

**CONTRASTING CONTRIBUTION OF 5-HT<sub>1A</sub> RECEPTOR ACTIVATION TO  
NEUROCHEMICAL PROFILE OF NOVEL ANTIPSYCHOTICS:  
FRONTOCORTICAL DOPAMINE AND HIPPOCAMPAL SEROTONIN RELEASE  
IN RAT BRAIN**

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Novel 5-HT<sub>1A</sub> antipsychotics: rat dopamine and 5-HT release

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Abbreviations: HPLC-EC, high pressure liquid chromatography with electrochemical detection; PET, positron emission tomography; EPS, extrapyramidal syndrome; (+)8-OH-DPAT, (+)-8-hydroxy-2-(di-n-propylamino)tetralin; SLV313, 1-(2,3-dihydrobenzo[1,4]dioxin-5-yl)-4-[5-(4-fluorophenyl)-pyridin-3-ylmethyl]-piperazine; SSR181507, (3-exo)-8-benzoyl-N-(((2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl)methyl)-8-azabicyclo(3.2.1)octane-3-methanamine; WAY100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide

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## Abstract

Several novel antipsychotics, such as aripiprazole, bifeprunox, SSR181507 and SLV313, activate serotonin 5-HT<sub>1A</sub> receptors. Such activity is associated with enhanced treatment of negative symptoms and cognitive deficits, which may be mediated by modulation of cerebral dopamine and serotonin levels. We employed microdialysis coupled to HPLC-EC, to examine 5-HT<sub>1A</sub> receptor activation in the modulation of extracellular dopamine in medial prefrontal cortex and serotonin in hippocampus of freely moving rats. The above compounds were compared with drugs that have less interaction with 5-HT<sub>1A</sub> receptors (clozapine, nemonapride, ziprasidone, olanzapine, risperidone and haloperidol). Hippocampal 5-HT was decreased by bifeprunox, SSR181507, SLV313, sarizotan, and nemonapride, effects similar to those seen with the 5-HT<sub>1A</sub> agonist, (+)8-OH-DPAT, consistent with activation of 5-HT<sub>1A</sub> autoreceptors. These decreases were reversed by the selective 5-HT<sub>1A</sub> antagonist, WAY100635. In contrast, haloperidol, risperidone, clozapine, olanzapine, ziprasidone and aripiprazole did not significantly modify hippocampal serotonin levels. In medial prefrontal cortex, dopamine levels were increased by SSR181507, SLV313, sarizotan and (+)8-OH-DPAT. These effects were reversed by WAY100635, indicating mediation by 5-HT<sub>1A</sub> receptors. In contrast, the increases in dopamine levels induced by clozapine, risperidone, olanzapine and ziprasidone were not blocked by WAY100635, consistent with predominant influence of other mechanisms in the actions of these drugs. Haloperidol, nemonapride and the D<sub>2</sub> partial agonists, aripiprazole and bifeprunox, did not significantly alter dopamine release. Taken together, these data demonstrate the diverse contribution of 5-HT<sub>1A</sub> receptor activation to the profile of antipsychotics and suggest that novel drugs selectively targeting D<sub>2</sub> and 5-HT<sub>1A</sub> receptors may present distinctive therapeutic properties.

## Introduction

Whilst conventional neuroleptics such as haloperidol, which selectively antagonize D<sub>2</sub>-like receptors (including the D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> subtypes) are effective in controlling positive symptomatology, they exhibit notable therapeutic shortcomings. These include lack of efficacy against negative symptoms, failure to attenuate cognitive deficits and induction of extrapyramidal symptoms (EPS) (Harvey and Bowie, 2003). 'Atypical' antipsychotics, such as clozapine, olanzapine and risperidone antagonize D<sub>2</sub>-like receptors but also act by mechanisms involving other neurotransmitter systems. Thus, actions at serotonin receptors, with emphasis on 5-HT<sub>2A/2C</sub> receptor subtypes, have gained prominence as targets for antipsychotics (Ichikawa and Meltzer, 1999). Recently, converging pre-clinical and clinical evidence has increasingly drawn attention to the role of 5-HT<sub>1A</sub> receptors, suggesting that combining antagonist activity at dopamine D<sub>2</sub> receptors with agonist activity at 5-HT<sub>1A</sub> receptors offers a promising strategy for the design of novel antipsychotics (see, Meltzer et al., 2003).

5-HT<sub>1A</sub> receptors are upregulated in frontal cortex of schizophrenics, as shown in post-mortem autoradiography and PET studies (Burnet et al., 1997; Tauscher et al., 2002) consistent with a role of 5-HT<sub>1A</sub> receptors in the pathophysiology of schizophrenia. Accordingly, antipsychotic drugs exhibiting 5-HT<sub>1A</sub> agonist properties are associated with several potential advantages. For example, 5-HT<sub>1A</sub> receptor activation lowers EPS liability: in animal studies, 5-HT<sub>1A</sub> agonists reverse the cataleptic effects of conventional neuroleptics (e.g., Prinssen et al., 1999) and 5-HT<sub>1A</sub> receptor agonists are active in models of anxiety and depression (Blier and Ward, 2003) suggesting enhanced treatment of negative symptoms. In addition, Sumiyoshi et al. (2001) have shown that adjunctive treatment with the 5-HT<sub>1A</sub> partial agonist, tandospirone, attenuates memory dysfunction in schizophrenia.

The neurochemical basis for beneficial properties of 5-HT<sub>1A</sub> receptor activation may be related to enhanced dopamine release in brain regions associated with cognitive function and consequent activation of D<sub>1</sub> receptors controlling acetylcholine release therein (Consolo et al., 1996). In fact, activation of 5-HT<sub>1A</sub> receptors preferentially enhances dopamine release in the prefrontal cortex over that in the striatum or nucleus accumbens (Arborelius et al., 1993) and efficacy against negative symptoms in schizophrenia is associated with a functional impairment of mesocortical dopaminergic transmission (Weinberger and Berman, 1996). Accordingly, marked increases in frontocortical dopamine release are found with atypical antipsychotics such as clozapine, olanzapine, ziprasidone and risperidone but not with conventional neuroleptics such as haloperidol (Volonte et al., 1997; Li et al., 1998; Rollema et al., 2000).

Among existing antipsychotics, clozapine, ziprasidone and aripiprazole possess 5-HT<sub>1A</sub> agonist properties (Newman-Tancredi et al., 2005), but also act at numerous other targets (Shapiro et al., 2003). In contrast, a new generation of antipsychotics is gaining prominence which selectively targets dopamine D<sub>2</sub> receptors and 5-HT<sub>1A</sub> receptors without appreciable interactions at other sites. These drugs, now undergoing clinical development, include bifeprunox (Wolf, 2003), SSR181507 (Claustre et al., 2003; Depoortere et al., 2003) and SLV313 (Glennon et al., 2002) as well as the anti-dyskinetic agent, sarizotan (Bartoszyk et al., 2004). However, only sparse information is available concerning the pharmacological properties of these drugs and the relative influence of their 5-HT<sub>1A</sub> agonist properties on the modulation of serotonergic and dopaminergic transmission remains unclear.

We therefore examined, in uniform experimental conditions, the effect of these drugs on two neurochemical measures. First, the 5-HT<sub>1A</sub> agonist properties of the drugs were examined by measuring the decrease in extracellular 5-HT in rat hippocampus, a region that has been widely used to assess activation of somatodendritic 5-HT<sub>1A</sub> receptors in the raphe (e.g., Assié

and Koek, 2000). Specific mediation of this response by 5-HT<sub>1A</sub> receptors was demonstrated by antagonism with the selective 5-HT<sub>1A</sub> antagonist WAY100635. Second, the influence of antipsychotics on dopaminergic neurotransmission was examined by measuring dopamine levels in medial prefrontal cortex. 5-HT<sub>1A</sub> receptor control of dopamine release in frontal cortex is complex and may involve both pre and post-synaptic 5-HT<sub>1A</sub> receptors via GABAergic and glutamatergic systems (Celada et al., 2001). By comparing the actions of the antipsychotics on these two neurochemical parameters, we demonstrate that 5-HT<sub>1A</sub> receptor activation plays widely differing roles in the actions of antipsychotics and suggest that novel drugs selectively targeted at D<sub>2</sub> and 5-HT<sub>1A</sub> receptors may display distinctive profiles.

## Materials and Methods

**Animals.** Male Sprague Dawley rats [Ico: OFA SD (I.O.P.S. Caw); Iffa Credo, France], weighing 260-340 g, were group housed (three rats per cage) in the animal-keeping facilities, under controlled conditions (12/12 light/dark cycle: lights on 07.00 a.m.; ambient temperature  $21 \pm 1^\circ\text{C}$ ; humidity  $55 \pm 5\%$ ), with rat food (AO4, UAR, France) and filtered ( $0.2 \mu\text{m}$ ) tap water available ad libitum. At least five days were allowed for adaptation before the rats were used in the experiments. The experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, US National Research Council, 1996), and were approved by the institutional Protocol Review Committee (protocol 222).

**Microdialysis procedure.** Rats were anaesthetized with chloral hydrate (400-500 mg/kg i.p.). A guide cannula with a dummy probe was stereotaxically implanted into the medial prefrontal cortex, stereotaxic coordinates: rostral +3.0 mm, lateral +0.8 mm, ventral -1.7 mm, from bregma or the hippocampus, stereotaxic coordinates: rostral -4.8 mm, lateral +4.6 mm, ventral -4.6 mm, from bregma and skull surface. Two additional holes were drilled for skull screws and the guide was secured with dental cement.

Following surgery and recovery from anaesthesia, animals were return to their home cage. At the end of the day, each rat was placed in a microdialysis cage. On the following day, the dummy probe was replaced by a microdialysis probe (3 mm length, 0.5 mm diameter, CMA, Microdialysis AB). The probe was continuously perfused ( $1.1 \mu\text{l}/\text{min}$ ) with artificial cerebrospinal fluid (140 mM NaCl, 3 mM KCl, 2.5 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 1.2 mM  $\text{Na}_2\text{HPO}_4$ , 0.27 mM  $\text{NaH}_2\text{PO}_4$ , 7.2 mM glucose). When measuring 5-HT in the hippocampus, basal levels amount to approximately 5 fmol/sample and decreases in 5-HT cannot be reliably determined. For this reason citalopram was included in the perfusion medium, thus

increasing basal 5-HT levels. Following a two-hour stabilization period, samples were collected every 20 min. After four baseline samples, saline or WAY100635 was injected s.c., followed 40 min later by administration of the antipsychotics and samples were collected for 140 min after administration of the latter drug.

At the end of the experiment, the animal was killed by injection of a lethal dose of pentobarbital (160 mg/kg, i.p.) and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

**Analytical procedure.** Analysis of dopamine or 5-HT was performed by means of an online HPLC system with electrochemical detection equipped with a reverse phase column (Merck, Lichrocart 125-2, Superspher 100 RP-18, length 119 mm, internal diameter 2 mm, granulometry 4  $\mu$ m). The mobile phase: 0.15 M  $\text{NaH}_2\text{PO}_4$ , 0.1 mM EDTA, 0.1 mM 1-octanesulfonic acid sodium salt, 14 % methanol pH 5.2 for the measure of dopamine and 0.15 M  $\text{NaH}_2\text{PO}_4$ , 0.1 mM EDTA, 0.7 mM 1-octanesulfonic acid sodium salt, 15 % methanol pH 4.6 for the measure of 5-HT) was pumped through the column at a rate of 0.2 ml/min (HPLC-118 solvent module, Beckman Coulter Inc., Fullerton CA, USA). Dopamine or 5-HT eluted from the column (retention time 5-6 min and 7-9 min, respectively) was measured with a glassy carbon working electrode maintained at a potential of +0.64 V versus Ag/AgCl reference electrode (DECADE detector, ANTEC Leyden BV, Leiden, The Netherlands). Data were acquired using a Beckman 32 Karats system. Concentrations of dopamine or 5-HT were estimated by comparing peak areas from the microdialysis samples with those of external standards of known concentration of the compounds. The limit of detection (three times baseline noise) was approximately 1 fmol/20  $\mu$ l sample.

**Data analysis.** Data were analyzed using repeated measures analysis of variance carried out with the mixed procedure of SAS 8.2 software. Mean AUC values for the 140 min period after administration of the compounds were analyzed by one-way ANOVA followed by



Dunnett's test were appropriate (SigmaStat, SPSS Chicago, USA). For each compound, the dose producing half-maximal effect ( $ED_{50}$ ) was determined by linear interpolation between the two doses that increase dopamine levels or decrease 5-HT levels with amounts bordering 50% (vehicle control as 0% and maximal effect of each compound as 100%).

**Drugs.** (+)8-OH-DPAT ((+)-8-hydroxy-2-(di-n-propylamino)tetralin) hydrobromide, haloperidol, risperidone, clozapine were purchased from Sigma - RBI (Saint Quentin Fallavier, France), and chloral hydrate from Acros (Geel, Belgium). Pentobarbital sodium was purchased from Ceva Santé Animale (Libourne, France). Citalopram hydrobromide and SLV313 (1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-[5-(4-fluorophenyl)-pyridin-3-ylmethyl]-piperazine) were kindly donated by Lundbeck (Copenhagen, Denmark) and Solvay-Duphar (Weesp, The Netherlands), respectively. Olanzapine, ziprasidone hydrochloride, nemonapride, aripiprazole, bifeprunox (DU127090) mesylate, sarizotan (EMD-128130) hydrochloride, SSR181507 ((3-exo)-8-benzoyl-N-(((2S)7-chloro-2,3-dihydro-1,4-benzodioxin-1-yl)methyl)-8-azabicyclo(3.2.1)octane-3-methanamine) monohydrochloride and WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide) hydrochloride were synthesized at the Centre de Recherche Pierre Fabre. The doses of compounds were expressed as the base. WAY100635, SSR181507 and (+)8-OH-DPAT were dissolved in distilled water; haloperidol, clozapine, olanzapine, risperidone and SLV313 were dissolved in distilled water with a drop of lactic acid, after which the pH was adjusted to 5-7 with sodium hydroxide 1N; all other compounds were suspended in distilled water by adding Tween 80 (2 drops/10 ml); the injection volume was 10 ml/kg.

## Results

**Extracellular concentration of dopamine in the medial prefrontal cortex.** The mean basal extracellular concentration of dopamine was  $6.5 \pm 0.2$  fmol/20  $\mu$ l ( $n = 297$ ).

Haloperidol (0.16 – 2.5 mg/kg), nemonapride (0.63 – 10 mg/kg), aripiprazole (2.5 – 40 mg/kg) and bifeprunox (0.63 – 10 mg/kg) did not significantly alter extracellular dopamine (Fig. 1 and 2). Clozapine (2.5- 40 mg/kg), olanzapine (0.63 – 40 mg/kg), risperidone (0.16 – 10 mg/kg), ziprasidone (0.16 – 10 mg/kg), sarizotan (0.63 – 40 mg/kg), SLV313 (0.04 - 0.63 mg/kg) and SSR181507 (2.5 – 40 mg/kg) like the 5-HT<sub>1A</sub> agonist (+)8-OH-DPAT (0.16 – 2.5 mg/kg) produced a dose-dependent increase in extracellular dopamine concentration (ED<sub>50</sub> values: Table 2). The effects of sarizotan (2.5 mg/kg), SLV313 (0.16 mg/kg) and SSR181507 (10 mg/kg), like those of (+)8-OH-DPAT (0.63 mg/kg) were reversed by the selective 5-HT<sub>1A</sub> antagonist, WAY100635 (0.16 mg/kg). In contrast, the effects of clozapine, olanzapine, risperidone and ziprazidone were not significantly antagonized by WAY100635.

**Extracellular concentrations of serotonin in the ventral hippocampus.** The mean basal extracellular concentration of 5-HT in the rat ventral hippocampus was  $38.9 \pm 0.9$  fmol/20  $\mu$ l ( $n = 227$ )

Haloperidol (2.5 mg/kg), risperidone (2.5 mg/kg), clozapine (2.5 – 40 mg/kg), olanzapine (40 mg/kg), ziprasidone (2.5 – 40 mg/kg), aripiprazole (2.5 – 40 mg/kg) produced no significant change in extracellular concentrations of 5-HT (Fig. 3 and 4). Nemonapride (0.63 – 10 mg/kg), bifeprunox (0.63 – 40 mg/kg), sarizotan (0.63 – 10 mg/kg), SLV313 (0.16 – 2.5 mg/kg) and SSR181507 (0.63 – 10 mg/kg), like (+)8-OH-DPAT (0.16 – 2.5 mg/kg), induced a dose-dependent, significant decrease in extracellular 5-HT levels (ED<sub>50</sub> values: Table 2). These effects were antagonized by WAY100635.

For a summary of the statistical analyses of data, see Table 1.

## Discussion

The major finding of the present study is that conventional and novel antipsychotics exhibit marked diversity in the contribution that 5-HT<sub>1A</sub> receptor activation makes to their neurochemical profile, as measured by increased dopamine levels in the medial prefrontal cortex and decreased 5-HT levels in the hippocampus.

Increases in medial prefrontal cortex dopamine levels are associated with attenuated cognitive dysfunction and negative symptoms in schizophrenia (Meltzer and McGurk, 1999). Correspondingly, several atypical antipsychotics, including clozapine, olanzapine, risperidone and ziprasidone, preferentially increase dopamine release in the prefrontal cortex (Volonte et al., 1997; Rollema et al., 2000; Li et al., 1998). Because 5-HT<sub>1A</sub> agonists preferentially increase dopamine release in this brain area (Arborelius et al., 1993; Ichikawa et al., 2001), the 5-HT<sub>1A</sub> agonist properties of certain antipsychotics may play a role in these effects. However, the contribution of 5-HT<sub>1A</sub> receptor activation to the actions of different antipsychotics is heterogeneous. Clozapine and ziprasidone, multireceptor agents which act as partial agonists at 5-HT<sub>1A</sub> receptors in vitro, may increase dopamine release in frontal cortex partly by actions at 5-HT<sub>1A</sub> sites (Rollema et al., 2000) although their actions at these targets are, at best, modest. In contrast, risperidone and olanzapine have little or no interaction at 5-HT<sub>1A</sub> receptors and enhance frontocortical dopamine release by indirect 5-HT<sub>1A</sub> receptor activation (Ichikawa et al., 2001). In comparison, recent drugs which selectively target both D<sub>2</sub>-like and 5-HT<sub>1A</sub> receptors, such as SLV313, SSR181507 and bifeprunox, would be expected to display accentuated serotonergic actions at 5-HT<sub>1A</sub> receptors (Newman-Tancredi et al., 2005). The present study investigated the role of 5-HT<sub>1A</sub> receptor activation in the neurochemical profile of conventional and new generation antipsychotics under uniform experimental conditions, and thus constitutes a powerful homogenous database to compare the various compounds.

### ***Influence of antipsychotics on serotonin release in hippocampus***

5-HT levels in the hippocampus were determined as a measure of somatodendritic 5-HT<sub>1A</sub> receptor activation. Indeed, 5-HT<sub>1A</sub> receptors located in the raphe nuclei exert inhibitory control of serotonergic neurotransmission and, consequently, reduce 5-HT release in terminal regions (Blier and Ward, 2003). Even drugs with low efficacy at 5-HT<sub>1A</sub> receptors, such as the partial agonist, buspirone (e.g., Assié and Koek, 2000), as a result of the high 5-HT<sub>1A</sub> autoreceptor reserve in the raphe (Cox et al., 1993). Here, the new putative antipsychotics, SSR181507, SLV313 and bifeprunox, like nemonapride, the antidyskinetic agent, sarizotan, and the prototypical 5-HT<sub>1A</sub> agonist (+)8-OH-DPAT, decrease extracellular 5-HT in the hippocampus (Fig. 1,2), actions that were reversed by 5-HT<sub>1A</sub> receptor blockade. These observations are consistent with the marked in vitro 5-HT<sub>1A</sub> agonist properties of these compounds (Newman-Tancredi et al., 2005). In contrast, aripiprazole, which exhibits only weak agonist properties at 5-HT<sub>1A</sub> receptors in vitro, showed little capacity to inhibit serotonin release whereas olanzapine, risperidone and haloperidol did not influence serotonin release, consistent with their absence of interaction with 5-HT<sub>1A</sub> receptors.

### ***Influence of antipsychotics on dopamine release in medial prefrontal cortex***

The present data on influence of established antipsychotics on medial prefrontal cortex dopamine release are generally similar to those reported in individual studies by other laboratories. Thus, the increase in dopamine release by ziprasidone is consistent with data from Rollema et al. (2000) although its effects here were not reversed by WAY100635 pre-treatment. In contrast, the increase in dopamine release induced by risperidone tended to be attenuated by WAY100635, in agreement with a previous report (Ichikawa et al., 2001). In view of the low affinity of risperidone for 5-HT<sub>1A</sub> receptors, an indirect mechanism involving the 5-HT<sub>2A</sub> antagonist properties of the compound has been proposed (Ichikawa et al., 2001).

Indeed, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors are co-localised in frontal cortex (Amargós-Bosch et al., 2004) and cross-talk between these receptors occurs at the signal transduction and electrophysiological level (Ashby et al., 1994). The effects of clozapine (14-fold increase of dopamine at 40 mg/kg) and olanzapine (8-fold increase of dopamine at 40 mg/kg) are considerably more pronounced than those of the other compounds studied here (maximal increase 2½ fold). In a previous study the effects of clozapine were attenuated by 5-HT<sub>1A</sub> receptor blockade (Rollema et al., 1997) but under present conditions and in agreement with other laboratories (Millan et al., 1998), the effects of clozapine and olanzapine were not reversed by WAY100635, reflecting their weak interaction at 5-HT<sub>1A</sub> receptors. In fact, clozapine and olanzapine interact at multiple receptor subtypes and their induction of dopamine release is related to antagonism of 5-HT<sub>2A/2C</sub> receptors (Ichikawa et al., 2001) and  $\alpha_2$  adrenoceptors (Devoto et al., 2003). Further, activation of muscarinic receptors by clozapine and its principal metabolite, desmethyl-clozapine, also contribute to release of dopamine (Li et al., 2004b).

The putative antipsychotics, SSR181507, SLV313 and bifeprunox, selectively target dopamine D<sub>2</sub>-like and 5-HT<sub>1A</sub> receptors (Newman-Tancredi et al., 2005), with little interaction with a range of other receptors including 5-HT<sub>2</sub> or adrenergic receptors (Claustre et al., 2003; Wolf, 2003; and unpublished observations Assié et al.). They therefore represent a new generation of antipsychotic agents and the present study provides the first comparative data of these drugs on 5-HT and dopamine release. In agreement with Claustre et al. (2003), SSR181507 increased dopamine levels in a dose-dependent manner and in a similar dose range as for inhibition of serotonin release in hippocampus (Table 2). In contrast, SLV313 potently increased dopamine release at doses 8-fold lower than those which inhibit serotonin release (Table 2) whereas sarizotan and (+)8-OH-DPAT, exerted their influence on dopamine and serotonin release at similar doses. All these responses in both hippocampus and medial

prefrontal cortex were abolished by pre-treatment with WAY100635, indicating mediation by 5-HT<sub>1A</sub> receptors. It may be speculated that the ligands differentially influence neurotransmitter release in different brain structures according to their balance of affinity / efficacy at 5-HT<sub>1A</sub> receptors and dopaminergic sites.

In contrast to these drugs, aripiprazole and bifeprunox did not increase dopamine release, consistent with their reported partial agonist properties at D<sub>2</sub> receptors and their only moderate efficacy and affinity, respectively, for 5-HT<sub>1A</sub> receptors (Wolf, 2003; Newman-Tancredi et al., 2005). The absence of effects of aripiprazole on dopamine release confirms previous observations (Jordan et al., 2004) although a recent report indicates that it slightly increased dopamine release at a low dose of 0.3 mg/kg (Li et al., 2004a). Under present conditions, aripiprazole failed to increase dopamine release even at this low dose (0.31 mg/kg, i.p., AUC value: 85 ± 10 %). In comparison, although nemonapride has high efficacy at 5-HT<sub>1A</sub> receptors (Assié et al., 1997), it did not increase dopamine release, likely because of its potent dopamine D<sub>2</sub> antagonist properties. Indeed, in measures of catalepsy in rats, nemonapride exhibits 5-HT<sub>1A</sub> properties at doses 60-fold greater than those which antagonize D<sub>2</sub> receptors (Prinssen et al., 1998).

### ***Summary and conclusions***

In summary, the present study provides a comparison of the influence of 5-HT<sub>1A</sub> receptor activation on the release of serotonin and dopamine by established and novel antipsychotic agents. Different groups of compounds may be defined. First, compounds such as clozapine, olanzapine, risperidone and ziprasidone, have weak or negligible actions at 5-HT<sub>1A</sub> receptors, as assessed by their absence of influence on serotonin release. These drugs nevertheless influence dopamine release in medial prefrontal cortex (Fig. 4), but do so predominantly via other receptor mechanisms, such as blockade of 5-HT<sub>2A</sub> receptors. Second, aripiprazole and

bifeprunox exert little influence on dopamine release, a consequence of their partial agonist properties at D<sub>2</sub> receptors. Third, SSR181507, SLV313 (and sarizotan) exhibit pronounced serotonergic properties, inhibiting 5-HT release in hippocampus in a manner similar to the 5-HT<sub>1A</sub> agonist, (+)8-OH-DPAT. These drugs, which interact with D<sub>2</sub>-like receptors but are devoid of marked interactions at other receptor subtypes, increase cortical dopamine release by direct 5-HT<sub>1A</sub> receptor activation. This distinctive profile of action on serotonin and dopamine release may translate into an innovative pattern of therapeutic properties. However, additional investigation would be desirable, in particular by extending these studies to other brain regions, such as nucleus accumbens and striatum. Further, no information is currently available concerning regulation of somatodendritic 5-HT<sub>1A</sub> autoreceptor function following chronic administration of these drugs. Electrophysiological studies demonstrated desensitization of raphe cells after chronic administration of 5-HT<sub>1A</sub> receptor agonists (Blier and Ward, 2003). In accordance, microdialysis studies have shown that the ability of a challenge dose of 5-HT<sub>1A</sub> agonist to decrease terminal 5-HT release was altered after chronic administration of 5-HT<sub>1A</sub> receptor agonists (Kreiss and Lucki, 1997; Casanovas et al., 1999). In addition, Hensler and Durgham (2001), using [<sup>35</sup>S]GTPγS autoradiography, have shown that the regulation of presynaptic 5-HT<sub>1A</sub> receptor function following repeated administration of 8-OH-DPAT involves changes in receptor-G protein interaction. Thus, the regulation of 5-HT and dopamine release following chronic administration of existing and novel antipsychotics may reveal additional complexity (Hernandez and Hoebel, 1995).

In conclusion, considerable diversity exists in the contribution that 5-HT<sub>1A</sub> receptor activation makes to the neurochemical profile of antipsychotic agents. A new generation of drugs is in clinical development which selectively targets dopamine D<sub>2</sub>-like and 5-HT<sub>1A</sub> receptors, whilst additional investigation is required to fully elucidate their actions, these

compounds may display distinctive properties in the treatment of mood deficits, negative symptoms and cognitive dysfunction in schizophrenic patients.

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**Footnotes.** A preliminary account of these data was presented at the XXIV<sup>th</sup> annual meeting of Collegium Internationale of Neuro-psychopharmacologicum: Assié M-B, Ravailhe V, Faucillon V and Newman-Tancredi A (2004) Effects of antipsychotics with diverse dopamine D<sub>2</sub> and serotonin 5-HT<sub>1A</sub> properties on cerebral dopamine and serotonin levels in rats. *Int J Neuropsychopharmacol* **7**:P02.506.

### Legends For Figures

**Fig.1.** Effects of new antipsychotics with 5-HT<sub>1A</sub> agonist properties on extracellular dopamine levels in the medial prefrontal cortex of freely moving rats. Data are expressed as a percentage of the mean absolute amount of dopamine in the four samples collected before treatment. The first arrow indicates injection of saline, the second indicates injection of the antipsychotic. Results are mean  $\pm$  S.E.M. for 3 to 6 animals per group.

**Fig.2.** Effects of various compounds on extracellular dopamine concentration in rat medial prefrontal cortex and reversal with the selective 5-HT<sub>1A</sub> antagonist WAY100635 (0.16 mg/kg, s.c.). Results, expressed as mean AUC for 140 min after administration of the compound, are mean  $\pm$  S.E.M. for 3 to 6 animals per dose. Data were analyzed using one-way ANOVA followed by Dunnett's test (\*  $P < 0.05$  compared to corresponding control animals) for the dose-response effects, and using Student's *t* test for the antagonism by WAY100635 (+  $P < 0.05$  compared to the corresponding dose of compound). Note differing x-axis according to drug tested. Compounds on the left-hand side of the figure have little or no interaction with 5-HT<sub>1A</sub> receptors. Compounds on the right-hand side of the figure act as 5-HT<sub>1A</sub> agonists.

**Fig.3.** Effects of new antipsychotics with 5-HT<sub>1A</sub> agonist properties on extracellular 5-HT levels in hippocampus of freely moving rats. Data are expressed as a percentage of the mean absolute amount of 5-HT in the four samples collected before treatment. The first arrow indicates injection of saline, the second indicates injection of the antipsychotic. Results are mean  $\pm$  S.E.M. for 3 to 6 animals per group.

**Fig.4.** Effects of various compounds on extracellular 5-HT concentration in rat hippocampus and reversal with the selective 5-HT<sub>1A</sub> antagonist WAY100635 (0.16 mg/kg, s.c.). Results,



expressed as mean AUC for 140 min after administration of the compound, are mean  $\pm$  S.E.M. for 3 to 6 animals per dose. Data were analyzed using one-way ANOVA followed by Dunnett's test (\*  $P < 0.05$  compared to corresponding control animals) for the dose-response effects, and using Student's *t* test for the antagonism by WAY100635 (+  $P < 0.05$  compared to the corresponding dose of compound). Note differing x-axis according to drug tested. Compounds on the left-hand side of the figure have little or no interaction with 5-HT<sub>1A</sub> receptors. Compounds on the right-hand side of the figure act as 5-HT<sub>1A</sub> agonists.

TABLE 1

Statistical analysis of the effects of the compounds: two-way ANOVA with repeated measures on dopamine levels in the frontal cortex (DA) and 5-HT levels in the hippocampus (5-HT)

Compound	parameter	DA	5-HT
Haloperidol	time	F(6,102) = 3.81; P = 0.0018	F(6,29) = 2.02; P = 0.096
	treatment	F(3,17) = 0.61; P = 0.62	F(1,5) = 0.18; P = 0.69
	interaction	F(18,102) = 1.69; P = 0.05	F(6,29) = 1.5; P = 0.21
Risperidone	time	F(6,198) = 11.9; P < 0.0001	F(6,30) = 2.12; P = 0.08
	treatment	F(7,33) = 9.27; P < 0.0001	F(1,5) = 0.82; P = 0.41
	interaction	F(42,198) = 2.80; P < 0.0001	F(6,30) = 1.15; P = 0.36
Clozapine	time	F(6,144) = 104; P < 0.0001	F(6,88) = 3.58; P = 0.003
	treatment	F(5,24) = 224; P < 0.0001	F(3,15) = 3.53; P = 0.041
	interaction	F(30,144) = 46.7; P < 0.0001	F(18,88) = 1.24; P = 0.25
Olanzapine	time	F(6,174) = 25.1; P < 0.0001	F(6,29) = 1.21; P = 0.33
	treatment	F(7,29) = 27.8; P < 0.0001	F(1,5) = 4.28; P = 0.09
	interaction	F(42,174) = 7.81; P < 0.0001	F(6,29) = 2.06; P = 0.09
Ziprasidone	time	F(6,216) = 6.21; P < 0.0001	F(6,60) = 0.49; P = 0.82
	treatment	F(8,36) = 8.68; P < 0.0001	F(3,10) = 0.88; P = 0.48
	interaction	F(48,216) = 1.66; P = 0.008	F(18,60) = 0.46; P = 0.97
Nemonapride	time	F(6,72) = 5.87; P < 0.0001	F(6,136) = 3.62; P = 0.002
	treatment	F(3,12) = 1.34; P = 0.31	F(5,24) = 10.7; P < 0.0001
	interaction	F(18,72) = 1.79; P = 0.04	F(30,136) = 0.92; P = 0.60
Aripiprazole	time	F(6,72) = 5.60; P < 0.0001	F(6,86) = 0.98; P = 0.45
	treatment	F(3,12) = 2.28; P = 0.13	F(3,15) = 3.70; P = 0.036
	interaction	F(18,72) = 1.23; P = 0.26	F(18,86) = 1.05; P = 0.41
Bifeprunox	time	F(6,66) = 3.92; P = 0.0021	F(6,158) = 7.59; P < 0.0001
	treatment	F(3,11) = 1.85; P = 0.20	F(6,27) = 9.42; P < 0.0001
	interaction	F(18,66) = 1.35; P = 0.19	F(36,158) = 1.66; P = 0.018
Sarizotan	time	F(6,182) = 4.17; P = 0.0006	F(6,144) = 1.81; P = 0.10
	treatment	F(7,31) = 8.50; P < 0.0001	F(5,24) = 15.1; P < 0.0001
	interaction	F(42,182) = 1.93; P = 0.0017	F(30,144) = 1.18; P = 0.26
SLV313	time	F(6,150) = 8.23; P < 0.0001	F(6,172) = 11.5; P < 0.0001
	treatment	F(5,25) = 13.1; P < 0.0001	F(6,30) = 5.47; P = 0.0006
	interaction	F(30,150) = 2.48; P = 0.0002	F(36,172) = 1.80; P = 0.0068
SSR181507	time	F(6,143) = 6.87; P < 0.0001	F(6,139) = 6.18; P < 0.0001
	treatment	F(5,24) = 11.7; P < 0.0001	F(5,24) = 11.1; P < 0.0001
	interaction	F(30,143) = 2.63; P < 0.0001	F(30,139) = 0.91; P = 0.61
(+)8-OH-DPAT	time	F(6,144) = 11.1; P < 0.0001	F(6,136) = 2.19; P = 0.047
	treatment	F(5,24) = 25.4; P < 0.0001	F(5,24) = 19.9; P < 0.0001
	interaction	F(30,144) = 3.23; P < 0.0001	F(30,136) = 1.99; P = 0.0042

TABLE 2

ED<sub>50</sub> values (mg/kg) for the various compounds at increasing extracellular dopamine levels in the medial prefrontal cortex or decreasing extracellular 5-HT levels in the hippocampus.

Compound	Frontal cortex dopamine levels	Hippocampus 5-HT levels
Haloperidol	-	-
Risperidone	0.36	-
Clozapine	17	-
Olanzapine	15	-
Ziprasidone	0.87	-
Nemonapride	-	2.4
Aripiprazole	-	-
Bifeprunox	-	3.0
Sarizotan	0.69	1.7
SLV313	0.05	0.40
SSR181507	4.0	1.5
(+)-8-OH-DPAT	0.2	0.33

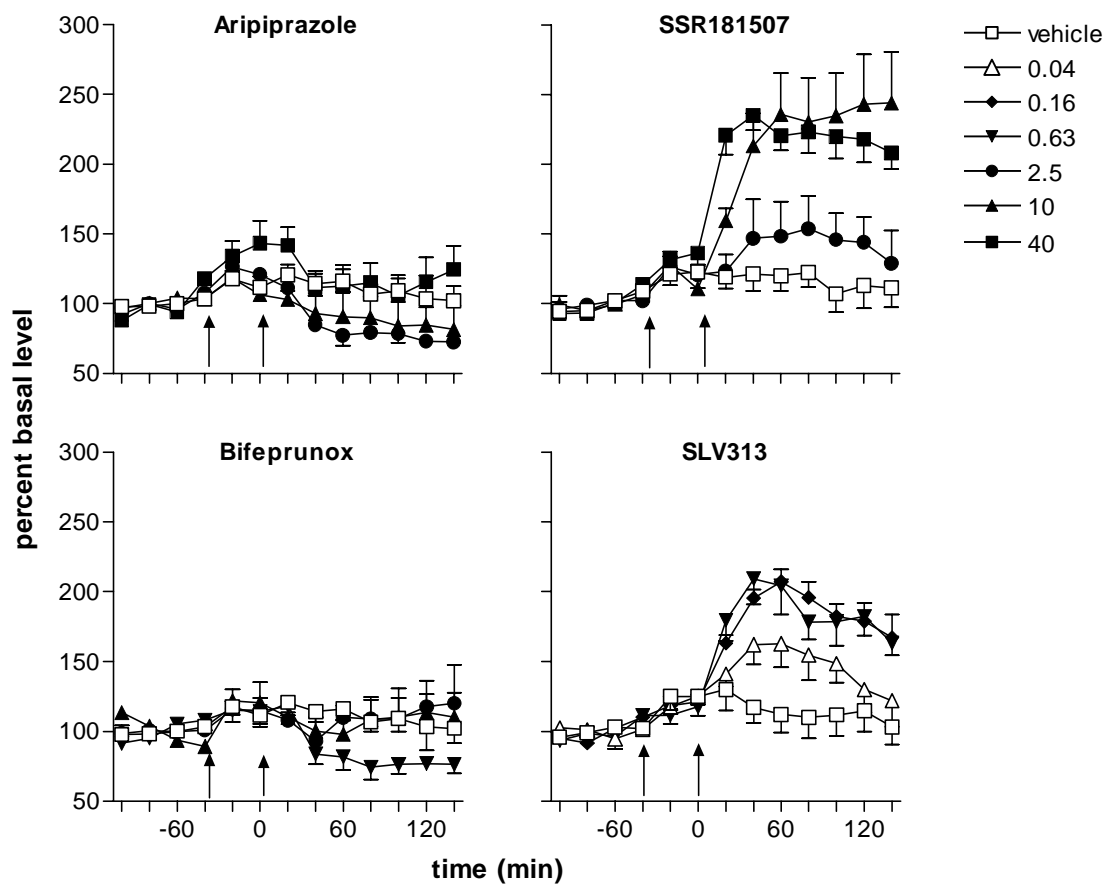


Fig 1

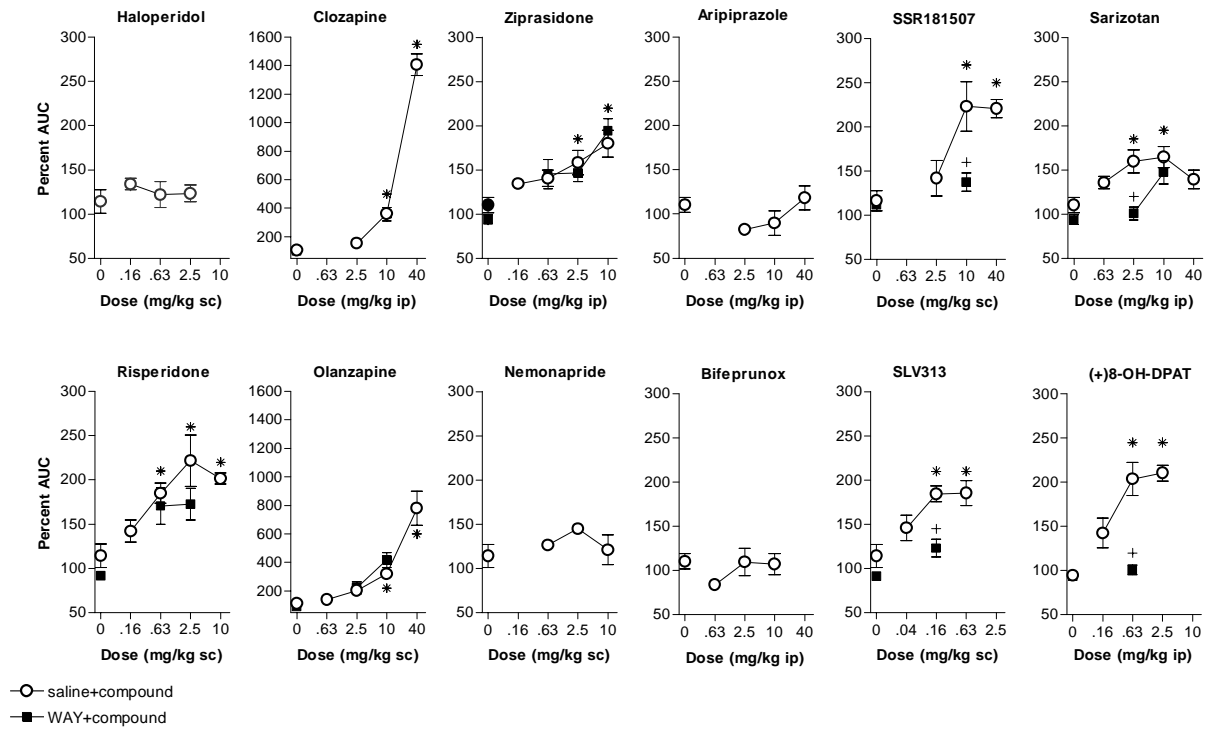


Fig 2

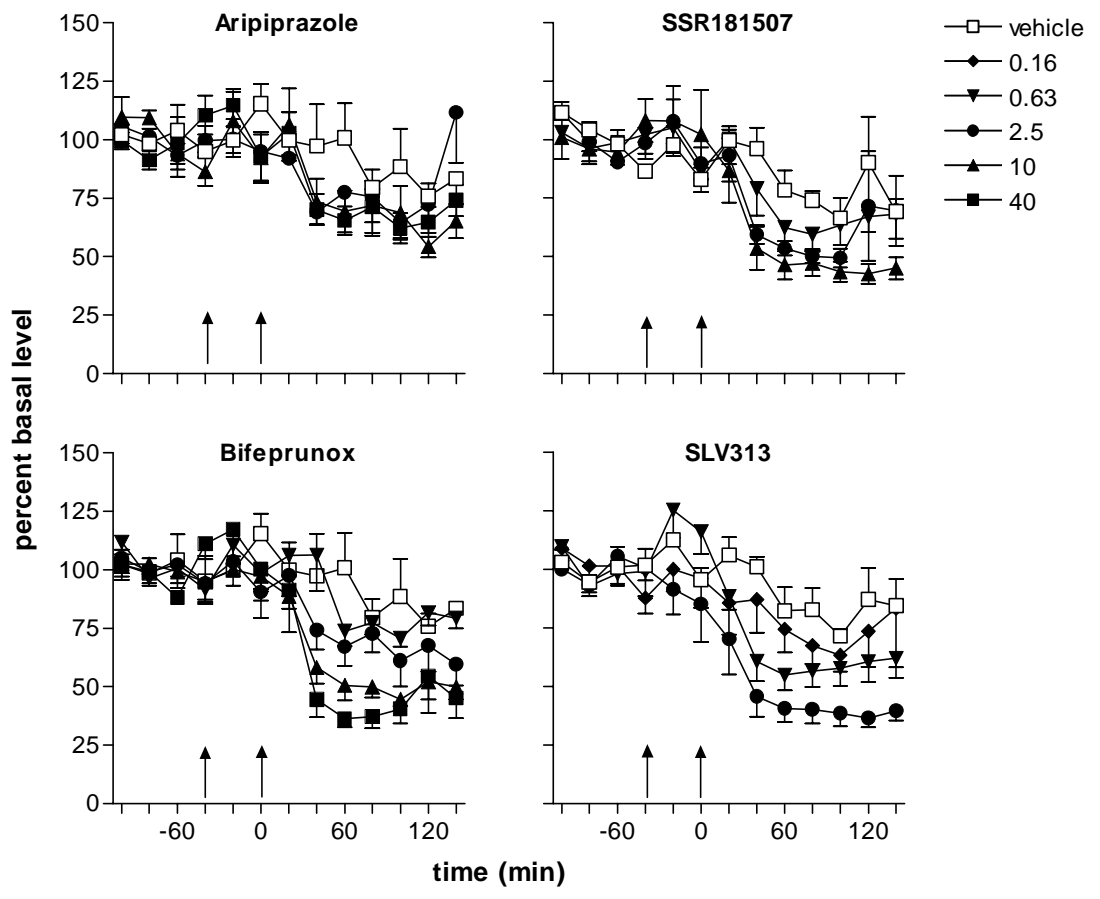


Fig 3

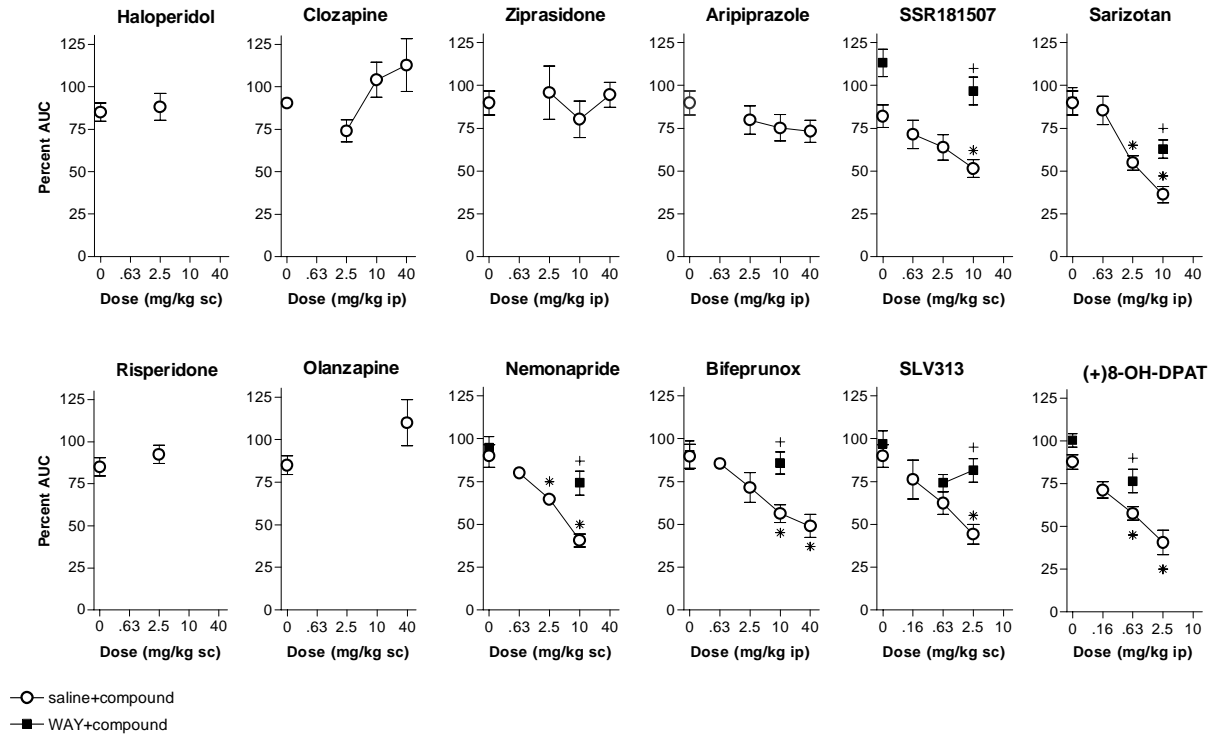


Fig 4