

## **UNRAVELING MONOAMINE RECEPTORS INVOLVED IN THE ACTION OF TYPICAL AND ATYPICAL ANTIPSYCHOTICS ON GLUTAMATERGIC AND SEROTONERGIC TRANSMISSION IN PREFRONTAL CORTEX**

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## INTRODUCTION

The NMDA receptor hypofunction hypothesis of schizophrenia was established after the observation that NMDA receptor antagonists such as phencyclidine (PCP) and ketamine can induce, in healthy individuals, altered behavioral states that resemble the positive and negative symptoms as well as the cognitive deficits seen in schizophrenia [1-3]. In addition, ketamine can heighten psychotic episodes in patients with schizophrenia [4-6]. In rodents, PCP and the more potent and selective noncompetitive NMDA receptor antagonist, dizocilpine (MK-801), cause hyperlocomotion and stereotypies [7-9]. The hyperactivity is considered to be due predominantly to activation of D<sub>2</sub> receptors in the nucleus accumbens, whereas stereotypies are caused by stimulation of D<sub>2</sub> receptors in the caudate-putamen [10]. These behaviors have been potentially related to positive symptoms of schizophrenia [11-13]. Acute NMDA receptor antagonism has also been reported to increase the release of glutamate [14-18], dopamine [19, 20], and serotonin (5-HT) [8, 16-18, 21, 22] in the medial prefrontal cortex (mPFC). It has been proposed that stimulation of NMDA receptors on the GABA inhibitory interneurons within the cortex leads to the release of GABA, which would inhibit glutamatergic neurons and the subsequent release of glutamate. Blockade of such NMDA receptors would therefore decrease GABAergic inhibitory tone and result in an enhanced activity of pyramidal neurons within the cortex [14, 23, 24] leading to downstream changes in other transmitters. Indeed, GABAergic interneurons in limbic cortex and hippocampus are more sensitive than pyramidal neurons to the action of NMDA receptor antagonists [25, 26]. In addition, reduced GABAergic function would alter the synchronous firing patterns of cortical neurons, which may underlie information-processing deficits present in patients with schizophrenia [27].

Various studies have demonstrated that the interactions among dopamine, 5-HT and glutamate play an important role in the pathophysiology of schizophrenia and that receptors for these transmitters are involved in the action of antipsychotic drugs. Increased activity of the mesolimbic dopamine system is believed to underlie the positive or psychotic symptoms of

schizophrenia and decreased activity within the mesocortical dopamine system is believed to reflect negative symptoms and cognitive function also seen in this illness [28-32]. All available antipsychotic drugs possess some degree of dopamine D<sub>2</sub>/D<sub>3</sub> receptor antagonism, and blockade of dopamine D<sub>2</sub>/D<sub>3</sub> receptors within the mesolimbic pathway reduces psychotic symptoms [33]. As a matter of fact, a good correlation exists between the clinical efficacy of antipsychotic drug and their affinity for D<sub>2</sub> receptor [34, 35]. Furthermore, most antipsychotic drugs display a dose-dependent threshold of D<sub>2</sub>/D<sub>3</sub> receptor occupancy for their therapeutic effects [36]. However, the necessity of D<sub>2</sub>/D<sub>3</sub> receptor blockade for therapeutic action has been challenged recently by the finding that drugs that attenuate glutamate release, without acting directly on dopamine receptors, are beneficial for positive and negative symptoms [37].

Several atypical antipsychotic drugs, on the other hand, are characterized by a lower affinity for D<sub>2</sub>/D<sub>3</sub> and higher affinity for 5-HT<sub>2A</sub> receptors, which has been proposed to confer a superior efficacy and tolerability [38, 39]. The lower occupancy of dopamine D<sub>2</sub>/D<sub>3</sub> receptors appears to be responsible for causing fewer extrapyramidal side-effects (EPS) [40]. In addition to the predominant role of the D<sub>2</sub>/D<sub>3</sub> receptor in the current treatment of psychotic symptoms, dopamine D<sub>1</sub> receptors have been implicated in schizophrenia [41, 42]. This line of evidence has been recently highlighted by the finding that cognitive/negative symptoms in schizophrenia are associated with a reduction of prefrontal dopamine D<sub>1</sub> receptor binding [43]. Some atypical antipsychotic drugs may also have affinity for other transmitter receptors such as serotonin 5-HT<sub>1A</sub>,  $\alpha$ -adrenergic, histamine H<sub>1</sub>, and muscarinic receptors, which may affect their efficacy and side-effect profile [44, 45]. Thus, there is some evidence for the importance of 5-HT<sub>1A</sub> receptor agonists in certain aspects of the pharmacotherapy of schizophrenia [46-49]. Furthermore, 5-HT<sub>1A</sub> receptor agonists have been shown to reduce the incidence of EPS in schizophrenia patients treated with haloperidol [50, 51]. With regard to  $\alpha$ -adrenergic mechanisms, many clinically effective antipsychotic drugs (both classical and atypical) possess  $\alpha_1$ -adrenoceptor antagonist properties [44, 52], which has been postulated to be clinically relevant [53]. However, although

central  $\alpha_1$ -adrenoceptors may regulate sensorimotor gating altered in schizophrenia [54], there are not studies dealing with the occupancy of  $\alpha_1$ -adrenoceptors in individuals under antipsychotic treatment.

In the animal setting, most studies have shown that atypical, but not typical, antipsychotics block the effects of NMDA receptor antagonists on prepulse inhibition (PPI) of the startle response [55]. In addition, atypical antipsychotic drugs, but not haloperidol, increases extracellular DA concentration in the PFC [56, 57] and prevent the brain increase in regional 2-deoxyglucose uptake induced by ketamine [58]. We have shown recently that the local perfusion of antipsychotic drugs decreased extracellular 5-HT in the mPFC [59, 60]. Stimulation of 5-HT<sub>1A</sub> and blockade of 5-HT<sub>2A</sub> receptors might contribute to this effect since both types of compounds were able to prevent the increases in 5-HT and glutamate, as well as cognitive deficits induced by NMDA receptor antagonists [16, 17, 61, 62]. In contrast, raclopride and eticlopride (dopamine D<sub>2</sub>/D<sub>3</sub> antagonists) and SKF 38393 (dopamine D<sub>1</sub>/D<sub>5</sub> agonist) prevented the increase of glutamate (but not that of 5-HT) induced by MK-801 [62]. Furthermore, clozapine and haloperidol attenuated PCP-induced increase in cortical glutamate [63], as well as the elevated firing of a population of mPFC pyramidal neurons elicited by PCP or MK-801 [64, 65]. Interestingly, clozapine exhibited a leveling effect on the firing of pyramidal cells in the mPFC, increasing the activity of neurons with low baseline firing rates and decreasing the activity of neurons with higher firing rates [64]. This fine-tuning effect might contribute to the unique therapeutic efficacy of clozapine in schizophrenia.

In a previous study we showed that both clozapine and haloperidol blocked the MK-801-induced increase in glutamate in rat mPFC, whereas only clozapine was able to block the increased efflux of 5-HT [18]. In addition, we also showed that antagonism at dopamine D<sub>2</sub> receptors and agonism at dopamine D<sub>1</sub> receptors resulted in blockade of the effects of MK-801 on mPFC glutamate but not 5-HT. In contrast, 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptor antagonists, as well as 5-HT<sub>1A</sub> receptor agonist, were able to prevent the increase in 5-HT and glutamate elicited by MK-801 [62]. In the present study we set out to replicate such findings using a wider range of concentrations and

extend them to other typical (chlorpromazine) and atypical (olanzapine) antipsychotic drugs. To this end, intracerebral microdialysis in the mPFC was used under the same experimental conditions described previously [18, 63]. The choice of prefrontal cortex was accounted for by its pivotal involvement in higher brain functions such as executive tasks and cognition [66] known to be disrupted in schizophrenia [67] and in animal models [68]. Despite the importance of mPFC in the etiology and pharmacotherapy of schizophrenia, it is worth noting that the effects of such compounds in other brain regions may also be crucial for the treatment of the illness. From a methodological point of view it is also important to note that antipsychotic drugs and selective compounds were delivered in the mPFC by reverse dialysis. Despite the *in vitro* affinity ( $10^{-8}$ - $10^{-9}$  M) of antipsychotics and selective compounds used in the present study for serotonergic and adrenergic receptors, the use of concentrations in the micromolar range is required in *in vivo* microdialysis to significantly affect neurotransmitter receptors (e.g. [69, 70]). This is due to the fact that effective concentration at receptors is limited by the low application rate (in the low nmol/h range) and the continuous clearance of the applied drug by the cerebrospinal fluid. Moreover, a substantial number of post-synaptic receptors in mPFC neurons must be recruited to activate/inhibit the mPFC-raphé circuit and elicit changes in mPFC 5-HT release [71, 130].

### **IS CORTICAL 5-HT TRANSMISSION RELATED TO “ATYPICALITY”?**

The term “atypical” referred to an antipsychotic drug was first applied to clozapine because this drug was devoid of the overt EPS and increased prolactin secretion seen in humans treated with neuroleptics. By comparison with the pharmacological profile of clozapine, it is considered that atypical antipsychotic drugs are relatively more potent serotonin 5-HT<sub>2A</sub> antagonists than dopamine D2 antagonists (see [39] for review). More recently, the multi-receptor pharmacology of atypical antipsychotics has included other features such as serotonin 5-HT<sub>1A</sub> and dopamine D1 agonism, dopamine D2 partial agonism as well as  $\alpha_1$ -adrenergic antagonism [46, 53, 72, 73].

The effects of antipsychotic drugs on extracellular 5-HT in the mPFC are far from conclusive [18, 61, 74, 75]. In general, both typical and atypical antipsychotics seem to decrease or leave unchanged cortical 5-HT although the particular effect might depend on the experimental conditions and/or dose of the drug. However, there seems to be a concurrence of results showing that risperidone increases cortical dialysate 5-HT, an effect which is probably related to its antagonistic action at  $\alpha_2$ -adrenoceptor, and seems to take place at the nerve terminal level [74]. In line with this assumption, Hertel and coworkers [76] demonstrated that idazoxan, an  $\alpha_2$ -adrenoceptor antagonist, in combination with raclopride (dopamine D<sub>2</sub>/D<sub>3</sub> receptor antagonist), exerted a clozapine-like antipsychotic effect on phencyclidine-treated animals. Thus, the role of mPFC 5-HT in the mechanism of action of atypical antipsychotic drugs appears equivocal. Nevertheless, a different issue that has been addressed in the present study is the role of cortical 5-HT in the action of antipsychotics in an animal model of schizophrenia.

Previous studies have demonstrated that NMDA receptor antagonists augment the firing rate of putative pyramidal neurons within the mPFC [65, 77, 78], which results in an increased efflux of glutamate and 5-HT. These effects are accounted for by an overstimulation of cortical AMPA-dependent glutamatergic transmission because they were abolished by intra-mPFC perfusion of an AMPA receptor antagonist [18, 79]. In line with previous research [8, 14, 15], MK-801 increases glutamate release onto AMPA/kainate receptors leading to an enhanced glutamatergic output from mPFC neurons (including those projecting to the dorsal raphe nucleus) thereby increasing serotonergic cell firing and cortical 5-HT release. Furthermore, it has been shown that blockade of AMPA/kainate receptors in the prefrontal cortex inhibited PCP-induced locomotion and stereotypy [80]. The antipsychotic action would therefore result from a prevention of the effects resulting from this cortical hyperglutamatergia.

In an initial study carried out in our lab we showed that pretreatment with atypical antipsychotic drugs such as clozapine and olanzapine suppressed the increased release of 5-HT in the mPFC elicited by phencyclidine (PCP) and

ketamine whereas haloperidol failed to do so [22]. Subsequently we demonstrated that clozapine and haloperidol were able to block the increase in cortical glutamate produced by MK-801, but only clozapine reduced the increase in 5-HT [18]. In addition, the mPFC appeared to be the preferential site of action of these drugs inasmuch as their effects were seen after local infusion through a dialysis probe. As shown in Fig. (1), this differential action has been replicated and confirmed also for chlorpromazine (typical antipsychotic) and olanzapine (atypical antipsychotic). The fact that all four antipsychotic drugs tested are able to block the effect of MK-801 on extracellular glutamate is in line with recent results showing that both clozapine and haloperidol possess the ability to markedly inhibit a subset of mPFC neurons [64]. The most prominent features of the pharmacological profile shared by these antipsychotics are dopamine D2 receptor and  $\alpha_1$ -adrenoceptor antagonism [44, 45, 52, 81-83]. However, since prazosin can also prevent the increase in dialysate 5-HT produced by NMDA antagonists ([22]; Fig. (7) of this study), it was suggested that glutamatergic transmission in the mPFC is predominantly dependent on dopamine D2 receptors, but not on  $\alpha_1$ -adrenoceptors. Unlike the actions of clozapine and olanzapine, the lack of effect of haloperidol and chlorpromazine on cortical 5-HT points to a different regulation of the pyramidal cells projecting to the dorsal raphe nucleus (DR). Because only 5% of pyramidal neurons in layer V of the mPFC project to the dorsal raphe nucleus [84], it is conceivable that, under conditions of increased 5-HT and glutamate transmission in the mPFC following the administration of NMDA antagonists, blockade of D2 receptors by antipsychotic drugs might be able to inhibit cortical output (blockade of increased glutamate efflux), although sparing mPFC  $\rightarrow$  DR projections. An alternative explanation could be that cortico-raphe projecting neurons might be inhibited by both typical antipsychotics, but not to the extent needed to suppress serotonergic firing distally in the DR and the subsequent cortical 5-HT release. On the other hand, we hypothesized that 5-HT concentration in the mPFC appears to be under the control of multiple monoamine receptors, which is coincident with the multi-receptor profile of atypical antipsychotics.

## EFFECTS OF DOPAMINERGIC COMPOUNDS

In accord with our hypothesis, we have shown that two different dopamine D<sub>2</sub> receptor antagonists, raclopride and eticlopride, elicited the same effect than that of haloperidol and chlorpromazine ([62]; present study). Raclopride binds with equal affinity to D<sub>2</sub> and D<sub>3</sub> receptors and has negligible affinity for D<sub>4</sub> receptors [85], and eticlopride possesses equal selectivity for D<sub>2</sub> and D<sub>3</sub> receptors [86], but 10-fold greater affinity than raclopride for both receptors [87]. Eticlopride also interacts with D<sub>4</sub> receptors [83]. It could be argued that the lack of effect of D<sub>2</sub>/D<sub>3</sub> antagonists on cortical 5-HT can be accounted for by the low concentration of antagonists used. However, as shown in Fig (2), a higher concentration of raclopride (100 μM) produced similar results, i.e. blockade of MK-801-induced effects on glutamate but not on 5-HT. Differences in the receptor populations present in layer V of the mPFC and the distinct pharmacological profile of clozapine, olanzapine, chlorpromazine and haloperidol (Table 1), could provide the anatomical substrate for this differential effect. Altogether, it 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 D<sub>2</sub> receptor activation. Further evidence of the regulation of glutamate release in the mPFC by dopamine D<sub>2</sub> receptors is provided by electrophysiological studies. Thus, the augmented efflux of dopamine elicited by the blockade of NMDA receptors [11, 19, 88] may promote dopamine D<sub>2</sub>-induced burst firing only in a small subset of pyramidal cells of the mPFC [89], possibly in those cells enriched in dopamine D<sub>2</sub> receptors. However, dopamine D<sub>2</sub> receptors are located not only in pyramidal cells, but also in GABA interneurons [90-93]. Further, tyrosine hydroxylase-positive terminals have been observed in apposition with GABA interneurons [94, 95]. Among the different types of cortical GABA interneurons, it has been shown that dopamine acts almost exclusively on those containing parvalbumin (PV) [92, 96], which target the perisomatic compartment of pyramidal cells [97, 98]. In addition, immunocytochemistry studies have reported that PV interneurons express both



dopamine D<sub>1</sub> and D<sub>2</sub> receptors [92]. From a physiological viewpoint, dopamine exerts a tonic inhibitory action on PV interneurons through D<sub>2</sub> receptors [99, 100]. In addition, evidence from a postmortem study has indicated that, in the anterior cingulate cortex of schizophrenics, there is a shift of dopaminergic terminals from pyramidal cells to GABA interneurons [101]. This dopamine could then act on D<sub>2</sub> receptors located in these interneurons thereby reducing the release of GABA with the subsequent disinhibition of pyramidal cells. In the animal model setting that we used, an excessive release of dopamine following MK-801 administration [19] might lead to a further reduction in GABAergic inhibition, which would result in an impairment of the intrinsic cortical circuitry. In fact, this has been postulated to occur in the brain of schizophrenics [102-104]. Dopamine D<sub>2</sub> antagonists would dampen the inhibitory action of dopamine overflow on GABA interneurons, thus elevating GABA release and reducing cortical glutamatergic output induced by MK-801. The relevance of PV interneurons in this process is underscored by two facts: 1) in rodents 50% of all GABA neurons contain PV [105] and 2) PV neurons are thought to play a predominant role in gamma oscillations [106-108]. A subtype of dopamine D<sub>2</sub> receptor that has received attention recently is the D<sub>4</sub> receptor. The dopamine D<sub>4</sub> receptor is of interest because of its localization in limbic structures associated with the regulation of mood and cognition, such as cerebral cortex and hippocampus [109, 110]. In addition, clozapine and olanzapine have significant affinities at the dopamine D<sub>4</sub> receptor (Table 1), which supports the role of this receptor as a potential target for antipsychotic drugs. For these reasons, it was deemed of importance to examine the effects of a dopamine D<sub>4</sub> receptor antagonist (L-745,870) on the MK-801-induced increases in cortical 5-HT and glutamate. Intracortical pretreatment with L-745,870 prevented the effects of MK-801 on glutamate, but not those on 5-HT (Fig (3)). Although there is histological evidence of the presence of dopamine D<sub>4</sub> receptors in the mPFC of rodents, controversy exists as regards to the level of expression [91, 111-115]. There is consensus, however, that the prefrontal cortex depicts a prominent expression of this receptor [91, 110, 114, 115], which is expressed in both pyramidal and GABAergic cells [91, 110, 114]. Our results are consistent

with a localization of dopamine D<sub>4</sub> receptors in GABAergic interneurons although it remains to be determined the phenotype of such cells.

The regulation of prefrontal function by dopamine involves not only D<sub>2</sub>, but also D<sub>1</sub> receptors. In fact, dopamine D<sub>1</sub> agonists enhance cortical dopaminergic transmission, which is postulated to be good against cognitive and negative symptoms [116]. Indeed, the systemic administration of a low dose of a dopamine D<sub>1</sub> receptor agonist ameliorates cognitive dysfunction induced by MK-801 although increases stereotypy and locomotor activity (model of psychotic symptoms) at higher doses [117]. Our results show that dopamine D<sub>1</sub> agonism elicits comparable effects to those of dopamine D<sub>2</sub> antagonists, i.e. a reduction of MK-801-induced increase of cortical glutamate [62]. However, when the concentration of the dopamine D<sub>1</sub> agonist, SKF-38393, was increased to 100  $\mu$ M, the elevation of 5-HT elicited by MK-801 was abolished (Fig (4)). However, it remains to be determined the potential clinical relevance of this effect. Although dopamine D<sub>1</sub> receptors are expressed by deep layer cortical pyramidal neurons [93, 118, 119,], they are also localized to GABA-containing interneurons [92, 93, 119, 120]. Since the effect of SKF-38393 is difficult to reconcile with an action on excitatory D<sub>1</sub> receptors located on pyramidal neurons, a more plausible explanation is that SKF-38393 would bind to dopamine D<sub>1</sub> receptors of GABAergic neurons, thereby turning on GABAergic inhibition, which would block the increase in glutamate efflux induced by MK-801. The dopamine D<sub>1</sub> receptor family is comprised of two subtypes: D<sub>1</sub> and D<sub>5</sub>. Inasmuch as the D<sub>1</sub> subtype seems to be predominant in cortical PV interneurons [120, 121], it is conceivable that, in the conditions of the present study, the blockade of MK-801-induced increase in cortical glutamate is accounted for by an action of SKF-38393 on D<sub>1</sub> receptors located in PV interneurons. However, the blockade of the effects on 5-HT might also suggest the presence of D<sub>1</sub> receptors in another subpopulation of GABAergic interneurons that influence mPFC output to the raphe nuclei (Fig (8)).

Altogether our results suggest that D<sub>2</sub> antagonists and D<sub>1</sub> agonists might end up with the same response, i.e. to restore cortical GABA efflux in order to prevent an excessive glutamatergic transmission following MK-801

administration. Furthermore, our results are also consistent with the proposal that D1 receptor activation requires phasic dopamine release whereas D2 receptors are continuously driven by basal, tonic dopamine release [122].

## **EFFECTS OF SEROTONERGIC COMPOUNDS**

In contrast to dopamine D2 receptor antagonists, the selective 5-HT<sub>2A</sub> antagonist, M100907 [123], prevented the increase in 5-HT and glutamate elicited by MK-801 in a concentration-dependent manner (Fig (5)). This agrees with prior work using a competitive NMDA receptor antagonist [16] and could reflect a reduction of a heightened prefrontal output (including that projecting to the DR) following MK-801. In fact, at the concentration of 300  $\mu$ M, M100907 decreased MK-801-induced 5-HT level below baseline values. Previous work from our lab has also shown that 5-HT<sub>2A</sub> antagonism is able to prevent other paradigms of higher serotonergic transmission in the mPFC, such as intra-mPFC perfusion of S- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid (S-AMPA), 2,5-dimethoxy-4-iodoamphetamine (DOI) and the  $\alpha_1$ -adrenoceptor antagonist cirazoline [59, 124], as well as thalamic disinhibition [60]. 5-HT<sub>2A</sub> receptors are largely localized to apical dendrites of pyramidal neurons [125, 126], a cellular zone that receives inputs from different cortical areas, allowing integration within cortical layers and between different cortical areas. For this reason, 5-HT<sub>2A</sub> antagonists are in a unique position to compensate for the increased cortico-cortical transmission that probably occurs after MK-801 administration and the pharmacological conditions mentioned above. Although there is evidence that 5-HT<sub>2A</sub> receptors are also present in cortical GABAergic interneurons of the rat mPFC [125, 127], our results points to a predominant effect of M100907 on the population of receptors located in pyramidal neurons following MK-801 administration.

Besides 5-HT<sub>2A</sub> receptor antagonism, 5-HT<sub>1A</sub> receptor agonism is also able to block the increase in 5-HT and glutamate elicited by MK-801 with a comparable potency (Fig (6)). Another study has shown that the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT also prevents the increase in cortical 5-HT and

glutamate evoked by a competitive NMDA antagonist [17]. As for the effects of M100907, the reduction of 5-HT is probably caused by a decrease in the activity of pyramidal cells projecting to the DR [128, 129], which would ease this nucleus from a tonic excitatory input, thereby decreasing the activity of 5-HT cells [130]. The 5-HT<sub>1A</sub> receptor agonist repinotan (BAY x 3702) [131] has proved also effective in blocking the increased 5-HT release induced by intra-mPFC perfusion of S-AMPA, DOI and cirazoline [59, 124], as well as thalamic disinhibition [60]. The additional blockade of increased glutamatergic transmission suggests that 5-HT<sub>1A</sub> receptor activation in the mPFC potently attenuates the action of agents that enhance the activity of pyramidal neurons, an effect shared by several different treatments and involving the stimulation of AMPA/kainate receptors in the mPFC [18, 79]. Furthermore, the crucial localization of 5-HT<sub>1A</sub> receptors in the perisomatic domain of cortical pyramidal neurons of the rat prefrontal cortex [132] might explain the powerful effects of 5-HT<sub>1A</sub> receptor agonists. Both 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors show a ~80% co-localization in the mPFC [124] with a high level of expression in pyramidal cells labeled by vGluT1 [127]. Altogether, this provides the anatomical support for an action of serotonergic compounds on pyramidal cells of the mPFC. Although 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> receptors are also expressed in cortical GABAergic interneurons of the rat [125, 127, 133], they do not seem to play a role in the control of cortical 5-HT and glutamate in the conditions of the present work.

## **EFFECTS OF $\alpha_1$ -ADRENOCEPTOR ANTAGONISTS**

As for 5-HT<sub>2A</sub> antagonists and 5-HT<sub>1A</sub> agonists, the intra-mPFC administration of the  $\alpha_1$ -adrenoceptor antagonist prazosin [134] also blocked the MK-801-induced increase in 5-HT and glutamate (Fig (7)). Although  $\alpha_1$ -adrenoceptors are largely co-expressed with 5-HT<sub>2A</sub> receptors (>80%) and are localized to both pyramidal and GABAergic cells (N. Santana, G. Mengod and F. Artigas, unpublished results) in the mPFC, the effects of prazosin seen in previous [62] and the present study are more congruous with a blockade of  $\alpha_1$ -adrenoceptors located on pyramidal neurons, including those in layer V that project to the

dorsal raphe nucleus. The mechanism of action of prazosin is most likely similar to that of M100907 inasmuch as both 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenoceptors are coupled to the same intracellular signaling mechanisms (phospholipase C) and mediate the excitatory actions of 5-HT and noradrenaline on pyramidal neurons of the mPFC [128, 135]. Moreover, the fact that 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenoceptors are localized to the same cortical areas [136-138] provides a neuroanatomical support for the parallel action of 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenoceptor antagonists. The main difference in the cortical laminar distribution between 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptors is that the latter are separated in two bands around layer V [139, 140]. In line with the present work, previous studies carried out in our laboratory have revealed that the perfusion of prazosin in the mPFC prevented the increase of 5-HT elicited by the cortical application of cirazoline, DOI and S-AMPA [60], as well as thalamic disinhibition [60]. Because all these compounds do not interact directly with each other's receptors their effects need to be interpreted not at receptorial level, but at cellular (pyramidal) level. This is correspondence with the role of pyramidal cells of the mPFC in integrating multiple inputs from cortical as well as subcortical areas [141-143].

## **IMPLICATIONS FOR ANTIPSYCHOTIC ACTION**

The findings of the present and previous [18, 62] work from our laboratory suggest that excessive glutamate transmission in the mPFC (resulting from NMDA receptor blockade) may be associated with some positive symptoms of schizophrenia, inasmuch as they are better treated by drugs that possess some degree of dopamine D2 receptor antagonism (a characteristic of the great majority of marketed antipsychotic drugs). In contrast, impairment of serotonergic pathways in the mPFC might rather be related to negative symptoms and/or cognitive deficits, conditions for which atypical antipsychotic drugs (e.g. clozapine and olanzapine) seem to display superior therapeutic efficacy. However, several recent studies have somehow challenged this purported higher efficacy in the clinic. Thus, the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study has shown that the typical drug

perphenazine is as effective as other atypical drugs in terms of PANSS scores. However, olanzapine was the medication that patients stayed on the longest in the trial [144]. In similar terms, the Cost Utility of the Latest Antipsychotics in Severe Schizophrenia (CUtLASS) study also concluded that second-generation (atypical) antipsychotics do not offer significant clinical benefits over first-generation (typical) drugs when prescribed to patients with schizophrenia [145]. Altogether these studies illustrate the difficulty of translating from experimentally-observable pharmacological differences in the preclinical setting to therapeutic advantages.

Thus, there is a clear need for targeting different transmitter receptors in order to achieve better treatment (as well as a lower side-effect profile) of schizophrenia. Pharmacological models of schizophrenia based on NMDA antagonism, dopaminergic stimulants or serotonergic agonists point to an exacerbated glutamatergic transmission, at least in the mPFC. Our results suggest that, antagonism at 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptors, as well as agonism at 5-HT<sub>1A</sub> receptors, are able to prevent the excessive glutamatergic transmission in the mPFC produced by such different conditions. In line with these results, it has been shown that 5-HT and selective receptor agonists modulate the excitability of cortical neurons and their discharge rate through the activation of several receptor subtypes: namely 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2A/2C</sub> and 5-HT<sub>3</sub> [128, 146-152]. Atypical antipsychotic drugs that exhibit 5-HT<sub>2A</sub> antagonism and/or 5-HT<sub>1A</sub> agonism, such as clozapine, increase dopamine efflux in the mPFC of rodents [57, 153-157], which is potentially involved in the improvement of negative symptoms and cognitive dysfunction in schizophrenia [154, 155, 158, 159]. This effect appears to be dependent on the presence of intact 5-HT<sub>1A</sub> receptors [57, 157]. Given that some of these drugs (e.g. olanzapine) do not depict affinity for 5-HT<sub>1A</sub> receptors (Table 1), it would seem that the 5-HT<sub>1A</sub> receptor has a permissive role in this action, rather than being directly responsible. However, the increase in dopamine efflux produced by atypical antipsychotic drugs, including olanzapine, is inhibited by the 5-HT<sub>1A</sub> receptor antagonist WAY 100635 [155] and absent in 5-HT<sub>1A</sub> receptor knockout mice [57], which suggests a pivotal role for 5-HT<sub>1A</sub> agonism in some of the key

properties desired for novel antipsychotic drugs. This concept is supported by clinical trials with partial 5-HT<sub>1A</sub> receptor agonists, which report the ability of these compounds not only to reduce the incidence of EPS [46] but also to enhance some domains of cognitive function [47]. However, inconsistent results have been found depending on the antipsychotic drug and the 5-HT<sub>1A</sub> receptor agonist used (reviewed in [160]).

With regard to  $\alpha_1$ -adrenoceptors, preclinical studies have revealed that the blockade of  $\alpha_1$ -adrenoceptors by prazosin potentiated the antipsychotic-like effect of dopamine D2 receptor antagonists [161]. In addition, all antipsychotic drugs used in this study display high *in vitro* affinity for  $\alpha_1$ -adrenoceptors (Table 1), which suggests a potential antipsychotic role of  $\alpha_1$ -adrenoceptor antagonism in the pharmacotherapy of schizophrenia. However, the use of drugs that possess a high  $\alpha_1$ -adrenoceptor antagonism *in vivo* is limited because their cardiovascular side-effects (hypotension, arrhythmia).

In summary, the intra-mPFC administration of M100907, repinotan and prazosin prevents MK-801-induced increase in 5-HT and glutamate, effects that are comparable to those obtained with clozapine and olanzapine [62; present study], and can be associated to a direct reduction the excitability of a subpopulation of pyramidal neurons in the mPFC (Fig (8)). Thus, it is possible that 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptor antagonism, as well as 5-HT<sub>1A</sub> receptor agonism, may relate to a better treatment of most symptoms of schizophrenia. In fact, preclinical studies have shown that 5-HT<sub>2A</sub> antagonists and 5-HT<sub>1A</sub> agonists can alleviate cognitive deficits induced by NMDA receptor antagonists [61]. Because each of these receptor components do not confer antipsychotic properties individually, it is conceivable that a combined effect is necessary to achieve this goal.

In a recent work, Homayoun and Moghaddam demonstrated that clozapine has the unique feature of increasing the activity of putative pyramidal neurons with low baseline firing rates and decreasing the activity of neurons with higher firing rates in prefrontal cortex [64]. Given that antagonists at D<sub>2</sub>/D<sub>3</sub>, D<sub>4</sub>, 5-HT<sub>2A</sub> receptors and a dopamine D1 receptor antagonist are all able to

increase basal extracellular glutamate on their own (Table 2), but they prevent the increase of glutamate release evoked by MK-801, the dual effect of clozapine can result from its interaction with such monoamine receptors, and this might be related to the pro-cognitive effects of the drug. It would be interesting to examine whether this effect of clozapine is shared by other atypical antipsychotic drugs with a similar pharmacological profile.

Unlike serotonergic and adrenergic compounds, the effects of dopaminergic compounds have been attributed to an increase in cortical inhibition responsible for a reduction of an excessive glutamatergic stimulation following NMDA antagonism ([62]; present study). This implies the localization of dopamine D<sub>1</sub>/D<sub>5</sub>, D<sub>2</sub>/D<sub>3</sub> and D<sub>4</sub> receptors in a subpopulation of GABA interneurons (possibly those containing PV). Interestingly, the finding that a high concentration of the SKF 38393 is also able to prevent the MK-801-induced increase of cortical 5-HT seems to suggest that D<sub>1</sub>/D<sub>5</sub> receptors are also located in a different set of GABA interneurons, perhaps those containing cholecystinin [162, 163]. Clozapine has been shown to enhance dopamine D1 receptor-mediated neurotransmission [72, 164]. Therefore, the pro-cognitive action of clozapine could also result from its action on dopamine D1 receptors. Alternatively, it has also been suggested that the action of clozapine could involve potentiation of NMDA transmission (see [165] for review). Our results support a similar view for olanzapine. On the other hand, blockade of dopamine D<sub>2</sub>/D<sub>3</sub> receptors may be in the front line of the pharmacological treatment of schizophrenia because dopamine D<sub>2</sub>/D<sub>3</sub> antagonists would increase GABA inhibition directly, thus restoring cortical synchrony.

The conclusions raised in previous [62] and the present study are established as a result of the analogies present in the action of antipsychotic drugs and selective agonists and antagonists for monoaminergic receptors. However, it remains to be determined if different effects may emerge from a combination of multiple interactions among these receptors. Furthermore, as a general rule for psychiatric illnesses, caution must be taken in extrapolating the results obtained in an animal model to a clinical setting.



## FUTURE TRENDS

From the beginning of pharmacotherapy of schizophrenia, medicine cases have been filled with drugs that shared the characteristic of being dopamine D<sub>2</sub> receptor antagonists. First generation typical antipsychotics like haloperidol and chlorpromazine potently block dopamine D<sub>2</sub>/D<sub>3</sub>/D<sub>4</sub> and  $\alpha_1$ -adrenergic receptors. The blockade of dopamine D<sub>2</sub> receptors in the PFC and the nucleus accumbens appears to be beneficial for psychotic symptoms (delusions, hallucinations). However, the same action in other areas of the brain can cause severe EPS and hyperprolactinemia [33]. Second generation atypical antipsychotic drugs like clozapine and olanzapine keep some degree of dopamine D<sub>2</sub>/D<sub>3</sub>/D<sub>4</sub> antagonism, but they display a higher antagonism at 5-HT<sub>2A/2C</sub> receptors. These features seem to be more effective for negative symptoms and cognitive deficits although some side-effects (weight gain, impairment of glucose and lipid metabolism) may appear [166]. However, this group of drugs is not homogeneous, and a recent meta-analysis of randomized controlled trials has shown that only four of these second-generation drugs (amisulpride, clozapine, olanzapine and risperidone) were better than first-generation antipsychotic drugs for overall efficacy [167]. The other second-generation drugs (quetiapine, sertindole, ziprasidone, zotepine) were not more efficacious than the first-generation drugs, even for negative symptoms. Therefore, efficacy on negative symptoms does not seem to be a core component of atypicality. Third generation antipsychotic drugs like aripiprazole and bifeprunox improve both positive and negative symptoms of schizophrenia without producing EPS or increases in serum prolactin [168, 169]. The unifying features of this third generation antipsychotics are the association of D<sub>2</sub>/D<sub>3</sub> interaction (antagonism or partial agonism) with 5-HT<sub>1A</sub> receptor activation without the requirement for 5-HT<sub>2A</sub> receptor blockade. More recently it has been shown that drugs that interact with mGluR also have potential for the treatment of schizophrenia [37, 170, 171]. However, a significant proportion of patients still do not experience complete remission of their positive symptoms and negative/cognitive symptoms remain poorly treated. In recent years, research

and development efforts have sought novel "atypical" antipsychotic drugs that would offer the therapeutic advantages of clozapine without the associated risk of side effects.

In the search for reliable biomarkers to be used in R+D of newer drugs, our results first suggest that the blockade of an exacerbated 5-HT release in the mPFC induced by NMDA antagonists can be a useful biochemical marker of "atypicality" of antipsychotic drugs. Although this has been established for clozapine and olanzapine (drugs that display a similar pharmacological profile), further research is needed to determine whether this is a distinct feature of other second generation antipsychotic drugs and, most interestingly, if this holds also true for third generation antipsychotics (which represents receptor mechanisms that include dopamine D<sub>2</sub>/D<sub>3</sub> receptor partial agonism).

GABAergic interneurons in prefrontal cortex play a critical role in cortical circuits by providing feedforward and feedback inhibition and synchronizing neuronal activity [172, 173]. In this regard, we also propose that blockade of dopamine D<sub>2</sub>/D<sub>3</sub>/D<sub>4</sub> as well as dopamine D<sub>1</sub> agonism in the mPFC may lead to a restoration of cortical GABA inhibition and synchrony alleged to be impaired in schizophrenia [102-104, 174]. Further support for this hypothesis has been obtained recently in our lab showing that both clozapine and haloperidol have the ability of reverse the effects of PCP on pyramidal cell firing and cortical synchronization [65]. However, it is also possible that the same effect is caused by different mechanisms, i.e. blockade of dopamine D<sub>2</sub>/D<sub>3</sub>/D<sub>4</sub> receptors (haloperidol) or 5-HT<sub>2A</sub> receptors (clozapine). Further research is needed to verify such different alternatives.

On the other hand, antagonism at 5-HT<sub>2A</sub> and  $\alpha_1$ -adrenergic receptors, as well as agonism at 5-HT<sub>1A</sub> receptors may also be helpful by suppressing an excessive stimulation of pyramidal cells. One of the most important issues in this field is the fact that all these monoamine receptors implicated in the pharmacological treatment of schizophrenia are present in both pyramidal cells and GABA interneurons in the mPFC [93, 127]. Therefore, the question arises as to what factors determine the binding of a drug predominantly to one of these

cellular populations. With this in mind, newer antipsychotics would target receptors responsible for stimulating cortical GABA inhibition and diminishing excessive excitability of pyramidal cells without the undesired effects of similar actions in other areas of the brain. In addition, the regulatory changes of the complex formed by 5-HT<sub>2A</sub> and mGluR2 receptors presumed to be involved in the altered cortical processes [175] also suggests that this receptor complex is a promising new target for the treatment of psychosis.

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**Table 1.** In vitro binding affinities of the four antipsychotics used in the present study, expressed in  $K_i$  values (nM).

<b>Receptor</b>	<b>Clozapine</b>	<b>Olanzapine</b>	<b>Haloperidol</b>	<b>Chlorpromazine</b>
D <sub>1</sub>	85	31	210	56
D <sub>2</sub>	125	11	1	2
D <sub>3</sub>	473	27	2	9
D <sub>4</sub>	35	21	5	12
5-HT <sub>1A</sub>	770	>10,000	2,600	116*
5-HT <sub>2A</sub>	12	2	78	2
5-HT <sub>2C</sub>	8	11	1,500	25
$\alpha_1$ -adrenergic	7	7	6	1
H <sub>1</sub> histamine	6	7	>730	9
Muscarinic	2	2	>1,500	60

Data taken from references [44, 45, 52, 81-83].

\*Data from human cloned 5-HT<sub>1A</sub> receptors.

**Table 2.** Changes in extracellular 5-HT and glutamate in the mPFC as a result of the action of intra-mPFC administration of drugs on different monoamine receptors. (=) unchanged, (↑) increase and (↓) decrease.

Receptor action	Changes in 5-HT	Changes in glutamate
D <sub>2</sub> /D <sub>3</sub> antagonism	=	↑
D <sub>4</sub> antagonism	=	↑
D <sub>1</sub> agonism	↓	↑
5-HT <sub>1A</sub> agonism	↓	=
5-HT <sub>2A</sub> antagonism	↓	↑
α <sub>1</sub> -adrenoceptor antagonism	↓	=

Data taken from reference [60, 62] and X. López-Gil, F. Artigas and A. Adell (unpublished results).

## FIGURE LEGENDS

**Figure 1.** Effects of the intra-mPFC perfusion (line) of 300  $\mu$ M of clozapine (CLZ), olanzapine (OLZ), chlorpromazine (CPZ) and haloperidol (HAL) 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. Analysis of variance (ANOVA) shows that all four antipsychotic drugs block the increase in glutamate produced by MK-801, but only clozapine and olanzapine are able to block the effect of MK-801 on 5-HT.

**Figure 2.** Effects of the perfusion of the dopamine D<sub>2</sub>/D<sub>3</sub> antagonist raclopride (RAC, 10, 30 and 100  $\mu$ M) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs. control group and  $\S$ p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA).

**Figure 3.** Effects of the perfusion of the selective dopamine D<sub>4</sub> antagonist L-745,870 (L, 10 and 30  $\mu$ M) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs. control group and  $\S$ p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA).

**Figure 4.** Effects of the perfusion of the dopamine D<sub>1</sub>/D<sub>5</sub> agonist SKF 38393 (SKF, 1, 10 and 100  $\mu$ M) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs.

control group and §p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA).

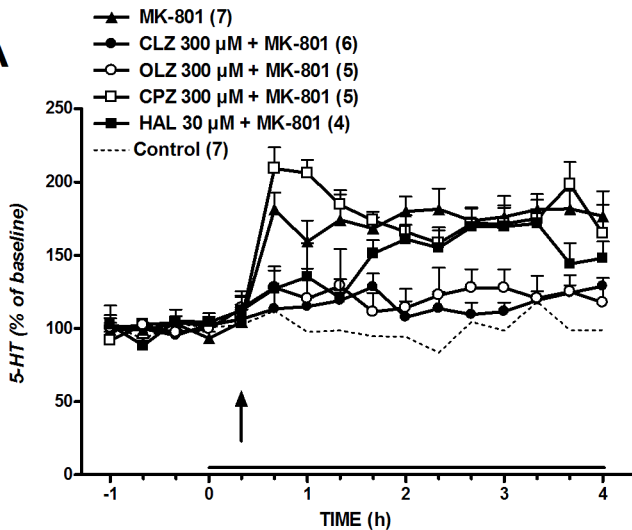
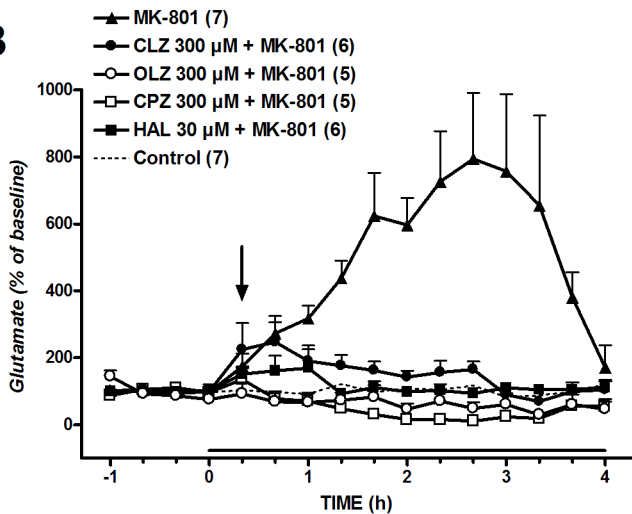
**Figure 5.** Effects of the perfusion of the selective 5-HT<sub>2A</sub> antagonist M100907 (M, 1, 10 and 300 µM) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs. control group and §p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA).

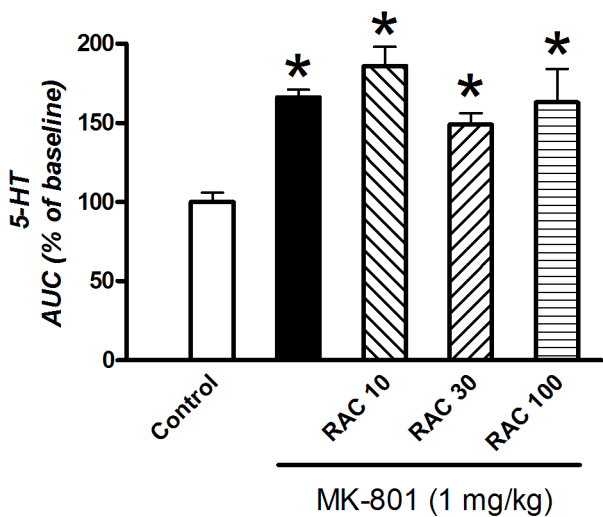
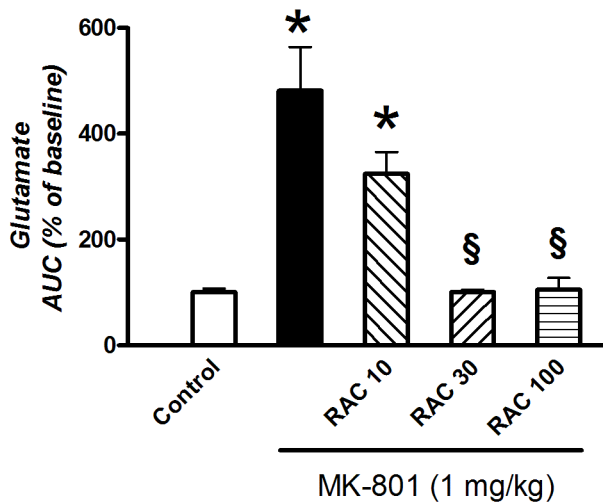
**Figure 6.** Effects of the perfusion of the selective 5-HT<sub>1A</sub> agonist repinotan (BAY x 3702; BAY, 1 and 30 µM) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs. control group and §p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA). Data taken and redrawn from reference [62], with permission.

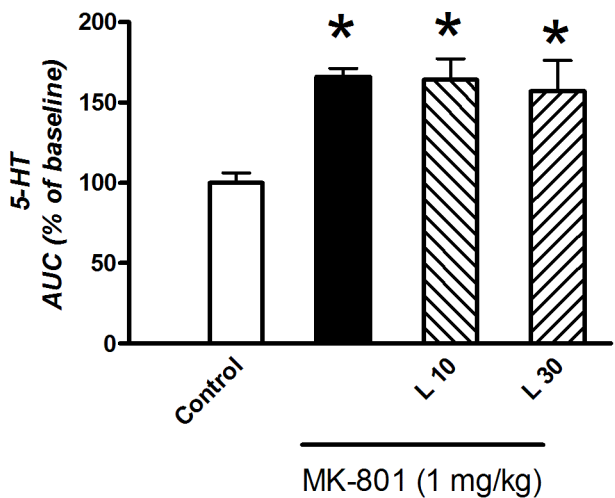
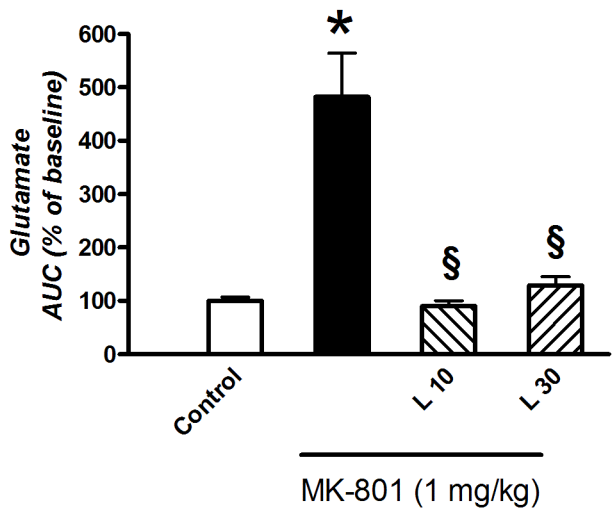
**Figure 7.** Effects of the perfusion of the selective α<sub>1</sub>-adrenoceptor antagonist prazosin (PRZ, 1, 10 and 100 µM) on the efflux of 5-HT (A) and glutamate (B) in the mPFC elicited by MK-801 (1 mg/kg, i.p.; arrow). Data are based on integrated area under the curve (AUC) analysis of 4-h perfusion, and expressed as the percentage change from the control group depicted as open bar. \*p < 0.01 vs. control group and §p < 0.01 vs. MK-801 alone (Newman-Keuls test following ANOVA).

**Figure 8.** The diagram illustrates the functional relationships between the mPFC and its dopaminergic and serotonergic projections from the VTA and the DR, respectively. Although all monoaminergic receptors depicted in this figure are present in both pyramidal cells and GABAergic interneurons, it is only

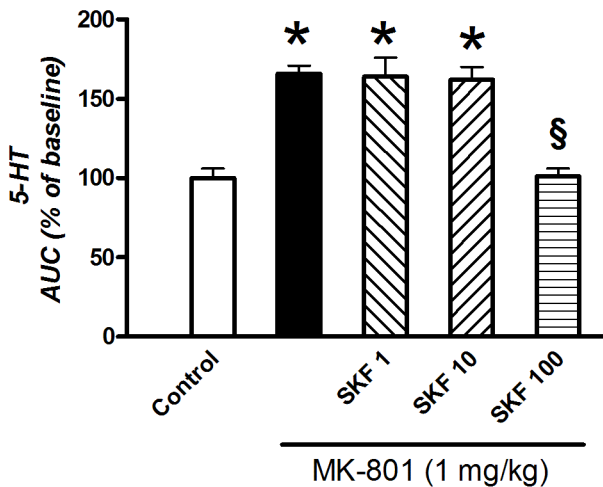
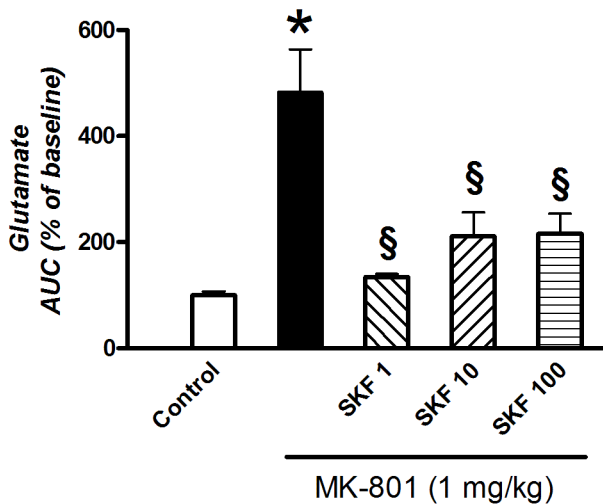
represented the localization that conforms to the results obtained in the present study.

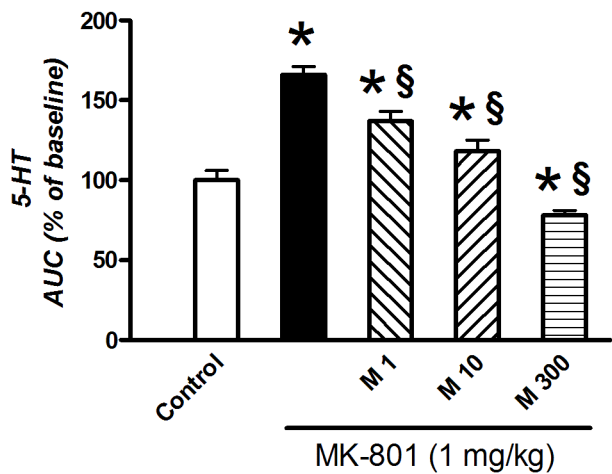
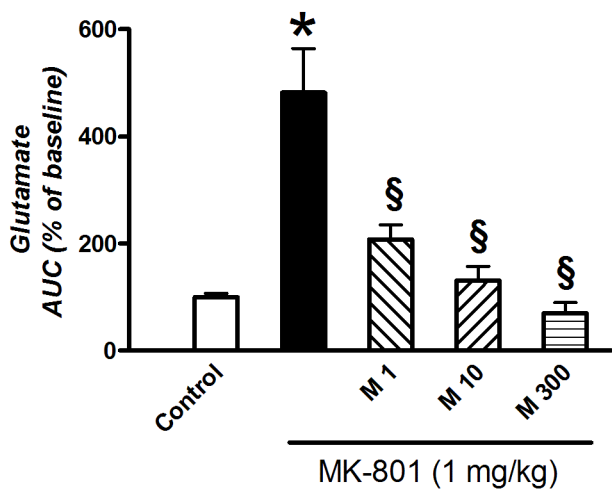
**A****B**

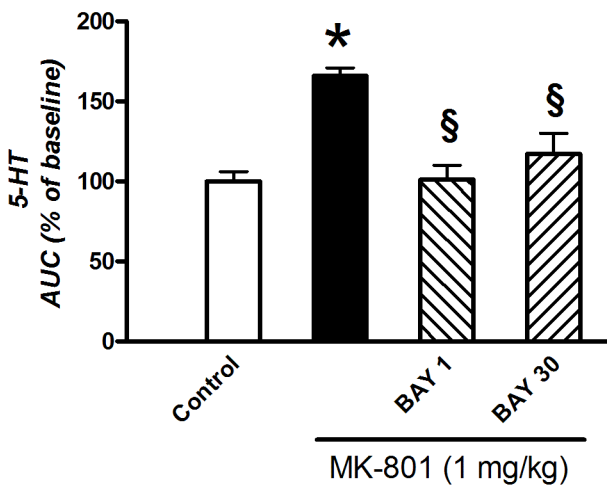
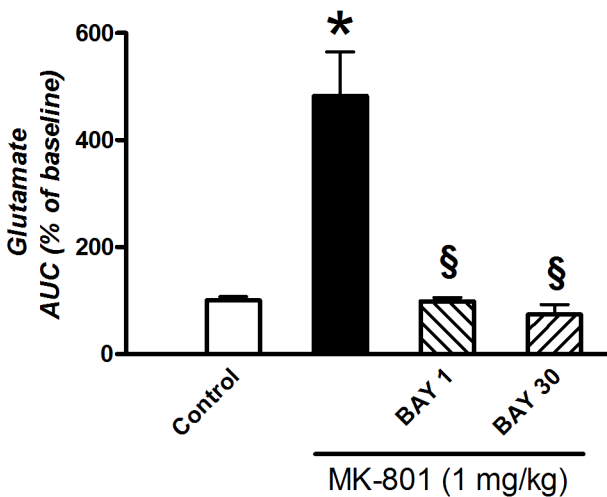
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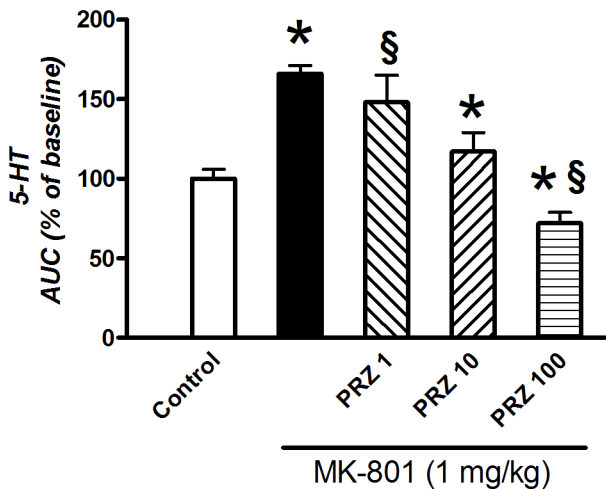
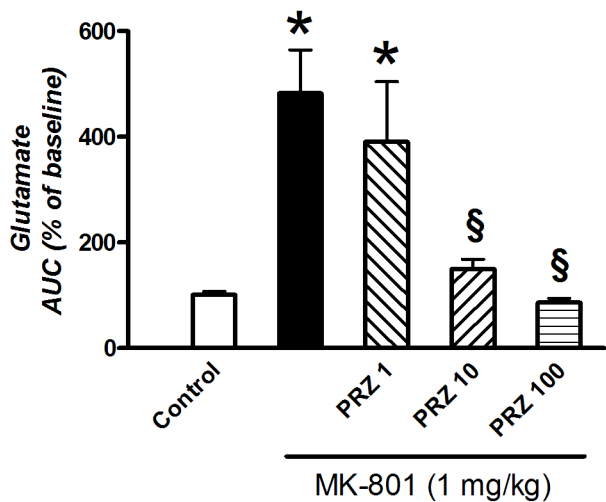
**A****B**



**A****B**

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**A****B**

# mPFC

