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The Serotonin 5-HT_{2A} Receptors Antagonist M100907 Prevents Impairment in Attentional Performance by NMDA Receptor Blockade in the Rat Prefrontal Cortex

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We investigated whether 5-HT_{2A} receptors contribute to the control of attentional performance by glutamate NMDA receptor mechanisms in the medial prefrontal cortex (mPFC). We examined the effects of NMDA receptor blockade in the mPFC on attentional performance by infusing a competitive glutamate NMDA receptor antagonist, 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) into the mPFC of rats performing a task of divided and sustained visual attention. The five-choice serial reaction time task provides indices of attentional functioning (% correct responses), executive control (measured by anticipatory and perseverative responses) and speed. A dose of 10 ng CPP injected bilaterally into the mPFC increased anticipatory and perseverative responding; 50 ng reduced accuracy. Increasing the stimulus duration alleviated the CPP-induced accuracy deficit but did not reduce its effects on anticipatory and perseverative responses. CPP at 50 ng caused motor hyperactivity whereas lower doses had no effect. [R-(+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (M100907) (M100907), a 5-HT_{2A} receptor antagonist, injected subcutaneously at 10 and 40 μg/kg, had no effect on accuracy but dose dependently reversed the impairment induced by 50 ng CPP. Both doses of M100907 completely abolished CPP-induced anticipatory but not perseverative over-responding. At the dose of 40 μg/kg M100907 reversed CPP-induced motor hyperactivity. This study provides evidence that the prefronto-cortical glutamate NMDA system may make an important contribution to the control of attention and executive functions. It also indicates that 5-HT_{2A} receptors may serve to optimize attentional selectivity and improve some aspects of executive control.

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INTRODUCTION

The prefrontal cortex has been implicated in a variety of cognitive functions, including working memory, visual attention, and executive functions (Shallice, 1982; Fuster, 1989; Corbetta *et al*, 1991; Duncan, 1995, 2001; Baddeley, 1996; Goldman-Rakic, 1998; Robbins, 1998). Lesions of the medial prefrontal cortex (mPFC) but not the cingulate, anterior-lateral or parietal cortex in rats impair attentional functioning and cause compulsive perseveration in a task of sustained and divided attention (Muir *et al*, 1996; Passetti *et al*, 2002, 2003a, b).

Cognitive deficits, including attention disorders and deficits in executive functions, are a central feature of schizophrenia (Frith, 1987; Kay and Sevy, 1990; Braff, 1993). Phencyclidine and ketamine, noncompetitive antagonists at glutamate NMDA receptors, exacerbate positive and negative symptoms in schizophrenic patients, and induce schizophrenia-like symptoms and cognitive deficits in healthy volunteers (Javitt and Zukin, 1991; Krystal *et al*, 1994). This is in line with findings in rodents that intracortical or peripheral administration of NMDA receptor antagonists causes deficits in sensory-motor gating (Jentsch and Roth, 1999), hyperactivity (O'Neill and Liebman, 1987; Jentsch *et al*, 1998), and working memory (Wesierska *et al*, 1990; Verma and Moghaddam, 1996; Moghaddam *et al*, 1997; Aura and Riekkinen, 1999; Romanides *et al*, 1999). Peripherally administered phencyclidine or dizocilpine, noncompetitive NMDA receptor antagonists, or the selective NMDA-R2B receptor antagonist Ro 63-1908, cause deficits in attentional performance in a five-choice serial reaction time (5-CSRT) task (Higgins *et al*, 2003; Le Pen *et al*, 2003).

Serotonin receptors are increasingly recognized as major targets for cognitive enhancement in schizophrenia (Meltzer

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et al, 2003; Roth *et al*, 2003). Indeed, all the currently available atypical antipsychotics such as clozapine, risperidone, olanzapine, quietapine, and ziprasidone, which are potent serotonin 5-HT$_{2A}$ receptor antagonists (Meltzer, 1999) consistently improve an aspect of attention such as vigilance and to some extent favor executive functions in patients with schizophrenia (Meltzer and McGurk, 1999; Harvey and Keefe, 2001; Harvey *et al*, 2003, 2004).

Serotonin 5-HT$_{2A}$ receptor subtypes are found in many regions of the CNS (Barnes and Sharp, 1999) and appear to be particularly rich in neo-cortical regions including layer V of the prefrontal cortex (Jakab and Goldman-Rakic, 1998, 2000). Serotonin, through 5-HT$_{2A}$ receptors, enhances glutamate-induced excitatory postsynaptic currents in the prefrontal cortex (Aghajanian and Marek, 1997). Stimulation of 5-HT$_{2A}$ receptors by [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) activates the glutamatergic-thalamo-cortical neurons, as revealed by Fos induction (Scruggs *et al*, 2000), increases cortical glutamate efflux (Scruggs *et al*, 2003), and indirectly activates AMPA/kainate receptors (Martin-Ruiz *et al*, 2001). Administration of NMDA antagonists or knocking out NMDA receptor functions raises extracellular 5-HT efflux in the mPFC (Martin *et al*, 1998; Miyamoto *et al*, 2001).

Agonists at 5-HT$_{2A}$ receptors impair attentional functioning and inhibitory response control in rats (Carli and Samanin, 1992; Koskinen *et al*, 2000). In contrast, the selective 5-HT$_{2A}$ receptor antagonist [R-(+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (M100907) (Kehne *et al*, 1996) reduces Fos expression induced by NMDA receptor antagonists (Habara *et al*, 2001). M100907 also reduces the elevated locomotor activity (Gleason and Shannon, 1997; Martin *et al*, 1997), forced swim immobility (Corbett *et al*, 1999), and deficit in prepulse inhibition (Varty *et al*, 1999) induced by NMDA receptor antagonists. In rats performing a 5-CSRT task, M100907 improved attentional performance (Winstanley *et al*, 2003; Passetti *et al*, 2003a) and reversed the deficit in inhibitory response control induced by systemic administration of the NMDA receptor antagonists, dizocilpine and Ro 63-1908 (Higgins *et al*, 2004).

The present study investigated whether 5-HT$_{2A}$ receptors contribute to the control of attentional performance exerted by glutamate NMDA receptor mechanisms in the mPFC. Rats' attentional performance was assessed in a 5-CSRT task, which measures different types of performance that include aspects of attention (measured by accuracy of detection) and executive control such as impulsive (anticipatory) and compulsive (perseverative) responding and provides various measures of speed of responding and motivation (Robbins, 2002). Thus in the present study, we infused various doses of a competitive NMDA receptor antagonist, 3-(R)-2-carboxypiperazin-4-propyl-l-phosphonic acid (CPP) directly into the mPFC, which is particularly rich in NMDA receptors (Cotman and Iversen, 1987). We also examined the effects of CPP in conditions of decreased attentional load by introducing 'challenge' sessions with longer stimulus duration. The effect of CPP on motor activation was also assessed. We investigated the contribution of 5-HT$_{2A}$ receptors to CPP-induced impairment in attentional performance using M100907, a selective 5-HT$_{2A}$ receptor antagonist, which has 300 times more affinity for 5-HT$_{2A}$ receptors than other receptor subtypes including 5-HT$_{2C}$ and α-1 adrenergic receptors (Kehne *et al*, 1996). Various doses of M100907 were injected subcutaneously to rats, which were given microinjections of CPP into the mPFC. We also examined the effect of M100907 on motor activation induced by CPP.

MATERIALS AND METHODS

Animals

Twenty-seven male Lister Hooded rats (Charles River, Italy) weighing between 300 and 350 g before surgery were used to examine the effects of various treatments on the performance of a 5-CSRT task. They were housed in pairs until surgery and then singly in a temperature-controlled room (21°C) with a day/night cycle (07:00–19:00). Water was available *ad libitum*. Limited access to food (about 15 g/rat of Altromin pellets) at the end of each day's testing kept the animals at 85–90% of their initial free-feeding weight. The male Lister Hooded rats ($n = 64$) (Charles River, Italy) used to assess the effects of treatments on motor activity had free access to food.

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national (D.L. n. 116, G.U., suppl., 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJ L 358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

Apparatus

The test apparatus has been described in detail previously (Carli *et al*, 1983). It consisted of two 25 × 25 cm aluminum chambers built in the Department of Experimental Psycho logy, University of Cambridge. The rear wall of each box was concavely curved, and had set into its full arc nine square holes, 4 cm deep and 2 cm above floor level. Each hole had an infrared beam crossing the entrance vertically and illuminating a photoelectric cell. A standard 3 W bulb at the rear of each hole provided illumination. Food pellets (Sandown Scientific, UK) were delivered to a tray at the front of the box. A hinged panel blocked the entrance to the tray. A 3 W house-light was installed centrally in the box roof. Each apparatus was controlled on-line and data were collected by a Control Universal Cube microcomputer system (Cambridge, UK), with software written in ONLI-BASIC.

Behavioral Procedures

5-CSRT task. Animals were trained on the 5-CSRT task to a stable performance as previously described (Carli *et al*, 1983). The start of the session was signaled by illumination of the house-light and the delivery of a single food pellet. Opening the panel to collect the pellet began the first trial. After a fixed delay (the intertrial interval, ITI), the light at the rear of one of the holes came on for a short period. The light stimulus was presented in each hole for an equal

number of times during the course of a complete session, with the order of presentation randomized by the computer. While the light was on, and for a short period afterwards (the limited hold), responses in the hole that was illuminated (correct response) resulted in the delivery of a food pellet. Responses in the holes that had not been illuminated (incorrect responses) or failure to respond within the limited hold (omissions) caused the houselights to be turned off for a short period (time out). Responses made in the holes while the house-light was off restarted the time out.

After the delivery of food, or at the end of time out, the rat started the next trial by opening the panel at the front of the chamber. Responses made in the holes after a correct response (perseverative responses), or after the end of time out before opening the panel, resulted in a period of time out. Responses made in the holes during the ITI (anticipatory responses) also resulted in a period of time out. After anticipatory responses, however, opening the panel re-started the current trial.

Each daily session consisted of 100 trials or 30 min of testing, whichever was completed sooner, after which all lights were turned off and further responses had no effect.

In the first session of the test schedule the stimulus and limited hold each lasted 1 min and, depending on individual performances, they were progressively reduced to 0.5 s. The ITI and time out both lasted 2 s during the first session and the ITI was raised to 5 s in subsequent sessions; time out was not changed. When the rats reached a stable performance with a mean of 80% correct responses and no more than 15% omissions, they were allocated to different treatment schedules. Each rat had only one session on the 5-CSRT task per day throughout the experiments.

Motor activity. Motor activity was assessed in activity cages ($40 \times 25 \times 18$ cm) equipped with infrared photocell beams running horizontally along the axis of the cage (6 cm from the cage-end and 1 cm above the floor). The apparatus was controlled and data were collected by an Acorn computer system equipped with SPIDER extension (Paul Fray, Cambridge, UK). Rats were implanted with cannulae in the mPFC. After 7 days of recovery the animals were habituated to the activity cages for 1 h after which they were taken out and after the appropriate treatment put back into the activity cage, and the photocell beam interruptions were recorded over a 2 h period in 5-min bins.

Surgery. Rats previously trained to a stable level of performance were anesthetized with Equithesin (9.7 mg/ml sodium pentobarbital in saline $+42.6$ mg/ml chloral hydrate in propylenglycol $+21.2$ mg/ml Mg_2SO_4 in ethanol; 3.0 ml/kg intraperitoneally, i.p.), and secured in a stereotaxic frame (Kopf Ins., USA) with the incisor bar set at -3.3 mm relative to the interaural line. Bilateral 23-gauge, stainless-steel guide cannulae (Cooper's Needles, UK) were implanted in the medial region of the prefrontal cortex using standard stereotaxic techniques and secured to the skull using three bone screws and dental cement. The co-ordinates used were: anterior–posterior $+3.8$ mm from bregma, lateral ± 0.8 mm from midline, and dorsal–ventral -3.2 from dura (Paxinos and Watson, 1982). Thirty-gauge stainless-steel stylets flush with the end of the guide cannulae were inserted in the guide cannulae. After surgery rats were housed singly and had 1 week of recovery without training on the 5-CSRT task. After recovery all rats were retrained on the task to re-establish a presurgery level of baseline performance.

Micro-infusion procedure. On testing days, while the rat was held, the stylets were removed and two injection units terminating 2 mm below the tip of the guides were inserted. A volume of 1 μl per hemisphere of various doses of CPP or saline was delivered at a rate of 0.5 μl/min by a 10 μl Hamilton syringe mounted in a CMA/100 infusion pump (CMA Microdialysis, Sweden), connected by PP10 tubing to the injection units. Injection units were left in place for 1 min to allow for diffusion.

Histology. After completion of the behavioral testing, rats were killed by a lethal dose of Equithesin and perfused transcardially with phosphate buffer saline followed by 4% formalin solution. Brains were removed and postfixed in 4% formalin solution. Before being cut, the brains were transferred to 20% sucrose in 0.2 M phosphate buffer saline. Coronal sections were cut at 30 μm in a Cryo-cut and stained with cresyl violet. Inspection of the stained slides under the light microscope and the trajectory of gliosis produced by the cannula allowed its location and tip to be estimated and mapped on figures of the atlas (Paxinos and Watson, 1982) (Figure 1). Only data from rats in which the cannulae were located in the desired area were included in the results. Three rats were excluded because of infection at the injection site.

Drugs and experimental design. CPP (Tocris, UK) was dissolved in 1 μl saline and injected into the mPFC at various doses 10 min before the test session. M100907 (Aventis, USA) was dissolved in 2 ml vehicle (sterile water with two to three drops of 90% lactic acid). Vehicle or M100907 was given subcutaneously 20 min before a microinjection of 1 μl saline or 50 ng/μl CPP into the mPFC.

Each rat used in the CPP dose–response study ($n=9$) received saline (1 μl) or 1, 10, and 50 ng/μl CPP. To examine the effects of CPP in conditions of increased stimulus duration, each rat ($n=9$) received 1 μl saline or 50 ng/μl CPP injected into the mPFC and was exposed to sessions in which stimulus duration was 0.5 or 1.0 s. A similar experimental design was used to examine the effects of various combinations of vehicle (2 ml/kg) and 10 or 40 μg/kg M100907 plus 1 μl saline or 50 ng/μl CPP injected into the mPFC ($n=9$). We also examined the effects of vehicle (2 ml/kg) or 10 and 40 μg/kg M100907 during 'challenge' sessions in which the ITI was increased from the standard 5 to 7 s.

On each test day drugs or the various combinations were administered according to a Latin-square design. At least 2 days were left between test days. Rats were always tested on these 'free' days to re-establish the baseline and check the lasting effects of drugs.

The effects of various doses of CPP on motor activity were assessed using 24 experimentally naïve rats implanted with cannulae in the mPFC. After habituation to the activity cages rats were injected with 1, 10, and 50 ng/μl CPP or 1 μl

vehicle and 10 min later were placed in the activity cages. The interaction between M100907 and CPP on motor activity was assessed in a group of 40 rats implanted with cannulae in the mPFC. After habituation to the activity cages the rats were injected subcutaneously with vehicle (2 ml/kg) or M100907 (10 and 40 μg/kg) and 20 min later received bilateral injections of CPP (50 ng/μl) or vehicle (1 μl) into the mPFC. After 10 min, they were transferred to the activity cages and their motor activity was recorded. The effects of CPP on motor activity decayed rapidly and after the first 30 min the effects were extremely variable. Thus, only the data collected during the first 30 min of testing were statistically analyzed and are presented in the Results section.

Statistical analysis. The main dependent variables selected for analysis were: (a) the percentage of correct responses (total correct responses/total correct + total incorrect responses × 100); (b) percentage of omissions (total omissions/total number of trials × 100); (c) mean correct response latency (measured to the nearest 0.01 s); (d) the number of anticipatory responses in the holes during the ITI; and (e) the number of perseverative responses in the holes after a correct response.

We also measured and analyzed the mean latency to make an incorrect response, mean latency to collect the earned food pellet (both measured to the nearest 0.01 s) and the number of panel-pushes during ITI. However, CPP, M100907 or their combinations had no effect on these measures and they are not presented or discussed.

Correct responses and omissions, as percentages, were transformed according to the formula 2 arcsin(SQRT (%X/100)). The mean latencies to respond correctly were transformed by log 10. These transformations were done in order to normalize the distributions in accordance with the ANOVA model (Winer, 1971).

The CPP dose–response results and the effects of M100907 in conditions of increased ITI were analyzed by within-subjects one-way ANOVA, and the means of individual treatments were compared with saline by Dunnett's *t*-test. The results of the experiments testing the effects of 50 ng/μl CPP in combination with different stimulus durations (0.5 and 1.0 s) or different doses of M100907 (10 and 40 μg/kg) were analyzed by within-subjects two-way ANOVA. The means of the individual treatment combinations were compared by Tukey's HDS test.

The motor activity data recorded in 5-min time bins were summed over the first 30 min of the test period and analyzed by between-subjects one-way or two-way ANOVA, as appropriate. The means of individual treatments were compared with controls using Dunnett's *t*-test. Tukey's HDS test was employed for multiple comparisons of the means for various individual treatments.

Statistical software (SAS Institute Inc., USA) was run on a Micro VAX 3500 computer (Digital, USA).

RESULTS

The gray areas in Figure 1 depict the location of the injector tips of rats included in the results. The majority was confined to the prelimbic area between bregma +4.2 and +3.7. In some rats the tips were between bregma +3.7 and +3.2. However, we did not observe any difference in the behavioral results of rats with injection tips confined to the more anterior (bregma 4.2–3.7) or the posterior (bregma 3.7–3.2) area of mPFC. Repeated injections did not cause extensive damage and only a few animals had signs of infection so were not included in the results.

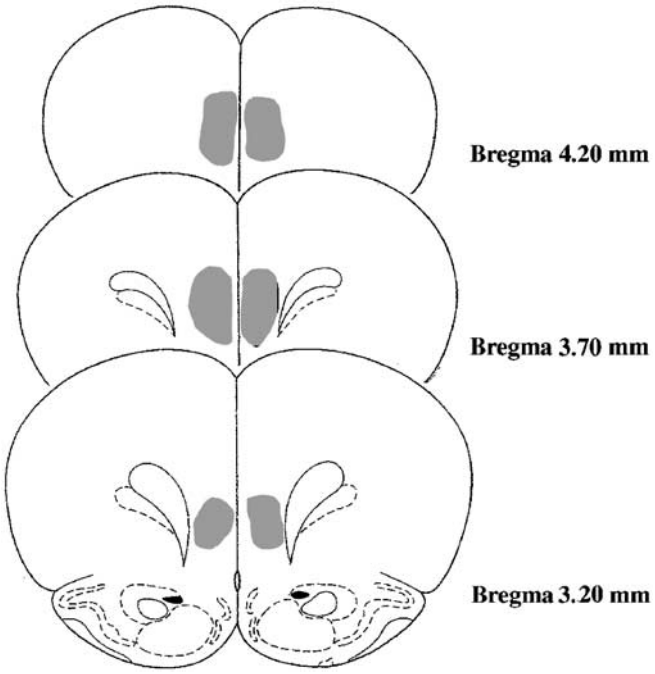

Figure 1 Schematic representation of the injection sites in the mPFC. The gray area indicates the location of the injection tips.

Effects of CPP on Performance of a 5-CSRT Task

Baseline condition. Figure 2a shows how CPP impaired rats' discriminative accuracy. Overall, CPP (1, 10, and 50 ng/μl per side) dose dependently lowered the percentage of correct responses ($F_{(3,21)} = 6.4$, $P = 0.003$). However, despite the apparent decrease with 10 ng/μl ($P > 0.05$, Tukey's test), the percentage was only significantly lower after 50 ng/μl ($P < 0.05$, Tukey's test). In parallel to the deleterious effects on accuracy, Table 1 shows that CPP increased omissions ($F_{(3,21)} = 10.59$, $P = 0.0002$) and correct response latencies ($F_{(3,21)} = 5.6$, $P = 0.005$). The omissions were significantly increased by 50 ng/μl ($P < 0.05$, Tukey's test) while both 10 and 50 ng/μl increased correct response latencies (both $P < 0.05$, Tukey's test).

In addition, CPP particularly impaired the rats' ability to withhold inappropriate nose-poke responses, as shown by increased number of anticipatory ($F_{(3,21)} = 5.3$, $P = 0.007$) (Figure 2b) and perseverative responses ($F_{(3,21)} = 3.95$, $P = 0.02$) (Figure 2c). These increases were already maximal at 10 ng/μl and did not rise further with 50 ng/μl (both Tukey's test $P < 0.05$). The dose of 1 ng/μl had no effect on any measure of attentional performance.

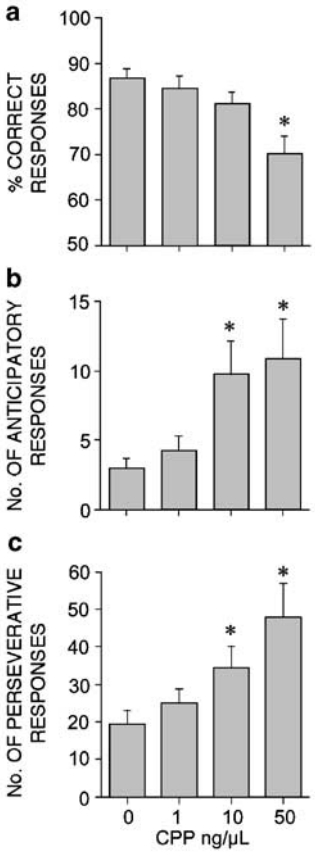

Figure 2 The effects of 1, 10, and 50 ng/μl of CPP or 1 μl vehicle injected into the mPFC 10 min before the test session on correct responses (a), anticipatory responses (b), and perseverative responses (c). CPP and vehicle were administered at least 48 h apart, according to a Latin-square design. The histograms show mean ± SEM of eight rats. *$P<0.05$ *vs* 0 (Tukey's test).

Table 1 Effect of Various Doses of CPP on Omissions and Correct Response Latency

Treatment (ng/μl)	Omissions (%)	Correct response latency (s)
Vehicle	16.6 ± 2.1	0.58 ± 0.02
CPP 1	15.1 ± 1.9	0.65 ± 0.05
CPP 10	25.3 ± 5.0	0.78 ± 0.05*
CPP 50	31.2 ± 5.7*	0.73 ± 0.03*

Each value is the mean ± SEM of eight rats. CPP was injected into the mPFC 10 min before the test session. Doses of 1, 10, and 50 ng/μl of CPP or vehicle (1 μl) (Vehicle) were injected bilaterally, at least 48 h apart, according to a Latin-square design.
*$P<$ 0.05 *vs* Vehicle (Dunnett's *t*-test).

Increased stimulus duration. The performance accuracy and omissions are shown in Figure 3a and Table 2, respectively. The deficits of rats injected with 50 ng/μl CPP were abolished when the stimulus duration was increased to 1.0 s from the standard 0.5 s (% correct responses: stimulus × CPP, $F_{(1,24)}=6.7$, $P=0.016$; CPP, $F_{(1,24)}=12.2$, $P=0.002$; stimulus, $F_{(1,24)}=32.1$, $P=0.0001$; % omissions: stimulus × CPP, $F_{(1,24)}=6.8$, $P=0.01$; CPP, $F_{(1,24)}=9.46$, $P=0.005$; stimulus, $F_{(1,24)}=1.3$, $P>0.05$). Table 2 shows that the longer stimulus, or CPP injections

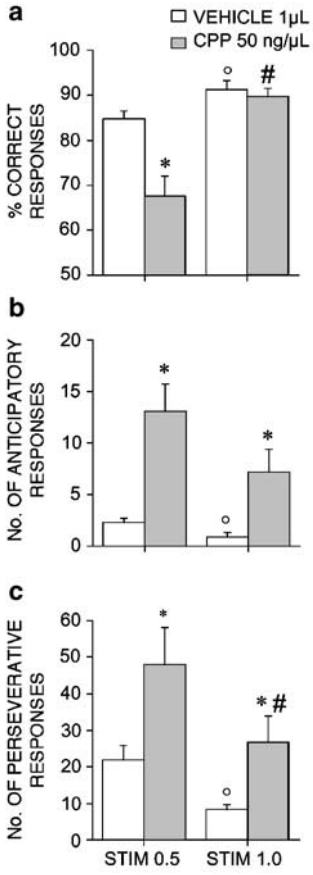

Figure 3 Effects of lengthening the stimulus from 0.5 to 1.0 s on correct responses (a), anticipatory responses (b), and perseverative responses (c) of rats injected with 50 ng/μl CPP or 1 μl vehicle into the mPFC 10 min before the test session. CPP or vehicle were administered at least 48 h apart, according to a Latin-square design. The histograms show mean ± SEM of nine rats. *$P<0.05$ *vs* Vehicle; °$P<0.05$ *vs* Vehicle (STIM 0.5); #$P<0.05$ *vs* CPP (STIM 0.5) (Tukey's test).

Table 2 Effects of CPP and Stimulus Duration on Omissions and Correct Response Latency

Treatment	Omissions (%)		Correct response latency (s)	
Stimulus	ST 0.5 s	ST 1.0 s	ST 0.5 s	ST 1.0 s
Vehicle	8.0 ± 1.7	11.1 ± 2.7	0.51 ± 0.03	0.65 ± 0.04
CPP 50 ng/μl	22.8 ± 4.1*	12.7 ± 2.8#	0.68 ± 0.05*	0.67 ± 0.04

Each value is the mean ± SEM of nine rats. In all, 50 ng/μl CPP (CPP 50) and 1 μl Vehicle were injected bilaterally into the mPFC 10 min before the test session. Various stimulus durations plus CPP were administered at least 48 h apart, according to a Latin-square design.
*$P<0.05$ *vs* Vehicle; #$P<0.05$ *vs* ST 0.5 s (Tukey's test).

in rats performing at the 0.5 s stimulus increased correct response latencies. However, these effects were not additive. CPP did not increase correct response latency when the rats performed the task with the 1.0 s stimulus (stimulus × CPP, $F_{(1,24)}=6.9$, $P=0.01$; CPP, $F_{(1,24)}=10.1$, $P=0.004$; stimulus, $F_{(1,24)}=1.5$, $P>0.05$) (Table 2).

Increasing the stimulus duration reduced the number of anticipatory (Figure 3b) and perseverative (Figure 3c)

responses in controls and CPP-treated rats ($F_{(1,24)} = 4.2$, $P = 0.05$; $F_{(1,24)} = 17.5$, $P = 0.002$; respectively). These nose-pokes were increased proportionally by CPP at both stimulus durations (anticipatory; stimulus × CPP, $F_{(1,24)} = 1.5$, $P > 0.05$; CPP, $F_{(1,24)} = 22.7$, $P = 0.0001$ and perseverative; stimulus × CPP, $F_{(1,24)} = 0.8$, $P > 0.05$; CPP, $F_{(1,24)} = 27.7$, $P = 0.0003$).

Effects of M100907 on CPP-Induced Impairments in Attentional Performance

Figure 4a shows how the effects of 50 ng/μl CPP in the mPFC on accuracy were modified by the selective 5-HT$_{2A}$ receptor antagonist, M100907 administered subcutaneously. Doses of 10 and 40 μg/kg M100907 by themselves had no effect on accuracy but dose dependently prevented the effects of CPP on the percentage of correct responses (M100907 × CPP, $F_{(2,40)} = 6.1$, $P = 0.005$; M100907, $F_{(2,40)} = 2.9$, $P = 0.07$; CPP, $F_{(1,40)} = 13.5$, $P = 0.0007$). M100907 10 μg/kg significantly reduced ($P < 0.05$; Tukey's test) and 40 μg/kg completely abolished the accuracy impairment induced by CPP ($P < 0.05$ Tukey's test). As shown in Table 3, the combination of CPP plus 40 μg/kg M100907 had additive effects on omissions (M100907 × CPP, $F_{(2,40)} = 0.87$, $P > 0.05$; M100907, $F_{(2,40)} = 6.4$, $P = 0.004$; CPP, $F_{(1,40)} = 43.0$, $P = 0.0001$) and correct response latency (M100907 × CPP, $F_{(2,40)} = 0.5$, $P > 0.05$; M100907, $F_{(2,40)} = 9.8$, $P = 0.0003$; CPP, $F_{(1,40)} = 25.7$ $P = 0.0001$).

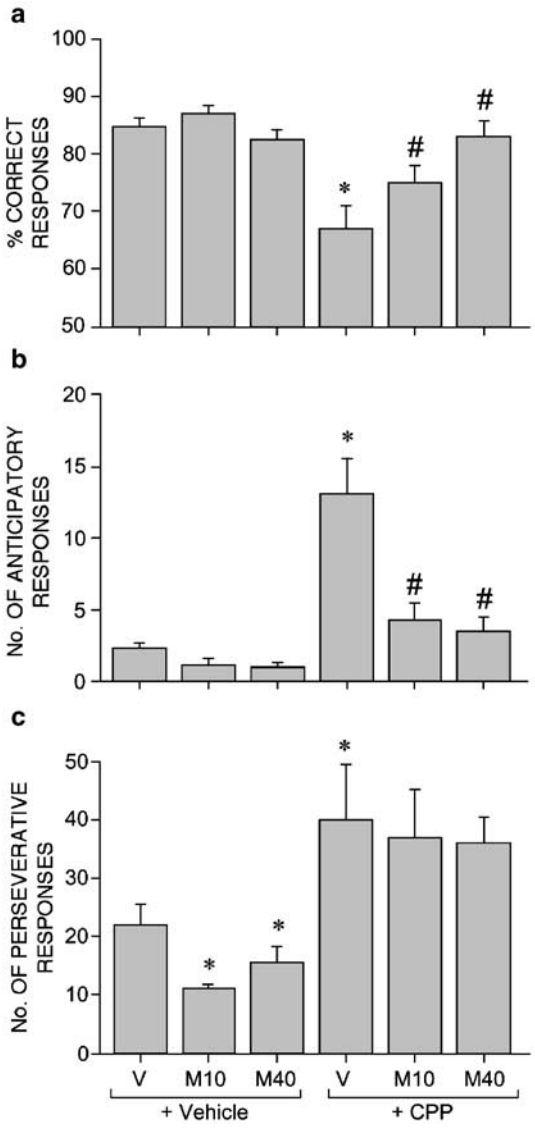

Figure 4 Effects of M100907 alone or with CPP on correct responses (a), anticipatory responses (b), and perseverative responses (c). Each rat was injected subcutaneously with vehicle (V), 10 or 40 μg/kg M100907 (M) 20 min before 1 μl vehicle (V) or 50 ng/μl CPP into the mPFC, 10 min before the test session. CPP and M100907 singly or combined were administered at least 48 h apart, according to a Latin-square design. The histograms show mean ± SEM of nine rats. *$P < 0.05$ *vs* V + V; #$P < 0.05$ *vs* V + CPP (Tukey's test).

The CPP-induced increase in anticipatory responses (Figure 4b) was completely abolished by 10 and 40 μg/kg of M100907 (M100907 × CPP, $F_{(2,40)} = 6.8$, $P = 0.003$; M100907, $F_{(2,40)} = 11.4$, $P = 0.0001$; CPP, $F_{(1,40)} = 27.8$, $P = 0.0001$). M100907 alone tended to reduce anticipatory responses, but not significantly probably, because the number of anticipatory responses in the control condition was already low. Increasing the ITI from 5 to 7 s resulted in an overall increase in anticipatory responses (ITI 5 s, vehicle = 3.3 ± 1.4; ITI 7 s, vehicle = 31.5 ± 5.2; $P < 0.05$ Student's *t*-test). Anticipatory responses significantly decreased with M100907 ($F_{(2,10)} = 15.1$, $P < 0.001$), from 31.5 ± 5.2 (vehicle) to 11.8 ± 2.6 (10 μg/kg) and 5.8 ± 1.2 (40 μg/kg). Figure 4c shows that M100907 by itself reduced the number of perseverative responses (both doses, $P < 0.05$, Tukey's test) but did not affect CPP-induced perseverative over-responding (M100907 × CPP, $F_{(2,40)} = 0.06$, $P > 0.05$; M100907, $F_{(2,40)} = 0.8$, $P = 0.47$; CPP, $F_{(1,40)} = 17.3$, $P = 0.0002$).

Motor activity. Motor activity was increased with CPP (vehicle 127 ± 17 ($n = 6$); CPP 1 ng/μl 122 ± 17 ($n = 6$); CPP 10 ng/μl 137 ± 31 ($n = 6$); CPP 50 ng/μl 205 ± 32 ($n = 6$)). Table 4 presents the effects of M100907 on CPP-induced motor hyperactivity. A two-way ANOVA on activity counts showed that 50 ng/μl of CPP injected into the mPFC

Table 3 Effects of M100907 and CPP on Omissions and Correct Response Latency

Treatment	Omissions (%)	Correct response latency (s)
VEH+VEH	7.9 ± 1.6	0.51 ± 0.03
M 10+VEH	15.2 ± 3.5*	0.67 ± 0.06*
M 40+VEH	15.6 ± 3.4*	0.62 ± 0.04*
VEH+CPP	22.8 ± 3.9*	0.67 ± 0.03*
M 10+CPP	25.3 ± 4.2°	0.84 ± 0.08
M 40+CPP	35.8 ± 3.5^{+}	0.85 ± 0.08$^{\$}$

Each value is the mean ± SEM of nine rats. M100907 at doses of 10 (M 10) and 40 μg/kg (M 40) were injected subcutaneously 20 min before bilateral injections of 1 μl vehicle (VEH) or 50 ng/μl CPP into the mPFC. After 10 min, the rats started the test sessions. The various doses were administered at least 48 h apart, according to a Latin-square design.
*$P <$ 0.05 *vs* VEH+VEH; °$P <$ 0.05 *vs* M 10+VEH; $^{+}P <$ 0.05 *vs* VEH+CPP; $^{\$}P < 0.05$ *vs* M 40+VEH; (Tukey's test).

Table 4 Effect of M100907 on CPP-Induced Motor Hyperactivity

Treatment	Activity counts
VEH+VEH	159.6 ± 13.1
M 10+VEH	89.3 ± 11.9*
M 40+VEH	97.5 ± 23.0*
VEH+CPP	246.0 ± 33.6*
M 10+CPP	191.0 ± 13.7
M 40+CPP	114.4 ± 15.5$^{\#}$

Each value is the mean ± SEM of six to seven rats. Motor activity is expressed as the total number of activity counts measured in the first 30 min of testing. CPP 50 ng/μl or vehicle (VEH) (1 μl) was injected bilaterally into the mPFC 10 min before the test session. M100907, 10 (M 10) and 40 μg/kg (M 40), or vehicle (2 ml/kg) was injected subcutaneously 20 min before CPP. *$P < 0.05$ *vs* VEH+VEH; $^{\#}P < 0.05$ *vs* VEH+CPP (Tukey's test).

significantly increased motor activity, as indicated by the significant main effect of CPP ($F_{(1,34)} = 17.0$, $P = 0.0002$). The main effect of M100907 was also significant ($F_{(2,34)} = 11.9$, $P = 0.0001$). *Post hoc* analysis showed that both doses of M100907 reduced the motor activity of control rats (Tukey's test, $P < 0.05$). The interaction between M100907 pretreatment and CPP was not significant ($F_{(2,34)} = 2.4$, $P = 0.10$). However, *post hoc* tests comparing the means of various individual treatments indicated that M100907 40 μg/kg significantly reduced the effects of CPP on motor activity (Tukey's test, $P < 0.05$).

DISCUSSION

This study found a functional interaction between serotonin 5-HT$_{2A}$ receptor mechanisms and medial prefronto-cortical NMDA receptors in the control of attentional performance. The selective and competitive glutamate NMDA receptor antagonist CPP (Lehmann *et al*, 1987), injected into the mPFC, had profound effects on rats' attentional performance. At 10 ng/μl it enhanced anticipatory and perseverative responding and increased the correct response latencies while 50 ng/μl impaired accuracy and omissions. M100907, a selective 5-HT$_{2A}$ receptor antagonist (Kehne *et al*, 1996), injected subcutaneously at 10 and 40 μg/kg, had no effect on accuracy but dose dependently prevented the impairment induced by 50 ng/μl CPP. The dose of 10 μg/kg M100907 already completely abolished CPP-induced anticipatory responding but perseverative over-responding was not affected by any dose. Both doses of M100907 decreased motor activity whereas 40 but not 10 μg/kg M100907 reversed CPP-induced motor hyperactivity.

The severe deficit in accuracy accompanied by the increases in omissions and latencies for correct detection indicate that CPP caused a pronounced attentional performance deficit. Similar deficits in accuracy, omissions, and correct response latencies in a 5-CSRT task have been reported after systemically administered noncompetitive NMDA receptor antagonists, dizocilpine, and phencyclidine (Higgins *et al*, 2003; Le Pen *et al*, 2003) and lesions of the mPFC (Muir *et al*, 1996; Passetti *et al*, 2002). At the dose that impaired accuracy of detection CPP injected into the mPFC increased motor activity. However, it is difficult to explain the accuracy impairment as a simple motor effect since correct and incorrect responses in this task have the same motor requirements. In addition, the accuracy deficit induced by the high dose of CPP was completely abolished when the attentional load on performance was reduced, by prolonging the stimulus. Omissions, which occur when the subject does not orient its attention on the stimulus presentation array in time might reflect motor or motivational factors. Again, prolonging the stimulus abolished CPP-induced increases in omissions. Therefore, these findings rule out the possibility that the CPP-induced impairment in accuracy was a consequence of hyperactivity, poor motivation or a failure to make associations or remember the general rules of the task.

Despite the profound impairment in attentional performance induced by blockade of NMDA receptors in the mPFC, the selective and potent 5-HT$_{2A}$ antagonist M100907 administered systemically dose dependently reversed the accuracy impairment induced by CPP. Systemic M100907 alone had no effect on accuracy in a 5-CSRT task (present result; Higgins *et al*, 2004; Winstanley *et al*, 2003) whereas when injected into the mPFC it boasted accuracy (Winstanley *et al*, 2003). This may be due to opposite effects of prefronto-cortical and subcortical 5-HT$_{2A}$ receptor blockade when the drug is administered by a systemic route. Previous work has shown that 5-HT lesion of the dorsal raphe (DR) nucleus improves attentional functioning (Harrison *et al*, 1997b). This 5-HT depletion, restricted to certain forebrain areas such as the cortex and striatum, presumably lowers 5-HT neurotransmission at all 5-HT receptor subtypes along the fronto-cortico-striatal loop but, as shown by Winstanley *et al* (2003), blockade of 5-HT$_{2A}$ receptors in the mPFC has effects similar to 5-HT lesions of the DR nucleus. On the other hand, stimulation of 5-HT$_{2A}$ receptors by DOI had no effect on accuracy (Koskinen *et al*, 2000). These findings suggest that serotonin, through 5-HT$_{2A}$ receptors, exerts a tonic control on attentional functioning, so reducing serotonergic function at 5-HT$_{2A}$ receptors might help preserve the attentional selectivity.

M100907 added its effects on the rate of omissions and correct response latencies to those of CPP, suggestive of some effects on motivation or motor activity. However, combined treatment did not cause a general disruption of performance and the majority of rats completed 100 trials within the allotted time (30 min). Although M100907 by itself reduced motor activity, thus supporting the interpretation that the increase in omissions and response latency might reflect some motor factors, it completely abolished CPP-induced hyperactivity. As a whole, these data again suggest that the effects of M100907 and CPP on omissions and correct response latency cannot be explained in terms of a simple change in motor activity.

CPP caused considerable impairment in executive control of the task, at 10 ng/μl a dose that did not affect motor activity or accuracy of detection. The failure in executive functions, as exemplified by the CPP-induced increase in anticipatory and perseverative responses, persisted even when the longer stimulus helped alleviate the accuracy deficit. This suggests that the deficits in anticipatory and perseverative responses were relatively independent from processes involving stimulus detection or motor activation.

An almost identical effect on anticipatory and perseverative responses was observed after systemic administration of NMDA antagonists (Higgins *et al*, 2003; Le Pen *et al*, 2003).

That the effects of CPP on attentional functioning may be dissociated from its effects on inhibitory response control is further suggested by the fact that 10 μg/kg M100907, a dose that only partially counteracted CPP' effects on accuracy, completely abolished the CPP-induced increase in anticipatory responses. Although in our study M100907 tended to reduce the anticipatory responses of rats performing under control conditions, the effect was not statistically significant, probably because of the small number of anticipatory responses by controls. However, we found that 10 and 40 μg/kg M100907 significantly reduced anticipatory responding when the ITI was increased from 5 to 7 s, thus allowing more anticipatory responses.

The effects of M100907 on CPP-induced anticipatory over-responding are similar to those reported recently by Higgins *et al* (2004) showing that M100907, although at doses 10 times those used in the present study, reversed the effects of the noncompetitive NMDA receptor antagonist dizocilpine and an NR2B-selective NMDA receptor antagonist Ro 63-1908 on anticipatory responding in a 5-CSRT task. It is interesting that the NMDA antagonists increased the release of 5-HT in the mPFC (Martin *et al*, 1998) and that poor inhibitory response control in a 5-CSRT task, measured by anticipatory responses, was associated with high 5-HT turnover (Puumala and Sirvio, 1998) or release in the mPFC (Dalley *et al*, 2002). Consistent with these findings is that stimulation of 5-HT$_{2A}$ receptors by a variety of nonselective 5-HT$_{2A}$ agonists increased while 5-HT$_{2A}$ receptor antagonists reduced anticipatory responses (Carli and Samanin, 1992; Ruotsalainen *et al*, 1997; Koskinen *et al*, 2000; Koskinen and Sirvio, 2001; Winstanley *et al*, 2003; Passetti *et al*, 2003a).

Therefore, over-activation of 5-HT$_{2A}$ receptors in the mPFC as a consequence of elevated 5-HT release in this cortical area may be an important mechanism that increases active responding in anticipation of reward. However, these findings challenge the view that loss of response control is necessarily mediated by diminished 5-HT function, since global forebrain 5-HT depletion consistently results in enhanced impulsivity in the rat (Harrison *et al*, 1997a). This apparent discrepancy may be explained by 5-HT exerting tonic inhibition on impulsivity through 5-HT$_{2C}$ receptors since blocking them greatly increased anticipatory responding in a 5-CSRT task (Higgins *et al*, 2004). Thus 5-HT, probably through opposite action on 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, contributes to the mechanisms responsible for preventing the disruptive consequences of loss of inhibitory response control on attentional performance.

Previous studies have strongly implicated the anterior cingulate cortex in the control of anticipatory responses (Muir *et al*, 1996). However, it is unlikely that diffusion of CPP into the anterior cingulate cortex contributed in some major way to the increased anticipatory responding. Doses of CPP (10 and 50 ng/μl) similar to those used in the present study had to be injected into the anterior cingulate cortex to induce anticipatory over-responding (M Carli and M Baviera, unpublished observation).

The increased perseveration, which is in line with that reported after excitotoxic lesions of the mPFC (Muir *et al*, 1996) could be the result of CPP preventing the suppression of responses once effective for obtaining reward. The enhanced tendency to perseverate appears to be a distinctive trait of frontal-lesion animals (Mishkin, 1964; Muir *et al*, 1996; Dias *et al*, 1997; Ragozzino *et al*, 1999; Killcross and Coutureau, 2003), and of frontal-lobe patients when required to inhibit previously reinforced responses (Owen *et al*, 1993). In addition, schizophrenic patients show increased perseverative responding in a two-choice visual task (Lyon and Gerlach, 1988) and in the Wisconsin Card Sorting Test, a task sensitive to prefronto-cortical dysfunction (Goldberg and Weinberger, 1994).

It is interesting that M100907 did not prevent the compulsive perseveration induced by CPP. This implies that the mechanisms of executive control impaired in perseveration may be different from those involved in anticipatory responding. Clearly, this double dissociation indicates that the two inhibitory processes can be differentiated at the level of the 5-HT$_{2A}$ receptor mechanisms.

Hyperactivity elicited by CPP injected into the mPFC was reduced by M100907 at the dose of 40 μg/kg. This dose is similar to the ED$_{50}$ of 30 μg/kg reported to block dizocilpine-induced hyperactivity in rats (Higgins *et al*, 2004). However, in contrast to published results we found that M100907 reduced spontaneous motor activity at doses 300 times lower than those previously reported in mice (Martin *et al*, 1997).

Behavioral deficits induced by acutely administered NMDA antagonists have been associated with enhanced glutamate release in the mPFC (Moghaddam *et al*, 1997; Moghaddam and Adams, 1998). Preliminary findings in our laboratory indicate that CPP in the mPFC increases glutamate efflux locally and this was prevented by systemic M100907 at doses similar to those used in the present study (Invernizzi *et al*, 2003). Therefore, it is likely that the suppression of glutamate release contributed to the mechanism by which M100907 prevents the effects of CPP on attentional performance. It would be of interest to study the effects of 5-HT$_{2A}$ blockade under other conditions that may alter cortical functions and impair attentional performance, such as selective cholinergic lesions of the nucleus basalis magnocellularis which complement those of CPP in the mPFC, thus providing further insights into the functional significance of the blockade of 5-HT$_{2A}$ receptors.

The failure in functions concerned with allocation of attentional resources (Shallice, 1982) is generally considered a mark of frontal lobe dysfunction (Owen *et al*, 1993) and attentional impairments and cognitive rigidity are well-known features of schizophrenia (Shallice *et al*, 1991; Elliott *et al*, 1998). Cognitive functions of the prefrontal cortex are modulated by an optimal level of mesocortical dopamine (DA) function (Arnsten, 1997; Zahrt *et al*, 1997). Attentional performance may be affected by fluctuations in prefrontal DA functions (Roberts *et al*, 1994; Granon *et al*, 2000). Thus, the increase in DA release in the mPFC as opposed to other brain areas such as the nucleus accumbens induced by some 5-HT$_{2A}$ antagonists, or their ability to enhance the effects of DA D$_2$ antagonists such as haloperidol on DA release in the mPFC (Bonaccorso *et al*, 2002; Liegeois *et al*, 2002) may be relevant to how atypical antipsychotics improve cognitive functions. The facilitation of attentional performance revealed by the present data also help explain why atypical

antipsychotics such as clozapine, risperidone, olanzapine, quietiapine, and ziprasidone—all potent 5-HT$_{2A}$ antagonists—have beneficial effects on attention and executive functions in schizophrenic patients (Harvey and Keefe, 2001; Harvey *et al*, 2003, 2004), beyond those of the typical antipsychotic drugs (Honey *et al*, 1999; Meltzer and McGurk, 1999).

Although it is problematic to extrapolate data from animal studies to complex human diseases such as schizophrenia, these results are compatible with the notion that dysfunctional glutamate NMDA neurotransmission within the PFC, and hyperfunction of 5-HT neuronal systems, are implicated in the pathophysiology of schizophrenia (Seeman *et al*, 1976; Javitt and Zukin, 1991; Meltzer, 1991; Tsai and Coyle, 2002).

The present study provides evidence that the prefrontocortical glutamatergic–NMDA system may make an important contribution to the control of attention and executive functions. It also shows that some aspects of executive functions such as inhibitory response control and compulsive repetition of responding may be differentiated at the level of 5-HT$_{2A}$ receptor function. Therefore, it could be concluded that 5-HT$_{2A}$ receptor function is relevant to processes that permit appropriate response selection and attentional selectivity in the face of interference induced by dysfunctional glutamate transmission in the prefrontal cortex.

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