Psychopharmacology (2000) 150:170–184
Digital Object Identifier (DOI) 10.1007/s002130000422

ORIGINAL INVESTIGATION

T.-J. Chiang · A.S.A. Al-Ruwaitea · S. Mobini
M.-Y. Ho · C.M. Bradshaw · E. Szabadi

The effect of *d*-amphetamine on performance on two operant timing schedules

Received: 24 September 1999 / Accepted: 15 February 2000 / Published online: 13 April 2000

Abstract *Rationale:* Previous experiments have shown that *d*-amphetamine disrupts timing behaviour in rats. It has been proposed that *d*-amphetamine's effects reflect a reduction in the period of the pacemaker of the hypothetical internal clock. However, some studies have obtained conflicting results. *Objective:* To examine the effects of *d*-amphetamine (0.2, 0.4, 0.8 mg kg^{-1} i.p.) on performance on two quantitative timing schedules: a free-operant schedule, in which rats were trained to distribute their responses differentially between two levers during the course of a 50-s trial (free-operant psychophysical procedure), and a discrete-trials schedule, in which rats were trained to discriminate the duration of light stimuli (interval bisection task). *Methods:* In experiment 1, rats were trained under the free-operant psychophysical procedure to respond on two levers (A and B) in 50-s trials in which reinforcement was provided intermittently for responding on A during the first half and on B during the second half of the trial. For one group, repetitive switching between levers was permitted; for another group, it was prevented. In experiment 2, rats were exposed to press lever A after a 2-s stimulus and lever B after an 8-s stimulus, and were then tested with stimuli of intermediate duration. For one group, a 'poke response' (depression of a central tray flap) was required after stimulus presentation to effect lever presentation; for the other group, this requirement did not operate. In both experiments, quantitative indices of timing were derived from the psychophysical functions (%B responding vs time). *Results:* In experiment 1, *d*-amphetamine increased the Weber fraction and displaced the psychophysical curve to the left in both versions of the schedule, as well as producing rate-dependent suppression of responding. In experiment 2, *d*-amphetamine increased the Weber fraction in both versions of the task without displacing the curve. *Conclusions:* These results confirm the disruptive effect of *d*-amphetamine on timing. The results of experiment 1 are consistent with the proposal that the drug reduces the period of the hypothetical pacemaker. However, the results of experiment 2 do not support this suggestion. Taken together, the results support the notion that different neural mechanisms may be involved in timing tasks involving temporal distribution of responding and discrimination of the duration of exteroceptive stimuli.

Key words *d*-Amphetamine · Timing · Free-operant psychophysical procedure · Interval bisection task

T.-J. Chiang · A.S.A. Al-Ruwaitea · S. Mobini · M.-Y. Ho (✉)
C.M. Bradshaw · E. Szabadi
Psychopharmacology Section, Division of Psychiatry,
University of Nottingham, Room B109, Medical School,
Queen's Medical Centre, Nottingham NG7 2UH, UK

Introduction

Most current theoretical models of interval timing behaviour assume the existence of an 'internal clock', consisting of a pacemaker which emits pulses at a constant mean rate and an accumulator which 'counts' these pulses (for reviews, see chapters in Bradshaw and Szabadi 1997; Rosenbaum and Collyer 1998). It has been proposed that activity within the central dopaminergic pathways determines the period of the hypothetical pacemaker (Meck 1996; Gibbon et al. 1997; Hinton and Meck 1997). This proposition first arose from early reports (Maricq et al. 1981; Maricq and Church 1983; Meck 1983) that a dopamine-releasing agent, methamphetamine, and a D_2 receptor antagonist, haloperidol, produced characteristic changes in performance in two quantitative timing schedules, the *interval bisection task* (Catania 1970; Church and Deluty 1977) and the *fixed-interval peak procedure* (Catania 1970; Roberts 1981) (see below).

In the interval bisection task, animals are first trained in a conditional discrimination task to respond on operandum A following a short stimulus (e.g. 2 s) and on operandum B following a longer stimulus (e.g. 8 s). When this temporal discrimination has been learnt, probe trials are used to assess relative preference for operandum B (%B) following presentation of stimuli of intermediate

duration. The resultant psychophysical function (%B plotted against stimulus duration) has a logistic form, and the bisection point (i.e. the duration at which %B=50) is located close to the geometric mean of the short and long standard durations (Church and Deluty 1977). Treatment with methamphetamine has been found to reduce the bisection point (i.e. to displace the psychophysical curve to the left), whereas haloperidol has the opposite effect (Maricq et al. 1981; Maricq and Church 1983; Meck 1983).

In the fixed-interval peak procedure, animals are trained under a discrete trials fixed-interval schedule. In 'standard' trials, reinforcer delivery follows the first response to be emitted after the expiry of a designated interval, timed from the start of the trial. In 'probe' trials, the reinforcer is omitted, and responding is allowed to continue for a period several times the length of the fixed interval. A plot of response rate against time in the probe trials yields a bell-shaped function whose peak lies close to the point in time when reinforcement occurs in the standard trials (Roberts 1981). Methamphetamine has been found to reduce the peak time (i.e. to displace the function to the left), whereas haloperidol and other D_2 receptor-blocking neuroleptics have the opposite effect (Meck 1986).

These results provide strong support for the hypothesis that dopaminergic mechanisms help to determine the period of the hypothetical pacemaker. According to pacemaker-based models of timing, an acute shortening of the pacemaker period should result in a leftward displacement of the bisection point (interval bisection task) and the peak time (fixed-interval peak procedure), whereas an acute lengthening of the pacemaker period should have the opposite effect (Meck 1996; Gibbon et al. 1997; Hinton and Meck 1997). Thus, the abovementioned findings with methamphetamine and haloperidol are consistent with a facilitatory action of dopaminergic neurotransmission on pacemaker function. However, some studies have yielded results that do not support this hypothesis. For example, Bayley et al. (1998) found no effect of *d*-amphetamine on peak time in the fixed-interval peak procedure, and there have been several reports of *d*-amphetamine and the D_2 receptor agonist quinpirole having inconsistent effects on temporal discrimination in conditional discrimination tasks or, in some cases, inducing a bias towards the operandum associated with the shorter duration, consistent with an increase, rather than a decrease, of the bisection point (Stubbs and Thomas 1974; Rapp and Robbins 1976; Lejeune et al. 1995; Santi et al. 1995; Stanford and Santi 1998).

It is important that these apparent inconsistencies in the literature be resolved, in order that a clear picture of dopamine's putative role in interval timing behaviour may emerge (Gibbon et al. 1997). It seems likely that methodological differences are largely responsible for the apparent discrepancies. Most timing schedules entail complex contingencies, and the performances that they engender are sensitive to alterations of a variety of behavioural functions other than 'pure' timing processes. For example, the quantitative timing indices derived from performance on inter-response time schedules (Zeiler 1977) in part reflect motivational factors and the capacity for response inhibition (Platt 1979); this complicates the interpretation of pharmacologically induced changes in these indices (Sanger and Blackman 1976; Wogar et al. 1992, 1993; Stephens and Voet 1994).

A series of experiments on the effects of central 5-hydroxytryptamine (5-HT) depletion on performance on various timing schedules has provided another line of evidence for the involvement of multiple behavioural processes in many commonly used timing tasks (Al-Ruwaitea et al. 1997a; Ho et al. 1998). One such process is the propensity to switch between alternative responses, which appears to influence timing performance to varying degrees in different timing paradigms (Al-Ruwaitea et al. 1997a; Ho et al. 1998). For example, the precision of temporal differentiation of responding under the free-operant psychophysical procedure (Stubbs 1976) was enhanced when the switching between two concurrently available operanda was experimentally constrained (Chiang et al. 1998). Facilitation of switching is one of the most robust effects of central 5-HT depletion on free-operant performance (Al-Zahrani et al. 1996; Al-Ruwaitea 1997b, 1999b; Chiang et al. 1999), which has been shown to influence the quantitative indices of interval timing in several free-operant timing schedules (Al-Zahrani et al. 1996; Al-Ruwaitea et al. 1997b, 1999a; Chiang et al. 1999). Facilitated switching has also been postulated to underlie the effect of central 5-HT depletion on performance on the interval bisection task (Morrissey et al. 1993; Ho et al. 1995), and the promotion of 'premature' responding on inter-response time schedules and the fixed-interval peak procedure (Wogar et al. 1992, 1993; Morrissey et al. 1994). For example, Morrissey et al. (1993) reported that central 5-HT depletion resulted in a reduction of the bisection point in the interval bisection task. Ho et al. (1995) showed that this effect was largely brought about by the facilitation of the rats' movement from the proximity of operandum A to the proximity of operandum B during the period of stimulus presentation. When this movement was restricted, by imposing a requirement to depress a flap midway between the levers after stimulus presentation, the effect of the lesion on the bisection point was diminished (Ho et al. 1995; see also Al-Ruwaitea et al. 1997a; Ho et al. 1998).

Facilitation of switching and the promotion of premature responding are also known effects of *d*-amphetamine and related drugs (Laties 1972; Sanger and Blackman 1976; Laties et al. 1981; Robbins and Watson 1981; Evenden and Robbins 1983; Harrison et al. 1997). However, it is not known whether these effects contribute to the observed effects of *d*-amphetamine on interval timing performance. One of the aims of the present experiments was to address this question. We examined the effects of acute treatment with *d*-amphetamine on the performance of rats on two timing schedules, the free-oper-

ant psychophysical procedure and the interval bisection task. In the former case, we used two versions of the task, the standard version, which permits unrestricted switching between the two operanda, and a modified version in which only one switch is permitted in each trial ("unconstrained switching" and "constrained switching"; Chiang et al. 1998, 1999). Likewise, we used two versions of the interval bisection task, the standard version, in which movement from one lever to the other during stimulus presentation is not restricted, and a modified version in which depression of a central flap is required in order for the rat to gain access to the two levers ("no-poke-requirement" and "poke-requirement"; Ho et al. 1995). Our aim was to replicate previous observations of the effects of amphetamine on timing performance using the standard versions of the schedules, and to examine whether these effects would be altered in the modified versions, when repetitive switching (free-operant psychophysical procedure) or movement across the chamber during stimulus presentation (interval bisection task) was prevented.

Experiment 1: effect of *d*-amphetamine on performance on the free-operant psychophysical procedure

Materials and methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals.

Subjects

Twenty-three experimentally naive 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). They 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.

Apparatus

The rats were trained in operant conditioning chambers (Campden Instruments Limited) of internal dimensions 20×23×22.5 cm. One wall of the chamber contained a recess into which a motor-operated dipper could deliver 50 µl of 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. A CUBE microcomputer (Paul Fray Ltd.) located in an adjoining room controlled the schedules and recorded the behavioural data.

Behavioural training

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-trial continuous reinforcement schedule, in which the two levers were presented in random sequence for three sessions. The rats were then randomly allocated to two groups: the "constrained switching" group (n=11) and the "unconstrained switching" group (n=12) (see below). The rats underwent 50-min training sessions, 7 days per week, at the same time each day during the light phase of the daily cycle (between 0700 hours and 1200 hours). The reinforcer, a 0.6-M solution of sucrose in distilled water, was prepared daily before each session.

Each session consisted of 50 trials each lasting 50 s, successive trials being separated by 10-s inter-trial intervals. Reinforcement was provided on a constant-probability variable-interval (VI) 30-s schedule (Catania and Reynolds 1968). At the start of the trial, the levers were inserted into the chamber, and they were withdrawn during the inter-trial interval. Thus, the inter-trial interval was signalled by withdrawal of the lever(s), which always occurred at the end of the 50-s trial, irrespective of the occurrence of a reinforcer. Except in the case when a trial ended during the delivery of a reinforcer, the lever(s) remained in the chamber during reinforcer delivery. During the first 25 s of the trial, reinforcers were delivered only for response 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 probe trials, in which no reinforcers were delivered, were interspersed randomly among the other, standard trials, with the constraint that at least one standard trial occurred between successive probe trials. Responses on the two levers were recorded in 5-s bins during each trial. Switches between the two levers were also recorded; a switch was defined as a response on lever B that immediately followed a response on lever A, or a response on lever A that immediately followed a response on lever B.

For the rats allocated to the "constrained switching" group, switching between levers was restricted to one switch per trial, from lever A to lever B. In each trial, the first response on lever B resulted in withdrawal of lever A until the start of the next trial. In the case of the rats in the "unconstrained switching" group, both lever A and lever B remained in the chamber throughout the trials, allowing switching from lever B to lever A as well as from lever A to lever B.

Drug treatment

The drug treatment regimen started after 60 sessions of preliminary training under the free-operant psychophysical procedure. Treatments were given by i.p. injection (2.5 ml/kg body weight) using a 25-gauge needle, 10 min before the start of the experimental session.

Injection of *d*-amphetamine sulphate was given on Tuesdays and Fridays, and injection of the vehicle alone (0.9% sodium chloride solution) on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. Each rat received three doses of *d*-amphetamine sulphate (0.2, 0.4 and 0.8 mg kg^{-1}). Each dose was administered on ten occasions in order to accrue a sufficient number of probe trials to obtain reliable estimates of the timing indices for individual rats (Chiang et al. 1998, 1999). The order of administration of the doses was counterbalanced across rats.

Data analysis

Only the data collected in the probe trials during the sessions in which injections had been given were used in the analysis.

Absolute response rates. For each rat and each treatment condition (vehicle alone, 0.2 mg kg^{-1}, 0.4 mg kg^{-1} and 0.8 mg kg^{-1} *d*-amphetamine), the mean response rates on each lever in successive time bins were analysed using a four-factor analysis of variance (ANOVA; group × treatment × lever × time bin) with repeated measures on the second, third and fourth factors.

Rate dependency. In order to examine the rate-dependent effect of *d*-amphetamine, the response rates on each lever in each time-bin

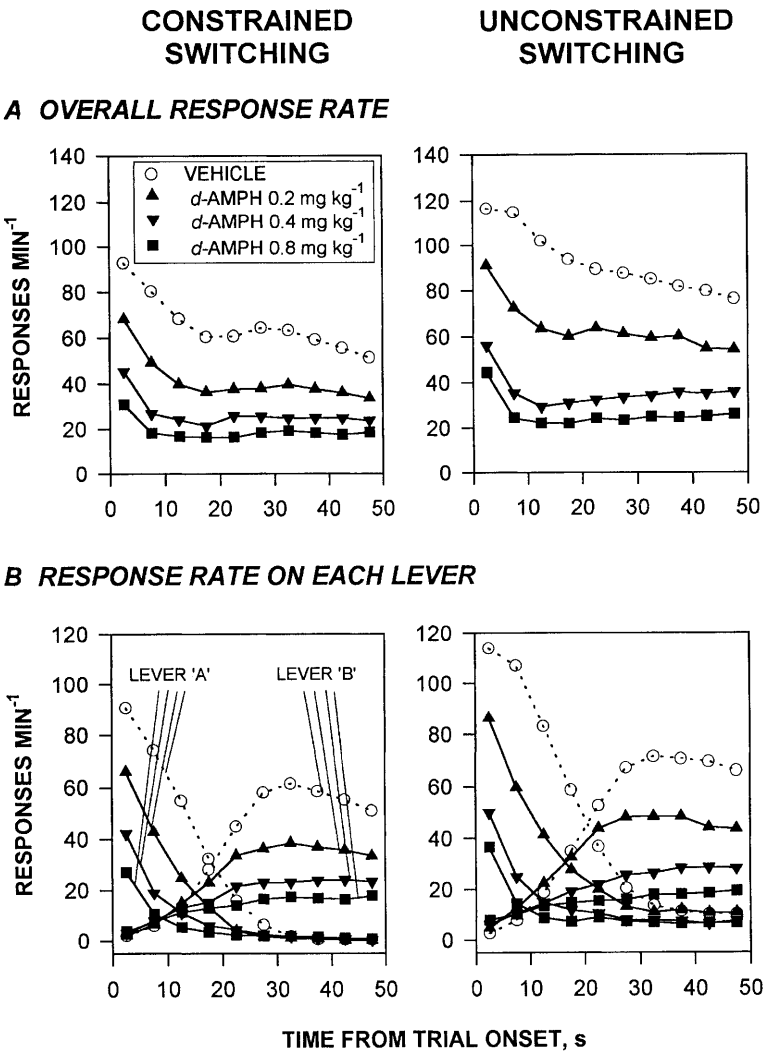

Fig. 1A–B Effect of *d*-amphetamine on absolute response rates (experiment 1). *Left-hand graphs*: "constrained-switching" group; *right-hand graphs*: "unconstrained-switching" group. **A** Group mean overall response rates on the two levers. **B** Group mean response rates on lever A (*descending curves*) and lever B (*ascending curves*). *Ordinates*: response rate (responses min^{-1}); *abscissae*: time from start of trial (s)

were pooled, yielding 20 data points under each treatment condition. Response rates under each active drug treatment condition, expressed as a percentage of those obtained under the vehicle-alone condition, were plotted against the response rates obtained under the vehicle-alone condition, in double-logarithmic coordinates (Dews and Wenger 1977). Linear functions were fitted to each rat's data; the slopes of these functions were analysed using two-factor ANOVA (group × treatment) with repeated measures on the latter factor.

Relative response rates and psychophysical function. The 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 analysed using a three-factor ANOVA (group × treatment × time bin) with repeated measures on the second and third factors. A two-parameter logistic function was fitted to the %B data obtained from each rat: $\%B=100/(1+[t/T_{50}]^{-\varepsilon})$, where t is time from the onset of the trial, T_{50} (the indifference point) is a parameter expressing the time at which %B=50%, and ε is the slope of the logistic function (Al-Zahrani et al. 1996). The curve-fitting procedure yields estimates (±SEM estimate) of the values of these parameters; goodness of fit of the logistic functions was expressed as p^2, the proportion of the data variance accounted for using the fitted function (Lewis 1960). The limen was defined as half the difference between T_{75} and T_{25}, where T_{75} and T_{25} are the values of t corresponding to %B=75% and %B=25%. The Weber fraction was calculated as the ratio of the limen to T_{50}. The values of T_{50}, ε and the Weber fraction were analysed using two-factor ANOVA (group × treatment) with repeated measures on the latter factor; post-hoc analyses of simple main effects were carried out when appropriate (Winer 1971). In the case of a significant effect of treatment condition, comparisons were made between each dose of *d*-amphetamine and the control (vehicle alone) condition using Dunnett's test (k=4; significance criterion, P<0.05).

Switching between levers. In the case of the "unconstrained switching" group only, the effects of *d*-amphetamine on the mean absolute rate of switching and the mean number of inter-switch responses were analysed using one-factor ANOVA (treatment condition) with repeated measures.

Results

Response rates

Absolute response rate. Figure 1A shows the combined response rates on both levers, and Fig. 1(B) the response rates on each lever, in successive 5-s time bins of the 50-s probe trials under each treatment condition. These data were analysed using a four-factor ANOVA (group ×

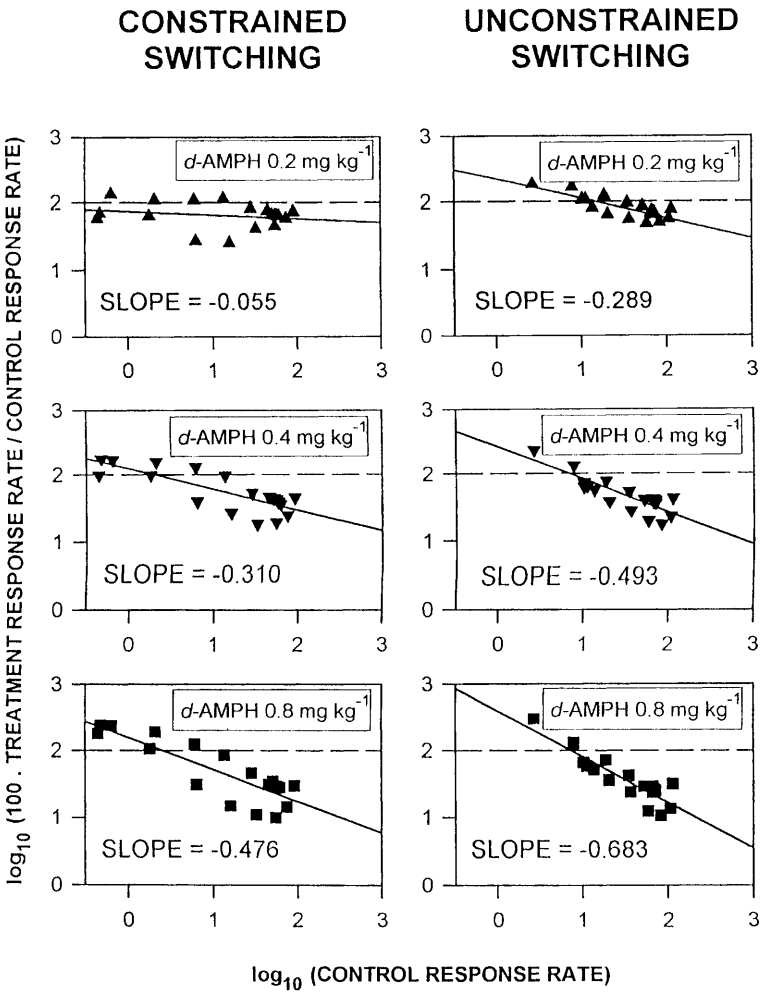

Fig. 2 Rate-dependent effects of *d*-amphetamine (experiment 1). *Left-hand graphs*: "constrained-switching" group; *right-hand graphs*: "unconstrained-switching" group.*Ordinates*: $\log_{10}$ response rate following treatment with *d*-amphetamine, expressed as a percentage of control response rate; *abscissae*: $\log_{10}$ control (vehicle alone) response rate. *Horizontal lines* indicate ordinate value of 2.0 (i.e. unchanged response rate); *points below the lines* represent response rates suppressed by *d*-amphetamine. Linear functions were fitted by the least squares method; *slopes* are shown in each graph

treatment × lever × time bin). In both groups, response rate on lever A declined and response rate on lever B increased as a function of time from trial onset, this being reflected in a significant lever × time-bin interaction ($F_{9,189}$=190.2, P<0.001). Overall response rate declined during the course of the trial, this being reflected in a significant main effect of time bin ($F_{9,189}$=21.7, P<0.001). Across the whole trial, a greater proportion of responding was directed at lever A than lever B, this being reflected in a significant main effect of lever ($F_{1,21}$=4.6, P<0.05). *d*-Amphetamine reduced the overall response rates and altered the distribution of responding between the two levers during the course of the trial, these effects being reflected in a significant main effect of treatment ($F_{3,63}$=84.2, P<0.001), and significant treatment × time-bin ($F_{27,567}$=10.9, P<0.001) and treatment × lever × time-bin ($F_{27,567}$=77.4, P<0.001) interactions; the treatment × lever interaction was not significant (F<1). Overall response rate was significantly higher in the "unconstrained switching" group than in the "constrained switching" group ($F_{1,21}$=6.9, P<0.02), and there was a significant group × treatment interaction ($F_{3,63}$=3.2, P<0.05). There were no other significant interactions involving the group factor (group × time bin, group × lever, group × lever × time bin: all F values <1; group × treatment × time bin: $F_{27,567}$=1.2, P>0.2; group × treatment × lever × time bin: $F_{27,567}$=1.5, P>0.05).

Rate-dependent effects. Figure 2 shows conventional rate-dependency plots (response rate following *d*-amphetamine treatment, expressed as a percentage of response rate following vehicle-alone treatment, in double-logarithmic co-ordinates) for the group mean data. In both groups, low response rates tended to be increased or unchanged, whereas higher response rates were suppressed by *d*-amphetamine. The linear functions fitted to these data had negative slopes, the steepness of which increased with increasing doses of *d*-amphetamine (values shown in Fig. 2). This trend was confirmed by the slopes of the regression lines fitted to the data from the individual rats (Table 1). ANOVA of these data (group × treatment) revealed a significant main effect of treatment ($F_{2,42}$=5.5, P<0.01), but no significant main effect of group (F<1), and no significant interaction ($F_{2,42}$=1.5, P>0.2).

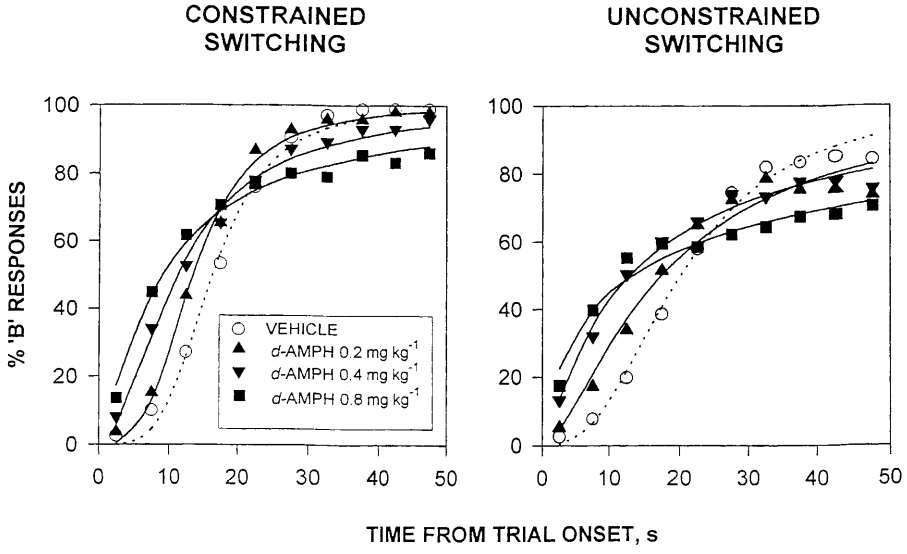

Fig. 3 Effect of *d*-amphetamine on relative response rate in successive 5-s time bins of the probe trials (experiment 1). *Left-hand graphs*: "constrained-switching" group; *right-hand graphs*: "unconstrained-switching" group. *Ordinates*: group mean response rate on lever B, expressed as a percentage of overall response rate; *abscissae*: time from the start of the trial. *Smooth curves* are best-fit logistic functions

Table 1 Slopes of rate-dependency functions (cf Fig. 2) fitted to the data from individual rats (mean±SEM)

Dose of *d*-amphetamine (mg kg^{-1})	"Constrained-switching" group (n=11)	"Unconstrained-switching" group (n=12)
0.2	–0.151±0.053	–0.255±0.052
0.4	–0.276±0.055	–0.384±0.087
0.8	–0.406±0.065	–0.348±0.057

Table 2 Parameters of logistic functions fitted to the data from individual rats in each group under the "free-operant psychophysical procedure" (group mean±SEM)

Parameters	Treatment condition			
	Vehicle alone	0.2 mg kg^{-1}	0.4 mg kg^{-1}	0.8 mg kg^{-1}
"Constrained switching" group (n=11)				
Slope, ε	4.86±0.45	4.41±0.61	2.80±0.32*	2.38±0.40*
T_{50} (s)	16.44±1.30	13.89±1.22*	11.87±1.82*	8.82±1.48*
p^2	0.995±0.001	0.983±0.004	0.948±0.017*	0.953±0.014*
Weber fraction	0.25±0.02	0.28±0.02	0.46±0.05*	0.60±0.07*
"Unconstrained switching" group (n=12)				
Slope, ε	3.02±0.29	2.08±0.28*	1.51±0.16*	1.04±0.14*
T_{50} (s)	20.78±1.19	17.62±1.47	13.87±2.44*	15.67±3.52*
p^2	0.981±0.004	0.931±0.025	0.918±0.023	0.822±0.058*
Weber fraction	0.43±0.06	0.67±0.07	0.92±0.10	1.93±0.39*

*Significant differences between drug treatment and vehicle alone: P<0.05

Relative response rates and psychophysical functions

Relative response rate. Figure 3 shows the relative response rates on lever B (%B) and logistic functions fitted to these data for the two groups under each treatment condition. In both groups, %B increased progressively as a function of time from the trial onset. %B appeared to increase less steeply in the "unconstrained switching" group than the "constrained switching" group. These trends were confirmed by the ANOVA (group × treatment × time bin), which revealed significant main effects of group ($F_{1,21}$=4.4, P<0.05) and time bin ($F_{9,189}$=295.5, P<0.001), and a group × time-bin interaction ($F_{9,189}$=5.2, P<0.001); there were no other significant interactions involving the group factor (group × treatment; group × treatment × time bin: F values <1). The main effect of treatment was not statistically significant ($F_{3,63}$=1.5, P>0.1); however, there was a significant treatment × time-bin interaction ($F_{27,567}$=34.5, P<0.001), reflecting a dose-dependent leftward displacement and 'flattening' of the %B functions. For each group, the logistic functions fitted to the group mean data accounted for more than 97% of the data variance across all treatment conditions of the experiment.

Table 2 shows the mean±SEM values of the parameters of the logistic functions fitted to the data from the individual rats.

Slope, ε. The values of ε were lower in the "unconstrained switching" group than in the "constrained switching" group. *d*-Amphetamine dose dependently reduced the value of ε in both groups. ANOVA (group × treatment) revealed a significant main effects of group ($F_{1,21}$=22.6, P<0.001) and treatment ($F_{3,63}$=25.6, P<0.001), but no significant interaction ($F_{3,63}$=1.5, P>0.2). Analysis of the simple effect within each group revealed that the effect of treatment was significant in both groups ("constrained switching" group: $F_{3,30}$=10.2, P<0.001; "unconstrained switching group": $F_{3,33}$=25.7, P<0.001). Multiple comparisons with the vehicle-alone condition (Dunnett's test; see Data analysis) showed that, in the "constrained switching" group, ε was significantly reduced by the 0.4-mg kg^{-1} and 0.8-mg kg^{-1} doses (t_{30}=3.9 and 4.6, respectively), but not by the 0.2-mg kg^{-1} dose (t<1), whereas all three doses had significant effects in the "unconstrained switching group (0.2 mg kg^{-1}: t_{33}=4.0; 0.4 mg kg^{-1}: t_{33}=6.3; 0.8 mg kg^{-1}: t_{33}=8.3).

Indifference point, T_{50}. d-Amphetamine dose dependently reduced the value of T_{50} in both groups, this being confirmed by a two-factor ANOVA (group × treatment) that revealed a significant main effect of treatment ($F_{3,63}$=12.8, P<0.001); there was no significant main effect of group ($F_{1,21}$=3.0, P>0.05), or group × treatment interaction ($F_{3,63}$=1.5, P>0.2). Multiple comparisons with the vehicle-alone condition within each group (Dunnett's test) showed that T_{50} was significantly reduced by all doses of *d*-amphetamine in the "constrained switching" group (0.2 mg kg^{-1}: t_{30}=2.7; 0.4 mg kg^{-1}: t_{30}=4.9; 0.8 mg kg^{-1}: t_{30}=8.1), whereas only 0.4 mg kg^{-1} and 0.8 mg kg^{-1} had significant effects in the "unconstrained switching" group (0.2 mg kg^{-1}: t_{33}=1.6; 0.4 mg kg^{-1}: t_{33}=3.4; 0.8 mg kg^{-1}: t_{33}=2.5).

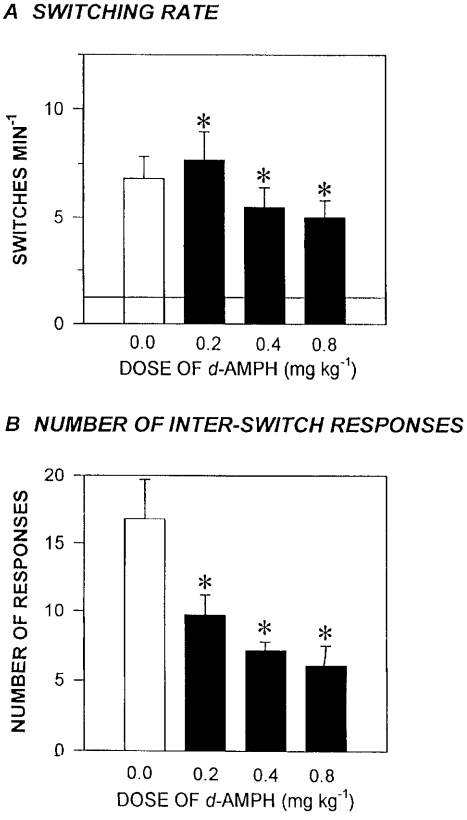

Fig. 4A,B Effect of *d*-amphetamine on rate of switching between levers in the "unconstrained switching" group (experiment 1). **A** Group mean rates of switching (switches min^{-1}); *vertical bars* indicate SEM. *Horizontal line* indicates the rate at which switching was constrained in the "constrained-switching" group. **B** Mean numbers of lever press responses between successive switches (conventions as in A). Significance of difference from vehicle treatment: *P<0.05

p^2. The proportion of the data variance accounted for by the logistic functions was somewhat lower in the "unconstrained switching" group than the "constrained switching" group. In both groups, the value of p^2 decreased following drug treatment. ANOVA revealed significant main effects of group ($F_{1,21}$=5.8, P<0.05) and treatment ($F_{3,63}$=6.8, P<0.001), but no significant interaction ($F_{3,63}$=2.5, P>0.05). There were significant simple main effects of treatment in both groups ("constrained switching" group: $F_{3,30}$=5.1; "unconstrained switching" group: $F_{3,33}$=5.0, P values <0.01). Multiple comparisons with the vehicle-alone condition (Dunnett's test) showed than p^2 was reduced by 0.4 mg kg^{-1} and 0.8 mg kg^{-1} *d*-amphetamine in the "constrained switching" group (0.2 mg kg^{-1}: t<1; 0.4 mg kg^{-1} and 0.8 mg kg^{-1}: t_{30} values >3.1), and by 0.8 mg kg^{-1} in the "unconstrained switching" group (0.2 mg kg^{-1}: t_{33}=1.2; 0.4 mg kg^{-1}: t_{33}=1.5; 0.8 mg kg^{-1}: t_{33}=3.9).

Weber fraction. The Weber fractions were higher in the "unconstrained switching" group than in the "constrained switching" group. *d*-Amphetamine dose dependently increased the Weber fraction in both groups. This was confirmed by two-factor ANOVA, which showed significant main effects of group ($F_{1,21}$=19.6, P<0.001) and treatment ($F_{3,63}$=15.8, P<0.001), and a significant interaction ($F_{3,63}$=6.1, P<0.001). Analysis of the simple effects of treatment revealed significant effects in both groups ("constrained switching" group: $F_{3,30}$=14.1, P<0.001; "unconstrained switching" group: $F_{3,33}$=11.8, P<0.001). Multiple comparisons with the vehicle-alone condition (Dunnett's test) showed that the 0.4-mg kg^{-1} and 0.8-mg kg^{-1} doses had significant effects in the "constrained switching" group (0.2 mg kg^{-1}: t<1; 0.4 mg kg^{-1}: t_{30}=3.5; 0.8 mg kg^{-1}: t_{30}=5.8), whereas only the 0.8-mg kg^{-1} dose had a significant effect in the "unconstrained switching" group (0.2 mg kg^{-1}: t_{33}<1; 0.4 mg kg^{-1}: t_{33}=1.8; 0.8 mg kg^{-1}: t_{33}=5.5).

Switching

Switching rate. The rates of switching are shown in Fig. 4A). The horizontal line indicates the switching rate in the "constrained switching" group, which was limited to one switch per trial (i.e. 1.2 switches min^{-1}). In the

"unconstrained switching" group, switching rate increased slightly under the lowest dose of *d*-amphetamine, and decreased under the 0.4-mg kg^{-1} and 0.8-mg kg^{-1} doses. ANOVA revealed a significant effect of treatment ($F_{3,33}$=5.1, P<0.01). Multiple comparisons with the vehicle-alone condition (Dunnett's test) revealed significant effects of all three doses (0.2 mg kg^{-1}: t_{33}=3.3; 0.4 mg kg^{-1}: t_{33}=5.3; 0.8 mg kg^{-1}: t_{33}=7.2).

Inter-switch responses. *d*-Amphetamine dose dependently reduced the number of responses emitted between successive switches in the "unconstrained switching" group (Fig. 4B; $F_{3,33}$=11.5, P<0.001).

Experiment 2: effect of *d*-amphetamine on performance on the interval bisection task

Materials and methods

The experiment was carried out in accordance with UK Home Office regulations.

Subjects

Twenty-four experimentally naive female Wistar rats aged 4 months and weighing 250–290 g at the start of the experiment were housed under the same conditions as in experiment 1.

Apparatus

The apparatus was the same as that used in experiment 1, except that the entrance to the recess was covered by a hinged transparent Perspex flap (the "tray-flap"), and the chamber was fitted with a 2.8-W white lamp located within the reinforcer recess.

Behavioural training

After gradual reduction to 80% of their free-feeding body weights, the rats were trained to press the levers and were exposed to a discrete-trial continuous reinforcement schedule for three sessions, as in experiment 1. Then they were randomly allocated to two groups, the "no-poke requirement" (n=12) and the "poke requirement" group (n=12) (see below). Thereafter, they underwent daily 50-min training sessions, 7 days per week, during the light phase of the daily cycle (between 0700 hours and 1200 hours). The reinforcer, a 0.6-M solution of sucrose in distilled water, was prepared daily before each session.

Preliminary training. Each session consisted of 120 30-s trials. Each trial was initiated by the illumination of the lamp within the reinforcer recess, either for 2 s or for 8 s. For the "no-poke-requirement" group, the levers were introduced into the chamber immediately after the light had been extinguished. For the "poke-requirement" group, the levers were not inserted at the end of the period of stimulus presentation, until the rat had depressed the tray flap ("poke" response). Following a response on either lever (or after 5 s, if no response had occurred), both levers were withdrawn and the chamber remained in darkness until the start of the next trial. In trials in which the duration on the light stimulus was 2 s, a response on lever A resulted in reinforcer delivery, whereas a response on lever B did not; conversely, in trials in which the duration of the light stimulus was 8 s, a response on lever B resulted in reinforcer delivery, whereas the response on lever A did not. Reinforcer delivery consisted of raising the dipper into the recess for 5 s. In each session, the stimulus duration was 2 s in 60 trials and 8 s in the other 60 trials. The sequence of trials was randomised, with the constraint that no more than four trials of one type occurred in succession. The position of levers A and B (left versus right) were counterbalanced across subjects. Training under this regimen continued for 30 sessions.

Testing phase. In each session, there were 100 "standard" trials identical to those described above (50 trials with the 2-s stimulus duration and 50 trials with 8-s stimulus duration). The remaining 20 trials were "probe" trials, in which the stimulus was presented for durations intermediate between 2 s and 8 s (2.5 s, 3.2 s, 4.0 s, 5.0 s, 6.4 s: four trials in each case). Reinforcers were never delivered in probe trials. The 20 probe trials were interspersed randomly among the standard trials, with the constraint that at least one standard trial of each type occurred between successive probe trials. The testing phase continued until the end of the experiment.

Drug treatment

The drug treatment regimen started at the beginning of the testing phase. The regimen was identical to that used in experiment 1. Each dose of *d*-amphetamine was administered on ten occasions. This was necessary in order that a substantial number of probe trials (40) could be cumulated under each treatment condition for computation of percentage choice at each stimulus duration (Ho et al. 1996; see Data analysis).

Data analysis

Only the data obtained during the sessions in which injections had been given were used in the analysis. The "poke" response data were collected only during the 2-s and 8-s stimulus presentations. The number of "poke" responses for each stimulus type were pooled across all the sessions under each treatment condition. These data were analysed using a three-factor ANOVA (group × treatment × stimulus type).

The lever-press data obtained in all the sessions were pooled, yielding 500 standard trials of each type (2 s and 8 s) and 200 probe trials (40 presentations of each intermediate stimulus duration) for each active drug treatment, and three times as many trials of each type for the control (vehicle alone) condition. In the case of each stimulus duration, the percentage of trials in which lever B was pressed (i.e. the percentage of responses appropriate to the 8-s stimulus: %B) was calculated. These data were analysed using three-factor ANOVA (group × treatment × stimulus duration) with repeated measures on the second and third factors. Post-hoc analyses of simple effects were carried out when appropriate (Winer 1971). Plots of %B responses against stimulus duration (d, in logarithmic units) were derived for each rat, and for the group mean data, under each treatment condition. Two-parameter logistic functions (cf. experiment 1) were fitted to these data: $\%B = 100/(1+[d/D_{50}]^{-\varepsilon})$; D_{50} and ε are free parameters expressing the bisection point (the stimulus duration at which %B=50%) and the slope of the function, respectively. Goodness of fit of the logistic functions was expressed as p^2. The values of the limen and the Weber faction were also calculated (see experiment 1). The values of D_{50} and the Weber fraction were analysed using two-factor ANOVA (group × treatment) with repeated measures on the latter factor; post-hoc analyses of simple main effects were carried out when appropriate (Winer 1971). In the case of a significant effect of treatment condition, comparisons were made between each dose of *d*-amphetamine and the vehicle-alone condition using Dunnett's test (k=4; significance criterion, P<0.05). As it has been proposed that *d*-amphetamine may induce "inattention" to the onset of the stimulus, and that this may contaminate the estimation of the parameters of the psychophysical function (Maricq et al. 1981; Maricq and Chruch 1983), an additional analysis was carried out on the data obtained under the 0.8-mg kg^{-1} conditions, after exclusion of trials in which the response latency was >3 s.

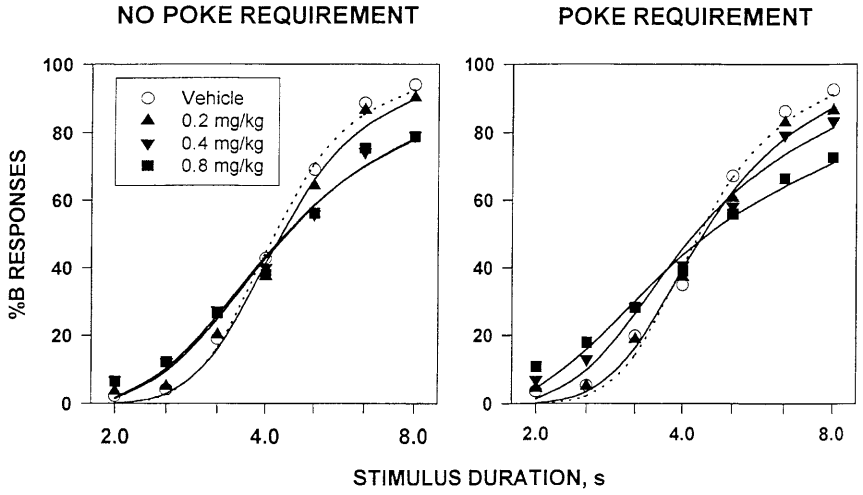

Fig. 5 Effect of *d*-amphetamine on performance under the interval bisection task (experiment 2). *Left hand graph*: "no-poke requirement" group; *right-hand graph*: "poke-requirement" group. *Ordinates*: percentage choice of lever B; *abscissa*: stimulus duration (s). *Points* are group mean data. *Curves* are best-fit logistic functions (see text)

Table 3 Parameters of logistic functions fitted to the data from individual rats in each treatment conditions of the two groups under the "interval bisestion task" (group mean±SEM)

Parameters	Treatment conditions			
	Vehicle alone	0.2 mg kg^{-1}	0.4 mg kg^{-1}	0.8 mg kg^{-1}
"No-poke-requirement" group (*n*=12)				
Slope, ε	5.13±0.34	5.87±0.89	3.95±0.67	3.42±0.52*
D_{50} (s)	4.17±0.07	4.34±0.14	4.75±0.42	4.91±0.69
p^2	0.991±0.002	0.959±0.012	0.967±0.005	0.953±0.072*
Weber fraction	0.23±0.02	0.25±0.04	0.57±0.19	0.88±0.38*
"Poke-requirement" group (*n*=11)				
Slope, ε	5.22±0.37	4.17±1.05	3.25±0.25*	2.51±0.32*
D_{50} (s)	4.33±0.10	4.48±0.23	4.23±0.19	4.52±0.28
p^2	0.982±0.005	0.952±0.029	0.950±0.009	0.867±0.045*
Weber fraction	0.24±0.03	0.29±0.06	0.40±0.05	1.02±0.38*

*Significant differences between drug treatment and vehicle alone: $P<0.05$

Each of the curve-fitting parameters from the "short-latency" trials (<3 s) was compared with that obtained from all the trials, using two-factor ANOVAs (group × trial type).

Results

Psychophysical functions

Both groups maintained accurate discrimination throughout the testing phase of the experiment (>85% correct responses on standard trials across all treatment conditions).

The group mean data obtained during the testing phase under each treatment condition are shown in Fig. 5. Both groups displayed sigmoid relationships between the percentage of trials in which lever B was pressed (%B) and stimulus duration plotted on a logarithmic scale. Three-factor ANOVA showed a significant main effect of stimulus duration ($F_{6,132}$=477.4, $P<0.001$), and treatment × stimulus duration interaction ($F_{18,396}$= 13.7, $P<0.001$). The main effects of group ($F<1$) and treatment ($F_{3,66}$=1.2, $P>0.2$) were not statistically significant, and there were no significant interactions involving group factor (group × treatment: $F<1$; group × treatment × stimulus duration: $F_{18,396}$=1.2, $P>0.2$). Logistic functions were fitted to the group mean data shown in Fig. 5. In each case, the fitted function accounted for more than 97% of the data variance.

Curve-fitting parameters

Logistic functions were fitted to the data obtained from each rat in each group under each treatment condition. One animal in the "poke-requirement" group was excluded from the analysis of the curve-fitting parameters, because the function could not be fitted to this animal's data under the highest dose of *d*-amphetamine (this rat's responding was markedly suppressed, and it failed to show any discrimination between the standard stimuli following the 0.8-mg kg^{-1} dose).

Table 3 shows the group mean (±SEM) values of the parameters of the fitted functions derived from the individual animals in each group.

Slope, ε There was no significant main effect of group ($F_{1,21}$=1.7, $P>0.2$). The effect of treatment was significant ($F_{3,63}$=9.5, $P<0.001$), reflecting the dose-dependent 'flattening' of the curves in both groups. The interaction term was not significant ($F_{1,21}$=3.0, $P>0.05$). Multiple

comparisons (Dunnett's test) showed that ε was significantly altered only by 0.8 mg kg^{-1} *d*-amphetamine in the "no-poke requirement" group (t_{33}=2.5), and by 0.4 mg kg^{-1} and 0.8 mg kg^{-1} in the "poke-requirement" group (0.4 mg kg^{-1}: t_{30}=2.8; 0.8 mg kg^{-1}: t_{30}=3.8).

Bisection point, D_{50}. D_{50} did not differ significantly between the two groups (F<1), and was not significantly affected by *d*-amphetamine (treatment and interaction effects: F values<1).

p^2. The goodness of fit of the logistic function did not differ significantly between the groups ($F_{1,21}$=3.0, P>0.05). There was a dose-dependent reduction of p^2 in both groups ($F_{3,63}$=6.3, P<0.001); the interaction term was of 'borderline' significance ($F_{3,63}$=2.3, P=0.05). Multiple comparisons (Dunnett's test) showed that p^2 was significantly reduced by 0.8 mg kg^{-1} *d*-amphetamine in each group ["poke-requirement": t_{30}=8.5; "no poke-requirement": t_{33}=2.9). (Note that the effect on p^2 would have been somewhat greater if the data from one rat had not been excluded from the analysis: see above.)

Weber fraction. The Weber fraction did not differ significantly between the two groups (F<1). The effect of treatment was significant ($F_{3,63}$=6.2, P<0.001); there was no significant interaction (F<1). Multiple comparisons (Dunnett's test) showed that the Weber fraction was significantly reduced by 0.8 mg kg^{-1} *d*-amphetamine in each group ("poke-requirement": t_{30}=3.0; "no poke-requirement": t_{33}=2.5).

All trials versus "short-latency" trails

In the case of the 0.8-mg kg^{-1} and the corresponding vehicle-treatment sessions, the analysis of the parameters of the psychophysical functions was repeated after exclusion of the data from all trials in which the response latency was longer than 3 s. ANOVAs (group × treatment) showed that *d*-amphetamine significantly reduced the slope (ε) ($F_{1,21}$=71.4; P<0.001), increased the Weber fraction ($F_{1,21}$; P<0.05) and reduced the value of p^2 ($F_{1,21}$=9.1; P<0.01). There was no significant effect of *d*-amphetamine on the bisection point ($F_{1,21}$=1.2; P>0.1). None of these analyses revealed a significant effect of group ("poke-requirement" vs "no poke-requirement") or a significant group × treatment interaction (P>0.1 in every case).

Separate ANOVAs comparing the values of the parameters from the short-latency trials and all the trials (group × response-type [all responses, short-latency responses]) failed to reveal any significant effect of response-type in the presence of either *d*-amphetamine or vehicle treatment (F<1 in each case).

"Poke" responses

As the rate of "poke" responding in both groups did not differ significantly between the stimulus types (2 s vs 8 s; $F_{1,22}$=2.8, P>0.1), the data from the two stimulus types were pooled and analysed using two-factor ANOVA (group × treatment). Figure 6 shows the mean rates of "poke" responding (±SEM) under each treatment condition. The rate of "poke" responding was significantly higher in the "poke-requirement" group than in the "no-poke-requirement" group across all treatment conditions, this being reflected in a significant main effect of group ($F_{1,22}$=15.6, P<0.001). "Poke" responding was dose dependently reduced by *d*-amphetamine in the "poke-requirement" group, but was not greatly affected in the "no-poke-requirement" group, these trends being reflected in a significant main effect of treatment ($F_{3,66}$=24.4, P<0.001) and a significant group × treatment interaction ($F_{3,66}$=8.5, P<0.001). Analysis of the simple effect of treatment within each group revealed a significant effect ($F_{3,33}$=27.3, P<0.001) in the "poke-requirement" group, but not the "no-poke-requirement" group ($F_{3,33}$=2.4, P>0.08). For the "poke-requirement" group, multiple comparisons with the vehicle-alone condition (Dunnett's test) showed that responding was significantly reduced by 0.4 mg kg $^{-1}$ and 0.8 mg kg $^{-1}$ *d*-amphetamine (0.2 mg kg^{-1}: t_{33}=1.7; 0.4 mg kg^{-1}: t_{33}=4.0; 0.8 mg kg^{-1}: t_{33}=8.5).

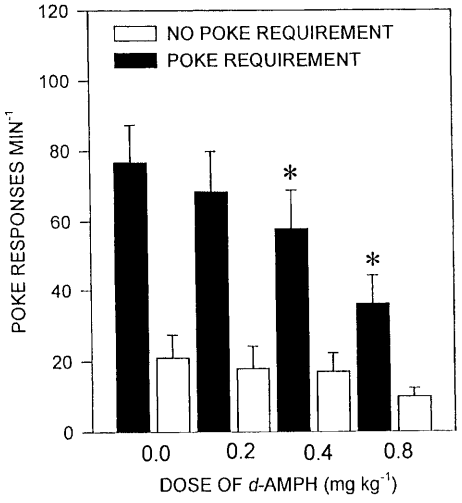

Fig. 6 Effect of *d*-amphetamine on rate of "poke" responding (depression of tray flap) during stimulus presentation. *Columns* show group mean data (poke responses min^{-1}); *vertical bars* indicate SEM. *Open columns*: "poke-requirement" group; *filled columns*: "no-poke requirement" group. Significance of difference from vehicle treatment: *P<0.05

Discussion

Free-operant psychophysical procedure

The pattern of responding seen in this experiment is consistent with numerous previous studies with this schedule (Stubbs 1976, 1979; Bizo and White 1994a, 1994b, 1997; Al-Zahrani et al. 1996, 1998; Chiang et al. 1998, 1999). In both the "unconstrained-switching" and "con-

strained-switching" versions of the schedule, response rate on lever A declined and response rate on lever B increased during the course of the trial, this being reflected in an increasing percentage of total responding being devoted to lever B (%B) as the trial progressed. The relationship between %B and time within the trial was well described by a logistic function, the goodness of fit of the function seen in this experiment being similar to that seen in many previous studies (Bizo and White 1994a, 1994b, 1997; Al-Zahrani et al. 1996, 1998; Chiang et al. 1998, 1999). In this experiment, as in several previous studies (Fetterman and Killeen 1995; Al-Zahrani et al. 1996, 1998; Chiang et al. 1998, 1999), there was a progressive decline in overall response rate during the course of the trial. The imposition of the constraint on switching resulted in a significant steepening of the slope of the psychophysical function and a reduction of the Weber fraction. This is in accord with previous experiments using this procedure (Chiang et al. 1998, 1999).

d-Amphetamine produced a dose-dependent suppression of response rates in both versions of the schedule. The high response rates on lever A at the start of the trial and on lever B at the end of the trial appeared to be particularly vulnerable to the rate-suppressant effect of *d*-amphetamine. This is consistent with the well-known rate-dependency hypothesis of *d*-amphetamine's behavioural effects (Dews 1958; Branch and Gollub 1974; Heffner et al. 1974; Sanger and Blackman 1976; Dews and DeWeese 1977; Dews and Wenger 1977; Seiden et al. 1993), which holds that the effect of *d*-amphetamine and related compounds upon operant behaviour depends on the rate of responding under control conditions. Formal analysis of the rate-dependent effects of *d*-amphetamine in the present experiment, using conventional double-logarithmic plots of proportional change in response rate against control response rate (Dews and Wenger 1977), revealed linear functions whose negative slope tended to become steeper with increasing doses of the drug. This is in agreement with many previous findings derived from a wide range of operant schedules (e.g. Branch and Gollub 1974; Sanger and Blackman 1976; Bradshaw et al. 1981; Robbins and Evenden 1985; see below for further comment).

As expected, the rats trained under the "unconstrained switching" condition showed rather high rates of switching under the control (vehicle alone) condition (cf. Al-Zahrani et al. 1996, 1998; Chiang et al. 1998, 1999). The lowest dose of *d*-amphetamine (0.2 mg kg^{-1}) produced a slight increase in switching rate; however, the higher doses markedly suppressed switching rate.

The reduction of switching rate produced by 0.4 mg kg^{-1} and 0.8 mg kg^{-1} *d*-amphetamine might, at first glance, appear to be inconsistent with reports that this drug increases the probability of switching in some discrete-trials schedules (Evenden and Robbins 1983; Robbins and Evenden 1985). However, the reduction of switching rate (switches min^{-1}) induced by *d*-amphetamine was accompanied by a proportionately greater suppression of operant responding, with the result that there was a dose-dependent decline in the number of inter-switch responses (Fig. 4B); in other words, *d*-amphetamine increased the frequency of switches per unit response. This result is entirely consistent with previous findings of enhanced probability of switching (switches $response^{-1}$) following treatment with *d*-amphetamine (Evenden and Robbins 1983; Robbins and Evenden 1985).

d-Amphetamine had two effects on the psychophysical function: (1) a dose-dependent leftward displacement of the curve, reflected in a reduction of the indifference point, and (2) a dose-dependent 'flattening' of the curve, reflected in a reduction of the slope parameter, ε, and an increase of the Weber fraction. To our knowledge, the effect of *d*-amphetamine on performance on the free-operant psychophysical procedure has not been examined previously. However, the effects seen here closely resemble previously reported effects of *d*-amphetamine and related drugs in another free-operant timing schedule, the fixed-interval peak procedure (Meck 1986, 1996; Frederick and Allan 1996; Kraemer et al. 1997; but see also Bayley et al. 1998). In terms of pacemaker/accumulator models of timing, the effects are consistent with a combination of two separate effects, reduction of the period of the pacemaker (reduced T_{50}) and an impairment of the precision of timing (increased Weber fraction) (Gibbon et al. 1997). However, before accepting an interpretation of *d*-amphetamine's effects in terms of a putative interaction with 'internal clock' mechanisms, two other possible explanations for *d*-amphetamine's effects on the timing indices should be considered: (1) might they be secondary to *d*-amphetamine's effect on switching? and (2) might they be a manifestation of a more general rate-dependent action of *d*-amphetamine, and not specifically related to the mechanisms of interval timing?:

1. The finding that *d*-amphetamine had similar effects on the timing indices in the "unconstrained switching" and "constrained switching" versions of the schedule argues against the possibility that the drug's effects on timing were subservient to its effect on switching. Although the constraint on switching had significant effects on the timing indices (most notably, reducing the Weber fraction: see above), *d*-amphetamine had similar effects on performance on both versions of the schedule. In this context, it may be noted that Al-Zahrani et al. (1996) predicted that changes in the propensity to switch between operanda would be reflected in changes in T_{50} under the "constrained switching" condition. However, Chiang et al. (1999) recently found that central 5-HT depletion, which markedly increased switching rate under the "unconstrained switching" condition, failed to affect the value of T_{50} under either the "constrained" or "unconstrained" versions of the task. It seems, therefore, that switching rate in the free-operant psychophysical procedure may be altered by pharmacological inter-

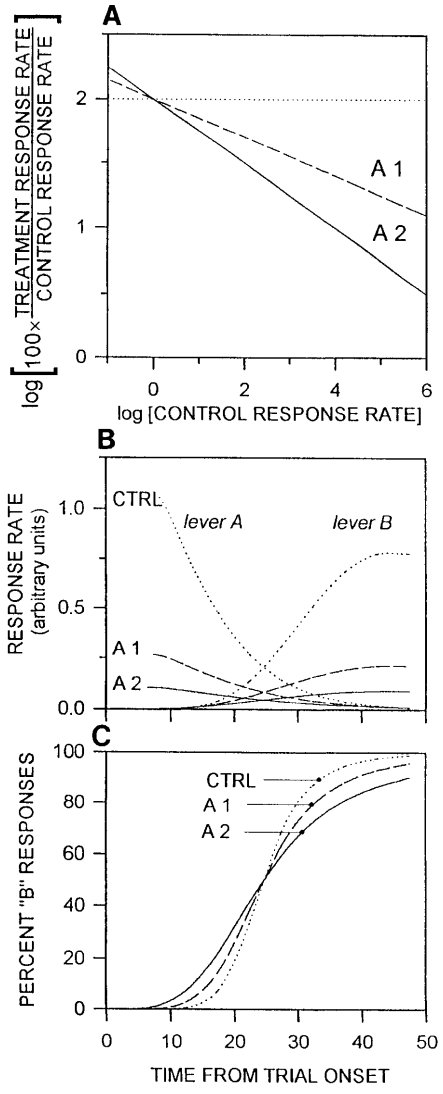

Fig. 7A–C Theoretical analysis of the relationship between the psychophysical curve and the rate-dependency function. **A** Hypothetical rate-dependency functions for two doses of *d*-amphetamine (A1 and A2) (cf Fig. 2). **B** Hypothetical response rates on lever A (*descending curves*) and lever B (*ascending curves*) under control (*CTRL*) conditions (*dotted lines*), and in the presence of the two doses of *d*-amphetamine (A1: *broken lines*; A2: *continuous lines*) (cf Fig. 1). CTRL curves were derived using the Poisson process equation proposed by Killeen and Fetterman (1988); A1 and A2 curves were derived using the same equation parameters, response rates being adjusted using the rate-dependency functions shown in **A**. **C** Psychophysical functions derived from the curves shown in **B** (cf Fig. 3). See text for explanation

ventions without there being any change in the value of T_{50}.

2. The possibility that *d*-amphetamine's effects on the timing indices were a manifestation of a more general rate-dependent action of the drug cannot be totally excluded on the basis of the present data. However, the theoretical analysis summarised in Fig. 7 renders this possibility rather implausible. The upper panel shows hypothetical linear rate-dependency functions for two doses of *d*-amphetamine (A1 and A2), plotted in the conventional way (Dews and Wenger 1977). The dotted curves in the middle panel show hypothetical response rates on the free-operant psychophysical procedure under control conditions, modelled using the equation for a Poisson process proposed by Killeen and Fetterman (1988; see also Bizo and White 1997). The other curves in the middle panel depict hypothetical response rates following treatment with the two doses of *d*-amphetamine, generated using the same model, with no change in any of the parameters of the timing function, and assuming only that the drug had rate-dependent effects as defined by the linear functions shown in the top panel. The lower panel shows the sigmoid psychophysical functions derived from the response rates shown in the middle panel. It is clear that the rate-dependent effect of the drug results in 'flattening' of the curve, but no shift in the indifference point. We conclude, therefore, that rate dependency may account for *d*-amphetamine's effects on the slope parameter (ε) and the Weber fraction; however it cannot readily account for the dose-dependent reduction of T_{50} seen in this experiment.

Interval bisection task

Performance on the interval bisection task in this experiment was in good agreement with many previous reports (Church and Deluty 1977; Fetterman and Killeen 1992; Morrissey et al. 1993; Ho et al. 1995, 1996). In both versions of the task, proportional choice of lever B (%B) increased as a function of stimulus duration, from approximately zero at the short standard stimulus duration (2 s) to nearly 100% at the long standard stimulus duration (8 s). The relationship between %B and stimulus duration was well described by a logistic function, whose goodness of fit was similar to that reported previously (Ho et al. 1995, 1996). Under control conditions (vehicle alone), the bisection point lay close to the geometric mean of the two standard durations (i.e. 4 s), and the Weber fraction (mean values: 0.23 and 0.24 for the "no poke requirement" and "poke requirement" groups, respectively) were similar to those reported previously for rats performing this task (Church and Deluty 1977; Morrissey et al. 1993; Ho et al. 1995, 1996).

Imposition of the "poke requirement" resulted in a significant increase in the rate of "poke" responding during the period of stimulus presentation. However, under the control (vehicle alone) condition, the parameters of the psychophysical function did not differ between the two groups. These findings are consistent with the results of a previous experiment in which this contingency was used (Ho et al. 1995). *d*-Amphetamine suppressed the high rates of "poke" responding seen under the "poke-requirement" condition, but had little effect on the lower rates seen under the "no poke-requirement" condition.

In both groups, *d*-amphetamine 'flattened' the psychophysical curve, reducing the value of ε and increas-

ing the Weber fraction. The bisection point was not significantly altered by *d*-amphetamine in either group. The presence/absence of the poke-requirement did not influence the effect of *d*-amphetamine on the timing indices. The increase of the Weber fraction produced by *d*-amphetamine is consistent with some previous findings with this drug and with some D_2 receptor agonists (Stubbs and Thomas 1974; Maricq et al. 1981; Maricq and Church 1983; Meck 1983; Stanford and Santi 1998). An increase of the Weber fraction signifies a reduction of the precision of temporal discrimination. It has been suggested that this effect of *d*-amphetamine and related drugs reflects an impairment of attention to the stimulus, reflected in long response latencies on some trials (Gibbon et al. 1997; Hinton and Meck 1997). Maricq et al. (1981), Maricq and Church (1983) and Meck (1983) found that exclusion of responses whose latencies were longer than 3 s removed the effect of methamphetamine on the slope of the psychophysical function. Stanford and Santi (1998), however, found that the apparent effects of the D_2 receptor agonist quinpirole on the slope and the Weber fraction were not altered by this ploy. The present results resemble those of Stanford and Santi (1998), in that elimination of all responses with latencies longer than 3 s did not alter the psychophysical function under control (vehicle alone) conditions, nor did it alter the apparent effect of *d*-amphetamine (0.8 mg kg^{-1}) on the function.

The failure of *d*-amphetamine to reduce the bisection point in this experiment stands in contrast to some previous studies (Maricq et al. 1981; Maricq and Church 1983; Meck 1983, 1996), although it is consistent with some others (Stubbs and Thomas 1974; Lejeune et al. 1995; Santi et al. 1995; Stanford and Santi 1998). From a theoretical point of view, the present results do not provide supporting evidence for the involvement of dopaminergic mechanisms in the hypothetical pacemaker which is purported to underlie interval bisection performance.

The basis for the different findings obtained with amphetamine-related drugs in the interval bisection task remains uncertain, because although there are a number of methodological differences between studies, there seems to be a complete overlap between the methods used in studies that found an alteration of the bisection point and in those that did not (see Stanford and Santi 1998, for discussion). One possibility that deserves consideration is that, in the present experiment, the rats may have 're-learned' the standard intervals during the drug-treatment sessions; if this had been the case, then, according to pacemaker-based theories of timing, the value of T_{50} would, after a short-lived leftward displacement, have reverted to its original locus, due to re-adjustment of the accumulator criterion (Gibbon et al. 1997). This possibility seems implausible, however, because many training sessions are usually required to establish stable interval bisection performance (Morrissey et al. 1993), whereas in the present experiment, *d*-amphetamine was administered intermittently, with two or three vehicle-alone or no-treatment sessions interposed between successive drug-treatment sessions. Moreover, it is difficult to see why 're-learning' should have occurred in this experiment, but not in the free-operant psychophysical procedure (experiment 1), in which T_{50} was dose dependently reduced by *d*-amphetamine, administered according to the same regimen.

Conclusions

The results of both experiments confirm that *d*-amphetamine can disrupt timing performance. In both cases, the drug flattened the psychophysical curve and increased the Weber fraction, consistent with a reduction of the precision of timing. However, the effects of *d*-amphetamine on the indifference point of the psychophysical function differed between the two experiments. The leftward shift of the psychophysical curve seen in experiment 1 is consistent with the proposal that this drug, by facilitating dopamine release in the basal ganglia, reduces the period of the pacemaker of the hypothetical internal clock (Gibbon et al. 1997; Hinton and Meck 1997). However, the failure of *d*-amphetamine to exert a similar effect on the psychophysical function in experiment 2 argues against this proposal. Taken together, the results of these two experiments are consistent with previous observations of the effects of central 5-HT depletion (Al-Ruwaitea et al. 1997a), in indicating that the same pharmacological intervention may have qualitatively different effects on timing behaviour in different types of timing schedule. According to the taxonomy proposed by Killeen and Fetterman (1988), the free-operant psychophysical procedure is an example of an immediate timing schedule (i.e. a schedule in which behaviour is controlled by the passage of time during an ongoing interval), whereas the interval bisection task is an example of a retrospective timing schedule (i.e. a schedule in which the organism is trained to emit different responses following stimuli of different duration). The finding that behaviour in these two types of timing schedule is differentially sensitive to pharmacological interventions suggests that different mechanisms may be involved in timing performance which involves temporal regulation of the organism's own behaviour (as in immediate timing schedules) and that which involves discrimination between the duration of external events (as in retrospective timing schedules) (Al-Ruwaitea et al. 1997a; Ho et al. 1998).

Finally, it may be of interest to compare the effects of *d*-amphetamine seen in these experiments with the effects of central 5-HT depletion on performance on the same tasks, as seen in our previous experiments. 5-HT and dopamine are known to exert opposing influences on a number of behavioural and neuroendocrine functions, and it has been suggested that 5-HTergic mechanisms may oppose the putative dopaminergic regulation of the hypothetical pacemaker (Hinton and Meck 1997). Although *d*-amphetamine has more than one pharmacological action (see below), most of its behavioural effects are

believed to be mediated by dopamine release (Seiden et al. 1993). One might therefore expect *d*-amphetamine's effects on timing performance to resemble those of central 5-HT depletion. In fact, the effects of these two interventions on performance on the two schedules used in the present experiments appear to be qualitatively different:

1. *d*-Amphetamine shortened the indifference point in the free-operant psychophysical procedure, whereas loss of central 5-HT had no effect on this parameter (Al-Zahrani et al. 1996; Chiang et al 1999).
2. *d*-Amphetamine increased the Weber fraction in both the free-operant psychophysical procedure and the interval bisection task, whereas loss of central 5-HT had no effect on the Weber fraction in either case (Morrissey et al. 1993; Ho et al. 1995; Al-Zahrani et al. 1996; Chiang et al. 1999).
3. *d*-Amphetamine did not affect the bisection point in the interval bisection procedure, whereas (in the case of the "no poke-requirement" condition) 5-HT depletion reduced it (Morrissey et al. 1993; Ho et al. 1995).

The dissimilar effects of *d*-amphetamine and central 5-HT depletion might reflect the different conditions of administration of the two interventions: central 5-HT depletion was induced by permanent destruction of the ascending 5-HTergic projection, whereas *d*-amphetamine was administered acutely. It will be of interest, in future studies, to examine the effects of acute treatment with drugs affecting the 5-HTergic system on performance on quantitative timing schedules. However, it should also be noted that *d*-amphetamine has other pharmacological actions in addition to the release of dopamine, including noradrenaline (Paton 1975; Wortley et al. 1999) and 5-HT (Reid 1970; Breese et al. 1974) release, which have been proposed to underlie some of its behavioural effects (Sloviter et al. 1978; Archer et al. 1986; Morley et al. 1987). It remains to be seen whether these actions contribute to the effects of *d*-amphetamine on timing performance reported here.

Acknowledgement We are grateful to Mrs S. DeBlaquiere for skilled technical help.

References

Al-Ruwaitea ASA, Al-Zahrani SSA, Ho M-Y, Bradshaw CM, Szabadi E (1997a) Effect of central 5-hydroxytryptamine depletion on performance in the 'time-left' procedure: further evidence for a role of the 5-hydroxytryptaminergic pathways in behavioural 'switching. Psychopharmacology 134:179–186

Al-Ruwaitea ASA, Al-Zahrani SSA, Ho M-Y, Bradshaw CM,
Szabadi E (1997b) 5-Hydroxytryptamine and interval timing. In: Bradshaw CM, Szabadi E (eds) Time and behaviour: psychological and neurobehavioural analyses. Elsevier, Amsterdam, pp 517–570

Al-Ruwaitea ASA, Chiang T-J, Al-Zahrani SSA, Ho M-Y, Bradshaw CM, Szabadi E (1999a) Effect of central 5-hydroxytryptamine depletion on tolerance of delay of reinforcement: evidence from performance in a discrete-trials "time-left" procedure. Psychopharmacology 141:22–29

Al-Ruwaitea ASA, Chiang T-J, Ho M-Y, Bradshaw CM, Szabadi E (1999b) Effect of central 5-hydroxytryptamine depletion on changeover behaviour in concurrent schedules of reinforcement. Psychopharmacology 144:264–271

Al-Zahrani SSA, Ho M-Y, Velazquez Martinez DN, Lopez Cabrera M, Bradshaw CM, Szabadi E (1996) Effect of destruction of the 5-hyroxytryptaminergic pathways on behavioural timing and "switching" in a free-operant psychophysical procedure. Psychopharmacology 127:346–352

Al-Zahrani SSA, Al-Ruwaitea ASA, Ho M-Y, Bradshaw CM, Szabadi E (1998) Effect of destruction of noradrenergic neurones with DSP4 on performance on a free-operant timing schedule. Psychopharmacology 136:235–242

Archer T, Fredriksson A, Jonsson G, Lewander T, Mohammed AK, Ross SB (1986) Central noradrenaline depletion antagonizes aspects of *d*-amphetamine-induced hyperactivity in the rat. Psychopharmacology 88:141–146

Bayley PJ, Bentley GD, Dawson GR (1998) The effects of selected antidepressant drugs on timing behaviour in rats. Psychopharmacology 136:114–122

Bizo LA, White KG (1994a) Pacemaker rate and the behavioral theory of timing. J Exp Psychol Anim Behav Process 20:308–321

Bizo LA, White KG (1994b) The behavioral theory of timing: reinforcer rate determines pacemaker rate. J Exp Anal Behav 61:19–33

Bizo LA, White KG (1997) Timing with controlled reinforcer density: implications for models of timing. J Exp Psychol Anim Behav Process 23:327–383

Bradshaw CM, Szabadi E (1997) (eds) Time and behaviour: psychological and neurobehavioural analyses. Elsevier, Amsterdam

Bradshaw CM, Ruddle HV, Szabadi E (1981) Relationship between response rate and reinforcement frequency in variable-interval schedules: III. Effect of *d*-amphetamine. J Exp Anal Behav 36:28–39

Branch MN, Gollub LR (1974) A detailed analysis of the effects of *d*-amphetamine on behavior under fixed-interval schedules. J Exp Anal Behav 21:519–539

Breese GR, Cooper BR, Mueller RA (1974) Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamine. Br J Pharmacol 52:307–314

Catania AC (1970) Reinforcement schedules and psychophysical judgements: a study of some temporal properties of behavior. In: Schoenfeld WM (ed) The theory of reinforcement schedules. Appleton-Century-Crofts, New York, pp 1–42

Catania AC, Reynolds GS (1968) A quantitative analysis of the responding maintained by interval schedules of reinforcement. J Exp Anal Behav 11:327–383

Chiang T-J, Al-Ruwaitea ASA, Ho M-Y, Bradshaw CM, Szabadi E (1998) The influence of 'switching' on the psychometric function in the free-operant psychophysical procedure. Behav Proc 44:197–209

Chiang T-J, Al-Ruwaitea ASA, Ho M-Y, Bradshaw CM, Szabadi E (1999) Effect of central 5-hydroxytryptamine depletion on performance in the free-operant psychophysical procedure: facilitation of switching, but no effect on temporal differentiation of responding. Psychopharmacology 143:166–173

Church RM, Deluty MZ (1977) Bisection of temporal intervals. J Exp Psychol Anim Behav Process 3:216–228

Dews PB (1958) Studies on behavior, IV. Stimulant actions of methamphetamine. J Pharmacol Exp Ther 122:137–147

Dews PB, DeWeese J (1977) Schedules of reinforcement. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of psychopharmacology, vol 7. Plenum Press, New York, pp 107–150

Dews PB, Wenger GR (1977) Rate dependency of the behavioral effects of amphetamine. In: Thompson T, Dews PB (eds) Advances in behavioral pharmacology, vol 1. Academic Press, New York, pp 167–227

Evenden JL, Robbins TW (1983) Increased response switching, perseveration and perseverative switching following d-amphetaine in the rat. Psychopharmacology 80:67–73

Fetterman JG, Killeen P (1992) Time discrimination in Columba livia and Homo sapiens. J Exp Psychol Anim Behav Process 18:80–94

Fetterman JG, Killeen PR (1995) Categorical scaling of time: implications for clock-counter models. J Exp Psychol Anim Behav Process 21:43–63

Frederick DL, Allen JD (1996) Effects of selective dopamine D1 and D2 agonists and antagonists on timing performance in rats. Pharmacol Biochem Behav 53:759–764

Gibbon J, Malapani C, Dale CL, Gallistel CR (1997) Toward a neurobiology of temporal cognition: advances and challenges. Curr Opin Neurobiol 7:170–184

Harrison AA, Everitt BJ, Robbins TW (1997) Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. Psychopharmacology 133:329–342

Heffner TG, Drawbaugh RB, Zigmond MJ (1974) Amphetamine and operant behavior in rats: relationship between drug effect and control response rate. J Comp Physiol Psychol 86:1031–1043

Hinton SC, Meck WH (1997) How time flies: functional and neural mechanisms of interval timing. In: Bradshaw CM, Szabadi E (eds) Time and behaviour: psychological and neurobehavioural analyses. Elsevier, Amsterdam, pp 409–458

Ho M-Y, Al-Zahrani SSA, Velazquez Martinez DN, Lopez Cabrera M, Bradshaw CM, Szabadi E (1995) The role of the ascending 5-hydroxytryptaminergic pathways in timing behaviour: further observations with the interval bisection task. Psychopharmacology 120:213–219

Ho M-Y, Al-Zahrani SSA, Velazquez Martinez DN, Lopez Cabrera M, Bradshaw CM, Szabadi E (1996) Effects of desipramine and fluvoxamine on timing behaviour investigated with the fixed-interval peak procedure and the interval bisection task. Psychopharmacology 125:274–284

Ho M-Y, Al-Zahrani SSA, Al-Ruwaitea ASA, Bradshaw CM, Szabadi E (1998) 5-Hydroxytryptamine and impulse control: prospects for a behavioural analysis. J Psychopharmol 12:68–78

Killeen P, Fetterman JG (1988) A behavioral theory of timing. Psychol Rev 95:274–295

Kraemer PJ, Randall CK, Dose JM, Brown RW (1997) Impact of *d*-amphetamine on temporal estimation in pigeons tested with a production procedure. Pharmacol Biochem Behav 58:165–173

Laties VG (1972) The modification of drug effects on behavior by external discriminative stimuli. J Pharmacol Exp Ther 183:1–13

Laties VG, Wood RW, Cooper Rees D (1981) Stimulus control and the effects of d-amphetamine in the rat. Psychopharmacology 75:277–282

Lejeune H, Hermans I, Mocaer E, Rettori M, Poignant JC, Richelle M (1995) Amineptine, response timing, and time discrimination in the albino rat. Pharmacol Biochem Behav 51:165–173

Lewis D (1960) Quantitative methods in psychology. Springer, Berlin Heidelberg New York

Maricq AV, Church RM (1983) The differential effects of haloperidol and methamphetamine on time estimation in the rat. Psychopharmacology 79:10–15

Maricq AV, Roberts S, Church RM (1981) Methamphetamine and time estimation. J Exp Psychol Anim Behav Process 7:18–30

Meck WH (1983) Selective adjustment of the speed of internal clock and memory storage processes. J Exp Psychol Anim Behav Process 9:171–201

Meck WH (1986) Affinity for the dopamine D_2 receptor predicts neuroleptic potency in decreasing the speed of an internal clock. Pharmacol Biochem Behav 25:1185–1189

Meck WH (1996) Neuropharmacology of timing and time perception. Cogn Brain Res 3:227–242

Morley MJ, Bradshaw CM, Szabadi E (1987) DSP4 alters the effect of *d*-amphetamine on variable-interval performance: analysis in terms of Herrnstein's equation. Psychopharmacology 92:247–253

Morrissey G, Wogar MA, Bradshaw CM, Szabadi E (1993) Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on timing behaviour investigated with an interval bisection task. Psychopharmacology 112:80–85

Morrissey G, Ho M-Y, Wogar MA, Bradshaw CM, Szabadi E (1994) Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on timing behaviour investigated with the fixed-interval peak procedure. Psychopharmacology 114:463–468

Paton DM (1975) Structure-activity relations for the acceleration of efflux of noradrenaline from adrenergic nerves in rabbit aorta by sympathomimetic amines. Can J Physiol Pharmacol 53:822–829

Platt JR (1979) Temporal differentiation and the psychophysics of time. In: Zeiler MD, Harzem P (eds) Advances in the analysis of behaviour, vol 1: reinforcement and the organization of behaviour. Wiley, Chichester, pp 1–29

Rapp DL, Robbins TW (1976) The effects of *d*-amphetamine on temporal discrimination in tyhe rat. Psychopharmacology 51:91–100

Reid WD (1970) Turnover rate of brain 5-hydroxytryptamine increased by amphetamine. Br J Pharmacol 40:483–491

Robbins TW, Evenden J (1985) Rate-independent approaches to the analysis of drug action. In: Lowe CF, Richelle M, Blackman DE, Bradshaw CM (eds) Behaviour analysis and contemporary psychology. Erlbaum, London, pp 217–256

Robbins TW, Watson BA (1981) Effects of *d*-amphetamine on response repetition and win-stay behaviour in the rat. In: Bradshaw CM, Szabadi E, Lowe CF (eds) Quantification of steady-state operant behaviour. Elsevier/North Holland, Amsterdam, pp 441–444

Roberts S (1981) Isolation of an internal clock. J Exp Psychol Anim Behav Process 7:242–268

Rosenbaum DA, Collyer CE (1998) (eds) Timing of behavior: neural, psychological and computational perspectives. MIT Press, Cambridge

Sanger DJ, Blackman DE (1976) Rate-dependent effects of drugs: a review of the literature. Pharmacol Biochem Behav 4:73–83

Santi A, Weise L, Kuiper D (1995) Amphetamine and memory for event duration in rats and pigeons: disruption of attention to temporal samples rather than changes in the speed of the internal clock. Psychobiology 23:224–232

Seiden LS, Sabol KE, Ricaurte GA (1993) Amphetamine: effects on catecholamine systems and behavior. Ann Rev Pharmacol Toxicol 32:639–677

Sloviter RS, Drust EG, Connor JD (1978) Evidence that serotonin mediates some behavioral effects of amphetamine. J Pharmacol Exp Ther 206:348–352

Stanford L, Santi A (1998) The dopamine D2 agonist quinpirole disrupts attention to temporal signals without selectively altering the speed of the internal clock. Psychobiology 26:258–266

Stephens DN, Voet B (1994) Differential effects of anxiolytic benzodiazepine receptor ligands on performance of a differential reinforcement of low rate (DRL) schedule. Behav Pharmacol 5:4–14

Stubbs DA (1976) Scaling of stimulus duration by pigeons. J Exp Anal Behav 26:15–25

Stubbs DA (1979) Temporal discrimination and psychophysics. In: Zeiler MD, Harzem P (eds) Advances in analysis of behaviour, vol 1: reinforcement and the organization of behaviour. Wiley, Chichester, pp 341–369

Stubbs DA, Thomas JR (1974) Discrimination of stimulus duration and *d*-amphetamine in pigeons: a psychophysical analysis. Psychopharmacologia 36:313–322

Winer BJ (1971) Statistical principles in experimental design, 2nd edn. McGraw-Hill, New York

Wogar MA, Bradshaw C, Szabadi E (1992) Impaired acquisition of temporal differentiation performance following lesions of the ascending 5-hydroxytryptaminergic pathways. Psychopharmacology 111:373–378

Wogar MA, Bradshaw CM, Szabadi E (1993) Does the effect of central 5-hydroxytryptamine depletion on timing depend on motivational change. Psychopharmacology 112:86–92

Wortley KE, Hughes ZA, Heal DJ, Stanford SC (1999) Comparison of changes in the extracellular concentrations of noradrenaline in rat frontal cortex induced by sibutramine or *d*-amphetamine: modulation by α_2-adrenoceptors. Br J Pharmacol 127:1860–1866

Zeiler MD (1977) Schedules of reinforcement. In: Honig WK, Staddon JER (eds) Handbook of operant behavior. Prentice-Hall, Englewood Cliffs, pp 201–232