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Antipsychotic drugs reverse the AMPA receptor-stimulated release of 5-HT in the medial prefrontal cortex

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Abstract

Background. The prefrontal cortex (PFC) is involved in the pathophysiology of schizophrenia. PFC neuronal activity is modulated by monoaminergic receptors for which antipsychotic drugs display moderate-high affinity. Conversely, PFC pyramidal neurons project to and modulate the activity of raphe serotonergic neurons and serotonin (5-HT) release.

Methods. We studied the effect of antipsychotic drugs on the *in vivo* 5-HT release evoked by increasing glutamatergic transmission in rat medial PFC (mPFC). This was achieved by applying S-AMPA in mPFC (reverse dialysis) or by disinhibiting excitatory afferents to mPFC through the intrathalamic application of bicuculline. Antipsychtoic drugs were locally (in mPFC) or systemically administered.

Results. The application of haloperidol, chlorpromazine, clozapine and olanzapine in mPFC by reverse dialysis (but not that of reboxetine or diazepam) reversed the S-AMPA-evoked 5-HT release in mPFC. Likewise, the local (in mPFC) or systemic administration of these antipsychotic drugs reversed the increased prefrontal 5-HT release produced by thalamic disinhibition. These effects were shared by the 5-HT_{2A} and α_1-adrenoceptor antagonists M100907 and prazosin, respectively, but not by raclopride.

Conclusion. These results suggest that, in addition to their action in limbic striatum, antipsychotic drugs may attenuate glutamatergic transmission in PFC, an effect possibly mediated by blockade of 5-HT_{2A} and/or α_1-adrenoceptors.

Abbreviations: 5-HT, 5-hydroxytryptamine or serotonin; CM, centromedial nucleus of the thalamus; iGluR, ionotropic glutamate receptors; MD, mediodorsal nucleus of the thalamus; mPFC, medial prefrontal cortex; PFC, prefrontal cortex

The prefrontal cortex (PFC) plays a key role in higher brain functions (Fuster, 2001). Many neurochemical, cellular and functional alterations have been reported in the PFC of schizophrenic patients (Weinberger et al., 1994; Andreasen et al., 1997; Bertolino et al., 2000; Lewis and Lieberman, 2000; Lewis et al., 2005). In particular, changes in prefrontal GABAergic and glutamatergic transmission have been reported (Lewis and Lieberman, 2000; Tsai and Coyle, 2002; Krystal et al., 2003; Mogaddham, 2003). Behavioural deficits induced by non-competitive NMDA receptor antagonists resemble schizophrenic symptoms, which suggests a glutamatergic hypofunction in schizophrenia. However, neurochemical (Mogaddham et al., 1997) and electrophysiological observations (Suzuki et al., 2002; Jackson et al., 2004) indicate that these agents increase glutamatergic transmission in mPFC, possibly by acting in afferent areas (Jodo et al., 2005).

The activity of projection (pyramidal) neurons -which make up ~75% of all neurons in PFC- depends on glutamatergic inputs from cortical and subcortical areas and is locally modulated by GABA interneurons. Main subcortical excitatory inputs arise from the mediodorsal/centromedial nuclei of the thalamus (MD/CM), the hippocampus and the amygdala, which are reciprocally connected with the PFC (Kuroda et al., 1998; Groenewegen and Uylings, 2000; Van der Werf et al., 2002). Interestingly, the PFC and the brainstem monoaminergic nuclei (ventral tegmental area, raphe nuclei and locus coeruleus) are also reciprocally connected (Groenewegen and Uylings, 2000). Catecholaminergic and serotonergic axons innervate the PFC and modulate neuronal activity through various inhibitory and excitatory receptors (Araneda and Andrade, 1991; Pompeiano et al., 1992; Pieribone et al., 1994; Aghajanian and Marek, 1997, 1999; O'Donnell, 2003; Amargós-Bosch et al., 2004; Puig et al., 2005). In turn, the activity of brainstem aminergic neurons is modulated by descending inputs from PFC (Aghajanian and Wang, 1977; Thierry et al., 1979; Jodo et al., 1998; Hajós et al., 1998; Celada et al., 2001). Consistent with this distal control of serotonergic neurons, the release of serotonin (5-HT) in PFC is modulated by the activation of postsynaptic receptors in PFC, including 5-$HT_{1A/2A}$, α_1-adrenoceptors and AMPA receptors (Celada et al., 2001; Martín-Ruiz et al., 2001; Puig et al., 2003; Amargós-Bosch et al., 2003, 2004).

Classical neuroleptics are believed to exert their therapeutic action by modulating excitatory inputs onto limbic striatum following the blockade of local dopamine (DA) D2 receptors (Moore et al., 1999; Grace, 2000). However, the presence of antipsychotic-sensitive monoaminergic receptors in PFC (e.g., 5-HT_{1A}, 5-$HT_{2A/2C}$ receptors, α_1-

adrenoceptors, among others) and the role of PFC in behavioural control suggest that antipsychotics may have additional actions in this cortical area.

Here we examined the effect of antipsychotic drugs on the glutamate-stimulated release of 5-HT in mPFC, under the working hypothesis that they may attenuate the excitatory drive to midbrain and hence, reduce the *in vivo* terminal 5-HT release. The activity of PFC neurons was enhanced by locally applying S-AMPA and by disinhibiting thalamic afferents to mPFC, a procedure that dramatically increases the activity of pyramidal neurons in mPFC (Puig et al., 2003).

Materials and methods

Animals

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

Chemicals

5-HT oxalate, (S)-AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate), bicuculline, chlorpromazine, diazepam, prazosin, reboxetine and raclopride were from Sigma (Tres Cantos, Spain). Haloperidol and clozapine were from Tocris (Bristol, UK). M100907 (R-(+)-alpha-(2,3-dimethoxyphenyl)-1-[4-fluorophenylethyl]-4-piperidinemethanol; Lilly code LY 368675) and olanzapine were from Eli Lilly & Co. Other materials and reagents were from local commercial sources. Drugs were dissolved in the perfusion fluid or water (except clozapine, dissolved in acetic acid, and olanzapine, dissolved in HCl). Concentrated solutions (1 mM; pH adjusted to 6.5-7 with $NaHCO_3$ when necessary) were stored at -80 ^{o}C and working solutions were prepared daily by dilution in artificial CSF. Concentrations are expressed as free bases. Control rats were

perfused for the entire experiment with artificial CSF. The bars in the figures show the period of local drug application (corrected for the void volume of the system).

Surgery and microdialysis experiments

An updated description of the microdialysis procedures used can be found in Adell and Artigas (1998) and Puig et al. (2003). Briefly, anesthetized rats (sodium pentobarbital, 60 mg/kg i.p.) were stereotaxically implanted with concentric microdialysis probes equipped with a Cuprophan membrane. The probes were perfused at 1.5 µL/min with artificial CSF (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) containing 1 µM citalopram. After one-hour stabilization period, four fractions were collected to obtain basal values before local (reverse dialysis) or systemic administration of drugs. Successive 20-min (30 µl) dialysate samples were collected. At the end of the experiments, rats were killed by an overdose of anesthetic. The placement of the dialysis probes was examined by perfusion of fast green dye and visual inspection of the probe track after cutting the brain at the appropriate levels.

In experiments involving the local application of S-AMPA in mPFC, rats were implanted with only one 4-mm probe in this area, at the following coordinates (in mm): AP +3.2, L -0.8, DV –6.0, taken from bregma and duramater (Paxinos and Watson, 1986). These microdialysis experiments were conducted in freely moving rats one day after implants. After collecting four baseline fractions, S-AMPA was applied in mPFC dissolved in the aCSF used to perfuse the probes (reverse dialysis) for twelve fractions (4 h). Two hours after beginning S-AMPA perfusion (6 fractions), the syringe was replaced by one containing S-AMPA plus the test drug (M100907, prazosin, antipsychotics, etc.) and 6 additional microdialysis fractions were collected.

In the experiments involving the disinhibition of thalamic inputs onto the mPFC, rats were implanted with two microdialysis probes, in mPFC (as above) and in a thalamic area sampling the mediodorsal (MD) and centromedial (CM) nuclei of the thalamus projecting to the mPFC (AP -3.5, L -0.5, DV -6.5; probe tip 1.5 mm). These experiments required the use of anesthetized rats in order to prevent an excessive behavioral activation produced by bicuculline application. Both the MD and CM nuclei give rise to a dense excitatory input onto mPFC (see Introduction). Previous studies showed that this procedure increases dramatically (~15-20-fold) the firing activity of pyramidal neurons in mPFC and doubles the release of 5-HT in this area (Martín-Ruiz et al., 2001; Puig et al., 2003).

On the day after probe implants, rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and supplemental doses of the anesthetic were given when appropriate until the end of the experiments. After collecting baseline dialysate values in mPFC (four fractions), the aCSF used to perfuse the thalamic probes was replaced by one containing 1 mM bicuculline until the end of the experiments (twelve more fractions). Two hours after bicuculline application in the CM + MD nuclei, the test drug was applied by reverse dialysis in mPFC or given systemically to examine its effects on prefrontal dialysate 5-HT values.

The concentration of 5-HT in dialysate samples was determined by HPLC, as described (Adell and Artigas, 1998). 5-HT was separated using a Beckman (San Ramon, CA) 3-µm particle size column and detected with a Hewlett Packard 1049 electrochemical detector at +0.6 V. Retention time was between 3.5-4 min and the limit of detection was typically 1-2 fmol/sample.

The concentrations of drugs used herein were taken from previous studies on the mPFC-raphe circuit (Martín-Ruiz et al., 2001; Bortolozzi et al., 2003; Puig et al., 2003; Amargós-Bosch et al., 2004). Despite the *in vitro* nanomolar affinity of antipsychotics for 5-HT_{2A} and α_1-adrenoceptors, the use of concentrations in the micromolar range is required in *in vivo* microdialysis in order to significantly affect neurotransmitter receptors or transporters (e.g., Tao et al., 2000; Hervás et al., 2000; Sakai and Crochet, 2001; West and Grace, 2002). This is due to the fact that effective concentrations at receptors is limited by the low application rate (typically in the range of few nmol/h), the continuous clearance of applied drug by the CSF and the fact that a substantial number of receptors must be recruited to activate the mPFC-raphe circuit. The specificity of these high nominal concentrations is shown by the fact that similar concentrations of 5-HT_{1A} agonists are without effect in 5-HT_{1A} receptor knockout mice (Amargós-Bosch et al., 2004).

Systemic administration of drugs was carried out s.c. at the stated doses. Drugs were dissolved in saline or water (except clozapine, dissolved in acetic acid, and olanzapine, dissolved in HCl). The pH of clozapine and olanzapine solutions was brought up to ~6 with $NaHCO_3$ before injection. Vehicles did not significantly affect the 5-HT output in mPFC.

Data and statistical analysis

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

Results

Local S-AMPA application

Baseline dialysate 5-HT values in the mPFC of freely moving rats were 31 ± 1 fmol/fraction (n = 87). The application of 300 µM S-AMPA in mPFC produced a persistent and stable ~100% increase in the local 5-HT release ($p < 0.0001$, time effect; n = 5; Fig. 1). Control rats perfused with aCSF for the whole experiment did not show any alteration of 5-HT levels (n = 5). Although behavioral ratings have not been performed during microdialysis experiments, we noted that the application of S-AMPA in mPFC elicited an overt behavioral activation of the freely moving rats but not seizure activity.

The application of 300 µM of the classical (chlorpromazine, haloperidol) and atypical (clozapine, olanzapine) antipsychotics in mPFC completely reversed the 5-HT elevation induced by the local S-AMPA application ($p < 0.001$ for both drugs, repeated measures ANOVA; n = 4-5 rats/group) (Fig. 1). This effect was particularly remarkable for haloperidol, which reduced 5-HT values to levels comparable to those produced by the suppression of nerve impulse with tetrodotoxin (e.g., Martín-Ruiz et al., 2001). This concentration of haloperidol had been shown to produce a similar decrease in dialysate 5-HT when administered alone (Amargós-Bosch et al., 2003). A lower haloperidol concentration (100 µM) also reversed the effect of S-AMPA and returned dialysate 5-HT values to baseline ($p < 0.001$, repeated measures ANOVA; n = 4; Fig. 1). When given alone, this haloperidol concentration reduced maximally dialysate 5-HT to 43 ± 5 % of baseline.

The S-AMPA-induced elevation of 5-HT release in mPFC could be also reversed by the co-perfusion of the selective 5-HT_{2A} receptor and α_1-adrenoceptor antagonists M100907 and prazosin, respectively (Fig. 2). Given the high affinity of the classical

antipsychotics for dopamine D2 receptors, we examined the ability of the DA $D_{2/3}$ receptor antagonist raclopride to reverse the S-AMPA-evoked 5-HT release. Raclopride application in mPFC (100 µM; n = 7) elicited a partial reversal of the effect of S-AMPA which was statistically significant ($p < 0.05$, repeated measures ANOVA) but of smaller size than that produced by haloperidol or chlorpromazine (Fig. 2).

Contrary to the antipsychotic drugs, neither the anxiolytic drug diazepam ($GABA_A$ receptor modulator; 10 and 100 µM, n = 4 each) nor the antidepressant drug reboxetine (noradrenaline reuptake inhibitor; 50 µM, n = 5) counteracted the S-AMPA-induced elevation of 5-HT release when co-perfused in mPFC. Actually, reboxetine significantly enhanced the 5-HT release over S-AMPA alone ($p < 0.03$, repeated measures ANOVA). Figure 2 shows the summary effects of the antipsychotic drugs, M100907, prazosin, raclopride, diazepam and reboxetine on the S-AMPA-induced elevation of 5-HT release in mPFC.

Figure 3 shows the effect of the local (in mPFC) and systemic administration of classical and atypical antipsychotics, M100907, prazosin and raclopride (selective antagonists of $5\text{-}HT_{2A}$ receptors, α_1-adrenoceptors and dopamine $D_{2/3}$ receptors, respectively) on the basal 5-HT release in mPFC. The local concentrations were as those in Figure 2. Systemic (s.c.) doses were as follows: haloperidol 0.1 and 1 mg/kg, chlorpromazine, clozapine, olanzapine and raclopride, 1 mg/kg, and M100907 and prazosin, 0.3 mg/kg. All drugs, except raclopride, significantly reduced the spontaneous 5-HT release in mPFC compared to baseline ($p < 0.05$, repeated measures ANOVA). Likewise, the local (but not systemic) administration of M100907 significantly reduced 5-HT release in mPFC.

Disinhibition of thalamic afferents to mPFC

The baseline dialysate 5-HT value in the mPFC of chloral hydrate anesthetized rats was 27 ± 1 fmol/fraction (n = 131). This value was significantly lower ($p < 0.005$; Student's *t*-test)) than that of freely moving rats (31 ± 1 fmol/fraction). As previously observed (Martín-Ruiz et al., 2001; Puig et al., 2003), the local application of bicuculline in the CM + MD nuclei of the thalamus induced a sustained elevation of the 5-HT release in mPFC which was very similar to that produced by S-AMPA application (maximal effect 200 ± 10 % of baseline; Fig. 4). The concurrent application of haloperidol in mPFC (300 µM, n = 4) completely reversed the 5-HT elevation and reduced dialysate 5-HT to a maximal value of 15 ± 2 % of baseline ($p < 0.001$, repeated

measures ANOVA). The application of chlorpromazine in mPFC (300 μM, n = 6) also reversed significantly the increase in 5-HT release produced by thalamic disinhibition and lowered 5-HT values to 77 ± 4% of baseline ($p < 0.001$, repeated measures ANOVA) (Fig. 4A). Likewise, the application of the atypical antipsychotics clozapine and olanzapine (300 μM each; n = 4 and 6, respectively) significantly reversed the 5-HT increase produced by thalamic disinhibition ($p < 0.001$ for both agents; repeated measures ANOVA) (Fig. 4B)

As previously observed for the local application of S-AMPA (Fig. 2) the local application of M100907 (300 μM, n = 5) and prazosin (100 μM, n = 5) in mPFC also reversed the 5-HT elevation in mPFC induced by the thalamic disinhibition (Fig. 4C). The application of raclopride (100 μM, n = 7) induced a smaller but statistically significant attenuation of the effect of thalamic disinhibition ($p < 0.001$, repeated measures ANOVA) (Fig. 4C).

We subsequently examined the effect of the systemic administration of classical and atypical antipsychotic drugs on the elevation of 5-HT release induced by thalamic disinhibition. A saline s.c. injection (n = 5) did not alter the effect of thalamic disinhibition on cortical 5-HT release (Fig. 5). However, the s.c. administration of 1 mg/kg of all antipsychotic drugs significantly attenuated the effect of thalamic disinhibition and returned 5-HT values to baseline ($p < 0.001$ for all drugs, repeated measures ANOVA). A lower haloperidol dose (0.1 mg/kg s.c., n = 4) induced a partial but statistically significant attenuation of the increase in 5-HT release ($p < 0.001$, repeated measures ANOVA) (Fig. 6).

The s.c. administration of M100907 (0.3 mg/kg, n = 5) and prazosin (0.3 mg/kg, n = 5) but not raclopride (1 mg/kg, n = 5) significantly reversed the effect of thalamic disinhibition on 5-HT release in mPFC (Fig. 5C). Figure 6 shows the summary effects of the local and systemic administration of antipsychotic drugs and receptor antagonists on the increase of 5-HT release in mPFC produced by thalamic disinhibition.

Discussion

Psychotic symptoms and cortical hyperglutamatergia

Numerous reports suggest that schizophrenia is associated with an abnormal glutamatergic transmission in PFC (Lewis and Lieberman, 2000; Tsai and Coyle, 2002;

Harrison and Lewis, 2003; Krystal et al., 2003; Moghaddam and Krystal, 2003). The reduced spine density and synaptic proteins, reduced glutamatergic markers and hypofrontality (Andreasen et al., 1997) suggest a decreased glutamatergic activity. However, hypofrontality appears to be mainly associated with negative symptoms (Potkin et al., 2002) and other studies have reported normal or high cortical activity in schizophrenic patients, particularly during hallucinations (Catafau et al., 1994; Dierks et al., 1999; Shergill et al., 2000). Likewise, proton magnetic resonance studies reported higher than normal glutamate/glutamine levels in PFC of neuroleptic-naïve schizophrenic patients (Bartha et al., 1997; Théberge et al., 2002). Concurrently, a reduction of GABAergic markers occurs in the PFC of schizophrenic patients (Lewis et al., 2005) which possibly results in a decrease of local inhibitory inputs and increased glutamatergic transmission. Moreover, NMDA receptor antagonists, used as pharmacological models of schizophrenia, increase glutamate outflow (Moghaddam et al., 1997; Ceglia et al., 2004) and pyramidal cell firing in rat mPFC (Suzuki et al., 2002; Jackson et al., 2004; Jodo et al., 2005). Finally, LY-254740, a mGluR2/3 agonist abolished the deleterious effects of ketamine on working memory (Krystal et al., 2005), an effect that may result from a reduction of glutamate release. Collectively, these data suggest that psychotic symptoms may be associated with an increased glutamatergic transmission in PFC, yet affective/negative symptoms may involve distinct neurotransmitter abnormalities.

Experimental models used

In agreement with this view, we tested the effects of conventional and atypical antipsychotics in two experimental conditions evoking an increased glutamatergic tone on mPFC neurons: a) local activation of AMPA receptors by S-AMPA application, and b) thalamic disinhibition. The latter procedure was achieved by applying bicuculline in the CM + MD nuclei, which project densely to mPFC and make synapses with pyramidal neuron spines (Berendse and Groenewegen, 1991; Kuroda et al., 1998; Van der Werf et al., 2002). Consistent with this connectivity, MD stimulation increased AMPA-mediated responses in mPFC pyramidal neurons (Pirot et al., 1994). Also, thalamic disinhibition increased c-fos expression in mPFC (Erdtsieck-Ernste et al., 1995; Bubser et al., 1998), as well as the activity of pyramidal neurons and 5-HT release in mPFC (Puig et al., 2003). The latter effect was antagonized by mGluR2/3 agonists and NBQX application in mPFC. Likewise, the increase in pyramidal cell firing was totally abolished by the selective mGluR2/3 agonist LY 379268 (Puig et al., 2003). These observations

suggest that thalamic disinhibition enhances glutamate release in mPFC, which results in an increased activation of AMPA receptors.

We employed the extracellular 5-HT concentration in mPFC as an *in vivo* index of the overall activity of PFC neurons activated by these procedures. This experimental approach is based on several observations (Fig. 7). First, anatomical and electrophysiological data indicate the presence of a very close relationship between the mPFC and the midbrain raphe nuclei (see introduction). The electrical stimulation of the mPFC elicited profound changes in most DR 5-HT neurons and vice-versa (Celada et al., 2001; Puig et al., 2005). Second, the activation of excitatory (5-HT_{2A}, α_1-adrenergic, AMPA) or inhibitory (5-HT_{1A}, μ-opioid, mGluR2/3) receptors in mPFC increased and decreased, respectively, the local 5-HT release (Celada et al., 2001; Martín-Ruiz et al., 2001; Puig et al., 2003; Amargós-Bosch et al., 2003, 2004). In particular, increasing PFC glutamatergic transmission by electrical stimulation or disinhibition of the CM+MD nuclei as well as blockade of glutamate reuptake in mPFC increased 5-HT release in mPFC (Martín-Ruiz et al., 2001; Puig et al., 2003). Third, the change in local 5-HT release produced by these procedures evoked a similar change in 5-HT cell firing or 5-HT release in the DR (Celada et al., 2001; Martín-Ruiz et al., 2001; Amargós-Bosch et al., 2003). Fourth, NMDA receptor antagonists, which increase pyramidal cell firing and glutamate release in mPFC, also increase 5-HT neuron activity (Lejeune et al., 1994) and 5-HT release in mPFC (Martin et al., 1998; Ceglia et al., 2004; Amargós-Bosch et al., 2006) an effect blocked by local NBQX application (X. López-Gil et al., in preparation). Altogether, these observations suggest that the 5-HT release in mPFC can reliably monitor *in vivo* local changes in excitatory transmission.

Notwithstanding these observations supporting the involvement of long loops to midbrain, a local effect of glutamate or S-AMPA cannot be excluded. Indeed, S-AMPA increased the local 5-HT release in areas not feeding back to the raphe (e.g., striatum; Maione et al., 1997) and presynaptic AMPA receptors modulate glutamate and GABA release in various CNS areas (Patel et al., 2001; Satake et al., 2000; Schenk et al., 2003, 2005). This raises the possibility that such receptors may be also present in 5-HT axons. In such a case, an increased glutamatergic transmission in mPFC might result in a local enhancement of 5-HT release. However, since none of the receptors for which antipsychotics exhibit high affinity (in particular 5-$HT_{2A/2C}$ and α_1-adrenergic) is present in 5-HT terminals, the observed drug effects must necessarily involve the blockade of postsynaptic receptors in prefrontal neurons (either pyramidal or GABAergic).

Effect of antipsychotic drugs

Classical and atypical antipsychotics reversed the increase in 5-HT release in mPFC produced by local S-AMPA application and thalamic disinhibition. This effect cannot be accounted for by a direct competition at iGluRs (Bymaster et al., 1996; Arnt and Skarsfeldt, 1998) and may likely result from summation of effects on prefrontal neurons. This view is supported by the reduction of the 5-HT output when drugs were applied alone, an observation which also suggests that the activity of raphe 5-HT neurons is tonically controlled by the mPFC. Indeed, pyramidal neurons integrate a large number of excitatory, inhibitory and modulatory signals and express most aminergic receptors (see introduction) for which antipsychotics have high affinity.

Interestingly, the antipsychotic effect 1) was common to classical and atypical drugs, 2) was observed after local (in mPFC) and systemic administration, and 3) was independent of the experimental model used (S-AMPA application or thalamic disinhibition). Moreover, neither diazepam nor reboxetine reversed the effect of S-AMPA on 5-HT release, emphasizing the specificity of the observed effect.

M100907 and prazosin also cancelled the effect of S-AMPA and thalamic disinhibition on dialysate 5-HT, which supports the involvement of 5-HT_{2A} and/or α_1-adrenergic receptors. In contrast, raclopride (dopamine $D_{2/3}$ antagonist) was partly or totally ineffective. Indeed, the excitatory effect of dopamine on PFC pyramidal neurons was insensitive to the $D_{2/3}$ receptor antagonist (-)sulpiride (Ceci et al., 1999), in agreement with the predominant role of D1 receptors in mediating the effect of dopamine on cortical transmission (Gonzalez-Islas and Hablitz, 2003; O'Donnell, 2003). This suggests that D2 receptor blockade does not play a major role in the observed effects despite full occupancy of D2 receptors by conventional antipsychotics at the doses used (Schotte et al., 1993).

The M100907 and prazosin reversal seems to argue against the specificity of the observed effect, since none is an antipsychotic drug. However, prazosin addition enhanced the antipsychotic effect of raclopride in rats (Wadenberg et al., 2000) and both M100907 and prazosin have been reported to block behavioral effects of hallucinogenic compounds such as DOI or non-competitive NMDA receptor antagonists in rats (Schreiber et al., 1995; Dursun and Handley, 1996M Varty et al., 1999; Mirjana et al., 2004). Hence, although M100907 and prazosin do not have full antipsychotic activity, blockade of 5-HT_{2A} receptors and α_1-adrenoceptors may contribute to the therapeutic

effects of classical and atypical antipsychotics. Hence, lacking a full cellular correlate of the present neurochemical observations, our interpretation of the data is that antipsychotic drugs may reverse the increase in glutamate- and S-AMPA-stimulated prefrontal activity through the blockade of postsynaptic 5-HT_{2A} and/or α_1-adrenoceptors in mPFC (Fig. 7B). This effect would attenuate excitatory transmission in mPFC and consequently, 5-HT release. This interpretation is supported by the complex interplay between 5-HT_{2A} receptors, α_1-adrenoceptors and glutamatergic transmission in mPFC.

Indeed, 5-HT_{2A} receptors are abundantly expressed by pyramidal neurons in mPFC (Santana et al., 2004) and mediate the excitatory actions of 5-HT *in vivo* (Amargós-Bosch et al., 2004; Puig et al., 2005) and *in vitro* (Aghajanian and Marek, 1999). The latter effects are blocked by AMPA antagonists and mGluR2/3 agonists. Likewise, the increases in pyramidal cell firing and 5-HT release in mPFC induced by the hallucinogen DOI (5-$HT_{2A/2C}$ agonist) were cancelled by AMPA receptor blockade and mGluR2/3 activation (Martín-Ruiz et al., 2001; Puig et al., 2003). Conversely, the effect of S-AMPA on 5-HT release was blocked by M100907 (Amargós-Bosch et al., 2003; this study). Furthermore, some behavioral effects of NMDA receptor antagonists are blocked by 5-HT_{2A} receptor antagonists (e.g., Varty et al., 1999; Mirjana et al., 2004) although it is controversial whether this effect implies a reduction of glutamate overflow in mPFC (Adams and Moghaddam, 2001; Ceglia et al., 2004).

On the other hand, α_1-adrenoceptors are also expressed in PFC (Pieribone et al., 1994; Day et al., 1997; Domyancic and Morilak, 1997) and, in common with 5-HT_{2A} receptors, their activation increases the activity of pyramidal neurons in mPFC (Araneda and Andrade, 1991; Marek and Aghajanian, 1999). α_1-Adrenoceptor blockade has been suggested to participate in the therapeutic action of antipsychotics in acute schizophrenia (Svensson, 2003), and prazosin augmented the effect of raclopride in a model of antipsychotic activity (Wadenberg et al., 2000). Interestingly, α_1-adrenoceptors and 5-HT_{2A} receptors share signal transduction pathways and their respective mRNAs are massively co-expressed in PFC (Santana et al., unpublished observations) suggesting a convergence of excitatory serotonergic and noradrenergic signals on PFC neurons. Hence, classical and atypical antipsychotics may attenuate the prefrontal activation in the two experimental models used, as well as in basal conditions (Fig. 3) by blocking such receptors. The *ex vivo* ED_{50} values of clozapine for 5-HT_2 and α_1-adrenoceptor occupancy in rat brain are 1.3 and 0.58 mg/kg s.c., respectively, whereas the

corresponding values for haloperidol are 2.6 and 0.4 mg/kg s.c. (Schotte et al., 1993). Similar occupancies have been reported elsewhere (Chaki et al., 1999). Therefore, it is likely that both compounds produce substantial occupancy of α_1-adrenoceptors at 1 mg/kg whereas clozapine and olanzapine can additionally occupy 5-HT_2 receptors. It is noteworthy that 1mg/kg s.c. clozapine is amongst the lowest doses of this compound proven effective in different pharmacological or behavioral models.

In summary, both classical (chlorpromazine and haloperidol) and atypical antipsychotics (clozapine and olanzapine) counteract the increase in 5-HT release produced by exogenous (S-AMPA application) and endogenous (thalamic disinhibition) increases in prefrontal glutamatergic transmission. This effect possibly involved the blockade of α_1-adrenergic and/or 5-HT_{2A} receptors, for which these drugs display high affinity. Since pyramidal neurons in PFC project to ventral striatum, an attenuation of prefrontal excitatory inputs onto accumbal neurons might add to the blockade of DA D2 receptors in this area, which is considered to underlie antipsychotic action.

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Figure legends

Figure 1. The application of S-AMPA (300 μM) by reverse dialysis in mPFC enhanced the local 5-HT release (n = 5). The co-perfusion of the classical antipsychotics chlorpromazine (CPZ, n = 5) and haloperidol (HAL 300 μM, n = 5; HAL 100 μM, n = 4) (panel A) or the atypical antipsychotics clozapine (CLZ, n = 4) and olanzapine (OLZ, n = 4) (panel B) fully reversed the S-AMPA-induced elevation in 5-HT release in mPFC. Bars indicate the period of drug application. See text for statistical analysis.

Figure 2. Bar diagram showing the effects of various drugs on the S-AMPA-evoked 5-HT release in mPFC. The black bar shows the effect of the perfusion of S-AMPA alone. The rest of bars show average values of the last three fractions (1 hr) of co-perfusion of each drug in combination with S-AMPA using the experimental procedure shown in figure 1. In addition to classical (haloperidol, HAL; 100 and 300 μM; chlorpromazine, CPZ 300 μM) and atypical antipsychotics (clozapine, CLZ and olanzapine, OLZ, both at 300 μM) the selective 5-HT_{2A} and α_1-adrenoceptor antagonists M100907 (300 μM) and prazosin (100 μM) respectively, completely reversed the effect of S-AMPA (the data of M100907 and prazosin were taken from Amargós-Bosch et al., 2003). In contrast, the dopamine $D_{2/3}$ receptor antagonist raclopride (RAC, 100 μM; n = 7) exerted a partial reversal whereas the anxiolytic drug diazepam (DZP, 10 and 100 μM, n = 4 each) and the antidepressant drug reboxetine (RBX, 50 μM, n = 5) did not attenuate the S-AMPA-evoked 5-HT release. Actually, reboxetine significantly enhanced the S-AMPA-induced elevation in 5-HT release. $^{a}p < 0.05$ vs. baseline; $^{*}p < 0.05$ vs. S-AMPA alone.

Figure 3. A) Effect of the local administration of various drugs on the basal 5-HT release in mPFC. Drugs were applied at varying concentrations, as in Figure 2. Bars show one-hour average 5-HT values expressed as percentage of baseline. B) Effect of the systemic administration of various drugs on the basal 5-HT release in mPFC. Doses used were haloperidol (HAL) 0.1 and 1 mg/kg, chlorpromazine (CPZ), clozapine (CZP) and olanzapine (OZP), 1 mg/kg, M100907 (MDL) and prazosin (PRA), 0.3 mg/kg and raclopride (RAC), 1 mg/kg. $^{*}p < 0.05$ vs. baseline.

Figure 4. The application of 1 mM bicuculline by reverse dialysis in the centromedial and mediodorsal nuclei of the thalamus (CM + MD) increases the 5-HT release in mPFC of chloral hydrate anesthetized rats (n = 7). The co-perfusion of 300 μM of the classical (panel A; HAL, haloperidol, n = 4; CPZ, chlorpromazine, n = 6) or atypical antipsychotics (panel B; CLZ, clozapine, n = 4; OLZ, olanzapine, n = 6) reversed this effect. Likewise, the local application in mPFC of 300 μM M100907 or 100 μM prazosin (n = 5 each; panel C) in mPFC reversed the 5-HT elevation induced by the thalamic disinhibition. However, the application of 100 μM raclopride (n = 7) exerted only a partial, though significant attenuation of the effect of thalamic disinhibition on prefrontal 5-HT release. Bars indicate the period of drug application in each area. See text for statistical details.

Figure 5. The s.c. administration of vehicle (n = 5; filled circles) did not alter the increase in 5-HT release produced by disinhibition of thalamic afferents to mPFC. In contrast, the administration of 1 mg/kg of classical (chlorpromazine, n = 7; haloperidol; n = 4; panel A) and atypical antipsychotics (clozapine, n = 5; olanzapine, n = 4; panel B) totally reversed the increase in 5-HT release produced by thalamic disinhibition. Likewise, the s.c. administration of the selective 5-HT_{2A} and α_1-adrenoceptor antagonists M100907 and prazosin, respectively (0.3 mg/kg; n = 5 each; panel C) attenuated the effect of thalamic disinhibition on prefrontal 5-HT release. However, the s.c. administration of the selective $D_{2/3}$ receptor antagonist raclopride (1 mg/kg) did not alter significantly 5-HT release. Arrows show the time of drug injection. See text for statistical analysis.

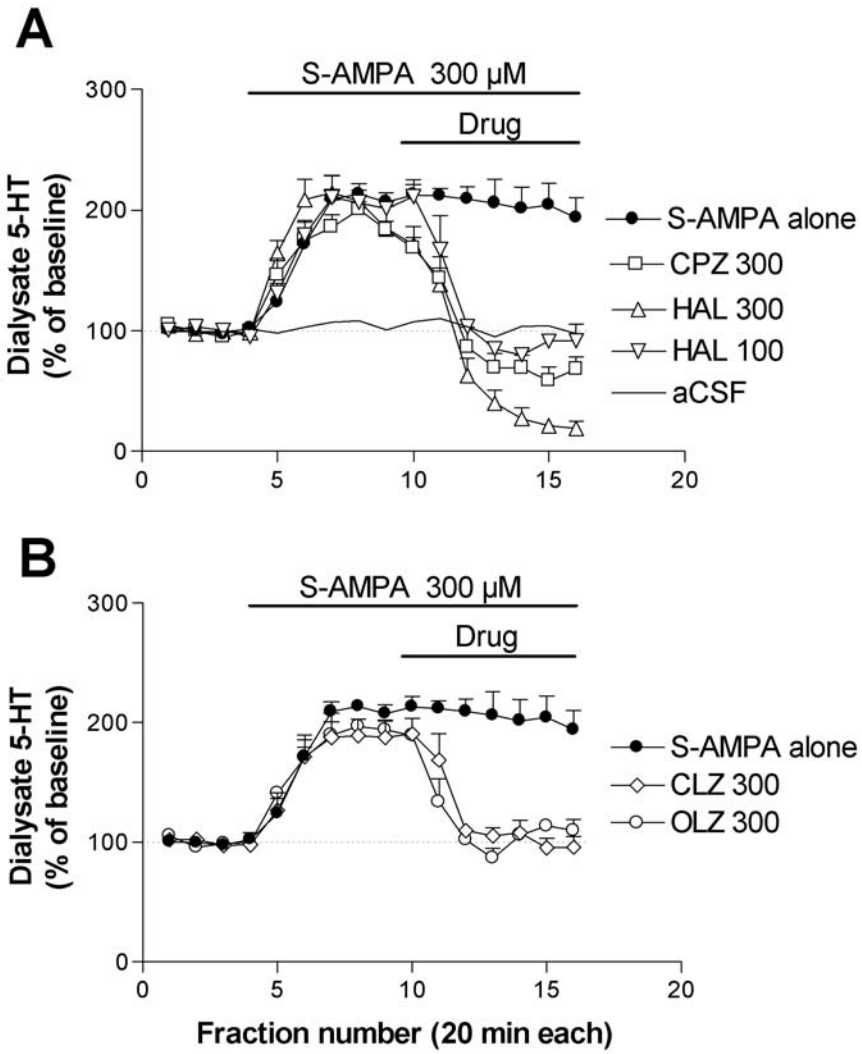
Figure 6. Bar diagram showing the effects of various drugs on the 5-HT release in mPFC evoked by the application of bicuculline in the mediodorsal and centromedial nuclei of the thalamus. Panel A shows the effects of drugs applied locally in mPFC, as shown in figure 4. Panel B shows the effects of systemically administered drugs, as in figure 5. Black bars show the average effect of the thalamic disinhibition in the control groups shown in Figs. 4 and 5. The rest of bars show average values of the last three fractions (1 hr) of administration (local or systemic) of each drug in combination with the thalamic disinhibition following the experimental procedure shown in figures 4 and 5. All drugs reduced significantly the increase in 5-HT when they were locally applied in mPFC or were systemically administered, except raclopride. This agent exerted a

moderate but significant reduction of 5-HT release after its local application but did not reduce 5-HT after systemic administration. Drug concentrations in A are as follows: haloperidol, chlorpromazine, clozapine, olanzapine and M100907 (300 μM), prazosin (100 μM) and raclopride (100 μM). Subcutaneous doses in B are 1 mg/kg for all antipsychotic drugs (plus 0.1 mg/kg haloperidol), 0.3 mg/kg for M100907 and prazosin and 1 mg/kg for raclopride. Bars show one-hour average 5-HT values expressed as percentage of baseline. $^{a}p < 0.05$ vs. baseline; $*p < 0.05$ vs. thalamic disinhibition alone.

Figure 7. Schematic diagrams of the experimental model used and the putative action of antipsychotic drugs in prefrontal cortex (PFC). **A**) The local application of S-AMPA in mPFC by reverse dialysis or the disinhibition of thalamic afferents to mPFC by applying bicuculline in the mediodorsal/centromedial (MD/CM) nuclei of the thalamus increased the extracellular 5-HT concentration in mPFC. Previous observations indicate that this effect can be blocked by the local application (in mPFC) of NBQX (AMPA receptor antagonist), mGluR II agonists (LY 379268 and 1S, 3S-ACPD) and DAMGO, a μ-opioid agonist (Puig et al., 2003). However, local application of MK-801 (non-competitive NMDA receptor antagonist) could not block this effect (Martín-Ruiz et al., 2001) suggesting the predominance of AMPA receptors in the evoked 5-HT release. The activation of pyramidal neurons produced by S-AMPA and thalamic disinhibition (the latter procedure increased 15-20-fold pyramidal cell firing; Puig et al., 2003) may be translated into a change in 5-HT release via distal afferents to the dorsal and median raphe nuclei (DR/MnR) or through local activation of putative AMPA receptors on 5-HT terminals. There is ample evidence on the existence of descending excitatory projections from mPFC to DR/MnR and functional control of 5-HT neurons by the mPFC (see Introduction). However, the presence of presynaptic AMPA receptors has been documented in glutamate and GABA but not in serotonergic axons. **B**) The administration of conventional and atypical antipsychotics (both systemically and in mPFC) occupies 5-HT_{2A} and α_1-adrenoceptors, which are abundantly expressed in PFC. The blockade of these receptors reverses the excitatory actions of 5-HT and noradrenaline on pyramidal neurons (Araneda and Andrade, 1991; Marek and Aghajanian, 1999; Amargós-Bosch et al., 2004). This effect may result in an attenuation of the activity of pyramidal neurons, and hence, of the glutamate-evoked 5-HT release in mPFC. It remains to be shown whether this effect is translated into a reduction of excitatory inputs in other areas relevant for the

antipsychotic action, such as nucleus accumbens, to which also pyramidal neurons in mPFC project. An additional action of antipsychotic drugs at raphe α_1-adrenoceptors to reduce 5-HT release cannot be disregarded when these compounds were systemically administered, since the activity of 5-HT neurons is tonically dependent on their activation. However, these receptors should not participate in the local effects of antipsychotics nor in local and systemic effects of the selective 5-HT_{2A} antagonist M100907.

Figure 1


A
S-AMPA 300 µM
Drug
Dialysate 5-HT (% of baseline)
300
200
100
0
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5
10
15
20
S-AMPA alone
CPZ 300
HAL 300
HAL 100
aCSF
B
S-AMPA 300 µM
Drug
Dialysate 5-HT (% of baseline)
300
200
100
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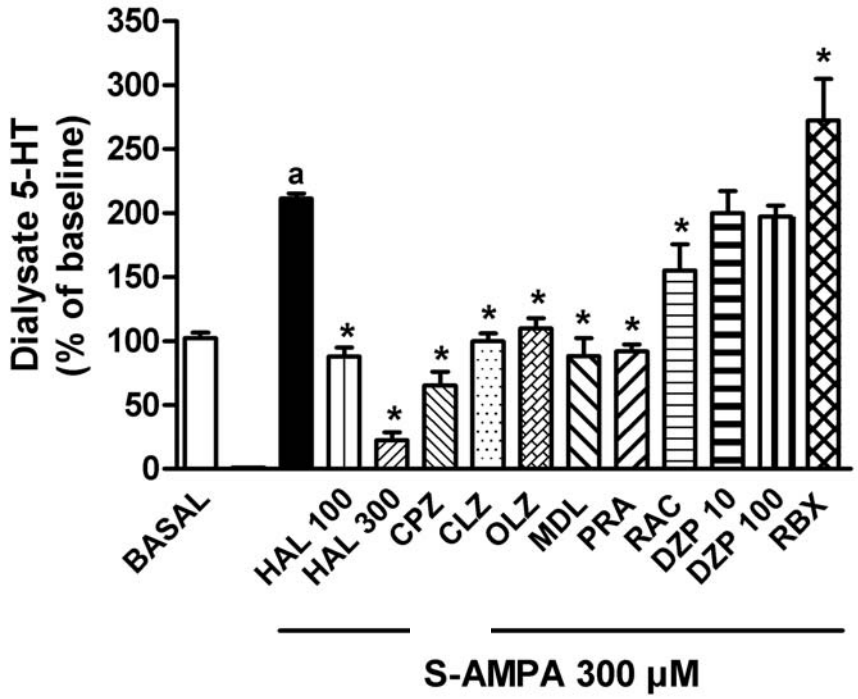
0
5
10
15
20
Fraction number (20 min each)
S-AMPA alone
CLZ 300
OLZ 300

Figure 2


Dialysate 5-HT
(% of baseline)
350
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HAL 100
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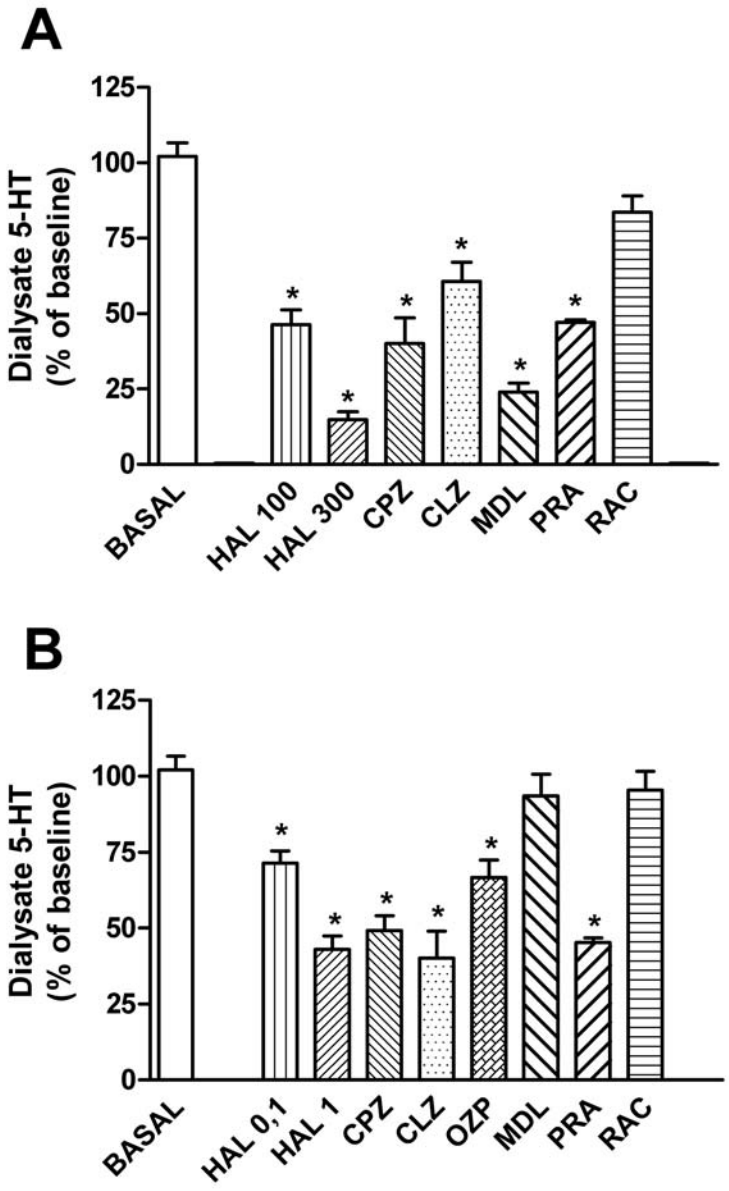
RAC
DZP 10
DZP 100
RBX
S-AMPA 300 µM

Figure 3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Dialysate 5-HT (% of baseline)
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Dialysate 5-HT (% of baseline)
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MDL
PRA
RAC

Figure 4

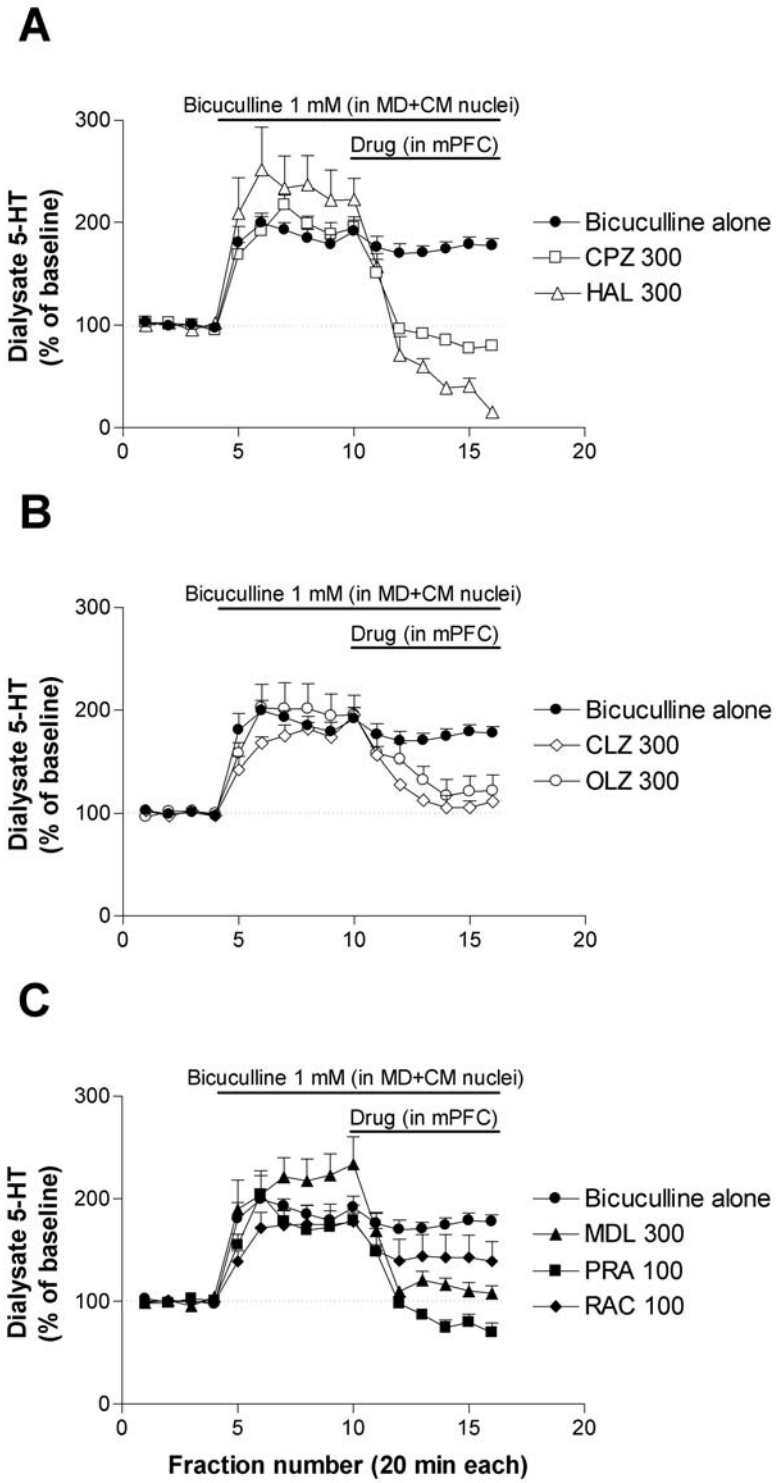

A
Bicuculline 1 mM (in MD+CM nuclei)
Drug (in mPFC)
Dialysate 5-HT (% of baseline)
300
200
100
0
0
5
10
15
20
Bicuculline alone
CPZ 300
HAL 300
B
Bicuculline 1 mM (in MD+CM nuclei)
Drug (in mPFC)
Dialysate 5-HT (% of baseline)
Bicuculline alone
CLZ 300
OLZ 300
C
Bicuculline 1 mM (in MD+CM nuclei)
Drug (in mPFC)
Dialysate 5-HT (% of baseline)
Bicuculline alone
MDL 300
PRA 100
RAC 100
Fraction number (20 min each)

Figure 5

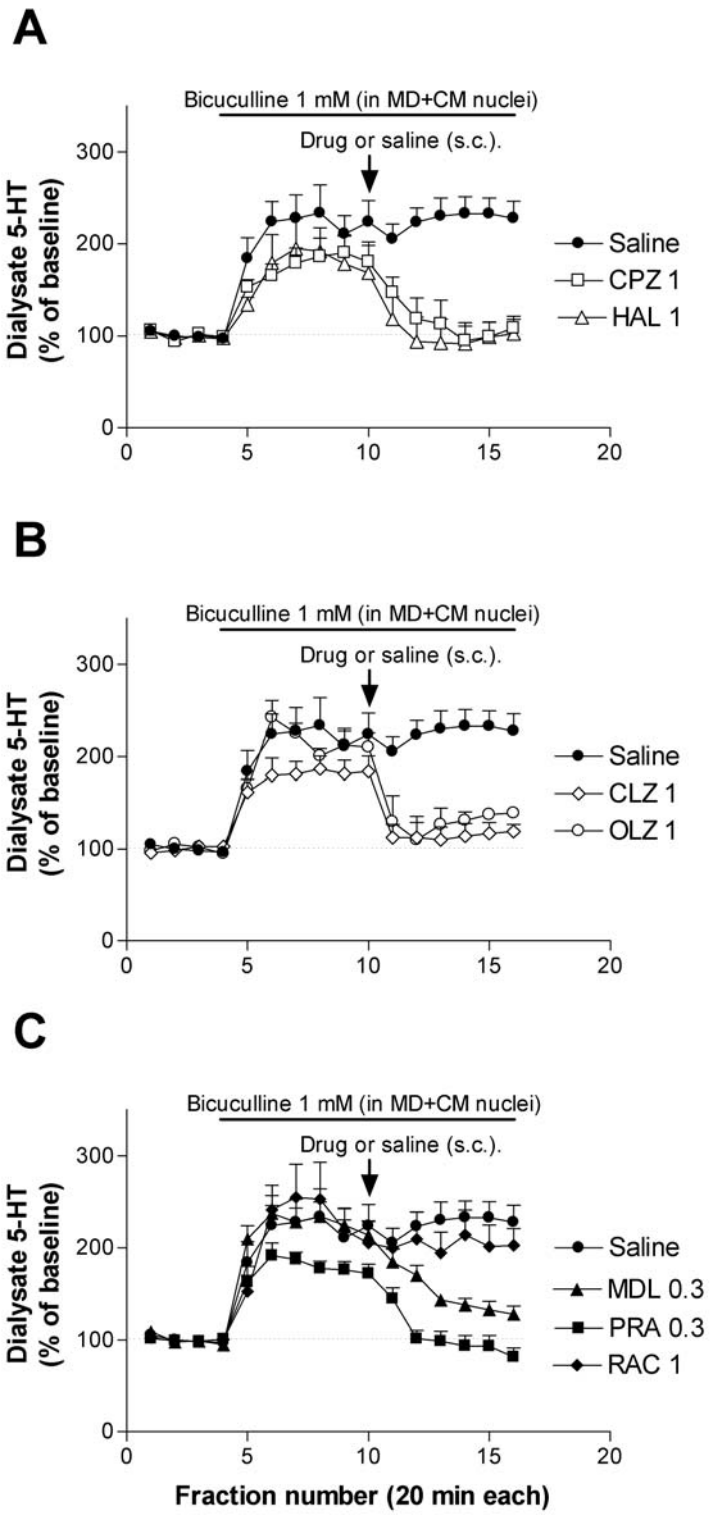

A
Bicuculline 1 mM (in MD+CM nuclei)
Drug or saline (s.c.).
Dialysate 5-HT (% of baseline)
300
200
100
0
0
5
10
15
20
Saline
CPZ 1
HAL 1
B
Bicuculline 1 mM (in MD+CM nuclei)
Drug or saline (s.c.).
Dialysate 5-HT (% of baseline)
Saline
CLZ 1
OLZ 1
C
Bicuculline 1 mM (in MD+CM nuclei)
Drug or saline (s.c.).
Dialysate 5-HT (% of baseline)
Saline
MDL 0.3
PRA 0.3
RAC 1
Fraction number (20 min each)

Figure 6

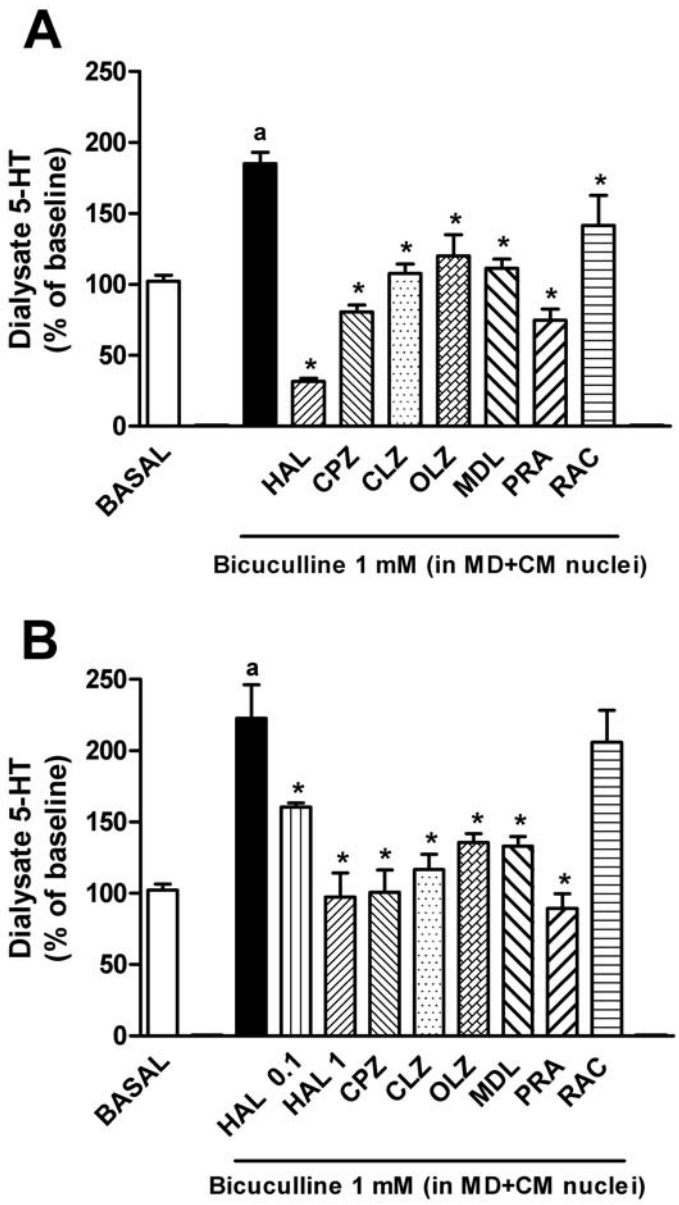
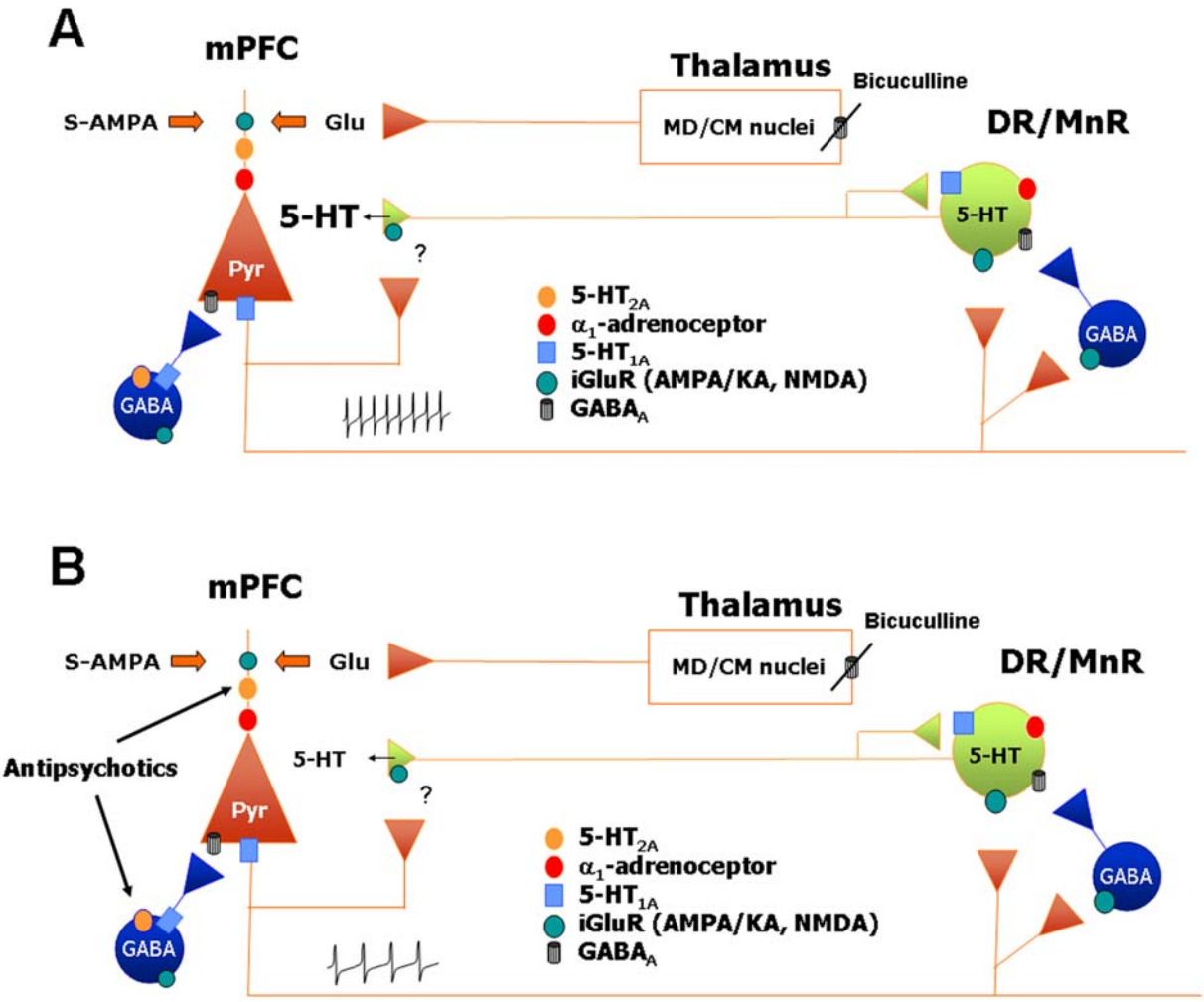

A
Dialysate 5-HT (% of baseline)
250
200
150
100
50
0
a
*
BASAL
HAL
CPZ
CLZ
OLZ
MDL
PRA
RAC
Bicuculline 1 mM (in MD+CM nuclei)
B
Dialysate 5-HT (% of baseline)
250
200
150
100
50
0
a
*
BASAL
HAL 0.1
HAL 1
CPZ
CLZ
OLZ
MDL
PRA
RAC
Bicuculline 1 mM (in MD+CM nuclei)

Figure 7


A
mPFC
Thalamus
Bicuculline
S-AMPA
Glu
MD/CM nuclei
DR/MnR
5-HT
5-HT
?
Pyr
GABA
GABA
5-HT2A
α1-adrenoceptor
5-HT1A
iGluR (AMPA/KA, NMDA)
GABAA
B
mPFC
Thalamus
Bicuculline
S-AMPA
Glu
MD/CM nuclei
DR/MnR
Antipsychotics
5-HT
5-HT
?
Pyr
GABA
GABA
5-HT2A
α1-adrenoceptor
5-HT1A
iGluR (AMPA/KA, NMDA)
GABAA