

Dissociable Contribution of 5-HT_{1A} and 5-HT_{2A} Receptors in the Medial Prefrontal Cortex to Different Aspects of Executive Control such as Impulsivity and Compulsive Perseveration in Rats

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Serotonin (5-HT) receptors are increasingly recognized as major targets for cognitive enhancement in schizophrenia. Several lines of evidence suggest a pathophysiological role for glutamate NMDA receptors in the prefrontal cortex in schizophrenia and associated disorders in attention and executive functioning. We investigated how the interactions between 5-HT_{1A} and 5-HT_{2A} and glutamate NMDA receptor mechanisms in the medial prefrontal cortex (mPFC) contribute to the control of different aspects of attentional performance. Rats were trained on a five-choice serial reaction time (5-CSRT) task, which provides indices of attentional functioning (percentage of correct responses), executive control (measured by anticipatory and perseverative responses), and speed. The competitive NMDA receptor antagonist CPP (50 ng/side) was infused directly into the mPFC 5 min after infusion of either 8-OH-DPAT (30 and 100 ng/side) or M100907 (100 and 300 ng/side) into the same brain area. Impairments in attentional functioning induced by CPP were completely abolished by both doses of 8-OH-DPAT or M100907. In addition, M100907 abolished the CPP-induced anticipatory responding but had no effects on perseverative over-responding, while 8-OH-DPAT reduced the perseverative over-responding but had no effects on anticipatory responding induced by CPP. The selective 5-HT_{1A} receptor antagonist WAY100635 (30 ng/side) antagonized the effects of 8-OH-DPAT (100 ng/side). 8-OH-DPAT at 30 ng/side reduced the latency of correct responses in controls and CPP-injected rats and lowered the percentage of omissions in CPP-injected rats. The data show that 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC exert opposing actions on attentional functioning and demonstrate a dissociable contribution of 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC to different aspects of executive control such as impulsivity and compulsive perseveration.

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INTRODUCTION

Cognitive deficits, including attention disorders and deficits in working memory and executive functions, are a central feature of schizophrenia (Braff, 1993; Frith, 1987; Kay and Sevy, 1990). Dysregulation of prefrontal glutamate NMDA receptor function may contribute to the pathophysiology of schizophrenia and to cognitive dysfunctions (Goff and Coyle, 2001; Konradi and Heckers, 2003). In normal humans, NMDA receptor antagonists cause cognitive deficits analogous to those in schizophrenic patients (Javitt

and Zukin, 1991; Krystal *et al*, 1994). In experimental animals, administration of NMDA receptor antagonists systemically or locally into the medial prefrontal cortex (mPFC) dysregulates the firing and bursting activity of pyramidal neurons (Jackson *et al*, 2004) and cortical glutamate release (Ceglia *et al*, 2004; Moghaddam and Adams, 1998; Verma and Moghaddam, 1996), and produce a range of behavioral impairments reminiscent of frontal lobe dysfunction (Carli *et al*, 2004; Egerton *et al*, 2005; Higgins *et al*, 2003a; Jentsch and Roth, 1999; Le Pen *et al*, 2003; Murphy *et al*, 2005; Rodefer *et al*, 2005).

Serotonin (5-HT) receptors are increasingly recognized as major targets for cognitive enhancement in schizophrenia (Meltzer *et al*, 2003). Indeed, the superior efficacy of current atypical antipsychotics on aspects of attention such as vigilance and to some extent executive functioning in patients with schizophrenia may be due partly, to their direct or indirect effects on serotonin 5-HT_{2A} and 5-HT_{1A}

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receptors (Harvey *et al*, 2003a,b; Harvey and Keefe, 2001; Meltzer *et al*, 2003; Meltzer and McGurk, 1999).

The mPFC receives extensive 5-HT innervation from the dorsal (DR) and median raphe (MR) nuclei and contains several 5-HT receptors, with a particular abundance of 5-HT_{1A} and 5-HT_{2A} subtypes (Azmitia and Segal, 1978; Barnes and Sharp, 1999; Blue *et al*, 1988; Steinbusch, 1984). These receptors are highly colocalized (80%) in nearly half of the glutamatergic pyramidal neurons of the PFC and they are also present in about 25% of GABA interneurons (Santana *et al*, 2004). The activation of 5-HT_{1A} receptors in the PFC inhibits the neuronal output of pyramidal neurons by activation of a hyperpolarizing potassium current; 5-HT_{2A} facilitates output through a reduction of potassium conductance, reduction of the after-hyperpolarization, and increase in excitatory postsynaptic currents and discharge rate (Aghajanian and Marek, 1997; Andrade and Nicoll, 1987; Araneda and Andrade, 1991; Tanaka and North, 1993). Inhibitory effects of 5-HT_{2A} receptors on pyramidal cell activity have also been reported (Ashby *et al*, 1994; Puig *et al*, 2003; Zhou and Hablitz, 1999).

The 5-HT_{1A} and 5-HT_{2A} receptors have an important role in cognitive processes related to frontal lobe functions such as attention, executive function, and working memory (Carli and Samanin, 1992, 2000; Koskinen *et al*, 2000; Williams *et al*, 2002; Winstanley *et al*, 2003). While activation of 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC has opposite effects on attentional functioning (Winstanley *et al*, 2003), the 5-HT_{2A} but not 5-HT_{1A} receptors appear particularly involved in processes controlling response inhibition (Koskinen *et al*, 2000; Winstanley *et al*, 2003). In addition, a selective antagonist at 5-HT_{2A} receptors, M100907, reverses the effects of systemic or intra-mPFC NMDA antagonists on attentional functioning and some aspects of executive function (Carli *et al*, 2004; Higgins *et al*, 2003b).

The five-choice serial reaction time (5-CSRT) task used to assess attentional and executive functions in rodents is functionally analogous to the continuous performance test in humans (Carli *et al*, 2004; Higgins *et al*, 2003b; Robbins, 2002) and schizophrenic subjects reliably show impairments in the task, compared to controls (Kurtz *et al*, 2001; Orzack and Kornetsky, 1966). The 5-CSRT task taxes attentional capacity, as indicated by accuracy of correctly reporting the location of a brief visual stimulus, in addition to inhibitory response control related to executive attentional processes that permit accurate response selection in the face of distraction and interference (Robbins, 1998; Shallice, 1982).

Two different aspects of inhibitory response control may be indexed (Robbins, 2002). First, anticipatory responding represents a failure of response inhibition during the orienting behavior in anticipation of the target stimulus, which occur in anticipation of visual stimulus. This behavior has been operationally defined as 'impulsive' after Soubrié (1986), although it is recognized that impulsivity, as it occurs clinically, may also take other forms. Second, perseverative responding represents a failure to stop an apparently aimless 'compulsive' repetition by which rats continue to respond in stimulus holes after a correct response has been made. This perseverative behavior is akin to that reported in the frontal-lobe and schizophrenic

patients (Owen *et al*, 1993 #76; Lyon and Gerlach, 1988 #167; Goldberg and Weinberger, 1994 #166), and animals with PFC lesions (Chudasama *et al*, 2003; Mishkin, 1964). Deficits of perseveration and impulsivity and inattention may all contribute to the dys-executive syndromes such as in schizophrenia as well as in obsessive-compulsive disorder, ADHD, and Parkinson's disease (Aron *et al*, 2003; Baxter, 1990; Cools *et al*, 2002; Frith, 1987).

The present study investigated the contribution of mPFC 5-HT_{1A} and 5-HT_{2A} receptor activity to various aspects of performance in the 5-CSRT task in conditions of disrupted glutamate NMDA neurotransmission in the PFC. Various doses of a selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetraline (8-OH-DPAT) (Hoyer *et al*, 1994; Peroutka, 1986) or 5-HT_{2A} receptor antagonist [R-(+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol] (M100907) (Kehne *et al*, 1996) were microinjected into the mPFC and their effects examined on attentional performance deficits induced by the competitive NMDA receptor antagonist 3-(*R*)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) (Lehmann *et al*, 1987) injected into the same cortical area. The selective 5-HT_{1A} antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide, WAY100635 (Forster *et al*, 1995) was used to antagonize any effect of 8-OH-DPAT on rats' performance.

Stimulation of 5-HT_{1A} or blockade of 5-HT_{2A} receptors in the mPFC prevented the CPP-induced deficit in attentional functioning. The CPP-induced loss of executive control such as the increase in impulsivity was prevented by M100907, while 8-OH-DPAT decreased compulsive perseveration.

MATERIALS AND METHODS

Animals

Male hooded Lister rats (Charles River, Italy) weighing between 300 and 350 g before surgery were used. They were housed in pairs until surgery and then singly in a temperature-controlled room (21°C) with a day/night cycle (0700–1900 h). Water was available *ad libitum*. Limited access to food (about 15 g of Altromin pellets for rats) at the end of each day's testing kept the animals at 85–90% of their initial free-feeding weight. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national (D.L. no. 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, December 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 (Carli *et al*, 1983). It consisted of two 25 × 25 cm aluminum chambers built in the Department of Experimental Psychology, 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 micro-computer system (Cambridge, UK), with software written in ONLIBASIC.

Behavioral Procedures

Animals were trained to a stable performance in the 5-CSRT task as previously described (Carli *et al.*, 1983). The start of the session was signalled by illumination of the house-light and delivery of a single food pellet. Opening the panel to collect the pellet began the first trial. After a fixed delay (the inter-trial interval, ITI), the light at the rear of one of the holes came on for a short period. The light stimulus was presented the same number of times in each hole 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 responses) 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 house-light 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 restarted 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 and 5 s, respectively. 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 per day on the 5-CSRT task throughout the experiments.

Surgery

Rats previously trained to a stable level of performance were anesthetized by an intraperitoneal (I.P.) injection (2 ml/kg) containing 40 mg/ml ketamine and 5 mg/ml xylazine. All animals received I.P. injections of 0.1 mg/kg atropine sulphate. The animals were secured in a stereotaxic frame (Kopf Ins., USA) with the incisor bar set at -3.3 mm relative to the inter-aural line. Bilateral 23-gauge, stainless-steel

guide cannulae (Cooper's Needles, UK) were implanted in the medial region of the prefrontal cortex (mPFC) using standard stereotaxic techniques and secured to the skull using three bone screws and dental cement. The coordinates used were: anteroposterior +3.1 mm from bregma, lateral \pm 0.7 mm from midline, and dorsoventral -2.8 mm from dura (Paxinos and Watson, 1982). Thirty-gauge stainless-steel stylets were inserted flush with the end of the guide cannulae. After surgery, rats were housed singly and had 1 week of recovery without training on the task. After recovery, all rats were retrained on the task to re-establish the presurgery level of baseline performance.

Microinfusion Procedure

On testing days, the rat was held and the stylets were removed and two injection units terminating 2 mm below the tip of the guides were inserted. A volume of 1 μ l per hemisphere of various doses of drugs or vehicle were delivered at a rate of 0.5 μ l/min by a 10- μ l 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 deeply anesthetized with chloral hydrate (400 mg/kg, I.P.) and killed by decapitation. Brains were removed and postfixed in 4% formalin solution. Subsequently, the brains were transferred to 20% sucrose in 0.2 M phosphate buffer saline. The next day the brains were frozen in *n*-pentane and stored at -20°C. Coronal sections were cut at 30 μ m 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 the atlas (Paxinos and Watson, 1982). Only data from rats in which the cannulae were 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), and 8-OH-DPAT (Tocris, USA) and WAY100635 (Pharmacia, Nerviano, Italy) were dissolved in the phosphate buffer saline (PBS composition in mM: NaCl 137, KCl 2.7, Na₂HPO₄ 8.0, KH₂PO₄ 1.8, pH 7.4). M100907 (Aventis, USA) was dissolved in vehicle (PBS containing a few drops of HCl 1 M). The pH of the solution was adjusted to 7 with NaOH 1 M.

A group of rats ($n = 11$) received 1 μ l vehicle (PBS) or 30 and 100 ng/ μ l 8-OH-DPAT into the mPFC 5 min before an injection of 1 μ l PBS or 50 ng/ μ l CPP into the same cortical area. At 10 min after the last cortical injection, rats were put into the box and the test session started. A different group of rats ($n = 14$) received a volume of 1 μ l vehicle (PBS) or 100 and 300 ng/ μ l M100907 into the mPFC 5 min before 1 μ l PBS or 50 ng/ μ l CPP into the same cortical region. At 10 min later rats were put into the box and the test session started.

Eight rats were used to assess the effects of various combinations of vehicle, WAY100635 and 8-OH-DPAT on

CPP-induced performance deficits. WAY100635 (30 ng/0.5 μ l) or vehicle (0.5 μ l) were mixed with the solution of 8-OH-DPAT (100 ng/0.5 μ l) or vehicle (0.5 μ l) and injected in a volume of 1 μ l into the mPFC 5 min before CPP (50 ng/ μ l). Using thin-layer chromatography, it was shown that WAY100635 and 8-OH-DPAT do not form stable complexes when mixed in a solution (Carli et al. 1998). On each test day, drugs solutions were administered according to a Latin-square design. At least 2 days were left between test days. Rats were always tested on these intervening days to re-establish the baseline and check for long-lasting effects of drugs.

Statistical Analysis

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

Correct responses and omissions, as percentages, were transformed according to the formula $2\arcsin(\sqrt{\%X/100})$. The mean latencies to respond correctly and to collect the reinforcement were transformed by \log_{10} . These transformations were performed in order to normalize the distributions in accordance with the ANOVA model (Winer, 1971). Data from 11 rats were used to examine the effects of 8-OH-DPAT combined with saline or CPP and data from 14 rats were used to test the effects of M100907 combined with saline or CPP. Data were analyzed by a within-subject 2×2 ANOVA with factors 8-OH-DPAT or M100907 and CPP. The data of eight rats testing the effects of WAY100635 plus 8-OH-DPAT were analyzed by a 2×2 repeated measure ANOVA. The mean values of individual treatments were compared using Tukey's HDS test. Statistical software (SAS Institute Inc., USA.) was run on a Micro VAX 3500 computer (Digital, USA).

RESULTS

Histology

Examination of stained coronal sections of the brains showed that multiple injections into the mPFC produced limited tissue damage. Additionally, in the between-injections days rats returned to perform at very high level of accuracy (80–90%), making less than 20% omissions and only a small number of anticipatory and perseverative responses. Thus, the various treatments and multiple injections did not have a permanent effect on performance. Figure 1 shows a photograph of the representative histological section of the rat brain.

Effects of the 5-HT_{1A} Receptor Agonist on CPP-Induced Deficits

Figure 2a shows that 8-OH-DPAT prevented the CPP-induced decrease in the percentage of correct responses

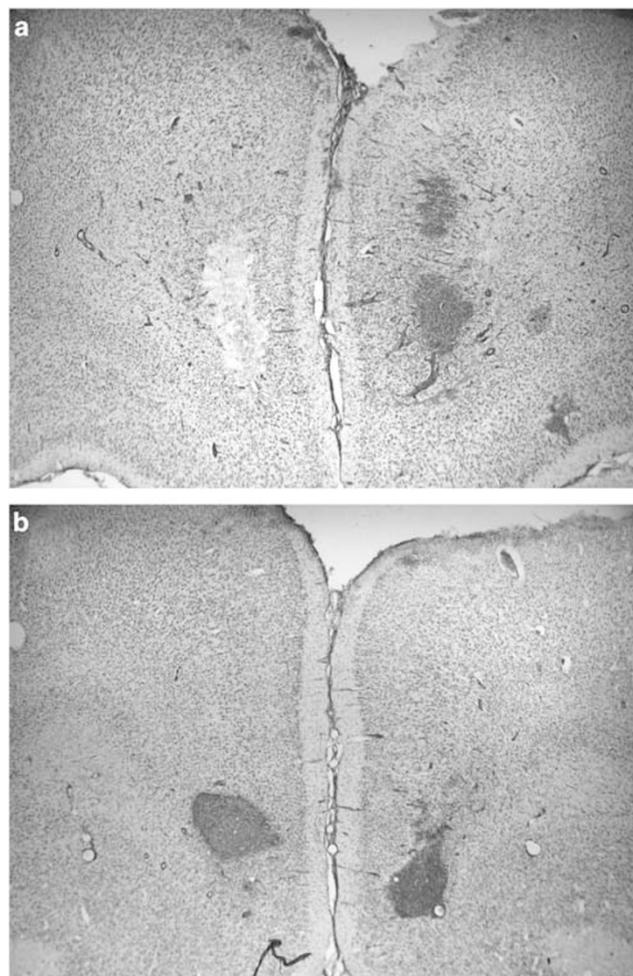


Figure 1 Some representative photographs of the histological sections showing the cannula tracks and the infusion sites in two (a and b) different rats.

(8-OH-DPAT \times CPP, $F_{2,50} = 3.6$, $P = 0.03$; 8-OH-DPAT, $F_{2,50} = 11.0$, $P = 0.0001$; CPP, $F_{1,50} = 47.8$, $P < 0.0001$). Multiple comparison of the various treatment group means by Tukey's HDS test indicated that 30 and 100 ng/ μ l 8-OH-DPAT (both $P < 0.05$) prevented the decrease in correct responses induced by CPP ($P < 0.05$). By itself 8-OH-DPAT had no such effect ($P > 0.05$).

The CPP-induced increase in the number of anticipatory responses (Figure 2b) was not affected by 8-OH-DPAT (8-OH-DPAT \times CPP, $F_{2,50} = 1.5$, $P = 0.2$; 8-OH-DPAT, $F_{2,50} = 0.3$, $P = 0.7$; CPP, $F_{1,50} = 5.6$, $P = 0.02$). By itself 8-OH-DPAT tended to increase anticipatory responses, but not significantly ($P > 0.05$). The CPP-induced perseverative over-responding (Figure 2c) was significantly reduced by 8-OH-DPAT (8-OH-DPAT \times CPP, $F_{2,50} = 3.9$, $P = 0.02$; 8-OH-DPAT, $F_{2,50} = 1.0$, $P = 0.4$; CPP, $F_{1,50} = 16.5$, $P = 0.0002$). Doses of 30 and 100 ng/ μ l 8-OH-DPAT by themselves had no effect on perseverative responses but dose-dependently lowered the effects of CPP ($P < 0.05$).

Table 1 shows the effects of 8-OH-DPAT on the CPP-induced increase in the proportion of omissions (8-OH-DPAT \times CPP, $F_{2,50} = 2.6$, $P = 0.08$; 8-OH-DPAT, $F_{2,50} = 3.5$, $P = 0.03$; CPP, $F_{1,50} = 30.5$, $P = 0.0001$) and the mean latency to make a correct response (8-OH-DPAT \times CPP, $F_{2,50} = 1.9$,

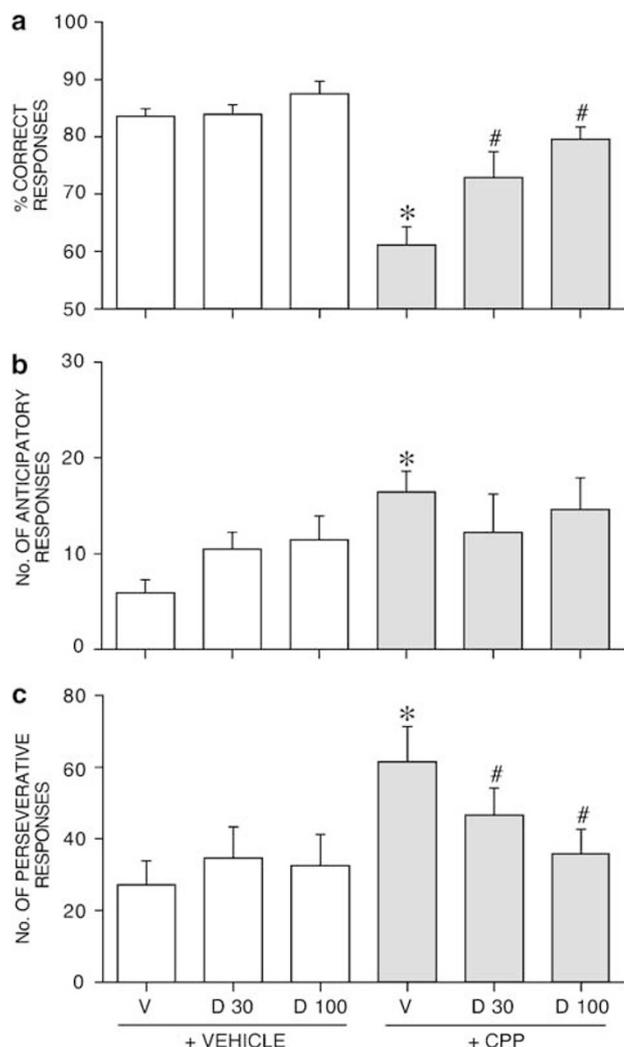


Figure 2 The effects of 8-OH-DPAT and CPP alone and in combination on the percentage of correct responses (a), the number of anticipatory (b) and the number of perseverative (c) responses. Vehicle 1 μ l (V) or 8-OH-DPAT at doses of 30 ng/ μ l (D 30) and 100 ng/ μ l (D 100) were injected into the mPFC 5 min before bilateral injections of 1 μ l vehicle (VEHICLE) or 50 ng/ μ l CPP (CPP) into the same area. After 10 min, the rats started the test sessions. The various treatment combinations were administered at least 48 h apart, according to a Latin-square design. The histograms represent the mean \pm SEM of 11 rats. * $P < 0.05$ vs V (+VEHICLE); # $P < 0.05$ vs V (+CPP) (Tukey's test).

$P = 0.15$; 8-OH-DPAT, $F_{2,50} = 5.3$, $P = 0.008$; CPP, $F_{1,50} = 22.3$, $P = 0.0001$). Further analysis by comparing individual treatment means indicated that by itself 8-OH-DPAT had no effect on omissions but reduced the effects of CPP on omissions, although only at 30 ng/ μ l ($P < 0.05$). In the control condition, 8-OH-DPAT speeded up correct responding, although only at 30 ng/ μ l ($P < 0.05$). Similarly, the CPP-induced increases in correct response latency were reduced by 30 ng/ μ l ($P < 0.05$) but not 100 ng/ μ l 8-OH-DPAT.

Effects of WAY100635 Plus 8-OH-DPAT on CPP-Induced Deficits

We examined the effects of 30 and 100 ng/ μ l WAY100635 on performance of rats injected with vehicle (1 μ l) or CPP

Table 1 Effects of 8-OH-DPAT, CPP, and their Combination on Omissions and Correct Response Latency

Treatment	Omissions (%)	Correct response latency (s)
VEH+VEH	10.1 \pm 2.2	0.61 \pm 0.02
DPAT 30+VEH	9.1 \pm 1.5	0.54 \pm 0.02*
DPAT 100+VEH	11.4 \pm 2.5	0.56 \pm 0.03
VEH+CPP	29.0 \pm 4.9*	0.93 \pm 0.11*
DPAT 30+CPP	15.9 \pm 3.9#	0.67 \pm 0.04#
DPAT 100+CPP	22.0 \pm 4.5	0.72 \pm 0.06

Each value is the mean \pm SEM of 11 rats. 8-OH-DPAT at doses of 30 ng/ μ l (DPAT 30) and 100 ng/ μ l (DPAT 100) were injected into the mPFC 5 min before bilateral injections of 1 μ l vehicle (VEH) or 50 ng/ μ l CPP into the same area. 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 VEH+CPP (Tukey's test).

(50 ng/ μ l) into the mPFC. WAY100635 had no effects on any measure of performance in rats receiving vehicle (1 μ l) into the mPFC (data not shown). However, 100 ng/ μ l WAY100635 interfered with the performance of rats given CPP (50 ng/ μ l); they stopped performing and made a large proportion of omissions (data not shown). Thus, 30 ng/ μ l WAY100635 was selected to examine the selectivity of 8-OH-DPAT's effects on CPP-induced performance deficit.

All animals received bilateral injections of 50 ng/ μ l CPP into the mPFC. This experiment showed that 8-OH-DPAT increased accuracy ($F_{1,21} = 27.7$, $P < 0.0001$) and that this effect was blocked by WAY100635 (WAY100635 \times 8-OH-DPAT, $F_{1,21} = 4.6$, $P = 0.04$; WAY100635, $F_{1,21} = 12.6$, $P = 0.002$). Multiple comparison of the treatment group means by Tukey's HSD test is illustrated in Table 2; after 100 ng/ μ l 8-OH-DPAT in the mPFC rats made a higher proportion of correct responses than after vehicle ($P < 0.05$) thus replicating in a new group of rats the results of the experiment presented in Figure 2a. WAY100635 30 ng/ μ l abolished the effect of 100 ng/ μ l 8-OH-DPAT on the percentage of correct responses since rats receiving both drugs made fewer correct responses than animals receiving vehicle with 8-OH-DPAT ($P < 0.05$). Similarly, WAY100635 blocked the effects of 8-OH-DPAT on the number of perseverative responses (WAY100635 \times 8-OH-DPAT, $F_{1,21} = 7.6$, $P = 0.05$). Table 2 shows that after 8-OH-DPAT rats made fewer perseverative responses than after vehicle ($P < 0.05$) whereas when WAY100635 was added to 8-OH-DPAT, rats made more perseverative responses than those receiving only 8-OH-DPAT ($P < 0.05$). Other measures of rats' performance such as anticipatory responses, proportion of omissions, and correct response latencies were not affected by 8-OH-DPAT, WAY100635, or the combination (data not shown) and are not commented further.

Effects of the 5-HT_{2A} Receptor Antagonist on CPP-Induced Deficits

Figure 3a shows that doses of 100 and 300 ng/ μ l M100907 by themselves had no effect on accuracy (% correct responses) but prevented the CPP-induced impairment in accuracy

Table 2 Effects of Intracortical Co-Infusion of WAY100635 Plus 8-OH-DPAT in Rats Receiving CPP in the mPFC

Treatment	% Correct	Perseverative
VEH+VEH	62.1 ± 4.6	62.1 ± 4.3
WAY 30+VEH	58.1 ± 1.8	53.7 ± 6.8
VEH+DPAT 100	83.1 ± 3.1*	41.0 ± 7.3*
WAY 30+DPAT 100	67.0 ± 3.9 [#]	57.5 ± 4.9 [#]

Each value is the mean ± SEM of eight rats. All animals received 50 ng/μl CPP into the mPFC. A solution containing WAY100635 30 ng/μl (WAY 30) plus 8-OH-DPAT 100 ng/μl (DPAT 100) was co-injected into the mPFC 5 min before CPP and 10 min later the rats started the test sessions. The various doses were administered at least 48 h apart, according to a Latin-square design.

% Correct, percentage of correct responses; Perseverative, number of perseverative responses.

* $P < 0.05$ vs VEH+VEH; [#] $P < 0.05$ vs VEH+DPAT 100 (Tukey's test).

(M100907 × CPP, $F_{2,65} = 5.8$, $P = 0.004$; M100907, $F_{2,65} = 5.4$, $P = 0.006$; CPP, $F_{1,65} = 31.7$, $P < 0.0001$). Both doses of M100907 were equally potent in preventing the accuracy impairment induced by CPP (both $P < 0.05$).

The CPP-induced increase in anticipatory responses (Figure 3b) was dose-dependently reduced by 100 and 300 ng/μl M100907 (M100907 × CPP, $F_{2,65} = 7.9$, $P = 0.0009$; M100907, $F_{2,65} = 13.2$, $P < 0.0001$; CPP, $F_{1,65} = 52.2$, $P < 0.0001$). In the control condition, 300 ng/μl M100907 tended to reduce anticipatory responses, but not significantly, possibly because the number of anticipatory responses was already low. Figure 3c shows that M100907 had no effect by itself on perseverative responses nor did it affect CPP-induced perseverative over-responding (M100907 × CPP, $F_{2,65} = 1.2$, $P = 0.3$; M100907, $F_{2,65} = 0.9$, $P = 0.4$; CPP, $F_{1,65} = 116.1$, $P < 0.0001$).

As shown in Table 3, in the control condition M100907 did not affect omissions or the speed of correct responding. The CPP-induced increases in omission (M100907 × CPP, $F_{2,65} = 0.6$, $P = 0.6$; M100907, $F_{2,65} = 1.4$, $P = 0.2$; CPP, $F_{1,65} = 32.9$, $P < 0.0001$) and mean latency to a correct response were unaffected by M100907 (M100907 × CPP, $F_{2,65} = 1.6$, $P = 0.2$; M100907, $F_{2,65} = 2.5$, $P = 0.08$; CPP, $F_{1,65} = 33.6$, $P < 0.0001$).

DISCUSSION

This is the first study to demonstrate a dissociable contribution of serotonin 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC to aspects of executive control such as impulsivity and compulsive perseveration. It also shows that these receptors exert opposite action on attentional functioning. The selective and competitive glutamate NMDA receptor antagonist CPP (Lehmann *et al*, 1987), injected into the mPFC, impaired accuracy and enhanced anticipatory and perseverative responses. These effects were accompanied by increases in omissions and latencies for correct detection, confirming previous reports (Mirjana *et al*, 2004; Murphy *et al*, 2005). As shown by Carli *et al* (Mirjana *et al*, 2004) the deficit may be recovered to a certain degree, when, by increasing stimulus duration, the attentional load on

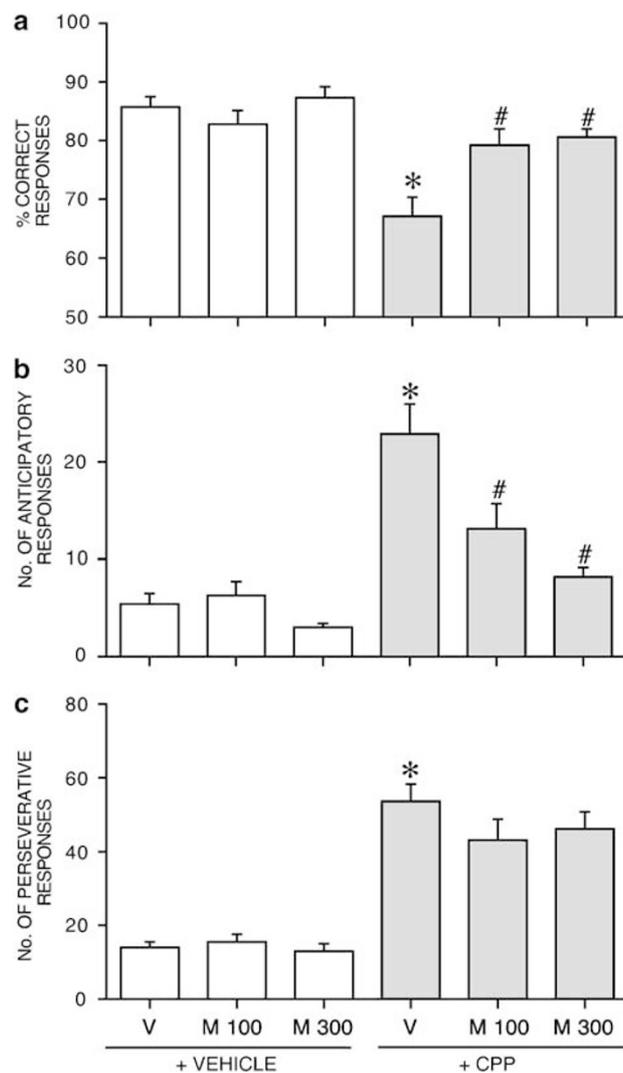


Figure 3 The effects of M100907 and CPP alone and in combination on the percentage of correct responses (a), the number of anticipatory (b) and the number of perseverative (c) responses. Vehicle 1 μl (V) or M100907 at doses of 100 ng/μl (M 100) and 300 ng/μl (M 300) were injected into the mPFC 5 min before bilateral injections of 1 μl vehicle (VEHICLE) or 50 ng/μl CPP (CPP) into the same area. After 10 min, the rats started the test sessions. The various treatment combinations were administered at least 48 h apart, according to a Latin-square design. The histograms represent the mean ± SEM of 14 rats. * $P < 0.05$ vs V (+VEHICLE); [#] $P < 0.05$ vs V (+CPP) (Tukey's test).

performance is reduced, suggesting that it is attentional in nature.

Like systemic M100907 (Mirjana *et al*, 2004), intra-mPFC injections of 100 and 300 ng/μl M100907 abolished the deficit in attentional accuracy and anticipatory but not perseverative responding induced by 50 ng/μl CPP. Various behavioral deficits induced by NMDA antagonists have been associated with enhanced glutamate release in the mPFC (Moghaddam *et al*, 1997; Moghaddam and Adams, 1998) and findings in our laboratory indicate that CPP in the mPFC increases glutamate efflux locally and that this was prevented by systemic or intra-mPFC M100907 (Ceglia *et al*, 2004). Thus, 5-HT_{2A} receptors in the mPFC play a major role in controlling CPP-induced glutamate release and some aspects of attentional performance in a 5-CSRT task.

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

Treatment	Omissions (%)	Correct response latency (s)
VEH+VEH	15.3±2.4	0.58±0.02
M 100+VEH	11.6±1.4	0.56±0.02
M 300+VEH	16.0±2.4	0.63±0.04
VEH+CPP	24.2±3.7*	0.74±0.06*
M 100+CPP	25.1±3.5	0.99±0.11
M 300+CPP	29.6±4.1	0.98±0.12

Each value is the mean ± SEM of 14 rats. M100907 at doses of 100 ng/μl (M 100) and 300 ng/μl (M 300) were injected into the mPFC 5 min before bilateral injections of 1 μl vehicle (VEH) or 50 ng/μl CPP into the same area. 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 (Tukey's test).

Impairments in attentional accuracy induced by CPP were completely abolished by 30 and 100 ng/μl 8-OH-DPAT injected into the mPFC. The 5-HT_{1A} receptors are found in a large proportion (50–60%) in glutamate neurons of the mPFC (Santana *et al*, 2004) thus raising the possibility that 5-HT_{1A} receptors may exert some of their functions by acting on glutamatergic signalling. Additionally, the 5-HT_{1A} receptor subtype can be considered functionally antagonistic to the 5-HT_{2A} receptors. They are highly colocalized (80%) in pyramidal neurons of the PFC (Santana *et al*, 2004) and electrophysiological studies have shown that M100907 potentiate 8-OH-DPAT suppression on firing rate (Ashby *et al*, 1994). In addition, 5-HT_{2A} receptor antagonists ICI 180,809 and ritanserin potentiate the 5-HT syndrome produced by 8-OH-DPAT (Backus *et al*, 1990; Sharp *et al*, 1990) whereas 8-OH-DPAT inhibits head twitching behavior induced by systemic DOI (Berendsen and Broekkamp, 1990; Darmani *et al*, 1990; Dursun and Handley, 1993) or microinjection into the mPFC of (–) DOB, a congener of DOI and a 5-HT_{2A/2C} receptors agonist (Granhoff *et al*, 1992). Clearly, the opposition between the two 5-HT receptor subtypes suggests that the improvement produced by M100907 and 8-OH-DPAT on CPP-induced accuracy deficit may result from a functionally antagonistic activity of these receptors on a common intracellular mechanism.

8-OH-DPAT but not M100907 had some additional effects on rats' attentional functioning but only at the low dose of 30 ng/μl; it speeded up correct response latencies and reduced CPP-induced omissions. Substantial evidence implicates the dopaminergic (DA) system in decision processes in this task (Robbins, 2002). The speeding up of correct responses and a decrease in omissions in a 5-CSRT task had been observed after systemic amphetamine and the dopamine D₁ receptor agonist SKF 38393 in the mPFC (Granon *et al*, 2000; Robbins, 2002). Thus, the fact that intra-mPFC 8-OH-DPAT increases DA efflux in this cortical region (Sakaue *et al*, 2000) may have contributed to its effect on speed and omissions.

In contrast to the effects of M100907, injections of 8-OH-DPAT into the mPFC did not have any effect on CPP-induced anticipatory responding. However, CPP-induced

perseverative over-responding was significantly reduced by pretreatment with 8-OH-DPAT. These results clearly demonstrate the selectivity of executive control processes and indicate that impulsivity and perseveration may be dissociated by 5-HT_{1A} and 5-HT_{2A} receptor mechanisms in the PFC. Evidently response inhibition operates independently for preparing responses and for monitoring performance, thus providing behavioral flexibility. This conclusion is generally consistent with emerging evidence of distinct neural systems in the control of 'impulsive' behavior, as, for example, after lesions of the infralimbic (IL) prefrontocortical region and in 'compulsive' behaviors associated with lesions of the prelimbic and orbitofrontal regions of the rat PFC (Chudasama and Muir, 2001; Chudasama *et al*, 2003; Passetti *et al*, 2002).

A selective 5-HT_{1A} receptor antagonist, WAY100635, blocked the effects of 8-OH-DPAT on CPP-induced accuracy deficits and perseverative over-responding suggesting that the effects of 8-OH-DPAT were due to selective activation of 5-HT_{1A} receptors in the mPFC.

Clinical and experimental evidence shows that the tendency to act without foresight, that is, 'impulsivity' may manifest itself in several ways (Evenden, 1999a, b). In reaction time tasks (such as the 5-CSRT task), it might be detected as enhanced errors or inappropriate anticipatory responding (Evenden, 1999b; Robbins, 2002). The 5-HT system has been implicated in the regulation of different forms of impulsive behavior (Evenden, 1999a, b; Mobini *et al*, 2000; Soubrié, 1986). In fact, impulsivity in a 5-CSRT task as measured by anticipatory responses was associated with high 5-HT turnover (Puumala and Sirvio, 1998) and release in the mPFC (Dalley *et al*, 2002). Stimulation of 5-HT_{2A} receptors by a variety of 5-HT_{2A} agonists increased whereas blockade of 5-HT_{2A} receptors (by antagonists) reduced anticipatory responses (Higgins *et al*, 2003b; Koskinen *et al*, 2000; Mirjana *et al*, 2004; Passetti *et al*, 2003b; Winstanley *et al*, 2003).

Interestingly, opposite behavioral effects of 8-OH-DPAT were often reported depending on whether the drug was administered directly into the DR or into its projecting areas (Carli *et al*, 1995, 1998; Warburton *et al*, 1997). Systemic 8-OH-DPAT increased impulsivity and impaired accuracy in a 5-CSRT task through stimulation of pre-synaptic 5-HT_{1A} autoreceptors in the DR nucleus (Carli and Samanin, 2000) whereas stimulation of postsynaptic 5-HT_{1A} receptors in the mPFC had no effect on impulsivity but improved accuracy (Winstanley *et al*, 2003; present results). However, facilitation of accuracy in various cognitive tasks has been reported after low brain concentrations of 8-OH-DPAT (Carli *et al*, 2000; Cole *et al*, 1994; Winstanley *et al*, 2003) and the selective 5-HT lesions of the DR nucleus (Harrison *et al*, 1997b).

The NMDA receptor antagonists either infused into the mPFC or injected systemically increase the release of 5-HT in the mPFC (Ceglia *et al*, 2004; Martin *et al*, 1998). Therefore, overactivation of 5-HT_{2A} but not 5-HT_{1A} receptors in the mPFC as a consequence of elevated 5-HT release may be an important mechanism that increases active responding in anticipation of reward. This view is challenged by findings that global 5-HT depletion consistently enhanced anticipatory responding in a 5-CSRT task in the rat (Harrison *et al*, 1997a, b).

The enhanced tendency to perseverate is presumably an expression of behavioral inflexibility after blockade of NMDA receptors in the mPFC, preventing the rats from suppressing irrelevant responses and shifting their attention to the next relevant response in a well-learned sequence. The 'compulsive' perseveration appears to be a distinctive trait of frontal-lesion animals (Mishkin, 1964; Muir *et al*, 1996), and frontal-lobe and schizophrenic patients when required to suppress previously reinforced responses (Owen *et al*, 1993 #76; Lyon and Gerlach, 1988 #167; Goldberg and Weinberger, 1994 #166).

That enhanced glutamate and 5-HT release may not be involved in CPP's effects on perseverative responses is indicated by studies showing that lowering CPP-induced glutamate release by M100907 does not abolish perseverative responding (Carli *et al*, 2004; Ceglia *et al*, 2004; the present results). In addition, reducing but not increasing 5-HT function in the PFC leads to response perseveration in tasks such as reversal learning (Clarke *et al*, 2004, 2005) and in some instances in a 5-CSRT (Winstanley *et al*, 2004).

CPP like other NMDA receptor antagonists increased DA release in the mPFC (Del Arco and Mora, 1999; Feenstra *et al*, 2002; Moghaddam *et al*, 1997). Accordingly, increasing DA transmission by systemic D-amphetamine increased perseverative responses in a 5-CSRT task (Baunez and Robbins, 1999). The exact mechanism by which 8-OH-DPAT might reduce CPP-induced perseverative responding is not clear. Both systemic and intra-mPFC 8-OH-DPAT increased mPFC DA efflux (Arborelius *et al*, 1993; Sakaue *et al*, 2000). However, 8-OH-DPAT actually reduced the rise in DA release in the mPFC induced by amphetamine, stress, and isolation rearing (Ago *et al*, 2002; Kuroki *et al*, 1996; Rasmusson *et al*, 1994) and attenuated the locomotor effects of amphetamine (Przegalinski and Filip, 1997). Therefore, the decrease in perseverative responding after intra-mPFC 8-OH-DPAT might be due to its effects on the increase in cortical DA release induced by CPP.

It can be speculated that the segregation of 5-HT_{2A} receptors to apical dendrites of glutamatergic pyramidal neurons (Jakab and Goldman-Rakic, 1998) and to GABAergic interneurons specialized in the perisomatic inhibition of pyramidal cells (Jakab and Goldman-Rakic, 2000) affects excitatory glutamate input (Aghajanian and Marek, 1997) whereas 5-HT_{1A} receptors in the axon hillock (Czyrak *et al*, 2003; DeFelipe *et al*, 2001) can suppress the generation of action potentials along the axon and influence the activity in their subcortical projection areas. Thus 5-HT, acting on 5-HT_{1A} and 5-HT_{2A} receptors, may finely tune the complex activity of glutamate pyramidal neurons, differently influencing various aspects of cognitive functions. It is of particular interest that mPFC neurons expressing 5-HT_{1A} and 5-HT_{2A} receptors simultaneously project to the 5-HT cells of DR nucleus and DA cells of the ventral tegmental areas (VTA) and influence their activity (Carr and Sesack, 2000; Celada *et al*, 2001; Hajos *et al*, 2003; Sesack and Bunney, 1989; Thierry *et al*, 1983). Cognitive functions of the prefrontal cortex are influenced by the 5-HT system (Robbins, 2000) and by an optimal level of mesocortical dopamine (DA) function (Arnsten, 1997; Granon *et al*, 2000; Roberts *et al*, 1994; Zahrt *et al*, 1997). The ability of some 5-HT_{2A} receptors antagonists to enhance the effects of DA D₂ antagonists such as haloperidol on DA function in the

mPFC has been attributed to their direct or indirect agonist activity on 5-HT_{1A} receptors (Ichikawa *et al*, 2001; Liegeois *et al*, 2002; Meltzer *et al*, 2003) and may be relevant to how atypical antipsychotics improve cognitive functions in schizophrenic patients (Harvey and Keefe, 2001; Meltzer and McGurk, 1999).

In conclusion, blockade of NMDA receptors in the mPFC, by inducing inhibitory deficits of impulsivity and perseveration as well as inattention, might offer a model of dys-executive syndrome of schizophrenia (Frith, 1987). The data indicate that 5-HT_{1A} and 5-HT_{2A} receptors in the mPFC exert opposing actions on the attentional impairment induced by blockade of NMDA receptors. Furthermore, this is the first demonstration that multiple executive mechanisms that cooperate to preserve accurate response selection can be dissociated at the levels of 5-HT_{2A} and 5-HT_{1A} receptor mechanisms. A complex interaction of glutamate NMDA receptor mechanisms with serotonergic and dopaminergic systems in the prefrontal cortex may be essential to preserve attentional selectivity and executive functioning. Such mechanisms may also help explain how atypical antipsychotics improve cognitive ability in schizophrenia.

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