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ORIGINAL INVESTIGATION

Evidence for a role of 5-HT_{2C} receptors in the motor aspects of performance, but not the efficacy of food reinforcers, in a progressive ratio schedule

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Abstract
Rationale 5-Hydroxytryptamine$_{2C}$ (5-HT_{2C}) receptor agonists reduce the breakpoint in progressive ratio schedules of reinforcement, an effect that has been attributed to a decrease of the efficacy of positive reinforcers. However, a reduction of the breakpoint may also reflect motor impairment. Mathematical models can help to differentiate between these processes.
Objective The effects of the 5-HT_{2C} receptor agonist Ro-600175 ((α*S*)-6-chloro-5-fluoro-α-methyl-1*H*-indole-1-ethanamine) and the non-selective 5-HT receptor agonist 1-(*m*-chlorophenyl)piperazine (mCPP) on rats' performance on a progressive ratio schedule maintained by food pellet reinforcers were assessed using a model derived from Killeen's Behav Brain Sci 17:105–172, 1994 general theory of schedule-controlled behaviour, 'mathematical principles of reinforcement'.
Method Rats were trained under the progressive ratio schedule, and running and overall response rates in successive ratios were analysed using the model. The effects of the agonists on estimates of the model's parameters, and the sensitivity of these effects to selective antagonists, were examined.
Results Ro-600175 and mCPP reduced the breakpoint. Neither agonist significantly affected *a* (the parameter expressing incentive value), but both agonists increased *δ* (the parameter expressing minimum response time). The effects of both agonists could be attenuated by the selective 5-HT_{2C} receptor antagonist SB-242084 (6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide). The effect of mCPP was not altered by isamoltane, a selective 5-HT_{1B} receptor antagonist, or MDL-100907 ((±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)methanol)), a selective 5-HT_{2A} receptor antagonist.
Conclusions The results are consistent with the hypothesis that the effect of the 5-HT_{2C} receptor agonists on progressive ratio schedule performance is mediated by an impairment of motor capacity rather than by a reduction of the incentive value of the food reinforcer.

Keywords Progressive ratio schedule · Mathematical principles of reinforcement · Mathematical model · 5-HT_{2C} receptors · mCPP · Ro-600175 · Incentive value · Motor capacity · Rats

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Introduction

5-Hydroxytryptamine$_{2C}$ (5-HT_{2C}) receptors are widely distributed in the brain and are known to play an important role in the regulation of dopaminergic function (see Alex et al. 2005; Alex and Pehek 2007; Dekeyne et al. 2008; Di Matteo et al. 2008; Filip and Bader 2009). They exert an inhibitory influence on the activity of dopaminergic neurones of the nigrostriatal and mesolimbic pathways and reduce dopamine release in the projection regions of both these pathways (Millan et al. 1998; Di Matteo et al. 1999, 2004; Di Giovanni et al. 2001; Porras et al. 2002; De Deurwaerdere et al. 2004; Alex et al. 2005; Invernizzi et al. 2007; Bubar et al. 2011).

5-HT_{2C} receptor agonists suppress operant behaviour maintained by many types of positive reinforcer, including

food (Grottick et al. 2000; Fletcher et al. 2010; Cunningham et al. 2011; Higgins et al. 2012, 2013), nicotine (Grottick et al. 2001; Fletcher et al. 2008; Levin et al. 2011; Higgins et al. 2012), ethanol (Tomkins et al. 2002) and cocaine (Grottick et al. 2000; Fletcher et al. 2004; Burbassi and Cervo 2008; Katsidoni et al. 2011). These effects, which are believed to reflect the inhibition of mesolimbic dopaminergic activity (Fletcher et al. 2004; Katsidoni et al. 2011; Filip et al. 2012), have led to the suggestion that 5-HT_{2C} receptor agonists may prove to be effective treatments for clinical obesity (Martin et al. 1998; Bickerdike 2003; Halford et al. 2005; Miller 2005; Nilsson 2006; Marston and Heisler 2009) and drug addiction (Bubar and Cunningham 2006; Filip et al. 2004, 2010, 2012; Cunningham et al. 2011).

A behavioural test that is often used to assess the effects of drugs on the efficacy of reinforcers is the progressive ratio schedule. In this schedule, the subject is required to emit a specified number of responses, *N*, to obtain a reinforcer. *N* is systematically increased, usually from one reinforcer to the next (Hodos 1961; Stafford and Branch 1998) but sometimes after batches of two or more reinforcers (Baunez et al. 2002; Randall et al. 2012) or between successive sessions (Griffiths et al. 1978; Czachowski and Samson 1999). Responding on progressive ratio schedules is usually rapid under low ratios but declines towards zero as *N* is increased. The ratio at which the subject stops responding, the breakpoint, is widely regarded as a measure of subject's motivation or the incentive value of the reinforcer (for review, see Ping-Teng et al. 1996; Killeen et al. 2009; see below for further discussion).

There is abundant evidence that 5-HT_{2C} receptor agonists, including relatively non-selective compounds such as *m*-chlorophenylpiperazine (mCPP) and MK-212 (6-chloro-2-(l-piperazinyl)pyrazine), and a range of newer, more selective compounds such as Ro-600175 ((α*S*)-6-chloro-5-fluoro-α-methyl-1*H*-indole-1-ethanamine), lorcaserin ((1R)-8-chloro-1-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) and CP-809101 (2-(3-chlorobenzyloxy)-6-(piperazin-1-yl)pyrazine), reduce the breakpoint in progressive ratio schedules using various types of positive reinforcer, including food (Wolff and Leander 2000; Fletcher et al. 2004, 2010, 2012; Ward et al. 2008; Higgins et al. 2012, 2013), cocaine (Grottick et al. 2000; Fletcher et al. 2004, 2008, 2012; Burbassi and Cervo 2008), ethanol (Tomkins et al. 2002) and nicotine (Grottick et al. 2001; Levin et al. 2011; Fletcher et al. 2012; Higgins et al. 2012). These effects can be blocked by the highly selective 5-HT_{2C} receptor antagonist SB-242084 (6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide) (Grottick et al. 2000; Tomkins et al. 2002; Fletcher et al. 2004, 2010, 2012; Higgins et al. 2012, 2013). Insofar as the breakpoint constitutes a valid measure of motivation or the incentive value of positive reinforcers, these findings constitute strong support for the proposal that 5-HT_{2C} receptors play an inhibitory role in reinforcement processes (Higgins and Fletcher 2003; Fletcher et al. 2010, 2012; Filip et al. 2012).

However, notwithstanding its compelling face validity as a measure of motivation or incentive value, several authors have counselled circumspection in the interpretation of the breakpoint. A major concern is the fact that the breakpoint is sensitive not only to changes in the incentive properties of reinforcers (Rickard et al. 2009) but also to non-motivational manipulations such as changes in the response requirement (Skjoldager et al. 1993; Aberman et al. 1998) and the ratio step size (Covarrubias and Aparicio 2008). Moreover, the breakpoint has some technical shortcomings as a behavioural measure: For example, it shows considerable variability, being derived from a single time point, data from the rest of the session being ignored (Arnold and Roberts 1997; Killeen et al. 2009), and its definition is arbitrary, there being no consensus as to the time that must elapse without a response before responding may be said to have stopped (Arnold and Roberts 1997; Killeen et al. 2009).

Quantitative analyses that take into account the response rate in each component ratio of the schedule avoid some of these pitfalls. In this paper, we used a model (Bradshaw and Killeen 2012) derived from Killeen's general theory of schedule-controlled behaviour, the mathematical principles of reinforcement (MPR; Killeen 1994), to analyse the effects of mCPP and Ro-600175 on performance on a progressive ratio schedule. The theoretical basis of this model is outlined below.

According to MPR, schedule-controlled responding is determined by an excitatory effect of reinforcers on behaviour, biological constraints on responding and the efficiency with which schedules couple responses to reinforcers. In addition, the model derived from MPR to account for performance on progressive ratio schedules (Bradshaw and Killeen 2012) invokes the linear waiting principle (Wynne et al. 1996) to predict the escalating post-reinforcement pause duration in successive ratios, enabling it to provide a dynamic account of performance on these schedules. The linear waiting principle expresses the empirical finding that the post-reinforcement pause on trial *i*, $T_{P,i}$, is linearly related to the total inter-reinforcement interval on trial *i*-1, $T_{TOT,i\text{-}1}$:

$$T_{P,i} = T_0 + k\, T_{TOT,i-1}, \tag{1}$$

where T_0 and *k* are parameters that define the minimum post-reinforcement pause and the slope of the linear waiting function. The model contains two key equations that define running response rate, R_{RUN}, and overall response rate, $R_{OVERALL}$:

$$R_{RUN,i} = \frac{1}{\delta\left(1 \quad + T_{TOT,i-1}/a\right)} \tag{2}$$

$$R_{OVERALL,i} = N_i / T_{TOT,i}. \tag{3}$$

The parameter *a* ('specific activation') is defined as the duration of behavioural activation induced by a single reinforcer and is regarded as an index of incentive value, and δ is the minimum time needed to execute a response (the reciprocal of the maximum response rate) and is regarded as a measure of the biological limitations on responding (Killeen 1994; Reilly 2003; Covarrubias and Aparicio 2008; Sanabria et al. 2008; Bradshaw and Killeen 2012).

The model has been used to analyse the effects of a number of interventions on progressive ratio schedule performance. Consistent with the interpretation of *a* as an index of incentive value, it has been found that this parameter is monotonically related to the volume of a sucrose-solution reinforcer (Rickard et al. 2009; data re-analysed by Bradshaw and Killeen 2012) and that values of *a* for corn oil and sucrose are concordant with extant evidence for the greater incentive value of the former reinforcer when equal volumes of the two reinforcers are compared (Olarte-Sánchez et al. 2013). Recently, Valencia-Torres et al. (2014) found that streptozotocin-induced diabetes reduces *a*, consistent with an anti-hedonic effect of this treatment (Nefs et al. 2012). D_1 and D_2 receptor antagonists also reduce *a* (Olarte-Sánchez et al. 2012a; data re-analysed by Bradshaw and Killeen 2012; Olarte-Sánchez et al. 2012a, b), while some drugs with known sedative properties, including clozapine and cyproheptadine, also increase the response-time parameter δ (Olarte-Sánchez et al. 2012a; data re-analysed by Bradshaw and Killeen 2012).

In the present experiments, we used the progressive ratio model to analyse the effects of two 5-HT_{2C} receptor agonists, mCPP and Ro-600175, on performance on a progressive ratio schedule. It was expected that these agonists would reduce the breakpoint, in keeping with previous findings (see above). The main purpose of the experiments was to examine whether the predicted reduction of the breakpoint would be associated with a reduction of *a* and/or an increase in δ, since, according to the model, a reduction of the breakpoint may reflect a reduction of the incentive value of the food reinforcer (represented by *a*) and/or an impairment of motor performance (represented by δ). The ability of the 5-HT_{2C} receptor antagonist SB-242084 to reverse the effects of mCPP and Ro-600175 was examined, and in the case of mCPP, which also has considerable affinity for 5-HT_{1B} and 5-HT_{2A} receptors, the ability of antagonists of these receptor subtypes (isamoltane and MDL-100907 ((±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)methanol)), respectively) to reverse the effect of the agonist was also examined.

Methods

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

Subjects

Female Wistar rats (Charles River, UK) approximately 4 months old 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 (light on 0600–1800 h) and were maintained at 80 % of their initial free-feeding body weights throughout the experiment by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages, and environmental enrichment (cardboard tunnels and wooden chew blocks) was provided, as prescribed by the local Ethics Committee.

Apparatus

The rats were trained in operant conditioning chambers of internal dimensions 20×23×22.5 cm (Campden Instruments Ltd., UK). One wall of the chamber contained a recess into which a motor-operated pellet dispenser could deliver 45-mg food pellets (TestDiet products, formula 5TUM). An aperture located 5 cm above and 2.5 cm to one side of the recess (left for half the subjects; right for the other half) allowed insertion of a motorised retractable lever into the chamber. The lever could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest with additional masking noise (approximately 80 dB[A]) generated by a rotary fan. No houselight was present during the sessions. An Acorn microcomputer programmed in Arachnid BASIC (CeNeS Ltd, Cambridge, UK) located in an adjacent room controlled the schedule and recorded the behavioural data.

Behavioural training

Two weeks before starting the experiment, the food deprivation regimen was introduced and the rats were gradually reduced to 80 % of their free-feeding body weights. The rats were first trained to press the lever for the food pellet reinforcer and were then exposed to a fixed ratio 1 schedule for 3 days followed by fixed ratio 5 for a further 3 days. Thereafter, they underwent daily training sessions under the progressive ratio schedule. The progressive ratio schedule was based on the exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, …, derived from the formula $(5\times e^{0.2n})-5$, rounded to the nearest integer, where *n* is the position in the ratio sequence (Roberts and Richardson 1992). Sessions took place at the same time each day during the light phase of the daily cycle (between 0800 and 1300 h) 7 days a week. Throughout all phases of the experiment, the lever was inserted into the chamber at the start of each session; the session was terminated by withdrawal of the lever 50 min later.

Drug treatment

The drug treatment regimen started after 100 sessions of preliminary training under the progressive ratio schedule. Injections of drugs were given on Tuesdays and Fridays and injections of the vehicle alone on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. The numbers of rats tested with each drug are listed below; each rat was tested five times with each dose of each drug, the order of treatments being counterbalanced across animals according to a Latin square design. Drugs were injected intraperitoneally (2.5 ml kg^{-1}; 25-gauge needle) or subcutaneously (1.0 ml kg^{-1}, 26 gauge needle) 15–40 min before the start of the experimental session (see below). The times of administration of the vehicles were matched to those of the drugs used in each experiment. The doses of the drugs were calculated from the weights of the salts. Five experiments are described in this report.

mCPP (n=16) mCPP HCl, 0.625, 1.25 and 2.5 mg kg^{-1}, was dissolved in 0.9 % NaCl solution. It was injected intraperitoneally 15 min before the start of the experimental session. The doses of mCPP were selected on the basis of previous behavioural experiments with rats using this drug (e.g. Khaliq et al. 2008; Papakosta et al. 2013; Body et al. 2014).

Interaction between mCPP and SB-242084 (n=16) mCPP 2.5 mg kg^{-1} was administered either alone or in combination with 6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide dihydrochloride (SB-242084) 0.3 mg kg^{-1}. SB-242084 was dissolved in a 0.9 % NaCl solution containing 10 % cyclodextrin with 25 mM citric acid; it was injected intraperitoneally 40 min before the start of the experimental session. mCPP was prepared and administered as described above. The dose of SB-242084 was selected on the basis of previous behavioural experiments with rats using this drug (e.g. Fletcher et al. 2006; Schepisi et al. 2013; Body et al. 2014) and pilot experiments carried out for the present experiment.

Interaction between mCPP and isamoltane (n=15) mCPP 2.5 mg kg^{-1} was administered either alone or in combination with isamoltane fumarate 8.0 mg kg^{-1}. Isamoltane was dissolved in a 0.9 % NaCl solution containing 10 % cyclodextrin with 25 mM citric acid; it was injected intraperitoneally 30 min before the start of the experimental session. mCPP was prepared and administered as described above. The dose of isamoltane was selected on the basis of previous behavioural experiments with rats using this drug (e.g. Ahlenius and Larsson 2000; Shimazoe et al. 2004; Body et al. 2014) and pilot experiments carried out for the present experiment.

Interaction between mCPP and MDL-100907 (n=15) mCPP 2.5 mg kg^{-1} was administered either alone or in combination with (±)2,3-dimethoxyphenyl-1-(2-(4-piperidine)-methanol) (MDL-100907) 0.5 mg kg^{-1}. MDL-100907 was dissolved in glacial acetic acid, buffered to pH 5.5 using NaOH and diluted to volume using 0.9 % NaCl solution; it was injected intraperitoneally 25 min before the start of the experimental session. mCPP was prepared and administered as described above. The dose of MDL-100907 was selected on the basis of previous behavioural experiments with rats using this drug (e.g. Fletcher et al. 2012; Body et al. 2006a, b, 2014).

Interaction between Ro-600175 and SB-242084 (n=13) (α*S*)-6-Chloro-5-fluoro-α-methyl-1*H*-indole-1-ethanamine fumarate (Ro-600175) 4.0 mg kg^{-1} administered either alone or in combination with SB-242084 0.3 mg kg^{-1}. Ro-60175 was dissolved in 0.9 % NaCl solution. It was injected intraperitoneally 30 min before the start of the experimental session. SB-242084 was prepared and administered as described above. The dose of Ro-600175 was selected on the basis of previous behavioural experiments with rats using this drug (e.g. Fletcher et al. 2008, 2012; Body et al. 2014) and pilot experiments carried out for the present experiment.

mCPP was obtained from Sigma Chemical Company (Poole, UK) and isamoltane from Tocris Cookson (Avonmouth, UK); Ro-600175, SB-242084 and MDL-100907 were a generous gift from Solvay Pharmaceuticals (Weesp, The Netherlands).

Data analysis

Overall response rate ($R_{OVERALL}$) was calculated for each ratio by dividing the number of responses by the total time taken to complete the ratio, including the post-reinforcement pause, measured from the end of the preceding reinforcer delivery until the emission of the last response of the ratio (Bizo and Killeen 1997). The first ratio (a single response) and any ratios that had not been completed at the end of the session were excluded from the analysis. Running rate (R_{RUN}) was calculated by dividing the number of responses by the 'run-time' (i.e. the time taken to complete the ratio, excluding the post-reinforcement pause; Bizo et al. 2001). Post-reinforcement pause duration was measured from the end of the reinforcer delivery until the emission of the first response of the following ratio.

The breakpoint was defined as the last ratio to be completed before 5 min elapsed without any responding or, in cases where this criterion was not met within the session, the highest completed ratio (Olarte-Sánchez et al. 2012a, b).

The PR model comprising Eqs. 2 and 3 was fitted to the running and overall response rate data obtained from individual rats, and estimates of the four parameters, T_0, k, a and δ, were derived using the 'Solver' facility of Excel (Microsoft

Corporation); goodness of the combined fit of Eqs. 2 and 3 to the overall and running response rate data was expressed as R^2 (see Bradshaw and Killeen 2012).

For each experiment, the model was fitted to the data obtained from each rat in the sessions in which injections of the drug or its corresponding vehicle were administered and estimates of the four parameters were derived. These estimates, and the breakpoint, were analysed by separate one-factor analyses of variance with treatment condition as a within-subject factor followed, in the case of a significant effect of treatment, by comparison of each active treatment with the vehicle-alone treatment using Dunnett's test. In the case of the drug interaction experiments, when a significant effect of the agonist was identified, planned comparisons were made between the values of the measures obtained in the agonist-alone condition and the agonist+antagonist condition using Student's *t* test for paired comparisons. The effect sizes revealed by the analyses of variance were expressed as partial η^2 (η^2_p). A significance criterion of $p<0.05$ was adopted in all statistical analyses (two-tailed comparisons in the case of the post hoc tests).

Results

Performance on the progressive ratio schedule was in good accord with the model. Running response rate declined monotonically towards zero, whereas overall response rate rose to a peak before declining towards zero. The model provided a good description of the group mean data (see Figs. 1, 2, 3, 4 and 5) and the data obtained from individual rats (see Tables 1, 2, 3, 4 and 5), as indicated by the values of R^2 which were in most cases >0.9.

Effect of mCPP

The group mean response rate data are shown in Fig. 1, and the measures derived from the individual rats under all treatment conditions are shown in Table 1.

Analysis of variance showed a significant effect of treatment on the value of δ [$F(3,45)=15.4$, $p<0.001$; $\eta^2_p=0.51$], reflecting a dose-related reduction of the maximum running response rate; the linear contrast effect was statistically significant [$F(1,15)=31.5$, $p<0.01$], and multiple comparisons indicated that the highest dose of mCPP significantly increased the value of δ. There was a significant effect of treatment on k [$F(3,45)=13.8$, $p<0.001$; $\eta^2_p=0.49$]; the linear contrast effect was statistically significant [$F(1,15)=27.2$, $p<0.001$], and multiple comparisons showed that the highest dose of mCPP significantly reduced this parameter. There were no significant effects of treatment on T_0 ($F<1$, not significant (*N.S.*); $\eta^2_p=0.06$) or a ($F<1$, *N.S.*; $\eta^2_p=0.05$).

Treatment with mCPP was associated with a reduction of the breakpoint [$F(3,45)=14.7$, $p<0.001$; $\eta^2_p=0.50$]; the linear contrast effect was statistically significant [$F(1,15)=19.7$, $p<0.001$], and multiple comparisons indicated that all three doses produced a significant reduction of the breakpoint.

Interaction between mCPP and SB-242084

The group mean response rate data are shown in Fig. 2, and the measures derived from the individual rats under all treatment conditions are shown in Table 2.

There was a significant effect of treatment on δ [$F(3,45)=10.1$, $p<0.001$; $\eta^2_p=0.40$], reflecting a reduction of the maximum running response rate by mCPP. Multiple comparisons with the vehicle-alone condition indicated that both mCPP-alone and the combined mCPP+SB-242084 treatment increased the value of this parameter; the planned comparison between the mCPP-alone and combined mCPP+SB-242084 treatment conditions indicated that the increase induced by mCPP was significantly attenuated by co-administration of SB-242084. There was a significant effect of treatment on k [$F(3,45)=4.9$, $p<0.01$ $\eta^2_p=0.25$]. Multiple comparisons showed that only the combined mCPP+SB-242084 treatment reduced the value of this parameter. There were no significant effects of treatment on T_0 [$F(3,45)=2.5$, *N.S.*; $\eta^2_p=0.14$] or a ($F<1$, *N.S.*; $\eta^2_p=0.03$).

There was a significant effect of treatment on the breakpoint [$F(3,45)=14.6$, $p<0.001$, $\eta^2_p=0.49$]; multiple comparisons indicated that both the mCPP-alone and the combined mCPP+SB-242084 treatments reduced the breakpoint; the planned comparison between the mCPP-alone and combined mCPP+SB-242084 treatment conditions indicated that the reduction of the breakpoint induced by mCPP was significantly attenuated by co-administration of SB-242084.

Interaction between mCPP and isamoltane

The group mean response rate data are shown in Fig. 3, and the measures derived from the individual rats under all treatment conditions are shown in Table 3.

There was a significant effect of treatment on δ [$F(3,42)=8.3$, $p<0.001$; $\eta^2_p=0.37$], reflecting a reduction of the maximum running response rate by mCPP. Multiple comparisons with the vehicle-alone condition indicated that both the mCPP-alone and the combined mCPP+isamoltane treatments increased the value of this parameter; the planned comparison between the mCPP-alone and combined treatment conditions showed no significant effect of isamoltane on the increase in δ induced by mCPP. There were no significant effects of treatment on T_0 ($F<1$, *N.S.*; $\eta^2_p=0.06$), k [$F(3,42)=2.1$, *N.S.*; $\eta^2_p=0.12$] or a ($F<1$, *N.S.*; $\eta^2_p=0.04$).

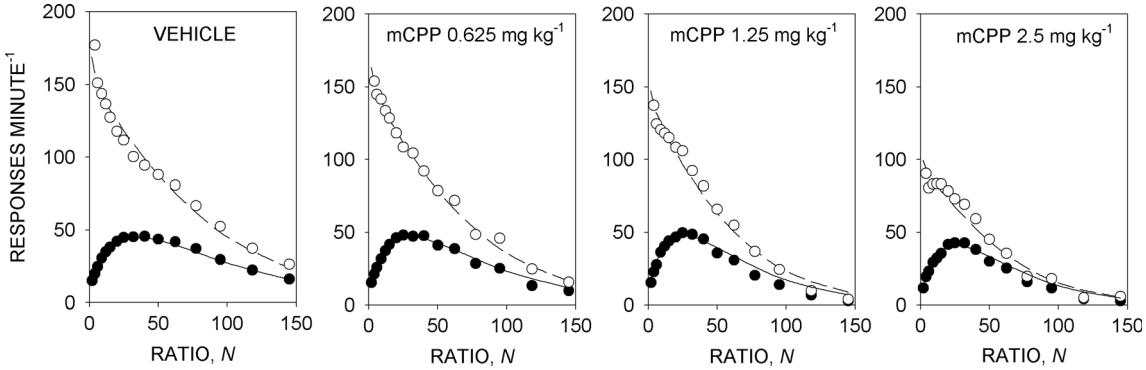

Fig. 1 Effect of mCPP on performance on the progressive-ratio schedule. *Ordinate*, response rate; *abscissa*, response/reinforcer ratio, *N*. Points are group mean data: *unfilled symbols* indicate running response rate, and *filled symbols* indicate overall response rate. The curves are best-fit functions defined by Eqs. 2 and 3 [R^2=0.991 (vehicle), 0.997 (mCPP 0.625 mg kg^{-1}), 0.992 (mCPP 1.25 mg kg^{-1}) and 0.982 (mCPP 2.5 mg kg^{-1})]

There was a significant effect of treatment on the breakpoint [$F(3,42)=21.0$, $p<0.001$; $\eta^2_p=0.60$]; multiple comparisons indicated that both mCPP-alone and the combined mCPP+SB-242084 treatments reduced the breakpoint, and the planned comparison between the mCPP-alone and combined treatment conditions showed no significant effect of isamoltane on the reduction of the breakpoint induced by mCPP.

Interaction between mCPP and MDL-100907

The group mean response rate data are shown in Fig. 4, and the measures derived from the individual rats under all treatment conditions are shown in Table 4.

There was a significant effect of treatment on δ [$F(3,42)=9.5$, $p<0.001$; $\eta^2_p=0.40$], reflecting a reduction of the maximum running response rate by mCPP. Multiple comparisons with the vehicle-alone condition indicated that mCPP alone and the combined treatment with mCPP and MDL-100907 increased the value of this parameter; the planned comparison between the mCPP and combined treatment conditions showed no significant effect of MDL-100907 on the increase in δ induced by mCPP. There were no significant effects of treatment on T_0 ($F<1$; *N.S.*, $\eta^2_p=0.01$), k [$F(3,42)=2.1$, *N.S.*; $\eta^2_p=0.12$] or a [$F(3,42)=1.4$, *N.S.*; $\eta^2_p=0.09$].

There was a significant effect of treatment on the breakpoint [$F(3,42)=15.9$, $p<0.001$; $\eta^2_p=0.53$]; multiple comparisons indicated that both mCPP alone and the combined treatment with mCPP and MDL-100907 reduced the breakpoint, and the planned comparison between the mCPP and combined treatment conditions showed no significant effect of MDL-100907 on the reduction of the breakpoint induced by mCPP.

Interaction between Ro-600175 and SB-242084

The group mean response rate data are shown in Fig. 5, and the measures derived from the individual rats under all treatment conditions are shown in Table 5.

There was a significant effect of treatment on δ [$F(3,36)=5.9$, $p<0.01$; $\eta^2_p=0.33$], reflecting a reduction of the maximum running response rate by Ro-600175. Multiple comparisons with the vehicle-alone condition indicated that only the Ro-600175-alone treatment increased the value of this parameter; the planned comparison

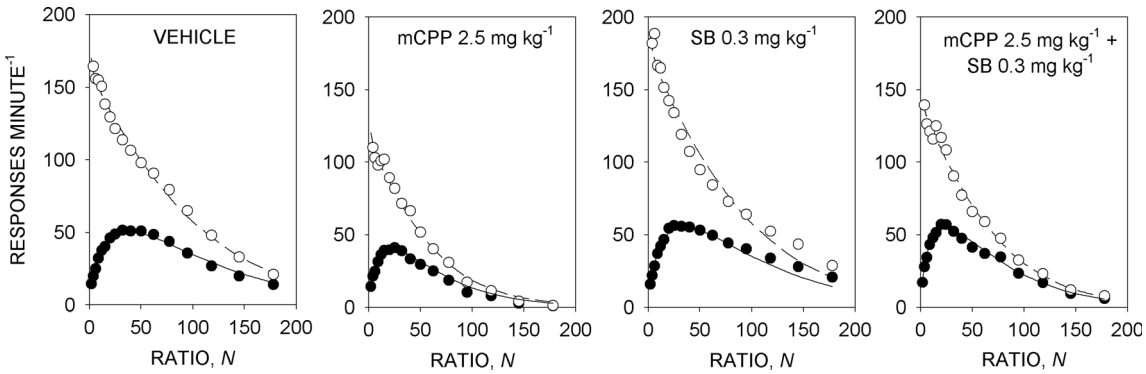

Fig. 2 Effects of mCPP, SB-242084 (*SB*) and combined treatment with mCPP and SB-242084 on performance on the progressive-ratio schedule. Conventions are as in Fig. 1 [R^2=0.997 (vehicle), 0.992 (mCPP 2.5 mg kg^{-1}), 0.987 (SB-242084 0.3 mg kg^{-1}) and 0.992 (mCPP 2.5 mg kg^{-1}+SB-242084 0.3 mg kg^{-1})]

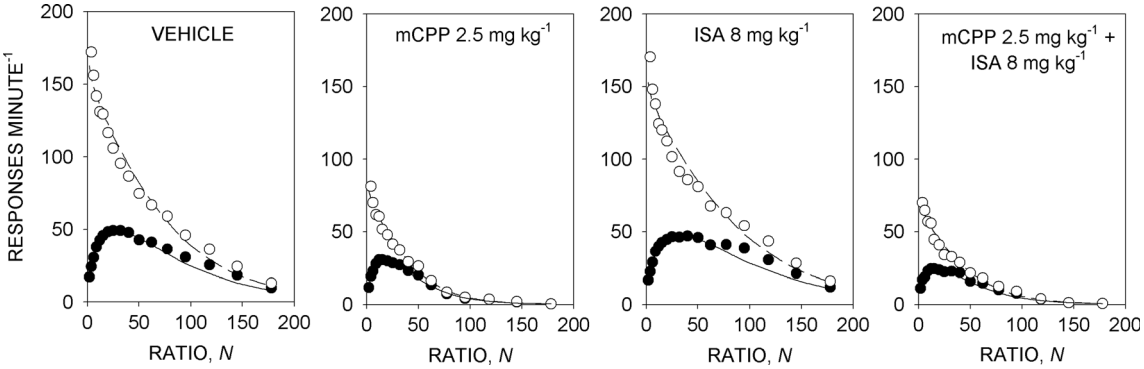

Fig. 3 Effects of mCPP, isamoltane (*ISA*) and combined treatment with mCPP and isamoltane on performance on the progressive-ratio schedule. Conventions are as in Fig. 1 [R^2=0.988 (vehicle), 0.990 (mCPP 2.5 mg kg^{-1}), 0.978 (isamoltane 8 mg kg^{-1}) and 0.984 (mCPP 2.5 mg kg^{-1}+isamoltane 8 mg kg^{-1})]

between the Ro-600175-alone and combined Ro-600175+SB-242084 treatment conditions indicated that the increase in δ induced by Ro-600175 was significantly attenuated by co-administration of SB-242084. There was a significant effect of treatment on T_0 [$F(3,36)=4.6$, $p<0.01$; $\eta^2_p=0.28$]. Multiple comparisons showed that all three active treatments reduced the value of this parameter; the planned comparison between the Ro-600175-alone and combined Ro-600175+SB-242084 treatment conditions indicated that the increase in T_0 induced by Ro-600175 was not altered by co-administration of SB-242084. There were no significant effects of treatment on k [$F(3,36)=1.6$, *N.S.*; $\eta^2_p=0.12$] or a [$F(3,36)=1.2$, *N.S.*; $\eta^2_p=0.09$].

There was a significant effect of treatment on the breakpoint [$F(3,36)=9.0$, $p<0.001$; $\eta^2_p=0.43$]; multiple comparisons indicated that only the Ro-600175-alone treatment reduced the breakpoint, and the planned comparison between the Ro-600175-alone and combined Ro-600175+SB-242084 treatment conditions indicated that the reduction of the breakpoint induced by Ro-600175 was significantly attenuated by co-administration of SB-242084.

Discussion

In agreement with many previous reports (Grottick et al. 2000; Wolff and Leander 2000; Tomkins et al. 2002; Fletcher et al. 2004, 2010, 2012; Ward et al. 2008), mCPP and Ro-600175 reduced the breakpoint in the progressive ratio schedule, and this effect was attenuated by the selective 5-HT$_{2C}$ receptor antagonist SB-242084. Although mCPP is an effective 5-HT$_{2C}$ receptor agonist, it also has substantial affinity for other 5-HT receptor subtypes, particularly 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors (Hoyer 1988; Hamik and Peroutka 1989; Dalton et al. 2004). Therefore, the ability of selective antagonists of these receptor subtypes to antagonise the effect of mCPP was also tested. Neither the 5-HT$_{1B}$ receptor antagonist isamoltane nor the 5-HT$_{2A}$ receptor antagonist MDL-100907 altered the effect of mCPP, suggesting that the effect of mCPP on the breakpoint was mediated mainly by 5-HT$_{2C}$ receptors. It is, of course, possible that the ineffectiveness of isamoltane and MDL-100907 reflected the use of inadequate doses; however, the doses used in these experiments were well within the range of doses that have proved adequate to reverse 5-HT$_{1B}$ and 5-

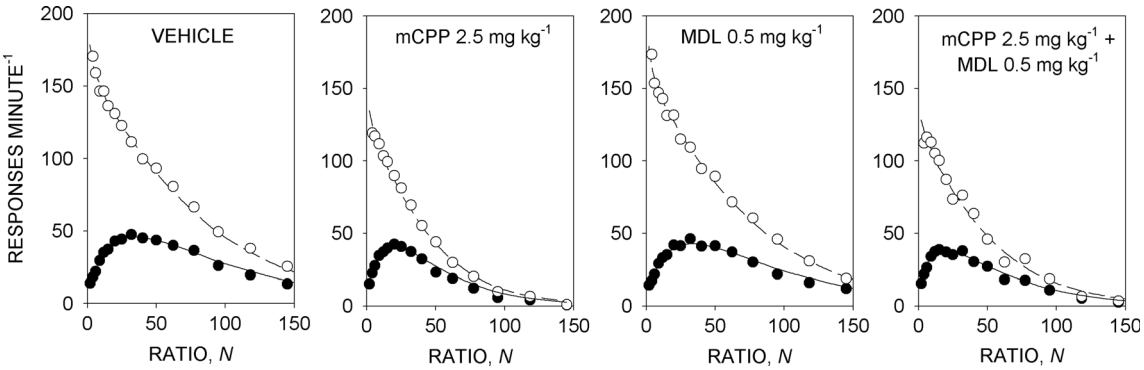

Fig. 4 Effects of mCPP, MDL-100907 (*MDL*) and combined treatment with mCPP and MDL-100907 on performance on the progressive-ratio schedule. Conventions are as in Fig. 1 [R^2=0.998 (vehicle), 0.996 (mCPP 2.5 mg kg^{-1}), 0.997 (MDL-100907 0.5 mg kg^{-1}) and 0.991 (mCPP 2.5 mg kg^{-1}+MDL-100907 0.5 mg kg^{-1})]

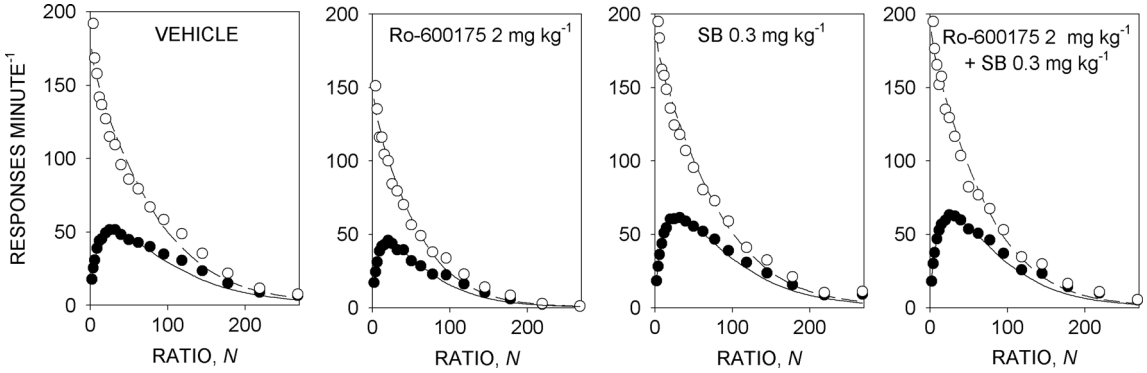

Fig. 5 Effect of Ro-600175, SB-242084 (*SB*) and combined treatment with Ro-600175 and SB-242084 on performance on the progressive-ratio schedule. Conventions are as in Fig. 1 [R^2=0.997 (vehicle), 0.992 (Ro-600175 4 mg kg^{-1}), 0.987 (SB-242084 0.3 mg kg^{-1}) and 0.992 (Ro-600175 4 mg kg^{-1}+SB-242084 0.3 mg kg^{-1})]

HT_{2A} receptor-mediated behavioural effects in previous experiments (e.g. Ahlenius and Larsson 2000; Shimazoe et al. 2004; Body et al. 2006b; Fletcher et al. 2012).

The reduction of the breakpoint by 5-HT_{2C} receptor agonists has generally been attributed to a motivational decrement, in keeping with the traditional interpretation of the breakpoint as an index of motivation or the incentive value of positive reinforcers (Hodos 1961; Hodos and Kalman 1963; see Ping-Teng et al. 1996; Killeen et al. 2009 for discussion). However, as mentioned in the 'Introduction', several authors have expressed misgivings about this interpretation of the breakpoint, in the light of a growing body of evidence that this measure is sensitive to ostensibly non-motivational manipulations such as changes in the response requirement (Skjoldager et al. 1993; Aberman et al. 1998) and the ratio step size (Covarrubias and Aparicio 2008). The possibility that a reduction of the breakpoint may arise from effects on motor functions as well as effects on motivation is especially pertinent in the case of drugs such as 5-HT_{2C} receptor agonists which have prominent effects on locomotor and other unconditioned behaviours (Kennett and Curzon 1988; Lucki et al. 1989; Kennett et al. 2000; Gleason et al. 2001; Higgins et al. 2001; Mosher et al. 2005; Steidl et al. 2007; Wright and Rodgers 2014). The mathematical model used in these experiments offers the prospect of discriminating between motivational and motor processes that may be confounded in the breakpoint (Bradshaw and Killeen 2012).

In agreement with previous findings (data re-analysed by Bradshaw and Killeen 2012; Olarte-Sánchez et al. 2012a, b; Valencia-Torres et al. 2014), response rates in successive ratios of the progressive ratio schedule were well described by the model. Running response rate declined monotonically as the schedule progressed (Eq. 2), while overall response rate followed an inverted U function, increasing in the early ratios and then declining in later ones (Eq. 3). As discussed elsewhere (Bradshaw and Killeen 2012), the model provides a more satisfactory description of performance than the earlier model designed to account for fixed-ratio schedule performance (Killeen 1994). Unlike the fixed-ratio model, which treats response rates in successive ratios as though they were independent of one another, the progressive ratio model takes the dynamic nature of the schedule into account and thereby correctly predicts a curvilinear descent of response rates in successive ratios, rather than the linear approximation provided by the fixed-ratio model. The progressive ratio model also distinguishes between two functionally important aspects of performance which are amalgamated in the fixed-ratio model: post-reinforcement pausing (represented by the linear waiting

Table 1 Effects of mCPP on the indices of performance on the progressive ratio schedule (group mean values±SEM)

Performance index	Vehicle	mCPP 0.625 mg kg^{-1}	mCPP 1.25 mg kg^{-1}	mCPP 2.5 mg kg^{-1}
Breakpoint	125.7±12.9	108.7±10.8[a]	89.1±6.9[a]	77.5±6.8[a]
Parameters of the PR model				
T_0, s	7.23±0.59	7.78±1.51	6.35±0.85	8.52±0.99
k	0.51±0.04	0.48±0.05	0.42±0.04	0.19±0.04[a]
a, s	39.7±7.2	33.6±5.1	31.1±4.0	35.0±5.1
δ, s	0.27±0.03	0.25±0.03	0.32±0.03	0.47±0.05[a]
R^2	0.96±0.01	0.93±0.01	0.91±0.01	0.84±0.02

mCPP *m*-chlorophenylpiperazine

[a] Significantly different from vehicle control (p<0.05)

Table 2 Effects of mCPP and SB-242084 on the indices of performance on the progressive-ratio schedule (group mean values±SEM)

Parameter	Vehicle	mCPP 2.5 mg kg^{-1}	SB-242084 0.3 mg kg^{-1}	SB-242084 0.3 mg kg^{-1}+mCPP 2.5 mg kg^{-1}
Breakpoint	136.3±12.4	77.7±6.6[a]	153.3±17.8	105.5±10.3[a,b]
Parameters of the PR model				
T_0, s	7.07±0.90	5.25±0.68	6.27±1.00	4.86±0.47
k	0.47±0.04	0.45±0.05	0.49±0.05	0.34±0.04[a,b]
a, s	44.5±7.7	39.6±5.5	40.4±8.4	33.9±5.7
δ, s	0.28±0.03	0.43±0.04[a]	0.24±0.03	0.34±0.02[a,b]
R^2	0.95±0.01	0.88±0.02	0.91±0.02	0.89±0.01

mCPP *m*-chlorophenylpiperazine

[a] Significantly different from vehicle control ($p<0.05$)

[b] Significantly different from mCPP 2.5 mg kg^{-1} ($p<0.05$)

parameters T_0 and k) and inter-response times during trains of responses (represented by the response time parameter δ) (for further discussion, see Bradshaw and Killeen 2012).

Neither mCPP nor Ro-600175 had a significant effect on the 'motivational' parameter, a, which has been proposed as a metric of the incentive value or efficacy of positive reinforcers (Reilly 2003; Sanabria et al. 2008). On the other hand, both agonists significantly increased the value of the 'motor' parameter, δ, reflecting suppression of the maximum running response rate. The effects of the agonists on δ mirrored their effects on the breakpoint, in that the increase in δ was attenuated by the 5-HT$_{2C}$ receptor antagonist SB-242084, whereas, in the case of mCPP, the effect was impervious to the 5-HT$_{1B}$ receptor antagonist isamoltane and the 5-HT$_{2A}$ receptor antagonist MDL-100907. These results are consistent with the notion that the increases in δ induced by the agonists were mediated by 5-HT$_{2C}$ receptor stimulation. From the theoretical standpoint of MPR, the pattern of effect of the agonists on δ and a indicates that the agonists induced a decrement of the motor aspects of performance but had no effect on the incentive value of food.

The exact nature of the effect of the agonists on motor functioning is uncertain. It is well known that 5-HT$_{2C}$ receptor agonists, including mCPP and Ro-600175, reduce spontaneous locomotion (Kennett and Curzon 1988; Lucki et al. 1989; Kennett et al. 1997, 2000; Gleason et al. 2001; Steidl et al. 2007; Higgins et al. 2001; Fletcher et al. 2006, 2009; Wright and Rodgers 2014) and operant response rates (Gommans et al. 1999; Grottick et al. 2000; Higgins et al. 2012; Body et al. 2014). They also promote various unconditioned behaviours such as grooming and oral stereotypies which may intrude on operant responding (De Deurwaerdere and Chesselet 2000; Graf et al. 2003; Wright and Rodgers 2014). The neural mechanisms underlying these effects are not fully understood. It is well established that 5-HT$_{2C}$ receptors exert inhibitory control over nigrostriatal and mesolimbic dopaminergic function, and there is evidence that this action makes a significant contribution to the effect of 5-HT$_{2C}$ receptor agonists on motor performance (Giorgetti and Teccot 2004; Alex and Pehek 2007; Filip et al. 2012). However, 5-HT$_{2C}$ receptors are present in many regions of the basal ganglia, and it is becoming increasingly apparent that the effect of 5-HT$_{2C}$ receptor agonists on motor function reflects disruption of multiple neural and behavioural processes (Graves et al. 2013; De Deurwaerdere et al. 2013). For example, stimulation of 5-HT$_{2C}$ receptors in the ventral prefrontal cortex has also been shown to affect locomotor behaviour, possibly by modifying corticofugal control of

Table 3 Effects of mCPP and isamoltane on the indices of performance on the progressive-ratio schedule (group mean values±SEM)

Parameter	Vehicle	mCPP 2.5 mg kg^{-1}	Isamoltane 8 mg kg^{-1}	Isamoltane 8 mg kg^{-1}+mCPP 2.5 mg kg^{-1}
Breakpoint	141.4±16.7	75.8±8.6[a]	145.6±20.3	68.1±8.0[a]
Parameters of the PR model				
T_0, s	6.53±0.95	7.00±0.37	8.34±1.16	7.52±0.80
k	0.48±0.04	0.34±0.06	0.49±0.07	0.46±0.09
a, s	33.0±7.7	34.6±5.7	40.6±9.5	43.7±9.1
δ, s	0.26±0.04	0.66±0.14[a]	0.26±0.04	0.73±0.13[a]
R^2	0.94±0.02	0.88±0.02	0.90±0.02	0.90±0.01

mCPP *m*-chlorophenylpiperazine

[a] Significantly different from vehicle control ($p<0.05$)

Table 4 Effects of mCPP and MDL-100907 on the indices of performance on the progressive-ratio schedule (group mean values±SEM)

Parameter	Vehicle	mCPP 2.5 mg kg^{-1}	MDL-100907 0.5 mg kg^{-1}	MDL-100907 0.5 mg kg^{-1}+mCPP 2.5 mg kg^{-1}
Breakpoint	113.2±11.0	67.9±6.8[a]	111.4±12.9	69.5±7.6[a]
Parameters of the PR model				
T_0	8.30±1.42	4.20±0.49[a]	8.33±1.06	4.80±0.81[a]
k	0.50±0.04	0.53±0.06	0.53±0.05	0.54±0.06
a, s	32.2±6.0	31.8±4.8	47.0±12.3	38.0±4.5
δ, s	0.23±0.04	0.38±0.03[a]	0.24±0.04	0.40±0.04[a]
R^2	0.94±0.01	0.91±0.01	0.94±0.01	0.90±0.01

mCPP *m*-chlorophenylpiperazine

[a] Significantly different from vehicle control ($p<0.05$)

the mesolimbic dopaminergic pathway (Filip and Cunningham 2003). There is also evidence that stimulation of 5-HT$_{2C}$ receptors, probably located on γ-aminobutyric acid (GABA)ergic interneurones in the locus coeruleus, reduces the activity of the dorsal ascending noradrenergic pathway, which may contribute to the locomotor suppressant effect of 5-HT$_{2C}$ receptor agonists (Millan et al. 1998; Gobert et al. 2000).

As well as increasing δ, both agonists had some effects on the parameters of the linear waiting function, although these were not so consistent as the effects on δ. The linear waiting function describes the dependence of post-reinforcement pausing on the prior inter-reinforcer interval. T_0 expresses the minimum post-reinforcement pause; it is affected by the viscosity of liquid reinforcers, presumably due to the occurrence of more protracted post-prandial orofacial grooming following the ingestion of more viscous solutions (Olarte-Sánchez et al. 2012a, b). k expresses the increase in the duration of the post-reinforcement pause as a function of the increasing inter-reinforcer interval in successive ratios. The sensitivity of k to experimental manipulations has yet to be fully explored; however, Valencia-Torres et al. (2014) recently reported that k was somewhat less stable than the other parameters of the model across a protracted training period. In the present experiments, T_0 was significantly reduced by treatment with mCPP and combined treatment with mCPP+MDL-100907 in one experiment (Table 4) and by Ro-600175, SB-242084 and combined treatment with Ro-600175+SB-242084 in another (Table 5). Treatment with mCPP 2.5 mg kg^{-1} was associated with an increase in k in one experiment (Table 1), but this was not replicated in any of the other experiments. k was also reduced by combined treatment with mCPP+SB-242084 (Table 2). It is difficult to know how to interpret these results; their lack of consistency across experiments raises the possibility that they were spurious. In any case, it is noteworthy that in no case was the effect of an agonist on either k or T_0 significantly attenuated by SB-242084, suggesting that the effects were not specifically related to the stimulation of 5-HT$_{2C}$ receptors.

The profile of effects of mCPP and Ro-600175 seen in these experiments differs from the profile seen with another operant task, temporal differentiation in the free-operant psychophysical procedure (Stubbs 1976). Body et al. (2014) recently found that mCPP reduced the indifference time in that procedure, displacing the psychometric timing function towards shorter durations. No such effect was seen with Ro-600175, and the effect of mCPP was antagonised by MDL-100907 but not by SB-242084. It seems that 5-HT$_{2C}$ but not 5-HT$_{2A}$ receptors may influence motor performance on the progressive ratio

Table 5 Effects of Ro-600175 and SB-242084 on the indices of performance on the progressive-ratio schedule (group mean values±SEM)

Performance index	Vehicle	Ro-600175 2 mg kg^{-1}	SB-242084 0.3 mg kg^{-1}	SB-242084 0.3 mg kg^{-1}+Ro-600175 2 mg kg^{-1}
Breakpoint	159.4±22.8	120.6±13.5[a]	168.2±24.5	170.8±21.2[b]
Parameters of the PR model				
T_0, s	6.92±0.71	4.47±0.46[a]	5.01±0.51[a]	4.59±0.52[a]
k	0.47±0.04	0.51±0.04	0.44±0.04	0.46±0.04
a, s	41.3±11.5	41.0±8.0	37.0±8.0	32.3±6.4
δ, s	0.27±0.04	0.33±0.05[a]	0.29±0.04	0.26±0.03[b]
R^2	0.94±0.02	0.93±0.01	0.90±0.03	0.93±0.01

[a] Significantly different from vehicle control ($p<0.05$)

[b] Significantly different from Ro-600175 2 mg kg^{-1} ($p<0.05$)

schedule, whereas the reverse is true of free-operant timing performance.

Finally, it may be appropriate to mention some limitations of these results. In the first place, it should be emphasised that the mathematical model of progressive ratio schedule performance is work in progress, and further research is needed into the sensitivity of the parameters of the model to a broader range of interventions affecting motor and motivational processes (for discussion, see Bradshaw and Killeen 2012). It will also be important to extend the present findings to other, more selective 5-HT_{2C} receptor agonists such as lorcaserin and vabicaserin (Filip et al. 2012; De Deurwaerdere et al. 2013), as well as testing a broader range of doses of the antagonists. Furthermore, it should be noted that the present experiments were concerned solely with food reinforced behaviour; it will be of interest, in future experiments, to apply the model to progressive ratio schedule performance maintained by cocaine and nicotine, in view of the known effects of 5-HT_{2C} receptor agonists on the breakpoint in schedule performance maintained by these reinforcers. These caveats notwithstanding the present results reinforce the reservations expressed by a number of workers concerning the viability of the breakpoint as a pure index of motivation or incentive value (Arnold and Roberts 1997; Bezzina et al. 2008; Killeen et al. 2009; Rickard et al. 2009) and emphasise the need for caution when interpreting this measure in the case of 5-HT_{2C} receptor agonists and other drugs with known effects on motor functions (Filip et al. 2012).

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