TREBALL 1

***In vivo* modulation of the activity of piramidal neurons in the rat medial prefrontal cortex by 5-HT_{2A} receptors: relationship to thalamocortical afferents.**

M. Victoria Puig, Pau Celada, Llorenç Díaz Mataix i Francesc Artigas

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En aquest treball es mostra que l'activació dels receptors de serotonina 5-HT_{2A} per l'agonista dels receptors 5-$HT_{2A/2C}$ DOI, incrementa la freqüència de descàrrega de les neurones piramidals de l'escorça prefrontal medial. La deshinibició dels nuclis CM i MD del tàlem mimetitza els efectes del DOI perquè incrementa l'activitat piramidal i l'alliberació de 5-HT a l'EPFm. L'activació de receptors μ-opiodes i mGlu II localment a l'EPFm reverteix l'efecte de la desinhibició talàmica però no l'efecte del DOI, a més aquest fàrmac fa el mateix efecte tant en rates controls com en rates amb el tàlem danyat.

Amb els resultats d'aquest article concloem que l'activació dels receptors 5-HT_{2A} pel DOI incrementa l'activitat del circuit EPFm-DR (Martín-Ruiz et al., 2001) per una acció en els receptors 5-HT_{2A} postsinàptics (localitzats a les neurones piramidals) però no els presents a les aferències talàmic – corticals.

In Vivo Modulation of the Activity of Pyramidal Neurons in the Rat Medial Prefrontal Cortex by 5-HT$_{2A}$ Receptors: Relationship to Thalamocortical Afferents

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The activation of 5-HT$_{2A}$ receptors in medial prefrontal cortex (mPFC) by the hallucinogen DOI increases the firing activity of dorsal raphe (DR) 5-HT neurons and prefrontal 5-HT release. Here we show that the i.v. administration of DOI markedly affected the firing rate of identified pyramidal neurons recorded extracellularly. DOI excited (481%) 21/56 neurons, inhibited (11%) 17/56 neurons and left the rest unaffected (overall 2.4-fold increase in firing rate). Both effects were antagonized by 5-HT$_{2A}$ receptor blockade. 5-HT$_{2A}$-mediated orthodromic excitations were recorded in pyramidal neurons projecting to DR after electrical stimulation of this nucleus. We also examined whether the effects of DOI in mPFC involve thalamic excitatory inputs. The disinhibition of the mediodorsal and centromedial nuclei of the thalamus by local bicuculline resembled the effects of DOI as it increased pyramidal cell firing and 5-HT release in mPFC. However, the selective activation of prefrontal μ-opioid and mGlu II receptors counteracted the effects of the thalamic disinhibition but not those of DOI. Moreover, extensive thalamic lesions did not alter the effect of DOI on pyramidal cell firing and 5-HT release. We conclude that DOI increases the activity of the mPFC-DR circuit by an action on postsynaptic 5-HT$_{2A}$ receptors unrelated to thalamocortical afferents.

Introduction

The prefrontal cortex is involved in a large number of higher brain functions (working memory, cognition, affect, behavioral inhibition, etc.) and controls hierarchically the activity of subcortical structures (Fuster, 1997; Miller and Cohen, 2001). Pyramidal neurons in cortical layer V integrate excitatory inputs from cortical and subcortical areas. The latter include, among others, several nuclei of the thalamus, such as the mediodorsal (MD) and centromedial (CM) nuclei (Berendse and Groenewegen, 1991; Fuster, 1997; Kuroda *et al.*, 1998; Van der Werf *et al.*, 2002). Modulatory inputs arise from the brainstem aminergic nuclei (raphe nuclei, ventral tegmental area and locus coeruleus) (Azmitia and Segal, 1978; Phillis, 1984; Kosofsky and Molliver, 1987; Durstewitz *et al.*, 2000; Lewis and O'Donnell, 2000). Signal integration in pyramidal neurons is exerted at various cellular levels, with a key role played by the large apical dendrites. These are highly enriched in serotonergic 5-HT$_{2A}$ receptors which are also present in large and medium-size GABAergic interneurons that control the activity of pyramidal neurons in local microcircuits (Willins *et al.*, 1997; Jakab and Goldman-Rakic, 1998, 2000; Jansson *et al.*, 2001; Martín-Ruiz *et al.*, 2001). Hallucinogens like LSD or DOI are agonists and atypical antipsychotics are antagonists at 5-HT$_{2A}$ receptors (Kroeze and Roth, 1998; Meltzer, 1999). Also, atypical antipsychotics can augment the effects of antidepressant drugs (Ostroff and Nelson, 1999; Shelton *et al.*, 2001; Marangell *et al.*, 2002). Moreover, although the physiological role of cortical 5-HT$_{2A}$ receptors remains largely unknown, recent data suggest their involvement in working memory (Williams *et al.*, 2002). Together, these observations suggest that 5-HT$_{2A}$ receptor blockade may be beneficial for the treatment of severe psychiatric disorders.

Recently, we showed that the selective activation of prefrontal 5-HT$_{2A}$ receptors by DOI increased the firing activity of 5-HT neurons in the dorsal raphe nucleus (DR) and the 5-HT release in mPFC (Martín-Ruiz *et al.*, 2001). These effects are presumably mediated by an increased excitatory transmission through pyramidal axons projecting to DR 5-HT neurons (Aghajanian and Wang, 1977; Sesack *et al.*, 1989; Takagishi and Chiba, 1991; Hajós *et al.*, 1998; Peyron *et al.*, 1998; Celada *et al.*, 2001). However, conflicting results have been reported in regards to the effects of 5-HT$_{2A}$ receptor activation on cortical pyramidal cells. Thus, the microiontophoretic application of DOI to anesthetized rats predominantly inhibited prefrontal neurons, although low ejection currents potentiated the excitatory effect of glutamate (Ashby *et al.*, 1989, 1990). Using *in vitro* intracellular recordings of pyramidal neurons, depolarizing and hyperpolarizing effects of 5-HT through the activation of 5-HT$_{2A}$ receptors have been reported (Araneda and Andrade, 1991; Tanaka and North, 1993; Aghajanian and Marek, 1997, 1999, 2000; Arvanov *et al.*, 1999; Zhou and Hablitz, 1999). DOI has been shown to prolong late EPSCs evoked in layer V pyramidal neurons by electrical stimulation of afferent fibers (Aghajanian and Marek, 1999). However, an examination of the *in vivo* systemic actions of DOI on the firing activity of pyramidal cells is lacking. Moreover, the cellular site(s) responsible for the above effects are not fully elucidated. Hence, despite the abundance of 5-HT$_{2A}$ receptors in pyramidal neurons compared to other cellular locations (Willins *et al.*, 1997; Jakab and Goldman-Rakic, 1998, 2000), the 5-HT-induced EPSCs in layer V pyramidal neurons have been attributed to the stimulation of terminal 5-HT$_{2A}$ receptors on thalamocortical afferents on the basis of lesion and pharmacological studies (Marek and Aghajanian, 1998; Marek *et al.*, 2001). Also, thalamic lesions partly prevented the increase in *c-fos* expression produced mainly in non-pyramidal neurons of somatosensory cortex by a large systemic dose of DOI (Scruggs *et al.*, 2000).

Using extracellular recordings of projection neurons and intracerebral microdialysis, in the present study we sought to examine whether DOI enhances the firing activity of projection pyramidal neurons in mPFC, and whether these effects are dependent on 5-HT$_{2A}$ receptors putatively located in thalamocortical afferents.

Materials and Methods

Experimental Design

To experimentally assess the above points, we examined the effects of the administration of DOI on the firing rate of projection pyramidal neurons and 5-HT release in mPFC. We also examined the effects of increasing the excitatory thalamic input onto mPFC neurons on the pyramidal firing rate and 5-HT release. Thalamic disinhibition was carried out by locally

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applying bicuculline in the mediodorsal (MD) and centromedial (CM) nuclei, which project to the cingulated and limbic parts of the mPFC (Berendse and Groenewegen, 1991; Fuster, 1997; Van der Werf *et al.*, 2002). Finally, we assessed the effects of drugs modulating glutamate release from thalamocortical afferents and of extensive thalamic lesions on the DOI-induced effects. Specifically, we tested the hypotheses that (i) DOI may enhance the firing rate of pyramidal neurons in mPFC *in vivo*, thus resulting in an increased excitatory output to subcortical structures, and (ii) this effect may be independent of thalamocortical inputs. The effects of the excitatory thalamic input on pyramidal cell firing and 5-HT release in mPFC have been studied using single unit recordings and dual probe microdialysis (mPFC and thalamus), respectively. To increase the excitatory input onto mPFC, we applied bicuculline by reverse dialysis through the thalamic probe in order to disinhibit thalamocortical afferents. Also, by performing lesions of the thalamic nuclei projecting to mPFC, we studied whether the effects of DOI on 5-HT release and on pyramidal cell firing depend on the integrity of thalamic inputs onto this cortical area.

Animals

Male albino Wistar rats (Iffa Credo, Lyon, France) weighing 250–320 g and kept in a controlled environment (12 h light–dark cycle and 22 ± 2°C room temperature) with food and water provided *ad libitum*, were used in *in vivo* experiments. Animal care followed the European Union regulations (OJ of EC L358/1 18/12/1986).

Drugs and Reagents

5-HT oxalate, (–)-bicuculline methiodide, DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]), (+)-MK-801 (dizocilpine) and picrotoxinin were from Sigma/RBI (Natick, MA). 1S,3S-Aminocyclopentane dicarboxylic acid (1S,3S-ACPD), DAMGO and NBQX (2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide) were from Tocris (Bristol, UK). Citalopram·HBr, LY-379268 {(–)-2-oxa-4-aminobicyclo-[3.1.0]hexane-4,6-dicarboxylate]} and M100907 [R-(+)-alpha-(2,3-dimethoxyphenil)-1-[4-fluorophenylethyl]-4-piperidinemethanol] (Lilly code LY 368675) were from Lundbeck A/S and Eli Lilly & Co., respectively. Other materials and reagents were from local commercial sources. For the assessment of local effects, drugs were dissolved in the perfusion fluid and applied by reverse dialysis at the stated concentrations. Concentrated solutions (1 mM; pH adjusted to 6.5–7 with $NaHCO_3$ when necessary) were stored frozen (–80°C) and working solutions were prepared daily by dilution. Concentrations are expressed as free bases. In microdialysis experiments, control rats were perfused for the entire experiment with artificial cerebrospinal fluid. The concentrations of DOI and other agents used in the present microdialysis experiments were determined in previous studies (Martín-Ruiz *et al.*, 2001) or in pilot studies. Nominal concentrations are much greater than the *in vitro* drug affinities for receptors in order to allow for sufficient stimulation of the desired receptors in the *in vivo* conditions. Indeed, only a small percentage of the drugs applied by reverse dialysis at the flow rate used enter the brain compartment and then, they are continuously cleared by the CSF and the systemic circulation. The bars in the figures show the period of drug application (corrected for the void volume of the system). In experiments involving systemic administration, the drugs were administered i.v. at the doses stated.

Electrophysiological Experiments

We carried out three types of electrophysiological experiments involving the extracellular recording of pyramidal neurons in mPFC. We examined effects of the i.v. administration of DOI (0.05–0.6 mg/kg in cumulative doses) on the firing rate of pyramidal cells. In some instances, the 5-HT_{2A} receptor antagonist M100907 (0.12–1 mg/kg i.v. in cumulative doses) was administered after DOI to examine the involvement of 5-HT_{2A} receptors in the action of DOI. Secondly, we assessed the effects of endogenous 5-HT on the activity of pyramidal neurons in mPFC. To this end, the DR was stimulated (0.15–2 mA, 0.2 ms square pulses, 0.9 Hz) and peristimulus time histograms (PSTH) were constructed in baseline conditions and after the administration of M100907. Finally, we assessed the effects of the application of bicuculline in the CM + MD nuclei of the thalamus. Bicuculline (0.1–0.3 mM; dissolved in aCSF) was infused through a stainless steel (32 g) cannula (n = 3) implanted in the thalamus (tip coordinates: AP –3.6, L –0.7, DV –6.5). The cannula was attached to a 10 µl Hamilton syringe by a Teflon tubing. A microinfusion pump (Bioanalytical Systems Inc., West Lafayette, IN) was used. After recording baseline spontaneous activity for at least 5 min, 0.2 µl of bicuculline (0.1–0.3 mM) were infused through the cannula over the course of 1 min. This volume has been reported to diffuse to a maximum effective diameter of 0.4–0.6 mm (Myers, 1971). In another group of rats, we applied bicuculline (1 mM, dissolved in the aCSF) in the CM + MD thalamic nuclei by reverse dialysis using a modified dialysis probe designed to minimize dead volume. Flow rate was adjusted at 3 µl/min to reduce the onset of delivery of bicuculline to the brain tissue. Basal firing activity of pyramidal cells was recorded and then the aCSF used to perfuse the probes was changed to one containing bicuculline.

Single-unit extracellular recordings of pyramidal neurons were performed as follows. Rats were anesthetized (chloral hydrate 400 mg/kg i.p.) and positioned in a stereotaxic apparatus. Additional doses of chloral hydrate (80 mg/kg) were administered i.v. through the femoral vein. Body temperature was maintained at 37°C throughout the experiment with a heating pad. All wound margins and points of contact between the animal and the stereotaxic apparatus were infiltrated with lidocaine solution (5%). In order to minimize pulsation, the atlanto-occipital membrane was punctured to release some CSF. Stimulating electrodes were stereotaxically positioned in the DR or ventral tegmental area (VTA) (coordinates: DR, AP –7.8, L –3.1, DV –6.8 with an angle of 30° to avoid the sinus; VTA, AP –6.0, L –0.5, DV –8.2) and were stimulated at 0.15–2 mA, 0.2 ms square pulses, 0.9 Hz. Pyramidal neurons were recorded extracellularly with glass micropipettes pulled from 2.0 mm capillary glass (WPI, Saratosa, FL) on a Narishige PE-2 pipette puller (Narishige Sci. Inst., Tokyo, Japan). Microelectrodes were filled with 2 M NaCl. Typically, impedance was between 4 and 10 MΩ. Constant current electrical stimuli were generated with a Grass stimulation unit S-48 connected to a Grass SIU 5 stimulus isolation unit. Single unit extracellular recordings were amplified with a Neurodata IR283 (Cygnus Technology Inc., Delaware Water Gap, PA), postamplified and filtered with a Cibertec amplifier (Madrid, Spain) and computed on-line using a DAT 1401plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Descents were carried out at AP +3.2 to +3.4, L –0.5 to –1.0, DV –1.1 to –4.8 below the brain surface. All recorded pyramidal neurons were identified by antidromic activation from DR and/or VTA and collision extinction with spontaneously occurring spikes (Fuller and Schlag, 1976). Neurons without antidromic activation or without spontaneous firing activity were discarded.

Surgery and Microdialysis Procedures

An updated description of the microdialysis procedures used can be found in Adell and Artigas (Adell and Artigas, 1998). Briefly, anesthetized rats (pentobarbital, 60 mg/kg i.p.) were stereotaxically implanted with concentric microdialysis probes equipped with a Cuprophan membrane. In experiments involving the local effect of DOI in mPFC, rats were implanted with one probe in mPFC (AP +3.2, L –0.8, DV –6.0; probe tip: 4 mm of active membrane, between DV –1.5 to –5.5) [coordinates in mm (Paxinos and Watson, 1986)]. In experiments involving thalamic disinhibition rats were implanted with two probes (see below). On the day following implants, microdialysis experiments were performed in freely moving (DOI effect) or chloral hydrate anesthetized rats (thalamic disinhibition). The probes in mPFC were perfused at 1.5 µl/min with artificial cerebrospinal fluid (aCSF; 125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) containing 1 µM citalopram. After a 1 h stabilization period, four fractions were collected to obtain basal values before local administration of drugs by reverse dialysis. Successive 20 min (30 µl) dialysate samples were collected. In most experiments, the partial 5-$HT_{2A/2C}$ receptor agonist DOI was applied alone for 2 h (six fractions), followed by its application in combination with other drugs for another 2 h period.

For experiments in chloral hydrate anesthetized rats, these were implanted with two microdialysis probes, one in mPFC (as above) and a second one in a thalamic area sampling parts of the CM an MD nuclei of the thalamus (AP –3.6, L –0.7, DV –6.5; probe tip 1.5 mm). Bicuculline (1 mM, dissolved in aCSF) was perfused through the probe implanted in the thalamus at 1.5 µl/min and the effects on the 5-HT release in mPFC

were analyzed. These experiments were aimed at disinhibiting thalamic excitatory afferents to mPFC and examining the effects on distal 5-HT release. We also examined the modulation of these effects on prefrontal 5-HT release by drugs perfused through the probe in mPFC (NBQX, 1S,3S-ACPD, LY-379268 and DAMGO).

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

Thalamic Lesions

Electrolytic lesions of several thalamic nuclei projecting to the mPFC (Berendse and Groenewegen, 1991; Van der Werf *et al.*, 2002) were performed by passing 1.5 mA (two pulses of 10 s each; Grass stimulation unit S-48 connected to a Grass SIU 5 stimulus isolation unit) at three different localizations: (a) AP −1.8, L −0.7, DV −5.7, (b) AP −2.7, L −0.7, DV −6.3, and (c) AP −3.6, L −0.7, DV −6.5 (Fig. 1). The tip of electrodes was peeled off (~0.5 mm) and poles were slightly separated (~0.5 mm) to affect a larger tissue area. The coordinates used were those employed by Marek *et al.* (Marek *et al.*, 2001) to perform chemical (NMDA) thalamic lesions and were intended to lesion the two main nuclei projecting densely to the mPFC, CM and MD as well as several neighboring nuclei which also project to the mPFC area sampled by microdialysis probes and recording electrodes in the present study. These included, among others, the paraventricular (PV), paracentral (PC) and paratenial (PT) nuclei. Sham controls were stereotaxically implanted with the electrodes but no current was passed. Single unit recordings and microdialysis experiments were conducted 8 days after the lesion. One microdialysis experiment was carried out in rats lesioned 3 days before.

Histological Examinations

At the end of the experiments, rats were killed by an overdose of anesthetic. The placement of the dialysis probes was examined by perfusion of Fast green dye and visual inspection of the probe track after cutting the brain at the appropriate level. In experiments involving disinhibition of thalamic afferents to mPFC the placement of the thalamic probes or cannulae was verified histologically with Neutral Red staining after transcardial perfusion with saline followed by 10% formalin solution (Sigma). Lesioned animals were examined in the same way. Brains were post-fixed, sagitally sectioned (80 μm) and stained with Neutral Red.

Data and Statistical Analysis

Microdialysis results are expressed as fmol/fraction (uncorrected for recovery) and shown in figures as percentages of basal values (individual means of four pre-drug fractions). The statistical analysis was carried out using one- or two-way ANOVA for repeated measures followed by Duncan test. The effects of DOI or thalamic disinhibition with bicuculline on the 5-HT release in mPFC were assessed using one-way ANOVA of the 5-HT values (four stable basal fractions plus all post-drug fractions). In experiments devised to reverse the effect of the thalamic disinhibition or DOI application on 5-HT release, a second drug was applied through the microdialysis probe in mPFC. The assessment of the effects of this second drug was done with fractions 7–16. These correspond to four fractions with an stabilized effect of DOI or thalamic disinhibition on 5-HT release in mPFC followed by the six fractions (2 h) in which additional drugs were applied in mPFC. The effect of DOI on pyramidal neuron firing was assessed using paired Student's *t*-test. Changes in firing rate were quantified by averaging the values after i.v. drug injection (omitting the first minute). Data are expressed as the mean ± SEM. Statistical significance has been set at the 95% confidence level (two-tailed).

Results

Firing Characteristics of Projection Neurons in mPFC

A total of 144 projection pyramidal neurons recorded in untreated rats have been included in this study (15 additional neurons were recorded in rats with thalamic lesions, whose characteristics are given below). All them were identified by

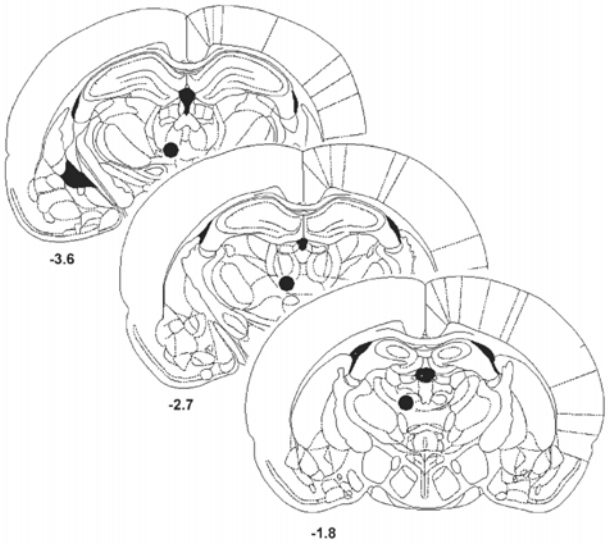

Figure 1. Schematic representation of the location (black dots) of the tip of bipolar electrodes used to deliver electrolytic lesions in the thalamus. Each plate [(Paxinos and Watson, 1997) CD edition, with permission from Elsevier Science] corresponds to each of the three AP coordinates used (–1.8, –2.7 and –3.6 mm from bregma). See the actual extent of the lesions in Figure 8.

antidromic activation from the DR (n = 104) or the VTA (n = 48) as described in Materials and Methods (Fig. 1*A*,*E*). In some instances, pyramidal neurons projecting to both midbrain structures were recorded (n = 10). However, since no systematic stimulation from both midbrain nuclei were performed, these figures are not representative of the actual proportion of mPFC neurons innervating the DR and VTA concurrently. Two additional neurons were identified by antidromic activation from MD nucleus. The mean firing rate was 1.52 ± 0.14 spikes/s (n = 144). According to previously reported data (Dégenètais *et al.*, 2002), we identified neurons with regular spiking and inactivating burst firing modes. Figure 2 shows typical examples of the waveform and firing characteristics of the recorded neurons as well as the identification by antidromic activation and collision test of a prefrontal neuron projecting to the DR. The calculated average conduction velocity (linear distance/latency of antidromic spikes) was 0.93 ± 0.03 m/s for the neurons projecting to the DR (n = 104) and 1.89 ± 0.15 m/s for those projecting to the VTA (n = 48). These values fall within the range of previously reported values for cortical pyramidal neurons (Thierry *et al.*, 1983; Peterson *et al.*, 1990).

Effects of DOI on the Firing Rate of Projection Pyramidal Neurons

The i.v. administration of DOI resulted in three types of responses in pyramidal neurons of the mPFC. Typically, pyramidal neurons responded to doses of DOI between 0.05 and 0.3 mg/kg i.v. One additional neuron responded to 0.6 mg/kg i.v. DOI enhanced the firing rate of 38% of the neurons examined (21/56). The mean firing rate rose from 1.34 ± 0.36 spikes/s (baseline) to 6.45 ± 0.97 spikes/s (481%; n = 21). When considering the individual change from baseline, DOI increased the firing rate to 1525 ± 549%. This difference (1525 versus 481%) is due to the low basal firing rate of some of the recorded units, which made the change (expressed as percentage of basal) much greater than the ratio between mean baseline and

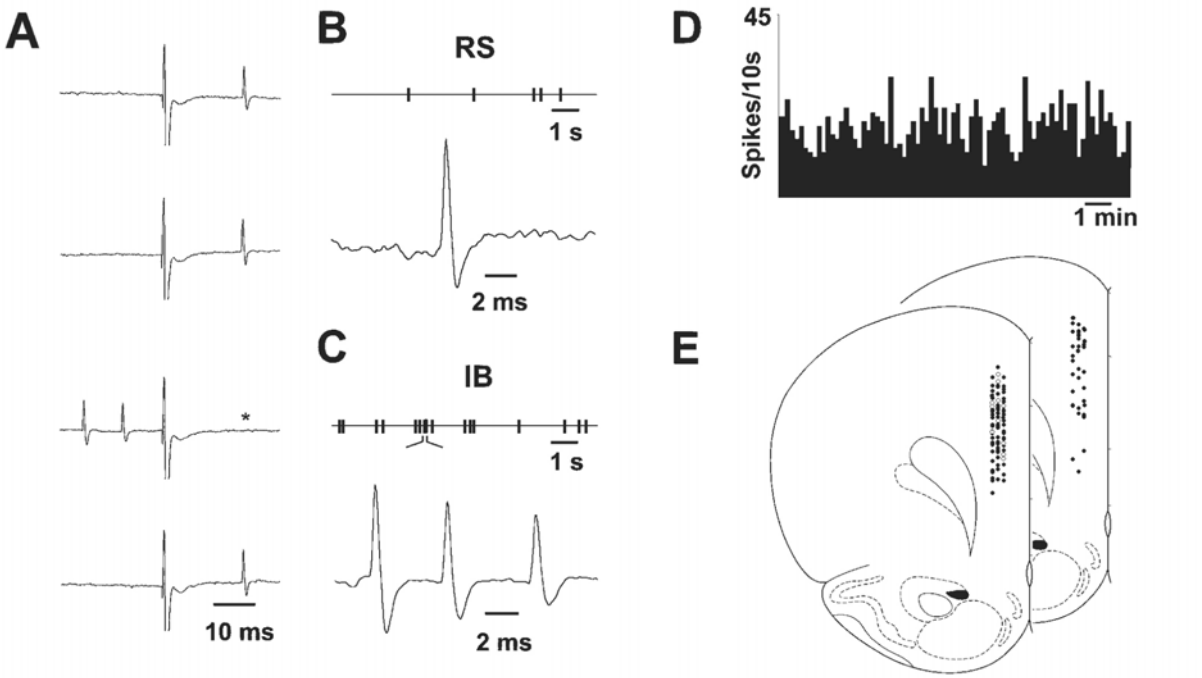

Figure 2. Extracellular recordings of projection neurons in the mPFC. (*A*) Recording of a cell projecting to the DR, as shown by antidromic stimulation from this midbrain area. The asterisk denotes an antidromic spike missing due to collision with ongoing spontaneous action potentials. (*B*) and (*C*) Representative spikes and firing pattern of projection neurons in mPFC exhibiting regular mode of firing (regular spiking, RS) or burst firing (inactivating burst firing, IB) (Dégenètais *et al.*, 2002). Note the attenuation in spike amplitude in the neuron firing in the IB mode. (*D*) A representative integrated firing rate histogram of a projection neuron in baseline conditions. (*E*) Schematic drawing of a frontal section of the rat brain at anterior + 3.2 [(Paxinos and Watson, 1997) CD edition, with permission from Elsevier Science] illustrating the area where pyramidal neurons were recorded. The section on the right shows the DV location of projecting neurons identified by antidromic stimulation from VTA (black dots). Left section shows the recording sites of pyramidal neurons projecting to DR (black dots) and the ones which are antidromically activated when stimulated from both DR and VTA (open dots).

post-DOI firing rates in the 21 neurons examined. Another 32% of projection neurons (18/56) were unaffected by DOI administration (up to 0.3–0.8 mg/kg i.v.) whereas the rest (17/56, a 30%) experienced reductions in cell firing (to 11 ± 3% on average). Baseline firing rate of the neurons excited or inhibited by DOI did not significantly differ (1.34 ± 0.36 spikes/s for excitations versus 1.20 ± 0.33 spikes/s for inhibitions). Summing over all neurons examined, the systemic administration of DOI significantly increased the output of mPFC projection neurons a 238%, from 1.23 ± 0.18 to 2.93 ± 0.53 spikes/s (n = 56; P < 0.002, paired t-test). Figure 3 shows representative examples of projection pyramidal neurons in mPFC excited and inhibited by the systemic administration of DOI.

The reduction in firing rate elicited by DOI was antagonized by the selective 5-HT_{2A} receptor antagonist M100907 (0.1–0.5 mg/kg; n = 6; Fig. 3*C*) in all cases examined. The inhibitory effect of DOI was also reversed by picrotoxinin administration (1–3 mg/kg i.v.; n = 5; Fig. 3*D*). The DOI-induced excitations were reversed by M100907 (0.1–0.5 mg/kg i.v.) in six out of nine cases examined. Because DOI is also a partial agonist at 5-HT_{2C} receptors, we cannot discard that its effects on some pyramidal neurons may be mediated by this receptor subtype. Alternatively, it may occur that M100907 cannot reverse the pyramidal excitation once these neurons become extensively depolarized, likely by activation of ionotropic glutamate receptors. Indeed, in one of these M100907-insensitive units, the effect of DOI was fully reversed by the mGluR II agonist LY-379268 (Fig. 3*B*). This agent could reverse the DOI-induced excitations of pyramidal neurons in four out of five neurons (up to 4 mg/kg i.v.).

Effects of DR Electrical Stimulation on mPFC Pyramidal Neurons

To examine whether the excitatory effect of DOI could be also elicited by endogenous 5-HT, we recorded pyramidal neurons in mPFC during the electrical stimulation of the DR (see Methods). Because we were interested on putative 5-HT_{2A}-mediated excitations, neurons that were only inhibited by DR stimulations were not considered. Peristimulus time histograms showed the presence of excitations (Fig. 4), which had a mean duration of 80 ± 8 ms and a mean latency of 82 ± 8 ms (n = 19). Six out of the 19 excitations recorded were preceded by marked inhibitions, as in the neuron shown in Figure 4. The success rate of orthodromic activation varied between neurons and was 51 ± 8% on average. In 18 out of the 19 pyramidal neurons examined, orthodromic and antidromic excitations were recorded, showing the existence of a strong reciprocal DR-mPFC connectivity (Fig. 4). The DR-induced excitations were reversed by the i.v. administration of M100907 in seven out of eight neurons tested (0.2–0.8 mg/kg i.v.) (P < 0.01 t-test, n = 7; Fig. 3*B*). The success rate before and after M100907 was significantly different (from 62 ± 12% to 15 ± 4%; P < 0.01). The duration and latency of the seven excitations inhibited by M100907 did not differ from those of the other 11 neurons in which reversal by M100907 was not attempted.

Effect of the Thalamic Disinhibition on the Activity of Pyramidal Neurons

The single-point application of bicuculline in the CM + MD nuclei (0.2 µl, 0.1–0.3 mM, over the course of 1 min) elicited a dramatic increase in the activity of projection neurons in mPFC.

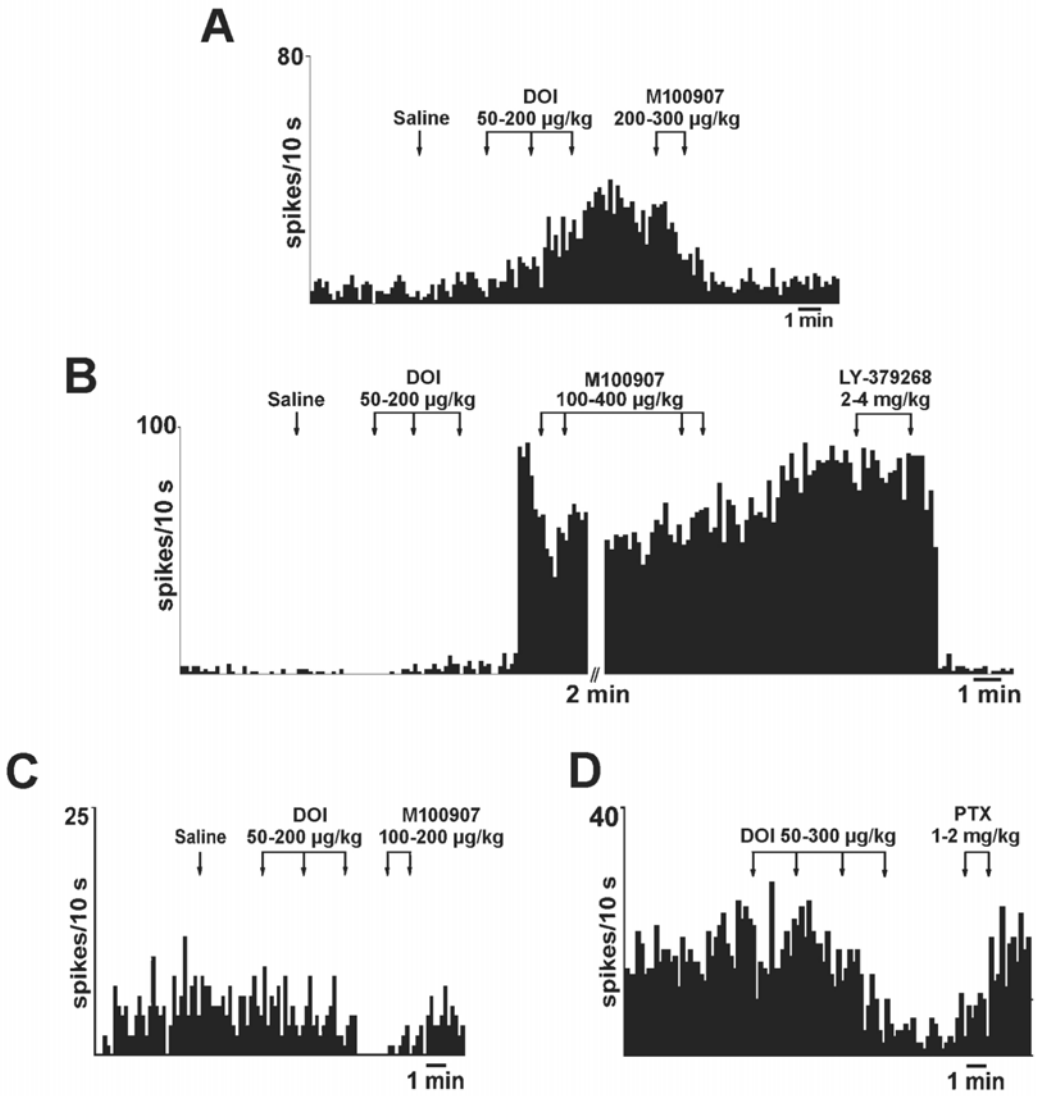

Figure 3. Effects of the intravenous administration of DOI on pyramidal projection neurons of the mPFC. (*A–D*) Integrated firing rate histograms showing the effects of DOI on four different neurons. (*A*, *B*) These neurons responded to the administration of cumulative doses of DOI (50–200 µg/kg i.v.) with an increase in firing rate. (*A*) The effect of DOI was antagonized by M100907 (200–300 µg/kg i.v.). (*B*) shows the inhibitory effect of the selective mGluR II agonist LY-379268 (2–4 mg/kg i.v.) on the firing rate of a pyramidal neuron excited by DOI. In this particular neuron, the 5-HT_{2A} antagonist M100907 (up to 400 µg/kg i.v.) was unable to antagonize the effect of DOI. The panels in *C–D* show the inhibitory effect of DOI. (*C*) DOI (50–200 µg/kg i.v.) suppressed the firing rate of a pyramidal neuron and the effect was antagonized by M100907 (100–200 µg/kg i.v.). (*D*) shows the reversal of the effect of DOI-induced inhibition (50–300 µg/kg i.v.) by the $GABA_A$ antagonist picrotoxinin (PTX; 1–2 mg/kg i.v.). Note the different ordinate scale in *A–B* and *C–D*.

On average, the increase in firing rate was 2423 ± 500% (n = 3). Figure 5*B* shows a representative example of a neuron excited by the application of bicuculline in the thalamus. In several other neurons depolarization block occurred soon after a rapid and dramatic increase in firing rate (data not shown). The data of these neurons was not included in the calculations. We then applied bicuculline 2 mM in the CM + MD nuclei by reverse dialysis, thus enabling a more controlled and stable delivery of the drug. The application of bicuculline by this method also resulted in a very marked increase of the firing rate of projection neurons in mPFC, which attained 1881 ± 564% of baseline on average (n = 9). Figure 5*A* shows a representative example of a projection neuron in mPFC whose firing rate increased from 0.34 to 5.21 spikes/s after the application of bicuculline by reverse dialysis in the CM and MD nuclei. When examined, the increase in pyramidal firing rate was reversed by the i.v. administration of the mGluR II agonist LY-379268 (0.5–3.5 mg/kg) (n = 2; Fig. 5*A*).

The application of 2 mM bicuculline by reverse dialysis in the CM and MD nuclei of chloral hydrate anesthetized rats, to exactly mimic the experimental conditions in single unit recording experiments, resulted in a 3-fold increase of the 5-HT release in the mPFC [$F(10,30) = 7.05$, $P < 0.00002$, one-way repeated measures ANOVA] (Fig. 5*C*).

Effect of the Thalamic Disinhibition on 5-HT Release in mPFC in Anesthetized Rats

The application by reverse dialysis of 1 mM bicuculline in the CM + MD nuclei of the thalamus increased significantly the 5-HT output in mPFC of chloral hydrate anesthetized rats to a

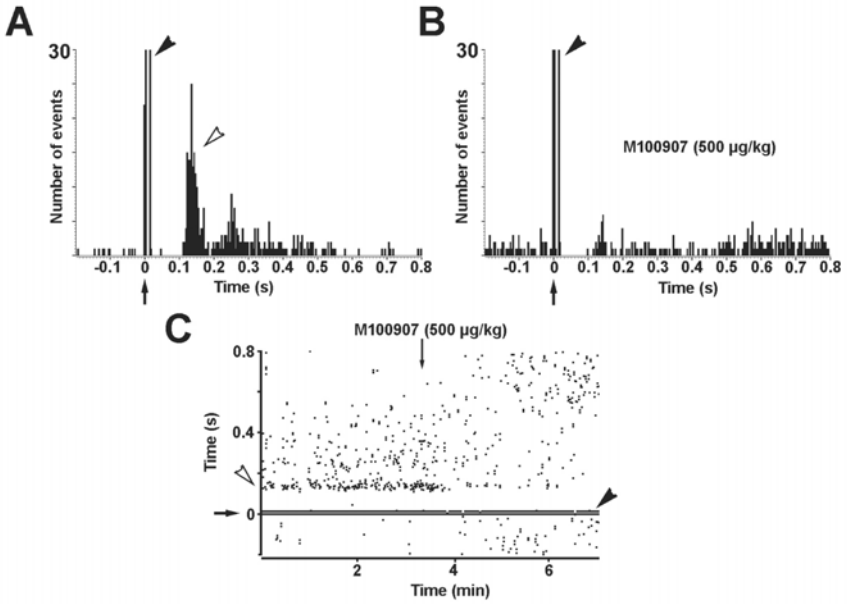

Figure 4. Peristimulus time histograms (PSTH) showing the excitatory response of a pyramidal projection neuron in mPFC to the electrical stimulation of the DR (0.2 ms, 2 mA, 0.9 Hz). The PSTH in (*A*) shows baseline antidromic (filled arrowhead) (latency 13 ms) and orthodromic (open arrowhead) activation (93% success rate; latency 107 ms, duration 75 ms) elicited by the stimulation of DR. (*B*) The orthodromic activation was blocked by the administration of the 5-HT_{2A} receptor antagonist M100907 (500 µg/kg i.v.). Each PSTH consists of 165 trials with a 4 ms bin width. (*C*) Raster display of the response showed in *A* and *B*. Note that the abscissa in *C* corresponds to the ordinate in *A* and *B*. Collisions are seen in *C* as white gaps in the line showing the antidromic response.

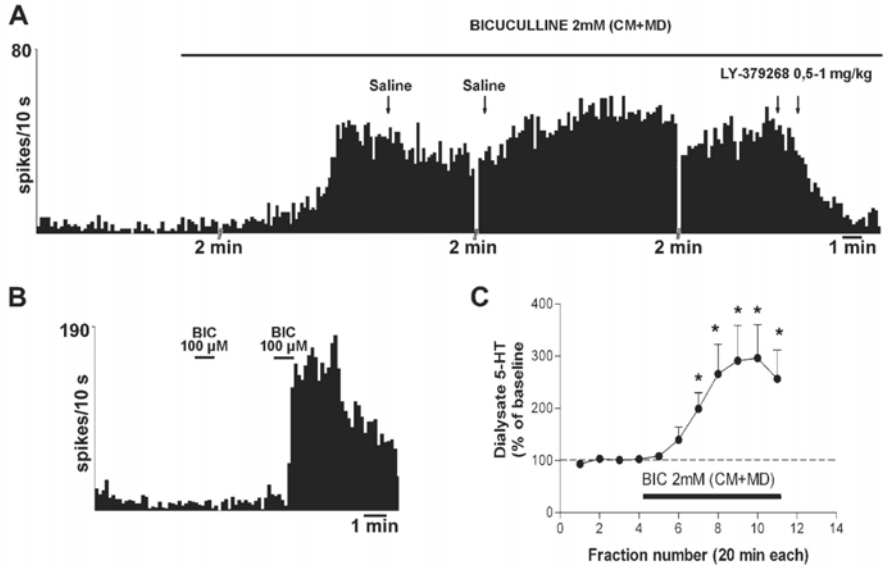

Figure 5. Modulation of the spontaneous firing rate of pyramidal neurons and 5-HT release in mPFC by bicuculline (BIC) application in the mediodorsal and centromedial thalamic nuclei (MD + CM). (*A*) Integrated firing rate histogram showing the response of a pyramidal neuron to the application of BIC (2 mM) by reverse dialysis in the CM and MD nuclei (shown by the horizontal bar). The effect of bicuculline was reversed by the mGluR II receptor agonist LY-379268 (0.5–1 mg/kg i.v.). Arrows show the points of i.v. administration. (*B*) Integrated firing rate histogram showing the response of a pyramidal neuron to single-point infusions of bicuculline (0.2 µl, 100 µM) through a cannula into the MD and CM nuclei. Each application is shown by a 1 min bar. (*C*) The application of 2 mM bicuculline by reverse dialysis in the MD + CM nuclei of anesthetized rats, in the same experimental conditions (as in *A*) markedly enhanced the 5-HT release in medial prefrontal cortex (mPFC) (*$P < 0.05$ versus baseline). The bars show the periods of drug application ($n = 4$).

maximal increase of 175 ± 11% of baseline [$F(15,45) = 5.86$, $P < 0.000002$, one-way repeated measures ANOVA; Fig. 6*A*]. This effect was reversed by the concurrent application of the µ-opioid agonist DAMGO (100 µM) in mPFC, which fully attenuated the increase in 5-HT elicited by the thalamic disinhibition [$F(9,27) = 6.62$, $P < 0.00006$ one-way ANOVA of fractions 7–16; Fig. 6*A*]. DAMGO alone (10, 30 and 100 µM) did not affect the basal 5-HT release (data not shown). Likewise, the application in mPFC of the AMPA/KA antagonist NBQX (300 µM) or the mGluR II/III agonist 1S,3S-ACPD (300 µM) also antagonized the increase

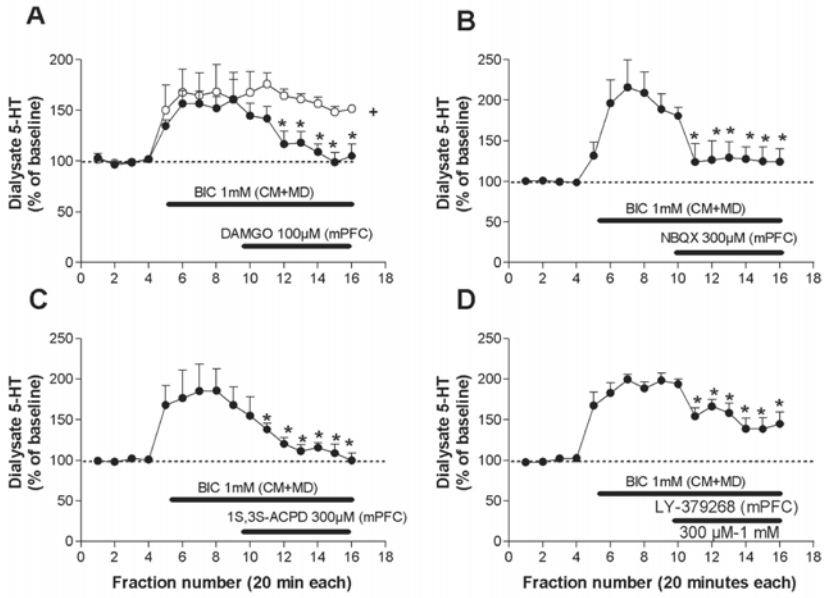

Figure 6. (*A*) The application of 1 mM bicuculline in the centromedial + mediodorsal nuclei of the thalamus (CM + MD) of chloral hydrate anesthetized rats induced a persistent and stable elevation of the 5-HT release in medial prefrontal cortex (mPFC) (open circles, $n = 4$). In a separate group of animals, the subsequent application of the μ-opioid agonist DAMGO by reverse dialysis in mPFC fully counteracted the effect of the disinhibition of thalamic afferents on 5-HT release (filled circles, $n = 4$). (*B*) The 5-HT increase was also reversed by the application in mPFC of the AMPA/KA antagonist NBQX (300 μM; $n = 4$) and (*C*) by the mGluR II/III agonist 1S,3S-ACPD ($n = 4$). (*D*) Partial reversal of this effect exerted by the application in mPFC of the selective mGluR II agonist LY-379268 ($n = 5$). The bars show the periods of drug application in each brain area. See text for detailed statistical analysis. A '+' sign denotes significant differences ($P < 0.05$) for all points during bicuculline application versus baseline; *$P < 0.05$ versus values during bicuculline alone.

in mPFC 5-HT release produced by thalamic disinhibition [$F(9,27) = 4.64$, $P < 0.00009$ and $F(9,27) = 7.16$, $P < 0.00003$, respectively, one-way repeated measures ANOVA; Fig. 6*B*,*C*]. The selective mGluR II agonist LY-379268 appeared to be less effective and elicited only a partial reversal at 0.3–1 mM (three fractions each) [$F(9,36) = 7.66$, $P < 0.00003$; Fig. 6*D*]. A higher concentration (3 mM) failed to antagonize the effect of thalamic disinhibition and increased 5-HT further (data not shown).

Effect of DOI on 5-HT Release in mPFC

As previously observed (Martín-Ruiz *et al.*, 2001) the application of DOI 100 μM in mPFC significantly increased the local 5-HT release [$F(9,63) = 11.5$, $P < 0.000001$, one-way repeated measures ANOVA] (Fig. 7). This effect is due to the selective activation of 5-HT_{2A} receptors, as it is fully blocked by co-perfusion of the selective 5-HT_{2A} antagonist M100907 (Martín-Ruiz *et al.*, 2001). Moreover, it is also reversed by NBQX and 1S,3S-ACPD (Martín-Ruiz *et al.*, 2001), as observed for the effects of thalamic disinhibition (Fig. 6). Since 100 μM DAMGO application in mPFC reversed the effect of thalamic disinhibition (Fig. 6*A*), we examined whether it could antagonize or reverse the effect of DOI on 5-HT release in mPFC. The concurrent application of DAMGO 100 μM did not prevent the increase in 5-HT release induced by DOI (Fig. 7*A*). Two-way ANOVA revealed a significant effect of time [$F(9,90) = 19.43$, $P < 0.00001$] but not of the group or time × group interaction. Also, DAMGO was equally ineffective to reverse the effect of DOI [$F(9,36) = 0.74$, $P = 0.66$; Fig. 7*B*]. The selective mGluR II agonist LY-379268 also failed to reverse the effect of DOI in a wide range of concentrations (100 μM–1 mM) (Fig. 7*C*). In fact, at 100 μM, LY-379268 further enhanced the 5-HT release induced by DOI [$F(9,27) = 4.91$, $P < 0.0006$]. This effect is possibly related to the reported enhancement of 5-HT release induced by this agent after systemic administration, although lower local doses (3–10 μM) did not elicit such effect (Cartmell *et al.*, 2001).

Influence of Thalamic Lesions on the Effects of DOI in mPFC

Figure 8 shows examples of the thalamus of control (sham-operated) and lesioned rats. These were subjected to electrolytic lesions 3 and 8 days before experiments, as described in Methods. All brain sections showed extensive destruction of the centromedial and mediodorsal nuclei and also of the paracentral and paratenial nuclei. Additionally, most animals (~70%) exhibited moderate to severe lesions of the anteromedial, centrolateral, parafascicular, paraventricular and reuniens nuclei, which also project to the cingulated and limbic portions of the mPFC.

The ability of DOI to modulate the firing rate of pyramidal neurons in mPFC was unaffected by the prior lesion (8 days) of the CM and MD nuclei of the thalamus ($n = 9$) (Fig. 9). All neurons recorded were also identified as projection neurons by antidromic stimulation from the DR or VTA, as in control rats. Baseline firing rate was significantly lower in pyramidal neurons from lesioned rats compared to the rest of pyramidal neurons (0.51 ± 0.12 spikes/s versus 1.52 ± 0.14 spikes/s, $n = 15$ and 144, respectively; $P < 0.00001$). Four projection neurons were excited by DOI administration (0.05–0.3 mg/kg i.v.), an effect antagonized by M100907 in all cases examined (3/3; 0.2–0.5 mg/kg i.v.) (Fig. 9*A*). Likewise, four neurons were inhibited by DOI (0.05–0.2 mg/kg i.v.), an effect also antagonized by M100907 (0.2–0.5 mg/kg i.v.) (Fig. 9*B*). One neuron was unaffected by DOI administration (up to 0.3 mg/kg i.v.).

The application of DOI 100 μM in the mPFC of rats subjected

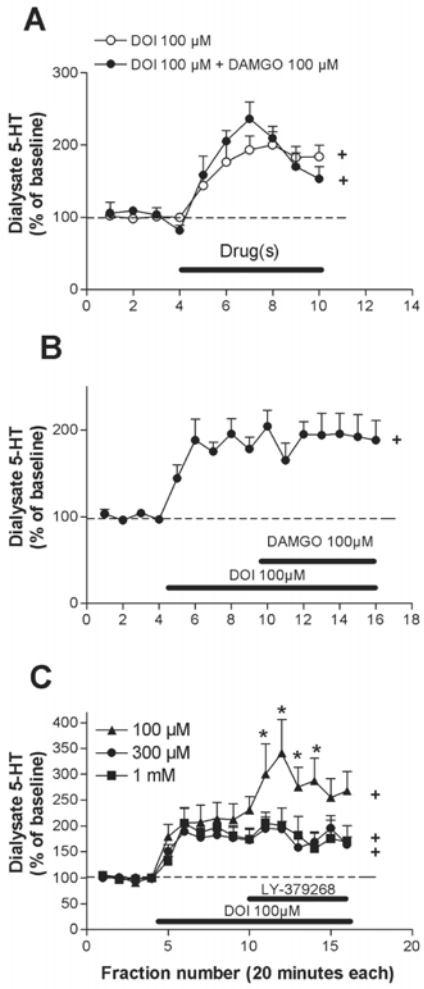

Figure 7. (*A*) The concurrent application of the μ-opioid agonist DAMGO 100 μM did not antagonize the increase in 5-HT release in mPFC elicited by the local application of DOI (DOI alone, open circles, $n = 8$; DOI + DAMGO, filled circles, $n = 4$). (*B*) DAMGO application (100 μM) did not reverse the increase in 5-HT release induced by DOI application in mPFC ($n = 5$). (*C*) The application of the selective mGluR II agonist LY-379268 (100 μM, 300 μM and 1 mM; $n = 4$, 7 and 5, respectively) failed to reverse the 5-HT increase induced by local DOI application (100 μM). The concentration of 100 μM significantly increased 5-HT release. The horizontal bars show the period of drug application. A '+' sign denotes significant ($P < 0.05$) differences versus baseline for points during DOI application. *$P < 0.05$ versus DOI alone.

to sham lesions of the thalamus increased the 5-HT release to an extent similar to that seen in naïve animals (~200% of baseline; non-significant difference with respect to naïve rats, two-way repeated measures ANOVA; Fig. 10). Likewise, DOI application elicited a comparable increase of 5-HT release in rats subjected to electrolytic lesions of the thalamus 3 and 8 days before (Fig. 10). Two-way repeated measures ANOVA revealed a significant effect of the time [$F(9,207) = 27.4$ $P < 0.000001$] but not of the group or time × group interaction at any of the lesion times versus sham rats. In two rats lesioned 8 days before, the subsequent co-perfusion of M100907 (300 μM) fully reversed the DOI-stimulated 5-HT release (to ~70% of baseline), indicating that the effect of DOI was due to the activation of 5-HT_{2A} receptors (data not shown).

Discussion

5-HT_{2A} receptors are widely expressed in prefrontal cortex of rodent and primate brain (see Introduction). Their involvement in cognition and mood is supported by the fact that hallucinogens (LSD, DOI or chemically related compounds) act as partial agonists whereas atypical antipsychotics and some antidepressant drugs, like nefazodone or trazodone, are 5-HT_{2A} receptor antagonists (Kroeze and Roth, 1998; Meltzer, 1999). More recently, direct evidence for the involvement of 5-HT_{2A} in cognition has been provided (Williams *et al.*, 2002). 5-HT_{2A} receptor activation has been shown to exert a variety of actions in prefrontal neurons *in vitro* (Araneda and Andrade, 1991; Tanaka and North, 1993; Aghajanian and Marek, 1997, 1999, 2000; Arvanov *et al.*, 1999; Zhou and Hablitz, 1999). The present study shows that DOI markedly affects the firing activity of prefrontal pyramidal neurons *in vivo*, and in particular, of neurons projecting back to the DR. Also, the physiological stimulation of the DR elicited 5-HT_{2A}-mediated excitations in such DR-projecting pyramidal neurons.

DOI markedly excited nearly 40% of the neurons examined. A lower proportion of neurons (30%) were inhibited and the rest were unaffected by the DOI doses used (in most instances, up to 300 μg/kg). This suggests that the prefrontal output to subcortical structures is affected by DOI in an uneven manner. However, the average effect of DOI is excitatory (2.4-fold increase in firing rate), which is consistent with the increase in 5-HT cell firing and 5-HT release produced by the local application of DOI in mPFC (Martín-Ruiz *et al.*, 2001). Given the existence of anatomical projections from the mPFC to the DR and the control exerted by the mPFC on DR 5-HT neurons (Hajós *et al.*, 1998; Celada *et al.*, 2001), the local effect of DOI on mPFC 5-HT release was interpreted as resulting from an enhanced excitatory output from the mPFC, but definite evidence that DOI might exert such an effect on projection neurons was lacking. However, in previous studies DOI was applied locally whereas here we examined its systemic effect on cell firing, which raises the possibility that, in addition to local (mPFC) 5-HT_{2A} receptors, other 5-HT_{2A}-rich cortical areas (Pazos *et al.*, 1985) which project to mPFC may also be involved.

The DOI-mediated increase of pyramidal activity involved 5-HT_{2A} receptors since they were reversed by M100907. However, M100907 failed to antagonize the excitatory effect of DOI in some units, which may indicate the additional involvement of pyramidal 5-HT_{2C} receptors (Clemett *et al.*, 2000) or the inability of M100907 to reverse a full-blown activation of pyramidal neurons once initiated.

Interestingly, the electrical stimulation of the DR also elicited orthodromic excitations in pyramidal neurons which were blocked by M100907, showing that endogenous 5-HT can activate pyramidal neurons through 5-HT_{2A} receptors *in vivo*. In most cases examined (18/19) the excited neurons projected to the DR, which indicates the existence of a strong reciprocal mPFC-DR interplay involving 5-HT_{2A} receptors. The DR-induced excitations of pyramidal neurons were evoked at a stimulation rate (0.9 Hz), close to the firing rate of 5-HT neurons (typically 1 Hz in the anesthetized rat), which suggests that prefrontal excitations may occur following the physiological activation of DR neurons by incoming excitatory stimuli. Interestingly, the activation of prefrontal neurons elicited by a delayed response task in monkey prefrontal cortex was reversed by M100907, indicating the involvement of this receptor in pyramidal excitations (Williams *et al.*, 2002).

The activation of 5-HT_{2A} receptors may increase the efficacy of AMPA-mediated inputs onto pyramidal neurons (see below).

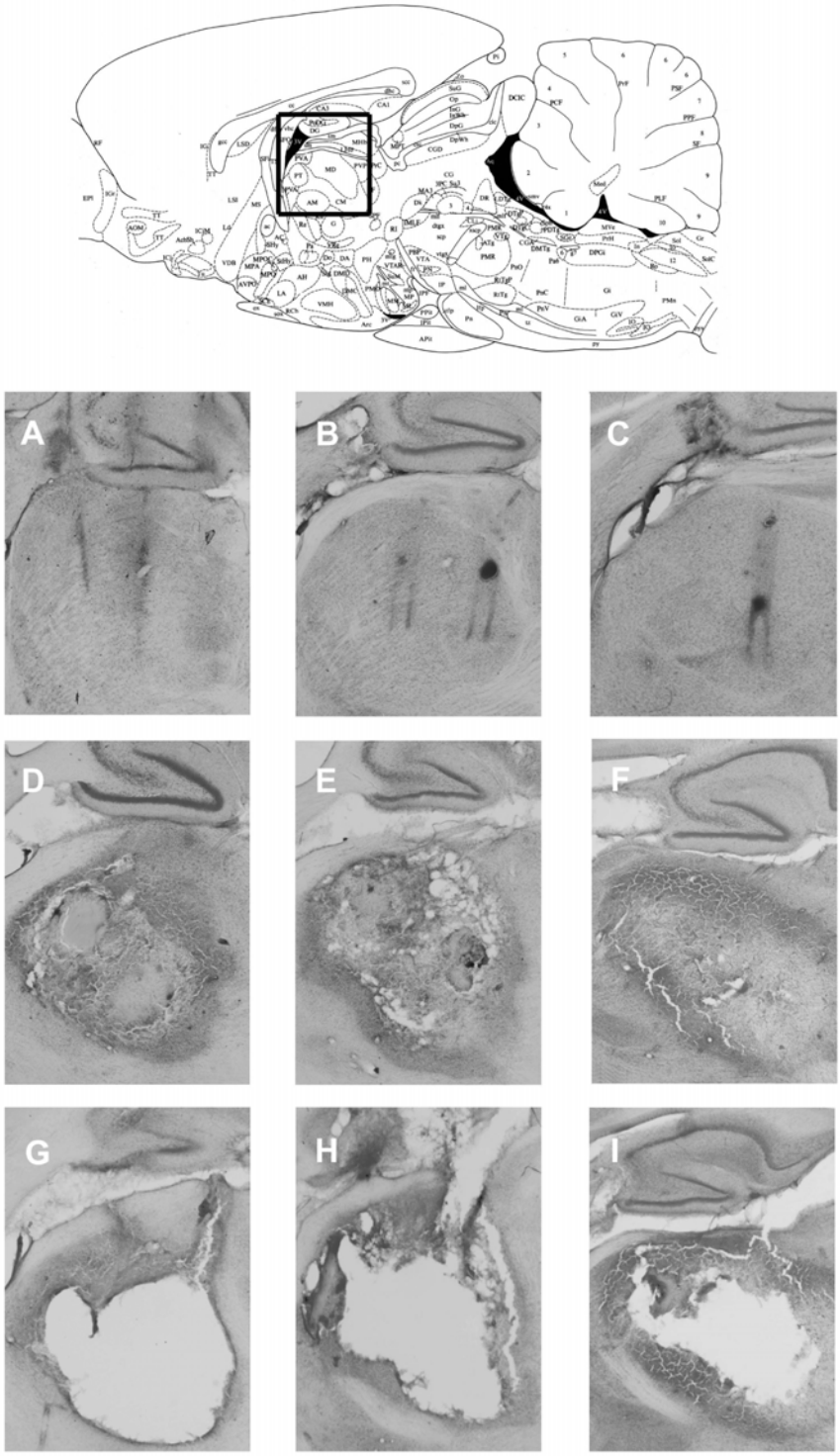

Figure 8. *Upper panel*. Sagittal section of rat brain taken from Paxinos and Watson (Paxinos and Watson, 1986). The rectangle shows the approximate location of sections *A–I*. *Lower panels* (*A–I*). Examples of the effect of sham electrode descents (see Materials and Methods) (*A–C*) and electrolytic lesions performed 3 or 8 days before the experiments (*D–I*) on the integrity of thalamic nuclei projecting to the mPFC area where *in vivo* experiments were performed. In sections from sham-operated animals, the tracks left by electrodes are clearly visible. (*D–I*) Sections of three different rats at two different lateral levels. *G–I* correspond to sections cut at the lateral coordinate of the electrode implant (approximately L –0.7 mm) whereas *D–F* are from the vicinity of the area where maximal brain damage occurred. Each pair of sections (*D–G*, *E–H*, *F–I*) correspond to the same animal, lesioned 8 days (*D*, *E*) or 3 days (*F*) before the experiments. All lesioned animals showed extensive destruction of the centromedial, mediodorsal, paracentral and paratenial nuclei. Additionally, in most instances, moderate to severe lesions of the anteromedial, centrolateral, parafascicular, paraventricular and reuniens nuclei were also observed.

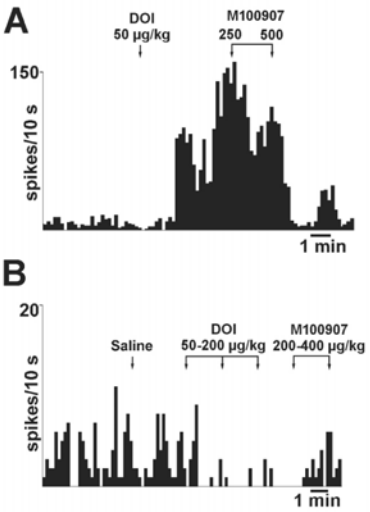

Figure 9. Effects of the intravenous administration of DOI on spontaneous firing rate recorded from pyramidal neurons in rats subjected to lesions of the thalamic nuclei projecting to mPFC 8 days before the experiment. The neuron in *A* responded with a marked increase in firing rate to the administration of DOI (50 µg/kg i.v.) whereas the neuron in *B* was inhibited by DOI (50–200 µg/kg i.v.). In both cases the effects of DOI were antagonized by the 5-HT_{2A} antagonist M100907 (*A*) (250–500 µg/kg i.v.) and (*B*) (200–400 µg/kg i.v.). Note the different ordinate scale in *A* and *B*.

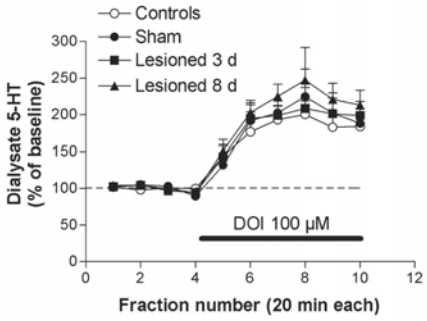

Figure 10. The local application of DOI 100 µM in mPFC enhanced the 5-HT release in this area in naïve rats (open circles, $n = 8$) and in rats subjected to sham lesions of the centromedial and mediodorsal nuclei of the thalamus (filled circles, $n = 11$). The application of DOI 100 µM was equally effective in rats lesioned 3 days (filled squares; $n = 9$) or 8 days (filled triangles, $n = 6$) before the microdialysis experiments. The horizontal bar shows the period of DOI application. See text for detailed statistical analysis.

Among other possible sources of glutamate, it could arise from both serotonergic and non-serotonergic neurons of the DR projecting to mPFC which express the vesicular glutamate transporter 3 (VGLUT3) (Gras *et al.*, 2002). Indeed, glutamate was identified as a co-transmitter with 5-HT in cell culture (Johnson, 1994). However, the latency of the DR-evoked excitations is greater than that reported for glutamatergic axons (Maurice *et al.*, 1999; Celada *et al.*, 2001) and in most instances, the excitations were reversed by the 5-HT_{2A} antagonist M100907. However, we cannot fully exclude that excitations were produced by co-release of 5-HT and glutamate from serotonergic axons in mPFC.

There is a substantial overlap in rat cortex between 5-HT axon terminals and 5-HT_2 receptors (Blue *et al.*, 1988). However, the cellular localization of 5-HT_{2A} receptors responsible for the excitatory effects of 5-HT and DOI is unclear. 5-HT_{2A} receptors are mainly localized to pyramidal neurons and GABAergic interneurons in mPFC (Willins *et al.*, 1997; Jakab and Goldman-Rakic, 1998, 2000; Martín-Ruiz *et al.*, 2001). The latter are large and medium-size parvalbumin- and calbindin-containing interneurons involved in the feed-forward inhibition of pyramidal neurons (Jakab and Goldman-Rakic, 2000). In the rat frontoparietal cortex, 5-HT axons have been observed that run in parallel with the apical dendrites of pyramidal neurons expressing 5-HT_{2A} receptors (Jansson *et al.*, 2001). Additionally, a lower proportion of 5-HT_{2A} receptors was found presynaptically (Jakab and Goldman-Rakic, 1998; Miner *et al.*, 2003). The activation of 5-HT_{2A} receptors by 5-HT or 5-$HT_{2A/2C}$ agonists (DOI, DOB) exerts complex effects on prefrontal neurons. Thus, the microiontophoretic application of DOI suppressed the firing activity of presumed pyramidal neurons in anesthetized rats but enhanced the excitatory effect of glutamate at low ejection currents (Ashby *et al.*, 1989, 1990). *In vitro* recordings of identified pyramidal neurons in prefrontal slices has revealed that 5-HT_{2A} receptor activation increases spontaneous EPSCs and depolarizes the recorded cells (Araneda and Andrade, 1991; Tanaka and North, 1993; Aghajanian and Marek, 1997, 1999, 2000; Zhou and Hablitz, 1999). In addition, 5-HT can elicit 5-HT_{2A}-mediated IPSCs through the activation of GABA synaptic inputs (Zhou and Hablitz, 1999). Hence, the inhibitory responses elicited by systemic DOI in the present study may be attributable to the activation of 5-HT_{2A} receptors in GABA interneurons that locally control pyramidal cell activity.

The excitatory effects of DOI appear to involve interaction with glutamatergic transmission. As mentioned above, DOI could increase the excitatory effects of glutamate on prefrontal neurons (Ashby *et al.*, 1989, 1990). Likewise, the 5-HT_{2A} receptor-mediated EPSCs evoked by 5-HT in prefrontal slices are canceled by blockade of AMPA receptors and mGluR II receptor activation (Aghajanian and Marek, 1997, 1999). Moreover, the modulation of prefrontal NMDA transmission by 5-HT and DOB appears to involve pre- and postsynaptic 5-HT_{2A} receptors (Arvanov *et al.*, 1999). Our observation that the selective mGluR II agonist LY-379268 reversed the excitatory effect of DOI on pyramidal neurons *in vivo* is consistent with these *in vitro* observations. Also, the local increase of prefrontal 5-HT release elicited by DOI was blocked by NBQX (AMPA/KA antagonist) and 1S,3S-ACPD (mGluR II/III agonist) and was mimicked by AMPA application and blockade of glutamate reuptake (Martín-Ruiz *et al.*, 2001). However, the NMDA receptor antagonist MK-801 failed to reverse the effect of DOI on 5-HT release.

In the present study, we used the disinhibition of the CM and MD nuclei of the thalamus which project densely to the mPFC (Berendse and Groenewegen, 1991; Fuster, 1997; Van der Werf *et al.*, 2002) to examine whether an increase in thalamocortical inputs to the mPFC would mimic the excitatory effects of DOI on cell firing and 5-HT release. We also sought to examine whether the action of DOI implied an increase in thalamocortical excitatory inputs through the activation of terminal 5-HT_{2A} receptors. Thalamic disinhibition increases *c-fos* expression in mPFC (Erdtsieck-Ernste *et al.*, 1995; Bubser *et al.*, 1998) by enhancing excitatory inputs from mediodorsal afferents that terminate on dendritic spines of pyramidal dendrites (Kuroda and Price, 1991; Kuroda *et al.*, 1998). Previous studies had determined that stimulation of the MD nucleus increases AMPA inputs onto prefrontal pyramidal neurons (Pirot *et al.*, 1994). Consistent with this synaptic connectivity, the thalamic application of bicuculline dramatically enhanced the firing rate of pyramidal neurons. This effect

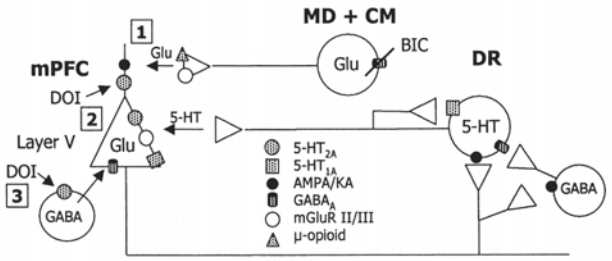

Figure 11. Schematic representation of the anatomical and functional relationships between the mPFC and the DR as well as the influence of thalamocortical afferents to mPFC on the control of the prefrontal-raphe circuit. (1) The disinhibition of thalamocortical afferents by bicuculline application in the MD + CM nuclei increased excitatory inputs onto mPFC, resulting in an increased activation of AMPA/KA receptors on pyramidal neurons and the subsequent increase in pyramidal firing rate and local 5-HT release (likely via activation of ascending DR neurons). Both effects were reversed by mGluR II/III agonists and the increase in 5-HT release was also canceled by the activation of μ-opioid receptors in mPFC by DAMGO. (2) and (3) Involvement of 5-HT_{2A} receptors in the effects of DOI. Excitatory and inhibitory actions of DOI on pyramidal cell firing are likely mediated by receptors located on pyramidal neurons (2; most abundant receptor subpopulation in mPFC) and GABA interneurons (3), respectively. Overall, DOI elicits an stimulatory action on the output of mPFC *in vivo*, as judged by the overall increase (3-fold) in pyramidal cell firing and the stimulation of 5-HT cell firing and 5-HT release seen in present and past studies (Martín-Ruiz *et al.*, 2001). Unlike the effect of thalamic disinhibition, the stimulatory effect of DOI on 5-HT release is not canceled by μ-opioid receptor activation. Likewise, extensive thalamic lesions do not prevent this effect. Both observations argue against 5-HT_{2A} receptors on thalamocortical afferents as mediators of the effect of DOI, as proposed for the 5-HT-induced, 5-HT_{2A}-mediated EPSCs recorded *in vitro* in layer V pyramidal cells.

was reversed by the mGluR II agonist LY-379268, which reduces glutamate release (Cartmell and Schoepp, 2000).

The thalamic disinhibition also increased the prefrontal 5-HT release in dual probe experiments performed in freely moving and anesthetized rats, paralleling the effects on pyramidal cell firing. This effect was reversed by the application in mPFC of agents that reduce/block glutamatergic transmission, such as 1S,3S-ACPD, LY-379268 and NBQX. This suggests that an increased excitatory input onto mPFC neurons may activate descending excitatory pathways to the DR, which eventually results in an enhanced 5-HT release in mPFC (see scheme in Fig. 11). The stimulatory effect of the thalamic disinhibition on prefrontal 5-HT release was also reversed by the application in mPFC of DAMGO, a μ-opioid agonist. The DAMGO-induced reduction is likely accounted for by a presynaptic suppression of excitatory inputs onto mPFC since μ-opioid receptors suppress inhibitory and excitatory transmission in brain (Jolas and Aghajanian, 1997; Lüscher *et al.*, 1997). Also, cortical μ-opioid receptors are considered to be present on thalamocortical afferents due to the mismatch between the receptor protein (mainly in cortex) and its encoding mRNA (mainly in thalamus) (Delfs *et al.*, 1994; Zastawny *et al.*, 1994). In agreement, lesions of the thalamic nuclei projecting to mPFC reduced cortical [^{3}H]DAMGO binding (Marek *et al.*, 2001).

The parallel effects of DOI and thalamic disinhibition (e.g. both increased 5-HT release and pyramidal output) raised the possibility that DOI might act by activating the release of glutamate from thalamocortical afferents, as observed for 5-HT in the *in vitro* slice preparation (Aghajanian and Marek, 1997, 1999). However, this view is discordant with several observations from the present study. First, the application of DAMGO and LY-379268 in mPFC fully and partly reversed, respectively, the 5-HT increase produced by thalamic disinhibition but failed to attenuate the effect of DOI on 5-HT release. The partial suppression of 5-HT release elicited by LY-379268 may seem at variance with the full reversal of the effect of the thalamic disinhibition on pyramidal cell firing, given the ability of this agent to reduce presynaptic glutamate release (Schoepp *et al.*, 1999; Cartmell and Schoepp, 2000). However, LY-379268 increases 5-HT release by itself (Cartmell *et al.*, 2001) (see also Fig. 7*C*), an action which may mask the 5-HT decrease elicited by reducing excitatory inputs. The dissimilar effects of 1S, 3S-ACPD and LY-379268 on the thalamic disinhibition paradigm and on DOI's effects may indicate the involvement of mGluR receptors other than mGluR II in the effect of 1S,3S-ACPD (Martín-Ruiz *et al.*, 2001). The lack of any effect of DAMGO on the DOI-stimulated 5-HT release (but reversal of the effects of thalamic disinhibition) also suggests that the effect of DOI does not involve an increase in excitatory inputs from thalamocortical afferents.

Second, the effects of DOI on 5-HT release and pyramidal cell firing were unaffected by lesions of the thalamic nuclei that project to mPFC (Berendse and Groenewegen, 1991; Van der Fuster, 1997; Werf *et al.*, 2002). Chemical lesions (NMDA) applied at the coordinates used herein partly attenuated the EPSCs induced by 5-HT in layer V pyramidal neurons of the mPFC (Marek *et al.*, 2001). Electrolytic lesions caused an extensive brain damage of the centromedial, mediodorsal, paracentral and paratenial nuclei, which project densely to subdivisions of the mPFC, and other thalamic nuclei in their vicinity (anteromedial, centrolateral, parafascicular, paraventricular and reuniens nuclei). The lesion probably affected ascending fibers from other thalamic nuclei as well. Eight days after the electrolytic lesions, the ability of DOI to modulate pyramidal cell firing and 5-HT release in mPFC was unaltered. This cannot be attributed to an insufficient tissue damage, since all animals included showed extensive loss of thalamic tissue. Also, 1 week appears to be a sufficient time to allow for degeneration of existing terminals in mPFC since electrolytic lesions performed at this time post-lesion altered the thalamic influence on a variety of brain areas, including the prefrontal cortex (Raos and Savaki, 1995; Raos *et al.*, 1995). Moreover, electrolytic lesions of the MD nucleus performed 4 days before markedly affected spatial memory, which suggests an impaired thalamocortical input (Stokes and Best, 1988). Interestingly, in our experiments, the baseline firing of pyramidal neurons in lesioned rats was one-third of that in untreated animals (0.51 versus 1.51 spikes/s), which likely reflects the marked loss of excitatory thalamic inputs.

Thus, on account of the above evidence, we suggest that DOI does not affect pyramidal cell firing (excitations and inhibitions) and 5-HT release by acting on 5-HT_{2A} receptors putatively located on thalamocortical afferents to mPFC. This view is consistent with a recent study on the ultrastructural localization of 5-HT_{2A} receptors in the rat mPFC. 5-HT_{2A} receptor immunoreactivity was mainly found postsynaptically in dendritic shafts and spines (73% of labeled profiles) whereas the rest was located presynaptically on axons that do not display the typical morphology of glutamatergic axons (Miner *et al.*, 2003).

In summary, the present study shows that the *in vivo* activation of 5-HT_{2A} receptors by DOI has profound effects on pyramidal cell activity and, by virtue of descending mPFC-DR excitatory afferents, on 5-HT neurons. In turn, the electrical stimulation of the DR can elicit 5-HT_{2A}-mediated excitations on pyramidal neurons. Taken together, these observations indicate the existence of a close functional relationship between the mPFC and the serotonergic system in rat brain (mPFC-DR circuit; Fig. 11). Conceivably, pyramidal 5-HT_{2A} receptors mediate the excitatory effects of DOI whereas those present in GABA

interneurons mediate inhibitory effects. Thalamic disinhibition resembled the excitatory effects of DOI, yet pharmacological differences (activation of μ-opioid receptors and mGluR II) suggest that DOI does not act by enhancing glutamatergic inputs from thalamocortical afferents, a view also supported by the absence of effects of the thalamic lesions on DOI's action. However, since the excitatory effect of DOI appears to depend on glutamatergic inputs (Martín-Ruiz *et al.*, 2001) (this study) it may involve a 5-HT_{2A}-mediated synergism with AMPA inputs [for instance, increasing Ca^{2+} entry (Porter *et al.*, 1999)] onto pyramidal neurons. These glutamatergic inputs may arise from different cortical or subcortical projections to the mPFC (Fuster, 1997), an issue deserving further investigation. The preservation of such inputs *in vivo*, even in rats with thalamic lesions may perhaps explain the apparent discrepancy between the present *in vivo* results and the *in vitro* observations by Aghajanian and Marek (Aghajanian and Marek, 1997, 1999, 2000) in the slice preparation. The present observations may help to clarify the neurobiological substrate of the action of hallucinogens and atypical antipsychotics, acting as agonists and antagonists, respectively, at cortical 5-HT_{2A} receptors.

Notes

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