ORIGINAL INVESTIGATION

Attenuation of the effects of *d*-amphetamine on interval timing behavior by central 5-hydroxytryptamine depletion

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Abstract

Rationale Interval timing in the free-operant psychophysical procedure is sensitive to the monoamine-releasing agent *d*-amphetamine, the D_2-like dopamine receptor agonist quinpirole, and the D_1-like agonist 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1*H*-3-benzepine (SKF-81297). The effect of *d*-amphetamine can be antagonized by selective D_1-like and $5-HT_{2A}$ receptor antagonists. It is not known whether *d*-amphetamine's effect requires an intact 5-hydroxytryptamine (5-HT) pathway.

Objective The objective of this study was to examine the effects of *d*-amphetamine, quinpirole, and SKF-81297 on timing in intact rats and rats whose 5-hydroxytraminergic (5-HTergic) pathways had been ablated.

Materials and methods Rats were trained under the free-operant psychophysical procedure to press 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 for derivation of timing indices (T_{50}, time corresponding to %B=50%; Weber fraction). The effects of *d*-amphetamine (0.4 mg kg^{-1} i.p.), quinpirole (0.08 mg kg^{-1} i.p.), and SKF-81297 (0.4 mg kg^{-1} s.c.) were compared between intact rats and rats whose 5-HTergic pathways had been destroyed by intra-raphe injection of 5,7-dihydroxytryptamine.

Results Quinpirole and SKF-81297 reduced T_{50} in both groups; *d*-amphetamine reduced T_{50} only in the sham-lesioned group. The lesion reduced 5-HT levels by 80%; catecholamine levels were not affected.

Conclusions *d*-Amphetamine's effect on performance in the free-operant psychophysical procedure requires an intact 5-HTergic system. 5-HT, possibly acting at $5-HT_{2A}$ receptors, may play a 'permissive' role in dopamine release.

Keywords Timing · Free-operant psychophysical procedure · D_1-like dopamine receptors · D_2-like dopamine receptors · 5-HTergic pathways · 5,7-dihydroxytryptamine · *d*-Amphetamine · SKF-81297 · Quinpirole

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Introduction

Interval timing behavior in animals can be divided into two broad categories—temporal discrimination, where an animal learns to emit different responses depending on the duration of an external stimulus (e.g., a light or tone), and temporal differentiation, where the animal's behavior comes under the control of time during an ongoing interval (Killeen and Fetterman 1988; Killeen et al. 1997). This paper is concerned with the psychopharmacology of temporal differentiation.

Temporal differentiation is revealed by immediate timing schedules, an example of which 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 performance 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 substantial body of evidence for the involvement of central dopaminergic mechanisms in the control of interval timing behavior (see Meck 1996, 2006; Gibbon et al. 1997; Hinton and Meck 1997; Meck and Benson 2002; MacDonald and Meck 2004;). In the free-operant psychophysical procedure, the psychostimulant d-amphetamine, which is known to release dopamine, produces a leftward shift in the psychophysical function, which is reflected as a reduction of T_{50} (Chiang et al. 2000a; Cheung et al. 2006; Body et al. 2006b). The effects of d-amphetamine and other dopamine-releasing agents have generally been attributed to the stimulation of postsynaptic D_2 dopamine receptors (Meck 1986, 1996).[1] Consistent with this hypothesis, it has been found that the D_2 dopamine receptor agonist quinpirole can reduce T_{50} in the free-operant psychophysical procedure (Cheung et al. 2007a). However, it has recently been demonstrated that the selective D_1 dopamine receptor agonist 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine (SKF-81927) has a qualitatively similar effect on performance in the free-operant psychophysical procedure to that of d-amphetamine (Cheung et al. 2007b). Moreover, the reduction of T_{50} produced by d-amphetamine can be antagonized by a selective D_1 dopamine receptor antagonist 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SKF-83566), but not by the D_2 receptor antagonist haloperidol (Cheung et al. 2007b), indicating an involvement of D_1 dopamine receptors in d-amphetamine's effects on timing behavior in this schedule.

Manipulation of the 5-hydroxytryptaminergic (5-HTergic) system has also been shown to influence timing performance in the free-operant psychophysical procedure. Administration of the 5-HT$_{2A/2C}$ receptor agonist DOI produces a leftward shift in the psychophysical curve, which can be attenuated by the 5-HT$_{2A}$ receptor antagonists ketanserin and MDL-100907 (Body et al. 2003, 2006a). Interestingly, the highly selective 5-HT$_{2A}$ receptor antagonist MDL-100907 is also able to attenuate the effect of d-amphetamine on timing performance in this schedule, suggesting a functional interaction between D_1 dopamine receptors and 5-HT$_{2A}$ receptors (Body et al. 2006b).

Displacement of the psychometric function in interval timing schedules is not the only behavioral effect of d-amphetamine that has been shown to depend upon 5-HTergic mechanisms. For example, 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 d-amphetamine-induced disruption of latent inhibition (Moser et al. 1996). Forebrain 5-HT depletion has been found to attenuate some of d-amphetamine's behavioral effects, including the disruption of performance in the five-choice serial reaction time task and the reduction of tolerance of delay of reinforcement (Harrison et al. 1997; Winstanley et al. 2003). However, some behavioral effects of d-amphetamine appear to be impervious to central 5-HT depletion; for example, Fletcher et al. (1999) found no effect of 5-HT depletion on the reinforcing effect of d-amphetamine using a variety of self-administration schedules. It is not known whether central 5-HT depletion alters the ability of d-amphetamine to displace the psychometric timing function.

The present experiment examined whether d-amphetamine's effect on temporal differentiation performance in the free-operant psychophysical procedure depends upon an intact 5-HTergic system. The effect of d-amphetamine on temporal differentiation was compared between intact rats and rats whose ascending 5-HTergic pathways had been ablated by injection of the selective neurotoxin 5,7-dihydroxytryptamine into the dorsal and median raphe nuclei. In addition to d-amphetamine, an 'indirect' dopamine receptor agonist, we also examined the effects of a 'direct' D_1 dopamine receptor agonist, SKF-81297, and a 'direct' D_2 dopamine receptor agonist, quinpirole.

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[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 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 D_2-*like* receptor agonists and antagonists (Alexander et al. 2008). Throughout this paper, for the sake of simplicity, they are referred to as D_1 and D_2 receptor agonists and antagonists.

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

Twenty-four female Wistar rats aged approximately 4 months and weighing 250–300 g at the start of the experiment were used. They were housed individually under a constant cycle of 12 h light and 12 h darkness (lights on at 0700–1900 h) and were maintained at 80% of their initial free-feeding body weights by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cage.

Surgery

The rats received either lesions of the dorsal and median raphe nuclei ($n=11$) or sham lesions ($n=13$). Our methods for the surgical preparation of the rats have been described in detail elsewhere (Wogar et al. 1992). Rats were anesthetized with 4% isoflurane in oxygen and placed in a stereotaxic apparatus; anesthesia was maintained with 2% isoflurane in oxygen during the surgery. The following stereotaxic coordinates (Paxinos and Watson 1998) were used to locate the median and dorsal raphe nuclei: AP +1.2, L, 0.0, DV +1.5 (dorsal) +3.5 (median), measured from the interaural line, with the incisor bar fixed 3.3 mm below the interaural line. The neurotoxin 5,7-dihydroxytryptamine hydrobromide (5,7-DHT; Sigma-Aldrich, Poole, UK) was injected into the median and dorsal raphe nuclei of the rats in the lesioned group; the control group received sham injections into the same areas. The dose of 5,7-DHT injected into the dorsal and median raphe nuclei was 4 μg base dissolved in 1 μl vehicle (0.9%, *w/v* sodium chloride solution with 0.1% ascorbic acid) in each case. The injection rate was 0.1 μl per 15 s, and the cannula was left in position for a further 3 min after the completion of the injection in each site.

Apparatus

The rats were trained in operant conditioning chambers (Campden Instruments Limited, Sileby, UK) of internal dimensions 20 cm×23 cm×22.5 cm. 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. Twelve chambers were used, and each rat was always tested in the same chamber. An Acorn microcomputer, programmed in Arachnid BASIC (CeNeS Ltd, Cambridge, UK), located in an adjoining room, controlled the schedules and recorded the behavioral data.

Behavioral training

Two weeks after surgery, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers and were exposed to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence, for three sessions. Thereafter, the rats underwent 50 min training sessions under the free-operant psychophysical procedure, 7 days a week, at the same time each day during the light phase of the daily cycle (between 0700 and 1400 h). 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. During the first 25 s of the trial, reinforcers were delivered only for responses on lever A, whereas during the last 25 s, reinforcers were delivered only for responses on lever B. The positions of lever A and lever B (left versus right) were counterbalanced across subjects. Four of the 50 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 two levers was restricted to one switch per trial: in each trial, the first response on lever B triggered 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. Injections of drugs were given on Tuesdays and Fridays and injections of 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, in a pseudorandom sequence. Subcutaneous (s.c.) injections were given using a 26-gauge needle at a volume of 1.0 ml kg^{-1}; intraperitoneal

(i.p.) injections were given using a 25-gauge needle at a volume of 2.5 ml kg^{-1}.

(−)-Quinpirole hydrochloride 0.08 mg kg^{-1}, d-amphetamine sulfate 0.4 mg kg^{-1}, and 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine HBr (SKF-81297) 0.8 mg kg^{-1} were dissolved in 0.9% NaCl solution. Quinpirole and d-amphetamine were injected i.p. and SKF-81297 s.c. All injections were given 15 min before the start of the experimental session.

The doses of the drugs were selected on the basis of previous studies which had shown that these doses produced approximately equivalent effects on the timing performance of rats trained under the free-operant psychophysical procedure (see "Discussion" for references). Quinpirole and SKF-81297 were obtained from Tocris Cookson (Avonmouth, UK); d-amphetamine was obtained from Sigma (Poole, UK).

Biochemical assays

At the end of the experiment, the rats were killed, their brains removed, and the following areas were dissected out on ice: parietal cortex, hippocampus, amygdala, nucleus accumbens, and hypothalamus. The concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline, and dopamine in each region were measured by high-performance liquid chromatography combined with electrochemical detection. The method was as described by Wogar et al. (1992), with the exception that the mobile phase for the indoleamine assay was 0.1 M sodium citrate buffered to pH 2.45 containing 75% (w/v) acetonitrile and 1 mM octanesulfonic acid (ion pair agent); N-ω-methyl-5-hydroxytryptamine was used as the internal standard in the indoleamine assay. The five brain regions included areas principally innervated by the median raphe nucleus (hippocampus), the dorsal raphe nucleus (nucleus accumbens), and by both nuclei (amygdala and neocortex; Imai et al. 1986).

Data analysis

Only the data collected from the probe trials were used in the analysis. The effects of the three drugs were analyzed separately. For each drug, comparisons were made between the data collected in the drug treatment sessions and the data collected in the immediately preceding sessions in which the vehicle alone was administered.

Relative response rate 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. The data were analyzed by three-factor analyses of variance (group [sham lesion, 5,7-DHT lesion] ×

treatment [drug, vehicle] × time-bin) with repeated measures on the second and third 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 functions). The curve-fitting procedure yields estimates of the values of T_{50} and ε, 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 %B$=25$%), and the Weber fraction was defined as limen/T_{50}. Goodness of fit of the functions was expressed as the index of determination, p^2. The values of T_{50}, ε, and the Weber fraction derived from the individual subjects were analyzed using two-factor analyses of variance (group [sham lesion, 5,7-DHT lesion] × treatment [drug, vehicle]) with repeated measures on the latter factor.

Overall response rate Overall response rate, calculated for each rat under each treatment condition, was analyzed in the same way as the parameter values (see above).

A significance criterion of $P<0.05$ was used in all analyses.

Results

In both groups, under all treatment conditions, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, and the proportion of responding allocated to lever B (%B) increased progressively as a function of time from trial onset. Logistic psychometric functions provided a good description of the data, the mean values of p^2 being >0.88 under all treatment conditions in both groups (Table 1–3).

Quinpirole

The relative response rate (%B) data are shown in Fig. 1. Quinpirole produced a leftward displacement of the timing function in both groups. Analysis of variance revealed significant main effects of treatment [$F(1, 22)=12.5$, $P<0.01$] and time-bin [$F(9, 198)=322.2$, $P<0.001$] and a significant treatment × time-bin interaction [$F(9, 198)=5.8$, $P<0.001$]. There was no significant main effect of group, nor any significant interaction involving the group factor [all Fs<1].

The group mean values of the parameters of the psychometric functions and overall response rate (±SEM)

Table 1 Effect of quinpirole on quantitative timing parameters and overall response rate (mean ± SEM)

Parameter	Sham lesion		5,7-DHT lesion	
	Vehicle	Quinpirole	Vehicle	Quinpirole
Indifference point, T_{50} (s)	14.7±1.8	9.6±1.0*	14.5±1.5	10.7±0.8*
Slope, ε	−4.3±0.4	−3.9±0.9*	−5.2±0.6	−3.1±0.3*
Weber fraction	0.31±0.05	0.40±0.05*	0.25±0.03	0.40±0.04*
p^2	0.97±0.02	0.98±0.01	0.98±0.01	0.98±0.01
Overall response rate (responses min^{-1})	47.0±7.8	29.2±4.2*	48.0±5.4	31.1±3.0*

*$P<0.05$, significant difference from vehicle-alone condition

are shown in Table 1 and the effects of quinpirole on each variable (drug − vehicle differences) in the two groups are shown in Fig. 2.

Indifference point, T_{50} Quinpirole reduced T_{50} in both groups. There was a significant main effect of treatment [$F(1, 22)=14.8$, $P<0.001$]; there was no significant effect of group and no significant group × treatment interaction [Fs<1].

Slope, ε Quinpirole increased ε in both groups (i.e., ε was less strongly negative under the drug treatment condition than under the vehicle condition). The main effect of treatment was significant [$F(1, 22)=5.1$, $P<0.05$], but there was no significant group effect [$F<1$] and no significant interaction [$F(1, 22)=2.1$, NS].

Weber fraction Quinpirole increased the Weber fraction in both groups. The main effect of treatment was significant

[$F(1, 22)=9.4$, $P<0.01$], but there was no significant group effect and no significant interaction [Fs<1].

Overall response rate Quinpirole reduced overall response rate in both groups. The effect of treatment was significant [$F(1, 22)=38.9$, $P<0.001$], but there was no significant group effect and no significant interaction [Fs<1].

d-Amphetamine

The relative response rate (%B) data are shown in Fig. 3. *d*-Amphetamine produced a leftward displacement on the timing function in the sham-lesioned group, but not in the 5,7-DHT-lesioned groups. Analysis of variance revealed significant main effects of treatment [$F(1, 22)=4.9$, $P<0.05$] and time-bin [$F(9, 198)=278.1$, $P<0.001$] and a significant treatment × time-bin interaction [$F(9, 198)=7.2$, $P<0.001$]. There was no significant main effect of group,
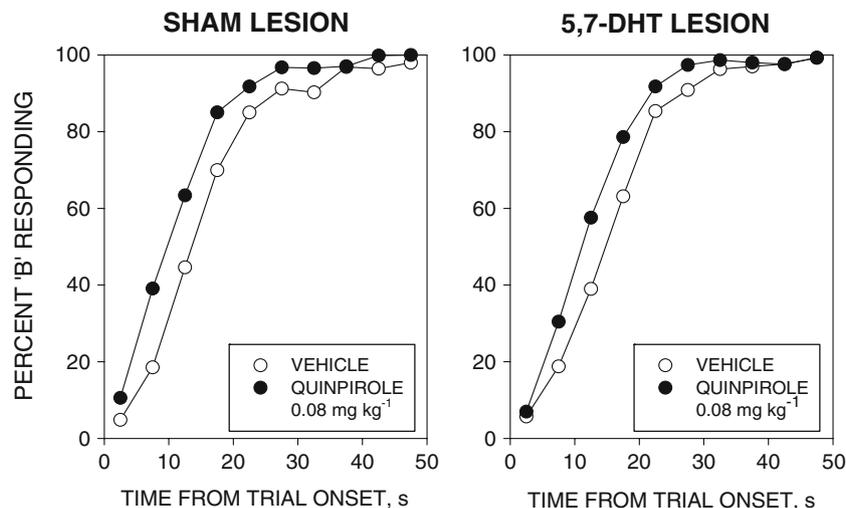

Fig. 1 Effects of quinpirole 0.08 mg kg^{-1} on performance in the free-operant psychophysical procedure. *Left-hand graph*, data from intact (sham-lesioned) rats; *right-hand graph*, data from rats whose ascending 5-HTergic pathways had been destroyed by intra-raphe nucleus injection of 5,7-dihydroxytryptamine (*5,7-DHT*). *Ordinates*, percent responding on lever B; *abscissae*, time from trial onset (s). *Points* show group mean data under each treatment condition (vehicle: *white circles*; quinpirole: *black circles*). Quinpirole displaced the psychometric function to the left in both groups

Fig. 2 Effects of quinpirole 0.08 mg kg^{-1} on the indifference time (T_{50}), the slope of the psychometric function (ε), and the Weber fraction derived from the psychometric functions and the overall response rates for the individual rats in the sham-lesioned group (*white columns*) and the 5,7-dihydroxytryptamine-lesioned group (*black columns*). *Bars* are group means + SEM. Quinpirole reduced T_{50}, increased ε and the Weber fraction, and reduced overall response rate in both groups. There were no significant differences between the two groups
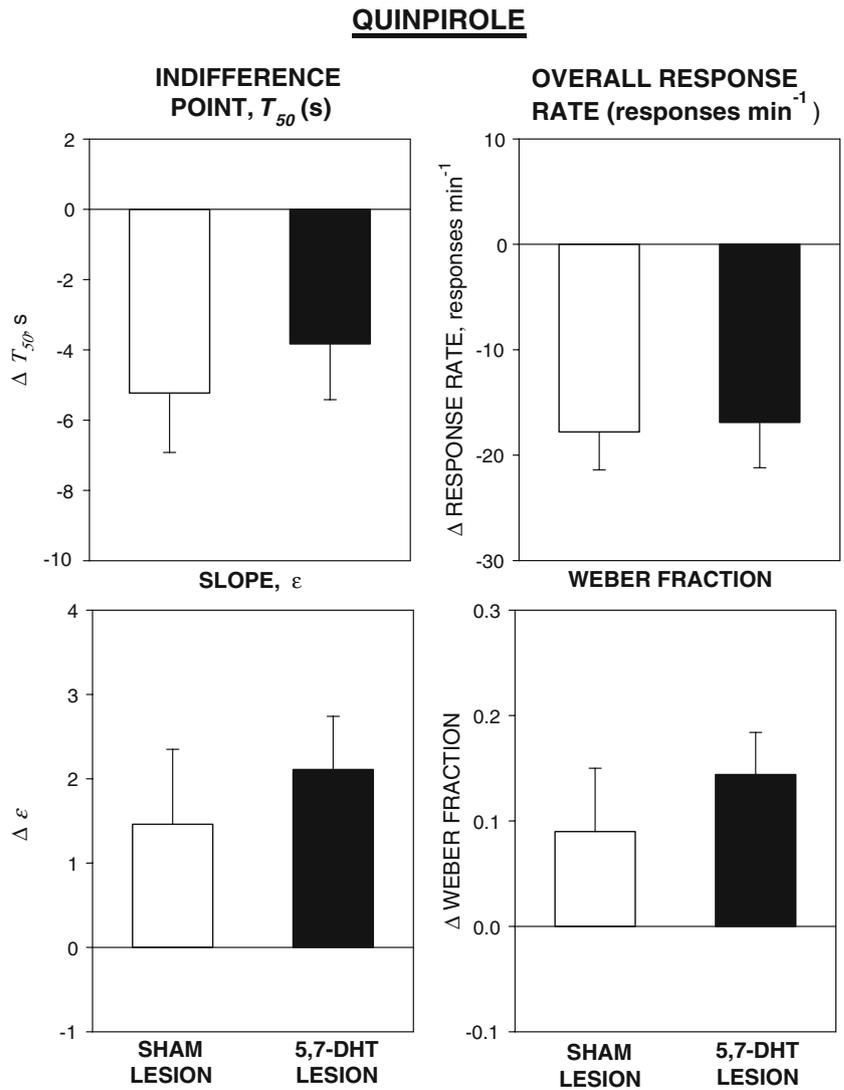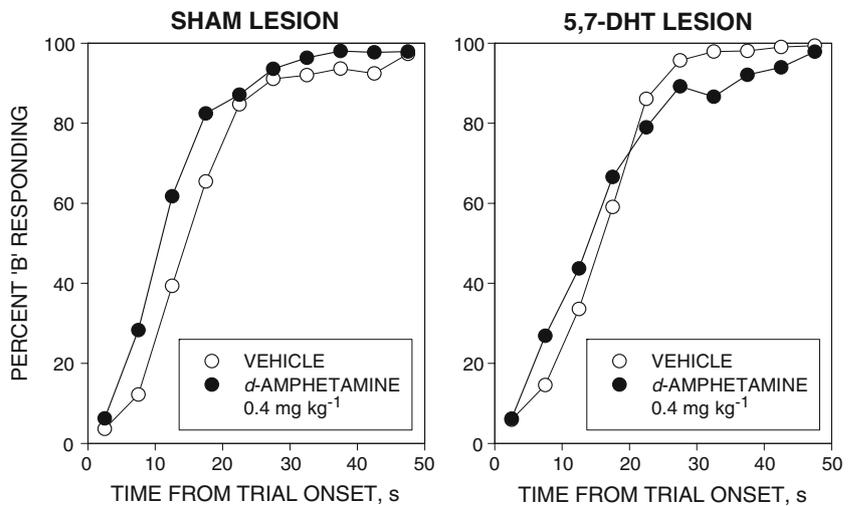

QUINPIROLE

Fig. 3 Effects of *d*-amphetamine 0.4 mg kg^{-1} on performance in the free-operant psychophysical procedure (conventions as in Fig. 1). *d*-Amphetamine displaced the psychometric function to the left in the sham-lesioned group. This effect was attenuated in the 5,7-DHT-lesioned group

but the group × treatment interaction was significant [$F(1, 22)=$ 5.3, $P<0.05$]. The group × time-bin [$F(9, 198)=1.6$, NS] and the three-way interaction [$F<1$] were not statistically significant.

The group mean values of the parameters of the psychometric functions and overall response rate (±SEM) are shown in Table 2 and the effects of d-amphetamine on each variable (drug – vehicle differences) in the two groups are shown in Fig. 4.

Indifference point, T_{50} d-Amphetamine reduced T_{50} in the sham-lesioned group; this effect was considerably attenuated in the 5,7-DHT-lesioned group. This was reflected in the analysis of variance, which showed a significant main effect of treatment [$F(1, 22)=11.8$, $P<0.01$] and a significant group × treatment interaction [$F(1, 22)=4.8$, $P<0.05$]. The main effect of group was not significant [$F<1$].

Slope, ε d-Amphetamine did not significantly alter the value of ε. There was no significant main effect of treatment [$F(1, 22)=3.7$, NS] or group [$F<1$], and no significant interaction [$F<1$].

Weber fraction d-Amphetamine did not significantly alter the Weber fraction. There was no significant main effect of treatment [$F(1, 22)=4.0$, NS] or group [$F<1$], and no significant interaction [$F<1$].

Overall response rate d-Amphetamine reduced overall response rate in both groups. The effect of treatment was significant [$F(1, 22)=38.0$, $P<0.001$], but there was no significant group effect and no significant interaction [Fs<1].

SKF-81297

The relative response rate (%B) data are shown in Fig. 5. SKF-81297 produced a leftward displacement on the timing

function in both groups. Analysis of variance revealed significant main effects of treatment [$F(1, 22)=20.0$, $P<0.001$] and time-bin [$F(9, 198)=307.7$, $P<0.001$] and a significant treatment × time-bin interaction [$F(9, 198)=11.4$, $P<0.001$]. There was no significant main effect of group, nor any significant interaction involving the group factor [all Fs<1].

The group mean values of the parameters of the psychometric functions and overall response rate (±SEM) are shown in Table 3 and the effects of SKF-81297 on each variable (drug − vehicle differences) in the two groups are shown in Fig. 6.

Indifference point, T_{50} SKF-81297 reduced T_{50} in both groups. There was a significant main effect of treatment [$F(1, 22)=21.6$, $P<0.001$]; there was no significant effect of group and no significant group × treatment interaction [Fs<1].

Slope, ε SKF-81297 increased ε in both groups. The main effect of treatment was significant [$F(1, 22)=10.4$, $P<0.01$], but there was no significant group effect and no significant interaction [Fs<1].

Weber fraction SKF-81297 increased the Weber fraction in both groups. The main effect of treatment was significant [$F(1, 22)=14.4$, $P<0.01$], but there was no significant group effect and no significant interaction [Fs<1].

Overall response rate SKF-81297 reduced overall response rate in both groups. The effect of treatment was significant [$F(1, 22)=64.4$, $P<0.001$], but there was no significant group effect and no significant interaction [Fs<1].

Biochemical data

Table 4 shows the concentrations of 5-HT and 5-HIAA, noradrenaline, and dopamine in the brain areas of the rats belonging to the two groups. The levels of 5-HT and 5-

Table 2 Effect of d-amphetamine on quantitative timing parameters and overall response rate (mean ± SEM)

Parameter	Sham lesion		5,7-DHT lesion	
	Vehicle	d-Amphetamine	Vehicle	d-Amphetamine
Indifference point, T_{50} (s)	15.9±1.9	11.4±1.3*	14.8±1.2	13.8±1.5
Slope, ε	−5.0±0.6	−4.0±0.4	−4.7±0.6	−3.6±0.7
Weber fraction	0.28±0.05	0.34±0.06	0.28±0.04	0.46±0.09
p^2	0.97±0.01	0.94±0.04	0.98±0.01	0.94±0.02
Overall response rate (responses min^{-1})	47.3±6.8	32.5±7.3*	47.0±5.5	27.8±4.7*

*$P<0.05$, significant difference from vehicle-alone condition

Fig. 4 Effects of *d*-amphetamine 0.4 mg kg^{-1} on the indifference time (T_{50}), the slope of the psychometric function (ε), and the Weber fraction derived from the psychometric functions and overall response rate (conventions as in Fig. 2). *d*-Amphetamine reduced T_{50} in the sham-lesioned group; this effect was significantly attenuated in the 5,7-DHT-lesioned group (significance of between group difference, *$P < 0.05$). Response rate was reduced in both groups
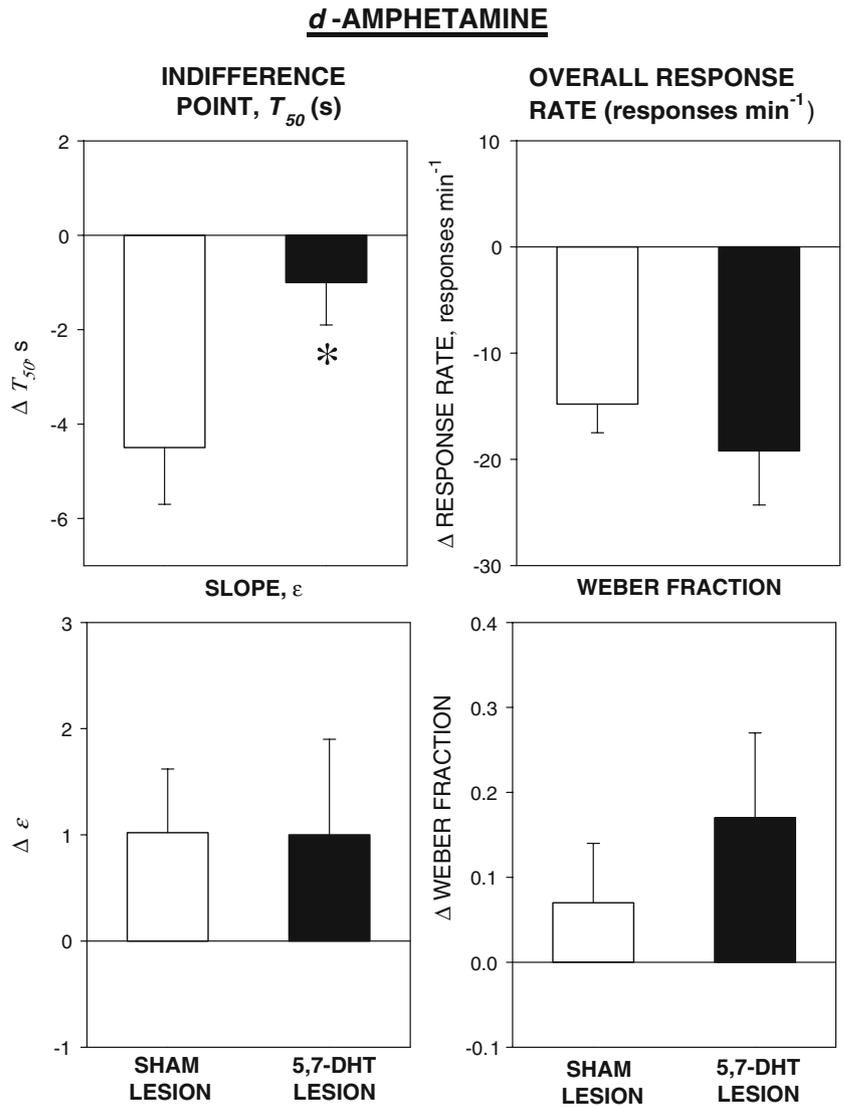
Fig. 5 Effects of SKF-81297 0.8 mg kg^{-1} on performance in the free-operant psychophysical procedure (conventions as in Fig. 1). SKF-81297 displaced the psychometric function to the left in both groups
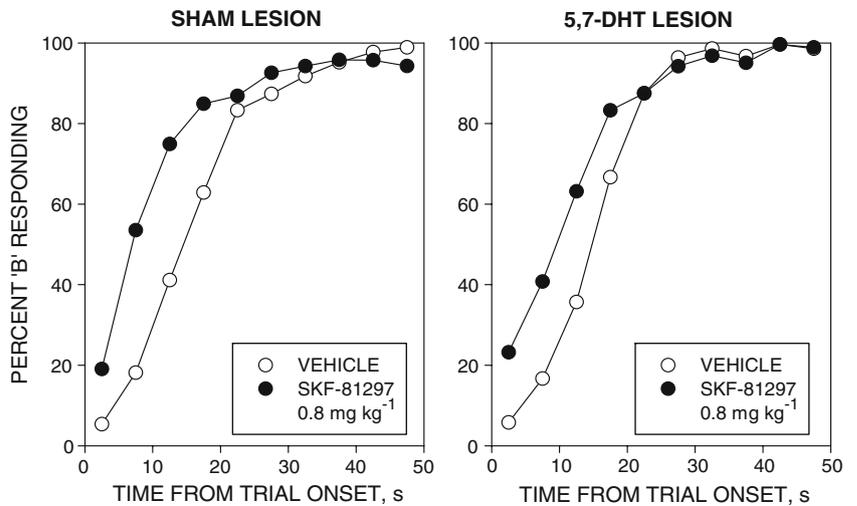
Table 3 Effect of SKF-81297 on quantitative timing parameters and overall response rate (mean ± SEM)

Parameter	Sham lesion		5,7-DHT lesion	
	Vehicle	SKF-81297	Vehicle	SKF-81297
Indifference point, T_{50} (s)	14.8±2.0	7.8±1.2*	14.1±1.1	9.0±1.4*
Slope, ε	−4.3±0.5	−3.1±0.5*	−4.6±0.5	−2.6±0.4*
Weber fraction	0.33±0.06	0.51±0.09*	0.28±0.04	0.56±0.07*
p^2	0.98±0.06	0.88±0.03	0.98±0.01	0.89±0.03
Overall response rate (responses min^{-1})	49.3±7.3	15.3±1.9*	47.2±5.6	15.8±1.8*

*$P<0.05$, significant difference from vehicle-alone condition

HIAA in the lesioned group reduced by >80% compared to the sham-lesioned group in all areas assayed [t test: $P<0.001$ in each case]. There was no significant effect of the lesion on the levels of the catecholamines in any brain region examined.

Discussion

In agreement with previous findings with Stubbs' free-operant psychophysical procedure (Stubbs 1976; Bizo and White 1994a, b; Chiang et al. 2000a, b; Machado and

Fig. 6 Effects of SKF-81297 0.8 mg kg^{-1} on the indifference time (T_{50}), the slope of the psychometric function (ε), and the Weber fraction derived from the psychometric functions and overall response rate (conventions as in Fig. 2). SKF-81297 reduced T_{50}, increased ε and the Weber fraction, and reduced overall response rate in both groups. There were no significant differences between the two groups

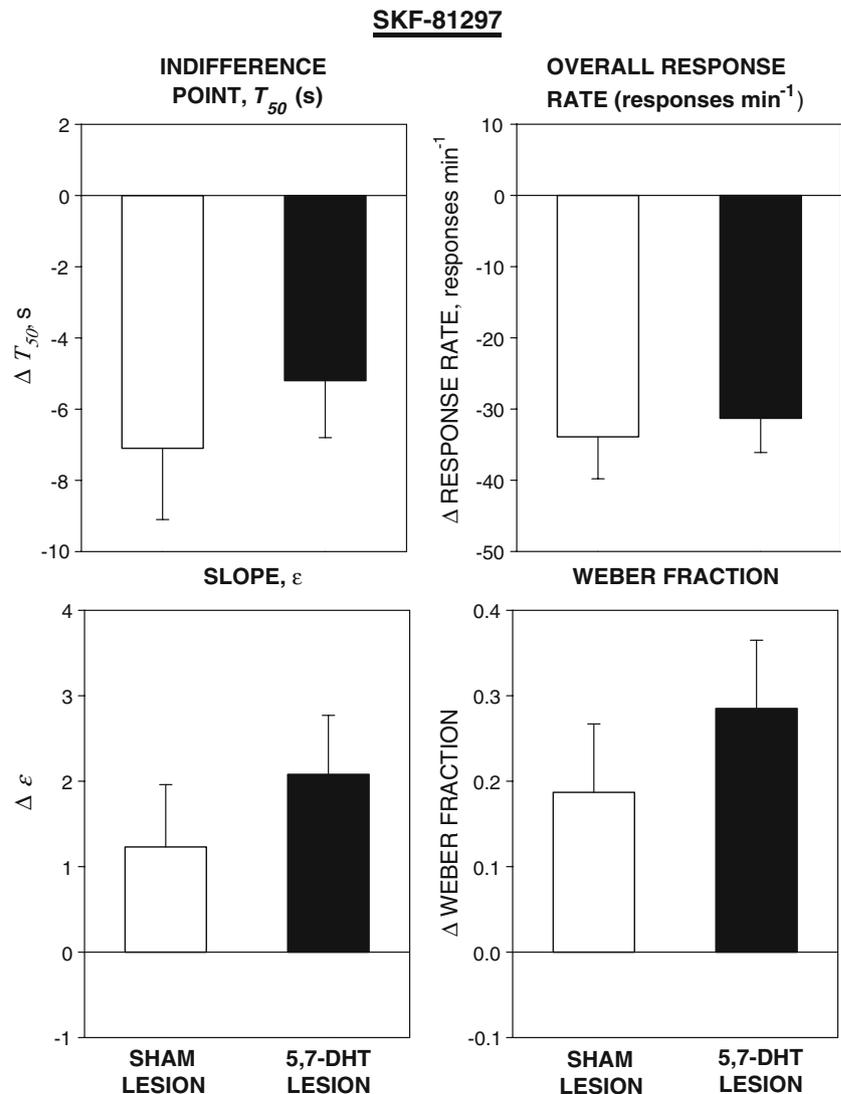**Table 4** Concentrations of monoamines in the brains of the 5,7-DHT-lesioned and sham-lesioned groups

Region	Concentration (ng g^{-1}, wet weight) ±SEM		
	Sham lesion	5,7-DHT lesion	Percent sham
5-HT			
Parietal cortex	235±15	43±10	18.2*
Hippocampus	315±19	43±9	13.7*
Amygdala	635±30	62±10	9.7*
Nucleus accumbens	769±56	63±16	8.2*
Hypothalamus	805±36	105±22	13.1*
Noradrenaline			
Parietal cortex	235±7	214±9	91.3
Hippocampus	331±9	310±9	93.6
Amygdala	435±9	425±11	97.6
Nucleus accumbens	197±19	186±15	94.7
Hypothalamus	1093±88	1149±70	105.1
5-HIAA			
Parietal cortex	190±9	17±5	9.1*
Hippocampus	327±17	27±5	8.1*
Amygdala	503±22	47±11	9.3*
Nucleus accumbens	635±30	48±13	7.5*
Hypothalamus	710±81	100±18	14.1*
Dopamine			
Parietal cortex	193±26	184±23	95.7
Hippocampus	35±4	32±4	89.7
Amygdala	553±36	546±31	98.7
Nucleus accumbens	5072±319	4936±296	97.3
Hypothalamus	367±19	393±16	107.1

*$P<0.001$, significant difference between groups

Guilhardi 2000; Body et al. 2003, 2004, 2006a, b; Cheung et al. 2006, 2007a, b, c), 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 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 to freely switch back and forth between the two levers throughout the trial (Chiang et al. 1998, 1999, 2000a, b). It is noteworthy that in these experiments the values of T_{50} are considerably shorter than the 'transition point' (the point at which reinforcer allocation was changed from lever A to lever B) of 25 s from trial onset. This appears to be a feature of the constrained switching version of this task as

other experiments using this schedule consistently report values of T_{50} that are shorter than the 'transition point' (Chiang et al. 1998, 1999, 2000a, b; Body et al. 2004, 2006a, b; Cheung et al. 2006, 2007a, b, c). The reason for this discrepancy between the value of T_{50} and the 'transition point' in the constrained switching version of the free-operant psychophysical procedure remains unclear. One possible explanation (Ho et al. 1998) is that the first reinforcer delivered for responding on lever B strengthens the act of switching from A to B, resulting in a tendency to switch 'early'; since switching from B to A is not permitted by the schedule, early switching is reflected in a shortening of T_{50}. Although intuitively reasonable, this interpretation fails to account for the finding of Chiang et al. (1998, 1999) that the value of T_{50} was not affected by removal and reinstatement of the constraint on switching, or for the finding by Chiang et al. (1999) and others (Al-Zahrani et al. 1996; Al-Ruwaitea et al. 1999; Body et al. 2001) that central 5-HT depletion markedly facilitates switching on a number of concurrent schedule procedures, including the free-operant psychophysical procedure, without significantly altering the steady-state value of T_{50}.

In both groups, quinpirole and SKF-81297 reduced the value of T_{50}; this is consistent with previous findings with these drugs (Cheung et al. 2007a, b). Quinpirole is a D$_2$-like dopamine receptor agonist with high affinity for D$_2$, D$_3$, and D$_4$ receptors and very little affinity for D$_1$ dopamine receptors or 5-HT receptors (Sokoloff et al. 1990; Van Tol et al. 1991; Levant et al. 1992; Seeman and Van Tol 1994; Tang et al. 1994; Millan et al. 2002; Moreland et al. 2004). Quinpirole's effect on temporal differentiation performance in the free-operant psychophysical procedure has previously been shown to be attenuated by the "D$_2$ receptor-preferring" antagonists haloperidol, eticlopride, and sulpiride, but not by the selective D$_3$ receptor antagonist nafadotride, the selective D$_4$ receptor antagonist L-745870, or the selective D$_1$ receptor antagonist SKF-83566 (Cheung et al. 2007a). SKF-81297 has also been previously shown to decrease the value of T_{50} in this schedule, an effect which is antagonized by the D$_1$ receptor antagonist SKF-83566, but not by haloperidol (Cheung et al. 2007b). The inability of 5-HT depletion to modify the effects of quinpirole and SKF-81297 suggests that the effect of direct stimulation of D$_1$ and D$_2$ dopamine receptors by agonists selective for these receptor subtypes is not dependent on an intact 5-HTergic system.

In the sham-lesioned group, *d*-amphetamine produced a leftward displacement of the psychometric function and a corresponding decrease in T_{50}, consistent with previous findings (Chiang et al. 2000a; Cheung et al. 2006; Body et al. 2006b). This effect of *d*-amphetamine was attenuated by depletion of forebrain 5-HT in the 5,7-dihydroxytryptamine-lesioned group, indicating that an intact 5-HTergic system is

required for d-amphetamine to exert its effects on temporal differentiation (see below).

The doses of the three agonists used in this experiment were selected on the basis of previous experiments with d-amphetamine (Chiang et al. 2000a; Cheung et al. 2006; Body et al. 2006b), quinpirole (Cheung et al. 2007a,c), and SKF-81297 (Cheung et al. 2007b), which indicated that these doses produced approximately equivalent effects on T_{50} in the free-operant psychophysical procedure. Visual inspection of the present data suggests that d-amphetamine had a somewhat smaller effect than the other two agonists (see Figs. 1, 3, and 5). It should be noted that only a single dose of each agonist was employed in this experiment, and therefore it is not possible to state whether the diminished ability of d-amphetamine to reduce T_{50} following 5-HT depletion would have been surmountable with higher doses.

The mechanisms underlying d-amphetamine's effect on temporal differentiation performance in the free-operant psychophysical procedure remain unclear at this time. The present results, together with the evidence reviewed above, indicate that both dopaminergic and 5-HTergic mechanisms are likely to be involved. It is well established that d-amphetamine promotes the release not only of dopamine but also of 5-HT and noradrenaline (Fuxe and Ungerstedt 1970; Kucenski and Segal 1989; Seiden and Sabol 1993; Rothman et al. 2001), raising the possibility that d-amphetamine's effect on T_{50} is brought about by the simultaneous release of dopamine and 5-HT. However, if that were the case, it might be expected that blockade of both D_1 dopamine receptors and 5-HT$_{2A}$ receptors would be needed in order to produce a complete antagonism of d-amphetamine's effect on T_{50}. The fact that d-amphetamine's effect on timing performance could be completely abolished by blockade of either D_1 receptors or 5-HT$_{2A}$ receptors (Body et al. 2006b; Cheung et al. 2006, 2007b) suggests a serial rather than a parallel arrangement of dopaminergic and 5-HTergic mechanisms. One possibility is that 5-HTergic mechanisms may play a "permissive" role in dopamine release. Such a mechanism could account for the loss of d-amphetamine's effect and the survival of quinpirole's and SKF-81297's effects following central 5-HT depletion. Thus, 5-HT depletion may reduce d-amphetamine's ability to release dopamine from presynaptic terminals, whereas it would not be expected to affect the interaction of directly acting agonists with postsynaptic D_1 and D_2 dopamine receptors. The involvement of such a mechanism in the regulation of interval timing is speculative. However, there is evidence that 5-HT$_{2A}$ receptor-mediated facilitation of dopamine release occurs in the striatum and medial prefrontal cortex (Sershen et al. 2000; Pehek et al. 2001; Alex and Pehek 2007).

The lesion employed in the present experiment entailed "global" depletion of 5-HT from the forebrain, and therefore does not provide information about the anatomical location of the 5-HT-dopamine interaction underlying d-amphetamine's effects on interval timing. Further work using local destruction of 5-HTergic terminals in different regions will be needed to address this question. Subregions of the corpus striatum and prefrontal cortex may be suitable initial targets for this endeavor, as these regions have been implicated in the control of interval timing (Gibbon et al. 1997; Meck and Benson 2002; Matell and Meck 2004; Meck 2006).

The present experiment did not seek to identify the species of 5-HT receptor that may be involved in d-amphetamine's effects on timing performance. Previous experiments have shown that 5-HT$_{2A}$ receptor stimulation can displace the psychometric timing function to the left in the free-operant psychophysical procedure and other immediate timing schedules (e.g., Body et al. 2004; Asgari et al. 2006). Our recent finding that d-amphetamine's effect on temporal differentiation could be antagonized by the selective 5-HT$_{2A}$ receptor antagonist MDL-100907 (Body et al. 2006b) is consistent with the notion that 5-HT$_{2A}$ receptor stimulation is an important component of 5-HT–dopamine interactions that may be involved in regulating interval timing behavior. The location of these 5-HT$_{2A}$ receptors is uncertain and requires further investigation. The dorsal striatum is an unlikely candidate, despite its putative key role in interval timing (Gibbon et al. 1997; Meck and Benson 2002; Matell and Meck 2004; Meck 2006), since direct injection of the 5-HT$_{2A/2C}$ receptor agonist DOI into this region was found not to affect timing performance (Body et al. 2006a).

5-HT$_{2A}$ receptors are not the only type of 5-HT receptor that mediate effects on timing performance; 5-HT$_{1A}$ receptor stimulation has been shown to exert a qualitatively similar effect to 5-HT$_{2A}$ receptor stimulation on temporal differentiation in the free-operant psychophysical procedure (Body et al. 2002, 2004) and the fixed-interval peak procedure (Asgari et al. 2006). Interestingly, it has been proposed that 5-HT$_{1A}$ receptors may regulate dopamine release in the prefrontal cortex, and that this may constitute a final common pathway of the action of atypical antipsychotics (Bantick et al. 2001; Ichikawa et al. 2001). The possible involvement of 5-HT$_{1A}$ receptors in d-amphetamine's effect on timing behavior has yet to be investigated.

Both quinpirole and SKF-812997 increased the Weber fraction, indicating a reduction of the precision of temporal differentiation. In neither case was there a differential effect in the two groups. d-Amphetamine appeared to have a qualitatively similar effect, although the change in the Weber fraction was not statistically significant in this case. It is doubtful whether this reflects a true difference between the effects of d-amphetamine and the direct receptor

agonists, as several previous studies have found a statistically significant effect of *d*-amphetamine on the Weber fraction (Chiang et al. 2000a; Cheung et al. 2006, 2007c).

In agreement with previous findings (Body et al. 2006b; Cheung et al. 2006, 2007a, b), *d*-amphetamine, quinpirole, and SKF-81297 all produced significant reductions of overall response rate. Interestingly, *d*-amphetamine's effect on response rate was not affected by 5-HT depletion. This suggests that while *d*-amphetamine's effect on temporal differentiation requires an intact 5-HTergic system, its effect on overall response rate does not. This finding also provides additional support for the claim that different mechanisms are involved in the effects of psychostimulants on timing performance and overall response rate (Chiang et al. 2000a; Odum et al. 2002; Body et al. 2004; Cheung et al. 2007a, b).

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