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5-HT_{2A} and 5-HT_{2C} receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-HT depletion

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Abstract *Rationale:* Global serotonin (5-HT) depletion increases the number of premature responses made on the five-choice serial reaction time task (5CSRT) in rats. In contrast, the 5-HT_{2A} receptor antagonist M100907 decreases this measure of impulsivity. Mounting evidence suggests that 5-HT_{2A} and 5-HT_{2C} receptors have opposing effects on behaviour, and that the 5-HT_{2C} receptor antagonist SB 242084 produces a pattern of behaviour similar to 5-HT depletion. *Objectives:* To assess the effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists on performance of the 5CSRT, to directly compare the effects of these drugs with those of ICV 5,7-dihydroxytryptamine (5,7-DHT) lesions and to investigate whether 5-HT depletion affects the action of these agents. *Methods:* The effects of M100907 (0, 0.01, 0.03, 0.1 mg/kg IP) and SB 242084 (0, 0.1, 0.25, 0.5 mg/kg IP) were investigated on performance of the 5CSRT in both ICV 5,7-DHT-lesioned and sham-operated rats. *Results:* ICV 5,7-DHT lesions, which significantly decreased forebrain levels of 5-HT by around 90%, increased levels of premature responding, decreased omissions and the latency to respond correctly, yet did not affect performance accuracy. M100907 decreased premature responding in sham-operated controls but not in 5-HT-depleted rats. In contrast, SB 242084 increased premature responding in all animals, and also decreased the latency to make a correct response in sham-operated controls. *Conclusions:* These data support the view that serotoner-

gic regulation of impulsive behaviour through different members of the 5-HT₂ receptor family is functionally heterogeneous. Although both 5-HT_{2A} and 5-HT_{2C} receptors participate in controlling this form of impulsive action, their relative contribution may depend on the endogenous state of the 5-HT system.

Keywords Serotonin · Impulsivity · 5-HT_{2A} receptor · 5-HT_{2C} receptor · 5,7-DHT

Introduction

The serotonergic system as a whole has been heavily implicated in the regulation of impulsivity (Linnoila et al. 1983; Soubrié 1986). In accordance with the hypothesis that decreasing serotonin (5-HT) function results in decreased impulse control, forebrain 5-HT depletion achieved through use of the selective toxin 5,7-dihydroxytryptamine (5,7-DHT) increases impulsive responding in a number of different tasks designed for use with rats, including the go/no-go task and DRL schedules (differential reinforcement of low-rate schedules) (Harrison et al. 1999; Fletcher 1995). Impulsive responding can also be assessed using the five-choice serial reaction time task (5CSRT), a task which provides relatively independent measures of attention, impulsivity, speed of responding and motivation (Carli et al. 1983). Intracerebroventricular (ICV) administration of 5,7-DHT has also been found to increase premature responding on this task, as well as decreasing omissions and decreasing the latency to make a correct response, without altering the accuracy of performance (Harrison et al. 1997).

Both systemic and intra-frontal administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT can decrease 5-HT release, either through activation of pre-synaptic autoreceptors located on serotonergic neurons (Bonvento et al. 1992) or through activation of a negative feedback loop projecting to the dorsal raphe nucleus (Celada et al. 2001; Hajos et al. 1999). However, it has recently been reported that such administration of 8-OH-DPAT improved atten-

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tional performance but did not alter the number of premature responses made on this task (Winstanley et al. 2003a). Hence, there appears to be a dichotomy between chronic decreases in 5-HT function caused by ICV 5,7-DHT lesions and acute decreases in 5-HT release caused by administration of a 5-HT_{1A} receptor agonist. This apparent paradox is more striking when considering that both intra-prefrontal and systemic administration of the 5-HT_{2A} receptor antagonist M100907 decreased premature responding on the 5CSRT (Winstanley et al. 2003a). It is of interest that an antagonist of the 5-HT system should produce opposing effects to a global decrease in serotonergic transmission. Such a dissociation may provide a useful demonstration of the behavioural specificity of 5-HT receptor subtypes. Alternatively, these different effects could reflect changes in chronic versus acute inhibition of 5-HT function. However, subcutaneous administration of 8-OH-DPAT, resulting in higher levels of drug in the brain than those that produced an increase in attentional performance, has been shown to increase impulsivity via its action at presynaptic 5-HT_{1A} receptors (Carli and Samanin 2000). Presumably, acute decreases in 5-HT function can therefore increase motoric impulsivity.

The 5-HT_{2A/2C} receptor agonist (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been reported to increase premature responding on the 5CSRT, an effect which has been attributed to activation of the 5-HT_{2A} receptor (Koskinen et al. 2000). Hence, both global decrease in 5-HT function and activation of 5-HT transmission through 5-HT receptor subtypes enhance impulsive responding on the 5CSRT. A growing body of evidence suggests that the 5-HT_{2A} and 5-HT_{2C} receptors have opposing roles. For example, 5-HT_{2C} receptors appear to inhibit, whereas activation of 5-HT_{2A} receptors enhance, the release of dopamine (DA) (Di Matteo et al. 2001, 2000; Millan et al. 1998). Furthermore, while antagonism of 5-HT_{2C} receptors potentiates some of the behavioural effects of cocaine, antagonism of 5-HT_{2A} receptors attenuates both cocaine-induced hypermotility and re-instatement of cocaine seeking (Fletcher et al. 2002). Decreasing 5-HT transmission through blockade of 5-HT_{2C} receptors could therefore have opposite effects on behaviour to those obtained through antagonising 5-HT_{2A} receptors. Indeed, it has recently been reported that the 5-HT_{2C} receptor antagonist SB 242084 increases premature responding on the 5CSRT, as well as decreasing the latency to respond correctly (Higgins et al. 2003).

Therefore, in this study, the contribution of 5-HT_{2A} and 5-HT_{2C} receptors to performance of the 5CSRT was investigated through systemic injections of the selective 5-HT_{2A} receptor antagonist M100907 (Kehne et al. 1996) and the selective 5-HT_{2C} receptor antagonist SB 242084 (Kennett et al. 1997) in order to provide a direct comparison between antagonism of these two 5-HT receptor subtypes on cognitive performance. Furthermore, whereas depleting 5-HT through ICV administration of 5,7-DHT dramatically reduces tissue levels of 5-HT to around 10% of sham-operated controls, the impact of any functional compensation occurring within the serotonergic

system for pharmacological studies and behavioural performance is uncertain (Kirby et al. 1995; Hall et al. 1999). Clearer understanding of these factors may help to explain some of the paradoxical findings observed when comparing data from ICV 5,7-DHT-lesioned animals and systemic drug challenges. M100907 and SB 242084 were therefore tested in animals with ICV 5,7-DHT lesions and in sham-operated controls in order to both replicate previous findings of increased impulsivity on this task and observe whether any alterations in task performance caused by drug administration were still evident in lesioned animals.

Materials and methods

Subjects were 24 male Lister Hooded rats (Charles River, Kent, UK) weighing 300–320 g at the start of each experiment and were maintained at 85% of their free-feeding weight. Water was available ad libitum. Animals were pair-housed under a reverse light cycle (lights on from 1900 hours to 0700 hours) and testing took place between 0900 hours and 1300 hours 5–6 days per week. All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

Behavioural apparatus

Details of the behavioural apparatus and training have been provided previously (Winstanley et al. 2003a). In brief, eight nine-hole operant chambers were used, each fitted with a house light, tray light and food magazine into which 45-mg pellets could be dispensed (Noyes dustless pellets, Sandown Scientific, UK). Each box was contained

Table 1 Tissue concentrations of serotonin (5-HT) in cortical, striatal and limbic areas of ICV 5,7-dihydroxytryptamine-lesioned and sham-operated rats. The data are averaged levels (\pm SEM) expressed as pmol/mg to two decimal places. *PrL* prelimbic cortex, *ACx* anterior cingulate, *NAC* nucleus accumbens, *DMS* dorsomedial striatum, *DLS* dorsolateral striatum, *Amyg* amygdala, *VHPC* ventral hippocampus, *DHPC* dorsal hippocampus, *SEP* septum, *HYP* hypothalamus

Region	5-HT	
	Sham	Lesion
PrL	0.41 (0.08)	0.00* (0.00)
ACx	0.28 (0.08)	0.01* (0.01)
NAC	0.60 (0.13)	0.06* (0.04)
DMS	0.29 (0.06)	0.01* (0.01)
DLS	0.29 (0.06)	0.03* (0.02)
Amyg	1.03 (0.34)	0.10* (0.07)
VHPC	0.32 (0.06)	0.03* (0.02)
DHPC	0.20 (0.06)	0.00* (0.00)
SEP	0.44 (0.09)	0.02* (0.02)
HYP	0.61 (0.15)	0.09* (0.04)

*A significant difference ($P < 0.05$) between sham and lesioned groups

within a ventilated and sound-attenuated chamber. Every other hole in the response array was blocked so that only five of the holes were accessible. An infra-red beam located at the entrance to each hole, and across the mouth of the food magazine, enabled detection of nose-poke responses. The boxes were controlled by software written in BBC BASIC (Paul Fray Ltd, Cambridge, UK) running on an Acorn Archimedes series computer (Cambridge, UK).

Behavioural training

Rats were trained on the 5CSRT to a stable level of performance as described previously (Granon et al. 2000). Briefly, subjects were trained to make nose-poke responses into the response apertures upon brief illumination (0.5 s) of the light therein. Animals received five to six sessions per week until a high level of stable performance was reached ($\geq 80\%$ accuracy, $\leq 20\%$ omissions). Each session consisted of 100 trials and lasted approximately 30 min. A correct response was rewarded with a food pellet, whereas an incorrect, premature or lack of response (omission) was punished by non-delivery of reward and a 5-s time-out period during which the house light was extinguished. Repeated responding in any hole during the presentation of the light stimulus or during the limited hold period was classified as perseverative responding and, whilst these responses were monitored, they were not punished.

Surgery

Subjects were matched for baseline performance across all variables measured and separated into two groups. One group received ICV 5,7-DHT lesions ($n=12$) and the other received ICV infusions of vehicle ($n=12$). All rats were treated 30 min before the start of surgery with 15 mg/kg desipramine HCl (Sigma Chemical Co., UK) dissolved in double distilled water in order to protect noradrenergic neurons from the neurotoxin. Rats were anaesthetised with Avertin [10 g 2,2,2-tribromoethanol (Sigma, Poole, UK) in 5 g tertiary amyl alcohol, diluted in a solution of 40 ml ethanol and 450 ml phosphate buffered saline] given at a dose of 1 ml/100 g (IP), and secured in a stereotaxic frame fitted with atraumatic earbars. Rats in the lesion group received bilateral ICV infusions of 80 μ g (free base) 5,7-DHT creatinine sulphate (Sigma Chemical Co., UK) dissolved in 10 μ l 0.1% ascorbic acid in saline, whilst the shams received bilateral ICV infusions of 10 μ l vehicle. Following each 8-min infusion, the injector was left in place for 2 min before withdrawal to allow the infusate to diffuse. The co-ordinates used were: AP -0.9 mm from bregma, L ± 1.5 mm from the midline, DV -3.5 mm from dura, calculated from a stereotaxic atlas (Paxinos and Watson 1998). The incisor bar was set at -3.3 mm relative to the interaural line in a flat skull position. After surgery, animals were given free access to

food for 10 days prior to behavioural testing to allow for the degeneration of serotonergic neurons (Bjorkland et al. 1975).

Effects of systemic M100907 and SB 242084 on 5CSRT performance of animals with ICV 5,7-DHT lesions and sham-operated controls

Following re-establishment of stable post-operative performance over 15 sessions, animals were separated into two equal groups and received SB 242084 (0, 0.1, 0.25 and 0.5 mg/kg IP) and M100907 (0, 0.01, 0.03, 0.1 mg/kg IP) according to a Latin-square crossover drug design. All drugs were administered 15 min prior to the start of the behavioural task. Drugs were given on Tuesdays and Fridays, and baseline testing occurred on Mondays and Thursdays. On other days, animals remained in their home cages.

Drugs

M100907 (Solvay, Weesp, The Netherlands) was dissolved in saline and the pH adjusted to 6.25 using 0.1 M NaOH and 0.1 M HCl. SB 242084 (Solvay, Weesp, The Netherlands) was dissolved in 25 mM citric acid in 8% cyclodextrine in 0.9% saline, and the pH adjusted to 6.4 using 0.1 M NaOH. Stock solutions (M100907, 0.1 mg/ml; SB 242084, 0.5 mg/ml) were prepared and aliquoted before being frozen at -80°C . On each experimental day, one aliquot of each drug was defrosted and diluted to give the range of concentrations required. Concentrations of both drugs were calculated as the salt. Systemic injections of drug were given in a volume of 1 ml/kg.

Ex vivo lesion analysis

At the end of the experiment, animals were sacrificed through exposure to increasing concentrations of carbon dioxide. The brains were then rapidly removed and frozen on dry ice. Thereafter, coronal sections were cut (150 μ m thickness) on a cryostat (-10°) from the frontal pole and mounted onto pre-chilled microscope slides. A stainless-steel micropunch (0.75 mm diameter) was used to remove 0.3- to 0.6-mg aliquots of tissue, as described by Palkovits (1973) from the following (left and right) brain regions: nucleus accumbens, prelimbic cortex, anterior cingulate cortex, dorsomedial striatum, dorsolateral striatum, amygdala, ventral hippocampus, dorsal hippocampus, septum and hypothalamus. Samples were homogenised in 75 μ l of 0.2 M perchloric acid to precipitate protein material. Following centrifugation at 6000 rpm for 20 min at 4°C , 50 μ l of the supernatant was decanted and placed into autoinjector microvials ready for analysis. Levels of DA, dihydroxyphenylacetic acid (DOPAC), noradrenaline (NA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined in brain samples by reversed-phase, high-

performance liquid chromatography (HPLC), as described previously (Matthews et al. 2001).

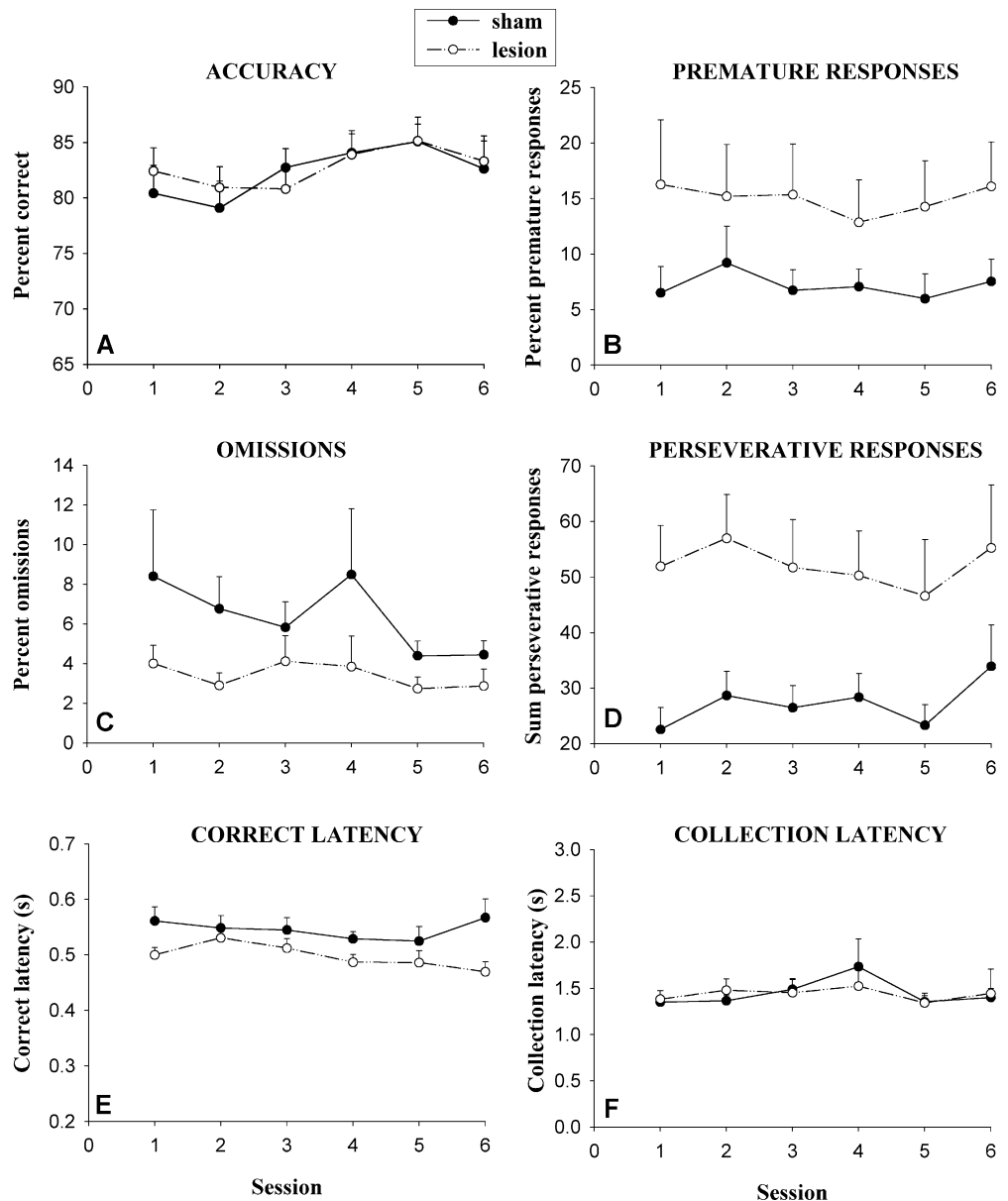
Data analyses

All analyses were conducted using SPSS for Windows (version 9.0; SPSS, Chicago, IL). Seven variables were analysed: the percentage of correct responses made [number of correct responses/(number of correct and incorrect responses) \times 100]; percentage of responses omitted [number of omissions/(total number of correct, incorrect and omitted responses) \times 100]; percentage of premature responses (number of premature responses/total number of trials \times 100), latency to make a correct response, latency to collect reward, the total number of perseverative responses and the total number of trials completed per session. Variables that were expressed as a

percentage were subjected to an arcsine transformation in order to limit the effect of an artificially imposed ceiling (i.e. 100%).

Baseline behavioural data were analysed by ANOVA with one within-subjects factor—day (six daily test sessions). Any effects of the ICV 5,7-DHT lesion were determined by means of analysis of variance (ANOVA) with one repeated-measures factor—day—and one between-subjects factor—lesion (two levels, sham and lesion). Results of drug studies were analysed by ANOVA with one repeated-measures factor—drug (four levels: three different doses of drug plus vehicle)—and one between-subjects factor—lesion (two levels: sham and lesion). If any analyses produced a significant effect of drug, mean values for individual doses were compared post-hoc to vehicle control values via paired sample *t*-tests. Data from both cycles of the crossover design were pooled to increase statistical power, but similar effects of

Fig. 1 Effects of ICV 5,7-dihydroxytryptamine (5,7-DHT) lesions on different measures of performance of the five-choice serial reaction time task (5CSRT). **a** Percentage of correct trials performed per session. **b** Percentage of premature responses performed per session. **c** Percentage of trials omitted per session. **d** Total number of perseverative responses performed per session. **e** Average latency to make a correct response per session. **f** Average latency to collect reward earned per session. Data shown are mean and SEM over six post-operative sessions from both sham-operated ($n=12$) and ICV 5,7-DHT-lesioned ($n=12$) rats



both SB 242084 and M100907 were observed in each cycle.

Results

Ex vivo analysis of the ICV 5,7-DHT lesion

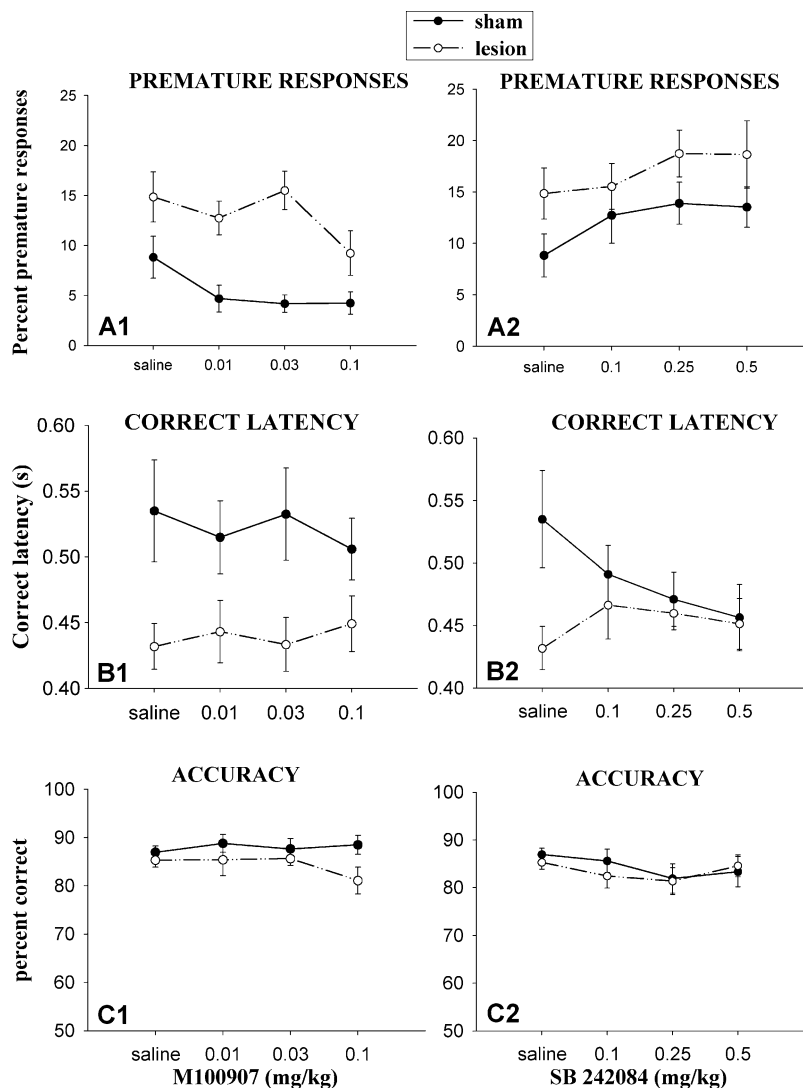
In keeping with previous reports (Harrison et al. 1997; Winstanley et al. 2003b), animals with ICV 5,7-DHT showed reductions of 85–90% of 5-HT in all regions tested compared with sham controls (Table 1). Levels of DA, NA, and DOPAC were not altered in lesioned animals (data not shown).

Effect of ICV 5,7-DHT lesions on performance of the 5CSRT

The pattern of effects caused by ICV 5,7-DHT lesions in this study on performance of the 5CSRT are generally in accordance with previous reports (Carli and Samanin

2000; Harrison et al. 1997). Post-operatively, animals with ICV 5,7-DHT lesions made significantly more premature responses (lesion: $F_{1,22}=10.387$, $P<0.003$, Fig. 1b) and omitted significantly fewer trials (lesion: $F_{1,22}=9.790$, $P<0.004$, Fig. 1c) than sham-operated controls. Lesioned animals were also faster to make a correct response (lesion: $F_{1,22}=6.824$, $P<0.014$, Fig. 1e). However, the lesion did not alter the percentage of correct responses that animals made (lesion: $F_{1,22}=0.005$, NS, Fig. 1a). All subjects completed over 100 trials within each session, and there was no change in the latency to collect rewards earned (lesion: $F_{1,22}=0.19$, NS, Fig. 1f), indicating that the lesion had not affected motivational processes. However, in contrast to the study by Harrison et al. (1997), an increase in perseverative responses was also observed in lesioned animals (lesion: $F_{1,22}=5.846$, $P<0.022$, Fig. 1d).

Fig. 2 Effects of M100907 and SB 242084 on performance of the five-choice serial reaction time task (5CSRT) in ICV 5,7-dihydroxytryptamine (5,7-DHT)-lesioned animals and sham-operated controls. On the left: the effect of M100907 in ICV 5,7-DHT-lesioned rats ($n=10$) and sham-operated controls ($n=12$) on (A1) the percentage of premature responses performed, (B1) the average latency to make a correct response per session and (C1) the percentage of correct trials performed. On the right: the effect of SB 242084 in ICV 5,7-DHT-lesioned rats ($n=9$) and sham-operated controls ($n=12$) on (A2) the percentage of premature responses performed, (B2) the average latency to make a correct response per session and (C2) the percentage of correct trials performed. Data shown are mean \pm SEM



Effect of systemic administration of M100907 and SB 242084 on performance of the 5CSRT performance in animals with ICV 5,7-DHT lesions and sham-operated controls

A complete data set was not obtained for all animals with ICV 5,7-DHT lesions, as three animals could not be tested due to ill health. Data from ten lesioned animals were obtained for M100907 and from nine for SB 242084.

Premature responses

In keeping with previous results (Winstanley et al. 2003a), M100907 significantly decreased premature responding (drug: $F_{3,60}=4.043$, $P<0.012$, Fig. 2 panel A1). However, the effect of the drug was significantly attenuated in animals with ICV 5,7-DHT lesions (drug \times lesion: $F_{3,60}=2.97$, $P<0.040$). Comparing the level of premature responding observed under individual doses of drug, lesioned rats still made significantly more responses than sham-operated controls (independent samples t -tests: 0.01 mg/kg: $t=-3.302$, $P<0.004$; 0.03 mg/kg: $t=-3.897$, $P<0.001$; 0.1 mg/kg: $t=-2.3$, $P<0.036$). If the data from the 5-HT-depleted rats only were examined, there was a trend for the drug to decrease premature responding, but this fell short of significance (drug: $F_{3,27}=2.670$, $P<0.079$), and none of the individual drug doses significantly altered the number of premature responses made relative to saline administration. In sham-operated animals, the higher two doses significantly attenuated premature responding (drug: $F_{3,33}=3.650$, $P<0.023$; 0.03 mg/kg: $t=-2.927$, $P<0.014$; 0.1 mg/kg: $t=-2.562$, $P<0.02$), whereas the lowest dose tended to have a similar effect (0.01 mg/kg: $t=-2.185$, $P<0.051$). Overall, lesioned animals still made significantly more premature responses than sham-operated controls (lesion: $F_{1,20}=37.596$, $P<0.0001$).

In contrast, SB 242084 significantly increased premature responding at the two highest doses tested (drug: $F_{3,57}=3.680$, $P<0.017$, Fig. 2 panel A2; 0.1 mg/kg: $t=-$

1.787 , $P<0.089$; 0.25 mg/kg: $t=-2.923$, $P<0.008$; 0.5 mg/kg: $t=-2.613$, $P<0.017$). The absence of a drug \times lesion interaction indicates that the drug had similar effects in both sham and lesioned animals (drug \times lesion: $F_{3,57}=1.280$, NS). Furthermore, there was no longer a main effect of lesion ($F_{1,19}=3.440$, $P<0.079$). Thus, it would appear that SB 242084 increased premature responses in the lesioned animals to the same extent as observed in sham-operated controls.

Correct latency

SB 242084 also decreased the latency to make a correct response (drug: $F_{3,57}=2.889$, $P<0.043$, Fig. 2 panel B2). There was a trend suggesting a difference between this response to the drug in sham and lesioned animals (drug \times lesion: $F_{3,57}=2.523$, $P<0.06$). Examining data from sham animals alone, the 5-HT_{2C} receptor antagonist dose dependently decreased the latency to respond correctly (drug: $F_{3,30}=3.970$, $P<0.017$), which reached significance at the highest dose tested (saline versus 0.5 mg/kg: $t=3.041$, $P<0.012$). Following administration of this dose of SB 242084, the latency to make a correct response was not significantly different in sham and lesioned animals (lesion: $F_{1,21}=0.954$, NS). However, when the lesioned animals were analysed separately, the drug did not significantly alter the correct latency (drug: $F_{3,33}=0.516$, NS), but lesioned animals were faster to make a correct response overall (lesion: $F_{1,21}=4.504$, $P<0.046$). In contrast, M100907 had no effect on the latency to respond correctly ($F_{3,60}=0.855$, NS), and this pattern was true for both lesioned animals and sham-operated controls ($F_{3,60}=0.254$, NS, Fig. 2 panel B1).

Accuracy and other variables

Neither M100907 nor SB 242084 affected accuracy of performance in either 5-HT-depleted rats or sham-operated controls (M100907: drug $F_{3,60}=0.987$, NS, drug \times lesion

Table 2 Effect of M100907 and SB 242084 on performance of the five-choice serial reaction time task (5CSRT) in ICV 5,7-dihydroxytryptamine (5,7-DHT)-lesioned and sham-operated rats. Data shown are: mean per session (SEM). Neither drug affected the number of perseverative responses, the latency to collect the reward

Treatment	Dose (mg/kg)	Perseverative responses		Collection latency (s)		Omissions	
		Sham	Lesion	Sham	Lesion	Sham	Lesion
Vehicle	–	20.60 (1.86)	44.77 (9.39)	1.61 (0.33)	1.17 (0.21)	5.45 (0.71)	2.08 (1.19)
M100907	0.01	22.40 (3.06)	48.15 (13.1)	1.33 (0.07)	1.09 (0.07)	4.18 (1.09)	2.13 (0.77)
	0.03	16.20 (3.89)	32.38 (10.61)	1.41 (0.10)	1.09 (0.07)	6.33 (3.24)	2.18 (0.56)
	0.1	27.40 (7.74)	39.78 (12.23)	1.96 (0.65)	1.37 (0.27)	4.62 (1.05)	3.87 (1.95)
Vehicle	–	25.56 (5.79)	41.94 (8.66)	1.40 (0.25)	1.37 (0.13)	6.01 (1.14)	3.11 (1.56)
SB242084	0.1	29.13 (7.91)	35.92 (8.35)	1.34 (0.09)	1.20 (0.14)	5.85 (0.53)	2.90 (1.72)
	0.25	27.00 (10.12)	40.83 (10.07)	1.31 (0.08)	1.20 (0.14)	4.71 (1.09)	3.05 (1.54)
	0.5	34.25 (7.83)	40.00 (10.85)	1.37 (0.10)	1.05 (0.11)	6.16 (0.66)	5.18 (2.71)

or the number of trials omitted in either ICV 5,7-DHT-lesioned rats or sham-operated controls, although lesioned animals made significantly more perseverative responses and omitted significantly fewer trials than sham-operated controls during baseline testing (Fig. 1)

$F_{3,60}=0.854$, NS; SB 242084: drug $F_{3,57}=0.066$, NS, drug \times lesion $F_{3,57}=0.284$, NS; Fig. 2 panels C1, C2). Likewise, neither drug altered the number of omissions and perseverative responses made, or the latency to collect food reward (Table 2).

Discussion

The results presented here demonstrate opposing and dissociable behavioural effects of 5-HT_{2A} and 5-HT_{2C} receptor antagonists. In particular, the 5-HT_{2A} receptor antagonist M100907 decreased premature responding, contradicting the hypothesis that general decreases in 5-HT function increase impulsivity, whereas the 5-HT_{2C} receptor antagonist SB 242084 increased this measure of impulsivity. Furthermore, SB 242084 decreased the latency to make a correct response, whereas M100907 had no effect on this aspect of performance. In keeping with previous reports (Harrison et al. 1997), ICV 5,7-DHT lesions increased premature responding and also decreased the number of trials omitted and the latency to respond correctly. While forebrain 5-HT depletion blocked the ability of M100907 to reduce premature responding, the effects of SB 242084 to increase this measure of impulsivity were still evident. The fact that ICV 5,7-DHT lesions produce a behavioural profile of effects more similar to antagonism of the 5-HT_{2C} than the 5-HT_{2A} receptor (or indeed any other so far tested) implies that the 5-HT_{2C} receptor plays a key role in the serotonergic regulation of behavioural inhibition.

The finding reported here that ICV 5,7-DHT lesions also increased perseverative responding has not been observed in other studies. In the current experiment, perseverative responses were not punished; yet, in the study by Harrison et al. (1997), such responses were penalized by a 5-s time out. This key difference in the behavioural consequences of emitting a perseverative response is likely to be the cause of the difference in results. Furthermore, any subsequent responses into any aperture following the correct response were classed as perseverative in the present study. Hence, this measure does not represent repeated responding at the aperture associated with reward delivery, but could reflect perseverative nose-poke activity at the array. Localised 5-HT depletion within the prefrontal cortex (PFC) of the marmoset has also been shown to increase perseverative errors during reversal learning (Clarke et al. 2003), and similar perseverative deficits have been observed following damage to the orbitofrontal cortex (Chudasama et al. 2003; Chudasama and Robbins 2003; Jones and Mishkin 1972; Schoenbaum et al. 2002; Rogers et al. 1999). In light of these data, the regulation of orbitofrontal function by 5-HT clearly merits further investigation.

It has been suggested that perseveration in a response associated with reward delivery may be related to both impulsive and compulsive behaviour, particularly when such an action is not rewarded or is punished (Soubrié 1986, Hollander and Rosen 2000). Recent data indicate

that different measures of impulsivity are not correlated within individuals, supporting the suggestion that they have independent biological mechanisms (McDonald et al. 2003, Winstanley et al. 2004). Neither M100907 nor SB 242084 affected the number of perseverative responses made in this study, despite altering the number of premature responses made, and a similar pattern of results has been observed previously following administration of these drugs (Winstanley et al. 2003a, Higgins et al. 2003). Such data demonstrate that these two forms of motoric disinhibition are differentially regulated by the 5-HT system, and support the view that different kinds of impulsivity differ in their neurobiological bases.

Whereas M100907 did not decrease impulsive responding in animals with ICV 5,7-DHT lesions, the ability of SB 242084 to increase premature responding was still apparent in lesioned animals, despite the fact that the lesion itself increased the number of premature responses. Not all the effects of SB 242084 were present in lesioned animals, notably the speeded latency to make a correct response. However, this could be attributed to a floor effect, as the decrease in the latency to respond correctly caused by the serotonergic lesion was of the same magnitude as that produced by the highest dose of SB 242084 in sham-operated controls. Furthermore, there is a physical limit as to how fast the rat can turn from the magazine, register the stimulus and respond.

If a 5-HT receptor antagonist is still producing a behavioural effect in animals with profound damage to the 5-HT system, it could be suggested that the effects of the drug are not due to its actions at 5-HT receptors. However, SB 242084 is a highly selective 5-HT_{2C} receptor antagonist. It has a high affinity for the 5-HT_{2C} receptor (pK_i 9.0), 100-fold, 158-fold selectivity over the 5-HT_{2B} and 5-HT_{2A} receptors, respectively, and also has over 100-fold selectivity for the 5-HT_{2C} receptor over a range of other serotonergic, dopaminergic and adrenergic receptors (Kennett et al. 1997). Therefore, it seems very unlikely that this drug is causing such marked effects on a behavioural dimension known to be sensitive to manipulations of the 5-HT system through its actions at non-serotonergic receptors. One further possibility is that SB 242084 is acting as an inverse agonist at 5-HT_{2C} receptors rather than as a neutral antagonist (Barker et al. 1994). Although some drugs, including antipsychotic agents, have been shown to possess inverse agonist properties at 5-HT_{2C} receptors (Herrick-Davis et al. 2000), there is no evidence to date demonstrating that SB 242084 can act as an inverse agonist *in vivo*.

Given that both ICV 5,7-DHT and systemic administration of a 5-HT_{2C} receptor antagonist both increase premature responding, it is possible that the elevated levels of impulsivity produced by global 5-HT depletion result from reduced activation of 5-HT_{2C} receptors. Although extensive lesions of the 5-HT system have profound effects on behaviour, they fail to completely abolish serotonergic function. Therefore, further increases in premature responding may be observed following administration of SB 242084 in lesioned animals because the

antagonist blocks the action of residual 5-HT released from the few surviving 5-HT neurons at 5-HT_{2C} receptors. Although ICV 5,7-DHT lesions produce 85–90% depletions in 5-HT tissue levels post-mortem, data from an *in vivo* microdialysis study indicate that significant 5-HT efflux can still be measured in the striatum due to compensatory release of 5-HT from remaining neurons, and limited increases in 5-HT efflux have still been observed in response to administration of fenfluramine (a 5-HT releasing agent) in lesioned animals (Hall et al. 1999; Kirby et al. 1995).

Unlike the effects of SB 242084, the ability of M100907 to alter the level of premature responding was blocked by ICV 5,7-DHT lesions, potentially indicating that reducing serotonergic tone differentially affects activation of the 5-HT_{2C} versus the 5-HT_{2A} receptor. A simpler interpretation of these effects is that the affinity of M100907 for the 5-HT_{2A} receptor may be lower than the affinity of SB 242084 for the 5-HT_{2C} receptor, or there may be differences in selectivity of 5-HT for 5-HT_{2C} over 5-HT_{2A} receptors. However, these explanations seem unlikely. First, the pK_i of M100907 for the 5-HT_{2A} receptor is 9.4, and the pK_i of SB 242084 for the 5-HT_{2C} receptor is 9.0 (see Barnes and Sharp 1999 for details). Second, comparing the functional activities of the two drugs, M100907 and SB 242084 are equipotent in antagonising the 5-HT_{2A} and 5-HT_{2C} receptors, respectively, and 5-HT shows equal affinity for 5-HT_{2A} and 5-HT_{2C} receptors as indicated by pEC_{50} values of 7.2 and 7.8, respectively (Glennon JC, unpublished observations). Furthermore, selective blockade of the effects of M100907 but not SB 242084 in ICV 5,7-DHT-lesioned rats is unlikely to be due to increased downregulation of 5-HT_{2A} relative to 5-HT_{2C} receptors. Although decreasing 5-HT does trigger downregulation of both 5-HT_{2A} and 5-HT_{2C} receptors (Van Oekelen et al. 2003; Compan et al. 1998), a greater decrease in 5-HT_{2A} receptors should lead to behavioural effects more typical of 5-HT_{2A} antagonism in 5-HT-lesioned rats, i.e. a decrease rather than an increase in premature responding, which is precisely the opposite of the results observed.

The differential effects of SB 242084 and M100907 in ICV 5,7-DHT-lesioned animals may, however, be explained with reference to the nature of their activation by 5-HT *in vivo*. Administration of 5-HT_{2C} receptor antagonists, including SB 242084, have been shown to increase basal levels of DA and NA efflux, whereas administration of 5-HT_{2C} receptor agonists have the opposite effect (Di Matteo et al. 2000; Millan et al. 1998; Gobert et al. 2000). In contrast, M100907 does not alter basal levels of DA or NA release, although it has been shown to reduce the increases in DA and NA efflux produced by the 5-HT_{2A/2C} agonist DOI (Gobert and Millan 1999) and to inhibit 3,4-methylenedioxymethamphetamine-induced DA release (Schmidt et al. 1994). Such data indicate that 5-HT_{2C} but not 5-HT_{2A} receptors are tonically activated, and that 5-HT_{2C} receptors are likely to be active under conditions of low serotonergic tone.

In contrast, some of the behavioural effects of M100907 have been shown to depend on the presence of a significant level of endogenous 5-HT. For example, the ability of M100907 to reduce hyperactivity produced by the *N*-methyl-D-aspartate receptor antagonist MK-801 is abolished following global decreases in 5-HT synthesis caused by administration of pCPA, yet is restored by acute administration of 5-HT directly into the brain of pCPA-treated rats (Martin et al. 1998). Such data indicate that 5-HT_{2A} receptor antagonists may decrease behavioural inhibition by unmasking the inhibitory effect of 5-HT at other 5-HT receptors, and therefore the strength of their behavioural effects is dramatically reduced under conditions of low endogenous 5-HT tone. Hence, in the current study, although SB 242084 is able to block the effect of low levels of 5-HT acting at 5-HT_{2C} receptors, thereby increasing the level of premature responses still further in ICV 5,7-DHT-lesioned animals, M100907 has very little effect on behaviour.

Administration of amphetamine causes a similar pattern of behavioural effects on the 5CSRT as ICV 5,7-DHT lesions, increasing the number of premature responses made and reducing the latency to make a correct response (Cole and Robbins 1987; Harrison et al. 1997). This amphetamine-induced increase in impulsive responding is dependent on the ability of amphetamine to increase DA release in the nucleus accumbens (Cole and Robbins 1987; Cole and Robbins 1989) and is also attenuated by serotonergic lesions (Harrison et al. 1997). In contrast, systemic administration of the D1 receptor antagonist SCH 23390 decreases premature responding on the task, and ameliorates the increase in premature responding produced by ICV 5,7-DHT (Harrison et al. 1997). These data implicate interactions between the 5-HT and DA system in regulating this form of impulsive behaviour.

M100907 and SB 242084 may therefore exert their opposite effects on impulsivity in this task via their contrasting modulation of the dopaminergic system. During performance of a simplified version of the 5CSRT, DA levels markedly increase in the mPFC, and a higher turnover of DA is observed in the frontal cortex of more impulsive animals *post-mortem* (Dalley et al. 2002). Enhanced DA release triggered by SB 242084 could lead to an increase in impulsive behaviour, whereas a decrease in task-related dopaminergic activation potentially caused by M100907 may account for the decrease in levels of premature responding observed. However, administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT has not always been found to increase premature responding (Winstanley et al. 2003a), yet even low doses of this serotonergic agent increase DA release in the nucleus accumbens and PFC (Arborelius et al. 1993; Gobert et al. 1998). Therefore, if SB 242084 and M100907 are affecting impulsive responding via their interactions with the DA system, then the functional effects of altering DA levels via 5-HT₂ receptors rather than via 5-HT_{1A} receptors produce very different behavioural changes. Whether such additional layers of complexity exist at the level of 5-HT:DA interactions, or whether 5-HT₂ receptor

agents alter impulsivity through a DA-independent mechanism, remains to be determined.

In summary, the demonstration that antagonism of 5-HT_{2A} and 5-HT_{2C} receptors can have opposite effects on a measure of impulsivity builds on previous work (Higgins et al. 2003; Winstanley et al. 2003a) and highlights the fact that 5-HT can exert dissociable effects on behaviour via these different receptor subtypes. The 5-HT system as a whole has been heavily implicated in numerous psychiatric illnesses, including depression, schizophrenia and anxiety, which do not appear to have many common factors or symptoms. Improved understanding of the regulation of behaviour by different 5-HT receptor subtypes—particularly when the state of the 5-HT system changes—and their relative roles in regulating other neurotransmitter systems may be critically important in unravelling the complexities of serotonergic modulation of both normal and pathological brain function.

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