

# Clozapine and Haloperidol Differently Suppress the MK-801-Increased Glutamatergic and Serotonergic Transmission in the Medial Prefrontal Cortex of the Rat

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The administration of noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine and ketamine has been shown to increase the extracellular concentration of glutamate and serotonin (5-HT) in the medial prefrontal cortex (mPFC). In the present work, we used *in vivo* microdialysis to examine the effects of the more potent noncompetitive NMDA receptor antagonist, MK-801, on the efflux of glutamate and 5-HT in the mPFC, and whether the MK-801-induced changes in the cortical efflux of both transmitters could be blocked by clozapine and haloperidol given systemically or intra-mPFC. The systemic, but not the local administration of MK-801, induced an increased efflux of 5-HT and glutamate, which suggests that the NMDA receptors responsible for these effects are located outside the mPFC, possibly in GABAergic neurons that tonically inhibit glutamatergic inputs to the mPFC. The MK-801-induced increases of extracellular glutamate and 5-HT were dependent on nerve impulse and the activation of mPFC AMPA/kainate receptors as they were blocked by tetrodotoxin and NBQX, respectively. Clozapine and haloperidol blocked the MK-801-induced increase in glutamate, whereas only clozapine was able to block the increased efflux of 5-HT. The local effects of clozapine and haloperidol paralleled those observed after systemic administration, which emphasizes the relevance of the mPFC as a site of action of these antipsychotic drugs in offsetting the neurochemical effects of MK-801. The ability of clozapine to block excessive cortical 5-HT efflux elicited by MK-801 might be related to the superior efficacy of this drug in treating negative/cognitive symptoms of schizophrenia. *Neuropsychopharmacology* (2007) **32**, 2087–2097; doi:10.1038/sj.npp.1301356; published online 14 March 2007

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## INTRODUCTION

The acute administration of noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine has been considered as a pharmacological model of schizophrenia because these drugs can evoke positive and negative symptoms as well as characteristic cognitive deficits that closely resemble those of the illness (Javitt and Zukin, 1991; Krystal *et al*, 1994; Malhotra *et al*, 1994; Newcomer *et al*, 1999). For this reason, schizophrenia has been associated with NMDA receptor hypofunction (Javitt and Zukin, 1991; Tamminga, 1998). In rats, PCP and the more potent and selective noncompetitive NMDA antagonist, dizocilpine (MK-801), bring about hyperlocomotion and stereotypies (Hertel *et al*,

1996; Adams and Moghaddam, 2001; Homayoun *et al*, 2004). These behaviors are thought to result from increased dopaminergic and serotonergic activities (Giros *et al*, 1996; Jentsch *et al*, 1998; Lucki, 1998), and have been potentially related to positive symptoms of schizophrenia (Moghaddam and Adams, 1998; Jentsch and Roth, 1999; Mohn *et al*, 1999). Furthermore, the behavioral deficits observed in humans and rodents are distinctly responsive to clozapine (Bakshi *et al*, 1994; Maurel-Remy *et al*, 1995; Malhotra *et al*, 1997). Acute NMDA receptor antagonism has also been reported to increase the release of glutamate (Moghaddam *et al*, 1997; Adams and Moghaddam, 2001; Lorrain *et al*, 2003), dopamine (Moghaddam and Adams, 1998; Mathé *et al*, 1999; Schmidt and Fadaye, 1996), and serotonin (5-HT) (Martin *et al*, 1998; Millan *et al*, 1999; Adams and Moghaddam, 2001; Amargós-Bosch *et al*, 2006) in the medial prefrontal cortex (mPFC) of rats. These effects are coincident with an enhanced spontaneous firing rate of putative pyramidal neurons of the mPFC (Suzuki *et al*, 2002; Jackson *et al*, 2004). The increase in cortical glutamatergic transmission elicited through the blockade of an excitatory glutamate receptor (NMDA) may seem contradictory.

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However, it has been postulated that noncompetitive NMDA receptor antagonists may actually inhibit GABAergic inputs to glutamatergic neurons in the mPFC (Olney and Farber, 1995; Moghaddam *et al*, 1997; Krystal *et al*, 2003). In fact, GABAergic interneurons in limbic cortex and hippocampus are extremely responsive to NMDA receptor antagonists in comparison with pyramidal neurons (Grunze *et al*, 1996; Li *et al*, 2002), and PCP is able to reduce cortical GABAergic function (Yonezawa *et al*, 1998). It remains to be determined whether this feature can be applied to other GABAergic cells throughout the brain. Thus, theoretically, this glutamatergic disinhibition could occur in the mPFC and/or in areas sending glutamatergic projections to the mPFC. However, the intra-mPFC administration of PCP or ketamine was unable to elevate cell firing and locomotion (Suzuki *et al*, 2002) or increase extracellular 5-HT (Amargós-Bosch *et al*, 2006). Therefore, it appears that the NMDA receptors responsible for these effects are located outside the mPFC, possibly in GABA neurons that would tonically inhibit glutamatergic efferents in areas that project densely to mPFC, such as hippocampus, thalamus, or amygdala.

In a previous work, we showed that acute PCP and ketamine enhanced extracellular 5-HT in the mPFC, and that this effect was blocked by systemic clozapine and olanzapine, but not by haloperidol (Amargós-Bosch *et al*, 2006). In the present study, we have tested the hypothesis that MK-801 would also increase serotonergic and glutamatergic transmission in the mPFC and examined the effects of clozapine and haloperidol, applied both systemically and intra-mPFC, on these neurochemical changes.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (Iffa-Credo, Lyon, France) weighing 250–280 g were used. They were maintained on a 12 h light/dark cycle (lights on at 0700) and housed three per cage before surgery and individually after surgery. Food and water were always freely available. All experimental procedures were carried out in strict accordance with European Communities Council Directive on ‘Protection of Animals Used in Experimental and Other Scientific Purposes’ of 24 November 1986 (89/609/EEC), and were approved by the Institutional Animal Care and Use Committees. To reduce the influence of between-day variations on drug effects, most experimental groups were not completed at once, but along the duration of the whole work instead.

### Drugs and Reagents

Dizocilpine maleate (MK-801), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX), 5-hydroxytryptamine oxalate, *o*-phthaldialdehyde (OPA) reagent (containing 1 mg OPA per ml solution with 2-mercaptoethanol as the sulphhydryl moiety), and glutamate were purchased from Sigma-Aldrich (Tres Cantos, Spain). Clozapine and tetrodotoxin (TTX) were from Tocris (Bristol, UK). Haloperidol was obtained as an injectable solution (5 mg/ml dissolved in 0.5% lactic acid) from Laboratorios Esteve (Barcelona,

Spain). Citalopram hydrobromide was generously donated by H Lundbeck A/S (Copenhagen-Valby, Denmark).

MK-801 was dissolved in saline for intraperitoneal (i.p.) administration or in artificial cerebrospinal fluid (see below for composition) for local application through dialysis probes. Clozapine was dissolved in a few drops of glacial acetic acid and further diluted with saline for subcutaneous (s.c.) administration, or artificial cerebrospinal fluid for local application. For s.c. administration, haloperidol (5 mg/ml) was brought to 1 mg/ml by diluting five times the injectable solution with saline. When needed, the pH of the final concentrations was adjusted to 6.5–7.0 with NaHCO<sub>3</sub>. Appropriate vehicles were administered in control groups.

### Microdialysis Procedures

Concentric dialysis probes with a 4-mm long membrane were implanted under sodium pentobarbital anesthesia (60 mg/kg, i.p.) in the mPFC (AP + 3.2 mm, L –0.8 mm, DV –6.0 mm; from bregma), according to Paxinos and Watson (1986). Microdialysis experiments were conducted 20–24 h after surgery in freely moving rats by continuously perfusing probes with artificial cerebrospinal fluid containing 125 mM NaCl, 2.5 mM KCl, 1.26 mM CaCl<sub>2</sub>, 1.18 mM MgCl<sub>2</sub>, and 1  $\mu$ M citalopram. The addition of an uptake inhibitor to the perfusion fluid is used in some microdialysis studies to decrease clearance from the extracellular space and magnify the release component of extracellular 5-HT (Adell *et al*, 2002). On the other hand, low concentrations of 5-HT uptake blockers, such as that used in the present study, are without effect on dialysate glutamate *in vivo* (Langman *et al*, 2006; Queiroz and Artigas, unpublished results). Interestingly, voltammetric studies have shown that MK-801 may interact with the serotonin transporter (Iravani *et al*, 1999; Callado *et al*, 2000), thereby underscoring the importance of including citalopram in the perfusion fluid.

The artificial cerebrospinal fluid was perfused at a rate of 1.5  $\mu$ l/min with a WPI model sp220i syringe pump (WPI, Aston, Stevenage, UK) attached to an overhead liquid swivel (Instech, Plymouth Meeting, PA). Dialysate samples of 30  $\mu$ l were collected every 20 min and divided into two fractions for the determination of 5-HT (20  $\mu$ l) and glutamate (10  $\mu$ l). The *in vitro* dialysis probe recoveries for 5-HT and glutamate were 15 and 17%, respectively. Owing to the incidental occurrence of chromatographic problems, for some rats data on only one transmitter (glutamate or 5-HT) were available. After a 100 min stabilization period, four dialysate samples were collected to obtain basal transmitter values (expressed as concentration of transmitter in a 30  $\mu$ l sample) before any pharmacological treatment. At the completion of dialysis experiments, rats were given an overdose of sodium pentobarbital and a fast green solution was perfused through the dialysis probes to stain the surrounding tissue for subsequent histological examination.

### Biochemical Determinations

The concentration of 5-HT in dialysate samples was determined by an HPLC system consisting of a Waters 717plus autosampler (Waters Cromatografia, Cerdanyola, Spain), a Hewlett-Packard series 1050 pump (Agilent

Technologies, Las Rozas, Spain), a 3- $\mu$ M octadecylsilica (ODS) column (7.5 cm  $\times$  0.46 cm; Beckman, San Ramon, CA), and an amperometric detector Hewlett-Packard 1049 (Agilent Technologies) set at an oxidation potential of 0.6 V. The mobile phase consisted of 0.15 M  $\text{NaH}_2\text{PO}_4$ , 1.8 mM octyl sodium sulfate, 0.2 mM EDTA (pH 2.8, adjusted with phosphoric acid), and 30% methanol and was pumped at 0.7 ml/min (Adell and Artigas, 1998). For the determination of glutamate, another HPLC system was used, which consisted of a Waters 717plus autosampler, a Waters 600 quaternary gradient pump, and a Nucleosil 5- $\mu$ M ODS column (10 cm  $\times$  0.4 cm; Teknokroma, Spain). Dialysate samples were precolumn derivatized with OPA reagent and all this process was carried out by the autosampler. Briefly, 90  $\mu$ l distilled water was added to the 10  $\mu$ l dialysate sample and this was followed by the addition of 15  $\mu$ l of the OPA reagent. After 2.5 min reaction, 80  $\mu$ l of this mixture was injected into the column. Detection was carried out with a Waters 470 Scanning Fluorescence Detector using excitation and emission wavelengths of 360 and 450 nm, respectively. The mobile phase consisted of 0.1 M sodium acetate, 5.5 mM triethylamine (pH 5.5) containing 10–70% acetonitrile and was pumped at 0.8 ml/min (Babot *et al*, 2005). The detection limits for 5-HT and glutamate were 4 fmol and 0.3 pmol, respectively (signal-to-noise ratio 3).

## Statistics

Data (mean  $\pm$  SEM) are expressed as fmol/30  $\mu$ l for 5-HT and pmol/30  $\mu$ l for glutamate, and shown in figures as percentages of basal values, averaged from four fractions collected before treatment. Unless otherwise stated, the changes in dialysate 5-HT and glutamate were analyzed by two-way repeated measures analysis of variance (ANOVA) with time and treatment as factors. When significant effects were found, *post hoc* Newman–Keuls multiple comparison tests were used to compare effects of different treatment groups. In some cases areas under the curve (AUC) of selected time intervals were calculated and compared using ANOVA followed by *post hoc* Newman–Keuls tests, where appropriated. The level of significance was set at  $p < 0.05$ .

## RESULTS

The basal (predrug) concentrations of 5-HT and glutamate in dialysate samples of the mPFC, not corrected for *in vitro* recovery, were  $50.3 \pm 1.8$  fmol/30  $\mu$ l ( $n = 155$ ) and  $7.2 \pm 0.6$  pmol/30  $\mu$ l ( $n = 164$ ), respectively.

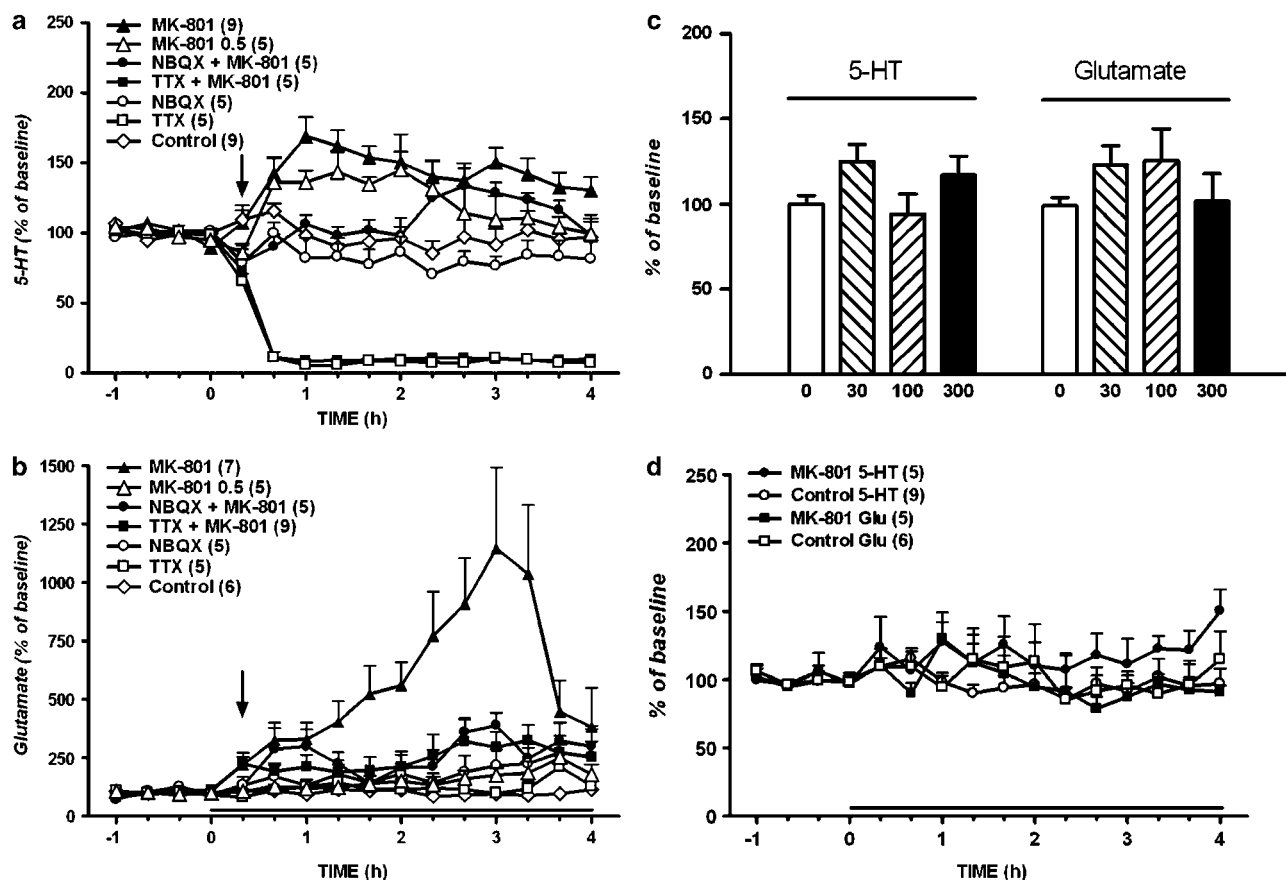
### Effects of MK-801

As described previously in the literature (Homayoun *et al*, 2004; Jackson *et al*, 2004) the systemic administration of MK-801 to rats evoked marked hyperlocomotion and stereotypies (behavioral observation). Two-way ANOVA showed that with respect to saline-injected group, the administration of MK-801 (0.5–1 mg/kg, i.p.) enhanced dose dependently the extracellular concentration of 5-HT (Figure 1a) as demonstrated by the significant effect of treatment ( $F_{2,20} = 12.5$ ,  $p < 0.0005$ ), time ( $F_{15,300} = 5.9$ ,  $p < 0.00001$ ), and treatment  $\times$  time interaction ( $F_{30,300} = 3.3$ ,  $p < 0.00001$ ). *Post hoc* comparisons showed that the

increase elicited by 1 mg/kg MK-801 was higher than that of 0.5 mg/kg ( $p < 0.05$ ; Newman–Keuls test). The systemic administration of MK-801 also increased extracellular glutamate (Figure 1b), as shown by the significant effect of treatment ( $F_{2,15} = 15.0$ ,  $p < 0.0003$ ), time ( $F_{15,225} = 5.0$ ,  $p < 0.00001$ ), and treatment  $\times$  time interaction ( $F_{30,225} = 4.6$ ,  $p < 0.00001$ ). However, only the dose of 1.0 mg/kg MK-801 significantly increased dialysate glutamate ( $p < 0.001$ ; Newman–Keuls test). For this reason, this latter dose was used for subsequent experiments. As described before for PCP (Adams and Moghadam, 2001), glutamate increased more slowly than 5-HT after MK-801 administration. On the contrary, the intra-mPFC perfusion of MK-801 at 30, 100, and 300  $\mu$ M for 80 min was without effect on dialysate 5-HT and glutamate (Figure 1c). To exclude any possible delayed effect of intracortical MK-801, a further experiment was carried out in which a concentration of 300  $\mu$ M of the compound was perfused through the dialysis probe for 4 h. This long perfusion of MK-801 did not alter the concentrations of 5-HT and glutamate in the mPFC (Figure 1d). The local application of the AMPA/kainate antagonist, NBQX (300  $\mu$ M), suppressed the MK-801-induced increase in 5-HT ( $F_{1,12} = 9.2$ ,  $p < 0.02$ ; Figure 1a) and glutamate ( $F_{1,10} = 6.7$ ,  $p < 0.03$ ; Figure 1b). When given alone, NBQX did not change 5-HT and glutamate levels in comparison to the corresponding control group. TTX (1  $\mu$ M) also suppressed MK-801-induced increase in 5-HT ( $F_{1,12} = 136.2$ ,  $p < 0.00001$ ; Figure 1a) and glutamate ( $F_{1,14} = 10.0$ ,  $p < 0.01$ ; Figure 1b) although with different efficacy. Thus, whereas TTX completely abolished the increase in 5-HT elicited by 1 mg/kg MK-801, it reduced glutamate levels to 40% of those induced by MK-801, as calculated using AUC from time = 0 to time = 4 h ( $t = 3.1$ ,  $p < 0.01$ ; Student's *t*-test, two-tailed). When perfused alone, TTX also abolished basal dialysate 5-HT ( $F_{1,10} = 95.5$ ,  $p < 0.00001$ ; Figure 1a), whereas it was without effect on basal dialysate glutamate (Figure 1b).

### Effects of Systemic Administration of Antipsychotic Drugs

The administration of haloperidol (1 mg/kg, s.c.) failed to block the increase in 5-HT elicited by MK-801 (Figure 2a), but effectively blocked that of glutamate (Figure 2b) as demonstrated by the significant effect of treatment ( $F_{3,20} = 12.5$ ,  $p < 0.0001$ ), time ( $F_{15,300} = 4.7$ ,  $p < 0.00001$ ), and treatment  $\times$  time interaction ( $F_{45,300} = 3.7$ ,  $p < 0.00001$ ). On the other hand, clozapine (1–5 mg/kg, s.c.) reduced MK-801-induced increase in dialysate 5-HT in a dose-dependent manner (Figure 3a), as demonstrated by the significant effect of treatment ( $F_{3,24} = 7.3$ ,  $p < 0.002$ ), time ( $F_{15,360} = 7.3$ ,  $p < 0.00001$ ), and the interaction between both factors ( $F_{45,360} = 4.2$ ,  $p < 0.00001$ ). Clozapine (1–5 mg/kg, s.c.) also decreased MK-801-induced increase in dialysate glutamate in a dose-dependent manner (Figure 3b), as demonstrated by the significant effect of treatment ( $F_{3,19} = 9.4$ ,  $p < 0.001$ ), time ( $F_{15,285} = 8.0$ ,  $p < 0.000001$ ), and treatment  $\times$  time interaction ( $F_{45,285} = 3.2$ ,  $p < 0.000001$ ). The effects of systemic clozapine were significant only at the dose of 5 mg/kg ( $p < 0.05$  for 5-HT and  $p < 0.005$  for glutamate; Newman–Keuls test), although the effect of the lowest dose almost reached significance for glutamate ( $p = 0.051$ ; Newman–Keuls test). When administered alone, haloperidol



**Figure 1** Effects of MK-801 (0.5 and 1 mg/kg, i.p.) and of the local perfusion of NBQX (300  $\mu$ M) and TTX (1  $\mu$ M) on the MK-801 (1 mg/kg, i.p.)-induced efflux of 5-HT (a) and glutamate (b) in the mPFC. Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses. NBQX or TTX was perfused from time 0 to time 4 h (line). The effects of the local perfusion for 80 min of three different concentrations of MK-801 in the mPFC on dialysate 5-HT and glutamate are depicted in (c). Data in (c) are expressed as percentage of AUC calculated from four consecutive samples of 4 (5-HT) and 5 animals (glutamate). The effects of the local perfusion of 300  $\mu$ M MK-801 for 4 h in the mPFC on dialysate 5-HT and glutamate (Glu) are depicted in (d).

(1 mg/kg, s.c.; Figure 2) and clozapine (1–5 mg/kg, s.c.; Figure 3) failed to alter dialysate 5-HT or glutamate.

### Effects of Local Application of Antipsychotic Drugs in the mPFC

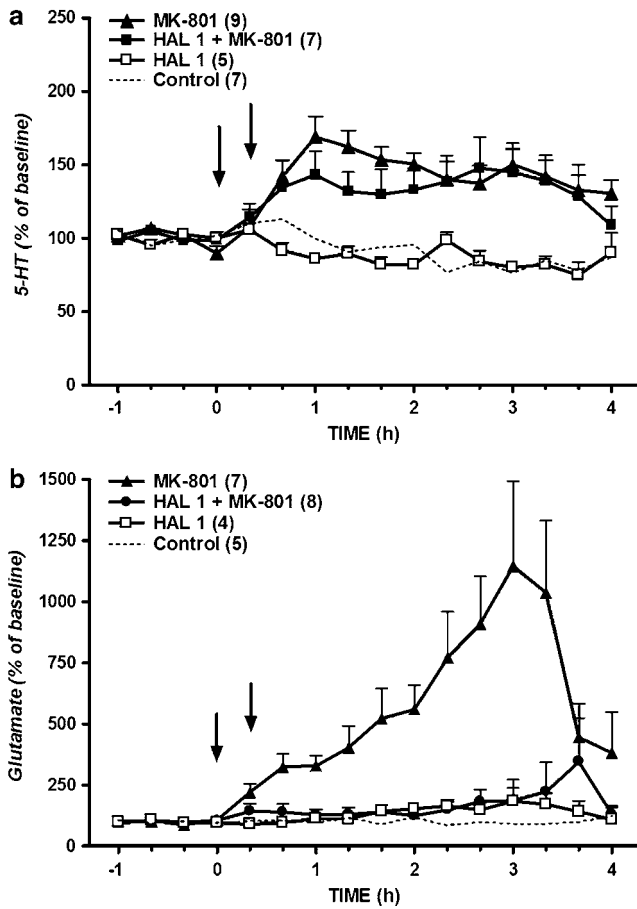
The intra-mPFC perfusion of haloperidol (3, 30  $\mu$ M) failed to modify the MK-801-induced increase in dialysate 5-HT (Figure 4a). However, the same concentrations of haloperidol suppressed the MK-801-induced increase in glutamate in a concentration-dependent manner (Figure 4b), as demonstrated by the significant effect of treatment ( $F_{2,19} = 13.2$ ,  $p < 0.001$ ), time ( $F_{15,285} = 11.0$ ,  $p < 0.00001$ ), and the interaction between both factors ( $F_{30,285} = 5.5$ ,  $p < 0.00001$ ). *Post hoc* comparisons showed that only 30  $\mu$ M haloperidol significantly blocked the effects of MK-801 on glutamate ( $p < 0.001$ ; Newman-Keuls test). Intra-mPFC perfusion of clozapine (30, 100, and 300  $\mu$ M) reduced MK-801-evoked increase in dialysate 5-HT in a concentration-dependent manner (Figure 5a), as demonstrated by the significant effect of treatment ( $F_{4,33} = 7.0$ ,  $p < 0.0005$ ), time ( $F_{15,495} = 15.9$ ,  $p < 0.00001$ ), and treatment  $\times$  time interaction ( $F_{60,495} = 4.3$ ,  $p < 0.00001$ ). *Post hoc* comparisons showed that only 300  $\mu$ M clozapine produced a significant effect ( $p < 0.05$ ; Newman-Keuls test). In contrast, the three

concentrations of clozapine were equipotent in blocking the MK-801-induced increase in glutamate (Figure 5b), as demonstrated by the significant effect of treatment ( $F_{4,29} = 12.7$ ,  $p < 0.00001$ ), time ( $F_{15,435} = 7.0$ ,  $p < 0.00001$ ), and their interaction ( $F_{60,435} = 4.9$ ,  $p < 0.00001$ ).

When perfused alone, haloperidol (3 and 30  $\mu$ M) had no effect on dialysate 5-HT (Figure 6a) and glutamate (Figure 6b). Intra-mPFC perfusion of clozapine (30, 100, and 300  $\mu$ M) decreased dialysate 5-HT in a concentration-dependent manner (Figure 7a), as demonstrated by the significant effect of treatment ( $F_{3,21} = 3.7$ ,  $p < 0.03$ ), time ( $F_{15,315} = 2.3$ ,  $p < 0.005$ ), and the interaction between both factors ( $F_{45,315} = 1.6$ ,  $p < 0.02$ ). Clozapine had also a significant effect on dialysate glutamate (Figure 7b), as demonstrated by the significant effect of treatment ( $F_{3,20} = 3.3$ ,  $p < 0.05$ ), time ( $F_{15,300} = 2.3$ ,  $p < 0.005$ ), and the interaction of both factors ( $F_{45,300} = 1.7$ ,  $p < 0.01$ ). *Post hoc* comparisons showed that only 300  $\mu$ M clozapine increased significantly the concentration of glutamate ( $p < 0.05$ ; Newman-Keuls test).

### DISCUSSION

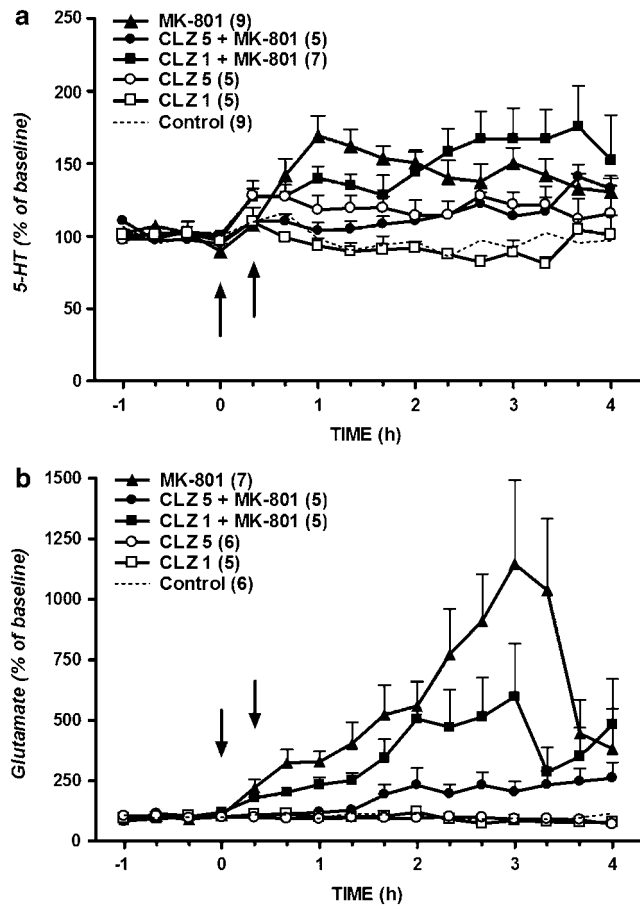
The first finding of the present study is that systemic administration of the NMDA receptor antagonist, MK-801,



**Figure 2** Effects of pretreatment (first arrow) with haloperidol (1 mg/kg, s.c.; HAL 1) on the efflux of 5-HT (a) and glutamate (b) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; second arrow). Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses. The control group received two injections (saline and vehicle) and, for the sake of clarity, is depicted as a dotted line.

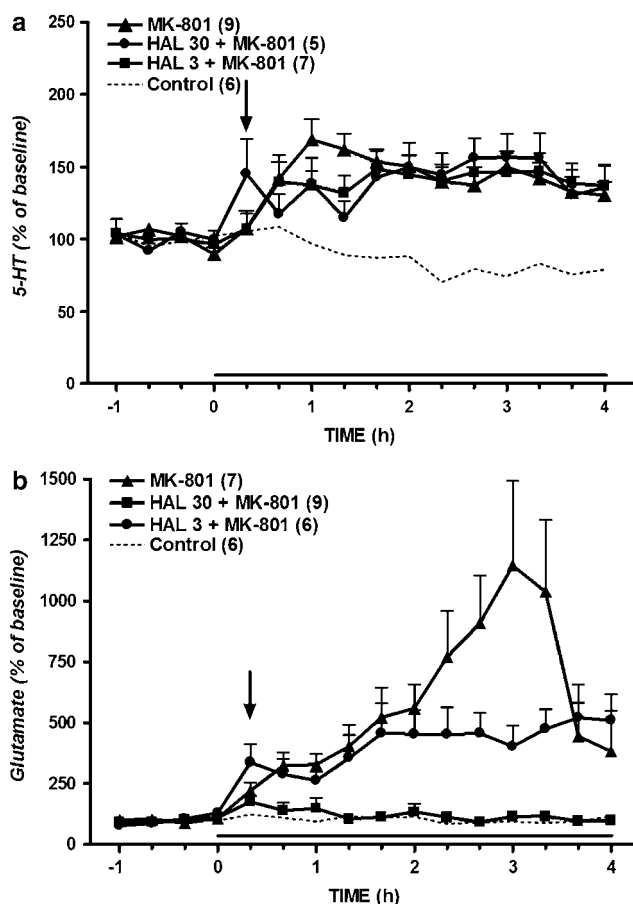
enhanced the extracellular concentration of 5-HT and glutamate in the mPFC. This was previously shown for PCP (Adams and Moghaddam, 2001). The kinetics of MK-801-induced changes in glutamate strongly paralleled those of induction of cortical c-fos mRNA, which also peaked at 3–4 h after the administration of MK-801 (Väisänen *et al*, 1999). This coincidence suggests that induction of c-fos mRNA synthesis would likely result from increased cortical glutamate efflux. These increases in 5-HT and glutamate are dependent on nerve impulse because they were inhibited by TTX, although to a different extent. Thus, basal and MK-801-induced elevation of 5-HT were both completely dependent on nerve impulse. However, basal dialysate glutamate in the mPFC was not blocked by TTX, in good agreement with prior work (Moghaddam, 1993; Timmerman *et al*, 1999), which pointed out that only a minor portion of basal extracellular glutamate is indeed exocytotically released (Timmerman and Westerink, 1997).

Nevertheless, the present study evidenced that MK-801-stimulated glutamate efflux is largely TTX dependent, that is released from neurons in an impulse-dependent manner. This is in line with previous work showing that the efflux of glutamate elicited by ketamine was also fully dependent on



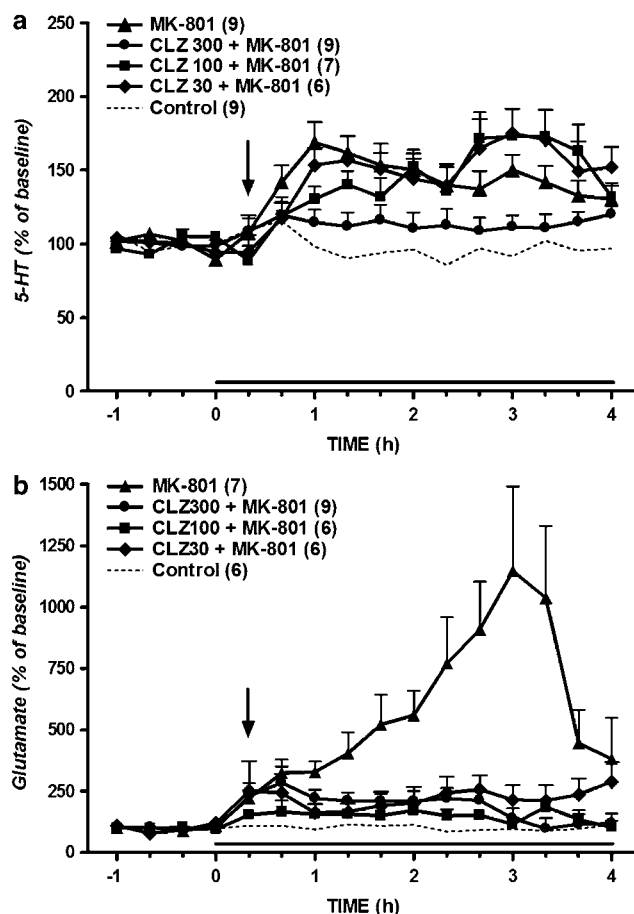
**Figure 3** Effects of pretreatment (first arrow) with clozapine (1 or 5 mg/kg, s.c.; CLZ 1 or CLZ 5) on the efflux of 5-HT (a) and glutamate (b) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; second arrow). Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses. The control group received two injections (saline and vehicle) and, for the sake of clarity, is depicted as a dotted line.

nerve impulse (Lorrain *et al*, 2003). It remains to be determined, however, the provenance of NMDA receptor antagonist-stimulated extracellular glutamate in the mPFC. The fact that local perfusion of PCP, ketamine, and MK-801 in the mPFC failed to elicit the increase in glutamate and/or 5-HT (Lorrain *et al*, 2003; Amargós-Bosch *et al*, 2006, this study) indicates that the NMDA receptors responsible for these effects are located outside the mPFC. Our results are consistent with other data showing that the increased locomotion and firing or EPSCs of putative pyramidal neurons of the mPFC following systemic administration of NMDA receptor antagonists (Suzuki *et al*, 2002; Jodo *et al*, 2003; Jackson *et al*, 2004) were not mimicked by intra-mPFC application of these compounds (Aghajanian and Marek, 2000; Suzuki *et al*, 2002; Jodo *et al*, 2005). What is the actual localization of these NMDA receptors and the source of the cortical hyperglutamatergic transmission induced by their blockade? Two of the well-known ascending glutamatergic inputs to the mPFC arise from the hippocampus (Carr and Sesack, 1996; Dégénétais *et al*, 2003) and the mediodorsal nucleus of the thalamus (Kuroda *et al*, 1998), and in both areas glutamatergic neurons are under the control of GABAergic cells (Gigg *et al*, 1994;



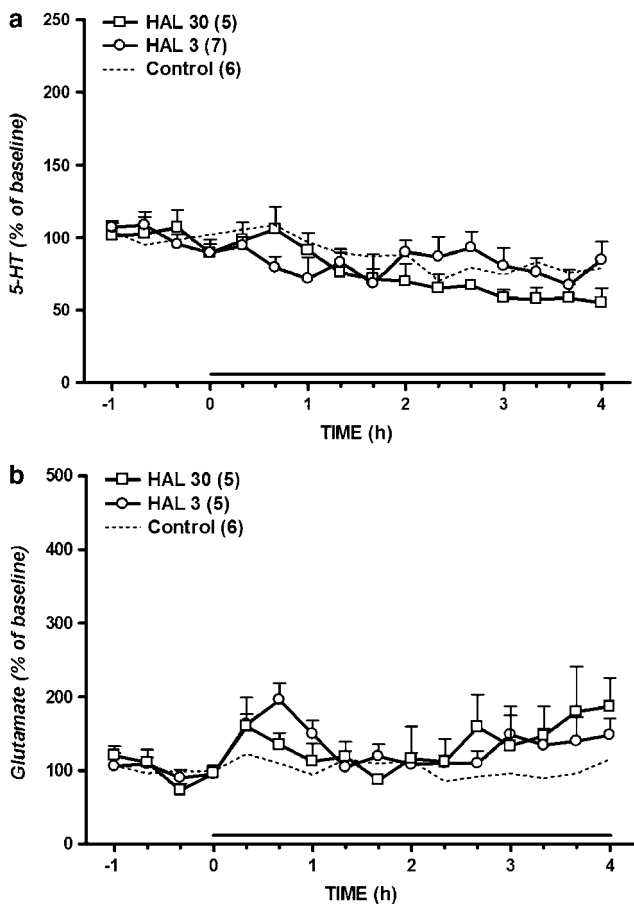
**Figure 4** Effects of the intra-mPFC perfusion (line) of haloperidol (3 and 30  $\mu$ M; HAL 3 and HAL 30, respectively) on the efflux of 5-HT (a) and glutamate (b) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses. The control group received an injection of saline and, for the sake of clarity, is depicted as a dotted line.

Krimer and Goldman-Rakic, 2001). In accordance with these findings, it has been shown that the local application of PCP into the hippocampus increases locomotion and the firing of pyramidal neurons of the mPFC in freely moving rats (Jodo *et al*, 2005). Furthermore, Sharp and coworkers have described that the blockade of NMDA receptors in the anterior thalamus by MK-801 resulted in injury of cortical pyramidal neurons measured by an increased synthesis of heat-shock protein 70 (HSP-70) in limbic cortex (Tomitaka *et al*, 2000; Sharp *et al*, 2001). Thus, all these findings suggest that NMDA receptor antagonists would attenuate the tonic activation of inhibitory (GABA) neurons (possibly, though not exclusively, in the hippocampus and/or the thalamus), which would result in a disinhibition of glutamatergic input to the mPFC (Olney and Farber, 1995; Moghaddam *et al*, 1997; Krystal *et al*, 2003). In fact, GABAergic neurons are very sensitive to NMDA antagonism (Grunze *et al*, 1996; Li *et al*, 2002), and PCP and MK-801 are able to reduce dialysate GABA in the mPFC (Yonezawa *et al*, 1998) and striatum (Hondo *et al*, 1995). Our results would suggest that the GABAergic control of glutamate efflux within the mPFC would not have a tonic nature.



**Figure 5** Effects of the intra-mPFC perfusion (line) of clozapine (30, 100, and 300  $\mu$ M; CLZ 30, CLZ 100, and CLZ 300, respectively) on the efflux of 5-HT (a) and glutamate (b) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses. The control group received an injection of saline and, for the sake of clarity, is depicted as a dotted line.

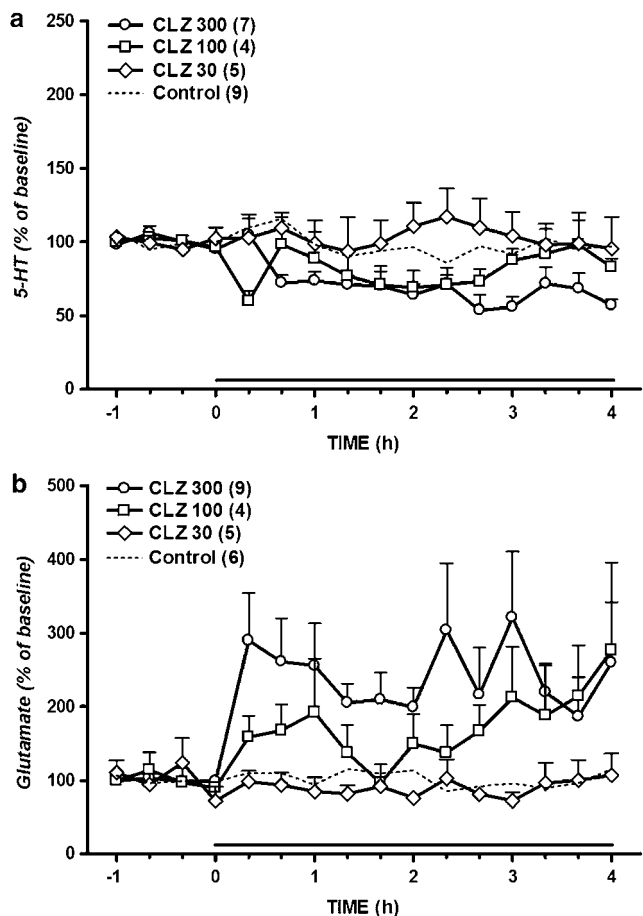
On the other hand, the increased extracellular concentrations of 5-HT and glutamate elicited by MK-801 are mediated by a stimulation of cortical AMPA receptors because they were suppressed by intra-mPFC perfusion of NBQX. Consistent with our findings, it has been shown that blockade of AMPA/kainate receptors in the prefrontal cortex inhibited PCP-induced locomotion and stereotypy (Takahata and Moghaddam, 2003). Thus, in line with previous reports (Moghaddam *et al*, 1997; Adams and Moghaddam, 2001; Lorrain *et al*, 2003), MK-801 increases glutamate release onto AMPA/kainate receptors, which, in turn, elicit an enhanced glutamatergic output from mPFC neurons, including those projecting to the dorsal raphe nucleus, thereby increasing serotonergic cell firing and cortical 5-HT efflux. Although this functional interplay between the mPFC and the dorsal raphe nucleus is well documented (Hajós *et al*, 1998; Celada *et al*, 2001; Martín-Ruiz *et al*, 2001; Amargós-Bosch *et al*, 2003; Lucas *et al*, 2005), we presently cannot rule out the possibility of a direct effect of MK-801 on serotonergic neurons of the dorsal raphe nucleus (Callado *et al*, 2000; Tao and Auerbach, 2000) and its blockade downstream by NBQX acting on AMPA receptors putatively located in serotonergic terminals



**Figure 6** Effects of the local perfusion of haloperidol (3 and 30  $\mu$ M; HAL 3 and HAL 30, respectively) on the efflux of 5-HT (a) and glutamate (b) in the mPFC. Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses.

(Maione *et al*, 1997). With regard to glutamate, however, although presynaptic AMPA receptors have been described in striatal glutamatergic axon terminals (Patel *et al*, 2001; Fujiyama *et al*, 2004), they do not seem to be present in the cortical counterparts (Fujiyama *et al*, 2004). Interestingly, the effect of NBQX on MK-801-induced increase in 5-HT appeared to be biphasic. Thus, NBQX was able to completely prevent the effect of MK-801 on 5-HT in the first phase (0–2 h), but not in the second (2–4 h). Although further research is warranted to know the mechanisms underlying these effects, it is possible that the late increase in cortical serotonergic transmission may result from a direct stimulation of 5-HT cells within the dorsal raphe nucleus by MK-801 (Callado *et al*, 2000; Tao and Auerbach, 2000), or from the activation of a different brain circuitry not including the mPFC (for instance hippocampus  $\rightarrow$  amygdala  $\rightarrow$  raphe).

Taken together that the effects of MK-801 mostly depends on glutamatergic projections from regions outside the mPFC, and that AMPA/kainate receptors are located predominantly in pyramidal cells, it was expected that NBQX would have blocked the MK-801-induced increase in 5-HT, but not that of glutamate. Unexpectedly, however, NBQX was able to block both actions of MK-801. It is conceivable that MK-801 could have elicited an initial subtle



**Figure 7** Effects of the local perfusion of clozapine (30, 100, and 300  $\mu$ M; CLZ 30, CLZ 100, and CLZ 300, respectively) on the efflux of 5-HT (a) and glutamate (b) in the mPFC. Data (mean  $\pm$  SEM) are expressed as percentage changes of the four basal predrug values. Number of animals is given in parentheses.

(not measurable in the present conditions) increase in glutamate from external sources. This glutamate could have been able to stimulate pyramidal cells immediately and the subsequent increase in glutamate measured would have resulted from a cascade of excitation within pyramidal cells, instead of directly from an external glutamatergic input. This proposal is consistent with our finding that a lower dose of MK-801 (0.5 mg/kg) was able to increase 5-HT without altering cortical glutamate. Therefore, the present results imply that the stimulation of cortical AMPA/kainate receptors can be achieved by subtle changes in glutamate level, which cannot be detected in the experimental conditions used because the stimulation of subcortical glutamatergic afferents immediately leads to the activation of pyramidal neurons. Overall, our results suggest that extracellular glutamate measured after MK-801 administration would partly arise from mPFC or at least depend on activation of mPFC output.

The second main finding of the present study is that clozapine and haloperidol differently blocked the MK-801-induced increase in 5-HT and glutamate. Thus, in line with our previous work with PCP and ketamine (Amargós-Bosch *et al*, 2006), the systemic administration of clozapine suppressed the increase of serotonin in the mPFC, whereas

haloperidol did not. The fact that a higher dose of clozapine (5 mg/kg) was needed to block the effects of MK-801 in comparison with those of PCP and ketamine (Amargós-Bosch *et al*, 2006) could be related to the higher potency of MK-801 in binding to the NMDA receptor channel. In contrast, systemic haloperidol completely abolished the MK-801-induced increase in glutamate. In a previous study, Adams and Moghaddam (2001) showed that clozapine (10 mg/kg) and haloperidol (0.1 mg/kg) failed to block the increase in dialysate glutamate elicited by PCP. However, in this study clozapine was administered i.p., which presumably result in a lower brain concentration of the drug, possibly insufficient to block the effect of PCP. On the other hand, it is possible that the blocking actions of clozapine on glutamate would be more prominent when the stimulated efflux is higher, which is the case of MK-801 (this study) compared to PCP (Adams and Moghaddam, 2001). In fact, both clozapine and haloperidol are able to block the effect of MK-801 on extracellular glutamate. In line with this, Homayoun and Moghaddam (2007) have recently shown that clozapine and haloperidol share the ability to markedly inhibit a small subset of mPFC neurons. Furthermore, only around 5% of projection neurons in the V layer of the mPFC project to the dorsal raphe nucleus, according to Gabbott *et al.* (2005). Thus, it is conceivable that under the conditions of the present study (increased 5-HT and glutamate transmission in the mPFC) haloperidol may be able to inhibit a subpopulation of pyramidal cells (blockade of increased glutamate efflux), though sparing cortico-raphe projections. Alternatively, it could be also possible that cortico-raphe projecting cells might be inhibited by haloperidol, but not to the extent needed to suppress serotonergic firing distally in the dorsal raphe nucleus and subsequent cortical 5-HT release. Differences in the receptor population present in pyramidal cells and the distinct pharmacological profile of clozapine, acting on different transmitter receptors particularly abundant in layer V mPFC neurons, could provide the anatomical substrate for this differential effect. It thus appears that serotonergic transmission in the mPFC is regulated by the concurrent participation of multiple transmitter receptors, whereas glutamatergic transmission is strongly dependent on dopamine D2 receptor activation. Further evidence of the regulation of glutamate release in the mPFC by dopamine D2 receptors is provided by electrophysiological studies. Thus, the augmented efflux of dopamine elicited by the blockade of NMDA receptors (Moghaddam and Adams, 1998; Mathé *et al*, 1999; Schmidt and Fadaye, 1996) may promote dopamine D2-induced burst firing only in a small subset of pyramidal cells of the mPFC (Wang and Goldman-Rakic, 2004), possibly in those cells enriched in dopamine D2 receptors. In summary, it appears that dopamine D2 antagonism in the mPFC may be pathway specific, affecting only a reduced number of pyramidal cells (Wang and Goldman-Rakic, 2004). In line with our results, both clozapine and haloperidol inhibit MK-801-induced induction of cortical c-fos mRNA (Väisänen *et al*, 1999), which further suggests that this effect may result from blockade of glutamate efflux. When administered alone, neither clozapine nor haloperidol, given systemically, altered the concentrations of 5-HT and glutamate, which is consistent with previous results (Daly and Moghaddam,

1993; Yamamoto *et al*, 1994; Cartmell *et al*, 2001; Heidbreder *et al*, 2001). Although recent data from our lab showed that the dose of 1 mg/kg of both antipsychotics elicited a maximal reduction of dialysate 5-HT in the mPFC to ~70% of basal values (Amargós-Bosch *et al*, 2006), such weak effect was comparable to that reported herein (~80%). The ability of clozapine to attenuate MK-801-evoked change in cortical 5-HT may be related to its superior effects in negative/cognitive symptoms. Actually, clozapine but not haloperidol effectively blocks the hyperactivity, metabolic activation, neurotoxic cell damage, as well as the deficits in prepulse inhibition and social interaction induced by NMDA receptor antagonists (Corbett *et al*, 1995; Gleason and Shannon, 1997; Abi-Saab *et al*, 1998; Duncan *et al*, 1998; Olney *et al*, 1999; Geyer *et al*, 2001; Farber *et al*, 2006). Further research is needed to ascertain the transmitter receptors responsible for such differences between clozapine and haloperidol.

In good accordance with the effects of systemic administration, the intra-mPFC perfusion of clozapine was able to block the increases of both 5-HT and glutamate evoked by MK-801, whereas haloperidol was only effective in blocking the increased efflux of glutamate. To the best of our knowledge, this is the first report that shows the effects of antipsychotic drugs applied locally into the mPFC on the neurochemical changes elicited by the systemic administration of noncompetitive NMDA receptor antagonists. The intra-mPFC action of clozapine and haloperidol paralleled those of their systemic administration, which underscores the important role of the mPFC in the pharmacological effects of these antipsychotic drugs. This was not unexpected given that the prefrontal cortex is involved in goal-directed actions, such as working memory and cognitive tasks (Goldman-Rakic, 1995; Ellenbroek *et al*, 1996; Carli *et al*, 2006), and these functions are disrupted after NMDA receptor antagonism (Abi-Saab *et al*, 1998; Olney *et al*, 1999; Geyer *et al*, 2001; Jackson *et al*, 2004) and in schizophrenia (Weinberger and Gallhofer, 1997; Park *et al*, 1999). The mechanisms underlying these differential effects of clozapine and haloperidol remain unknown, but might be the basis of their differential effectiveness on negative/cognitive symptoms (Lieberman, 1996). The finding that clozapine produces opposite effects on glutamatergic transmission depending on the activity of mPFC neurons is not new. In fact, clozapine has been shown to increase the activity of neurons with low baseline firing rates and reduce that of neurons with higher firing rates (Homayoun and Moghaddam, 2007). It is, thus, possible that the local reduction of 5-HT in the mPFC may be mediated through the action of clozapine on a multiplicity of receptors, such as dopamine D1, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>,  $\alpha$ -adrenergic, histamine H1, and/or muscarinic M1 receptors (Bymaster *et al*, 1996; Arnt and Skarsfeldt, 1998). On the other hand, the clozapine-induced increase in glutamate efflux (albeit only at high concentration) has been postulated to result from its potentiation of NMDA transmission through an inhibition of glycine reuptake and/or a direct agonist action on the glycine recognition site of NMDA receptors (see Millan, 2005 for review). Alternatively, extracellular concentration of glutamate may also be secondary to inhibition of GABA efflux elicited by clozapine (Bourdelaïs and Deutch, 1994). Altogether, these findings suggest that abnormally high



glutamate levels in the mPFC (caused by a primary NMDA receptor hypofunction) may be related, at least in part, with some of the psychotic/positive symptoms of schizophrenia that are generally alleviated by all antipsychotics, probably through a blockade of dopamine D2 receptors (Coyle and Tsai, 2004). In contrast, a disruption of 5-HT in the mPFC could rather be associated to negative symptoms and/or cognitive impairment, conditions for which clozapine depicts a superior efficacy. Although further research is needed to verify this hypothesis, it is interesting to note that some of the atypical antipsychotic drugs (characterized by their antiserotonergic properties together with a relatively weaker dopamine D2 antagonism) have been reported to display a better outcome in the control of negative symptoms compared with typical antipsychotics (Meltzer, 1999).

In summary, the results of the present study indicate that MK-801 (and possibly other noncompetitive NMDA receptor antagonists) increases the efflux of glutamate and 5-HT in the mPFC through the blockade of NMDA receptors located outside the mPFC (or at least, outside the area sampled by dialysis probes), and the activation of AMPA receptors present in the mPFC. The local effects of clozapine and haloperidol paralleled those observed after systemic administration, which emphasizes the relevance of the mPFC as a site of action of these antipsychotic drugs in offsetting the neurochemical effects of MK-801. Both clozapine and haloperidol blocked the effects of MK-801 on cortical glutamate, but only clozapine showed a unique ability in suppressing an excessive serotonergic activity in the mPFC. Although this latter action is shared by olanzapine (Amargós-Bosch *et al*, 2006), further research is needed to determine whether this is a distinct feature of atypicality of antipsychotic drugs.

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## DISCLOSURE/CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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