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The activation of 5-HT_{2A} receptors in prefrontal cortex enhances dopaminergic activity

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Abstract

Atypical antipsychotics show preferential 5-HT_{2A} versus dopamine (DA) D_2 receptor affinity. At clinical doses, they fully occupy cortical 5-HT_2 receptors, which suggests a strong relationship with their therapeutic action. Half of the pyramidal neurones in the medial prefrontal cortex (mPFC) express 5-HT_{2A} receptors. Also, neurones excited through 5-HT_{2A} receptors project to the ventral tegmental area (VTA). We therefore hypothesized that prefrontal 5-HT_{2A} receptors can modulate DA transmission through excitatory mPFC–VTA inputs. In this study we used single unit recordings to examine the responses of DA neurones to local (in the mPFC) and systemic administration of the 5-$HT_{2A/2C}$ agonist 1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane] (DOI). Likewise, using microdialysis, we examined DA release in the mPFC and VTA (single/dual probe) in response to prefrontal and systemic drug administration. The local (in the mPFC) and systemic administration of DOI increased the firing rate and burst firing of DA neurones and DA release in the VTA and mPFC. The increase in VTA DA release was mimicked by the electrical stimulation of the mPFC. The effects of DOI were reversed by M100907 and ritanserin. These results indicate that the activity of VTA DA neurones is under the excitatory control of 5-HT_{2A} receptors in the mPFC. These observations may help in the understanding of the therapeutic action of atypical antipsychotics.

Keywords: atypical antipsychotics, dopamine, prefrontal cortex, schizophrenia, serotonin receptors, ventral tegmental area.

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The ventral tegmental area (VTA) gives rise to the mesocortical and mesolimbic dopamine (DA) systems, which are involved in cognition, memory, reward and behavioural control (Glowinski *et al*. 1984; Williams and Goldman-Rakic 1995; Robbins 2000; Tzschentke and Schmidt 2000; Schultz 2004). Psychotic and cognitive/negative symptoms in schizophrenia appear to be associated with an overactivity of the mesolimbic pathway and a hypofunction of the mesocortical pathway, respectively (Carlsson 1988; Weinberger *et al*. 1994; Laruelle *et al*. 1996; Abi-Dargham *et al*. 2000).

The activity of DA and non-DA neurones in the VTA is modulated, among other areas, by the medial prefrontal cortex (mPFC) (Thierry *et al*. 1979, 1983; Tong *et al*. 1996, 1998; Carr and Sesack 2000a,b). This control is exerted via direct excitatory afferents as well as indirectly through the laterodorsal/pedunculopontine tegmentum or the nucleus accumbens/ventral pallidum pathways (Tzschentke and Schmidt 2000; Floresco *et al*. 2003; Adell and Artigas 2004; Omelchenko and Sesack 2005). The prefrontal cortex (PFC) plays a key role in higher brain functions (Fuster 2001) and prefrontal function and metabolism is altered in patients with schizophrenia (Weinberger *et al*. 1994; Andreasen *et al*. 1997). Likewise, cognitive deficits in schizophrenic patients are mediated by disruptions in brain circuits involving the PFC (Bertolino *et al*. 2000).

Classical neuroleptics used to treat schizophrenia block DA D_2 receptors (Seeman and Lee 1975; Creese *et al*. 1976), an action which also evokes extrapyramidal motor symptoms and hyperprolactinaemia. With few exceptions (e.g. amisulpride) second generation (atypical) antipsychotics display a

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Abbreviations used: DA, dopamine; DOI, 1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; VTA, ventral tegmental area.

preferential 5-HT_2 versus DA D_2 receptor affinity and occupancy in brain (Nyberg *et al*. 1998; Meltzer 1999) although 'atypicality' may encompass more than one mechanism (Roth *et al*. 2003). Several previous studies have reported that the administration of 5-HT_{2A} receptor ligands such as DOI (5-$HT_{2A/2C}$ receptor agonist) or M100907 (selective 5-HT_{2A} receptor antagonist) modulates the DA system (Gobert and Millan 1999; Ichikawa *et al*. 2001a; Minabe *et al*. 2001; Pehek *et al*. 2001; Porras *et al*. 2002). However, the location of 5-HT_{2A} receptors involved in these effects is presently unknown.

The neocortex of the rodent, primate and human brain contains 5-HT_{2A} receptors, with an enrichment in frontal regions (Pompeiano *et al*. 1994; Burnet *et al*. 1995; López-Giménez *et al*. 1998; Amargós-Bosch *et al*. 2004). Recent data indicate that a large population (50–66%) of pyramidal neurones and a smaller proportion of GABA interneurones (~20%) in the rat mPFC, an area projecting densely to the VTA, express 5-HT_{2A} receptors (Santana *et al*. 2004). Consistent with this dual location, 5-HT_{2A} receptor activation depolarizes or hyperpolarizes pyramidal neurones depending on experimental conditions (Araneda and Andrade 1991; Tanaka and North 1993).

In a previous study, we noticed that pyramidal neurones in the mPFC excited through the activation of 5-HT_{2A} receptors project to the VTA, as assessed by antidromic stimulation (Puig *et al*. 2003). Likewise, the 5-$HT_{2A/2C}$ agonist DOI markedly enhanced the activity of pyramidal neurones in the mPFC, including those projecting to the VTA (Puig *et al*. 2003). This raised the possibility that the aforementioned effects of 5-HT_{2A} receptor ligands on VTA DA neurones are mediated by the activation of such receptors in the mPFC, thereby affecting the activity of pyramidal neurones in the mPFC projecting to the VTA.

Hence, we conducted the present study to specifically assess whether 5-HT_{2A} receptors in the mPFC modulate the activity of DA neurones in the VTA and the release of DA in the mesocortical pathway.

Materials and methods

Animals and treatments

Male albino Wistar rats (230–300 g; Iffa Credo, Lyon, France) were kept in a controlled environment (12 h light/dark cycle and 22 ± 2°C room temperature) with food and water provided *ad libitum*. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee. Stereotaxic coordinates (in mm) were taken from bregma and duramater using the atlas of Paxinos and Watson (1998).

All drugs were from Sigma/RBI (Natick, MA, USA) except M100907 which was from Eli Lilly and Co. Indianapolis, IN, USA (LY 368675). For the assessment of local or distal effects in microdialysis experiments, drugs were dissolved in the perfusion fluid or water (except M1009007 which was dissolved in acetic acid) and diluted to appropriate concentrations in artificial CSF (aCSF). Concentrated solutions (pH adjusted to 6.5–7.4 with $NaHCO_3$ when necessary) were stored at −80°C and working solutions were prepared daily by dilution in aCSF at the stated concentrations and were applied by reverse dialysis (uncorrected for drug recovery). Control rats were perfused with aCSF. The bars in the figures show the period of drug application (corrected for the void volume of the system). Typically, the final concentrations of compounds used in microdialysis experiments were: DOI, MK-801 and ritanserin, 300 μM; bicuculline, 10, 30 and 100 μM; M100907, 30, 100 and 300 μM and tetrodotoxin, 1 μM.

For recordings of VTA DA neurones, drugs were administered i.v. or applied through a stereotaxically implanted cannula in the mPFC (tip coordinates: AP, +3.2; L, −0.8; DV, −3.5) following a described procedure (Martín-Ruiz *et al*. 2001). Briefly, 40 pmol of DOI (0.2 μL, 200 μM, dissolved in aCSF) was applied through a 32g stainless steel cannula (Small Parts Inc., Miami, FL, USA). The cannula was attached to a 10-μL Hamilton syringe by a Teflon tubing. A microinfusion pump (Bioanalytical Systems Inc., West Lafayette, IN, USA) was used. After a baseline recording of at least 5 min, DOI was infused over the course of 1 min. Depending on the effect obtained, a second DOI infusion was administered 5 min later. In all experiments, only one neurone per rat was recorded. After experimental procedures were completed, animals were killed by an overdose of anaesthetic and a careful histological verification of the correctness of the implants was carried out.

Single unit recordings of dopamine neurones in ventral tegmental area

Two sets of experiments were performed to examine the responses of VTA DA neurones to the local (in the mPFC) and systemic administration of DOI. Rats were anaesthetized (chloral hydrate 400 mg/kg i.p.) and positioned in a David Kopf stereotaxic frame. Thereafter, chloral hydrate was continuously administered i.p. at a dose of 50–70 mg/kg·h using a perfusion pump to avoid fluctuations in the degree of anaesthesia that can affect the activity of DA neurones (Fa *et al*. 2003). Body temperature was maintained at 37°C with a heating pad. DA neurones were recorded extracellularly with glass micropipettes pulled from 2.0-mm capillary glass (WPI, Sarasota, FL, USA) on a Narishige PE-2 pipette puller (Narishige Scientific Instruments Lab, Tokyo, Japan). Microelectrodes were filled with 2 M NaCl. Typically, impedance was 4–10 MΩ. Single unit extracellular recordings were amplified with a Neurodata IR283 (Cygnus Technology Inc., Delaware Water Gap, PA, USA), post-amplified and filtered with an amplifier (Cibertec, Madrid, Spain) and computed on-line using a DAT 1401 plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Descents in the VTA were carried out at AP, −5.0 to −5.6; L, −0.5 to −1 and DV, −7.5 to −9.0 to record DA neurones in both the parabrachial and paranigral subdivisions. The identification of DA neurones and burst firing analysis were carried out according to the criteria of Grace and Bunney (1984), as previously used by Celada *et al*. (1999). Briefly, neurones were considered dopaminergic if they possessed the following characteristics: (1) action potential duration greater than 2.5 ms; (2) typical bi- or triphasic waveform often with a notch in the initial rising phase; (3) slow firing rate (recorded neurones fired at 1–7 spikes/s) and (4) frequent presence

Table 1 Effect of the local application of DOI in the medial prefrontal cortex (mPFC) on dopamine cell firing in the ventral tegmental area

	All neurones		DOI-sensitive neurones	
	Baseline	DOI	Baseline	DOI
Firing rate (Hz)	3.17 ± 0.26	3.70 ± 0.32**	3.44 ± 0.34	4.30 ± 0.39**
% of basal firing rate	100	119 ± 7*	100	132 ± 9**
% spikes in burst	10.0 ± 2.3	23.0 ± 3.9**	13.1 ± 3.2	32.5 ± 4.5**
No. of spikes per burst	2.5 ± 0.1 (24)	2.9 ± 0.1(27)**	2.6 ± 0.1 (17)	3.2 ± 0.1(19)**
No. of bursts in 3 min	24 ± 6	56 ± 12**	31 ± 8	80 ± 15**
Spikes in burst in 3 min	67 ± 22	192 ± 46**	89 ± 31	281 ± 61**
N	29	29	19	19

Data are from 29 or 19 neurones, except values shown in parentheses (some neurones did not exhibit basal burst firing).Values are the maximal effect (average of 3 min) produced by the application of DOI in the mPFC. *$p < 0.01$, **$p < 0.005$ vs. pre-drug values, paired Student's *t*-test.

Table 2 Effect of the systemic administration of DOI on dopamine cell firing in the ventral tegmental area

	Baseline	DOI (50 μg/kg)	DOI (100 μg/kg)	M100907 (250–500 μg/kg)
Firing rate (Hz)	3.38 ± 0.49	3.64 ± 0.41	4.04 ± 0.47*	1.72 ± 0.48**,*
% of basal firing rate	100	115 ± 7	128 ± 9*	56.1 ± 8.1**,*
% spikes in burst	10.9 ± 4.0	16.4 ± 4.5	22.5 ± 6.1*	0.86 ± 0.86***
No. of spikes per burst	2.3 ± 0.1	2.3 ± 0.1	2.7 ± 0.2*	2(1)
No. of burst in 3 min	30 ± 13	46 ± 15	60 ± 18*	0.8 ± 0.8**
Spikes in burst in 3 min	79 ± 38	115 ± 41	178 ± 57*	2 ± 2**
N	12	12	12	5

Values are the effect of DOI or M100907 at the specified i.v. dose measured during 3 min. Repeated measures ANOVA showed a significant effect of DOI on all variables examined (*$p < 0.05$ vs. baseline, Duncan test following repeated measures ANOVA). The effect of M100907 was examined in a subgroup of five neurones activated by DOI. Only one neurone showed burst firing after M100907 administration. **$p < 0.05$ vs. DOI, ***$p = 0.07$ vs. DOI and baseline, paired Student's *t*-test.

of bursts. The structure of bursts was defined as starting with a first interspike interval of <80 ms and ending with an interspike interval of 160 ms or greater (Grace and Bunney 1984). Recorded neurones had 10–11% of spikes fired in bursts during baseline conditions (Tables 1 and 2). When appropriate, additional pharmacological identification was carried out with i.v. apomorphine followed by haloperidol reversal.

Microdialysis procedures

Microdialysis experiments in the mPFC were conducted essentially as described previously (Martín-Ruiz *et al*. 2001). Rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and implanted with 4-mm concentric dialysis probes (Cuprophan) in the mPFC at AP, +3.2; L, −0.8 and DV, −6.0. Groups of rats were also implanted with a second microdialysis probe (tip 1.5 mm) in the VTA (coordinates: AP, −5.3; L, −2.1 and DV, −8.9 with a vertical angle of 10° which placed the probe tip at L, −0.6 and DV, −8.7). Microdialysis experiments were performed in freely-moving rats >20 h after surgery except in experiments involving the electrical stimulation of the mPFC (see below). Probes were perfused with aCSF pumped at 1.5 μL/min. Following an initial 100-min stabilization period, four baseline samples were collected (20 min each) before local (reverse dialysis) or systemic drug administration and then successive dialysate samples were collected. In most experiments, the 5-HT$_{2A/2C}$ agonist DOI was locally perfused alone for six fractions followed by its application in combination with other drugs for another six fractions.

In the experiments examining the effects of the electrical stimulation of the mPFC on DA release in the VTA, rats were anaesthetized with chloral hydrate and 10-min fractions were collected (flow rate 3.0 μL/min). Bipolar stimulating electrodes consisted of two stainless steel enamel-coated wires (California Fine Wire, Grover Beach, CA, USA) with a diameter of 150 μm and a tip separation of ∼200 μm and *in vitro* impedances of 10–30 KΩ. A stimulating electrode was stereotaxically implanted in the mPFC (AP, −3.2; L, −0.8 and DV, −3.0) and secured to the skull with glue and dental cement. Constant current electrical stimuli were generated with a Grass stimulation unit S-48 connected to a Grass SIU 5 stimulus isolation unit. Three stimulating conditions were used: S1 (0.9 Hz, 1.5 mA, 0.2 ms), S2 (10 Hz, 0.5 mA, 1 ms) and S3 (20 Hz, 0.5 mA, 1 ms) (Celada *et al*. 2001).

The concentration of DA in dialysate samples was determined by HPLC with amperometric detection, using a modification of a previously described method (Ferré *et al*. 1994). Brain dialysates were collected on microvials containing 5 μL of 10 mM perchloric acid and were rapidly injected into the HPLC. DA was

amperometrically detected at 5–7.5 min with a limit of detection of 3 fmol/sample using an oxidation potential of +0.75 V.

Data and statistical analysis

Changes in firing rate or the proportion of burst firing in DA neurones were assessed using repeated measures ANOVA or paired Student's *t*-tests, as appropriate. These values were quantified by averaging the values during 3 min after local or i.v. administration (omitting the first minute).

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). Statistical analysis was carried out using one- or two-way ANOVA for repeated measures of the DA values during specified periods followed by post-hoc *t*-test when appropriate. Typically, the effect of DOI was assessed by one-way repeated measures ANOVA comparing baseline DA values with those attained during DOI perfusion. The effect of drugs on the DOI-induced DA elevation was assessed with one-way repeated measures ANOVA comparing DA values during DOI infusion with those attained during the infusion of DOI plus test drug. The *p*-values shown in the Results correspond to the time effect in the case of one-way repeated measures ANOVA and to the time–treatment interaction for two-way repeated measures ANOVA (e.g. stimulated vs. control rats). Data are expressed as the mean ± SEM. Statistical significance has been set at the 95% confidence level (two tailed).

Results

Electrical and pharmacological stimulation of the medial prefrontal cortex–ventral tegmental area pathway. Effects on dopamine release

Baseline extracellular DA values in the VTA of chloral hydrate-anaesthetized rats was 8 ± 1 fmol/10-min sample ($n = 24$). In freely-moving rats, the corresponding baseline concentrations in the VTA and mPFC (20-min fractions) were 19 ± 1 and 15 ± 1 fmol/20-min fraction ($n = 49$ and 79, respectively).

We examined the effect of the electrical stimulation of the mPFC on extracellular DA concentration in the ipsilateral VTA. Control (sham) rats in these experiments were equally implanted with stimulation electrodes in the mPFC but no current was passed. In a first experiment, the electrical stimulation of the mPFC at low frequency (S1; see Materials and methods) did not increase the extracellular DA concentration in the VTA. Higher stimulation frequencies (10 and 20 Hz; S2 and S3 conditions) significantly elevated the DA release in the VTA to 145 ± 12% ($n = 5$; $F_{17,68} = 6.20$; $p < 0.0001$; one-way repeated measures ANOVA; data not shown). In a second experiment (Fig. 1a), the stimulation at S2 and S3 conditions also significantly elevated extracellular DA compared with sham-stimulated rats, reaching a level similar to that seen in the previous experiment (153 ± 18%; $n = 7$ for stimulated rats, $n = 6$ for sham controls; $F_{1,11} = 16.90$, $p < 0.0015$ group effect; $F_{21,231} = 4.38$, $p < 0.00001$ time effect; $F_{21,231} = 2.70$ $p < 0.0015$ time–group interaction; two-way repeated measures ANOVA). This effect was completely prevented by the local perfusion of the NMDA receptor antagonist MK-801 (300 μM) in the VTA ($n = 6$; $F_{1,11} = 11.81$, $p < 0.0005$ group effect; $F_{21,231} = 2.94$, $p < 0.0001$ time effect; $F_{21,231} = 3.86$, $p < 0.0001$ time–group interaction; two-way repeated measures ANOVA).

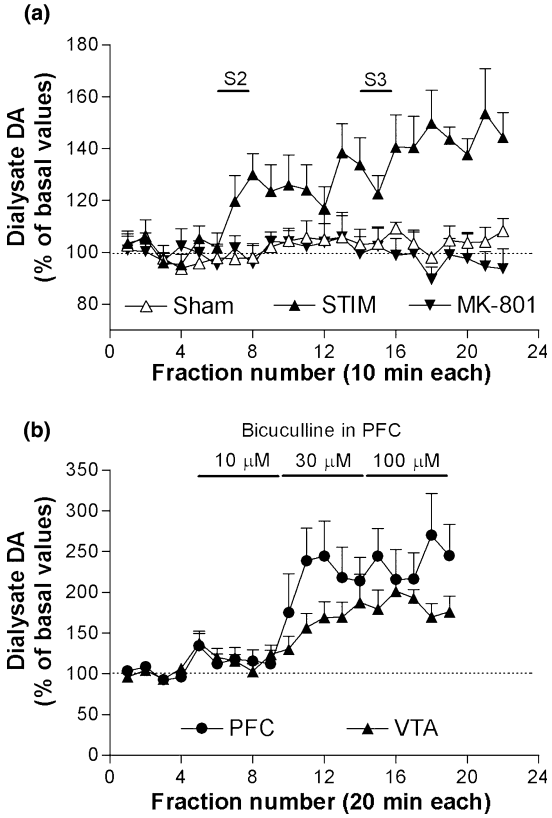

Fig. 1 (a) The electrical stimulation of the medial prefrontal cortex (mPFC) for 10-min periods at S2 and S3 conditions (S2, 10 Hz, 1 ms, 0.5 mA; S3, 20 Hz, 1 ms, 0.5 mA) significantly increased the dopamine (DA) release in the ventral tegmental area (VTA). This effect persisted until the end of the collection period (70 min after the end of S3) and was prevented by the perfusion of MK-801 (300 μM) administered by reverse dialysis in the VTA ($n = 6$, sham; $n = 7$, stimulation; $n = 6$, stimulation + MK-801). (b) Effect of the local application in the mPFC of the $GABA_A$ receptor antagonist bicuculline (10, 30 and 100 μM, shown by horizontal bars) on DA release in the mPFC and VTA ($n = 6$). See text for statistical analysis.

To examine the effect of the pharmacological stimulation of the mPFC, another group of rats was implanted with two microdialysis probes (mPFC and VTA). After collection of baseline values, the $GABA_A$ antagonist bicuculline was perfused in the mPFC at increasing concentrations (10, 30 and 100 μM; 100 min each) in order to disinhibit pyramidal neurones from local tonic GABAergic inputs and to increase the excitatory output to the VTA. Following bicuculline infusion in the mPFC, DA release was significantly elevated in both areas, reaching 269 ± 50% of baseline in the mPFC

($n = 6$; $F_{18,90} = 6.59$; $p < 0.00001$ vs. baseline, one-way repeated measures ANOVA) and 201 ± 16% of baseline in the VTA ($F_{18,90} = 6.02$; $p < 0.00001$ vs. baseline, one-way repeated measures ANOVA; Fig. 1b). Rats showed an increase in motor activity at 30–100 μM bicuculline but no seizure activity was observed.

In another dual-probe experiment, bicuculline application in the VTA (30–100 μM, 120 min each) elevated DA release locally (229 ± 25%; $F_{15,175} = 8.89$, $p < 0.00001$ vs. baseline, one-way repeated measures ANOVA) and in the mPFC (167 ± 16%; $F_{15,175} = 8.63$, $p < 0.00001$ vs. baseline, one-way repeated measures ANOVA) ($n = 6$; data not shown). Notwithstanding observations supporting a noradrenergic origin of the DA release in the PFC (Devoto *et al*. 2002) these results agree with previous observations showing a VTA dependence of cortical DA release (Mathé *et al*. 1999).

Effects of the activation of 5-HT$_{2A}$ receptors in medial prefrontal cortex on dopamine neurone activity and dopamine release

The application of DOI (0.2 μL, 40 pmol) in the mPFC enhanced (>30%) the activity of 65% of the recorded DA neurones in the VTA (19/29). The rest were unaffected ($n = 9$) or decreased ($n = 1$). The application of aCSF in the mPFC did not elevate burst firing ($n = 4$). Considering all neurones, DOI significantly increased DA activity (Table 1; Fig. 2). On average, the percentage of spikes in bursts, the total number of bursts and the total number of spikes fired in

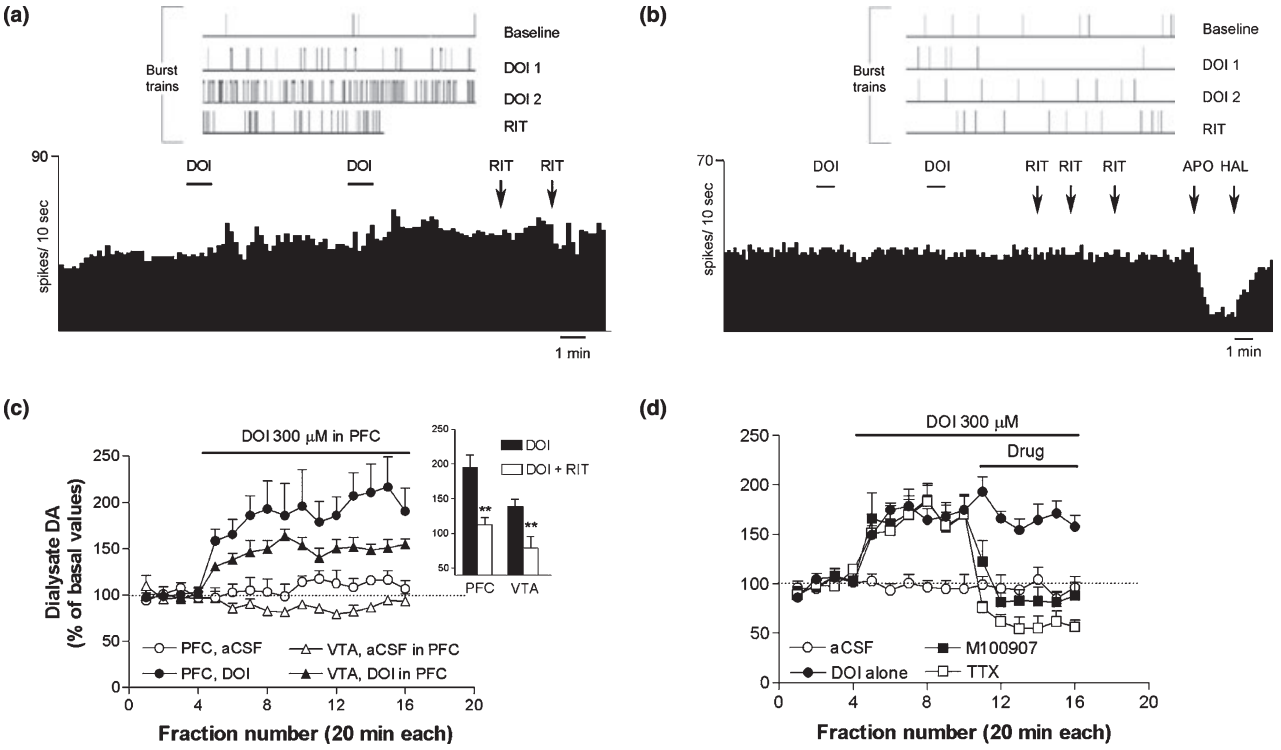

Fig. 2 (a) Representative example of a ventral tegmental area (VTA) dopamine (DA) neurone whose burst firing was increased by the local application of DOI in the medial prefrontal cortex (mPFC). Shown is the integrated firing rate histogram (abcissa, spikes/10 s; ordinate, min). The four upper traces show burst trains in baseline conditions, after cortical DOI application (first and second doses) and after systemic ritanserin (RIT) administration. This unit had a baseline 1% of burst firing. The application of DOI (0.2 μL, 40 pmol, denoted by one 1-min horizontal bar) in the mPFC increased burst firing to 9 and 35% (first and second applications). The administration of ritanserin (1 + 1 mg/kg i.v.) reduced burst firing to 14%. (b) Example of a VTA DA neurone insensitive to the application of DOI in the mPFC. The unit activity was suppressed by the administration of apomorphine (APO; 36 μg/kg i.v.) and reversed by the subsequent administration of haloperidol (HAL; 0.4 mg/kg i.v.). (c) In dual-probe microdialysis experiments, application of DOI in the mPFC increased DA release in the mPFC and VTA ($n = 8$; ● and ▲, respectively). Controls ($n = 4$; ○ and △) were perfused with artificial CSF (aCSF) for the entire experiment. Inset: reversal by ritanserin (300 μM, applied in the mPFC) of the elevation of DA release in the mPFC and VTA produced by the application of DOI (300 μM) in the mPFC. (d) In single probe experiments, the application of 300 μM DOI in the mPFC increased the local DA release (●; $n = 9$). This effect was reversed by co-perfusion of 1 μM tetrodotoxin (TTX, $n = 5$; □) and the selective 5-HT$_{2A}$ receptor antagonist M100907 ($n = 6$; ■). Controls ($n = 5$; ○) were perfused with aCSF for the entire experiment. Horizontal bars show the time of drug application (corrected for the void volume of the system). See text and Table 1 for statistical analysis. **$p < 0.001$ vs. DOI alone. PFC, prefrontal cortex.

bursts were significantly increased by DOI application in the mPFC ($n = 29$). This change was more marked and significant in the subgroup of neurones sensitive to DOI ($n = 19$; Table 1). Likewise, DOI slightly but significantly increased the overall firing rate of DA neurones (to 119 ± 7% of baseline for all neurones, $p < 0.01$, and 132 ± 9% for DOI-sensitive neurones, $p < 0.005$; paired Student's *t*-test). The increase in burst firing induced by DOI in the mPFC was reversed by the systemic administration of the 5-$HT_{2A/2C}$ antagonist ritanserin (1–2 mg/kg i.v.). In the three neurones where reversal was attempted, ritanserin reduced the number of spikes fired in bursts (2 min) from 218 ± 37 to 36 ± 34 ($n = 3$; $p < 0.05$; paired Student's *t*-test). Figure 2 shows representative examples of DA neurones excited by the application of DOI in the mPFC and reversed by ritanserin (Fig. 2a) and insensitive to DOI (Fig. 2b).

In pilot microdialysis experiments, DOI was perfused in the mPFC at increasing concentrations to examine whether it could modulate the local DA release. The maximal effect was attained at 300 μM (220 ± 12% of baseline; data not shown) and this concentration was used subsequently. In rats implanted with two microdialysis probes (in the mPFC and VTA) the application of 300 μM DOI in the mPFC significantly increased DA release in both areas ($n = 8$; $F_{15,105} = 7.39$ for the mPFC; $F_{15,105} = 12.43$ for the VTA; $p < 0.00001$ vs. baseline for both areas; one-way repeated measures ANOVA; Fig. 2c). Maximal elevations in the mPFC and VTA were 217 ± 32 and 163 ± 7% of baseline, respectively. The co-perfusion of ritanserin (300 μM) in the mPFC significantly reversed the elevation produced by DOI in both areas ($n = 5$; $F_{9,36} = 7.23$, $p < 0.0001$ vs. DOI alone in the mPFC; $F_{9,36} = 7.57$, $p < 0.0001$ vs. DOI alone in the VTA, one-way repeated measures ANOVA; see inset in Fig. 2c).

In single probe experiments, the application of DOI in the mPFC increased the local DA release to a similar extent (maximal effect 192 ± 14% of baseline, $n = 9$; $F_{15,120} = 9.20$, $p < 0.0001$ vs. baseline, one-way repeated measures ANOVA). The DA increase was reversed by the co-perfusion of 1 μM tetrodotoxin (to 55 ± 13% of baseline; $n = 5$; $F_{15,60} = 2.38$, $p < 0.00001$, one-way repeated measures ANOVA; $p < 0.05$ DOI vs. baseline; $p < 0.05$ tetrodotoxin vs. DOI; Duncan test post-ANOVA) and by the selective 5-HT_{2A} receptor antagonist M100907 (300 μM, to 81 ± 10% of baseline; $n = 6$; $F_{15,75} = 8.71$, $p < 0.00001$, one-way repeated measures ANOVA; $p < 0.05$ DOI vs. baseline; $p < 0.05$ M100907 vs. DOI; Duncan test post-ANOVA; Fig. 2d). The increase in DA release produced by 300 μM DOI was similar in awake and chloral hydrate-anaesthetized rats (to 207 ± 32%; $n = 4$; data not shown). The local application of M100907 at 30, 100 and 300 μM (four fractions each) by itself reduced DA release to 56 ± 7, 54 ± 4 and 37 ± 5% of baseline, respectively ($n = 5$; $F_{15,60} = 16.45$, $p < 0.0001$ repeated measures ANOVA) (data not shown).

Systemic administration of DOI. Effect on dopamine cell firing and dopamine release

The i.v. administration of DOI (0.05–0.1 mg/kg) significantly increased the activity of DA neurones in the VTA ($n = 12$; $F_{2,22} = 5.90$ for firing rate; $F_{2,22} = 6.42$ for burst firing; $p < 0.01$ vs. baseline; one-way repeated measures ANOVA; Fig. 3a; Table 2). At the lower dose used, the effect of DOI did not reach statistical significance. However, 0.1 mg/kg i.v. significantly increased the total firing rate and burst firing. The latter effect was observed in terms of the percentage of spikes fired in bursts (from 10.9 ± 4.0 to 22.5 ± 6.1%) as well as the total number of bursts and the number of spikes per burst (Table 2). In five out of six neurones, the 5-HT_{2A} receptor antagonist M100907 (0.25–0.5 mg/kg i.v.) counteracted the effect of DOI on cell firing, reducing it to 56% of baseline (1.7 ± 0.5 vs. 3.1 ± 0.8 spikes/s, $n = 5$; $p < 0.05$, paired Student's *t*-test). Likewise, M100907 significantly reversed the effect of DOI and reduced the percentage of burst firing at a dose not affecting DA neurone activity in the VTA (Minabe *et al.* 2001). In four out of five units, M100907 reduced the proportion of burst firing to below baseline, as in the example shown in Fig. 3(a). The other neurone did not show baseline burst firing (Table 2).

These observations were paralleled by the corresponding changes in DA release in microdialysis experiments. The administration of DOI (0.5 mg/kg s.c.) significantly increased DA release in the VTA ($n = 4$) (134 ± 5% of baseline), an effect reversed by the subsequent administration of 0.5 mg/kg s.c. M100907 (to 108 ± 9% of baseline; $n = 4$) ($F_{15,45} = 2.52$, $p < 0.01$; repeated measures ANOVA; $p < 0.05$ DOI vs. baseline; $p < 0.05$ M100907 vs. DOI, non-significant differences between M100907 and baseline; post-hoc Duncan test; Fig. 3b). Systemic M10097 administration did not change the DA release by itself (Fig. 3c).

Likewise, in rats implanted with two microdialysis probes (mPFC and VTA), the systemic administration of DOI (0.5 mg/kg s.c.) significantly increased DA release in both areas (maximal effect 222 ± 23 and 158 ± 19% in the mPFC and VTA, respectively; $n = 4$; $F_{11,33} = 7.80$, $p < 0.00001$ vs. baseline for the mPFC; $F_{11,33} = 4.41$, $p < 0.0005$ vs. baseline for the VTA, one-way repeated measures ANOVA; Fig. 3c). This effect was prevented by the prior administration of M100097 (0.5 mg/kg s.c.) in both areas ($n = 4$ in the mPFC, $n = 3$ in the VTA; $F_{1,6} = 12.86$, $p < 0.02$ group effect; $F_{11,66} = 4.67$, $p < 0.000001$ time effect; $F_{11,66} = 3.79$, $p < 0.0005$ time–group interaction in the mPFC; $F_{1,5} = 10.4$, $p < 0.025$ group effect; $F_{11,55} = 2.94$, $p < 0.005$ time–group interaction in the VTA, two-way repeated measures ANOVA; Fig. 3c).

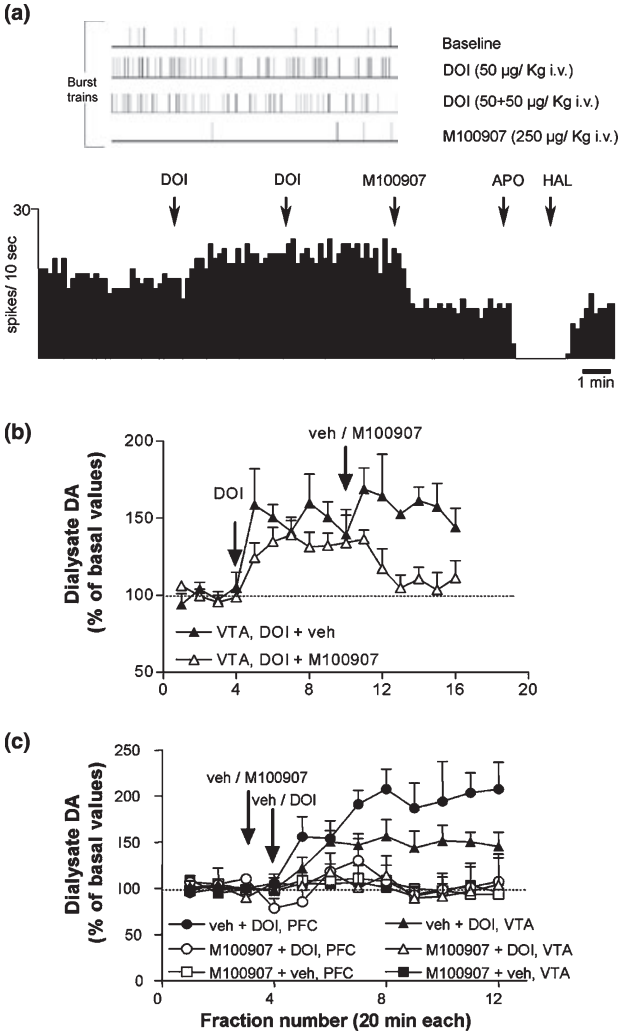

Fig. 3 (a) Representative example of a ventral tegmental area (VTA) dopamine (DA) neurone whose burst firing was increased by the i.v. administration of DOI. Shown is the integrated firing rate histogram (abcissa, spikes/10 s; ordinate, time in min). The four upper traces show burst trains in baseline conditions, after DOI application (0.05 and 0.1 mg/kg, injections shown by vertical arrows) and after systemic M100907 administration. This unit had a baseline 9% of burst firing, which was increased to 35 and 31% after the first and second doses of DOI. The administration of M100907 (250 μg/kg i.v.) reduced the overall firing rate and completely suppressed the increase in burst firing induced by DOI (to 4%). (b) The subcutaneous administration of DOI (0.5 mg/kg) increased DA release in the VTA, an effect reversed by M100907 administration (0.5 mg/kg s.c.) ($n = 4$). A vehicle injection did not alter the increase in DA release produced by DOI ($n = 4$). (c) Likewise, in rats implanted with two microdialysis probes, the administration of M100907 (0.5 mg/kg s.c.) before DOI (0.5 mg/kg s.c.) prevented the increase in DA release produced by this agent in the medial prefrontal cortex and VTA ($n = 4$, vehicle + DOI; $n = 4$, M100907 + DOI; $n = 4$, M100907 + vehicle, in both areas). Arrows show the time of drug administration (first arrow, vehicle or M100907; second arrow, vehicle or DOI). See text and Table 2 for statistical analysis. APO, apomorphine; HAL, haloperidol.

Discussion

The present results indicate that the activity of DA neurones in the VTA and the release of DA in the VTA and mPFC are modulated by 5-HT$_{2A}$ receptors in the mPFC. These observations are consistent with the existence of anatomical connections between the mPFC and VTA as well as the excitatory role of 5-HT$_{2A}$ receptors on pyramidal cell activity (see Introduction). Indeed, previous reports showed that high frequency stimulation of the mPFC (e.g. 60 Hz) increased DA release in nucleus accumbens (Taber and Fibiger 1995; Jackson *et al*. 2001). To examine the effect of the mPFC on VTA DA release, we used a lower, more physiological frequency (10–20 Hz) to mimic the excitatory effect of DOI on mPFC pyramidal neurones (Puig *et al*. 2003). The local application of MK-801 in the VTA completely suppressed the mPFC-evoked stimulation of DA release, supporting the involvement of NMDA-dependent inputs. This observation agrees with the fact that (1) mPFC stimulation induced burst firing in VTA DA neurones (Gariano and Groves 1988; Murase *et al*. 1993; Tong *et al*. 1996); (2) this effect depends on the activation of NMDA receptors (Overton and Clark 1997) and (3) burst firing is associated with a greater DA release (Chergui *et al*. 1994). Likewise, the elevation in VTA DA release induced by bicuculline application in the mPFC agrees with the notion that an increased mPFC activity increases that of DA neurones (Murase *et al*. 1993). Conceivably, bicuculline blocked (at least partially) local GABA$_A$ inputs onto pyramidal neurones, thus increasing their firing activity and therefore the impulse flow through axons projecting to midbrain.

Various brain areas are involved in the control of the activity of VTA DA neurones (Fig. 4). The present results cannot establish whether the increased DA release in the VTA produced by electrical stimulation of the mPFC is due to the activation of a direct mPFC–VTA pathway or whether long loops are also involved. In support of the first possibility are the observations that the PFC projects densely to the VTA and PFC stimulation induced short-latency excitations in VTA DA neurones (Thierry *et al*. 1979; Carr and Sesack 2000a).

A second possibility is that mPFC stimulation can also increase DA release in the VTA via afferents to other brain areas that in turn project and modulate the activity of VTA neurones (Tzschentke and Schmidt 2000; Sesack *et al*. 2003; Adell and Artigas 2004). Among these inputs the PFC → laterodorsal/pedunculopontine tegmentum → VTA and PFC → nucleus accumbens → ventral pallidum → VTA pathways may be responsible (Fig. 4). Both pathways have been shown to modulate DA neurone activity via phasic and tonic inputs, respectively (Floresco *et al*. 2003). These indirect pathways may perhaps account for the complex biphasic responses of DA neurones to mPFC stimulation (Tong *et al*. 1996).

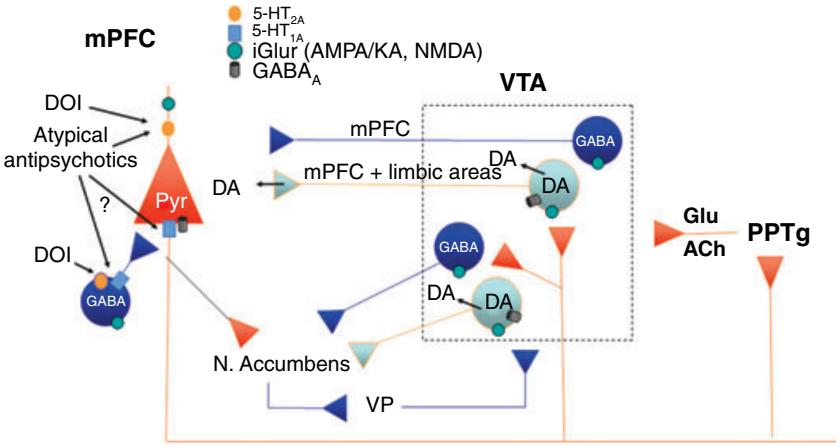

Fig. 4 Schematic representation of the direct and indirect connectivity between the medial prefrontal cortex (mPFC) and ventral tegmental area (VTA). The dotted frame marks the VTA, with known inputs from the mPFC onto dopamine (DA) and GABAergic neurones according to Carr and Sesack (2000a) (local GABA → DA inputs within the VTA are not shown). Inhibitory GABAergic afferents from ventral pallidum (VP) and excitatory [glutamate, acetylcholine (ACh)] afferents from the pedunculopontine tegmentum (PPTg) are not shown to synapse on DA or GABAergic neurones in the VTA. The electrical or pharmacological (bicuculline) activation of the mPFC output enhanced DA release in the VTA. Likewise, the selective activation of 5-HT_{2A} receptors in the mPFC by local DOI application increased DA cell activity and DA release in the VTA and mPFC, an action mimicked by systemic DOI administration. The prefrontal facilitation of VTA DA neurones may be direct, via an excitatory pathway to VTA DA neurones projecting to the prefrontal cortex (PFC) and unidentified areas (but not nucleus accumbens; Carr and Sesack 2000a), or indirect. The latter pathways may include the above inputs from VP and PPTg. Atypical antipsychotics may act on various receptors present in the PFC, such as 5-HT_{2A} or 5-HT_{2C}, to modulate DA cell transmission via these pathways. Likewise, the colocalization of 5-HT_{1A} with 5-HT_{2A} receptors in the PFC (Amargós-Bosch *et al.* 2004) and the evidence that prefrontal DA release is modulated by atypical antipsychotic drugs via 5-HT_{1A} receptors (Rollema *et al.* 1997; Ichikawa *et al.* 2001b) suggest that the latter receptors in the PFC may also be involved. Glu, glutamate; iGluR, ionotropic glutamate receptors; KA, kainate; Pyr, pyramidol neurons.

The application of DOI in the mPFC increased the activity of VTA DA neurones, indicating that the activation of 5-$HT_{2A/2C}$ receptors in the mPFC modulates the activity of a substantial proportion of VTA neurones (19 out of 29 DA neurones were sensitive to DOI). Detailed tracing studies have revealed a dense innervation of the VTA by the mPFC (Carr and Sesack 2000a,b), which is consistent with the present data. A relatively small proportion of DA neurones projects back to the mPFC, closing the mesocortical VTA–mPFC circuit, whereas the rest project to subcortical areas other than nucleus accumbens as mesoaccumbal DA neurones do not appear to be innervated by mPFC afferents (Carr and Sesack 2000a). Hence, it is likely that DOI-sensitive neurones project to limbic areas in addition to the mPFC, which emphasizes the need for further work to examine the role of prefrontal 5-HT_{2A} receptors on DA release in limbic areas.

The increased dopaminergic activity was accompanied by an increase in local (in the mPFC) and distal (in the VTA) DA release when DOI was applied by reversed dialysis in the mPFC. These effects were reversed by the co-perfusion of the non-selective and selective 5-HT_{2A} receptor antagonists ritanserin and M100907, respectively, and by tetrodotoxin. These results suggest that DOI increased the activity of the mesocortical DA pathway and the terminal and somatodendritic DA release through an impulse-dependent mechanism. Likewise, the reversal of the action of DOI on mPFC DA release by M100907 supports this effect being due to the activation of 5-HT_{2A} receptors. However, as DOI is also a 5-HT_{2C} receptor agonist, the involvement of the latter receptor cannot be fully discarded (see below).

Similarly, the systemic administration of DOI increased DA neurone activity and DA release. Previous reports also observed a similar effect of systemic DOI on prefrontal DA release (Gobert and Millan 1999; Pehek *et al.* 2001). However, to our knowledge, the systemic effect of DOI on DA cell firing and DA release in the VTA has not been examined before. The enhanced burst firing produced by DOI was accompanied by an enhanced DA release in the mPFC and VTA, an effect blocked by M100907.

Interestingly, 5-HT_{2A} receptors involved in the effect of DOI may be tonically activated as M100907 reduced the firing rate of some DA neurones to below baseline (Fig. 3a). However, this possibility needs to be examined in more detail as local (but not systemic) M100907 administration reduced DA release in the mPFC (Pehek *et al.* 2001; this work). Unfortunately, the limited availability of M100907 prevented us from examining its effect on VTA cell firing when given alone.

The similarity between the effect of the local and systemic DOI administration suggests that the dense population of 5-HT_{2A} receptors in the mPFC (Pompeiano *et al*. 1994; Amargós-Bosch *et al*. 2004; Santana *et al*. 2004) plays a major role in the modulation of DA neurones, notwithstanding the presence of a smaller receptor subset in the VTA (Nocjar *et al*. 2002). Indeed, the parallel increase in pyramidal (Puig *et al*. 2003) and DA neurone activity (this study) produced by the same doses of DOI suggests a close relationship between both effects. As discussed above for the effect of electrical stimulation, it is unclear whether the action of DOI is mediated by direct or indirect inputs to the VTA (Fig. 4).

The cancellation of the effect of DOI on DA neurone activity and on prefrontal DA release by M100907 suggests that it was due to interaction with 5-HT_{2A} receptors. However, a limitation of the present study is that the local and distal effects of DOI on DA release were reversed by the 5-$HT_{2A/2C}$ antagonist ritanserin. This was a necessity due to the limited availability of M100907, which is not commercially available. It is unlikely that 5-HT_{2C} receptors participate in the ritanserin reversal as: (1) the systemic effect of DOI was reversed by M100907; (2) DOI was applied in an mPFC area very rich in pyramidal neurones containing 5-HT_{2A} receptors (Santana *et al*. 2004) and (3) 5-HT_{2C} receptor activation inhibits, rather than enhances, DA transmission (Gobert *et al*. 2000; Di Matteo *et al*. 2001; De Deurwaerdere *et al*. 2004), which is in keeping with the presence of 5-HT_{2C} receptors in GABAergic interneurones in the PFC (Liu *et al*. 2004). However, DOI is a potent 5-HT_{2C} receptor agonist and the stimulatory effect of 5-HT_{2C} receptors on DA transmission has been observed after systemic drug administration. Therefore, we cannot rule out that the stimulation of 5-HT_{2C} receptors in the mPFC can also contribute to the increase in DA cell activity and DA release produced by DOI.

The putative mechanism by which DOI may modulate DA neurone activity and somatodendritic and terminal DA release is depicted in Fig. 4. Taking into account previous data together with the present results obtained with the local application of DOI in the mPFC, we suggest that the activation of 5-HT_{2A} receptors in the mPFC enhanced the excitatory cortical output to midbrain, resulting in an increased activity of VTA DA neurones which subsequently resulted in an increased DA release. A similar mechanism has previously been reported for raphe 5-HT neurones (Martín-Ruiz *et al*. 2001). Additionally, given the presence of a small subset of 5-HT_{2A} receptors in mPFC axons possibly on catecholaminergic terminals (Miner *et al*. 2003), DOI might also modulate DA release in a local manner.

In summary, the present results support the view that 5-HT_{2A} receptors in the mPFC modulate distally the activity of DA neurones in the VTA. The activation of such receptors markedly increases DA neurone activity and DA release in the mesocortical pathway but it is still unknown whether this excitatory influence is exerted via direct or indirect inputs to the VTA. Given the widespread influence of prefrontal 5-HT_{2A} receptors on VTA DA neurones (of which a majority projects to limbic areas) we suggest that atypical antipsychotic drugs may modulate the activity of ascending DA pathways by a cortically based action. Hence, the blockade of 5-HT_{2A} receptors in the PFC may attenuate a putative DA overactivity in schizophrenia. This effect would reduce DA cell activity and DA release in target areas without the massive blockade of post-synaptic D_2 receptors induced by classical neuroleptics, which is responsible for the severe side-effects of these compounds.

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