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Opposing Roles for 5-HT_{2A} and 5-HT_{2C} Receptors in the Nucleus Accumbens on Inhibitory Response Control in the 5-Choice Serial Reaction Time Task

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Serotonin (5-HT) is thought to play an important role in the regulation of behavioral inhibition. Studies manipulating 5-HT function in the rodent brain indicate that 5-HT receptors regulate distinct forms of impulsive behavior, including impulsive responding in the 5-choice serial reaction time task (5CSRTT). The present study investigates the loci of effects mediated by 5-HT_{2A} and 5-HT_{2C} receptors in attention and inhibitory response control using microinfusions targeted at the nucleus accumbens (NAc), prelimbic cortex (PL) and infralimbic cortex (IL). Rats were implanted with bilateral guide cannulas and received infusions of the selective 5-HT_{2A} receptor antagonist M100907 (0.1 and 0.3 μg) or selective 5-HT_{2C} receptor antagonist SB242084 (0.1 and 0.5 μg) immediately prior to testing. The results show that intra-NAc infusions of M100907 significantly decrease impulsive responding on the 5CSRTT and at the highest dose increased omissions as well. By contrast, infusions of SB242084 into the NAc selectively and dose-dependently increased impulsivity. Neither M100907 nor SB242084 significantly altered impulsive responding following either intra-PL or intra-IL administration. However, SB242084 significantly decreased omissions following intra-PL administration (0.5 μg only). These data reveal opposing effects on impulsivity following 5-HT_{2A} and 5-HT_{2C} blockade in the NAc. Our results suggest that the NAc, but not the PL or IL, is implicated in the mediation of the effects of M100907 and SB242084 on inhibitory response control during baseline 5CSRTT performance.
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INTRODUCTION

Deficits in impulse control and attention are core features of several psychiatric disorders including attention deficit hyperactivity disorder (ADHD), schizophrenia, and substance abuse. Clinical and preclinical research suggests that dysregulation within serotonergic (5-HT) systems is involved in impulsive behavior. Furthermore, 5-HT projections to medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) and indirect actions via 5-HT-receptors regulating PFC projections to the ventral tegmental area (VTA), are thought to be involved in attentional function and executive processes including inhibitory response control (Robbins, 2000; Chudasama and Robbins, 2004; Winstanley *et al*, 2006). However, the 5-HT system is complex, with at least 15 subtypes of receptor expressed in mammalian systems (for detailed review see Barnes and Sharp, 1999; Hoyer *et al*, 2002).

The relationship between 5-HT function and behavioral inhibition has been studied using a number of different animal models combined with manipulations of the 5-HT system (Soubrié, 1986; Robbins, 2002; Chudasama and Robbins, 2004; Winstanley *et al*, 2006). Depletion of forebrain 5-HT induces impulsive responding in rats when assessed using go/no-go or DRL paradigms (Fletcher, 1995; Harrison *et al*, 1999). Impulsivity, as measured by the 5-choice serial reaction time task (5CSRTT), has also been shown to be sensitive to 5-HT manipulations. The 5CSRTT assesses aspects of impulse control as well as attentional performance, speed of responding and motivation (Carli *et al*, 1983; Robbins, 2002). Harrison *et al* (1997a) showed that global 5-HT depletion increases premature responding on the 5CSRTT with concurrent decreases in omissions and latency to make a correct response. Intracerebral

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administration of the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) or specific lesions of serotonergic forebrain projections also increase impulsivity as well as impair attentional accuracy (Carli and Samanin, 1992; Jakala *et al*, 1992; Harrison *et al*, 1997a, b; Puumala and Sirviö, 1998; Winstanley *et al*, 2004). The relationship between 5-HT and premature responding in the 5CSRTT is not straightforward however, as Dalley *et al* (2002) also showed a significant positive correlation between PFC 5-HT and impulsivity consistent with previous reports of a positive correlation between serotonin utilization and premature responding (Puumala and Sirviö, 1998). These data suggest that 5-HT differentially regulates impulsive responding depending on the level of activation of different 5-HT receptors possibly involving different brain regions.

This hypothesis is further supported by studies investigating 5-HT_1 and 5-HT_2 receptor function and 5CSRTT performance (Carli and Samanin, 1992, 2000; Koskinen *et al*, 2000a, b, Koskinen and Sirviö, 2001; Ruotsalainen *et al*, 1997; Passetti *et al*, 2003; Winstanley *et al*, 2003, 2004). Several studies have shown that 5-HT_{2A} and 5-HT_{2C} receptors mediate opposing effects on behavioral inhibition, which may relate to their differential effects on dopamine (DA) function (Carli and Samanin, 1992; Koskinen *et al*, 2000a, b; Ruotsalainen *et al*, 1997; Passetti *et al*, 2003; Winstanley *et al*, 2003, 2004, 2005). Recent findings also imply a role for 5-HT_{2A} receptors in regulating glutamate function in PFC (Carli *et al*, 2004, Ceglia *et al*, 2004).

The present study investigates the role of 5-HT_{2A} and 5-HT_{2C} receptors in the NAc and the prelimbic (PL) and infralimbic (IL) subregions of the mPFC in mediating the opposing effects on premature responding previously observed following systemic blockade of these receptors (Winstanley *et al*, 2004). Given the important links between NAc and cortical DA, as well as cortical glutamate, and performance in the 5-CSRTT (Robbins, 2002; Murphy *et al*, 2005), the role of 5-HT_{2A} and 5-HT_{2C} receptors within these regions were investigated using targeted infusions of the subtype selective antagonists, M100907 and SB242084, respectively.

MATERIALS AND METHODS

Subjects

The subjects were 32 male hooded Lister rats weighing approximately 250 g at the start of training and 350–450 g at the start of infusions (Charles River, UK), housed in pairs under temperature-controlled conditions and alternating 12-h light–12-h dark cycle (lights off at 0700 h). They were maintained at approximately 85% of their free feeding weight by restricting access to laboratory chow (Purina, UK) to approximately 18 g per day per rat. Water was provided *ad libitum*. All procedures were conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 and in accordance with local institutional guidelines. Behavioral testing was carried out between 0800 and 1700 hours during the animals' active phase.

5-Choice Serial Reaction Time Task

A detailed description of the nine-hole apparatus (Carli *et al*, 1983) and procedures (Dalley *et al*, 2004) has been provided previously. The boxes were controlled by Whisker software (Cardinal and Aitken, 2001). Subjects were trained to detect the location of a brief visual stimulus (0.5 s in duration) presented pseudo-randomly in one of five apertures over a large number of trials, as described previously (Dalley *et al*, 2004). A number of performance measures were recorded, including choice accuracy (the proportion of correct responses to the total number of correct and incorrect responses), omissions (a failure to respond within the 5 s limited hold period postpresentation of the stimulus), premature responding (responses made before the target stimulus), correct response latency (the time from the stimulus onset to a correct response), and magazine latency (the time from a correct response to the collection of food in the magazine). Errors of commission (ie responses to the incorrect aperture when the visual target is presented) or premature responses were punished with a 5 s time-out when all lights were extinguished. Perseverative nosepokes (additional nosepokes made postpresentation of the stimulus in any nosepoke aperture) and perseverative panel pushes (additional responses made at the food magazine before or after food retrieval) were recorded although not punished. Subjects were considered to have acquired the task when their accuracy was greater than 80% and omissions were fewer than 20%.

Surgical Procedures

Rats were anesthetized with ketamine (Ketaset, 100 mg/kg i.p.; Vet Drug, Bury St, Edmunds, UK) and xylazine (Rompun 10 mg/kg i.p.; Vet Drug, Bury St, Edmunds, UK) and secured in a stereotaxic frame with the incisor bar set at −3.3 mm relative to the interaural line in flat skull position. Bilateral 22-gauge guide cannulas (Plastics One, Sevenoaks, UK) were implanted in the NAc or mPFC according to the following stereotaxic coordinates: mPFC, anteroposterior + 3.0 mm (from bregma), lateral ± 0.75 mm and dorsoventral −2.2 mm from dura; NAc, anteroposterior, + 1.5 mm from bregma; lateromedial, ± 1.9 mm from midline; and dorsoventral, −2.0 mm from dura (Paxinos and Watson, 1998). Cannulas were secured to the skull with dental acrylic and stainless steel screws, and a wire stylet occluded the guide to maintain patency. After surgery, the animals were housed individually and allowed 5–7 days recovery.

Infusion Procedure

Following reestablishment of stable postoperative performance over 5–7 sessions (Figure 2), rats were habituated to the infusion procedure during one session where animals were lightly restrained and the stylet removed and then replaced. Infusion studies were run in 3-day cycles, starting with a baseline session. The following day, rats received a drug or vehicle infusion (phosphate-buffered saline; PBS) 5 min before testing in the 5CSRTT. On the third day, animals were not tested and remained in their home cage. All studies used a within-subject fully randomized Latin square design.

During infusions, the rats were gently restrained while the stylets were removed and 28-gauge bilateral injectors extending 5, 1.5 or 3 mm beyond the length of the guide cannulas were inserted into the NAc, PL or IL, respectively.

The injectors were left in place for 1 min prior to infusions of vehicle or drug (0.5 μl) over 1 min. The injector was left in place for 2 min to allow diffusion of the drug into the tissue surrounding the injector. The injector was then removed and the stylet replaced.

Experimental Summary

During the baseline sessions and testing days, the parameters were identical to those used in training (stimulus duration 0.5 s, ITI 5 s, limited hold 5 s). Four separate groups of rats were used. Two groups received prefrontal cannulas for the cortical infusion studies and were infused with either M100907 (PBS, 0.1, 0.3 μg) or SB242084 (PBS, 0.1, 0.5 μg). Two further groups received intra-NAc cannulas and either M100907 (PBS, 0.1, 0.3 μg) or SB242084 (PBS, 0.1, 0.5 μg). For the cortical studies subjects received infusions of vehicle or drug into the PL in a counterbalanced manner. Following PL infusions and a period of 2 days, a second set of infusions targeting the IL cortex was given to the same rats, again vehicle and drug infusions being given in a counterbalanced manner.

Drugs

M100907 (R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol, Solvay, Weesp, The Netherlands) was dissolved in 0.01 M PBS and 0.1 M HCl and the pH adjusted to 6.2 using 0.1 M NaOH. SB242084 (6-chloro-5-methyl-1-[2(2methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline, Solvay, Weesp, The Netherlands) was dissolved in 25 mM citric acid and 8% cyclodextrine in 0.9% saline and the pH adjusted to 6.4 using 0.1 M NaOH. Stock solutions of M100907 and SB242084 were prepared and aliquoted before being frozen at -80°C.

Histology

Following the completion of the experiments, subjects were anesthetized with a lethal dose of sodium pentobarbitone (1.5 ml Euthatal, 200 mg/ml, Genus Express, UK) and perfused via the left ventricle with 0.01 M PBS followed by 4% paraformaldehyde. The brains were removed and postfixed in paraformaldehyde. Prior to being cut, the brains were transferred to 20% sucrose in 0.2 M PBS and left overnight. Coronal sections were cut at 60 μm on a freezing microtome and stained with Cresyl Violet. Locations of the final injector tip positions in the NAc and IL cortex were mapped onto standardized coronal sections of a rat brain stereotaxic atlas (Paxinos and Watson, 1998).

Statistical Analysis

All analyses were conducted using SPSS for Windows (version 11.0; SPSS, Chicago, IL). Seven variables were analyzed: the percentage of correct responses made (number of correct responses/total correct and incorrect responses); percentage of responses omitted (number of omissions/total number of correct, incorrect, and omitted responses); percentage of premature responses (number of premature responses/total number of correct, incorrect, and omitted responses), latency to make a correct response, latency to collect reward and perseverative nosepokes and perseverative panel responses. Homogeneity of variance was verified using Levene's test and, if necessary, data were log or arcsine transformed prior to analysis to conform to the requirements for parametric statistics. Behavioral data were subjected to analysis of variance (ANOVA) using a general linear model, within-subjects design with one within subjects factor, dose, for the NAc studies and two within-subject factors, dose and area for the PL and IL. All tests of significance were performed at $\alpha = 0.05$; full factorial models were used unless otherwise stated. For repeated-measures analyses, Mauchly's test of sphericity of the covariance matrix was applied and the degrees of freedom corrected to more conservative values using the Huynh–Feldt epsilon for any terms involving factors in which the sphericity assumption was violated. Following repeated-measures analyses, *post hoc* comparisons were made using LSD test in SPSS.

RESULTS

Figure 1 shows the actual position of injector tips in the NAc and IL. Since it was difficult to ascertain the precise location of injectors in the PL due to subsequent track damage caused by the IL injectors we determined the position of the PL injectors by extrapolation from the actual position of injector tips in the IL. It can be seen that PL injectors had been located in the intended target region. Animals were excluded from the study if the cannula position was not correct or if histological examination revealed tissue damage beyond local physical damage around from the injector tract. Following surgery, all animals returned to presurgical baseline performance within the 5 days of postoperative rebaseline sessions (Figure 2). As a further index of comparable baseline performance between the various treatment groups, and to rule out any nonspecific effects of tissue damage on behavior, we also assessed task performance on days preceding each drug infusion (data shown in Figure 3). It can be seen that there were no significant effects on any of the main behavioral variables across the entire duration of the study.

Experiment 1: Effect of Intra-NAc Infusions of M100907

M100907 induced a significant decrease in percent premature responding when infused into the NAc ($F_{(2,14)} = 4.67$, $p \leqslant 0.028$, Figure 4). Relative to vehicle, this effect was significant for the 0.3 μg dose ($p \leqslant 0.028$) and close to being significant for the 0.1 μg dose ($p \leqslant 0.055$). The total number of premature responses was reduced by approximately 50%, comparable with previous data obtained following systemic administration of M100907 (Winstanley *et al*, 2004). A significant increase in percent omissions was also observed ($F_{(2,14)} = 4.80$, $p \leqslant 0.035$, Figure 4) although *post hoc* analysis revealed that this effect was only significant for the 0.3 μg dose ($p \leqslant 0.04$). No significant effect of Dose was observed for any of the other parameters analyzed ($F < 3.2$, $p > 0.11$) (Figure 4 and Table 1).

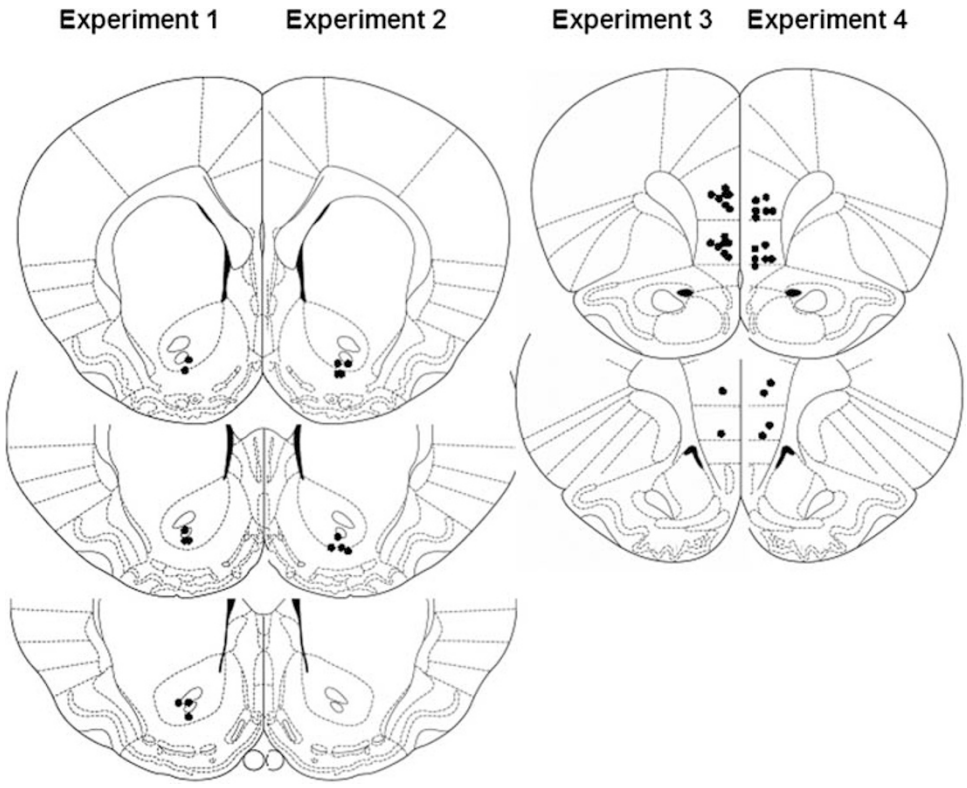

Figure 1 Schematic diagrams showing the location of the injector tips in the NAc (experiments 1 and 2) and IL cortex (experiments 3 and 4). The position of the prelimbic injector has been estimated from the final position in the IL cortex. Reconstructed from Paxinos and Watson (1998).

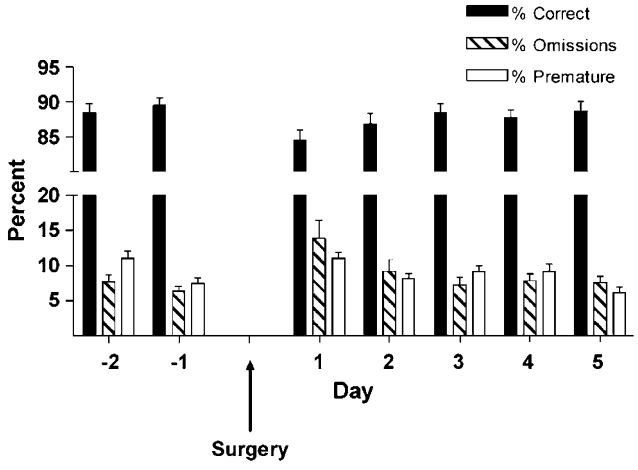

Figure 2 Pre- and postoperative baseline data ($n = 32$) for percent correct, omissions and premature responding. Data presented for all experimental groups as mean ± SEM.

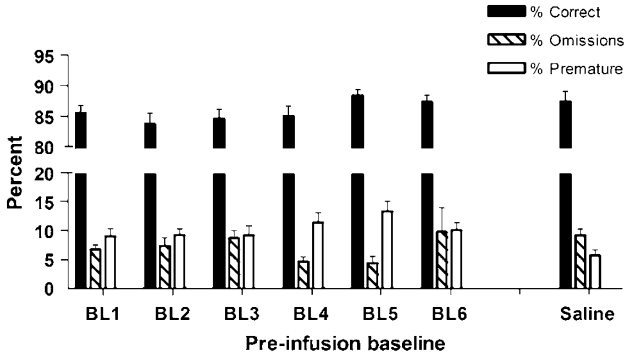

Figure 3 Results for percent correct, omissions and premature responses during the preinfusion baseline sessions and saline control infusion. Data presented for all experimental groups as mean ± SEM. However, statistical analysis was carried out separately for each individual experimental group ($n = 8$ per experiment). No significant differences were found across the baseline sessions or control saline infusion (ANOVA, % correct: $F > 2.3$, $p > 0.05$; % omissions: $F > 2.7$, $p > 0.07$; % premature: $F > 3.0$, $p > 0.08$, other variables (data not shown): $F > 2.1$, $p > 0.08$) and responses were comparable with pre- and postinfusion data (Figure 2).

Experiment 2: Effect of Intra-NAc Infusions of SB242084

SB242084 produced a dose-dependent increase in premature responding following infusion into the NAc ($F_{(2,14)} = 5.129$, $p \leqslant 0.021$, Figure 4). *Post hoc* analysis revealed a significant difference for the 0.1 μg ($p \leqslant 0.049$) and 0.5 μg, doses ($p < 0.018$). The total number of premature responses was increased by approximately 50%, comparable with previous data obtained following systemic administration of SB242084 (Winstanley *et al*, 2004). No significant effect of Dose was observed for any of the other parameters analyzed ($F < 1.6$, $p > 0.24$) (Figure 4 and Table 1).

Experiment 3: Effect of PL and IL Infusions of M100907

M100907 produced no significant effects on performance on the 5CSRTT following infusion into either the PL or IL (Area, $F < 3.4$, $p > 0.10$; Dose, $F < 3.2$, $p > 0.07$; or

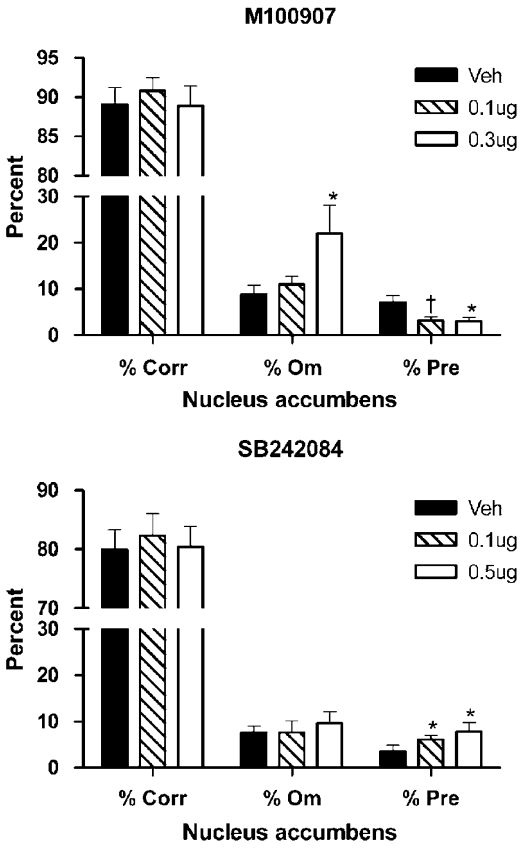

Figure 4 Effects of NAc infusions of the 5-HT$_{2A}$-receptor antagonist, M100907 (top) and the 5-HT2C-receptor antagonist, SB242084 (bottom), on the percent correct, omission, and premature responses ($n=8$). Data shown as mean ± SEM. *Denotes $p<0.05$, $^{\dagger}p=0.055$ compared with vehicle infusion.

Table 1 Summary of the Effects of M100907 and SB242084 on 5-CSRTT Performance Following Intra-NAc Infusions

M100907	Vehicle	0.1 μg	0.3 μg
% Correct	89.1 ± 2.1	90.8 ± 1.7	88.9 ± 2.5
% Omission	8.9 ± 2.0	11.0 ± 1.8	22.0 ± 6.2*
% Premature	7.1 ± 1.5	3.1 ± 0.9†	3.0 ± 0.9*
Perseverative nosepoke	41.8 ± 7.1	47.1 ± 9.9	48.6 ± 5.7
Perseverative panel push	25.5 ± 7.9	28.0 ± 10.0	21.5 ± 5.6
Correct latency (ms)	508.2 ± 20.4	532.2 ± 29.8	571.7 ± 29.8
Collection latency (ms)	1270.1 ± 103.9	1531.1 ± 123.8	1417.5 ± 95.6
SB242084	**Vehicle**	**0.1 μg**	**0.5 μg**
% Correct	79.9 ± 3.3	82.3 ± 3.7	80.4 ± 3.5
% Omission	7.6 ± 1.4	7.6 ± 2.5	9.6 ± 2.6
% Premature	3.5 ± 1.3	6.1 ± 0.9	7.8 ± 2.0
Perseverative nosepoke	55.1 ± 10.9	1.9 ± 10.8	67.4 ± 13.6
Perseverative panel push	55.5 ± 14.8	62.9 ± 17.6	60.9 ± 15.8
Correct latency (ms)	681.5 ± 76.0	627.2 ± 57.1	597.2 ± 44.5
Collection latency (ms)	1493.6 ± 78.3	1583.9 ± 311.0	1481.7 ± 54.5

Results shown as mean ± SEM ($n=8$), *$p<0.05$, $^{\dagger}p=0.055$ compared with vehicle infusion.

Area × Dose $F<2.9$, $p>0.10$). However, a trend towards an increase in the latency to make a correct response was observed ($F_{2,14}=3.2$, $p\leqslant 0.068$). Results for the effects of M100907 infused into the PL and IL are summarized in Figure 5 and Table 2.

Experiment 4: Effect of PL and IL Infusions of SB242084

SB242084 also failed to produce clear changes in 5-CSRTT performance following infusions into the PL and IL (Figure 6). However, for percent omissions, SB242084, ANOVA revealed a significant Area × Dose interaction ($F_{2,14}=5.3$, $p=0.019$) but no significant effects of Dose ($F_{2,13}=2.7$, $p=0.111$) or Area ($F_{1,7}=3.4$, $p=0.107$). *Post hoc* pair-wise comparisons revealed a significant reduction in percent omissions following infusion of 0.5 μg SB242084 into the PL ($p\leqslant 0.03$).

Analysis of percent premature responses suggested that SB242084 also tended to increase premature responding. Thus, there was a trend for a main effect of Dose ($F_{2,13}=3.0$, $p\leqslant 0.088$), but there was no main effect of Area ($F_{1,7}=1.2$, $p=0.31$) or Area × Dose interaction ($F_{2,14}=0.86$, $p=0.445$). No significant effects for Area ($F<1.7$, $p>0.239$), Dose ($F<2.3$, $p>0.148$), or Area × Dose interaction ($F<1.5$, $p>0.264$) were observed for any of the other parameters analyzed (Figure 6 and Table 3).

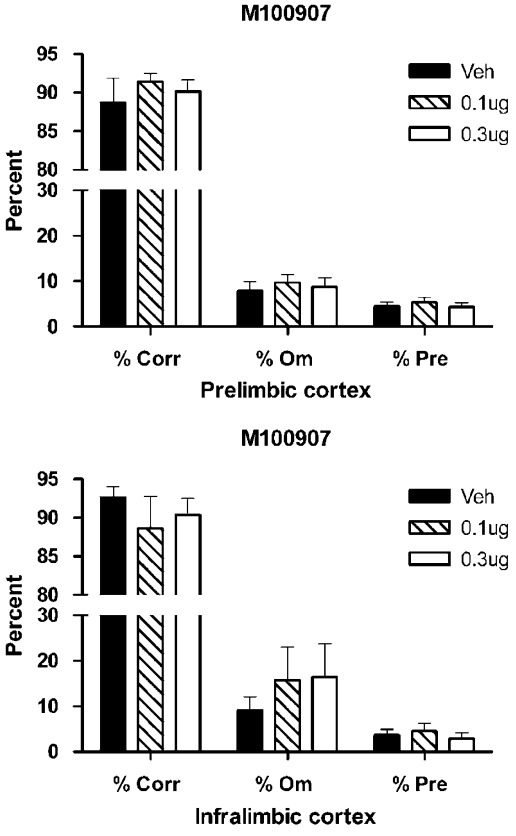

Figure 5 Effects of PL (top) and IL (bottom) infusions of the 5-HT$_{2A}$-receptor antagonist, M100907 on the percent correct, omission, and premature responses ($n=8$). Data shown as mean ± SEM.

Table 2 Summary of the Effects of M100907 on 5-CSRTT Performance Following Intraprelimbic or Intrainfralimbic Infusions

Prelimbic cortex	Vehicle	0.1 μg	0.3 μg
% Correct	88.7 ± 3.2	91.4 ± 1.0	90.2 ± 1.5
% Omission	7.9 ± 2.1	9.7 ± 1.8	8.8 ± 2.1
% Premature	4.5 ± 0.9	5.4 ± 1.1	4.4 ± 1.0
Perseverative nosepoke	43.3 ± 8.6	65.3 ± 23.3	56.8 ± 14.6
Perseverative panel push	49.0 ± 18.4	43.5 ± 15.3	55.3 ± 18.7
Correct latency (ms)	512.8 ± 30.3	583.6 ± 74.7	554.5 ± 39.9
Collection latency (ms)	1353.0 ± 77.2	1321.4 ± 70.4	1357.6 ± 78.3
Infralimbic cortex	**Vehicle**	**0.1 μg**	**0.3 μg**
% Correct	92.6 ± 1.4	88.6 ± 4.1	90.4 ± 2.1
% Omission	9.1 ± 3.1	15.8 ± 7.2	16.5 ± 7.3
% Premature	3.6 ± 1.3	4.5 ± 1.8	2.9 ± 1.2
Perseverative nosepoke	42.6 ± 6.6	35.8 ± 6.9	46.9 ± 9.6
Perseverative panel push	39.3 ± 8.6	51.6 ± 15.4	41.4 ± 11.9
Correct latency (msec)	510.8 ± 31.0	539.9 ± 36.1	610.2 ± 48.9
Collection latency (msec)	1317.6 ± 98.8	1397.8 ± 129.4	1521.4 ± 134.5

Results shown as mean ± SEM ($n = 8$).

Table 3 Summary of the Effects of SB242084 on 5-CSRTT Performance Following Intraprelimbic or Intrainfralimbic Infusions

Prelimbic cortex	Vehicle	0.1 μg	0.5 μg
% Correct	92.4 ± 1.3	89.5 ± 1.5	90.5 ± 1.4
% Omission	9.0 ± 1.9	6.6 ± 1.2	3.9 ± 0.5*
% Premature	5.4 ± 1.1	6.5 ± 1.3	7.8 ± 2.2
Perseverative nosepoke	55.4 ± 10.0	51.8 ± 9.2	48.8 ± 7.5
Perseverative panel push	18.0 ± 5.2	21.6 ± 7.5	22.8 ± 6.8
Correct latency (ms)	522.8 ± 30.9	515.3 ± 38.4	532.4 ± 37.7
Collection latency (ms)	1383.0 ± 108.2	1351.9 ± 93.7	1348.6 ± 88.6
Infralimbic cortex	**Vehicle**	**0.1 μg**	**0.5 μg**
% Correct	89.1 ± 2.6	90.3 ± 1.7	89.4 ± 2.2
% Omission	5.1 ± 1.3	4.5 ± 0.8	5.4 ± 0.9
% Premature	3.0 ± 0.7	8.0 ± 1.6	6.8 ± 1.6
Perseverative nosepoke	53.1 ± 8.1	52.4 ± 8.0	54.5 ± 9.2
Perseverative panel push	19.4 ± 5.4	25.3 ± 9.0	26.3 ± 8.1
Correct latency (ms)	549.2 ± 46.5	489.5 ± 25.5	484.2 ± 27.3
Collection latency (ms)	1375.2 ± 92.0	1649.0 ± 180.9	1396.7 ± 73.1

Results shown as mean ± SEM ($n = 8$), *$p < 0.05$ compared with vehicle infusion.

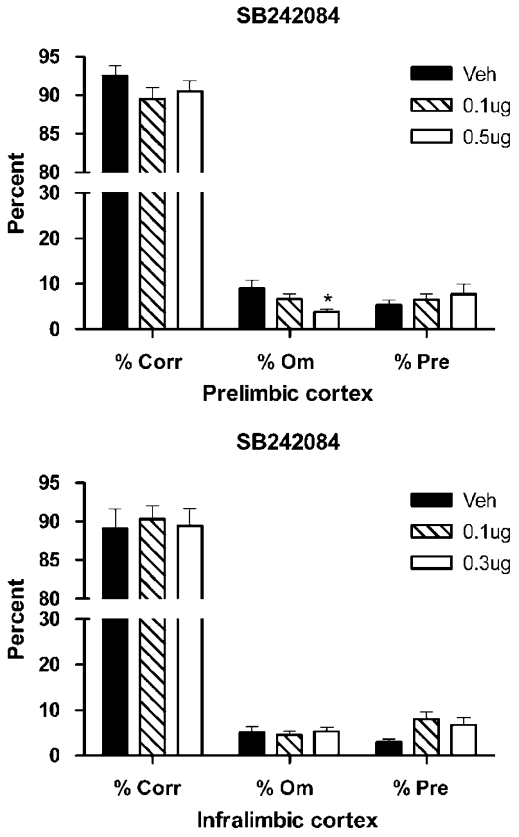

Figure 6 Effects of PL (top) and IL (bottom) infusions of the 5-HT_{2C}-receptor antagonist, SB242084 on the percent correct, omission, and premature responses ($n = 8$). Data shown as mean ± SEM. *Denotes $p < 0.05$ compared with vehicle infusion.

DISCUSSION

These data reveal that 5-HT_{2A} and 5-HT_{2C} receptor blockade in the NAc induces opposite effects on impulsive responding in the 5CSRTT with no effects on accuracy. Intra-PFC infusions did not reveal any major effects following 5-HT_{2A} or 5-HT_{2C} receptor blockade, although SB242084 reduced omissions (PL) and tended to increase premature responding (IL). The results from the present study demonstrate that local blockade of 5-HT_{2A} and 5-HT_{2C} receptors in the NAc induces a decrease and increase in premature responding, respectively; suggesting opposing effects on the neurochemical processes that underlie this behavioral variable. The results following mPFC infusions suggest that 5-HT_{2A} and 5-HT_{2C} receptors within this region play only a minor role in 5CSRTT performance under baseline task conditions. From this analysis it appears that effects on accuracy and premature responding produced by 5-HT receptor manipulations within the PFC in other studies only occur when task requirements are altered.

Blockade of 5-HT_{2C} receptors induces performance deficits that generally reflect the results obtained following global 5-HT depletion; however, the effects with M100907 were opposite. These opposing roles may explain why 5-HT depletion induces an increase in impulsivity (Winstanley *et al*, 2004) yet premature responding in the 5CSRTT positively correlates with mPFC 5-HT levels (Dalley *et al*, 2002). The ability of these two receptors to induce opposite effects implies that the actions of 5-HT within different brain areas depend on the relative level of activation of 5-HT_{2A} *vs* 5-HT_{2C} receptors. Actions at 5-HT_{1A} and other receptor subtypes are also likely to be involved and adaptive changes in the level of expression of postsynaptic 5-HT receptors following 5-HT depletion should be considered. Relevant to our findings is recent research showing that the

5-HT_2 receptor family is constitutively active; thus a change in the relative expression of 5-HT_{2A} *vs* 5-HT_{2C} receptor expression could theoretically alter impulsive responding, even in the absence of endogenous 5-HT (Berg *et al*, 2005; Millan, 2005).

Pharmacological and Neuroanatomical Specificity

M100907 and SB242084 are selective antagonists at the 5-HT_{2A} and 5-HT_{2C} receptors, respectively (Sorensen *et al*, 1993; Kehne *et al*, 1996; Kennett *et al*, 1997). M100907 has high affinity for the 5-HT_{2A} receptor with moderate affinity for 5-HT_{2C} and α_1 adrenoceptors (Kehne *et al*, 1996). Similarly, SB242084 is a high affinity antagonist with more than 100 fold selectivity for 5-HT_{2C}-receptors relative to 5-HT_{2B} or 5-HT_{2A} receptors (Kennett *et al*, 1997). The cannula placements, infusion volumes and rates used were based on previous studies (Murphy *et al*, 2005; Pezze *et al*, 2007). Infusion of 0.5 μl over 1 min restricts the spread of the drug to approximately 1 mm^3 limiting the effects of the drug to the areas of interest, (Myers, 1966; Myers *et al*, 1971; Routtenberg, 1972). The NAc contains two anatomically and functionally distinct subregions: a medioventral shell and a dorsolateral core (Zahm and Brog, 1992). The infusion sites were located in the NAc core close to the anterior commissure, and adjacent to the NAc shell. While previous studies (Pezze *et al*, 2007) have shown that the observed effects are unlikely to involve diffusion of the compounds into the dorsolateral striatum, an action within the NAc shell cannot be excluded.

Comparison with Previous Studies

The data from the present study concur with previous reports on effects of the drugs when administered systemically but provide important additional information on the localization of the receptors involved. Systemic administration of M100907 decreased premature responding, while SB242084 increased impulsivity (Winstanley *et al*, 2004) consistent with the present results following intra-NAc infusions. In contrast to the effects of systemic administration, the present data reveal that 5-HT_{2A} and 5-HT_{2C} receptors in the NAc do not play a significant role in performance accuracy or alter motivation to perform the task (Winstanley *et al*, 2004). At the higher dose, M100907 increased omissions and tended to increase response latency suggesting that excessive blockade of 5-HT_{2A} receptors may disrupt animals' ability to perform the task. Previous reports using the intra-NAc infusion of the non-selective 5-$HT_{2A/2C}$ agonist 2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) failed to alter premature responding (Koskinen and Sirviö, 2001). Its combined actions at 5-HT_{2A} and 5-HT_{2C} receptors, however, may induce activation of both subtypes with physiological antagonism counteracting effects on impulsivity. Systemic administration of the 5-$HT_{2A/2C}$ antagonist, ketanserin tends to reduce premature responding (Ruotsalainen *et al*, 1997; Passetti *et al*, 2003). Considering the present findings, the effects with ketanserin presumably result from a greater action at 5-HT_{2A} receptors (pKi 8.9 *vs* 7.0 5-HT_{2A} *vs* 5-HT_{2C}, respectively, Barnes and Sharp, 1999).

Previous studies investigating 5-HT_{2A} and 5-HT_{2C} receptor blockade in the mPFC have also shown a lack of effect of both M100907 and ketanserin on response inhibition under baseline conditions (Winstanley *et al*, 2003; Passetti *et al*, 2003). Although 5-HT_{2A} and 5-HT_{2C} receptors are expressed within the mPFC and function to regulate output via pyramidal neurons, the impact of blockade of these receptors on baseline 5CSRTT variables appears minimal. However, it is important to note that M100907 does decrease impulsive responding when the baseline level is altered through modification of task requirements, eg following reduced stimulus duration (Winstanley *et al*, 2003; Passetti *et al*, 2003). Studies have also shown that intra-mPFC M100907 attenuates NMDA antagonism-induced glutamate release and premature responding in the 5CSRTT (Carli *et al*, 2004, 2006). To date, studies investigating the involvement of 5-HT_{2C} receptors during similar conditions have not been carried out. The present study does not preclude the involvement of 5-HT_{2A} or 5-HT_{2C} receptors within the mPFC in attention and response inhibition but suggests that in highly trained rats, performing the 5CSRTT task under baseline conditions, 5-HT_{2A} or 5-HT_{2C} receptors do not contribute significantly to attention or response inhibition. A number of alternative methods could be used in future studies to increase the level of baseline premature responses, thereby enhancing the sensitivity of the task for detecting reductions in premature responding. Previous studies using intraprefrontal infusion of M100907 used a reduced stimulus duration (Winstanley *et al*, 2004), however, other strategies include using the forced choice version of the task with a single stimulus location, increasing the ITI or using a variable ITI (Robbins, 2002).

Serotonin-Dopamine Interactions

There is considerable evidence implicating DA-ergic mechanisms in inhibitory response control (Cole and Robbins, 1989; Robbins, 2002; Winstanley *et al*, 2006; Pezze *et al*, 2007; Dalley *et al*, 2007). Neurochemical and behavioral studies indicate that 5-HT_2 receptor subtypes differentially modulate DA as well as noradrenaline function within the NAc (Gobert and Millan, 1999; Di Matteo *et al*, 2001; Porras *et al*, 2002; Bubar and Cunningham, 2006). For example, differential effects for M100907 and SB242084 on NAc DA have also been shown following *in vivo* microdialysis experiments (Gobert and Millan, 1999; Di Matteo *et al*, 2001; Porras *et al*, 2002; De Deurwaerdére *et al*, 2004). M100907 does not affect baseline DA levels but attenuates amphetamine-induced hyperactivity (Sorensen *et al*, 1993) and amphetamine or DOI-induced DA release (Porras *et al*, 2002). Studies investigating the role of 5-HT_{2C} receptors in the NAc are complex and appear to depend on phasic *vs* tonic activity of DA neurones (Yan, 2000; Porras *et al*, 2002; De Deurwaerdére *et al*, 2004; Navailles *et al*, 2004, 2006). These effects are further complicated by constitutive activity of 5-HT_{2C} receptors, with neurochemical studies finding facilitatory and inhibitory roles for 5-HT_{2C} receptors and NAc DA (De Deurwaerdére *et al*, 2004; Navailles *et al*, 2004, 2006). Overall, a possible explanation for the findings in the present investigation is that M100907 and SB242084 differentially modulate NAc DA resulting in decreases and increases in premature responding, respectively.

In conclusion, these data demonstrate selective yet opposing effects for 5-HT_{2A} and 5-HT_{2C} receptors within the NAc on impulsivity in the 5CSRTT. The results concur with previous findings following systemic administration of M100907 or SB242084 (Winstanley *et al*, 2003, 2004) and suggest the NAc is an important site of action of these compounds. These data thus add to a growing body of evidence for an involvement of multiple neurotransmitter systems in the mediation and control of impulsive behavior, including in addition to 5-HT and DA, the noradrenergic (Robinson *et al*, 2007; Blondeau and Dellu-Hagedorn, 2007) and histaminergic (Day *et al*, 2007) systems. The present results indicate that selective 5-HT_{2A} receptor antagonists and/or 5-HT_{2C} receptor agonists may have beneficial effects in psychiatric disorders where co-existing impulsivity is often present, including ADHD, schizophrenia, and substance abuse.

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DISCLOSURE/CONFLICT OF INTEREST

ESJR, JWD, DEHT, MAP, ERM declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

JCG declares his part employment with Solvay Pharmaceuticals (Weesp, The Netherlands).

TWR declares that over the past 3 years he has received honorariums from Solvay Pharmaceuticals (Weesp, The Netherlands), Microsoft, Merck, Sharp and Dohme, Lundbeck, and as Editor of Psychopharmacology. TWR also acts a consultant for Glaxo Smith Kline, Lilly Inc., and Allon Therapeutics, and has shares and share options in CeNeS, Cambridge Cognition and Allon Therapeutics. TWR holds research grants with Pfizer and Glaxo Smith Kline.

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