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ORIGINAL INVESTIGATION

Effects of *d*-amphetamine and DOI (2,5-dimethoxy-4-iodoamphetamine) on timing behavior: interaction between D_1 and 5-HT_{2A} receptors

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Abstract
Rationale The dopamine-releasing agent *d*-amphetamine and the 5-HT_2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) have similar effects on free-operant timing behavior. The selective D_1 dopamine receptor antagonist 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol (SKF-83566), but not the D_2 dopamine receptor antagonist haloperidol, can antagonize the effect of *d*-amphetamine, and the selective 5-HT_{2A} receptor antagonist (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol (MDL-100907) can antagonize the effect of DOI. However, it is not known whether the effect of *d*-amphetamine can be reversed by MDL-100907 and the effect of DOI by dopamine receptor antagonists.
Objective The objective of this work is to examine the interactions of *d*-amphetamine and DOI with MDL-100907, SKF-83566, and haloperidol on timing performance.
Materials and methods Rats (n=12–15 per experiment) were trained under the free-operant psychophysical procedure to press two levers (A and B) in 50-s trials in which reinforcement was provided intermittently for responding on A in the first half, and B in the second half of the trial. Percent responding on B (%*B*) was recorded in successive 5-s epochs of the trials; logistic functions were fitted to the data from each rat for the derivation of timing indices [T_{50} (time corresponding to %*B*=50); Weber fraction]. Rats were treated systemically with *d*-amphetamine or DOI, alone and in combination with haloperidol, SKF-83566, or MDL-100907.
Results *d*-Amphetamine (0.4 mg kg^{-1}) reduced T_{50} compared to vehicle; this effect was antagonized by SKF-83566 (0.03 mg kg^{-1}) and MDL-100907 (0.5 mg kg^{-1}), but not by haloperidol (0.05, 0.1 mg kg^{-1}). DOI (0.25 mg kg^{-1}) also reduced T_{50}; this effect was reversed by MDL-100907 (0.5 mg kg^{-1}), but not by SKF-83566 (0.03 mg kg^{-1}) or haloperidol (0.05 mg kg^{-1}).
Conclusions The results suggest that both 5-HT_{2A} and D_1 receptors, but not D_2 receptors, are involved in *d*-amphetamine's effect on timing behavior in the free-operant psychophysical procedure. DOI's effect on timing is mediated by 5-HT_{2A} receptors, but neither D_1 nor D_2 receptors are involved in this effect.

Keywords Timing · Free-operant psychophysical procedure · D_1 dopamine receptors · D_2 dopamine receptors · 5-HT_{2A} receptors · *d*-amphetamine · DOI · SKF-83566 · MDL-100907 · Haloperidol

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Introduction

Temporal differentiation is a form of interval timing, revealed by immediate timing schedules, in which the organism's behavior comes under the control of time during

an ongoing interval (Killeen and Fetterman 1988; Killeen et al. 1997). An example of an immediate timing schedule is the free-operant psychophysical procedure (Stubbs 1976, 1980), in which reinforcement is provided intermittently for responding on two operanda, A and B; responding on A is reinforced in the first half and responding on B in the second half of each trial. Temporal differentiation is assessed quantitatively from the psychophysical function relating proportional responding on B (%B) to time measured from the onset of the trial. This function has a logistic form, characterized by the indifference point, T_{50} (the time at which %B=50), and a slope parameter, ε. These parameters may be used to derive the Weber fraction, an index of the precision of temporal differentiation (see Killeen and Fetterman 1988; Gibbon et al. 1997; Ho et al. 2002). In the free-operant psychophysical procedure, the Weber fraction is generally expressed as the ratio of the limen ($[T_{75}-T_{25}]/2$, i.e., half the difference between the times corresponding to %B=75% and %B=25%) to T_{50}. Thus, a low Weber fraction signifies a relatively steep psychometric function, in other words precise temporal differentiation.

There is a considerable body of evidence supporting the proposition that the dopaminergic system plays a major role in the regulation of interval timing behavior (see Meck 1996; Gibbon et al. 1997; Hinton and Meck 1997; Meck and Benson 2002; MacDonald and Meck 2004). Drugs that release dopamine, for example the psychostimulant *d*-amphetamine, have been found to produce a leftward displacement of the psychophysical function in some immediate timing schedules. In the case of the free-operant psychophysical procedure, this is reflected in a reduction of the value of T_{50} (Chiang et al. 2000a; Cheung et al. 2006). The effects of dopamine-releasing agents on temporal differentiation have generally been attributed to the stimulation of D_2 dopamine receptors (Meck 1986, 1996). However, Cheung et al. (2006) recently reported that *d*-amphetamine's effect on performance in the free-operant psychophysical procedure could be antagonized by a selective D_1 dopamine receptor antagonist 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol (SKF-83566), but not by the D_2 receptor antagonist haloperidol, indicating that *d*-amphetamine's effect on temporal differentiation in this schedule may be mediated mainly by D_1 dopamine receptors.[1]

[1]Most drugs acting at D_1 dopamine receptors do not discriminate between D_1 and D_5 dopamine receptors and are, therefore, more precisely designated as D_1-like receptor agonists and antagonists. Similarly, most drugs acting at D_2 receptors do not discriminate between D_2, D_3, and D_4 receptors and are, therefore, designated as D_2-like receptor agonists and antagonists (Seeman and van Tol 1994; Strange 2001). Throughout this paper, for the sake of simplicity, they are referred to as D_1 and D_2 receptor agonists and antagonists.

Temporal differentiation is also sensitive to stimulation of 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors. For example, the 5-HT$_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) reduces the value of T_{50} in the free-operant psychophysical procedure (Body et al. 2003, 2004, 2006), an effect that can be antagonized by the selective 5-HT$_{2A}$ receptor antagonist (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol) (MDL-100907; Body et al. 2006).

The findings discussed above indicate that stimulation of either D_1 or 5-HT$_{2A}$ receptors can disrupt temporal differentiation, reducing the value of T_{50} in the free-operant psychophysical procedure. However, it is not clear whether the effects of D_1 and 5-HT$_{2A}$ receptor agonists are fully independent of one another. There are several potential sources of interaction between dopamine and 5-HT receptor-mediated mechanisms in the control of timing behavior. For example, there is good evidence that 5-HT$_{2A}$ receptor stimulation may influence dopamine release in the striatum and prefrontal cortex (Ennis et al. 1981; Westfall and Tittermary 1982; Gudelsky et al. 1994; Schmidt et al. 1994; Ichikawa and Meltzer 1995; Schmidt and Fadayel 1995; Ng et al. 1999; Porras et al. 2002). Moreover, 5-HT$_{2A}$ receptors are present on dopaminergic neurones of the ventral tegmental area (VTA; Doherty and Pickel 2000; Nocjar et al. 2002) and may help to regulate the activity of these neurones (Sorenson et al. 1993; Olijslagers et al. 2004, 2005). At a behavioral level, 5-HT$_{2A}$ receptor antagonists have been shown to antagonize *d*-amphetamine-induced hyperlocomotion (Sorenson et al. 1993; Moser et al. 1996; O'Neill et al. 1999) and to reverse *d*-amphetamine's effect on latent inhibition (Moser et al. 1996).

The aim of the present experiments was to address the question of a possible interaction between dopamine and 5-HT$_{2A}$ receptors in the regulation of temporal differentiation. In particular, the study examined whether the effect of the 5-HT$_{2A/2C}$ receptor agonist DOI could be reversed by D_1 and D_2 dopamine receptor antagonists (SKF-83566 and haloperidol), and whether the effect of *d*-amphetamine could be reversed by the 5-HT$_{2A}$ receptor antagonist, MDL-100907.

Materials and methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals and was approved by the local ethical review committee.

Subjects

Female Wistar rats, aged approximately 4 months and weighing 250–290 g at the start of the experiment, were

housed individually under a constant cycle of 12 h light and 12 h darkness (lights on 0700–1900 hours) and were maintained at 80% of their initial free-feeding body weights throughout the experiment by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages. The rats were approximately 12 months old at the end of the experiment.

Apparatus

The rats were trained in operant conditioning chambers (Campden Instruments, Sileby, UK), with of internal dimensions 20×23×22.5 cm. Twelve identical chambers were used; each subject was always tested in the same chamber. One wall of the chamber contained a recess into which a motor-operated dipper could deliver 50 μl of a liquid reinforcer. Apertures were situated 5 cm above and 2.5 cm on either side of the recess; a motor-driven retractable lever could be inserted into the chamber through each aperture. Each lever could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest; masking noise was provided by a rotary fan. An Acorn microcomputer programmed in Arachnid BASIC (CeNeS, Cambridge, UK), located in an adjoining room, controlled the schedules and recorded the behavioral data.

Behavioral training

Two weeks before starting the experiment, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. Then they were trained to press the levers for the sucrose reinforcer and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence, for three sessions. The rats then underwent 50-min training sessions in the free-operant psychophysical procedure for 7 days a week at the same time each day during the light phase of the daily cycle (between 0800 and 1300 hours) for the remainder of the experiment. The reinforcer, a 0.6-M solution of sucrose in distilled water, was prepared daily before each session.

The free-operant psychophysical procedure was identical to that used by Chiang et al. (1998, 2000a,b). Each session consisted of fifty 50-s trials, successive trials being separated by 10-s intertrial intervals. In 40 of the 50 trials, reinforcement was provided on a constant probability, variable-interval 30-s schedule (Catania and Reynolds 1968). The levers were inserted into the chamber at the start of each trial and were withdrawn during the intertrial interval. Reinforcers were delivered only for responses on lever A during the first 25 s and lever B in the last 25 s of the trials. The positions of levers A and B (left vs right) were counterbalanced across subjects. Four trials in each session were probe trials, in which no reinforcers were delivered. The remaining six trials were forced-choice trials, in which only one lever was present in the chamber (lever A, three trials; lever B, three trials). The probe and forced-choice trials were interspersed randomly among the standard trials, with the constraint that at least one standard trial occurred between successive probe or forced-choice trials. In the standard and probe trials, switching between the levers was restricted to one switch per trial: in each trial, the first response on lever B resulted in the withdrawal of lever A until the start of the next trial (Chiang et al. 1998).

Drug treatment

The drug treatment regimen started after >90 sessions of preliminary training under the free-operant psychophysical procedure when the rats were approximately 8 months old. Injections of drugs were given on Tuesdays and Fridays, and injections of the vehicle alone on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays, or Sundays. In order to accrue a sufficient number of probe trials to obtain reliable estimates of the timing indices for individual rats, each active treatment was administered on five occasions according to a Latin Square design. Depending on the number of active treatments used (three or four; see below), each treatment series lasted for 50–70 days; each group of rats was used for a maximum of two treatment series (see below). Drugs were given by subcutaneous (s.c., 1.0 ml kg^{-1}) or intraperitoneal (i.p., 2.5 ml kg^{-1}) injections. The doses of the drugs were selected on the basis of previous behavioral studies with rats (see "Discussion" for references).

Interaction between d-amphetamine and haloperidol (n=14) *d*-Amphetamine sulfate 0.4 mg kg^{-1}, dissolved in 0.9% NaCl, was injected i.p. 15 min before the start of the session. It was administered alone or in combination with haloperidol. Haloperidol, dissolved in 0.1 M tartaric acid in 0.9% NaCl and buffered to pH 5.5 with NaOH, was injected i.p. 30 min before the start of the session. Two treatment series were carried out, in which the doses of haloperidol were 0.05 and 0.1 mg kg^{-1}.

Interaction between d-amphetamine, MDL-100907, and SKF-83566 (n=15) *d*-Amphetamine sulfate 0.4 mg kg^{-1} was administered alone or in combination with either MDL-100907 0.5 mg kg^{-1} or SKF-83566 0.03 mg kg^{-1}. MDL-100907 was dissolved in glacial acetic acid and sterile water, buffered to pH 5.5, and diluted to volume with 0.9% NaCl; it was injected i.p. 25 min before the start of the session. SKF-83566 was dissolved in 0.9% NaCl mixed with a few drops of tartaric acid and buffered to pH 5.5

with NaOH; it was injected s.c. 30 min before the start of the session.

Interaction between DOI and haloperidol (n=14) DOI 0.25 mg kg^{-1}, dissolved in 0.9% NaCl, was injected s.c. 15 min before the start of the session. It was administered alone or in combination with haloperidol 0.05 mg kg^{-1} as described above.

Interaction between DOI, MDL-100907, and SKF-83566 (n=14) DOI 0.25 mg kg^{-1} was administered alone or in combination with MDL-100907 0.5 mg kg^{-1} or SKF-83566 0.03 mg kg^{-1}, with the vehicles and administration times described above.

d-Amphetamine, haloperidol, and DOI were obtained from Sigma (Poole, UK), and SKF-83566 from Tocris Cookson (Avonmouth, UK); MDL-100907 was a generous gift from Solvay Pharmaceuticals (Weesp, The Netherlands).

Data analysis

Only the data collected from the probe trials were used in the analysis. Separate analyses were carried out on the data from each experiment.

Relative response rates Each 50-s trial was divided into 5-s time bins. The mean response rate on each lever in successive time bins was calculated for each rat under each treatment condition. Relative response rate on lever B (%*B*), defined as the response rate on lever B divided by the combined response rate on both levers, was analyzed by a two-factor analysis of variance (treatment × time bin) with repeated measures on both factors.

Psychometric functions A two-parameter logistic function was fitted to the relative response rate data: $\%B = 100/(1 + [t/T_{50}]^{\varepsilon})$, where t is time from trial onset, T_{50} (the indifference point) is a parameter expressing the time at which %*B*=50%, and ε is the slope of the function (Al-Zahrani et al. 1996; ε has a negative value in the case of ascending sigmoid functions). The curve-fitting procedure yields estimates (±SEest) of the values of T_{50} and the slope, from which the Weber fraction was determined as follows. The limen was defined as half the difference between T_{75} and T_{25} (T_{75} and T_{25} are the values of t corresponding to %*B*=75 and 25%, respectively), and the Weber fraction was calculated as the ratio of the limen to T_{50}. Goodness of fit of the logistic functions was expressed as the index of determination, p^2. The values of T_{50}, ε, and the Weber fraction were analyzed by one-factor analyses of variance (treatment) with repeated measures. In the case of a significant effect of treatment, comparisons were made between each active treatment and the control (vehicle alone) condition using Dunnett's test. In addition, planned comparisons were made between the agonist treatment condition (*d*-amphetamine or DOI) and the combined agonist + antagonist treatment condition (significance criterion, $P<0.05$).

Switching time Switching time was defined as the time, measured from the start of the trial, of the first response on lever B. Mean switching time was calculated for each rat under each treatment condition, and the data were subjected to one-factor analysis of variance (treatment) with repeated measures followed by multiple comparisons, as described above.

Overall response rates Overall response rate was calculated for each rat under each treatment condition. For each series of treatments, the data were analyzed using a one-factor analysis of variance (treatment) with repeated measures followed by multiple comparisons, as described above.

Results

Under each treatment condition in each experiment, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset (left-hand panels of Figs. 1, 2, 3 and 4). The proportion of responding allocated to lever B (%*B*) increased progressively as a function of time from trial onset (right-hand panels of Figs. 1, 2, 3 and 4). The two-parameter logistic function provided a good description of the data in each condition, with the group mean values of p^2 being ≥0.9 in each case (see Tables 1, 2, 3 and 4).

Interaction between *d*-amphetamine and haloperidol

A preliminary analysis of the data obtained after vehicle and *d*-amphetamine treatment indicated that there were no significant differences between the relative response rate (%*B*) data or the parameter values obtained in the two treatment series; therefore, the data from the two series were pooled in all subsequent analyses. The group mean response rates on the two levers are shown in the left-hand panel and the group mean %*B* data in the right-hand panel of Fig. 1. Analysis of variance of the %*B* data revealed significant main effects of time bin [$F(9,351)=325.1$, $P<0.001$] and treatment [$F(3,39)=5.0$, $P<0.01$], and a significant treatment × time bin interaction [$F(27,351)=4.5$, $P<0.001$]. *d*-Amphetamine displaced the psychometric function to the left compared to the function derived for the vehicle treatment. Haloperidol did not reverse this effect of *d*-amphetamine.

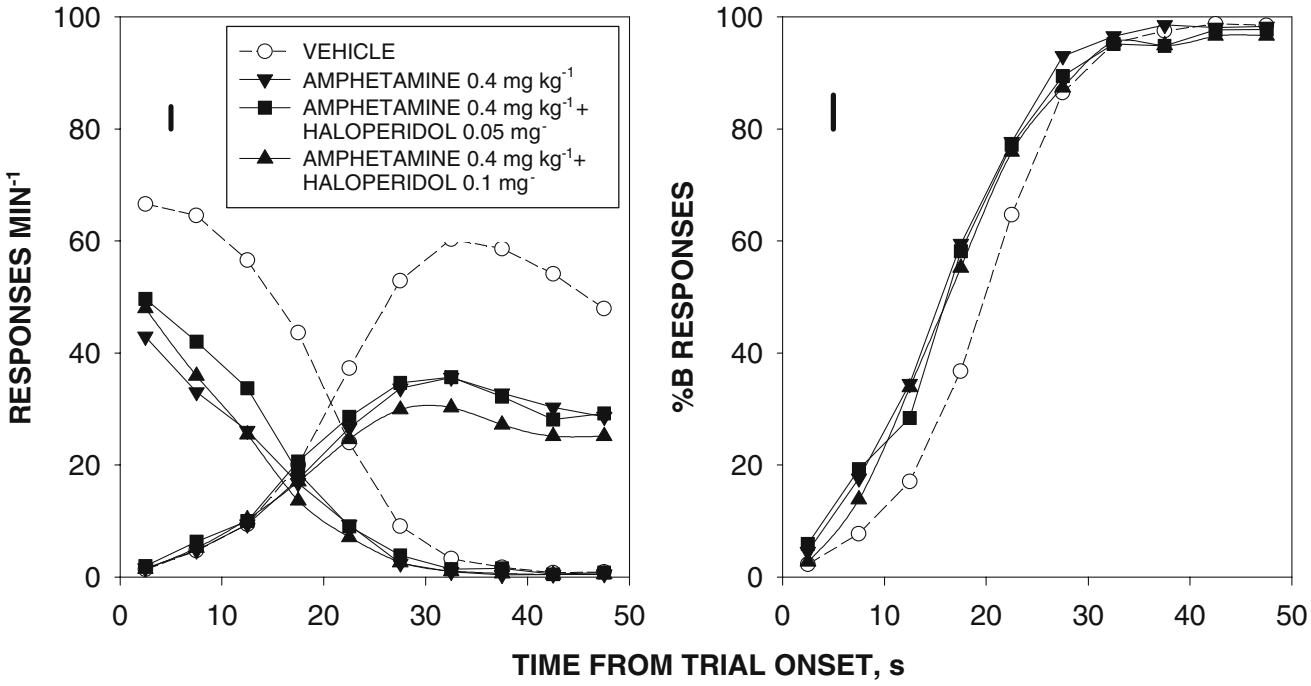

Fig. 1 Interaction between *d*-amphetamine and haloperidol on performance in the free-operant psychophysical procedure. *Left-hand graph* Absolute response rates on levers A (*descending curves*) and B (*ascending curves*), *ordinate* response rate (responses per minute), *abscissa* time from trial onset (seconds). *Right-hand graph* Relative response rate on lever B ("psychometric function"), *ordinate* percent responding on lever B, *abscissa* time from trial onset (seconds). *Points* indicate group mean data under each treatment condition (see *inset*). *Vertical bars* indicate 2 SEDs, derived from the error term of the analyses of variance. *d*-Amphetamine displaced the psychometric function to the left; neither dose of haloperidol altered this effect (see text for details)

The parameters of the logistic functions are shown in Table 1. There was a significant effect of treatment on T_{50} [$F(3,39)=6.5$, $P<0.01$]. Multiple comparisons showed that T_{50} was significantly reduced by *d*-amphetamine alone, compared to the vehicle-alone condition and that this effect was not significantly attenuated by co-administration of haloperidol.

There was no significant effect of treatment on the slope, ε [$F(3,39)=1.7$, $P>0.1$]. There was a significant effect of treatment on the Weber fraction [$F(3,39)=3.4$, $P<0.05$]; multiple comparisons showed that the combination of *d*-amphetamine + haloperidol 0.05 mg kg^{-1} produced a significant increase in the Weber fraction compared to the vehicle-alone treatment.

The group mean switching time (±SEM) under each treatment condition are shown in Table 1. There was a significant effect of treatment [$F(3,39)=4.1$, $P<0.05$]. Multiple comparisons showed that *d*-amphetamine signifi-

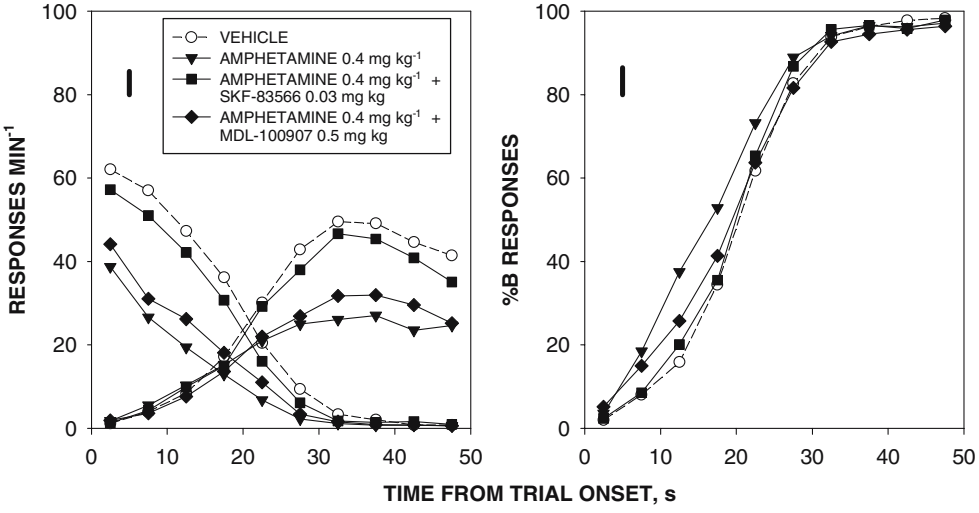

Fig. 2 Interaction between *d*-amphetamine, MDL-100907, and SKF-83566 on performance in the free-operant psychophysical procedure (conventions as in Fig. 1). *Left-hand graph* Absolute response rates, *right-hand graph* relative response rate on lever B. *d*-Amphetamine displaced the psychometric function to the left; this effect was antagonized by both MDL-100907 and SKF-83566 (see text for details)

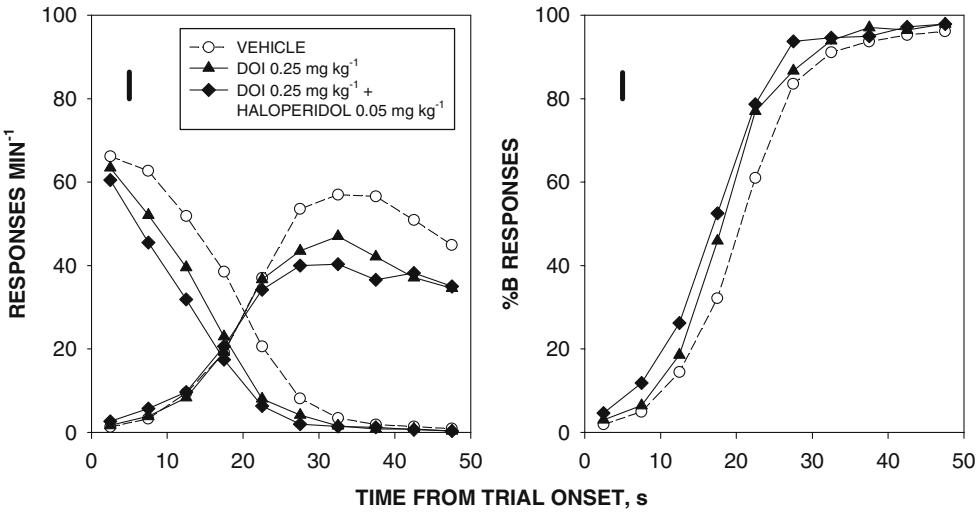

Fig. 3 Interaction between DOI and haloperidol on the free-operant psychophysical procedure (conventions as in Fig. 1). *Left-hand graph* Absolute response rates, *right-hand graph* relative response rate on lever B. DOI displaced the psychometric function to the left; haloperidol did not alter this effect (see text for details)

cantly reduced switching time compared to the vehicle-alone treatment and that this effect was not attenuated by either dose of haloperidol.

The group mean overall response rates (±SEM) under each treatment condition are shown in Table 1. There was a significant effect of treatment [$F(3,39)=36.3$, $P<0.001$]. Multiple comparisons showed that *d*-amphetamine alone and *d*-amphetamine in combination with each dose of haloperidol significantly decreased response rate compared to the vehicle-alone treatment.

Interaction between *d*-amphetamine, MDL-100907, and SKF-83566

The group mean response rates on the two levers are shown in the left-hand panel and the group mean %*B* data in the right-hand panel of Fig. 2. Analysis of variance of the %*B* data revealed significant main effects of time bin [$F(9,126)=435.1$, $P<0.001$] and treatment [$F(3,42)=3.5$, $P<0.05$] and a significant treatment × time bin interaction [$F(27,378)=3.0$, $P<0.001$]. *d*-Amphetamine displaced the psychometric function to the left compared to the function derived for the vehicle-alone treatment. Both MDL-100907 and SKF-83566 reversed this effect of *d*-amphetamine, in that the curves derived for the combined *d*-amphetamine + MDL-100907 and *d*-amphetamine + SKF-83566 treatments were close to the curve derived for the vehicle-alone treatment.

The parameters of the logistic functions are shown in Table 2. There was a significant effect of treatment on T_{50} [$F(3,42)=4.5$, $P<0.01$]. Multiple comparisons showed that *d*-amphetamine significantly reduced T_{50}, whereas there was no significant difference between the values of T_{50} derived for the vehicle, *d*-amphetamine + MDL-100907 (0.5 mg kg^{-1}), and *d*-amphetamine + SKF-83566 (0.03 mg kg^{-1}) treatments. The reduction in T_{50} produced by *d*-amphetamine was significantly reversed by combined treatment with either MDL-100907 or SKF-83566.

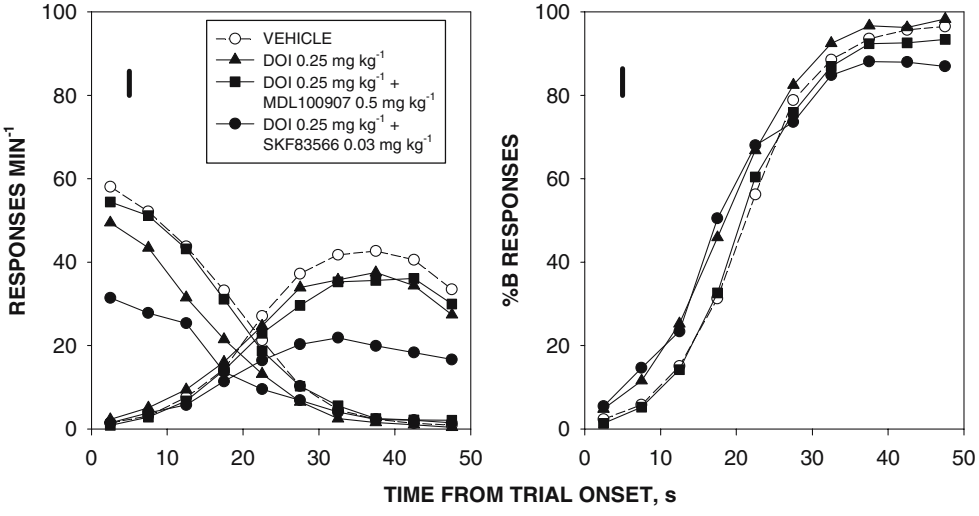

Fig. 4 Interaction between DOI, MDL-100907, and SKF-83566 on the free-operant psychophysical procedure (conventions as in Fig. 1). *Left-hand graph* absolute response rates, *right-hand graph* relative response rate on lever B. DOI displaced the psychometric function to the left; this effect was antagonized by MDL-100907 but not by SKF-83566

Table 1 Interaction between *d*-amphetamine and haloperidol on quantitative timing parameters and overall response rate (mean±SEM)

Treatment	Parameters of psychometric function					Overall response rate (responses min^{-1})
	T_{50} (s)	ε	p^2	Weber fraction	Mean switching time (s)	
Vehicle	19.2±0.8	−5.11±0.33	0.99±0.00	0.23±0.02	18.4±0.7	61.8±5.5
d-Amphetamine 0.4 mg kg^{-1}	15.1±1.3*	−4.39±0.47	0.98±0.00	0.31±0.04	15.3±0.8*	35.1±4.7*
d-Amphetamine 0.4 mg kg^{-1}+ haloperidol 0.05 mg kg^{-1}	15.5±1.5*	−3.97±0.41	0.96±0.01	0.35±0.06*	16.4±0.9*	47.5±4.6*
d-Amphetamine 0.4 mg kg^{-1}+ haloperidol 0.1 mg kg^{-1}	16.1±1.3*	−4.81±0.70	0.98±0.00	0.29±0.04	16.5±1.1*	33.2±4.7*

*$P<0.05$, significantly different from vehicle

There was no significant effect of treatment on ε [$F<1$] or the Weber fraction [$F(3,42)=2.4$, $P>0.05$].

There was a significant effect of treatment on switching time [$F(3,42)=6.1$, $P<0.01$; Table 2]. *d*-Amphetamine significantly reduced switching time compared to the vehicle-alone treatment. SKF-83566 and MDL-100907 significantly attenuated the effect of *d*-amphetamine.

The overall response rates under each treatment condition are shown in Table 2. There was a significant effect of treatment [$F(3,42)=20.9$, $P<0.001$] on overall response rate. Multiple comparisons showed that *d*-amphetamine alone and *d*-amphetamine in combination with MDL-100907 significantly reduced overall response rate compared to the vehicle-alone condition. SKF-83566 significantly attenuated the reduction in overall response rate induced by *d*-amphetamine.

Interaction between DOI and haloperidol

The group mean response rates on the two levers are shown in the left-hand panel and the group mean %*B* data in the right-hand panel of Fig. 3. Analysis of variance of the %*B* data revealed significant main effects of time bin [$F(9,117)=392.8$, $P<0.001$] and treatment [$F(2,28)=16.1$, $P<0.001$], and a significant treatment × time bin interaction [$F(18.234)=3.2$, $P<0.001$]. DOI displaced the psychometric function to the left compared to the vehicle-alone treatment, as did the combined DOI + haloperidol treatment.

The parameters of the logistic functions are shown in Table 3. There was a significant effect of treatment on T_{50} [$F(2,26)=10.9$, $P<0.001$]. Multiple comparisons showed that DOI alone and DOI + haloperidol reduced T_{50}. The reduction of T_{50} induced by DOI was not significantly altered by combined treatment with haloperidol.

There was no significant effect of treatment on ε [$F<1$] or the Weber fraction [$F<1$].

There was a significant effect of treatment on switching time[$F(2,26)=19.1$, $P<0.001$; Table 3]. DOI alone and DOI + haloperidol significantly reduced switching time compared to the vehicle-alone treatment. The effect of DOI + haloperidol was significantly greater than the effect of DOI alone.

The overall response rates under each treatment condition are shown in Table 3. There was a significant effect of treatment [$F(2,26)=18.4$, $P<0.001$] on overall response rate. Multiple comparisons showed that DOI alone and DOI + haloperidol significantly reduced the response rate compared to the vehicle-alone condition.

Table 2 Interaction between *d*-amphetamine, SKF-83566, and MDL-100907 on quantitative timing parameters and overall response rate (mean±SEM)

Treatment	Parameters of psychometric function					Overall response rate (responses min^{-1})
	T_{50} (s)	ε	p^2	Weber fraction	Mean switching time (s)	
Vehicle	19.2±0.7	−4.92±0.32	0.98±0.00	0.24±0.02	18.9±0.6	52.8±5.4
d-Amphetamine 0.4 mg kg^{-1}	15.8±1.4[a]	−4.45±0.85	0.96±0.00	0.33±0.04	15.3±0.9[a]	29.0±3.6[a]
d-Amphetamine 0.4 mg kg^{-1}+ SKF-83566 0.03 mg kg^{-1}	18.9±1.0[b]	−5.21±0.52	0.98±0.01	0.25±0.03	18.1±1.0[b]	47.5±4.9[b]
d-Amphetamine 0.4 mg kg^{-1}+ MDL-100907 0.5 mg kg^{-1}	18.5±1.4[b]	−4.76±0.69	0.94±0.01	0.32±0.05	16.8±0.9[ab]	33.2±4.6[a]

[a] Significantly different from vehicle ($P<0.05$)
[b] Significantly different from *d*-amphetamine alone ($P<0.05$)

Table 3 Interaction between DOI and haloperidol on quantitative timing parameters and overall response rate (mean±SEM)

Treatment	Parameters of psychometric function					Overall response rate (responses min^{-1})
	T_{50} (s)	ε	p^2	Weber fraction	Mean switching time (s)	
Vehicle	20.6±1.3	−5.52±0.62	0.99±0.00	0.23±0.02	18.4±0.7	58.8±6.9
DOI 0.25 mg kg^{-1}	18.0±1.1[a]	−5.87±0.64	0.98±0.00	0.22±0.02	17.1±0.8[a]	46.8±5.2[a]
DOI 0.25 mg kg^{-1}+ haloperidol 0.05 mg kg^{-1}	16.6±1.0[a]	−5.19±0.53	0.97±0.01	0.25±0.03	15.4±0.7[ab]	43.0±5.0[a]

[a] Significantly different from vehicle ($P<0.05$)
[b] Significantly different from DOI alone ($P<0.05$)

Interaction between DOI, MDL-100907, and SKF-83566

The group mean response rates on the two levers are shown in the left-hand panel and the group mean %*B* data in the right-hand panel of Fig. 4. Analysis of variance of the %*B* data revealed significant main effects of time bin [$F(9,117)=447.0$, $P<0.001$] and treatment [$F(3,39)=3.7$, $P<0.001$], and a significant treatment × time bin interaction [$F(27,351)=3.5$, $P<0.001$]. DOI displaced the psychometric curve to the left compared to the function derived for the vehicle-alone condition. SKF-83566 did not attenuate this effect of DOI, in that the curve derived for the DOI + SKF-83566 treatment was similar to the DOI-alone curve. MDL-100907 reversed the effect of DOI, the curve derived for the DOI + MDL-100907 treatment being close to that derived for the vehicle-alone treatment.

The parameters of the logistic functions are shown in Table 4. There was a significant effect of treatment on T_{50} [$F(3,39)=4.5$, $P<0.01$]. Multiple comparisons showed that DOI alone and DOI + SKF-83566 reduced T_{50}; the reduction in T_{50} induced by DOI was significantly reversed by combined treatment with MDL-100907.

There was a significant effect of treatment on ε [$F(3,39)$, $P<0.001$]. Multiple comparisons showed that only DOI + SKF-83566 significantly reduced ε. There was also a significant effect of treatment on the Weber fraction [$F(3,39)=15.9$, $P<0.001$]. Multiple comparisons showed that DOI + SKF-83566 produced a significant increase in the Weber fraction compared to the vehicle-alone treatment.

There was a significant effect of treatment on switching time [$F(3,39)=10.2$, $P<0.001$; Table 4]. DOI alone and DOI + SKF-82566 significantly reduced switching time compared to the vehicle-alone treatment. MDL-100907, but not SKF-83566, significantly attenuated the effect of DOI on switching time.

The overall response rates under each treatment condition are shown in Table 4. There was a significant effect of treatment [$F(3,39)=13.7$, $P<0.001$]. Multiple comparisons showed that DOI alone and DOI + SKF-83566 significantly decreased response rate compared to the vehicle-alone treatment. Response rate after combined treatment with DOI + MDL-100907 was not significantly different from the vehicle-alone condition or the DOI-alone condition, indicating a partial reversal of the DOI-induced reduction of response rate by MDL-100907. DOI + SKF-83566 reduced overall response rate significantly more than DOI alone.

Table 4 Interaction between DOI, MDL-100907, and SKF-83566 on quantitative timing parameters and overall response rate (mean±SEM)

Treatment	Parameters of psychometric function					Overall response rate (responses min^{-1})
	T_{50} (s)	ε	p^2	Weber fraction	Mean switching time (s)	
Vehicle	21.1±0.9	−4.64±0.30	0.99±0.00	0.26±0.02	18.3±0.8	47.7±6.2
DOI 0.25 mg kg^{-1}	18.1±1.2[a]	−4.73±0.62	0.97±0.00	0.27±0.03	15.8±0.9[a]	39.7±4.2[a]
DOI 0.25 mg kg^{-1}+MDL-100907 0.5 mg kg^{-1}	21.2±1.1[b]	−4.33±0.29	0.97±0.01	0.28±0.03	18.9±0.9[b]	43.5±5.3
DOI 0.25 mg kg^{-1}+SKF-83566 0.003 mg kg^{-1}	18.1±1.3[a]	−2.86±0.29[a]	0.90±0.01	0.46±0.05[a]	14.1±1.2[a]	26.1±3.4[ab]

[a] Significantly different from vehicle
[b] Significantly different from DOI alone ($P<0.05$)

Discussion

In agreement with previous findings with Stubbs' free-operant psychophysical procedure (Stubbs 1976; Bizo and White 1994a,b; Chiang et al. 1998; Machado and Guilhardi 2000), response rate on lever A declined, while response rate on lever B increased, as a function of time from trial onset, this being reflected in an increasing percentage of total responding being devoted to lever B (%*B*) as the trial progressed. The schedule employed in these experiments was the "constrained-switching" version of the free-operant psychophysical procedure, in which the first response on lever B in each trial results in the removal of lever A from the operant chamber; this has the advantage of precluding repetitive switching between the levers (Chiang et al. 1998). This version of the schedule results in more precise temporal differentiation (steeper psychometric functions and lower Weber fractions) than the conventional ("unconstrained switching") version, in which the subject is able freely to switch back and forth between the two levers throughout the trial (Chiang et al. 1998–2000a,b).

The aim of these experiments was to compare the effects of a selective 5-HT_{2A} receptor antagonist MDL-100907, a D_1 dopamine receptor antagonist SKF-83566, and a D_2 dopamine receptor antagonist haloperidol, on the changes in free-operant timing performance induced by the 5-HT_2 receptor agonist DOI and the dopamine-releasing agent *d*-amphetamine. The present experiments did not examine the effects of the antagonists administered alone. However, in previous experiments we have found that these antagonists, in the same doses as were used in the present experiments, did not significantly affect timing performance on the free-operant psychophysical procedure (Body et al. 2006; Cheung et al. 2006).

In agreement with previous findings with this schedule (Body et al. 2003, 2004, 2006), DOI reduced T_{50}. DOI has previously been shown to produce a dose-dependent effect on T_{50} within the range of 0.0625–0.25 mg kg^{-1} (Body et al. 2003). In agreement with previous findings (Body et al. 2006), DOI's effect on T_{50} was attenuated by co-administration of the selective 5-HT_{2A} receptor antagonist MDL-100907. The dose of MDL-100907 used in this experiment (0.5 mg kg^{-1}) was the same as that which has been found to be effective in reversing the effect of DOI in other behavioral experiments, including previous experiments on timing performance (Body et al. 2006; Asgari et al. 2006). In contrast, the effect of DOI was not significantly altered by the dopamine receptor antagonists haloperidol (0.05 mg kg^{-1}) and SKF-83566 (0.03 mg kg^{-1}). MDL-100907 is a highly selective 5-HT_{2A} receptor antagonist with minimal affinity for the other 5-HT_2 receptor subtypes (Sorenson et al. 1993; Kehne et al. 1996; Johnson et al. 1996). Thus, results from the present experiments indicate that DOI's effect on performance on the free-operant psychophysical procedure is mediated principally by the stimulation of 5-HT_{2A} receptors.

In previous experiments, it was found that DOI's effect on performance in the free-operant psychophysical procedure was not abolished by destruction of the ascending 5-HTergic pathways, suggesting that the effect is mediated by a postsynaptic receptor population (Body et al. 2004). Interestingly, destruction of the 5-HTergic pathways had little impact on steady-state performance in the free-operant psychophysical procedure (Al-Zahrani et al. 1996; Chiang et al. 1999; Body et al. 2004). This suggests that, while acute 5-HT_{2A} receptor stimulation can significantly disrupt timing performance, the basic mechanisms of free-operant timing do not depend on an intact 5-HTergic projection.

In agreement with previous findings with this schedule (Chiang et al. 2000a), *d*-amphetamine reduced T_{50}. Although only one dose of *d*-amphetamine was tested in the present experiments (0.4 mg kg^{-1}), Chiang et al. (2000a) found that the effect of *d*-amphetamine on T_{50} was dose-dependent within the range of 0.2–0.8 mg kg^{-1}. In agreement with previous findings (Cheung et al. 2006), the effect of *d*-amphetamine on T_{50} was almost completely abolished by the co-administration of SKF-83566 (0.03 mg kg^{-1}), but was not significantly altered by the co-administration of haloperidol (0.05 and 0.1 mg kg^{-1}). *d*-Amphetamine's effect on T_{50} is consistent with many previous reports that dopamine-releasing agents can cause a leftward shift of the psychophysical function in immediate timing schedules, including the fixed-interval peak procedure (Maricq et al. 1981; Meck 1996; Buhusi and Meck 2002; Saulsgiver et al. 2006) and the free-operant psychophysical procedure (Chiang et al. 2000a; Cheung et al. 2006). This effect of psychostimulants has traditionally been attributed to stimulation of D_2 dopamine receptors (Meck 1986, 1996; Buhusi and Meck 2002). However, in the present experiment, as in a previous study (Cheung et al. 2006), the D_2 receptor antagonist haloperidol failed to attenuate the effect of *d*-amphetamine's on T_{50}, whereas the selective D_1 receptor antagonist SKF-83566 fully reversed *d*-amphetamine's effect. It is unlikely that the ineffectiveness of haloperidol in the present experiment was due to the use of inadequate doses, because the same doses have been found to reverse the stimulant effect of *d*-amphetamine on locomotion (Cheung et al. 2006) and other behavioral tests that are believed to entail D_2 receptor stimulation (Spyraki et al. 1982; Velazquez Martinez et al. 1995; Herrera and Velazquez Martinez 1997). SKF-83566 is a selective D_1 receptor antagonist (Molloy et al. 1986; Waddington 1986; O'Boyle et al. 1989; Vieira-Coelho and Soares-da-Silva 2000), whereas haloperidol is a relatively selective D_2 receptor antagonist with approximately 80-fold higher

affinity for D_2 receptors than for D_1 receptors (see Seeman and van Tol 1994). Therefore the results of the present experiments are consistent with the notion that *d*-amphetamine's effect on temporal differentiation in the free-operant psychophysical procedure is mediated principally by the stimulation of D_1 dopamine receptors via endogenous dopamine release (Cheung et al. 2006).

Interestingly, *d*-amphetamine's effect on T_{50} was attenuated by the co-administration of MDL-100907 (0.5 mg kg^{-1}). The finding that a 5-HT_{2A} receptor antagonist can antagonize behavioral effects of psychostimulants is not unprecedented. For example, 5-HT_{2A} receptor antagonists have been found to suppress *d*-amphetamine-induced hyperlocomotion (Sorenson et al. 1993; Kehne et al. 1996; Moser et al. 1996; O'Neill et al. 1999; Broderick et al. 2004) and disruption of latent inhibition (Moser et al. 1996). However, not all behavioral effects of *d*-amphetamine can be reversed by MDL-100907. For example, Moser et al. (1996) found that MDL-100907 did not block *d*-amphetamine's stimulus properties in a drug-discrimination paradigm, nor did it block *d*-amphetamine-induced reduction of rewarding brain stimulation threshold. In the present experiment, MDL-100907 did not reverse *d*-amphetamine's suppressant effect on overall response rate, suggesting that different mechanisms may be involved in *d*-amphetamine's effects on temporal differentiation and operant response rate (see below).

The mechanism underlying MDL-100907's ability to reverse *d*-amphetamine's effect on temporal differentiation is unclear at this stage. In addition to its catecholamine-releasing action, *d*-amphetamine also promotes the release of 5-HT (Raiteri et al. 1975; Ziance 1977; Rothman et al. 2001; Munoz et al. 2003; Alexander et al. 2005), raising the possibility that its effect on T_{50} was mediated by stimulation of 5-HT_{2A} receptors via the release of endogenous 5-HT. However, the ability of SKF-83566 to antagonize the effect of *d*-amphetamine, but not DOI, on T_{50} argues against this possibility. A more plausible explanation may lie in the putative role of 5-HT_{2A} receptors in regulating dopaminergic neurotransmission. 5-HT_{2A} receptors occur on dopaminergic neurones of the VTA and substantia nigra (Nocjar et al. 2002). They are also present in the principal target regions of the dopaminergic pathways, including the prefrontal cortex, and nucleus accumbens (Pazos et al. 1985; Mengod et al. 1990; Morilak et al. 1993; Pompeiano et al. 1994; Nocjar et al. 2002). There is growing evidence that 5-HT_{2A} receptors can regulate dopamine release. 5-HT_{2A} receptor antagonists antagonize the increase in dopamine release induced by cocaine, amphetamine, and 3,4-methylenedioxymethamphetamine (Schmidt et al. 1992; Kehne et al. 1996; Gudelsky et al. 1994; Pehek et al. 2001; Porras et al. 2002; Broderick et al. 2004), and also the increase in dopamine release in the nucleus accumbens induced by electrical stimulation of the dorsal raphe nucleus (De Deurwaerdere and Spampinato 1999). The 5-HT_2 receptor agonist DOI can facilitate dopamine release and increase the activity of dopaminergic neurones, effects which can be antagonized by 5-HT_{2A} receptor antagonists (Gudelsky et al. 1994; Pessia et al. 1994; Gobert and Millan 1999; Lucas and Spampinato 2000; Yan 2000). Interestingly, however, 5-HT_{2A} receptor antagonists alone have generally been found to have little effect on basal dopaminergic function (Schmidt et al. 1992; Sorenson et al. 1993; Schmidt and Fadayel 1996; Di Matteo et al. 1998; De Deurwaerdere and Spampinato 1999; Lucas and Spampinato 2000; Yan 2000; Ichikawa et al. 2001; McMahon et al. 2001; Bonaccorso et al. 2002; Liegeois et al. 2002; Kuroki et al. 2003), although there have been some contradictory reports (Ugedo et al. 1989; Devaud et al. 1992; Schmidt and Fadayel 1995). It seems that 5-HT_{2A} receptor stimulation has a facilitatory effect on dopamine release under "stimulated" conditions but has little effect on basal dopaminergic activity (De Deurwaerdere and Spampinato 1999). This may account for the present finding that MDL-100907 alone did not affect temporal differentiation.

Interestingly, while 5-HT_{2A} receptor antagonists can block some behavioral effects of dopamine-releasing agents (Schmidt et al. 1992; Martin et al. 1997; present results), they generally do not alter the behavioral effects of directly acting D_1 receptor agonists (Molloy and Waddington 1987; O'Neill et al. 1999; Scalzitti et al. 1999). It will be of interest, in future experiments, to examine whether *d*-amphetamine's effects on temporal differentiation can be mimicked by directly acting D_1 receptor agonists, and whether MDL-100907 and other 5-HT_{2A} receptor antagonists can discriminate between the effects of direct and indirect D_1 receptor stimulation.

DOI and *d*-amphetamine had relatively little effect on the Weber fraction in these experiments. Although both drugs tended to increase the fraction, suggesting some reduction of the precision of temporal differentiation (see Ho et al. 2002), this was not statistically significant in either case. Previous experiments have yielded mixed findings on the effects of these drugs on the Weber fraction, although substantial increases have been seen in some studies (DOI, Body et al. 2006; *d*-amphetamine, Chiang et al. 2000a). *d*-Amphetamine's effect on the Weber fraction appeared to be reversed by SKF-83566. Interestingly, however, the combination of DOI and SKF-83566 produced a marked increase in the Weber fraction, although the antagonist did not alter DOI's effect on T_{50}. Since the Weber fraction is an index of the precision of temporal differentiation, this observation suggests that the combination of 5-HT_{2A} receptor stimulation and D_1 dopamine receptor blockade produced a general disruption of temporal control over and

above the effect 5-HT_{2A} receptor stimulation on the locus of the psychometric function. In contrast to SKF-83566, MDL-100907 had no effect on the Weber fraction, in combination with either DOI or *d*-amphetamine; similarly, Body et al. (2006) found no effect of MDL-100907 on the Weber fraction.

DOI reduced the overall rate of responding on this timing schedule, as reported before (Body et al. 2003, 2004, 2006). In a previous experiment, MDL-100907 completely reversed DOI's effect on response rate (Body et al. 2006); however, in the present experiment only partial antagonism was seen. The reason for the discrepancy between the results of the two experiments is not clear; however, both results suggest that DOI's effect on response rate is at least partially mediated by 5-HT_{2A} receptors. Neither haloperidol nor SKF-83566 attenuated DOI's effect on response rate; indeed the combination of DOI + SKF-83566 actually potentiated the effect of DOI. SKF-83566 has previously been found to reduce overall response rate when given alone, albeit at a higher dose than that used in the present experiment (Cheung et al. 2006). It is possible that the effect of the combined drug treatment reflects an accumulation of the suppressant effect of DOI and a subthreshold suppressant effect of SKF-83566 on the response rate.

d-Amphetamine also reduced the overall rate of responding on this timing schedule, as has been noted before (Chiang et al. 2000a; Cheung et al. 2006). This effect is unlikely to reflect motor debilitation, because *d*-amphetamine in the same dose range as that used in these experiments produces marked increases in locomotor behavior (e.g., Cheung et al. 2006). The effect of *d*-amphetamine on the response rate seen in these experiments was presumably mediated by D_1 rather than D_2 dopamine receptors, as it was reversed by SKF-83566 but not by haloperidol. MDL-100907 had no effect on the reduction of overall responding induced by *d*-amphetamine. The finding that MDL-100907 reversed *d*-amphetamine's effect on T_{50} but not it its effect on response rate suggests that different mechanisms may underlie these two effects of *d*-amphetamine, 5-HT_{2A} receptors contributing to the former effect, but not to the latter. The finding of a dissociation between the effects of *d*-amphetamine on timing parameters and response rate is consistent with a growing body of evidence showing that drug-induced changes in timing parameters are not secondary to changes in the response rate (Chiang et al. 2000a; Body et al. 2003, 2004; Cheung et al. 2006).

In conclusion, the present studies are in agreement with previous evidence that DOI's effect on temporal differentiation in the free-operant psychophysical procedure is mediated by 5-HT_{2A} receptors (Body et al. 2003, 2004, 2006). *d*-Amphetamine's effect on temporal differentiation appears to be mediated not only by D_1 dopamine receptor stimulation, as indicated by previous experiments (Cheung et al. 2006), but also by an interaction with 5-HT_{2A} receptors. The exact nature of this interaction remains uncertain; however, the present results are consistent with existing evidence that 5-HT_{2A} receptors have a phasic facilitatory effect on dopaminergic function (Schmidt et al. 1992; Sorenson et al. 1993; De Deurwaerdere and Spampinato 1999).

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