Treball 4:

Stimulation of α_1-adrenoceptors in the rat medial prefrontal cortex increases the local *in vivo* 5-hydroxytryptamine release: reversal by antipsychotic drugs.

Amargós-Bosch M., Adell A., Bortolozzi A., Artigas F.

Journal of Neurochemistry 87, 831-842 (2003).

Journal of Neurochemistry, 2003, **87**, 831–842 doi:10.1046/j.1471-4159.2003.02044.x

Stimulation of α_1-adrenoceptors in the rat medial prefrontal cortex increases the local *in vivo* 5-hydroxytryptamine release: reversal by antipsychotic drugs

Mercè Amargós-Bosch, Albert Adell, Analía Bortolozzi and Francesc Artigas

Department of Neurochemistry, Institut d' Investigacions Biomèdiques de Barcelona (CSIC), IDIBAPS, Barcelona, Spain

Abstract

Pyramidal neurons of the medial prefrontal cortex (mPFC) project to midbrain serotonergic neurons and control their activity. The stimulation of prefrontal 5-HT_{2A} and AMPA receptors increases pyramidal and serotonergic cell firing, and 5-hydroxytryptamine (5-HT) release in mPFC. As the mPFC contains abundant α_1-adrenoceptors whose activation increases the excitability of pyramidal neurons, we examined the effects of their stimulation on local 5-HT release, using microdialysis. The application of the α_1-adrenoceptor agonist cirazoline by reverse dialysis increased the prefrontal 5-HT release in a concentration-dependent manner, an effect antagonized by coperfusion of TTX, prazosin (α_1-adrenoceptor antagonist), BAY × 3702 (5-HT_{1A} agonist), NBQX (AMPA/KA antagonist) and 1*S*,3*S*-ACPD (mGluR II/III agonist), but not by MK-801 (NMDA antagonist). Cirazoline also enhanced the increase in 5-HT release induced by DOI (5-$HT_{2A/2C}$ agonist) and AMPA. In addition, M100907 (5-HT_{2A} antagonist) but not SB-242084 (5-HT_{2C} antagonist) reversed the cirazoline- and AMPA-induced 5-HT release. These results suggest that the stimulation of prefrontal α_1-adrenoceptors activates pyramidal afferents to ascending serotonergic neurons. The effect of cirazoline was also reversed by coperfusion of classical (chlorpromazine, haloperidol) and atypical (clozapine, olanzapine) antipsychotics, which suggests that a functional antagonism of the α_1-adrenoceptor-mediated activation of prefrontal neurons may partly underlie their therapeutic action.

Keywords: α_1-adrenoceptors, glutamate receptors, 5-HT release, 5-HT_{2A} receptors, medial prefrontal cortex, microdialysis.

J. Neurochem. (2003) **87,** 831–842.

The prefrontal cortex is involved in a large number of higher brain functions and controls neuronal activity in subcortical structures (Fuster 1997; Miller and Cohen 2001). A reduction of the prefrontal glucose metabolism has been found in psychiatric conditions such as depression or schizophrenia (Andreasen *et al.* 1997; Drevets *et al.* 1997). Pyramidal neurons play a key role in prefrontal function, by integrating excitatory inputs from other cortical and subcortical areas, such as the mediodorsal nucleus of the thalamus (Berendse and Groenewegen 1991; Kuroda *et al.* 1998; Van der Werf *et al.* 2002). They also receive a dense innervation from the monoaminergic nuclei of raphe, ventral tegmental area and locus coeruleus, which play a modulatory role (Azmitia and Segal 1978; Thierry *et al.* 1983; Kosofsky and Molliver 1987; Durstewitz *et al.* 2000; Lewis and O'Donnell 2000). Signal integration in pyramidal neurons is exerted at various cellular levels, with a key role played by the large apical dendrites which, in addition to ionotropic glutamate receptors, contain abundant 5-HT_{2A} receptors (Willins *et al.* 1997; Jakab and Goldman-Rakic 1998, 2000; Martín-Ruiz *et al.* 2001). Hallucinogens like LSD or DOI are partial agonists and atypical antipsychotics are antagonists at 5-HT_{2A} receptors (Kroeze and Roth 1998; Meltzer 1999). Likewise, the neocortex is enriched in various subtypes (α_{1A}, α_{1B} and α_{1D}) of α_1-adrenoceptors (Palacios *et al.* 1987; McCune *et al.* 1993; Pieribone *et al.* 1994; Day *et al.* 1997). The stimulation

Received March 6, 2003; revised manuscript received June 17, 2003; accepted July 14, 2003.

Address correspondence and reprint requests to Dr Francesc Artigas, Department of Neurochemistry, Institut d' Investigacions Biomèdiques de Barcelona (CSIC), IDIBAPS, Rosselló, 161, 6th floor, 08036 Barcelona, Spain. E-mail: fapnqi@iibb.csic.es

Abbreviations used: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid; CIR, cirazoline; DOI, 1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]; 5-HT, 5-hydroxytryptamine or serotonin; KA, kainic acid; mPFC, medial prefrontal cortex; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; PRA, prazosin; TTX, tetrodotoxin.

of 5-HT_{2A} receptors and α_1-adrenoceptors activates phospholipase C, which results in IP3 production and mobilization of Ca^{2+} stores (Bylund and U'Prichard 1983; Molinoff 1984; Claro *et al.* 1993; Bartrup and Newberry 1994; Berg *et al.* 1998; Hagberg *et al.* 1998; Porter *et al.* 1999). 5-HT_{2A} and α_1-adrenoceptors mediate the excitatory actions of 5-hydroxytryptamine (5-HT) and noradrenaline, respectively, on pyramidal neurons of the medial prefrontal cortex (mPFC) (Araneda and Andrade 1991; Marek and Aghajanian 1999).

The axons of prefrontal pyramidal neurons project to the brainstem monoaminergic nuclei and controls their activity (Aghajanian and Wang 1977; Thierry *et al.* 1983; Sesack *et al.* 1989; Takagishi and Chiba 1991; Sesack and Pickel 1992; Murase *et al.* 1993; Sara and Hervé-Minvielle 1995; Hajós *et al.* 1998; Jodo *et al.* 1998; Peyron *et al.* 1998; Au-Young *et al.* 1999). In particular, the mPFC controls the activity of brainstem serotonergic neurons (Hajós *et al.* 1998; Celada *et al.* 2001). Pyramidal 5-HT_{1A} and 5-HT_{2A} receptors are involved in the distal feed-back control of serotonergic activity, as their activation decreased and increased, respectively, the firing rate of dorsal raphe (DR) serotonergic cells and the 5-HT release in mPFC and DR (Casanovas *et al.* 1999; Celada *et al.* 2001; Martín-Ruiz *et al.* 2001). Moreover, the physiological increase of the thalamic excitatory input onto AMPA receptors in the rat mPFC increased the firing rate of pyramidal cells and the local 5-HT release (Martín-Ruiz *et al.* 2001; Puig *et al.* 2003). Based on these anatomical and functional data, we postulate the existence of a mPFC-DR circuit in which prefrontal and serotonergic neurons exert a reciprocal control that involves 5-HT_{1A} and 5-HT_{2A} receptors. These receptors would modulate the excitatory inputs onto pyramidal neurons, thus controlling the propagation of nerve impulses through pyramidal axons.

Given the similar laminar distribution of 5-HT_{2A} receptors and α_1-adrenoceptors in mPFC (Pazos *et al.* 1985; Palacios *et al.* 1987) and their similar excitatory action on pyramidal neuron activity (Araneda and Andrade 1991; Marek and Aghajanian 1999), we tested the hypothesis that α_1-adrenoceptor stimulation in mPFC might modulate the *in vivo* 5-HT release. We also examined the effect of classical and atypical antipsychotics on this effect. These agents are used for the treatment of schizophrenia and treatment-resistant depression (Kroeze and Roth 1998; Meltzer 1999; Ostroff and Nelson 1999; Shelton *et al.* 2001; Marangell *et al.* 2002) and show high affinity for receptors present in pyramidal neurons, such as 5-HT_{2A} and α_1-adrenoceptors (Sebban *et al.* 1999; Arnt and Skarsfeldt 1998; Bymaster *et al.* 1999).

Materials and methods

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 280–320 g at the time of the experiments were used. The animals were housed in groups of four per cage until the onset of the experiments and kept under a controlled temperature of 22 ± 2°C and a 12-h lighting cycle (lights on at 07 : 00 h). After surgery, rats were housed individually. Food and water were always freely available throughout the experiments. All experimental procedures were in strict compliance with the Spanish legislation and the European Communities Council Directive on 'Protection of Animals Used in Experimental and Other Scientific Purposes' of 24 November 1986 (86/609/EEC).

Drugs and reagents

5-HT oxalate, (*S*)-AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate), chlorpromazine, cirazoline, DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]), (+)-MK-801 (dizocilpine), NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline), SB 242084 (6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy)pyridin-3-ylcarbamoyl]indoline), prazosin and tetrodotoxin (TTX), were from Sigma/RBI (Natick, MA, USA). 1S,3S-ACPD (1*S*,3*S*-aminecyclopentane dicarboxylic acid), haloperidol and clozapine were from Tocris (Bristol, UK). BAY × 3702 (*R*-(–)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1,1-dioxo-benzo[d]isothiazolone·HCl), citalopram·HBr, M100907 (*R*-(+)-alpha-(2,3-dimethoxyphenyl)-1-[4-fluorophenylethyl]-4-piperidinemethanol; Lilly code LY 368675) and olanzapine were from Bayer AG, Lundbeck A/S and Eli Lilly & Co, respectively. Other materials and reagents were from local commercial sources. Drugs were dissolved in the perfusion fluid or water (except clozapine, dissolved in acetic acid, and olanzapine, dissolved in HCl). Concentrated solutions (1 mM; pH adjusted to 6.5–7 with $NaHCO_3$ when necessary) were stored at − 80°C and working solutions were prepared daily by dilution in artificial CSF. Concentrations are expressed as free bases. Control rats were perfused for the entire experiment with artificial CSF. The bars in the figures show the period of drug application (corrected for the void volume of the system).

Surgery and microdialysis procedures

An updated description of the microdialysis procedures used can be found in Adell and Artigas (1998). Briefly, anesthetized rats (sodium pentobarbital, 60 mg/kg i.p.) were stereotaxically implanted with concentric microdialysis probes equipped with a Cuprophan membrane. In most experiments rats were implanted with one probe in mPFC at the following coordinates (in mm): AP +3.2, L −0.8, DV −6.0 (probe tip 4 mm) taken from bregma and dura mater (Paxinos and Watson 1986). To determine whether the perfusion of cirazoline in the mPFC increased the activity of the ascending serotonergic system, as we hypothesized, an additional experiment was conducted, in which rats were implanted with two probes, i.e. one in the mPFC, as above, and the other in the dorsal raphe nucleus (DR) at AP −7.4, L −3.1, DV − 7.5 with a lateral angle of 30° (probe tip 1.5 mm) taken also from bregma and dura mater (Paxinos and Watson 1986). All microdialysis experiments were performed in freely moving rats on the day following implants. The probes were perfused at 1.5 μL/min with artificial CSF (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) containing 1 μM citalopram. After 1 h stabilization period, four fractions were collected to obtain basal values before local administration of drugs by reverse dialysis. Successive 20-min (30 μL) dialysate samples were collected. At the end of the experiments, rats were killed by an overdose of anesthetic. The placement of the dialysis probes was examined by perfusion of fast green dye and visual inspection of the probe track after cutting the brain at the appropriate level.

The concentrations of cirazoline and prazosin were determined in pilot experiments. Those of atypical antipsychotics were from Bortolozzi *et al.* (2003) whereas the rest of the drugs were used at concentrations known to reverse the increase in prefrontal 5-HT release induced by DOI (Martín-Ruiz *et al.* 2001). Given the *in vitro* nanomolar affinity of cirazoline and prazosin for α_1-adrenoceptors, the use of micromolar concentrations used may appear non-selective. However, effective concentrations applied by reverse microdialysis to stimulate/block brain receptors or transporters differ typically 3–4 orders of magnitude from *in vitro* affinities (see for instance Hervás *et al.* 2000; Tao *et al.* 2000; Sakai and Crochet 2001; West and Grace 2002). This difference is due mainly to the low application rates used together with the continuous clearance of applied drugs via the brain capillaries and the CSF so that only a very small drug fraction reaches the target receptors. This factor is particularly important in the present study as the effect of cirazoline on 5-HT release requires the stimulation of a substantial receptor population in projection neurons to the DR in order to elicit a measurable increase in terminal 5-HT release.

The concentration of 5-HT in dialysate samples was determined by HPLC, as described by Adell and Artigas (1998). 5-HT was separated using a Beckman (San Ramon, CA, USA) 3-μm particle size column and detected with a Hewlett-Packard 1049 electrochemical detector at + 0.6 V. Retention time was between 3.5 and 4 min and the limit of detection was typically 1 fmol/sample.

Data and statistical analysis

Data (mean ± SEM) are expressed as fmol/fraction (uncorrected for recovery) and shown in figures as percentages of basal values, averaged from four predrug fractions. Statistical analysis of drug effects on dialysate 5-HT was performed using analysis of variance (ANOVA) for repeated measures with time as repeated factor and drug concentration as independent factor. Average values of selected time periods were also calculated and compared using paired *t*-test. Statistical significance was set at the 95% confidence level (two tailed).

Results

Baseline 5-HT values were 26.2 ± 0.7 fmol/fraction in mPFC and 44.4 ± 7.9 in DR (n = 196 and 8, respectively). The perfusion of artificial CSF for 4 h did not alter the 5-HT release in mPFC (Fig. 1). The local application of cirazoline (30, 100 and 300 μM) by reverse dialysis increased dialysate 5-HT in a concentration-dependent manner compared with controls receiving artificial CSF ($F_{3,212} = 19.06$, $p < 0.00001$, group effect; $F_{15,315} = 32.2$, $p < 0.00001$, time effect; $F_{45,315} = 6.1$, $p < 0.00001$, time–group interaction). The mean elevation once the effect of cirazoline had stabilized was 110 ± 6%, 171 ± 9% and 223 ± 14% for 30, 100 and 300 μM, respectively (Fig. 1). In fact, the two groups of eight rats correspond to two different experiments with four animals each carried out 10 months apart (Fig. 1b).

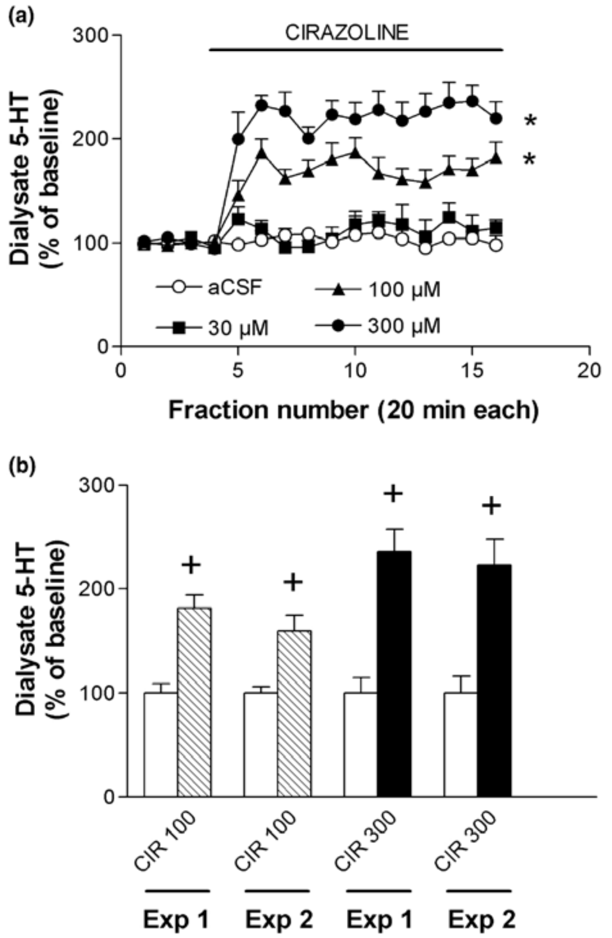

Fig. 1 (a) The local application of cirazoline 30 μM (n = 5), 100 μM (n = 8), and 300 μM (n = 8) increased the 5-HT output in medial prefrontal cortex in a concentration-dependent manner. The perfusion of both concentrations of cirazoline was carried out in two different experiments of four animals each (see b). $^*p < 0.05$ vs. artificial CSF (two-way ANOVA). (b) Bar graph showing the effect of 100 and 300 μM cirazoline on mPFC 5-HT release in two different experiments (n = 4 each) carried out 10 months apart. No significant differences were noted and the data were pooled. $^+p < 0.001$ vs. the corresponding basal values depicted as open bars (paired *t*-test). See also the similar increase in 5-HT produced in the experiment shown in Fig. 3.

Both experiments yielded the same results and the data was therefore pooled. In pilot experiments, the perfusion of increasing concentrations of cirazoline (100 and 300 μM, 2 h each) also elicited a concentration-dependent increase in 5-HT (141 ± 18% at 100 μM and 194 ± 28% at 300 μM; data not shown). The coperfusion of 1 μM TTX completely canceled the increase in 5-HT release induced by cirazoline and reduced 5-HT levels to below baseline ($F_{9,36} = 24.9$; $p < 0.00001$) (Fig. 2).

In double probe microdialysis experiments, the perfusion of cirazoline 300 μM in the mPFC elevated significantly the 5-HT release in both areas, although the effects was more

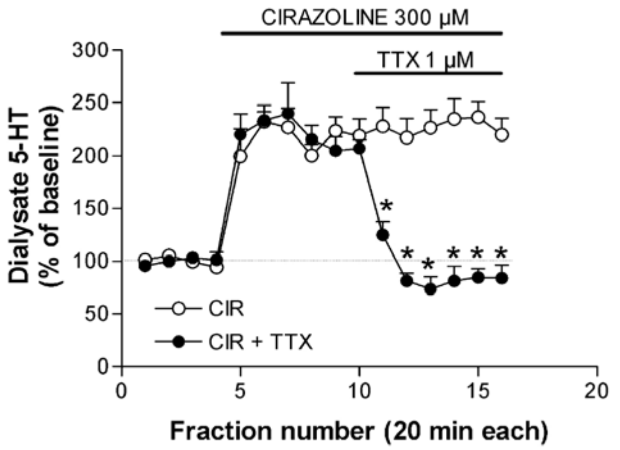

Fig. 2 The local application of cirazoline 300 μM ($n = 8$) increased the 5-HT output in medial prefrontal cortex. The coperfusion of 1 μM TTX completely reversed the elevation in 5-HT release elicited by the local perfusion of 300 μM cirazoline in medial prefrontal cortex ($n = 5$). *$p < 0.05$ vs. cirazoline alone.

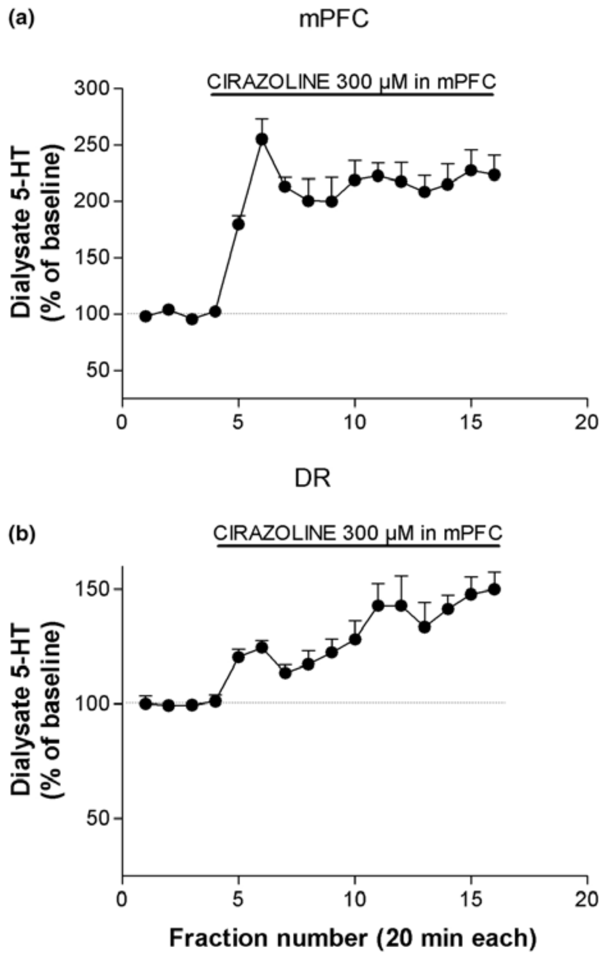

Fig. 3 In rats with dual-probe implants, the perfusion of 300 μM cirazoline increased the release of 5-HT not only locally in mPFC (a) but also in the DR (b). The perfusion of aCSF in the mPFC did not alter the release of 5-HT in the mPFC or in the DR (Celada *et al.* 2001).

marked in mPFC ($F_{15,105} = 31.6$; $p < 0.001$) than in the DR ($F_{15,105} = 6.9$; $p < 0.000001$) (Fig. 3a,b). The increase in dialysate 5-HT produced by the perfusion of 300 μM cirazoline in these animals was the same as that observed in animals implanted with a single probe. On the other hand, a previous dual-probe study showed that the perfusion of a CSF in the mPFC did not alter the release of 5-HT in the DR (Celada *et al.* 2001).

The coperfusion of the selective α_1-adrenoceptor antagonist prazosin (100 and 300 μM) reversed the 5-HT increase elicited by cirazoline 300 μM ($F_{9,36} = 6.2$, $p < 0.0001$ at 100 μM; $F_{9,36} = 26.4$, $p < 0.00001$ at 300 μM). Both concentrations of prazosin were equally effective and produced a slow decline in 5-HT which nearly reached baseline values at the end of the prazosin perfusion (Fig. 4a). The infusion of 100 μM prazosin rapidly and completely reversed the 5-HT increase induced by the application of 100 μM cirazoline ($F_{9,36} = 17.6$, $p < 0.00001$; Fig. 4b).

Previous observations indicate that the coperfusion of the selective 5-HT_{1A} receptor agonist BAY × 3702 reverses the increase in 5-HT release induced by the local application of DOI and AMPA in mPFC (Martín-Ruiz *et al.* 2001; Bortolozzi *et al.* 2003). This led us to examine the effect of BAY × 3702 on the effect of cirazoline. The coperfusion of 30 μM BAY × 3702 significantly reversed the increase in 5-HT release induced by 300 μM cirazoline ($F_{9,45} = 3.6$, $p < 0.002$; Fig. 5a). A higher concentration of BAY × 3702 (100 μM) elicited a similar antagonism (data not shown). However, 30 μM BAY × 3702 rapidly and completely reversed the effect of 100 μM cirazoline, and reduced 5-HT release to slightly below baseline ($F_{9,27} = 7.1$, $p < 0.00005$; Fig. 5b).

The coperfusion of cirazoline 300 μM enhanced the 5-HT elevation induced by the perfusion of DOI 100 μM ($F_{9,63} = 9.7$, $p < 0.00001$; Fig. 6). The stimulatory effect of DOI on 5-HT release in mPFC depends on glutamatergic transmission through AMPA receptors (Martín-Ruiz *et al.* 2001). We therefore examined whether the effect of cirazoline was also dependent on glutamatergic inputs in mPFC. The increase in 5-HT release elicited by 300 μM cirazoline was reversed by the coperfusion of the AMPA/KA receptor antagonist NBQX (300 μM) ($F_{9,27} = 9.8$, $p < 0.00001$; Fig. 7a) but not by the NMDA receptor antagonist MK-801 (Fig. 7b). Also, the non-selective mGluR II/III agonist 1S,3S-ACPD partially reversed the cirazoline-induced 5-HT increase at 3 but not at 1 mM ($F_{9,36} = 12.0$, $p < 0.00001$; Fig. 7c). Also, as previously shown (Martín-Ruiz *et al.* 2001), the local perfusion of AMPA 300 μM increased the 5-HT release (Fig. 7d). This effect was potentiated by the coperfusion of cirazoline 300 μM, which elevated 5-HT to 438 ± 34% of baseline ($F_{9,36} = 26.8$, $p < 0.00001$; Fig. 7d).

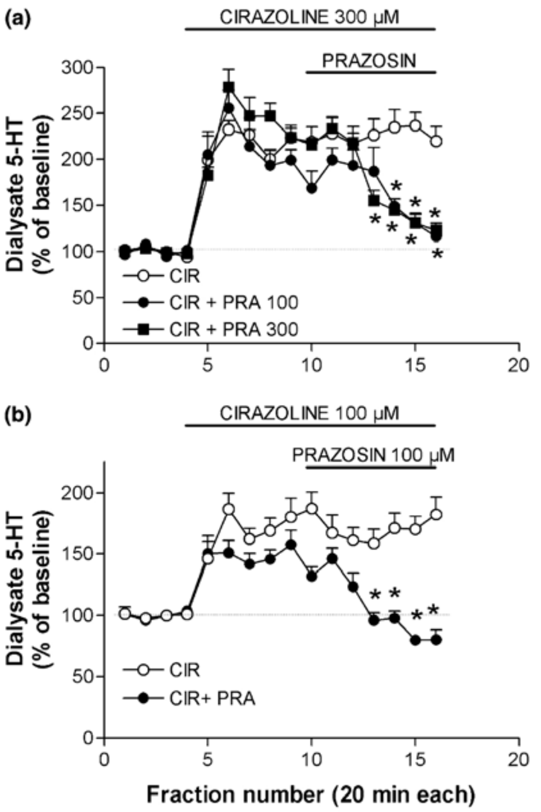

Fig. 4 (a) Reversal of the increase in 5-HT release induced by cirazoline 300 μM by the coperfusion of the α_1-adrenoceptor antagonist prazosin (PRA) at 100 μM ($n = 5$) and 300 μM ($n = 5$). (b) The coperfusion of 100 μM prazosin ($n = 5$) fully reversed the increase in 5-HT release elicited by the application of cirazoline 100 μM ($n = 8$). *$p < 0.05$ vs. cirazoline alone ($n = 8$).

The close relationship between the AMPA-mediated transmission, α_1-adrenoceptors and 5-HT_{2A} receptors was also illustrated by the functional antagonism of the *S*-AMPA-induced 5-HT release exerted by prazosin (100 μM) and M100907 (300 μM). The coperfusion of either antagonist reversed the increase in 5-HT release produced by 300 μM *S*-AMPA ($F_{9,36} = 12.0$, $p < 0.00001$ prazosin effect; $F_{9,27} = 10.6$, $p < 0.0001$, M100907 effect; Fig. 8a). The perfusion of prazosin 100 μM totally reversed the 5-HT elevation induced by the application of DOI 100 μM ($F_{9,81} = 40.2$, $p < 0.00001$; Fig. 8b). Likewise, the coperfusion of the selective 5-HT_{2A} receptor antagonist M100907 (300 μM) antagonized the 5-HT increase induced by cirazoline. This antagonism was partial at 300 μM cirazoline ($F_{9,36} = 9.8$, $p < 0.00001$; Fig. 8c) and total at 100 μM cirazoline ($F_{9,36} = 11.2$, $p < 0.00001$; Fig. 8d). However, the selective 5-HT_{2C} receptor antagonist SB 242084 (100 μM) failed to significantly alter the effect of cirazoline (Fig. 8c).

The increase in 5-HT release induced by cirazoline 100 μM was also reversed by the coperfusion of 300 μM of the

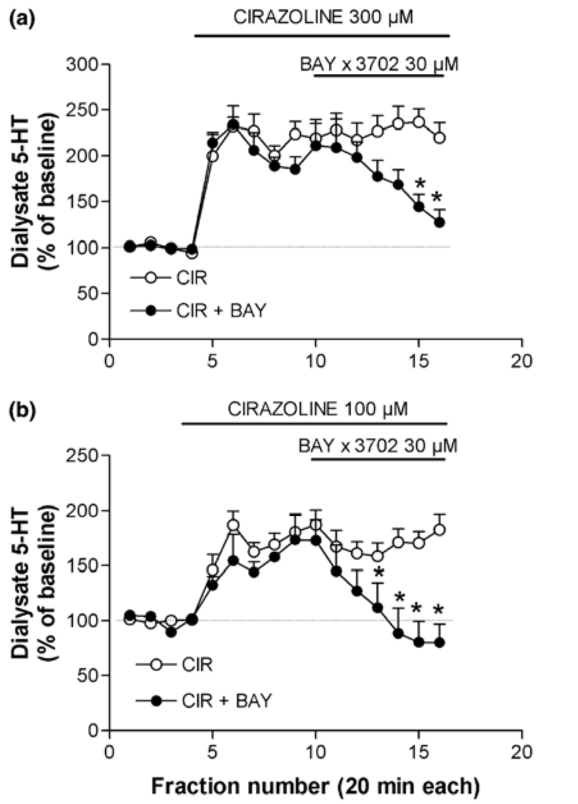

Fig. 5 (a) The coperfusion of the selective 5-HT_{1A} agonist BAY × 3702 (BAY, 30 μM) partially attenuated the increase in 5-HT release induced by cirazoline 300 μM ($n = 6$). (b) The same concentration of BAY × 3702 fully reversed the increase in 5-HT release elicited by 100 μM cirazoline ($n = 4$). Shown are in both graphs the effects of the perfusion of cirazoline alone (a, 300 μM, $n = 8$; b, 100 μM; $n = 8$). *$p < 0.05$ vs. cirazoline alone.

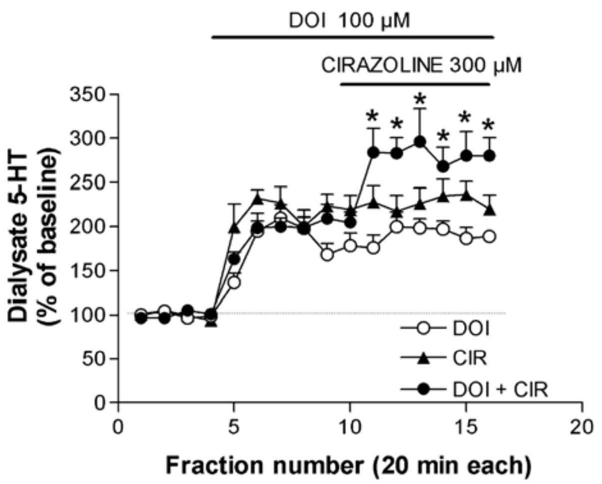

Fig. 6 Additive effects of the stimulation of 5-HT_{2A} receptors and α_1-adrenoceptors on the 5-HT release in medial prefrontal cortex. The coperfusion of cirazoline 300 μM enhanced the 5-HT release produced by the local application of DOI 100 μM ($n = 8$). Shown is also the effect of DOI alone ($n = 6$); and cirazoline alone ($n = 8$). *$p < 0.05$ vs. DOI alone.

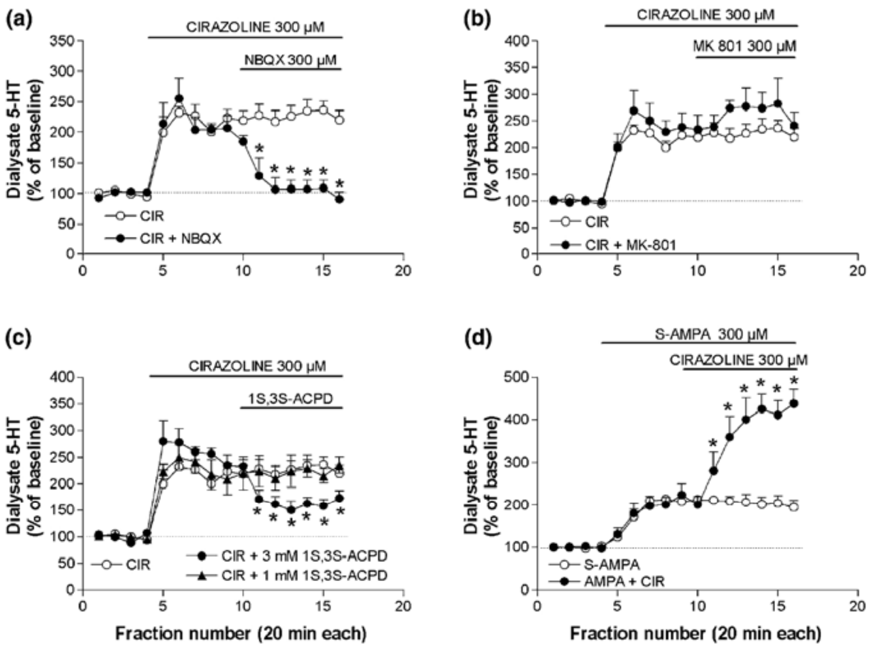

Fig. 7 The increase in 5-HT release elicited by the application of cirazoline in medial prefrontal cortex ($n = 8$) was completely attenuated by the coperfusion of the AMPA/KA receptor antagonist NBQX ($n = 4$; graph in a) but not by the NMDA receptor antagonist MK-801 ($n = 5$, graph in b). Likewise, the coperfusion of the non-selective mGluR II/III agonist 1*S*,3*S*-ACPD significantly attenuated the effect of 300 μM cirazoline at 3 but not at 1 mM ($n = 5$ each; graph in c). The graph in (d) shows the elevation in 5-HT release produced by the local application of *S*-AMPA (300 μM; $n = 5$) and its potentiation by the coperfusion of cirazoline 300 μM ($n = 5$). *$p < 0.05$ vs. cirazoline or S-AMPA alone.

classical antipsychotics haloperidol and chlorpromazine ($F_{9,36} = 14.9$, $p < 0.00001$ and $F_{9,45} = 14.8$, $p < 0.00001$ for haloperidol and chlorpromazine, respectively; Fig. 9a,b). Likewise, the atypical antipsychotics clozapine and olanzapine (300 μM each) significantly reduced 5-HT levels to or below baseline ($F_{9,36} = 10.2$, $p < 0.00001$ and $F_{9,27} = 23.2$, $p < 0.00001$ for clozapine and olanzapine, respectively; Fig. 9c,d).

In additional experiments we determined the effects of the administration of the different compounds that reduced dialysate 5-HT when perfused in combination with cirazoline. For this purpose BAY × 3702 (30 μM), M100907 (300 μM), NBQX (300 μM), prazosin (100 μM), haloperidol (300 μM), chlorpromazine (300 μM) and clozapine (300 μM) were perfused alone. The response of dialysate 5-HT was averaged over the last four samples, once the maximal effect was stabilized, and expressed as the percentage change from the corresponding basal (predrug) values. Paired *t*-test revealed that each of these compounds, except NBQX, reduced significantly ($p < 0.01$) the release of 5-HT (Fig. 10).

Discussion

Three main findings derive from the present study. First, the activation of α_1-adrenoceptors in mPFC increases the local release of 5-HT by an impulse-dependent mechanism. Second, this effect is dependent on AMPA-mediated inputs. Finally, antipsychotic drugs reduce the basal 5-HT release and reverse the effect of α_1-adrenoceptor activation, an observation possibly related to their therapeutic actions.

We would like to stress two different points relevant to the discussion of the data of this study. First, the fact that antipsychotics reverse the cirazoline-induced increase in prefrontal 5-HT release does not imply that psychotic states are necessarily associated to an increase in cortical serotonergic transmission. We used the stimulation of α_1-adrenoceptors in mPFC as a mean to activate the mPFC-raphe circuit in order to explore drug interactions *in vivo*. Second, because the mPFC has essentially an associative role, these drug interactions need to be interpreted at cellular (pyramidal) and not at receptor level, because several drugs used to reverse the effect of cirazoline (M100907, BAY × 3702, NBQX) are not expected to interact with α_1-adrenoceptors in the experimental conditions used.

The effect of cirazoline likely involves the activation of α_1-adrenoceptors on pyramidal neurons projecting to the DR, as previously observed for 5-HT_{2A} receptors (Fig. 11). This assumption is based on (i) the common signal transduction mechanisms activated by 5-HT_{2A} and α_1-adrenoceptors (see Introduction); (ii) the great abundance of both receptors in the prelimbic and infralimbic areas of the mPFC (Pazos *et al.* 1985; Palacios *et al.* 1987) which project to the DR (Hajós *et al.* 1998; Peyron *et al.* 1998); (iii) the increase in the DR 5-HT release produced by cirazoline application in mPFC; and (iv) the reversal of the effect of cirazoline by agents acting on pyramidal neurons (see below).

To our knowledge, there is no direct immunohistochemical evidence on the presence of α_1-adrenoceptors in pyramidal cells, although autoradiographic and *in situ* hybridization studies revealed abundant $\alpha_{1A/B/D}$-adrenoceptors at various cortical layers rich in pyramidal cells (Palacios *et al.* 1987; McCune *et al.* 1993; Pieribone *et al.* 1994; Day *et al.* 1997; Domyancic and Morilak 1997). In common with other cortical areas (Sato *et al.* 1989; Mouradian *et al.* 1991;

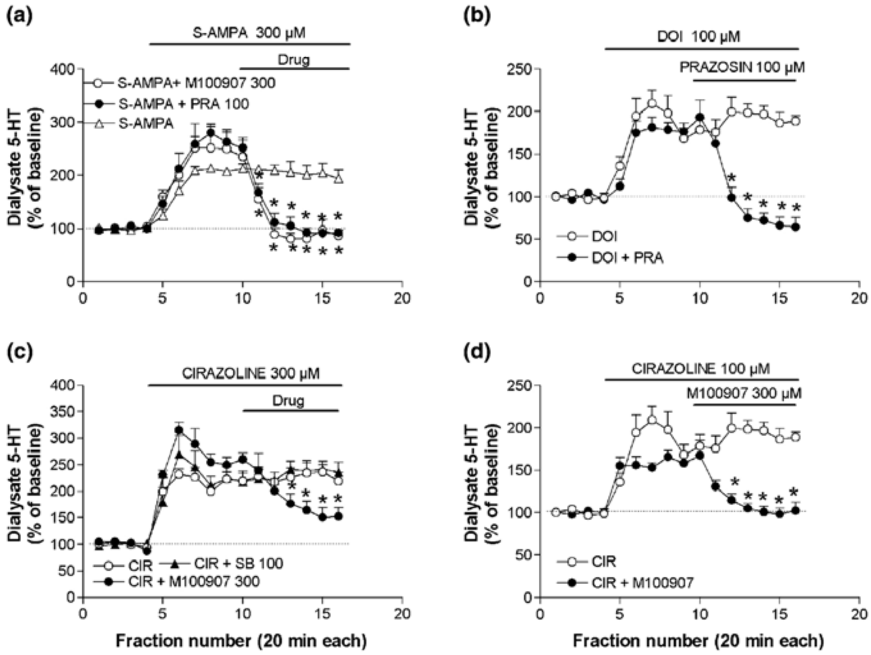

Fig. 8 (a) AMPA (300 μM, $n = 5$) enhanced dialysate 5-HT in the medial prefrontal cortex. The coperfusion of the α_1-adrenoceptor antagonist prazosin (PRA, 100 μM, $n = 5$) or the selective 5-HT_{2A} antagonist M100907 (300 μM, $n = 4$) completely reversed the elevation in prefrontal 5-HT release induced by the local application of S-AMPA (300 μM). (b) The perfusion of DOI 100 μM elicited a persistent increase of prefrontal 5-HT release for the whole sampling period ($n = 6$). The coperfusion of prazosin 100 μM reversed the 5-HT elevation induced by DOI 100 μM ($n = 10$). (c) Cirazoline (300 μM, $n = 8$) increased dialysate 5-HT. The application of M100907 300 μM partly reversed the elevation produced by 300 μM cirazoline ($n = 5$). However, the selective 5-HT_{2C} receptor antagonist SB 242084 (SB; 100 μM, $n = 6$) did not reverse the effect of cirazoline. (d) The perfusion of M100907 (300 μM) was able to fully counteract the increase in 5-HT release evoked by 100 μM cirazoline ($n = 5$). $^*p < 0.05$ vs. cirazoline or *S*-AMPA alone.

McCormick *et al.* 1993; Devilbiss and Waterhouse 2000), the stimulation of α_1-adrenoceptors in mPFC elicits excitatory responses (Araneda and Andrade 1991; Marek and Aghajanian 1999). Hence, a cirazoline-induced activation of mPFC pyramidal neurons, including those projecting to the midbrain raphe, is the most likely cause of the increase in 5-HT release. This view is strengthened by the increase of 5-HT release in the DR induced by cirazoline application in mPFC. The smaller effect in DR (compared to mPFC) may be due to a different sensitivity of 5-HT release to nerve impulse in both areas. However, it was similar to that produced by the electrical stimulation of the mPFC (Celada *et al.* 2001). Indeed, the DR probe may not be sampling exactly the neuronal population activated by mPFC afferents.

Cirazoline is not entirely selective for α_1-adrenoceptors and displays affinity for imidazoline receptors and α_2-adrenoceptors, where it behaves as an antagonist (Ruffolo and Waddell 1982). However, the comparatively lower affinity for these sites (Molderings *et al.* 1998) suggests that its effects are mediated by α_1-adrenoceptors. Moreover, the blockade of its effect by prazosin suggests that cirazoline acts via α_1-adrenoceptors although its similar affinity for the various subtypes does not allow to clarify which one(s) were involved. Two areas projecting to the mPFC (thalamus and midbrain raphe) express abundant α_{1B}-adrenoceptor mRNA. The good correspondence between receptor protein and mRNA suggests a somatodendritic location (Palacios *et al.* 1987; McCune *et al.* 1993; Pieribone *et al.* 1994; Day *et al.* 1997; Domyancic and Morilak 1997) and appears to exclude the possibility that putative terminal α_{1B}-adrenoceptors mediate the effect of cirazoline. Terminal 5-HT_{2A} receptors (Jakab and Goldman-Rakic 1998) in thalamocortical afferents to the mPFC have been suggested to mediate the 5-HT_{2A} receptor-dependent increase in the spontaneous excitability of pyramidal neurons in mPFC (Aghajanian and Marek 1999; Marek *et al.* 2001). However, terminal 5-HT_{2A} receptors in mPFC do not seem to be located in glutamatergic axons and extensive thalamic lesions left unaltered the effect of DOI on pyramidal cell firing (Miner *et al.* 2003; Puig *et al.* 2003), which suggests that postsynaptic 5-HT_{2A} receptors are involved in the excitatory effect of 5-HT_{2A} receptor stimulation. The analogy of effects of 5-HT and noradrenaline on pyramidal excitability (Marek and Aghajanian 1999) suggests a similar location for α_1-adrenoceptors. Moreover, the effect of cirazoline was canceled by the coapplication of NBQX, BAY × 3702 and antipsychotic drugs, which act on receptors located on intrinsic neurons of the prefrontal cortex (Petralia and Wenthold 1992; Kia *et al.*

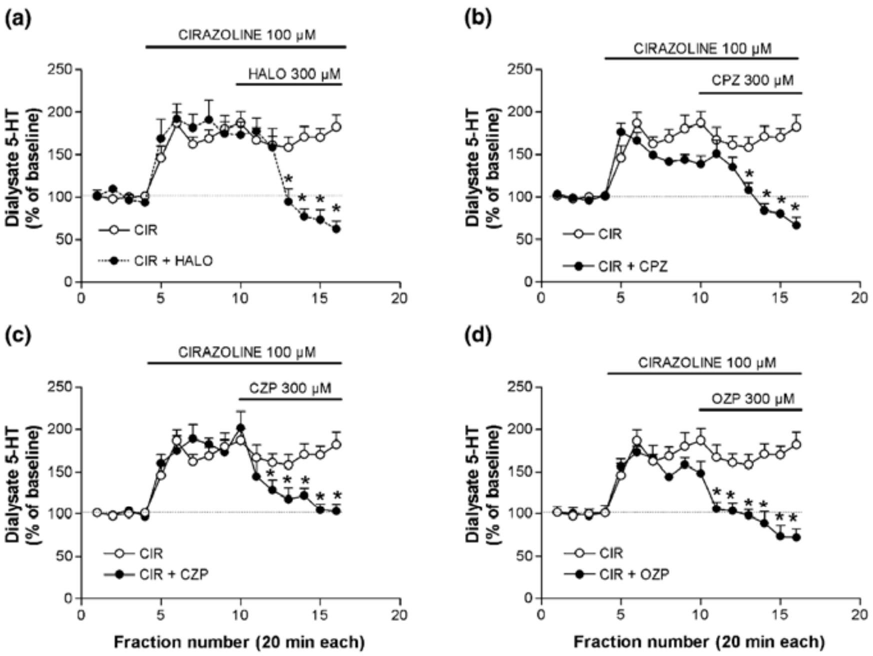

Fig. 9 Reversal of the increase in prefrontal 5-HT release produced by cirazoline 100 µM by the coperfusion of 300 µM of the classical antipsychotics haloperidol (HALO; graph in a, $n = 5$), chlorpromazine (CPZ; graph in b, $n = 6$) and the atypical antipsychotics clozapine (CZP; graph in c, $n = 5$) and olanzapine (OZP; graph in d, $n = 4$). Shown is also the effect of cirazoline alone ($n = 8$). $^*p < 0.05$ vs. cirazoline alone.

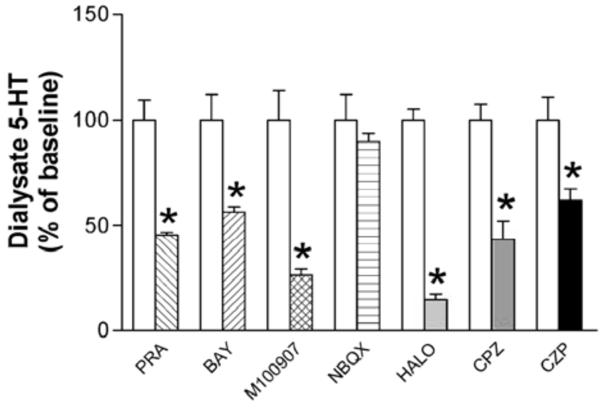

Fig. 10 Effect of the perfusion of BAY × 3702 (BAY, 30 µM, $n = 4$), M100907 (300 µM, $n = 5$), NBQX (300 µM, $n = 5$), prazosin (PRA, 100 µM, $n = 5$), haloperidol (HALO, 300 µM, $n = 4$), chlorpromazine (CPZ, 300 µM, $n = 4$), and clozapine (CLZ, 300 µM; $n = 4$) on dialysate 5-HT. Data are averaged 5-HT values over the last four samples (once the effect was stabilized) and expressed as the percentage change from the corresponding basal (predrug) values depicted as open bars. $^*p < 0.01$, paired *t*-test.

1996; Vysokanov *et al.* 1998; De Felipe *et al.* 2001). Given the complex pharmacological profile of the mGluR II/III agonist 1S,3S-ACPD, it is unclear whether this agent may act presynaptically (i.e. by reducing glutamate release) and/or postsynaptically, by activating postsynaptic inhibitory mGluRs.

As observed with the action of DOI (Martín-Ruiz *et al.* 2001), the 5-HT-increasing action of cirazoline depends on glutamatergic transmission in mPFC as it was reversed by AMPA/KA (but not NMDA) receptor blockade and mGluR II/III activation, and was mimicked by the local application of *S*-AMPA. Indeed, the 5-HT- and noradrenaline-induced increase in pyramidal excitability was also abolished by AMPA receptor blockade (Marek and Aghajanian 1999), suggesting a dependence on glutamatergic inputs onto mPFC.

The activation of 5-HT_{1A} receptors by the pre- and postsynaptic 5-HT_{1A} agonist BAY × 3702 (De Vry *et al.* 1998; Casanovas *et al.* 1999, 2000) counteracted the effect of DOI and cirazoline on 5-HT release (Martín-Ruiz *et al.* 2001; this study). 5-HT_{1A} receptors have been reported to occur in the somatodendritic compartment and axon hillock of pyramidal neurons (Kia *et al.* 1996; De Felipe *et al.* 2001) and their activation results in neuronal hyperpolarization and reduction of firing rate (Araneda and Andrade 1991; Ashby *et al.* 1994). Hence, BAY × 3702 may oppose to the increase in excitability produced by the activation of α_1-adrenoceptors, thus reducing the excitatory input onto midbrain 5-HT neurons and, hence, 5-HT release (see scheme in Fig. 11). The specificity of BAY × 3702 is supported by its total lack of action in the mPFC of 5-HT_{1A} receptor knockout mice at the concentration used herein (Amargós-Bosch *et al.*, unpublished results).

The reciprocal antagonism between 5-HT_{2A} and α_1-adrenoceptors (M100907 of cirazoline's effect and prazosin of DOI's effect) appeared surprising. These neurochemical results parallel behavioral data showing that the 5-HT_{2A}-mediated, DOI-induced head shakes in rodents were suppressed by prazosin and a number of ligands acting at cortical receptors, such as 5-HT_{1A} agonists, $5\text{-HT}_{2A/2C}$ antagonists or classical antipsychotics such as haloperidol, among others (Schreiber *et al.* 1995; Dursun and Handley 1996), an

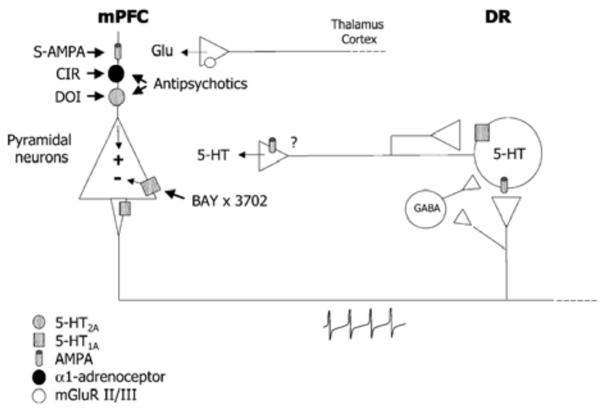

Fig. 11 Schematic representation of the interactions between the medial prefrontal cortex (mPFC) and the midbrain raphe 5-HT neurons, with some of the receptors and neurotransmitters involved. Pyramidal neurons express 5-HT_{2A} receptors and α_1-adrenoceptors whose activation increases the excitability and/or firing activity of prefrontal pyramidal neurons. Anatomical and functional studies indicate the existence of marked reciprocal interactions between the mPFC and the midbrain raphe nuclei. The selective activation of AMPA, 5-HT_{2A} and α_1-adrenoceptors in mPFC by local application of agonists (*S*-AMPA, DOI, cirazoline -CIR-, respectively) increased the local 5-HT release (Martín-Ruiz *et al.* 2001; this study) whereas that of 5-HT_{1A} receptors (e.g. by BAY × 3702) decreased local 5-HT release and counteracted the 5-HT-increasing action of DOI, AMPA and cirazoline (Casanovas *et al.* 1999; Celada *et al.* 2001; Martín-Ruiz *et al.* 2001; Bortolozzi *et al.* 2003; this study). 5-HT_{1A} receptors have been reported to occur in the somatodendritic region of cortical pyramidal neurons as well as in the axon hillock (Kia *et al.* 1996; De Felipe *et al.* 2001). The changes in 5-HT release are likely to be mediated by a modulation of the activity of pyramidal neurons in prelimbic and infralimbic mPFC that project densely to the DR (Hajós *et al.* 1998; Peyron *et al.* 1998), and control the activity of 5-HT neurons (Celada *et al.* 2001) and GABA interneurons (Celada *et al.* 2001; Varga *et al.* 2001) in midbrain (for simplicity, GABA receptors are not depicted). Antipsychotic drugs would possibly counteract the increased activity of pyramidal neurons by an action at α_1-adrenoceptors (classical antipsychotics) and at α_1-adrenoceptors and 5-HT_{2A} receptors (atypical antipsychotics), thus reducing the activity of pyramidal cells and, thus, the increase in 5-HT release produced by the activation of α_1-adrenoceptors in projection (pyramidal) neurons of the mPFC.

observation for which no clear neurobiological basis has been provided so far. The present data suggest that pyramidal neurons may play an integrative role for these actions to modulate motor output. Indeed, our observations suggest a close association between 5-HT_{2A}, α_1-adrenoceptors and AMPA receptors to regulate the activity of projection neurons in mPFC, which is the driving force of the observed changes in 5-HT release (Fig. 11). Also, cortical 5-HT_{2A}, α_1-adrenoceptors (but not AMPA receptors) appear to tonically control basal 5-HT release, given the reduction in 5-HT release produced by their local perfusion (α_1-adrenoceptors in the raphe also control tonically the activity of 5-HT cells and its local and terminal release; Baraban and Aghajanian 1980; Rouquier *et al.* 1994; Adell and Artigas 1999; Bortolozzi and Artigas 2003).

The effects of 5-HT_{2A} receptor and α_1-adrenoceptor activation on pyramidal cell excitability are consistent with a postsynaptic location. Indeed, most cortical 5-HT_{2A} receptors in mPFC are located postsynaptically (Miner *et al.* 2003). The tonic activation of both receptors can elicit a phospholipase C-mediated increase in Ca^{2+} signaling (see Introduction), which may facilitate AMPA-mediated transmission. This is also consistent with the α_1-adrenoceptor-mediated facilitation of the excitatory action of glutamate on cortical neurons (Mouradian *et al.* 1991; McCormick *et al.* 1993). The removal of the tone on either receptor by the respective antagonist may result in a loss of synergism and a subsequent reduction of pyramidal activity and of the descending excitatory input onto 5-HT neurons, which might explain the effect on basal and cirazoline-stimulated 5-HT release. Interestingly, prazosin and M100907 application completely reversed the 5-HT-increasing action of *S*-AMPA, an observation, which further supports the interaction between these receptors. However, we cannot clarify whether M100907 and prazosin act as pure antagonists *in vivo* as at least prazosin has been reported to be an inverse agonist in artificial cell systems (Zhu *et al.* 2000; Hein *et al.* 2001).

Interestingly, the basal and cirazoline-stimulated 5-HT release was also reversed by classical (chlorpromazine, haloperidol) and atypical antipsychotics (clozapine, olanzapine). All these agents display high *in vitro* affinity for α_1-adrenoceptors (in the low nanomolar range), whereas the only the atypical drugs have such high affinity for 5-HT_{2A} receptors (Arnt and Skarsfeldt 1998; Bymaster *et al.* 1999; Sebban *et al.* 1999). Both prazosin and M100907 reversed the elevation in mPFC 5-HT release produced by cirazoline (this study) and DOI (Bortolozzi *et al.* 2003). Similarly, chlorpromazine, haloperidol, clozapine and olanzapine also counteracted the increase in 5-HT produced by cirazoline (this study) and DOI (Bortolozzi *et al.* 2003). Based on the relative affinities of the four antipsychotic drugs tested, we postulate that only α_1-adrenoceptor blockade participates in the reversal of the effect of cirazoline by classical antipsychotics whereas both 5-HT_{2A} receptors and α_1-adrenoceptors may be involved in the action of atypical antipsychotics. Given the complex pharmacological profile of these drugs, it is likely that only the use of murine knockout models can clarify which receptor is involved in this reversal.

Atypical antipsychotics are 5-HT_{2A} receptor antagonists (Meltzer 1999). Likewise, the blockade of α_1-adrenoceptors by prazosin potentiated the antipsychotic-like effect of dopamine D2 receptor antagonists (Wadenberg *et al.* 2000) and there is increasing interest in the role played by 5-HT_{1A} receptors in the activity of atypical antipsychotics (Millan 2000; Ichikawa *et al.* 2001). It is noteworthy that these three properties (5-HT_{2A} receptor and α_1-adrenoceptor blockade, stimulation of 5-HT_{1A} receptors) converge in the same effect in mPFC, i.e. a reduction of the 5-HT release, which likely parallels the change in activity of pyramidal neurons. This suggests that, in addition to their antidopaminergic action, antipsychotics may partly exert their palliative effect by reducing the activity of prefrontal pyramidal neurons by any of these mechanisms. This would agree with the key role of the frontal lobe in the pathophysiology of schizophrenia and its treatment (for review, see Weinberger *et al.* 1994; Arnt and Skarsfeldt 1998; Lidow *et al.* 1998; Lewis and Lieberman 2000). Further work is required to examine the neuronal distribution of these three receptors in mPFC in order to clarify the cellular site(s) of interaction.

Acknowledgements

Work supported by grants SAF2001-2133 and FIS 2001–1147. Financial support from Bayer S.A and Eli Lilly & Co is also acknowledged. The authors thank Leticia Campa for help in HPLC analyses and the pharmaceutical companies for generous drug supply. AB is recipient of a postdoctoral fellowship from the Fundación Carolina. MA-B is recipient of a predoctoral fellowship from IDIBAPS.

References

Adell A. and Artigas F. (1998) A microdialysis study of the *in vivo* release of 5-HT in the median raphe nucleus of the rat. *Br. J. Pharmacol.* **125**, 1361–1367.

Adell A. and Artigas F. (1999) Regulation of the release of 5-hydroxytryptamine in the median raphe nucleus of the rat by catecholaminergic afferents. *Eur. J. Neurosci.* **11**, 2305–2311.

Aghajanian G. K. and Marek G. J. (1997) Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **36**, 589–599.

Aghajanian G. K. and Marek G. J. (1999) Serotonin, via 5-HT_{2A} receptors, increases EPSCs in layer v pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. *Brain Res.* **825**, 161–171.

Aghajanian G. K. and Wang R. Y. (1977) Habenular and other midbrain raphe afferents demonstrated by a modified retrograde tracing technique. *Brain Res.* **122**, 229–242.

Andreasen N. C., O'Leary D. S., Flaum M., Nopoulos P., Watkins G. L., Boles Ponto L. L. and Hichwa R. D. (1997) Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. *Lancet* **349**, 1730–1734.

Araneda R. and Andrade R. (1991) 5-Hydroxytryptamine$_2$ and 5-hydroxytryptamine$_{1A}$ receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* **40**, 399–412.

Arnt J. and Skarsfeldt T. (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* **18**, 63–101.

Ashby C. R., Edwards E. and Wang R. Y. (1994) Electrophysiological evidence for a functional interaction between 5-HT(1A) and 5-HT(2A) receptors in the rat medial prefrontal cortex: An iontophoretic study. *Synapse* **17**, 173–181.

Au-Young S. M., Shen H. and Yang C. R. (1999) Medial prefrontal cortical output neurons to the ventral tegmental area (VTA) and their responses to burst-patterned stimulation of the VTA: neuroanatomical and *in vivo* electrophysiological analyses. *Synapse* **34**, 245–255.

Azmitia E. C. and Segal M. (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J. Comp. Neurol.* **179**, 641–668.

Baraban J. M. and Aghajanian G. K. (1980) Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. *Neuropharmacology* **19**, 355–363.

Bartrup J. T. and Newberry N. R. (1994) 5-HT_{2A} receptor-mediated outward current in C6 glioma cells is mimicked by intracellular IP_3 release. *Neuroreport* **5**, 1245–1248.

Berendse H. W. and Groenewegen H. J. (1991) Restricted cortical termination fields of the midline and intralaminar thalamic nuclei in the rat. *Neuroscience* **42**, 73–102.

Berg K. A., Maayani S., Goldfarb J., Scaramellini C., Leff P. and Clarke W. P. (1998) Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* **54**, 94–104.

Bortolozzi A. and Artigas F. (2003) Control of 5-hydroxytryptamine release in the dorsal raphe nucleus by the noradrenergic system in rat brain: role of α-adrenoceptors. *Neuropsychopharmacology* **28**, 421–434.

Bortolozzi A., Amargós-Bosch M., Adell A., Díaz-Mataix L., Serrats J., Pons S. and Artigas F. (2003) *In vivo* modulation of 5-HT release in mouse prefrontal cortex by local 5-HT_{2A} receptors: effect of antipsychotic drugs. *Eur. J. Neurosci.* **18**, 1235–1246.

Bylund D. B. and U'Prichard D. C. (1983) Characterization of alpha 1- and alpha 2-adrenergic receptors. *Int. Rev. Neurobiol.* **24**, 343–431.

Bymaster F., Perry K. W., Nelson D. L., Wong D. T., Rasmussen K., Moore N. A. and Calligaro D. O. (1999) Olanzapine: a basic science update. *Br. J. Psychiatry Suppl.* **37**, 36–40.

Casanovas J. M., Hervás I. and Artigas F. (1999) Postsynaptic 5-HT_{1A} receptors control 5-HT release in the rat medial prefrontal cortex. *Neuroreport* **10**, 1441–1445.

Casanovas J. M., Berton O., Celada P. and Artigas F. (2000) *In vivo* actions of the selective 5-HT_{1A} receptor agonist BAY x, 3702 on serotonergic cell firing and release. *Naunyn Schmied. Arch. Pharmacol.* **362**, 248–254.

Celada P., Puig M. V., Casanovas J. M., Guillazo G. and Artigas F. (2001) Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABA(A), and glutamate receptors. *J. Neurosci.* **21**, 9917–9929.

Claro E., Fain J. N. and Picatoste F. (1993) Noradrenaline stimulation unbalances the phosphoinositide cycle in rat cerebral cortical slices. *J. Neurochem.* **60**, 2078–2086.

Day H. E., Campeau S., Watson S. J. and Akil H. (1997) Distribution of alpha-1A, alpha-1B and alpha-1D-adrenergic receptor mRNA in the rat brain and spinal cord. *J. Chem. Neuroanat.* **13**, 115–139.

De Felipe J., Arellano J. I., Gomez A., Azmitia E. C. and Munoz A. (2001) Pyramidal cell axons show a local specialization for GABA and 5-HT inputs in monkey and human cerebral cortex. *J. Comp. Neurol.* **433**, 148–155.

Devilbiss D. M. and Waterhouse B. D. (2000) Norepinephrine exhibits two distinct profiles of action on sensory cortical neuron responses to excitatory synaptic stimuli. *Synapse* **37**, 273–282.

De Vry J., Schohe-Loop R., Heine H. G., Greuel J. M., Mauler F., Schmidt B., Sommermeyer H. and Glaser T. (1998) Characterization of the aminomethylchroman derivative BAY x, 3702 as a highly potent 5-hydroxytryptamine (1A) receptor agonist. *J. Pharmacol. Exp. Ther.* **284**, 1082–1094.

Domyancic A. V. and Morilak D. A. (1997) Distribution of alpha1A adrenergic receptor mRNA in the rat brain visualized by *in situ* hybridization. *J. Comp. Neurol.* **386**, 358–378.

Drevets W. C., Price J. L., Simpson J. R., Todd R. D., Reich T., Vannier M. and Raichle M. E. (1997) Subgenual prefrontal cortex abnormalities in mood disorders. *Nature* **386**, 824–827.

Durstewitz D., Seamans J. K. and Sejnowski T. J. (2000) Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. *J. Neurophysiol.* **83**, 1733–1750.

Dursun S. M. and Handley S. L. (1996) Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest $5HT_{2A}$ receptors are active under physiological conditions. *Psychopharmacology* **128**, 198–205.

Fuster J. M. (1997) *The Prefrontal Cortex. Anatomy, Physiology and Neuropsychology of the Frontal Lobe.* Lipincott Raven, Philadelphia, New York.

Hagberg G. B., Blomstrand F., Nilsson M., Tamir H. and Hansson E. (1998) Stimulation of 5-HT_{2A} receptors on astrocytes in primary culture opens voltage-independent Ca^{2+} channels. *Neurochem. Int.* **32**, 153–162.

Hajós M., Richards C. D., Szekely A. D. and Sharp T. (1998) An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience* **87**, 95–108.

Hein P., Goepel M., Cotecchia S. and Michel M. C. (2001) A quantitative analysis of antagonism and inverse agonism at wild-type and constitutively active hamster alpha1B-adrenoceptors. *Naunyn. Schmied. Arch. Pharmacol.* **363**, 34–39.

Hervás I., Queiroz C. M., Adell A. and Artigas F. (2000) Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. *Br. J. Pharmacol.* **130**, 160–166.

Ichikawa J., Ishii H., Bonaccorso S., Fowler W. L., O'Laughlin I. A. and Meltzer H. Y. (2001) 5-HT_{2A} and D-2 receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.* **76**, 1521–1531.

Jakab R. L. and Goldman-Rakic P. S. (1998) 5-$Hydroxytryptamine_{2A}$ serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc. Natl Acad. Sci. USA* **95**, 735–740.

Jakab R. L. and Goldman-Rakic P. S. (2000) Segregation of serotonin 5-HT_{2A} and 5-HT_3 receptors in inhibitory circuits of the primate cerebral cortex. *J. Comp. Neurol.* **417**, 337–348.

Jodo E., Chiang C. and Aston-Jones G. (1998) Potent excitatory influence of prefrontal cortex activity on noradrenergic locus coeruleus neurons. *Neuroscience* **83**, 63–79.

Kia H. K., Brisorgueil M. J., Hamon M., Calas A. and Vergé D. (1996) Ultrastructural localization of 5-$hydroxytryptamoine_{1A}$ receptors in the rat brain. *J. Neurosci. Res.* **46**, 697–708.

Kosofsky B. E. and Molliver M. E. (1987) The serotoninergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse* **1**, 153–168.

Kroeze W. K. and Roth B. L. (1998) The molecular biology of serotonin receptors: therapeutic implications for the interface of mood and psychosis. *Biol. Psychiatry* **44**, 1128–1142.

Kuroda M., Yokofujita J. and Murakami K. (1998) An ultrastructural study of the neural circuit between the prefrontal cortex and the mediodorsal nucleus of the thalamus. *Prog. Neurobiol.* **54**, 417–458.

Lewis D. A. and Lieberman J. A. (2000) Catching up on schizophrenia: natural history and neurobiology. *Neuron* **28**, 325–334.

Lewis B. L. and O'Donnell P. (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. *Cereb. Cortex* **10**, 1168–1175.

Lidow M. S., Williams G. V. and Goldman-Rakic P. S. (1998) The cerebral cortex: a case for a common site of action of antipsychotics. *Trends Pharmacol. Sci.* **19**, 136–140.

Marangell L. B., Johnson C. R., Kertz B., Zboyan H. A. and Martinez J. M. (2002) Olanzapine in the treatment of apathy in previously depressed participants maintained with selective serotonin reuptake inhibitors: an open-label, flexible-dose study. *J. Clin. Psychiatry* **63**, 391–395.

Marek G. J. and Aghajanian G. K. (1999) 5-HT_{2A} receptor or $alpha_1$-adrenoceptor activation induces excitatory postsynaptic currents in layer V pyramidal cells of the medial prefrontal cortex. *Eur. J. Pharmacol.* **367**, 197–206.

Marek G. J., Wright R. A., Gewitz J. C. and Schoepp D. D. (2001) A major role for thalamocortical afferents in serotonergic hallucinogen receptor function in neocortex. *Neuroscience* **105**, 379–392.

Martín-Ruiz R., Puig M. V., Celada P., Shapiro D. A., Roth B. L., Mengod G. and Artigas F. (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. *J. Neurosci.* **21**, 9856–9866.

McCormick D. A., Wang Z. and Huguenard J. (1993) Neurotransmitter control of neocortical neuronal activity and excitability. *Cereb. Cortex* **3**, 387–398.

McCune S. K., Voigt M. M. and Hill J. M. (1993) Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience* **57**, 143–151.

Meltzer H. Y. (1999) The role of serotonin in antipsychotic drug action. *Neuropsychopharmacology* **21**, S106–S115.

Millan M. J. (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT)(1A) receptors. *J. Pharmacol. Exp. Ther.* **295**, 853–861.

Miller E. K. and Cohen J. D. (2001) An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.* **24**, 167–202.

Miner L. A. H., Backstrom J. R., Sanders-Bush E. and Sesack S. R. (2003) Ultrastructural localization of serotonin-2A receptors in the middle layers of the rat prelimbic prefrontal cortex. *Neuroscience* **116**, 107–117.

Molderings G. J., Donecker K., Burian M., Simon W. A., Schroder D. W. and Gothert M. (1998) Characterization of I2 imidazoline and sigma binding sites in the rat and human stomach. *J. Pharmacol. Exp. Ther.* **285**, 170–177.

Molinoff P. B. (1984) Alpha- and beta-adrenergic receptor subtypes properties, distribution and regulation. *Drugs* **28**, 1–15.

Mouradian R. D., Sessler F. M. and Waterhouse B. D. (1991) Noradrenergic potentiation of excitatory transmitter action in cerebrocortical slices: evidence for mediation by an alpha-1 receptor-linked second messenger pathway. *Brain. Res.* **546**, 83–95.

Murase S., Grenhoff J., Chouvet G., Gonon F. G. and Svensson T. H. (1993) Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied *in vivo*. *Neurosci. Lett.* **157**, 53–56.

Ostroff R. B. and Nelson J. C. (1999) Risperidone augmentation of selective serotonin reuptake inhibitors in major depression. *J. Clin. Psychiatry* **60**, 256–259.

Palacios J. M., Cortés R. and Hoyer D. (1987) $Alpha_1$-adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. *Brain Res.* **419**, 65–75.

Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney.

Pazos A., Cortes R. and Palacios J. M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain: II. serotonin-2 receptors. *Brain Res.* **346**, 231–249.

Petralia R. S. and Wenthold R. J. (1992) Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *J. Comp. Neurol.* **318**, 329–354.
Peyron C., Petit J. M., Rampon C., Jouvet M. and Luppi P. H. (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. *Neuroscience* **82**, 443–468.
Pieribone V. A., Nicholas A. P., Dagerlind A. and Hökfelt T. (1994) Distribution of alpha$_1$ adrenoceptors in rat brain revealed by *in situ* hybridization experiments utilizing subtype-specific probes. *J. Neurosci.* **14**, 4252–4268.
Porter R. H. P., Benwell K. R., Lamb H., Malcolm C. S., Allen N. H., Revell D. F., Adams D. R. and Sheardown M. J. (1999) Functional characterization of agonists at recombinant human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in CHO-K1 cells. *Br. J. Pharmacol.* **128**, 13–20.
Puig M. V., Celada P., Díaz-Mataix L. and Artigas F. (2003) *In vivo* modulation of the activity of pyramidal neruons in the rat medial prefrontal cortex by 5-HT$_{2A}$ receptors: relationship to thalamocortical afferents. *Cereb. Cortex* **13**, 870–882.
Rouquier L., Claustre Y. and Benavides J. (1994) α_1-Adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study. *Eur. J. Pharmacol.* **261**, 59–64.
Ruffolo R. R. and Waddell J. E. (1982) Receptor interactions of imidazolines: IX. cirazoline is an alpha-1 adrenergic agonist and an alpha-2 adrenergic antagonist. *J. Pharmacol. Exp. Ther.* **222**, 29–36.
Sakai K. and Crochet S. (2001) Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake–sleep states. *Neuroscience* **104**, 1141–1155.
Sara S. J. and Hervé-Minvielle A. (1995) Inhibitory influence of frontal cortex on locus coeruleus neurons. *Proc. Natl Acad. Sci. USA* **92**, 6032–6036.
Sato H., Fox K. and Daw N. W. (1989) Effect of electrical stimulation of locus coeruleus on the activity of neurons in the cat visual cortex. *J. Neurophysiol.* **62**, 946–958.
Schreiber R., Brocco M., Audinot V., Gobert A., Veiga S. and Millan M. J. (1995) (1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)$_{2A}$ receptors: modulation by novel 5-HT$_{2A/2C}$ antagonists, D$_1$ antagonists and 5-HT$_{1A}$ agonists. *J. Pharmacol. Exp. Ther.* **273**, 101–112.
Sebban C., Tesolin-Decros B., Millan M. J. and Spedding M. (1999) Contrasting EEG profiles elicited by antipsychotic agents in the prefrontal cortex of the conscious rat: antagonism of the effects of clozapine by modafinil. *Br. J. Pharmacol.* **128**, 1055–1063.
Sesack S. R. and Pickel V. M. (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J. Comp. Neurol.* **320**, 145–160.
Sesack S. R., Deutch A. Y., Roth R. H. and Bunney B. S. (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol.* **290**, 213–242.
Shelton R. C., Tollefson G. D., Tohen M., Stahl S., Gannon K. S., Jacobs T. G., Buras W. R., Bymaster F. P., Zhang W., Spencer K. A. *et al.* (2001) A novel augmentation strategy for treating resistant major depression. *Am. J. Psychiatry* **158**, 131–134.
Takagishi M. and Chiba T. (1991) Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. *Brain Res.* **566**, 26–39.
Tao R., Ma Z. Y. and Auerbach S. B. (2000) Differential effect of local infusion of serotonin reuptake inhibitors in the raphe versus forebrain and the role of depolarization-induced release in increased extracellular serotonin. *J. Pharmacol. Exp. Ther.* **294**, 571–579.
Thierry A. M., Deniau J. M., Chevalier G., Ferron A. and Glowinski J. (1983) An electrophysiological analysis of some afferent and efferent pathways of the rat prefrontal cortex. *Prog. Brain Res.* **58**, 257–261.
Van der Werf Y. D., Witter M. P. and Groenewegen H. J. (2002) The intralaminar and midline nuclei of the thalamus: anatomical and functional evidence for participation in processes of arousal and awareness. *Brain Res. Rev.* **39**, 107–140.
Varga V., Szekely A. D., Csillag A., Sharp T. and Hajos M. (2001) Evidence for a role of GABA interneurones in the cortical modulation of midbrain 5-hydroxytryptamine neurons. *Neuroscience* **106**, 783–792.
Vysokanov A., Flores-Hernandez J. and Surmeier D. J. (1998) mRNAs for clozapine-sensitive receptors co-localize in rat prefrontal cortex neurons. *Neurosci. Lett.* **258**, 179–182.
Wadenberg M. L., Hertel P., Fernhom R., Hygge Blakeman K., Ahlenius S. and Svensson T. H. (2000) Enhancement of antipsychotic-like effects by combined treatment with the α_1-adrenoceptor antagonist prazosin and the dopamine D$_2$ receptor antagonist raclopride in rats. *J. Neural Transm.* **107**, 1229–1238.
Weinberger D. R., Aloia M. S., Goldberg T. E. and Berman K. F. (1994) The frontal lobes and schizophrenia. *J. Neuropsychiatry Clin. Neurosci.* **6**, 419–427.
West A. R. and Grace A. A. (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining *in vivo* intracellular recordings and reverse microdialysis. *J. Neurosci.* **22**, 294–304.
Willins D. L., Deutch A. Y. and Roth B. L. (1997) Serotonin 5HT$_{2A}$ receptors are expressed on pyramidal neurons and interneurons in the rat cortex. *Synapse* **27**, 79–82.
Zhu J., Taniguchi T., Takauji R., Suzuki F., Tanaka T. and Muramatsu I. (2000) Inverse agonism and neutral antagonism at a constitutively active alpha-1A adrenoceptor. *Br. J. Pharmacol.* **131**, 546–552.