histogram bar on *left*) indicating bursting and a second mode at longer IRT durations indicating pausing. This second mode shows that the rats have learned to wait between responses (although not quite long enough). The connected dots indicate the corresponding negative exponential (random performance). 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 single dot at the far right indicates the relative frequency of IRTs greater than 72 s predicted to occur by the corresponding negative exponential. Similarly, the single histogram bar at the far right indicates the relative frequency of obtained IRTs greater than 72 s. Histograms in **B–F** show the effects of selected doses of the five substituted amphetamines. Comparison of **B–F** with control performance in **A** shows that all five amphetamine analogs disrupted the IRT distribution profiles. The histograms were made from the averaged relative frequency of all the rats that responded at the indicated drug dose. Abbreviations: *AMPH* amphetamine, *METH* methamphetamine, *MDMA* methylenedioxymethamphetamine, PCA para-chloroamphetamine, *FEN* fenfluramine

*dl*-Methylenedioxymethamphetamine

MDMA caused small increases in response rate at the 1.0 and 2.0 mg/kg doses and decreases in reinforcement rate at the 2.0 and 4.0 mg/kg doses. The PkA was decreased in a dose-dependent fashion by MDMA. The PkL was significantly decreased only at the highest dose of MDMA (4.0 mg/kg) (Fig. 2). The BR metric was not

significantly changed by MDMA. Disruption of the IRT distribution by MDMA is graphically shown in Fig. 3E.

*dl*-Fenfluramine

In contrast to the other compounds tested FEN did not significantly alter either response or reinforcement rate or PkL. The BR metric was not significantly changed by FEN. Importantly, PkA was decreased in a dose-dependent fashion by FEN demonstrating that the doses tested had effects on DRL schedule performance (Fig. 2). The disruptive effects of FEN on DRL schedule performance are shown in Fig. 3F.

Comparison of the effects of the five substituted amphetamines on the PkA and PkL metrics

Figure 4 shows PkL plotted against PkA. For AMPH, METH, MDMA and PCA, changes in PkL are positively correlated with changes in PkA. In contrast, for FEN there is no correlation between changes in PkL and PkA. This indicates that increasing doses of FEN have qualitatively different effects compared to increasing doses of the other four substituted amphetamines.

## Discussion

Differential effects of the five substituted amphetamines on DRL 36-s performance

Three distinctive patterns of effects on DRL 36-s performance were observed. In pattern one, AMPH and METH induced large increases in response rate, and decreases in PkL and PkA. In pattern two, PCA and MDMA induced small, but significant, increases in response rate, and decreases in PkL and PkA. In pattern three, FEN induced no change in response rate or PkL but decreased PkA. The fact that the dose of all five compounds was increased until more than half of the rats failed to respond insures that an adequate range of doses was studied (see Fig. 1). The differences between the AMPH-METH effect, and the PCA-MDMA effect is quantitative: AMPH and METH had a much greater effect on response rate. The differences between FEN and the other four compounds were qualitative in that FEN caused no increase in response rate or decrease in PkL. The difference between FEN and the four other compounds tested is further illustrated by Fig. 4. For AMPH, METH, MDMA and PCA, but not for FEN, changes in PkL are positively correlated with changes in PkA.

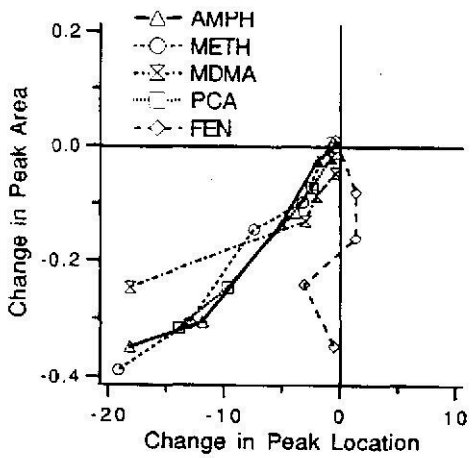


**Fig. 4** This plot visualizes the qualitative difference between the effects of FEN and the other four substituted amphetamines (AMPH, METH, MDMA and PCA). For the five substituted amphetamines, the change from control in PkA is plotted against the change from control in PkL at each dose, including saline. For AMPH, METH, MDMA and PCA, dose-dependent decreases in PkL correspond to dose-dependent decreases in PkA. In contrast, for FEN there is no dose-dependent decrease in PkL although PkA is dose-dependently decreased

Using DRL schedule performance in conjunction with peak deviation analysis to study drug effects

Training on DRL schedules of reinforcement results in a highly organized and characteristic behavioral pattern. This pattern of performance can be visualized by examining IRT distributions of animals well trained on the DRL schedule (see Fig. 3A). The characteristic pattern of behavior resulting from training on DRL schedules provides a useful baseline from which to measure the effects of drugs on behavior (Richards and Seiden 1991). The peak deviation analysis metrics (BR, PkA, and PkL) were developed to quantify the effects of drugs on DRL IRT distributions. These metrics allow detection of drug effects that otherwise might not be seen. For example, in the present paper 0.5 mg/kg PCA shifted the PkL toward shorter IRT durations and decreased the PkA. These statistically significant effects would not have been very convincing if only IRT histograms were offered as evidence of the effects. In another result, PkA was shown to be dose-dependently decreased by FEN while there was no significant effect on response and reinforcement rate. Without the PkA metric no effect of the drug would have been detected demonstrating that the peak deviation analysis metrics are a useful addition to the standard response and reinforcement rate measures.

Differential behavioral effects with AMPH derivatives

A distinction has been made between the stimulant effects of AMPH and the qualitatively different experience reported by human users of MDMA. In animal studies, MDMA has been shown to substitute for methylenedioxyamphetamine (MDA) in drug discrimination, but AMPH does not (Broadbent et al. 1992). Both MDMA and AMPH induce locomotion;

**Table 1** Effects of amphetamine analogs on DA vs. 5HT release. Based on experiments from the references cited below, the differential DA vs. 5HT releasing abilities of AMPH, METH, MDMA, PCA and FEN were compared. In order to standardize the results from the referenced papers, a release ratio[a] was calculated. This ratio indicates the degree of DA or 5HT selectivity that is demonstrated by the test drugs

| | DA release | | 5HT release | | |
|---|---|---|---|---|---|
| Drug | Ratio[a] | Drug dose/ concentration: DA increase | Ratio[a] | Drug dose/ Concentration: 5HT increase | Technique/reference |
| AMPH | 79 | $10^{-6}$M: 22% | 21 | $10^{-6}$M: 06% | In vitro synaptosomal release[c] |
| | 54 | $10^{-5}$M: 38% | 46 | $10^{-5}$M: 32% | |
| | 94 | 2 mg/kg: 3000% | 6 | 2 mg/kg: 200% | In vivo dialysis, % of basal[b,f] |
| | 66 | $10^{-5}$M: 0.10 | 33 | $10^{-5}$M: 0.05 | In vitro slices, fractional release[b,g] |
| Mean | 73 | | 27 | | |
| METH | 52 | $10^{-6}$M: 180% | 48 | $10^{-6}$M: 165% | In vitro synaptosomes, % of basal[c,h] |
| | 61 | $10^{-6}$M: 0.02 | 39 | $10^{-6}$M: 0.013 | In vitro slices, fractional release[b,i] |
| | 70 | $10^{-5}$M: 0.11 | 30 | $10^{-5}$M: 0.047 | |
| | 43 | $10^{-5}$M: 0.10 | 57 | $10^{-5}$M: 0.130 | In vitro slices, fractional release[b,g] |
| Mean | 57 | | 43 | | |
| MDMA | 46 | $10^{-6}$M: 155% | 54 | $10^{-6}$M:185% | In vitro synaptosomes, % of basal[c,h] |
| | 40 | $10^{-6}$M: 115% | 60 | $10^{-6}$M: 170% | In vitro synaptosomes, % of basal[d,h] |
| | 11 | $10^{-6}$M: 0.005 | 89 | $10^{-6}$M: 0.040 | In vitro slices, fractional release[b,i] |
| | 29 | $10^{-5}$M: 0.030 | 71 | $10^{-5}$M: 0.075 | |
| | 12 | $10^{-5}$M: 0.03 | 88 | $10^{-5}$M: 0.220 | In vitro slices, fractional release[b,g] |
| | 51 | $10^{-6}$M: 128% | 49 | $10^{-6}$M: 125% | In vitro slices, % of control[k] |
| | 43 | $10^{-5}$M: 164% | 57 | $10^{-5}$M: 221% | |
| | 51 | $10^{-6}$M: 163% | 49 | $10^{-6}$M: 159% | In vitro slices, % of control[c,k] |
| | 72 | $10^{-6}$M: 466% | 31 | $10^{-5}$M: 201% | |
| Mean | 39 | | 61 | | |
| PCA | 47 | 5 mg/kg: 614% | 53 | 5 mg/kg: 701% | In vivo dialysis, % of basal[j] |
| | 49 | $10^{-6}$M: 183% | 51 | $10^{-6}$M: 190% | In vitro synaptoosmes, % of basal[h] |
| | 16 | $10^{-6}$M: 0.005 | 84 | $10^{-6}$M: 0.027 | In vitro slices, fractional release[b,i] |
| | 33 | $10^{-5}$M: 0.035 | 67 | $10^{-5}$M: 0.070 | |
| | 7 | $10^{-5}$M: 0.030 | 93 | $10^{-5}$M: 0.400 | In vitro slices, fractional release[b,g] |
| Mean | 30 | | 70 | | |
| FEN | 6 | $10^{-6}$M: 02% | 94 | $10^{-6}$M: 32% | In vitro synaptosomal release[c] |
| | 11 | $10^{-5}$M: 05% | 89 | $10^{-5}$M: 41% | |
| | 40 | $10^{-6}$M: 120% | 60 | $10^{-6}$M: 180% | In vitro synaptosomes, % of basal[h] |
| | 4 | $10^{-5}$M 0.01 | 96 | $10^{-5}$M 0.22 | In vitro slices, fractional release[b,g] |
| | 21 | 12.5 mg/kg: 180% | 79 | 12.5 mg/kg: 658% | In vivo dialysis, % of basal[l] |
| Mean | 16 | | 84 | | |

[a] The DA/(DA+5HT) and 5HT/(DA+5HT) ratios represent a standardization across the different studies of the effects of AMPH analogs on DA release vs. 5HT release. For DA, this ratio is computed by summing the drug's DA and 5HT releasing effects, then dividing into the DA releasing effect. For example, the Kannengiesser et al. (1976) AMPH data in the first row of the table has a DA release ratio of 79. This value was arrived at by adding 22% and 6%, and dividing 22% by the total. The 5HT release ratio was arrived at through the same calculation, except the 5HT release effect is in the numerator of the equation

[b] Values are approximations, read from graphs in the referenced articles

[c] (+) isomer

[d] (−) isomer

[e] Kannengiesser et al. (1976). % release represents % radioactivity lost relative to controls; both pellet and supernatant were measured

[f] Kuczenski and Segal (1989)

[g] Schmidt et al. (1991). Fractional release refers to the following ratio: radioactivity released into the superfusion media divided by radioactivity remaining in the slice. Each slice was treated with 2–3 different concentrations of drug with 20-min washout intervals between drug

[h] McKenna et al. (1991). % basal release was defined as follows: [100–amount of radioactivity remaining on filter (in synaptosomes)]+100

[i] Schmidt et al. (1987). Fractional release refers to the following ratio: radioactivity released into the superfusion media divided by radioactivity remaining in the slice. Each slice was treated with 2–3 different concentrations of drug with 20-min washout intervals between drug

[j] Sharp et al. (1986)

[k] Johnson et al. (1986)

[l] Sabol and Seiden, in preparation

however, rearing and holepokes are seen in AMPH-treated rats but not in MDMA-treated rats (Paulus and Geyer 1992). The differences in response rate increases after AMPH and MDMA treatment reported here are consistent with these other observed differences.

## Behavioral differences and dopamine-serotonin interactions induced by the AMPH derivatives

One explanation for the different behavioral effects observed for the AMPH-like compounds tested here is

derived from their relative abilities to release dopamine and serotonin. For example, the $EC_{50}$ for (+) AMPH-induced release of [$^3$H]DA in vitro is 4.4 μM; for FEN-induced [$^3$H]DA release in vitro the $EC_{50}$ is 260 μM (Liang and Rutledge 1982). Table 1 summarizes studies which compared the ability of AMPH analogs to release DA and 5HT. These studies made use of a variety of techniques including in vitro synaptosomal release of preloaded [$^3$H]DA or [$^3$H]5HT, in vitro release of labeled amine from slices, and in vivo dialysis. Most of the data in this table come from in vitro experiments; it is therefore difficult to extrapolate directly from these data to the behavioral effects reported here. More in vivo dialysis experiments, and perhaps the use of serotonergic receptor antagonists in combination with the amphetamine-like drugs on the DRL 36-s schedule, would both provide stronger support for the ideas presented here. In the absence of these direct supports, however, we feel that the data reviewed in Table 1 do provide us with a reasonable basis for interpreting our behavioral data.

From the results summarized in Table 1 it can be seen that AMPH is the most selective DA releasing agent, and FEN is the most selective 5HT releasing agent. The differential effects of the amphetamine analogs on DA and 5HT release correspond to their effects on DRL 36-s schedule performance. The greatest increase in response rate was induced by AMPH, which was also the most selective dopamine releaser. There was no increase in response rate induced by FEN, which is the most selective serotonin releaser. Similarly, AMPH, METH, MDMA and PCA, which all cause increases in dopamine release, also shifted the PkL toward shorter IRT durations. In contrast, FEN, which has the weakest effect on DA release, had no effect on PkL (see Table 1, and Fig. 2).

DA and 5HT often have different roles in the mediation of behavior. For example, decreased serotonergic activity has been shown to increase the motor activity induced by AMPH (Breese et al. 1974; Hollister et al. 1976; Segal 1976); or conversely, increases in serotonergic activity have been shown to decrease AMPH-induced locomotion (Carter and Pycock 1978; Warbritton et al. 1978). This difference in the roles of DA and 5HT in the mediation of behavior suggests that dopaminergic and serotonergic agents should have different effects on the performance of the DRL 36-s schedule. The results we obtained are consistent with this interpretation.

It is more difficult to predict what should happen to behavior with drugs that release both DA and 5HT. One possibility is that the released transmitters antagonize each other at the cellular level. Evidence against this comes from in vivo dialysis experiments. Benloucif and Galloway (1991) found that when FEN was perfused through the dialysis probe, dopamine was dose-dependently increased. The increase in extracellular dopamine was blocked by fluoxetine (a 5HT uptake inhibitor) suggesting that the FEN-induced increase in dopamine release was indirectly mediated by a FEN-induced increase in serotonin. This conclusion is supported by Parsons and Justice (1993), who showed that local administration of serotonin causes an increase in extracellular levels of dopamine in vivo. A second possibility is that both dopaminergic and serotonergic behaviors are stimulated, and compete for expression. The serotonin syndrome of head weaving, forepaw treading, flat body posture, locomotion (Trulson and Jacobs 1976) is different from the stereotypies induced by AMPH (locomotion and sniffing at low doses, and focussed licking, gnawing, and biting at higher doses). When both dopamine and serotonin release are increased simultaneously, they may induce behaviors which compete with each other. The dominant behavioral response may in fact be incompatible with the task requirements (Lyon and Robbins 1975; Robbins 1981).

In the experiments reported here, the difference in the amount of DA versus 5HT release induced by the different amphetamine analogs may be reflected in the degree of response rate increase, as well as the degree of peak shift. The larger the DA contribution (relative to the 5HT contribution), the greater the increase in response rate, and the greater the shift in the peak toward shorter IRT durations. Increased DA release may disrupt DRL performance by increasing the probability of occurrence of behaviors which are *compatible* with bar pressing resulting in increased response rate and a decreased ability to wait between bar press responses (decreased PkL). Increased 5HT release may disrupt DRL performance by increasing the probability of occurrence of behaviors which are *incompatible* with bar pressing. Thus, an increasing 5HT contribution should act to counteract the response rate increasing effects of DA resulting in less of an increase in response rate, and less of a shift in PkL as is shown in Fig. 2. Kuzcinski and Segal (1989) made a similar suggestion regarding AMPH-induced behaviors. The locomotor increase (seen only at low doses) may be dopaminergic; however, when significant increases in serotonin release occur (2.0 mg/kg) there is a transition from stereotypic locomotion to stereotypic licking, biting and gnawing. Kuzcinski and Segal (1989) suggested that 5HT plays a role in the behavioral transition from stereotypic locomotion to stereotypic oral movements (see also Hollister et al. 1976; Segal 1976; Warbritton et al. 1978). In contrast, Paulus and Geyer (1992) analyzed the effects of several substituted amphetamines on spontaneous motor behavior. They used a complex monitoring device which analyzed the intensity, as well as pattern of drug-induced motor activity. According to their analysis, a drug's DA versus 5HT releasing ability did not explain the behavioral differences they measured; rather, the 5HT1B receptor seemed to be the relevant factor.

In summary, AMPH, METH, MDMA, PCA, and FEN have differential effects on the DRL 36-s

schedule. These results are interpreted to parallel differences between the five drugs tested in their relative abilities to release DA and 5HT.

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