



# Dopamine release induced by atypical antipsychotics in prefrontal cortex requires 5-HT<sub>1A</sub> receptors but not 5-HT<sub>2A</sub> receptors

Analia Bortolozzi<sup>1,2</sup>, Mercè Masana<sup>1,2</sup>, Llorenç Díaz-Mataix<sup>1</sup>, Roser Cortés<sup>1,3</sup>,  
María Cecilia Scorza<sup>1</sup>, Jay A. Gingrich<sup>4</sup>, Miklos Toth<sup>5</sup> and Francesc Artigas<sup>1,2</sup>

<sup>1</sup> Department of Neurochemistry and Neuropharmacology, IIBB – CSIC (IDIBAPS), Barcelona, Spain

<sup>2</sup> CIBERSAM, Barcelona, Spain

<sup>3</sup> CIBERNED, Barcelona, Spain

<sup>4</sup> Department of Psychiatry, Columbia University, NY, USA

<sup>5</sup> Department of Pharmacology, Weill Medical College, Cornell University, NY, USA

## Abstract

Atypical antipsychotic drugs (APDs) increase dopamine (DA) release in prefrontal cortex (PFC), an effect probably mediated by the direct or indirect activation of the 5-HT<sub>1A</sub> receptor (5-HT<sub>1A</sub>R). Given the very low *in-vitro* affinity of most APDs for 5-HT<sub>1A</sub>R and the large co-expression of 5-HT<sub>1A</sub>R and 5-HT<sub>2A</sub>R receptors (5-HT<sub>2A</sub>R) in the PFC, this effect might result from the imbalance of 5-HT<sub>1A</sub>R and 5-HT<sub>2A</sub>R activation after blockade of these receptors by APDs, for which they show high affinity. Here we tested this hypothesis by examining the dependence of the APD-induced DA release in medial PFC (mPFC) on each receptor by using *in-vivo* microdialysis in wild-type (WT) and 5-HT<sub>1A</sub>R and 5-HT<sub>2A</sub>R knockout (KO) mice. Local APDs (clozapine, olanzapine, risperidone) administered by reverse dialysis induced a dose-dependent increase in mPFC DA output equally in WT and 5-HT<sub>2A</sub>R KO mice whereas the DA increase was absent in 5-HT<sub>1A</sub>R KO mice. To examine the relative contribution of both receptors to the clozapine-induced DA release in rat mPFC, we silenced G-protein-coupled receptors (GPCRs) *in vivo* with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) while 5-HT<sub>1A</sub>R or 5-HT<sub>2A/2C</sub>R in the mPFC were selectively protected with the respective antagonists WAY-100635 or ritanserin. The inactivation of GPCRs while preserving ~70% of 5-HT<sub>2A/2C</sub>R prevented the clozapine-induced DA rise in mPFC. In contrast, clozapine increased DA in mPFC of EEDQ-treated rats whose 5-HT<sub>1A</sub>R were protected (~50% of control rats). These results indicate that (1) 5-HT<sub>1A</sub>R are necessary for the APDs-induced elevation in cortical DA transmission, and (2) this effect does not require 5-HT<sub>2A</sub>R blockade by APDs.

Received 13 July 2009; Reviewed 10 August 2009; Revised 12 January 2010; Accepted 13 January 2010;

First published online 17 February 2010

**Key words:** Antipsychotic drugs, dopamine, prefrontal cortex, 5-HT<sub>1A</sub> receptor, 5-HT<sub>2A</sub> receptor.

## Introduction

Cognitive impairment, including attention disorders, deficits in working memory and executive functions, is a central feature of schizophrenia lacking adequate treatment (Braff, 1993; Elvevag & Goldberg, 2000; Green *et al.* 2000). Negative symptoms are also poorly treated with current medications (Weickert *et al.* 2000), which, by themselves, induce this type of

symptomatology (Artaloytia *et al.* 2006). In particular, atypical antipsychotic drugs (APDs) improve negative symptoms and quality of life more than classical antipsychotics (Sumiyoshi *et al.* 2006; Woodward *et al.* 2005). Moreover, some APDs (amisulpride, clozapine, olanzapine, risperidone) proved superior to first-generation drugs when considering their overall efficacy (Leucht *et al.* 2009).

The ability of some APDs to improve negative/cognitive symptoms (Bubenikova-Valesova *et al.* 2008; Grayson *et al.* 2007; Harvey *et al.* 2008) has been attributed to their capacity to increase dopamine (DA)-mediated transmission in the medial prefrontal cortex (mPFC) and hippocampus of experimental animals

Address for correspondence: F. Artigas, Ph.D., Department of Neurochemistry and Neuropharmacology, IIBB-CSIC (IDIBAPS), Rosselló 161, 6th floor, 08036 Barcelona, Spain.

Tel.: (+34) 93-363-8315 Fax: (+34) 93-363-8301

Email: fapnqi@iibb.csic.es

(Assié *et al.* 2005; Bortolozzi *et al.* 2007b; Chung *et al.* 2004; Díaz-Mataix *et al.* 2005; Elsworth *et al.* 2008; Ichikawa *et al.* 2001; Kuroki *et al.* 1999; Li *et al.* 2009; Millan, 2000; Rollema *et al.* 1997, 2000; Youngren *et al.* 1999). Indeed, an optimal DA transmission is fundamental for the execution of PFC-dependent cognitive tasks (Vijayraghavan *et al.* 2007; Williams & Goldman-Rakic, 1995) and, among other anatomical and neurochemical abnormalities in PFC, schizophrenia patients show a reduced dopaminergic innervation (Akil *et al.* 1999; Lewis & Lieberman, 2000).

APDs share a higher *in-vitro* affinity and *in-vivo* occupancy of 5-HT<sub>2A</sub> receptors (5-HT<sub>2A</sub>Rs) vs. DA D<sub>2</sub> receptors (Meltzer *et al.* 1989; Nyberg *et al.* 1998; Stockmeier *et al.* 1993). 5-HT<sub>2A</sub>Rs are densely expressed in PFC, mainly in projection pyramidal neurons (Amargós-Bosch *et al.* 2004; López-Giménez *et al.* 1997; Pazos *et al.* 1985; Santana *et al.* 2004), including those projecting to the ventral tegmental area (VTA) (Vázquez-Borsetti *et al.* 2009). 5-HT<sub>2A</sub>R stimulation in PFC enhances the activity of pyramidal neurons projecting to the VTA (Puig *et al.* 2003, 2005) and of VTA dopaminergic neurons (Bortolozzi *et al.* 2005), leading to an increased mesocortical DA release (Bortolozzi *et al.* 2005; Gobert & Millan, 1999). Further, the 5-HT<sub>2A</sub>R antagonist M100907 reduced the firing of DA neurons and DA release in mPFC (Bortolozzi *et al.* 2005; Minabe *et al.* 2001; Pehek *et al.* 2001). Overall, these observations are consistent with the above anatomical finding showing the existence of (a) closed mPFC-VTA loops (Carr & Sesack, 2000) and (b) the expression of 5-HT<sub>2A</sub>Rs in mPFC pyramidal neurons projecting to the VTA (Vázquez-Borsetti *et al.* 2009). However, 5-HT<sub>2A</sub>R blockade has been suggested to be necessary for APDs to enhance DA release in the mPFC (Bonaccorso *et al.* 2002; Ichikawa *et al.* 2001; Liégeois *et al.* 2002).

In addition to 5-HT<sub>2A</sub>R blockade, APDs display variable, but often high affinity for other monoamine receptors (see <http://kidb.case.edu/pdsp.php>; Roth *et al.* 2003). Agonist activity at 5-HT<sub>1A</sub> receptors (5-HT<sub>1A</sub>Rs) by APDs appears to contribute to their superior efficacy in treating non-psychotic symptoms (Bantick *et al.* 2001; Meltzer & Sumiyoshi, 2008; Millan, 2000; Sumiyoshi *et al.* 2001a,b; but see Rényi *et al.* 2001; Yasuno *et al.* 2003). Hence, although APDs show little or no *in-vitro* affinity for 5-HT<sub>1A</sub>Rs ( $K_i$  = 770 nM for clozapine, >1000 nM for olanzapine and 490 nM for risperidone; Arnt & Skarsfeldt, 1998; Bymaster *et al.* 1996), these agents increase cortical DA release through 5-HT<sub>1A</sub>R activation (Díaz-Mataix *et al.* 2005; Rollema *et al.* 1997).

Given the large co-expression of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in PFC (Amargós-Bosch *et al.* 2004) and their opposite role in modulating pyramidal neuron activity (Aghajanian & Marek, 1997; Amargós-Bosch *et al.* 2004; Araneda & Andrade, 1991; Ashby *et al.* 1994; Puig *et al.* 2005), the apparent *in-vivo* action of APDs at 5-HT<sub>1A</sub>Rs might be due to blockade of 5-HT<sub>2A</sub>Rs in cells co-expressing both receptors, thus enhancing 5-HT<sub>1A</sub>R-mediated neurotransmission. Here we examined this possibility using control mice and mice lacking 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptors. We also used an *in-vivo* rat model consisting in the inactivation of G-protein-coupled receptors (GPCRs) with the alkylating agent *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), while selectively protecting 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors in the mPFC (Amargós-Bosch *et al.* 2004).

## Materials and methods

### Animals

Male albino Wistar rats (250–320 g) were from Iffa Credo (France). Male homozygous 5-HT<sub>1A</sub>R knockout (KO) mice were generated at Princeton University (Parks *et al.* 1998) and 5-HT<sub>2A</sub>R KO mice were generated at Columbia University (Fiorica-Howells *et al.* 2002). Both genotypes were gradually backcrossed to the C57BL/6 background. From these initial sources, some 5-HT<sub>1A</sub>R KO and 5-HT<sub>2A</sub>R KO mice were transferred to develop a stable colony in our animal facilities. Wild-type (WT) mice of the same genetic background (C57BL/6) were also used. Mice were aged 10–15 wk at the time of experiments. Animals were maintained in a temperature-controlled room with a 12-h light/dark cycle (lights on 08:00 hours). Food and water were available *ad libitum*. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee of the School of Medicine, University of Barcelona.

### Drugs and reagents

All reagents used were of analytical grade and were obtained from Merck (Germany). 5-HT oxalate, clozapine, 1-[2,5-dimethoxy-4-iodophenyl-2-amino-propane] (DOI), dopamine hydrochloride, EEDQ, risperidone, ritanserin, 6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbamoyl] indoline (SB-242084), spiperone and *N*-[2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl]-*N*-(2-pyridyl) cyclohexane carboxamide 3 HCl (WAY-100635) were from Sigma/RBI (Spain). BAY x 3792, citalopram HBr

and olanzapine were from Bayer, Lundbeck A/S (Denmark) and Eli Lilly (USA), respectively. To assess local effects in microdialysis experiments, drugs were dissolved in perfusion fluid [aCSF (artificial cerebrospinal fluid, mM): NaCl, 125; KCl, 2.5; CaCl<sub>2</sub>, 1.26 and MgCl<sub>2</sub>, 1.18] and administered by reverse dialysis at the stated concentrations (uncorrected for membrane recovery). Clozapine, olanzapine and risperidone were initially dissolved in a drop of acetic acid and diluted to appropriate concentrations in aCSF. All other drugs were dissolved in distilled water, saline or aCSF, as required. Concentrated solutions (1 mM; pH adjusted to 6.5–7 with NaHCO<sub>3</sub> when necessary) were stored at –80 °C and working solutions were prepared daily by dilution in aCSF. Control mice and rats were perfused with aCSF. Bars in the figures show the period of drug administration, corrected for the void volume of the system.

### Microdialysis procedures

Microdialysis experiments in rats and mice were conducted as described previously (Amargós-Bosch *et al.* 2004; Bortolozzi *et al.* 2003). Rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and implanted with 4-mm concentric dialysis probes (Cuprophane) in the mPFC [coordinates in mm: AP +3.2, L –0.8, DV –6.0 (Paxinos & Watson, 2005)]. Experiments were performed in awake animals ~20 h after surgery. Probes were perfused with aCSF at 1.5 µl/min. After an initial 100 min stabilization period, four baseline samples were collected (20 min each) before local drug administration, and successive dialysate samples were collected. For mice, the surgical and microdialysis procedures were identical to those described for rats, except for the dose of anaesthesia (40 mg/kg i.p.), length of dialysis membrane (2 mm), and the brain coordinates (in mm) of the mPFC: AP +2.2, L –0.2, DV –3.4 (Franklin & Paxinos, 1997).

Monoamine concentration in dialysate samples was determined by HPLC with electrochemical detection (Hewlett Packard 1049; +0.75 for DA, +0.6 V for 5-HT) as described previously (Bortolozzi *et al.* 2003; Díaz-Mataix *et al.* 2005). Detection limits were 2–3 fmol for DA and 5-HT.

At the end of the experiments, animals were killed by an overdose of anaesthetic. Brains were quickly removed and frozen in dry ice before being sectioned (40 µm) with a cryostat (HM500-Om Microm, Germany) in coronal planes. Brain sections were stained with Neutral Red to verify the correct placement of probes.

### Silencing of GPCRs in vivo with selective protection of 5-HT<sub>1A</sub>Rs or 5-HT<sub>2A/2C</sub>Rs in mPFC

We used a previously described strategy to selectively protect 5-HT<sub>1A</sub>Rs or 5-HT<sub>2A/2C</sub>Rs in mPFC from the overall inactivating effect of EEDQ on GPCRs (Amargós-Bosch *et al.* 2004). EEDQ was systemically administered to inactivate GPCRs while selectively protecting one or other receptor by the local perfusion of selective antagonists (WAY-100635 for 5-HT<sub>1A</sub>Rs, ritanserin for 5-HT<sub>2A/2C</sub>Rs) through the microdialysis probes. EEDQ alkylates several GPCRs and inactivates their function (Gozlan *et al.* 1994), except those whose binding pockets are occupied. Thus, the perfusion of WAY-100635 or ritanserin in mPFC confers a selective protection of 5-HT<sub>1A</sub>Rs or 5-HT<sub>2A/2C</sub>Rs in mPFC, respectively, during EEDQ treatment. These two experimental groups are designated as GPCR-silenced + 5-HT<sub>1A</sub>R-protected and GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected, respectively.

Three to four hours after implantation, microdialysis probes were perfused with WAY-100635 (300 µM) for 3 h at 1.5 µl/min (5-HT<sub>1A</sub>R protection). One hour after starting the perfusion, EEDQ (dissolved in ethanol/water 1:1) was administered at 6 mg/kg i.p. The same procedure was applied to protect 5-HT<sub>2A/2C</sub>Rs using the 5-HT<sub>2A/2C</sub>R antagonist ritanserin (300 µM). Control rats received vehicle intraperitoneally and aCSF through the dialysis probes. On the following day, histological or microdialysis experiments were performed. For autoradiographic studies, 14-µm-thick coronal sections were cut, thaw-mounted onto 3-aminopropyltriethoxysilane (APTS; Sigma/RBI, Spain) coated slides, and kept at –20 °C until required. All experiments with control and EEDQ-treated rats were run in parallel.

### Receptor autoradiography

To determine the extent of regional 5-HT<sub>1A</sub>R or 5-HT<sub>2A/2C</sub>R protection in EEDQ-treated rats we performed receptor autoradiography for 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptors using the ligands [<sup>3</sup>H]8-OH-DPAT (227.0 Ci/mmol) and [<sup>3</sup>H]mesulergine (83.0 Ci/mmol), respectively, from Amersham (GE Healthcare, Spain). Fresh frozen coronal sections of PFC from control and EEDQ-treated rats were used. Incubation conditions for [<sup>3</sup>H]8-OH-DPAT were as previously described (Mengod *et al.* 1996). Non-specific binding was defined as that remaining in presence of 10<sup>–5</sup> M 5-HT. Incubation conditions for [<sup>3</sup>H]mesulergine were as previously described (López-Giménez *et al.* 2002; Pazos *et al.* 1985). Non-specific binding was defined as that remaining in the presence of 10<sup>–5</sup> M

mianserin. After incubation and washing, tissue sections were dipped in distilled, ice-cold water and dried rapidly under a cold air stream. Tissues were exposed to tritium-sensitive film (Kodak Biomax MR; Kodak, USA) together with plastic  $^3\text{H}$  standards for 60 d at 4 °C. All tissue sections used for quantification of receptor sites were processed simultaneously under the same conditions.

5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors were examined in mice brain by receptor autoradiography as previously described (López-Giménez *et al.* 2002; Mengod *et al.* 1996; Pazos *et al.* 1985) using (a) [ $^3\text{H}$ ]8-OH-DPAT for 5-HT<sub>1A</sub>Rs, (b) [ $^3\text{H}$ ]mesulergine (plus  $10^{-7}$  M of the selective 5-HT<sub>2C</sub>R antagonist SB242084) for 5-HT<sub>2A</sub>Rs, and (c) [ $^3\text{H}$ ]mesulergine (plus  $10^{-7}$  M of the 5-HT<sub>2A</sub>R antagonist spiperone) for 5-HT<sub>2C</sub>Rs. Quantitative analysis of the autoradiograms was done with AIS computerized image analysis system (Imaging Research Inc., Canada).

#### Data and statistical analysis

Microdialysis results are expressed as fmol/30- $\mu\text{l}$  fraction for DA and 5-HT and shown in the Figures as percentages of baseline (individual means of four pre-drug fractions). Area under the curve (AUC) of selected time periods (fractions 6–16) was also calculated. Statistical analysis was performed using one- or two-way ANOVAs for repeated measures or AUC of DA or 5-HT values followed by Newman–Keuls *post-hoc* test.

Quantitative autoradiographic measurements obtained from the different radioligands were analysed using one-way ANOVA followed by Newman–Keuls *post-hoc* test or Student's *t* test, as appropriate. Data are expressed as means  $\pm$  S.E.M. Statistical significance has been set at the 95% confidence level (two tailed).

## Results

### Basal values of DA and 5-HT in mPFC dialysates

Basal extracellular levels of DA and 5-HT in dialysates from mPFC of mice and rats are shown in Table 1. Non-significant differences were found between mice genotype or between control and EEDQ-pretreated rats.

### 5-HT<sub>1A</sub>R and 5-HT<sub>2A</sub>R KO mice: receptor autoradiography and neurochemical analysis

The lack of 5-HT<sub>1A</sub>Rs in these KO mice was previously assessed by autoradiography, electrophysiology and microdialysis procedures (Amargós-Bosch *et al.* 2004). Here, we extended previous autoradiographic

**Table 1.** Basal DA and 5-HT dialysate values in the mPFC of mice and rats

Group	Baseline DA (fmol/20-min fraction)	Baseline 5-HT (fmol/20-min fraction)
WT mice	6.3 $\pm$ 0.8 ( <i>n</i> = 54)	17.2 $\pm$ 1.9 ( <i>n</i> = 25)
5-HT <sub>1A</sub> R KO mice	6.6 $\pm$ 0.8 ( <i>n</i> = 30)	19.7 $\pm$ 1.9 ( <i>n</i> = 9)
5-HT <sub>2A</sub> R KO mice	5.7 $\pm$ 0.6 ( <i>n</i> = 49)	16.5 $\pm$ 1.4 ( <i>n</i> = 24)
Control rats	8.9 $\pm$ 1.0 ( <i>n</i> = 27)	n.e.
EEDQ pretreated rats	11.2 $\pm$ 1.6 ( <i>n</i> = 17)	n.e.

n.e., Not examined.

Data are means  $\pm$  S.E.M. of the number of animals shown in parentheses.

observations. [ $^3\text{H}$ ]8-OH-DPAT binding to 5-HT<sub>1A</sub>Rs showed a high density in PFC, hippocampus and raphe nuclei of WT mice (Fig. 1, panels A1–A3). Homozygous 5-HT<sub>1A</sub>R KO mice showed no specific binding in either region (Fig. 1, panels A4–A6).

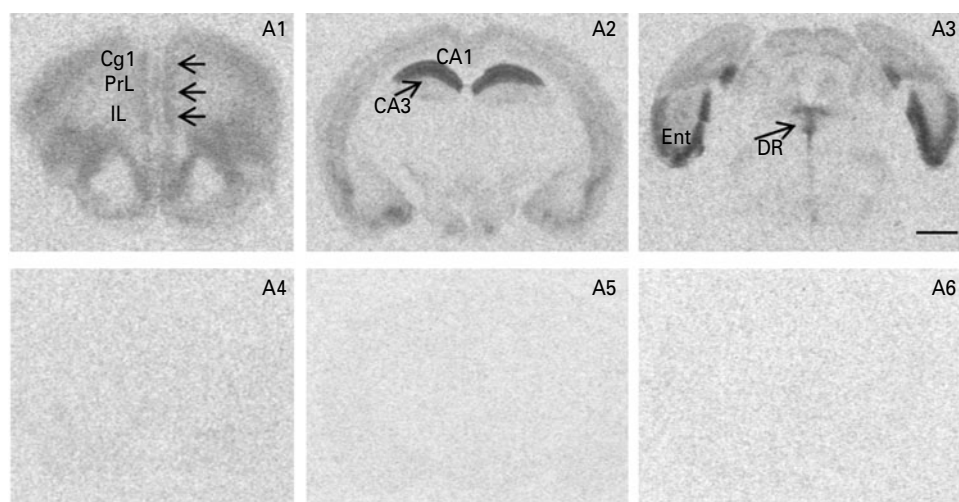
The absence of 5-HT<sub>2A</sub>Rs in these KO mice was evaluated by receptor autoradiography and by the neurochemical response to the preferential 5-HT<sub>2A</sub>R agonist DOI. Autoradiographic analysis of 5-HT<sub>2A</sub>Rs revealed the presence of a strong signal in the frontal cortex and claustrum of WT mice (Fig. 2, panels A1–A2). Homozygous 5-HT<sub>2A</sub>R KO mice showed no specific binding in either region (Fig. 2, panels A3–A4). Quantitative assessments of 5-HT<sub>1A</sub>R and 5-HT<sub>2C</sub>R density are shown in Table 2. No genotype differences between the densities of 5-HT<sub>2C</sub>Rs and 5-HT<sub>1A</sub>Rs were found in receptor-rich areas such as the choroid plexus and PFC, respectively (Fig. 2*b, c*).

Perfusion of aCSF did not significantly alter DA and 5-HT output in the mPFC of WT and 5-HT<sub>2A</sub>R KO mice (DA, *n* = 6 and 5, respectively; 5-HT, *n* = 5 for each genotype) (Fig. 3*a, b*). Local administration of DOI (100  $\mu\text{M}$  for 5-HT and 300  $\mu\text{M}$  for DA; see Bortolozzi *et al.* 2003, 2005) enhanced 5-HT and DA output in the mPFC of WT mice (Fig. 3*c, d*). DOI induced a maximal elevation of the 5-HT output to 205  $\pm$  19% of baseline (*n* = 10) [ $F(15, 135) = 7.71$ ,  $p < 0.001$ ]. Similarly, DOI elevated DA output to 192  $\pm$  23% of baseline (*n* = 10) [ $F(15, 135) = 6.46$ ,  $p < 0.001$ ]. Neither of these effects was observed when DOI was perfused in the mPFC of 5-HT<sub>2A</sub>R KO mice (Fig. 3*c, d*).

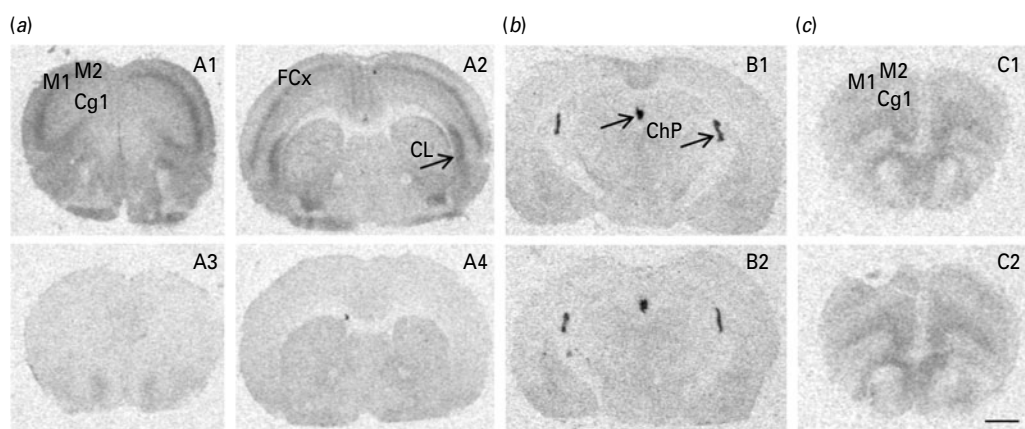
### Effect of atypical antipsychotics on mPFC DA output in 5-HT<sub>1A</sub>R and 5-HT<sub>2A</sub>R KO mice

These experiments were conducted to examine whether the increase in DA output induced by APDs





**Fig. 1.** Representative autoradiograms of [ $^3\text{H}$ ]8-OH-DPAT binding in the brains of wild-type (WT) (A1–A3, upper panels) and 5-HT $_{1A}$ R knockout (KO) mice (A4–A6, lower panels). In WT mice, note the high density for 5-HT $_{1A}$ Rs in (A1) prefrontal cortex [cingulated (Cg1), prelimbic (PrL), infralimbic (IL); AP: 1.78 mm], (A2) hippocampus (AP:  $-2.06$  mm) and (A3) dorsal raphe nuclei (DR) and entorhinal cortex (Ent) (AP:  $-4.36$  mm). Parallel sections of the corresponding null mutant mice (KO) show a lack of [ $^3\text{H}$ ]8-OH-DPAT binding (A4–A6). Scale bar, 1 mm.



**Fig. 2.** Representative autoradiograms of [ $^3\text{H}$ ]mesulergine (*a, b*) and [ $^3\text{H}$ ]8-OH-DPAT (*c*) binding in the brains of wild-type (WT) (upper panels) and 5-HT $_{2A}$ R knockout (KO) mice (lower panels). In WT mice, note the expected high density for 5-HT $_{2A}$ Rs as visualized with [ $^3\text{H}$ ]mesulergine plus  $10^{-7}$  M SB242084 in (A1) prefrontal cortex [cingulated (Cg1). Motor (M1, M2), AP: 2.10 mm] and, (A2) frontal cortex (FCx) and claustrum (CL) (AP: 0.74 mm). Parallel sections of the corresponding null mutant mice (KO) show a conspicuous lack of binding (A3–A4). No differences between genotypes were noted for 5-HT $_{2C}$ Rs as visualized with [ $^3\text{H}$ ]mesulergine plus  $10^{-7}$  M spiperone in the choroid plexus (ChP) (B1–B2) nor for 5-HT $_{1A}$ Rs, as visualized with [ $^3\text{H}$ ]8-OH-DPAT in prefrontal cortex (C1–C2) in WT and 5-HT $_{2A}$ R KO mice, respectively. Scale bar, 1 mm.

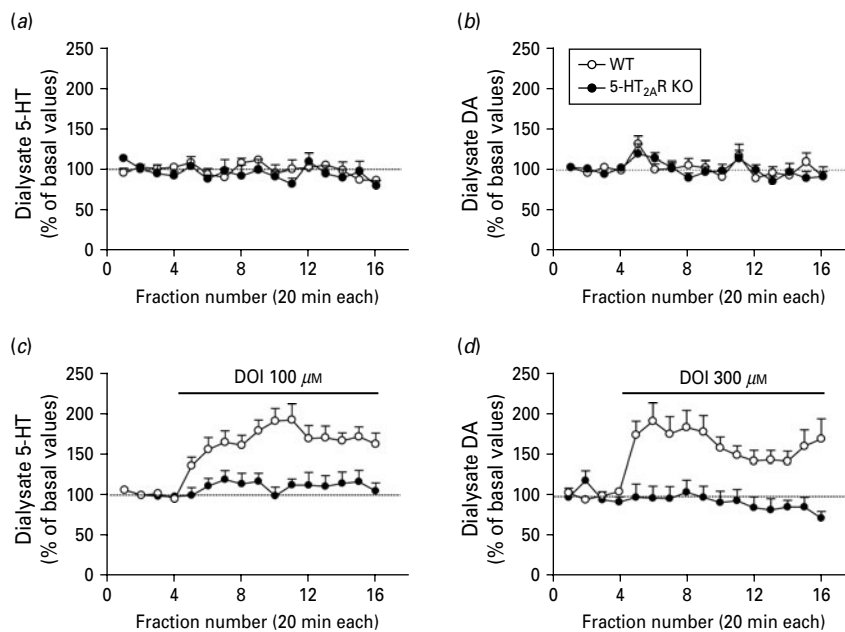
in mPFC is primarily associated with activation of 5-HT $_{1A}$ Rs or with blockade of 5-HT $_{2A}$ Rs. Local administration of clozapine (300  $\mu\text{M}$ ), olanzapine (100  $\mu\text{M}$ ) (Díaz-Mataix *et al.* 2005) and risperidone (100  $\mu\text{M}$ ) by reverse dialysis increased the DA concentration similarly in the mPFC of WT and 5-HT $_{2A}$ R KO mice. Two-way ANOVAs revealed a significant effect of time and non-significant effects of genotype and time  $\times$  genotype interaction (Fig. 4*a–c*). The maximal

effect induced by clozapine on mPFC DA output was  $428 \pm 52\%$  of baseline in WT mice ( $n=9$ ) and  $442 \pm 54\%$  of baseline in 5-HT $_{2A}$ R KO mice ( $n=9$ ) [time effect:  $F(15, 240)=37.22$ ,  $p<0.0001$ ]. Olanzapine perfusion increased DA output to  $363 \pm 61\%$  of basal values in mPFC of WT mice ( $n=6$ ) and to  $348 \pm 75\%$  in 5-HT $_{2A}$ R KO mice ( $n=7$ ) [time effect:  $F(15, 165)=10.84$ ,  $p<0.0001$ ], and the maximal elevation of mPFC DA release produced by risperidone was  $283 \pm 71\%$  of

**Table 2.** 5-HT<sub>1A</sub>R and 5-HT<sub>2C</sub>R labelling in different brain regions from WT and 5-HT<sub>2A</sub>R KO mice

	5-HT <sub>1A</sub> R [ <sup>3</sup> H]8-OHDPAT (fmol/mg tissue)		5-HT <sub>2C</sub> R [ <sup>3</sup> H]Mesulergine + 10 <sup>-7</sup> M spiperone (fmol/mg tissue)		
	Prefrontal cortex	Frontal cortex	Frontal cortex	Clastrum	Choroid plexus
WT	34.1 ± 0.5	23.1 ± 1.1	18.4 ± 3.2	18.0 ± 4.0	127.5 ± 4.2
5-HT <sub>2A</sub> R KO	32.5 ± 1.0	23.2 ± 1.7	13.6 ± 4.7	21.3 ± 4.6	128.6 ± 9.0

5-HT<sub>1A</sub>R labelling by [<sup>3</sup>H]8-OHDPAT and 5-HT<sub>2C</sub>R labelling by [<sup>3</sup>H]mesulergine + 10<sup>-7</sup> M spiperone were measured in different brain regions including prefrontal cortex (AP: 2.1 mm), frontal cortex and claustrum (AP: ~0.74 mm) and lateral and medial choroid plexuses (AP: about -1.58 mm). Results, expressed as fmol/mg tissue, are the means ± s.e.m. of 4–8 observations per mouse (*n* = 4) (one or two observations for each hemisphere of two consecutive sections per animal and four animals per group). Non-significant differences were observed between both genotypes (Student's *t* test).



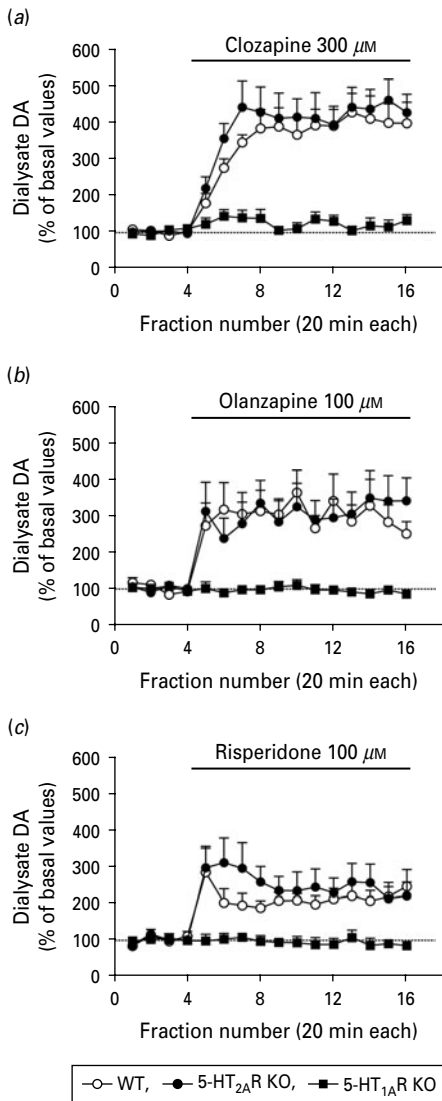
**Fig. 3.** Local effect of the 5-HT<sub>2A/2C</sub>R agonist DOI (100–300 μM) on the output of 5-HT (c) and DA (d) in the mPFC of wild-type (WT) and 5-HT<sub>2A</sub>R knockout (KO) mice. The perfusion of DOI increased 5-HT and DA levels in mPFC of WT mice (*n* = 10). Both effects were absent in 5-HT<sub>2A</sub>R KO mice (*n* = 7–10). The administration of aCSF did not alter prefrontal 5-HT (a) and DA (b) in either genotype (*n* = 5–6). Data are expressed as mean ± s.e.m. See Results section for statistical analysis.

baseline in WT mice (*n* = 5) and 310 ± 68% of baseline in 5-HT<sub>2A</sub>R KO mice (*n* = 5) [time effect:  $F(15, 120) = 10.85$ ,  $p < 0.0001$ ].

However, clozapine (300 μM), olanzapine (100 μM) and risperidone (100 μM) were unable to increase DA output in the mPFC of 5-HT<sub>1A</sub>R KO mice (*n* = 4–6) (Fig. 4a–c). Two-way ANOVAs revealed significant differences in the effects of APDs between the strains of mice: (a) clozapine [genotype effect:  $F(2, 19) = 7.83$ ,  $p < 0.01$ ; time effect:  $F(15, 285) = 21.88$ ,  $p < 0.0001$ ; time × genotype interaction:  $F(30, 285) = 3.79$ ,

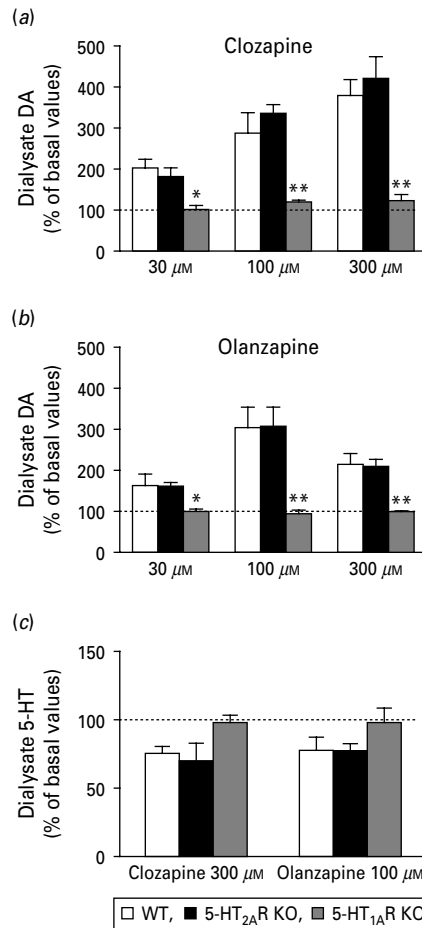
$p < 0.0001$ ], (b) olanzapine [genotype effect:  $F(2, 14) = 5.65$ ,  $p < 0.05$ ; time effect:  $F(15, 210) = 7.42$ ,  $p < 0.0001$ ; time × genotype interaction:  $F(30, 210) = 2.05$ ,  $p < 0.001$ ] and (c) risperidone [genotype effect:  $F(2, 13) = 8.66$ ,  $p < 0.01$ ; time effect:  $F(15, 195) = 10.41$ ,  $p < 0.0001$ ; time × genotype interaction:  $F(30, 195) = 3.73$ ,  $p < 0.0001$ ].

In addition, the local perfusion of clozapine and olanzapine at increasing concentrations (30–100–300 μM) significantly raised DA concentration in mPFC of both WT and 5-HT<sub>2A</sub>R KO, but not in 5-HT<sub>1A</sub>R KO mice in a concentration-dependent



**Fig. 4.** The local administration of (a) clozapine (300  $\mu$ M,  $n=9$ ), (b) olanzapine (100  $\mu$ M,  $n=6-7$ ) and (c) risperidone (100  $\mu$ M,  $n=5$ ) increased similarly DA levels in mPFC of wild-type (WT) and 5-HT<sub>2A</sub>R knockout (KO) mice. This effect was not observed when APDs were infused in the mPFC of 5-HT<sub>1A</sub>R KO mice ( $n=4-6$ , a-c). Data are expressed as mean  $\pm$  S.E.M. See Results section for statistical analysis.

manner (Fig. 5a,b). Two-way ANOVAs of AUC revealed significant differences in the effects of APDs on the different genotypes: (a) clozapine [concentration effect:  $F(2,40)=8.42$ ,  $p<0.001$ ; genotype effect:  $F(2,40)=16.02$ ,  $p<0.0001$ ] and (b) olanzapine [concentration effect:  $F(2,31)=5.40$ ,  $p<0.01$ ; genotype effect:  $F(2,31)=11.60$ ,  $p<0.001$ ]. Unlike DA, the concentration of 5-HT in mPFC dialysates was similarly affected by clozapine (300  $\mu$ M) and olanzapine (100  $\mu$ M) in the three genotypes. A marginal reduction was

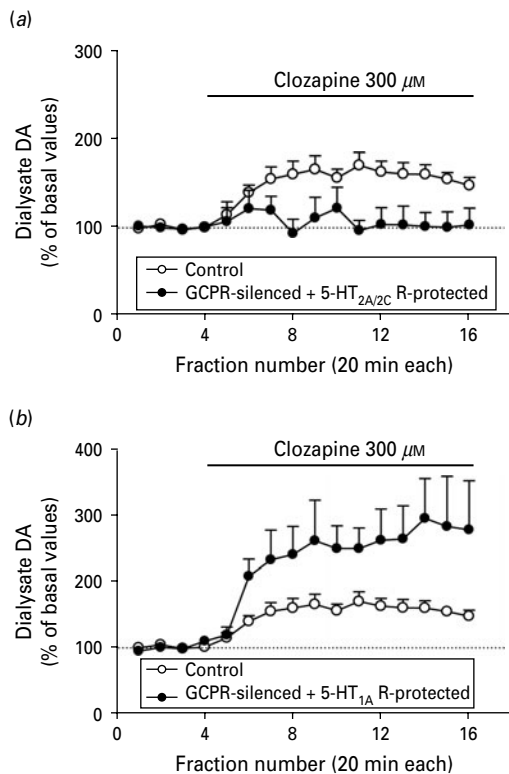


**Fig. 5.** Local administration of (a) clozapine and (b) olanzapine at increasing concentrations (30–100–300  $\mu$ M) raised a similar DA output in mPFC of wild-type (WT) and 5-HT<sub>2A</sub>R KO mice. This effect was not observed when APDs were perfused in the mPFC of 5-HT<sub>1A</sub>R KO mice. (c) Local effect of clozapine (300  $\mu$ M) and olanzapine (100  $\mu$ M) on 5-HT output in mPFC of WT, 5-HT<sub>2A</sub>R KO and 5-HT<sub>1A</sub>R KO mice. Data are AUCs (fractions 6–16) expressed as percentage of baseline.  $n=4-9$  mice for all groups, except for 300  $\mu$ M olanzapine in mPFC of 5-HT<sub>2A</sub>R KO mice, where  $n=3$ . See Results section for statistical analysis. \*  $p<0.05$ , \*\*  $p<0.01$  vs. WT and 5-HT<sub>2A</sub>R KO mice.

noted in WT and 5-HT<sub>2A</sub>R KO, but not in 5-HT<sub>1A</sub>R KO mice ( $p=0.09$  for clozapine;  $p=0.17$  for olanzapine; one-way ANOVA of AUCs) (Fig. 5c).

#### Effect of clozapine on mPFC DA output after GPCR silencing with selective protection of 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors in rats

To assess the involvement of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the clozapine-induced increase of DA output in rat PFC, we examined its effect in control



**Fig. 6.** (a) The perfusion of 300  $\mu\text{M}$  clozapine in mPFC of control rats increased local DA output ( $n=10$ ). This effect was absent in the mPFC of rats whose GPCRs were silenced by a prior EEDQ injection (6 mg/kg i.p.) and their prefrontal 5-HT<sub>2A/2C</sub>Rs had been protected by ritanserin (300  $\mu\text{M}$ ) administration through the microdialysis probe ( $n=5$ ) (see Methods section). (b) Conversely, clozapine administration (300  $\mu\text{M}$ ) elicited a significant DA elevation in mPFC of rats treated with EEDQ whose 5-HT<sub>1A</sub>Rs were preserved by prior WAY-100635 (300  $\mu\text{M}$ ) administration ( $n=6$ ). This effect was significantly greater than in control rats ( $n=10$ ). Data are expressed as mean  $\pm$  S.E.M. See Results section for statistical analysis.

rats and in rats whose GPCRs were previously inactivated by EEDQ together with a selective protection of 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors in mPFC (Amargós-Bosch *et al.* 2004, see Methods section).

Clozapine perfusion (300  $\mu\text{M}$ ) increased the DA output in control rats ( $n=10$ ) to  $170 \pm 14\%$  of baseline but to a much lower extent (a transient increase to  $121 \pm 24\%$  of baseline) in rats with preserved 5-HT<sub>2A/2C</sub>Rs ( $n=5$ ) (Fig. 6a). Two-way ANOVAs revealed a significant group effect [ $F(1, 13)=9.41$ ,  $p<0.009$ ], time effect [ $F(15, 195)=3.98$ ,  $p<0.0001$ ] and time  $\times$  group interaction [ $F(15, 195)=3.23$ ,  $p<0.0001$ ].

We also tested the role of mPFC 5-HT<sub>1A</sub>Rs in the clozapine-induced DA release in rats using the EEDQ

model. 5-HT<sub>1A</sub>Rs were unilaterally protected in rat mPFC by local administration of WAY-100635 (300  $\mu\text{M}$ ) during EEDQ treatment (see Methods section). In this group of rats, clozapine (300  $\mu\text{M}$ ) elicited a significant DA elevation ( $299 \pm 57\%$  of baseline,  $n=6$ ) which was greater than in control rats ( $170 \pm 14\%$  of baseline,  $n=10$ ; see above) (Fig. 6b). Two-way ANOVAs revealed a significant effect of group [ $F(1, 14)=8.17$ ,  $p<0.01$ ], time [ $F(15, 210)=14.08$ ,  $p<0.0001$ ] and time  $\times$  group interaction [ $F(15, 210)=3.51$ ,  $p<0.0001$ ].

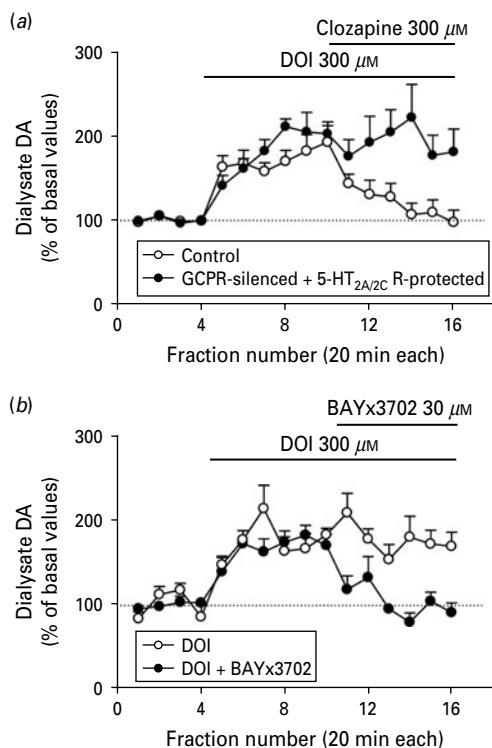
Previous studies have shown that clozapine reversed the increase of cortical 5-HT release induced by DOI (Bortolozzi *et al.* 2003). Since DOI also elevates DA release in mPFC by a 5-HT<sub>2A</sub>R-dependent mechanism (Bortolozzi *et al.* 2005), we examined whether clozapine was able to counteract the increase in DA output induced by DOI, despite its ability to increase DA output by itself. DOI (300  $\mu\text{M}$ ) was locally administered in the mPFC of control rats and of EEDQ-treated rats with protected 5-HT<sub>2A/2C</sub>Rs ( $n=6$  and 8, respectively). DOI increased DA output in control rats and in those treated with EEDQ (with protected 5-HT<sub>2A/2C</sub>Rs). Two-way ANOVAs revealed a significant effect of time [time effect:  $F(9, 108)=18.88$ ,  $p<0.0001$ ; non-significant effects of the group or time  $\times$  group interactions; fractions 1–10] (Fig. 7a). The co-perfusion of clozapine (300  $\mu\text{M}$ ) reversed the effect of DOI on DA output in the mPFC of control rats but not of those treated with EEDQ with protected 5-HT<sub>2A/2C</sub>Rs [group effect:  $F(1, 12)=11.58$ ,  $p<0.005$ ; time effect:  $F(15, 180)=8.87$ ,  $p<0.0001$ ; time  $\times$  group interaction:  $F(15, 180)=3.69$ ,  $p<0.0001$ ; fractions 1–16] suggesting that this effect does not involve blockade of 5-HT<sub>2A</sub>Rs.

To examine the involvement of 5-HT<sub>1A</sub>Rs in the clozapine-mediated reversal of DOI action on PFC DA output, we conducted additional experiments in which we evaluated the ability of the 5-HT<sub>1A</sub> agonist BAY  $\times$  3702 to antagonize the DOI-mediated DA increase. The local perfusion of 30  $\mu\text{M}$  BAY  $\times$  3702 reversed the DA elevation in PFC induced by local DOI administration [group effect:  $F(1, 7)=31.60$ ,  $p<0.0008$ ; time effect:  $F(15, 105)=7.26$ ,  $p<0.0001$ ; time  $\times$  group interactions:  $F(15, 105)=3.04$ ,  $p<0.0001$ ] (Fig. 7b).

#### Autoradiographic examination of 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptors in the GPCR-silencing model

We performed additional autoradiographic experiments in rats not subjected to drug infusion (except for WAY-100635 or ritanserin administration during EEDQ treatment) to determine the site and extent of 5-HT<sub>1A</sub>R and 5-HT<sub>2A/2C</sub>R protection in the





**Fig. 7.** (a) The perfusion of 300  $\mu\text{M}$  DOI (5-HT<sub>2A/2C</sub> agonist) increased DA output in the mPFC of control rats and of rats treated with EEDQ and whose mPFC 5-HT<sub>2A/2C</sub>Rs were unilaterally preserved by prior ritanserin administration. The perfusion of clozapine (300  $\mu\text{M}$ ) reversed the effect of DOI only in control rats ( $n=6-8$ ) but not in those with protected 5-HT<sub>2A/2C</sub>R. (b) In control rats, the local perfusion of BAY x 3702 (30  $\mu\text{M}$ ) antagonized the increase of DA output in mPFC induced by the local administration of DOI (300  $\mu\text{M}$ ) ( $n=5$ ). Data are expressed as mean  $\pm$  S.E.M. See Results section for statistical analysis.

EEDQ-treated rats. EEDQ evoked a massive reduction of 5-HT<sub>1A</sub>R and 5-HT<sub>2A/2C</sub>R density (Figs. 8 and 9).

Local, unilateral ritanserin perfusion by reverse dialysis in the mPFC partially avoided 5-HT<sub>2A/2C</sub>R inactivation by EEDQ. Densities of 5-HT<sub>2A/2C</sub>R-binding sites in the ipsilateral (protected) mPFC at AP coordinates (in mm) 3.20–3.70 ranged from 61% to 83% (mean  $71 \pm 4\%$ ,  $n=4$  rats) relative to mPFC of control rats in the same hemisphere ( $n=2$ ). In the contralateral (unprotected) side, 5-HT<sub>2A/2C</sub>R-binding sites were  $31 \pm 2\%$  ( $n=4$  rats) relative to the mPFC in the same hemisphere of control rats ( $n=2$ ). One-way ANOVA indicated that [<sup>3</sup>H]mesulergine binding in ipsilateral mPFC of the GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected group was significantly different from contralateral mPFC ( $p < 0.001$ ) (Fig. 8a, b). Coronal sections from an AP coordinate distant from local ritanserin

administration (e.g. 4.20 mm), revealed a marginally significant difference between ipsilateral and contralateral sides ( $68 \pm 3$  vs.  $43 \pm 3\%$  relative to PFC in the same hemispheres of control rats, respectively,  $p=0.058$ ).

Figure 9a shows autoradiograms of 5-HT<sub>1A</sub>Rs in PFC at AP 3.20–3.70 mm from different groups of rats: control ( $n=2$ ), GPCR-silenced ( $n=2$ ), GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected ( $n=4$ ) and GPCR-silenced + 5-HT<sub>1A</sub>R-protected ( $n=4$ ). WAY-100635 perfusion partially protected 5-HT<sub>1A</sub>Rs from inactivation by EEDQ. One-way ANOVA indicated that [<sup>3</sup>H]8-OH-DPAT binding in ipsilateral PFC of the GPCR-silenced + 5-HT<sub>1A</sub>R-protected group was significantly different from contralateral PFC ( $51 \pm 7$  vs.  $17 \pm 4\%$  relative to ipsilateral and contralateral cortices of control rats, respectively,  $p < 0.001$ ) (Fig. 9b). In this case, 5-HT<sub>1A</sub>R density was also significantly different between both ipsilateral and contralateral mPFC at AP  $\sim 4.20$  mm of GPCR-silenced + 5-HT<sub>1A</sub>R-protected rats (data not shown).

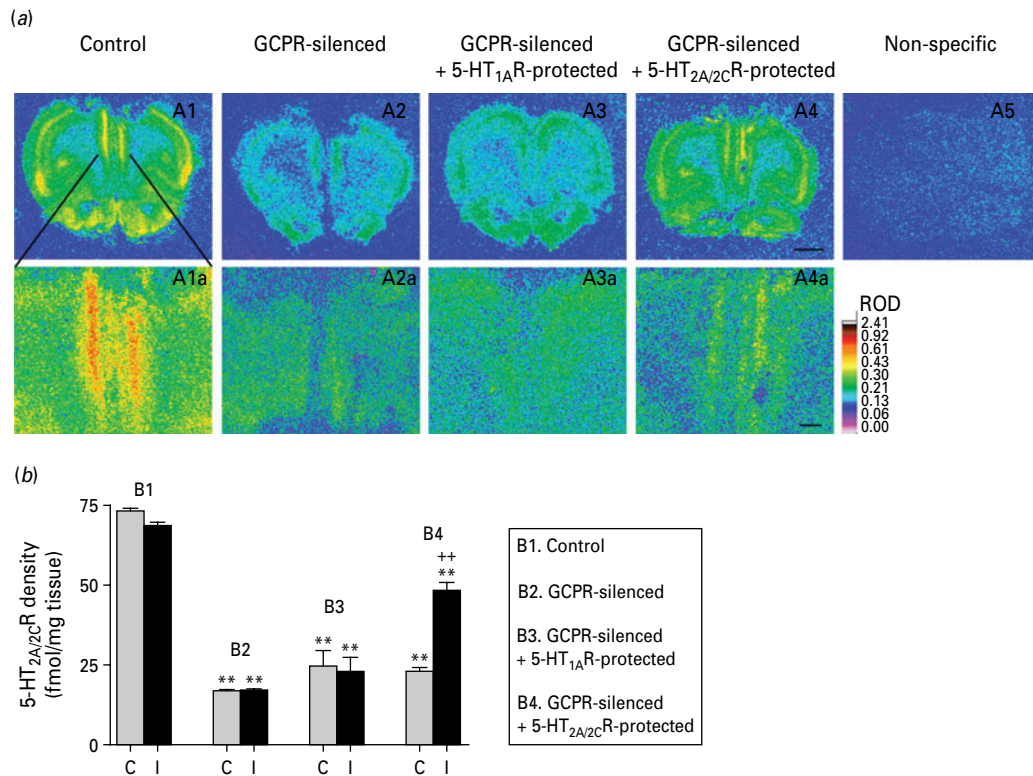
## Discussion

The main finding of the present study is that APDs such as clozapine, olanzapine and risperidone do not require interaction with 5-HT<sub>2A</sub>Rs to elevate DA release in rodent mPFC. This observation is relevant to understanding the neurobiological basis of the superior therapeutic action of these APDs in schizophrenia (Leucht *et al.* 2009) and may help to develop new drugs overcoming the limitations of existing treatments.

### Methodological considerations

Two experimental models have been used in the present study: (a) mice lacking 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptors, and (b) rats, whose GPCRs were inactivated by EEDQ using selective protection of 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors.

The lack of 5-HT<sub>1A</sub>Rs in KO mice (Parks *et al.* 1998) was assessed by receptor autoradiography, electrophysiology and microdialysis (Amargós-Bosch *et al.* 2004; present study). Here we extend these observations to 5-HT<sub>2A</sub>R KO mice (Fiorica-Howells *et al.* 2002). A preliminary account of these data has been presented previously (Bortolozzi *et al.* 2007a). We show the absence of compensatory changes of 5-HT<sub>1A</sub>R and 5-HT<sub>2C</sub>R proteins in mice lacking 5-HT<sub>2A</sub>Rs, similarly to Popa *et al.* (2005) who reported an unaltered 5-HT<sub>2C</sub>R mRNA expression in 5-HT<sub>2A</sub>R KO mice. Consistent with the autoradiographic data, the preferential



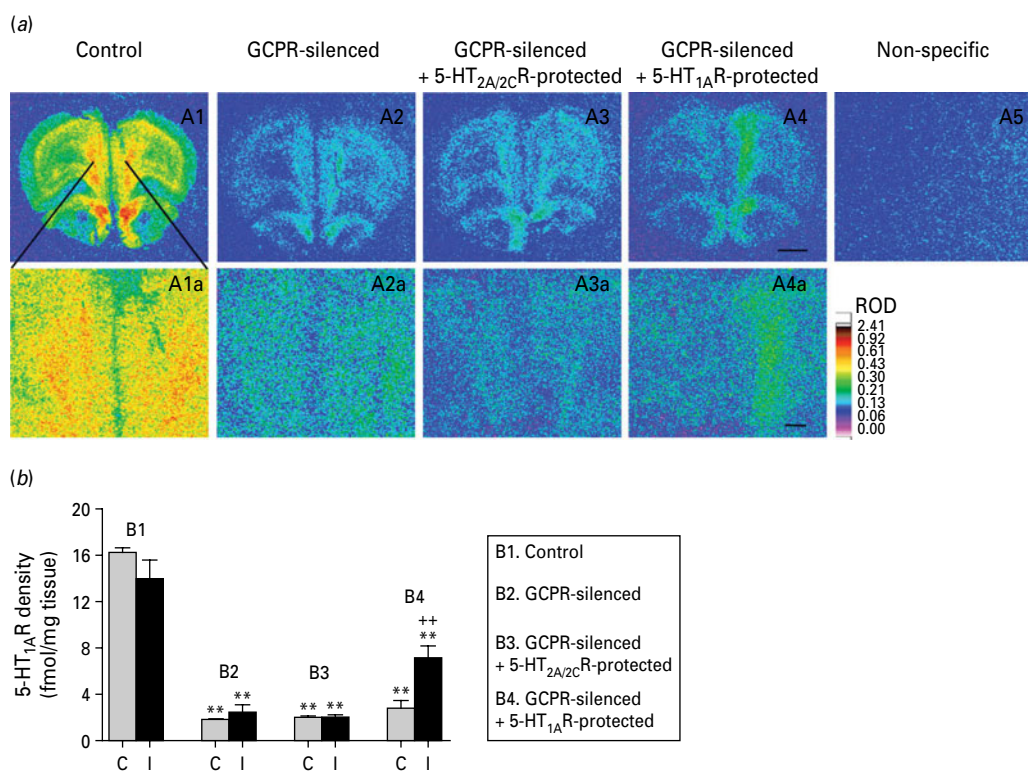
**Fig. 8.** (a) Representative autoradiograms showing the density of 5-HT<sub>2A/2C</sub>Rs labelled with [<sup>3</sup>H]mesulergine in coronal sections of PFC (AP in mm: 3.70–3.20) from different pretreatment groups: (A1) controls (rats received the EEDQ vehicle i.p. and aCSF through the dialysis probe), (A2) GPCR-silenced rats (injected with 6 mg/kg i.p. EEDQ and perfused with aCSF through the dialysis probe), (A3) GPCR-silenced + 5-HT<sub>1A</sub>R-protected rats (treated with 6 mg/kg i.p. EEDQ while 300 μM WAY-100635 was perfused through the dialysis probe), and (A4) GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected rats (treated with 6 mg/kg i.p. EEDQ while 300 μM ritanserin was perfused through the dialysis probe). A5 shows non-specific binding. Panels A1a–A4a are photomicrographs showing enlargements of the marked area in panels A1–A4. Note the higher 5-HT<sub>2A/2C</sub>R binding in ipsilateral (protected) mPFC with respect to the contralateral (unprotected) side of panel A4 and the very low occupancy for 5-HT<sub>2A/2C</sub>R in both hemispheres of panels A2 and A3. Scale bars, 2 mm (A1–A4) and 500 μm (A1a–A4a). (b) Densitometric quantification of 5-HT<sub>2A/2C</sub>R binding in mPFC including cingulate, prelimbic and infralimbic cortices of the different group of rats (B1–B4). Bars represent mean 5-HT<sub>2A/2C</sub>R fmol/mg tissue ± S.E.M. of 4–8 observations (two or four observations at left and right hemispheres of two consecutive sections per animal and two to four animals per group). \*\* *p* < 0.001 significantly different from corresponding contralateral (C) and ipsilateral (I) mPFC of control rats, ++ *p* < 0.001 significantly different from contralateral mPFC of GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected rats, using one-way ANOVA followed by Newman–Keuls *post-hoc* test.

5-HT<sub>2A</sub>R agonist DOI did not increase 5-HT and DA release in the mPFC of 5-HT<sub>2A</sub>R KO mice, an effect requiring the activation of post-synaptic 5-HT<sub>2A</sub>R on pyramidal cells projecting to the midbrain monoaminergic nuclei (Bortolozzi et al. 2005; Martín-Ruiz et al. 2001; Vázquez-Borsetti et al. 2009).

To examine the role of 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptors in rat PFC, we used a previously described model (Amargós-Bosch et al. 2004), consisting in the selective protection of one or other receptor from the inactivating action of EEDQ (Battaglia et al. 1987; Gozlan et al. 1994; Keck & Lakoski, 2000) through the local administration of antagonists (WAY-100635

or ritanserin, respectively) to occupy 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors in mPFC during EEDQ treatment. This model is far from the specificity of KO mice yet it allows for a preliminary examination of the involvement of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors on the effects of APDs in the rat brain.

The present autoradiographic data indicate that (1) EEDQ produces a massive loss of 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptors *in vivo*, and (2) the local protection of 5-HT<sub>1A</sub> or 5-HT<sub>2A/2C</sub> receptors by the respective antagonists was relatively successful, as shown by differences in receptor density between (a) ipsilateral (protected) and contralateral (unprotected) mPFCs,



**Fig. 9.** (a) Representative autoradiograms showing the density of 5-HT<sub>1A</sub>Rs labelled with [<sup>3</sup>H]8-OH-DPAT in coronal sections of PFC (AP in mm: 3.70–3.20) from different pretreatment groups: (A1) controls (rats received the EEDQ vehicle i.p. and aCSF through the dialysis probe), (A2) GPCR-silenced rats (injected with 6 mg/kg i.p. EEDQ and perfused with aCSF through the dialysis probe), (A3) GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected rats (injected with 6 mg/kg i.p. EEDQ while 300  $\mu$ M ritanserin was perfused through the dialysis probe), and (A4) GPCR-silenced + 5-HT<sub>1A</sub>R-protected rats (injected with 6 mg/kg i.p. EEDQ while 300  $\mu$ M WAY-100635 was perfused through the dialysis probe). A5 shows non-specific binding. Panels A1a–A4a are photomicrographs showing enlargements of the marked area in panels A1–A4. Note the higher 5-HT<sub>1A</sub>R binding in ipsilateral (protected) mPFC with respect to the contralateral (unprotected) side of A4 and the very low occupancy for 5-HT<sub>1A</sub>Rs in both hemispheres of panels A2 and A3. Scale bars, 2 mm (A1–A4) and 500  $\mu$ m (A1a–A4a). (b) Densitometric quantification of 5-HT<sub>1A</sub>R binding in mPFC including cingulate, prelimbic and infralimbic cortices of the different group of rats (B1–B4). Bars represent mean 5-HT<sub>1A</sub>R fmol/mg tissue  $\pm$  S.E.M. of 4–8 observations (two or four observations at left and right hemispheres of two consecutive sections per animal and two to four animals per group). \*\*  $p < 0.001$  significantly different from corresponding contralateral (C) and ipsilateral (I) mPFC of control rats, ++  $p < 0.001$  significantly different from contralateral mPFC of GPCR-silenced + 5-HT<sub>1A</sub>R-protected rats, using one-way ANOVA followed by Newman–Keuls *post-hoc* test.

and (b) the ipsilateral side in EEDQ-treated and control rats. The fact that receptor densities in protected sides were lower than in control rats may be partly due to the damage caused by the dialysis probe, which forced us to use coronal sections relatively distant from the administration site, and thus, with lower antagonist occupancy than sites close to the microdialysis probes receiving a higher antagonist concentration.

5-HT<sub>1A</sub>Rs have a great sensitivity to EEDQ *in vitro* (Gozlan *et al.* 1994). The present *in-vivo* data are consistent with this view, since EEDQ reduced 5-HT<sub>1A</sub> and 5-HT<sub>2A/2C</sub> receptor densities to 15% and 30% of controls, respectively. Similar differences have been noted for DA D<sub>1</sub> and D<sub>2</sub> receptors (see Cox &

Waszczak, 1993; Hemsley & Crocker, 2001 and references therein). Interestingly, despite measured receptor densities in protected sides being lower than 100% of controls, DOI increased DA release to the same extent in the mPFC of control rats and of those receiving EEDQ + ritanserin, indicating that local 5-HT<sub>2A/2C</sub>Rs remained entirely functional using this experimental paradigm. Similar results have been reported for 5-HT<sub>1A</sub>R agonists (Amargós-Bosch *et al.* 2004).

#### *Role of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the APD-induced DA release*

Despite the diverse pharmacological profiles of APDs (Arnt & Skarsfeldt, 1998), they share the ability



to increase DA release in rodent mPFC through 5-HT<sub>1A</sub>R activation (Bortolozzi et al. 2007b; Díaz-Mataix et al. 2005; Ichikawa et al. 2001; Li et al. 2009; Rollema et al. 1997, 2000). This effect was attributed to simultaneous blockade of 5-HT<sub>2A</sub> and D<sub>2</sub> receptors (Ichikawa et al. 2001) yet it seems to depend exclusively on the activation of 5-HT<sub>1A</sub>Rs in mPFC (Bortolozzi et al. 2007b; Díaz-Mataix et al. 2005). In the present study, we further confirm these previous observations in 5-HT<sub>1A</sub>R KO mice and show that 5-HT<sub>2A</sub>R blockade is not a requirement. Further, we extend these observations to rat mPFC, where clozapine increased local DA release in presence of ~50% of mPFC 5-HT<sub>1A</sub>Rs but not in rats whose 5-HT<sub>1A</sub>Rs were inactivated to 15% of controls by EEDQ treatment. The greater DA increase induced by clozapine in rats whose GPCRs were silenced by EEDQ – yet with preserved 5-HT<sub>1A</sub>Rs – suggests an additional regulatory role of other receptors in the clozapine-evoked DA release (e.g. DA D<sub>2</sub>,  $\alpha_2$ -adrenoceptors) once the DA increase has been induced by 5-HT<sub>1A</sub>R stimulation. It has been suggested that WAY-100635 may also bind to DA D<sub>4</sub> receptors in addition to 5-HT<sub>1A</sub>Rs (Chemel et al. 2006; Martel et al. 2007) and therefore, some protection for D<sub>4</sub> receptors may exist in the EEDQ + WAY-100635 model. Thus, it cannot be excluded that D<sub>4</sub> receptors play a role in the clozapine-induced cortical DA release using this model in rat mPFC despite its effects being totally absent in 5-HT<sub>1A</sub>R KO mice (Díaz-Mataix et al. 2005; present study).

The apparent bell-shaped dose–effect relationship of olanzapine on DA release (Fig. 5b) suggests the involvement of other prefrontal monoaminergic receptors (e.g.  $\alpha_1$ -adrenoceptors; Amargós-Bosch et al. 2003) for which olanzapine shows nm affinity (Arnt & Skarsfeldt, 1998).

The similar *in-vivo* DA increases in PFC produced by several APDs (Díaz-Mataix et al. 2005; Ichikawa et al. 2001), does not bear a relationship with their *in-vitro* affinities for 5-HT<sub>1A</sub>Rs, e.g. high for ziprasidone, low for clozapine and risperidone (yet clozapine occupies 5-HT<sub>1A</sub>Rs *in vivo*; Chou et al. 2003) or negligible for olanzapine (Arnt & Skarsfeldt, 1998; Bymaster et al. 1996; Newman-Tancredi et al. 1998). The exact way by which APDs lacking *in-vitro* affinity interact *in vivo* with 5-HT<sub>1A</sub>R-mediated neurotransmission is unclear. The DA output induced by APDs and 5-HT<sub>1A</sub> agonists was cancelled by co-perfusion with the GABA<sub>A</sub> antagonist bicuculline, suggesting the involvement of 5-HT<sub>1A</sub>Rs in GABA interneurons (Díaz-Mataix et al. 2005). Given the inhibitory nature of 5-HT<sub>1A</sub>Rs, a preferential action of APDs on 5-HT<sub>1A</sub>Rs located on GABA interneurons would eventually result in an

increased excitatory cortical output to the VTA to enhance DA neuron activity (Gessa et al. 2000), an effect qualitatively similar to that of selective 5-HT<sub>1A</sub> agonists (Díaz-Mataix et al. 2005, 2006).

The increase in mPFC DA release produced by APDs might theoretically result from an interaction between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in neurons co-expressing these receptors (Amargós-Bosch et al. 2004). Thus, 5-HT<sub>2A</sub>R blockade by APDs might alter the physiological balance between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, resulting in an increase of 5-HT<sub>1A</sub>R-mediated neurotransmission. However, the present data do not support this possibility, since the APDs clozapine, olanzapine and risperidone increased a similar DA release in the mPFC of mice lacking 5-HT<sub>2A</sub>Rs (with no alteration of 5-HT<sub>1A</sub>R density) and in WT controls. These results in mice were confirmed by rat data showing that clozapine was ineffective in enhancing DA output in the mPFC of rats whose 5-HT<sub>1A</sub>Rs were inactivated (~15% of controls), yet whose 5-HT<sub>2A/2C</sub>Rs were protected (~70% of controls).

Interestingly, and despite its ability to stimulate DA release when given alone, clozapine counteracted the increase in PFC DA output induced by DOI, suggesting a different effect in PFC in basal or stimulated conditions. This pattern is similar to that observed in some electrophysiological studies, where clozapine displays a state-dependent action, reducing neuronal hyperactivity (e.g. Homayoun & Moghaddam, 2007; Kargieman et al. 2007; Schwieler & Erhardt, 2003). Thus, clozapine would activate the mesocortical DA system from basal conditions but would dampen cortical hyperactivity.

Clozapine could not reverse the effect of DOI in GPCR-silenced + 5-HT<sub>2A/2C</sub>R-protected rats. In this group, 5-HT<sub>2A</sub>Rs were entirely functional, as indicated by the local effect of DOI on DA output, comparable to that in controls. The inability of clozapine to counteract DOI's effect in these rats suggests the involvement of other receptors, different from 5-HT<sub>2A</sub>Rs, to reverse the action of DOI in control rats. Although we could not perform a systematic study, the comparable effect of clozapine and the selective 5-HT<sub>1A</sub>R agonist BAY x 3702 suggests the involvement of 5-HT<sub>1A</sub>Rs.

Overall, these findings indicate that the stimulation of mesocortical DA release by APDs does not require the presence of 5-HT<sub>2A</sub>Rs and suggest that these drugs activate 5-HT<sub>1A</sub>Rs to enhance cortical DA neurotransmission. Alternatively, it is conceivable that other receptor–receptor interactions might explain this marked discrepancy between *in-vitro* and *in-vivo* actions of APDs at 5-HT<sub>1A</sub>Rs.



### Functional consequences

The present data, obtained in mice and rats using two different experimental models (permanent 5-HT receptor KO mice and GPCR inactivation with selective protection of 5-HT receptors) indicate that blockade of 5-HT<sub>2A</sub>Rs by APDs is not a necessary step to elevate DA release and that this effect is mediated by indirect activation of 5-HT<sub>1A</sub>Rs in PFC. The molecular/cellular basis of the present *in-vivo* results is not known.

These observations do not preclude at all that 5-HT<sub>2A</sub>R blockade by APDs participates in their therapeutic action. Our conclusions are restricted to the role of 5-HT receptors required to enhance mesocortical DA, an effect potentially important for the actions of APDs on negative symptoms and cognitive deficits of schizophrenia patients. Given the lack of adequate treatment of these problems, further detailed studies are required to examine the ability of APDs – and in particular, of clozapine – to stimulate cortical 5-HT<sub>1A</sub>R-mediated neurotransmission despite their low or negligible *in-vitro* affinity.

### Acknowledgements

This work was supported by grants SAF 2007-62378 and SENY Fundació. A.B. is recipient of a Ramón y Cajal contract from MICINN-IDIBAPS. M.M. is a recipient of a predoctoral fellowship from CSIC (I3P program). We thank the pharmaceutical companies for drug supply. We also thank Leticia Campa for her skilful maintenance of HPLC equipment and dialysate analyses. We acknowledge the skilful technical assistance of Mrs Verónica Paz. We thank Dr M. Hamon and Dr L. Lanfumey (Paris, France) for their help with 5-HT<sub>2A</sub>R KO mice.

### Statement of Interest

None.

### References

- Aghajanian GK, Marek GJ (1997). Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. *Neuropharmacology* **36**, 589–599.
- Akil M, Pierri JN, Whitehead RE, Edgar CL, et al. (1999). Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *American Journal of Psychiatry* **156**, 1580–1589.
- Amargós-Bosch M, Bortolozzi A, Adell A, Artigas F (2003). Stimulation of  $\alpha_1$ -adrenoceptors in the rat medial prefrontal cortex increases the local *in vivo* 5-hydroxytryptamine release. *Reversal by antipsychotics drugs. Journal of Neurochemistry* **87**, 831–842.
- Amargós-Bosch M, Bortolozzi A, Puig MV, Serrats J, et al. (2004). Co-expression and *in vivo* interaction of serotonin1A and serotonin2A receptors in pyramidal neurons of prefrontal cortex. *Cerebral Cortex* **14**, 281–299.
- Araneda R, Andrade R (1991). 5-Hydroxytryptamine-2 and 5-hydroxytryptamine1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* **40**, 399–412.
- Arnt J, Skarsfeldt T (1998). Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology* **18**, 63–101.
- Artaloytia JF, Arango C, Lahti A, Sanz J, et al. (2006). Negative signs and symptoms secondary to antipsychotics: a double-blind, randomized trial of a single dose of placebo, haloperidol, and risperidone in healthy volunteers. *American Journal of Psychiatry* **163**, 488–493.
- Ashby CR, Edwards E, Wang RY (1994). Electrophysiological evidence for a functional interaction between 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the rat medial prefrontal cortex: an iontophoretic study. *Synapse* **17**, 173–181.
- Assié MB, Ravailhe V, Faucillon V, Newman-Tancredi A (2005). Contrasting contribution of 5-hydroxytryptamine1A receptor activation to neurochemical profile of novel antipsychotics: frontocortical dopamine and hippocampal serotonin release in rat brain. *Journal of Pharmacology and Experimental Therapeutics* **315**, 265–272.
- Bantick RA, Deakin JF, Grasby PM (2001). The 5-HT<sub>1A</sub> receptor in schizophrenia: a promising target for novel atypical neuroleptics? *Journal of Psychopharmacology* **15**, 37–46.
- Battaglia G, Norman AB, Creese I (1987). Differential serotonin2 receptor recovery in mature and senescent rat brain after irreversible receptor modification: effect of chronic reserpine treatment. *Journal of Pharmacology and Experimental Therapeutics* **243**, 69–75.
- Bonaccorso S, Meltzer HY, Li Z, Dai J, et al. (2002). SR46349-B, a 5-HT<sub>2A/2C</sub> receptor antagonist, potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. *Neuropsychopharmacology* **27**, 430–441.
- Bortolozzi A, Amargós-Bosch M, Adell A, Díaz-Mataix L, et al. (2003). *In vivo* modulation of 5-hydroxytryptamine release in mouse prefrontal cortex by local 5-HT<sub>2A</sub> receptors: effect of antipsychotic drugs. *European Journal of Neuroscience* **18**, 1235–1246.
- Bortolozzi A, Díaz-Mataix L, Cortés R, Scorza C, et al. (2007a). Clozapine increases dopamine output in medial prefrontal cortex by a 5-HT<sub>2A</sub> receptor-independent mechanism. *Society for Neuroscience*, Abstract 499.12.
- Bortolozzi A, Díaz-Mataix L, Scorza MC, Celada P, et al. (2005). The activation of 5-HT<sub>2A</sub> receptors in prefrontal cortex enhances dopaminergic activity. *Journal of Neurochemistry* **95**, 1597–1607.
- Bortolozzi A, Díaz-Mataix L, Toth M, Celada P, et al. (2007b). *In vivo* actions of aripiprazole on serotonergic and dopaminergic systems in rodent brain. *Psychopharmacology (Berlin)* **191**, 745–758.

- Braff DL** (1993). Information processing and attention dysfunctions in schizophrenia. *Schizophrenia Bulletin* **19**, 233–259.
- Bubenikova-Valesova V, Stuchlik A, Svoboda J, Bures J, et al.** (2008). Risperidone and ritanserin but not haloperidol block effect of dizocilpine on the active allothetic place avoidance task. *Proceedings of National Academic of Sciences USA* **105**, 1061–1066.
- Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, et al.** (1996). Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology* **14**, 87–96.
- Carr DB, Sesack SR** (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *Journal of Neuroscience* **20**, 3864–3873.
- Chemel BR, Roth BL, Armbruster B, Watts VJ, et al.** (2006). WAY-100635 is a potent dopamine D4 receptor agonist. *Psychopharmacology (Berlin)* **188**, 244–251.
- Chou YH, Halldin C, Farde L** (2003). Occupancy of 5-HT<sub>1A</sub> receptors by clozapine in the primate brain: a PET study. *Psychopharmacology (Berlin)* **166**, 234–240.
- Chung YC, Li Z, Dai J, Meltzer HY, et al.** (2004). Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: role of 5-HT<sub>1A</sub> receptor agonism. *Brain Research* **1023**, 54–63.
- Cox RF, Waszczak BL** (1993). Inhibition of substantia nigra dopamine cell firing by R(-)-N-n-propylnorapomorphine: electrophysiological and autoradiographic studies after regional inactivation of dopamine receptors by microinjection of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. *Brain Research* **613**, 32–42.
- Díaz-Mataix L, Artigas F, Celada P** (2006). Activation of pyramidal cells in rat medial prefrontal cortex projecting to ventral tegmental area by a 5-HT<sub>1A</sub> receptor agonist. *European of Neuropsychopharmacology* **16**, 288–296.
- Díaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, et al.** (2005). Involvement of 5-HT<sub>1A</sub> receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical antipsychotic action. *Journal of Neuroscience* **25**, 10831–10843.
- Elsworth JD, Jentsch JD, Morrow BA, Redmond Jr. DE, et al.** (2008). Clozapine normalizes prefrontal cortex dopamine transmission in monkeys subchronically exposed to phencyclidine. *Neuropsychopharmacology* **33**, 491–496.
- Elvevag B, Goldberg TE** (2000). Cognitive impairment in schizophrenia is the core of the disorder. *Critical Reviews in Neurobiology* **14**, 1–21.
- Fiorica-Howells E, Hen R, Gingrich J, Li Z, et al.** (2002). 5-HT<sub>2A</sub> receptors: location and functional analysis in intestines of wild-type and 5-HT<sub>2A</sub> knockout mice. *American Journal of Physiology, Gastrointestinal and Liver Physiology* **282**, G877–993.
- Franklin KBJ, Paxinos G** (1997). *The Mouse Brain in Stereotaxic Coordinates*. San Diego, USA: Academic Press.
- Gessa GL, Devoto P, Diana M, Flore G, et al.** (2000). Dissociation of haloperidol, clozapine, and olanzapine effects on electrical activity of mesocortical dopamine neurons and dopamine release in the prefrontal cortex. *Neuropsychopharmacology* **22**, 642–649.
- Gobert A, Millan MJ** (1999). Serotonin (5-HT)<sub>2A</sub> receptor activation enhances dialysate levels of dopamine and noradrenaline, but not 5-HT, in the frontal cortex of freely-moving rats. *Neuropharmacology* **38**, 315–317.
- Gozlan H, Laporte AM, Thibault S, Schechter LE, et al.** (1994). Differential effects of N-ethoxycarbonyl-2-ethoxy-12-dihydroquinoline (EEDQ) on various 5-HT receptor binding sites in the rat brain. *Neuropharmacology* **33**, 423–431.
- Grayson B, Idris NF, Neill JC** (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behavioural Brain Research* **184**, 31–38.
- Green MF, Kern RS, Braff DL, Mintz J** (2000). Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the ‘right stuff’? *Schizophrenia Bulletin* **26**, 119–136.
- Harvey PD, Sacchetti E, Galluzzo A, Romeo F, et al.** (2008). A randomized double-blind comparison of ziprasidone vs. clozapine for cognition in patients with schizophrenia selected for resistance or intolerance to previous treatment. *Schizophrenia Research* **105**, 138–143.
- Hemsley KM, Crocker AD** (2001). Changes in muscle tone are regulated by D1 and D2 dopamine receptors in the ventral striatum and D1 receptors in the substantia nigra. *Neuropsychopharmacology* **25**, 514–526.
- Homayoun H, Moghaddam B** (2007). Fine-tuning of awake prefrontal cortex neurons by clozapine: comparison with haloperidol and N-desmethylclozapine. *Biological Psychiatry* **61**, 679–687.
- Ichikawa J, Ishii H, Bonaccorso S, O’Laughlin IA, et al.** (2001). 5-HT<sub>2A</sub> and D2 receptor blockade increases cortical DA release via of 5-HT<sub>1A</sub> receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *Journal of Neurochemistry* **76**, 1521–1531.
- Kargieman L, Santana N, Mengod G, Celada P, et al.** (2007). Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine. *Proceeding of National Academic of Science USA* **104**, 14843–14848.
- Keck BJ, Lakoski JM** (2000). Regional heterogeneity of serotonin(1A) receptor inactivation and turnover in the aging female rat brain following EEDQ. *Neuropharmacology* **39**, 1237–1246.
- Kuroki T, Meltzer HY, Ichikawa J** (1999). Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics* **288**, 774–781.
- Leucht S, Corves C, Arbter D, Engel RR, et al.** (2009). Second-generation versus first generation antipsychotic drugs for schizophrenia: a meta-analysis. *Lancet* **373**, 31–41.

- Lewis DA, Lieberman JA (2000). Catching up on schizophrenia: natural history and neurobiology. *Neuron* **28**, 325–334.
- Li Z, Prus AJ, Dai J, Meltzer HY (2009). Differential effects of M1 and 5-HT<sub>1A</sub> receptors on atypical antipsychotics drug-induced dopamine efflux in the medial prefrontal cortex. *Journal of Pharmacology and Experimental Therapeutics* **330**, 948–955.
- Liégeois JF, Ichikawa J, Meltzer HY (2002). 5-HT<sub>2A</sub> receptor antagonism potentiates haloperidol-induced dopamine release in rat medial prefrontal cortex and inhibits that in the nucleus accumbens in a dose-dependent manner. *Brain Research* **947**, 157–165.
- López-Giménez JF, Mengod G, Palacios JM, Vilaró MT (1997). Selective visualization of rat brain 5-HT<sub>2A</sub> receptors by autoradiography with [<sup>3</sup>H]MDL 100,907. *Naunyn-Schmiedeberg's Archives of Pharmacology* **356**, 446–454.
- López-Giménez JF, Tecott LH, Palacios JM, Mengod G, et al. (2002). Serotonin 5-HT<sub>2C</sub> receptor knockout mice: autoradiographic analysis of multiple serotonin receptors. *Journal of Neuroscience Research* **67**, 69–85.
- Martel JC, Leduc N, Ormière AM, Faucillon V, et al. (2007). WAY-100635 has high selectivity for serotonin 5-HT<sub>1A</sub> versus dopamine D(4) receptors. *European Journal of Pharmacology* **574**, 15–19.
- Martín-Ruiz R, Puig MV, Celada P, Shapiro DA, et al. (2001). Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate dependent mechanism. *Journal of Neuroscience* **21**, 9856–9866.
- Meltzer HY, Matsubara S, Lee JC (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D1, D2 and serotonin 2 pKi values. *Journal of Pharmacology and Experimental Therapeutics* **251**, 238–246.
- Meltzer HY, Sumiyoshi T (2008). Does stimulation of 5-HT<sub>1A</sub> receptors improve cognition in schizophrenia. *Behavioral Brain Research* **195**, 98–102.
- Mengod G, Vilaró MT, Raurich A, López-Giménez JF, et al. (1996). 5-HT receptors in mammalian brain: receptor autoradiography and in situ hybridization studies of new ligands and newly identified receptors. *Histochemical Journal* **28**, 747–758.
- Millan MJ (2000). Improving the treatment of schizophrenia: focus on serotonin 5-HT<sub>1A</sub> receptors. *Journal of Pharmacology and Experimental Therapeutics* **295**, 853–861.
- Minabe Y, Hashimoto K, Watanabe KI, Ashby Jr. CR (2001). Acute and repeated administration of the selective 5-HT<sub>2A</sub> receptor antagonist M100907 significantly alters the activity of midbrain dopamine neurons: an in vivo electrophysiological study. *Synapse* **40**, 102–112.
- Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, et al. (1998). Agonist and antagonist actions of antipsychotic agents at 5-HT<sub>1A</sub> receptors: a [<sup>35</sup>S]GTPγS binding study. *European Journal of Pharmacology* **355**, 245–256.
- Nyberg S, Dencker SJ, Malm U, Dahl ML, et al. (1998). D2 and 5-HT<sub>2</sub> receptor occupancy in high-dose neuroleptic-treated patients. *International Journal of Neuropsychopharmacology* **1**, 95–101.
- Parks CL, Robinson PS, Sibille E, Shenk T, et al. (1998). Increased anxiety of mice lacking the 5-HT<sub>1A</sub> receptor. *Proceedings of National Academic of Sciences USA* **95**, 10734–10739.
- Pazos A, Cortés R, Palacios JM (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Research* **346**, 231–249.
- Paxinos G, Watson C (2005). *The Rat Brain in Stereotaxic Coordinates*, 5th edn. Sydney, Australia: Academic Press.
- Pehek EA, McFarlane HG, Maguschak K, Price B, et al. (2001). M100,907, a selective 5-HT<sub>2A</sub> antagonist, attenuates dopamine release in the rat medial prefrontal cortex. *Brain Research* **888**, 51–59.
- Popa D, Léna C, Fabre V, Prenat C, et al. (2005). Contribution of 5-HT<sub>2</sub> receptor subtypes to sleep-wakefulness and respiratory control, and functional adaptations in knock-out mice lacking 5-HT<sub>2A</sub> receptors. *Journal of Neuroscience* **25**, 11231–11238.
- Puig MV, Artigas F, Celada P (2005). Modulation of the activity of pyramidal neurons in rat prefrontal cortex by raphe stimulation in vivo: involvement of serotonin and GABA. *Cerebral Cortex* **15**, 1–14.
- Puig MV, Celada P, Díaz-Mataix L, Artigas F (2003). In vivo modulation of the activity of pyramidal neurons in the rat medial prefrontal cortex by 5-HT<sub>2A</sub> receptors: relationship to thalamocortical afferents. *Cerebral Cortex* **13**, 870–882.
- Rënyi L, Evenden JL, Fowler CJ, Jerning E, et al. (2001). The pharmacological profile of (R)-3,4-dihydro-N-isopropyl-3-(N-isopropyl-N-propylamino)-2H-1-benzopyran arboxamide, a selective 5-hydroxytryptamine(1A) receptor agonist. *Journal of Pharmacology and Experimental Therapeutics* **299**, 883–893.
- Rollema H, Lu Y, Schmidt AW, Sprouse JS, et al. (2000). 5-HT<sub>1A</sub> receptor activation contributes to ziprasidone-induced dopamine release in the rat prefrontal cortex. *Biological Psychiatry* **48**, 229–237.
- Rollema H, Lu Y, Schmidt AW, Zorn SH (1997). Clozapine increases dopamine release in prefrontal cortex by 5-HT<sub>1A</sub> receptor activation. *European Journal of Pharmacology* **338**, R3–R5.
- Roth BL, Sheffler D, Potkin SG (2003). Atypical antipsychotic drug actions: unitary or multiple mechanisms for 'atypicality'? *Clinical Neuroscience Research* **3**, 108–117.
- Santana N, Bortolozzi A, Serrats J, Mengod G, et al. (2004). Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. *Cerebral Cortex* **14**, 1100–1109.
- Schwieler L, Erhardt S (2003). Inhibitory action of clozapine on rat ventral tegmental area dopamine neurons following increased levels of endogenous kynurenic acid. *Neuropsychopharmacology* **28**, 1770–1777.
- Stockmeier CA, DiCarlo JJ, Zhang Y, Thompson P, et al. (1993). Characterization of typical and atypical antipsychotic drugs based on in vivo occupancy of

- serotonin<sub>2</sub> and dopamine<sub>2</sub> receptors. *Journal of Pharmacology and Experimental Therapeutics* **266**, 1374–1384.
- Sumiyoshi C, Sumiyoshi T, Roy A, Jayathilake K, et al.** (2006). Atypical antipsychotic drugs and organization of long-term semantic memory: multidimensional scaling and cluster analyses of category fluency performance in schizophrenia. *International Journal of Neuropsychopharmacology* **9**, 677–683.
- Sumiyoshi T, Matsui M, Nohara S, Yamashita I, et al.** (2001a). Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *American Journal of Psychiatry* **158**, 1722–1725.
- Sumiyoshi T, Matsui M, Yamashita I, Nohara S, et al.** (2001b). The effect of tandospirone, a serotonin<sub>1A</sub> agonist, on memory function in schizophrenia. *Biological Psychiatry* **49**, 861–868.
- Yasuno F, Suhara T, Nakayama T, Ichimiya T, et al.** (2003). Inhibitory effect of hippocampal 5-HT<sub>1A</sub> receptors on human explicit memory. *American Journal of Psychiatry* **160**, 334–340.
- Youngren KD, Inglis FM, Pivrotto PJ, Jedema HP, et al.** (1999). Clozapine preferentially increases dopamine release in the rhesus monkey prefrontal cortex compared with the caudate nucleus. *Neuropsychopharmacology* **20**, 403–412.
- Vázquez-Borsetti P, Cortés R, Artigas F** (2009). Pyramidal neurons in rat prefrontal cortex projecting to ventral tegmental area and dorsal raphe nucleus express 5-HT<sub>2A</sub> receptors. *Cerebral Cortex* **19**, 1678–1686.
- Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, et al.** (2007). Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nature Neuroscience* **10**, 376–384.
- Weickert TW, Goldberg TE, Gold JM, Bigelow LB, et al.** (2000). Cognitive impairments in patients with schizophrenia displaying preserved and compromised intellect. *Archives of General Psychiatry* **57**, 907–913.
- Williams GV, Goldman-Rakic PS** (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* **376**, 572–575.
- Woodward ND, Purdon SE, Meltzer HY, Zal DH** (2005). A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. *International Journal of Neuropsychopharmacology* **8**, 457–472.