

# Involvement of 5-HT<sub>1A</sub> Receptors in Prefrontal Cortex in the Modulation of Dopaminergic Activity: Role in Atypical Antipsychotic Action

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Atypical antipsychotics increase dopamine (DA) release in the medial prefrontal cortex (mPFC), an effect possibly involved in the superior effects of atypical versus classical antipsychotics on cognitive/negative symptoms. We examined the role of 5-HT<sub>1A</sub> receptors in the mPFC on the modulation of dopaminergic activity and the mesocortical DA release *in vivo*. The highly selective 5-HT<sub>1A</sub> agonist BAY x 3702 (BAY; 10–40 μg/kg, i.v.) increased the firing rate and burst firing of DA neurons in the ventral tegmental area (VTA) and DA release in the VTA and mPFC. The increase in DA release in both areas was potentiated by nomifensine coperfusion. The selective 5-HT<sub>1A</sub> antagonist WAY-100635 reversed the effects of BAY in both areas, and the changes in the VTA were prevented by frontocortical transection.

The application of BAY in rat and mouse mPFC by reverse dialysis increased local extracellular DA at a low concentration (3 μM) and reduced it at a higher concentration (30 μM). Both effects disappeared in 5-HT<sub>1A</sub> knock-out mice. In the presence of bicuculline, BAY reduced DA release at all concentrations. The atypical antipsychotics clozapine, olanzapine, and ziprasidone (but not haloperidol) enhanced DA release in the mPFC of wild-type but not 5-HT<sub>1A</sub> knock-out mice after systemic and local (clozapine and olanzapine) administration in the mPFC. Likewise, bicuculline coperfusion prevented the elevation of DA release produced by local clozapine or olanzapine application. These results suggest that the activation of mPFC 5-HT<sub>1A</sub> receptors enhances the activity of VTA DA neurons and mesocortical DA release. This mechanism may be involved in the elevation of extracellular DA produced by atypical antipsychotics.

**Key words:** antipsychotic; dopamine; prefrontal cortex; schizophrenia; serotonergic 1A receptor; ventral tegmental area

## Introduction

The ventral tegmental area (VTA) gives rise to the mesocortical and mesolimbic dopamine (DA) systems, involved in higher brain functions such as cognition, memory, reward, and behavioral 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 seem 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). Classical neuroleptics used to treat schizophrenia block DA D<sub>2</sub> receptors (Seeman and Lee, 1975; Creese et al., 1976), an action that also evokes extrapyramidal motor symptoms and hyperprolactinemia. With few exceptions (e.g., amisulpride), second-generation (atypical) antipsychotics display a preferential 5-HT<sub>2</sub> versus DA D<sub>2</sub> receptor affinity and occupancy in the brain (Meltzer, 1999), although “atypicality” may encompass more than one mechanism (Roth et al., 2003).

Among the various aminergic receptors, there is growing interest in 5-HT<sub>1A</sub> receptors as potential targets for antipsychotic drug action (Millan, 2000). These receptors seem to contribute to the ability of atypical (but not classical) antipsychotics to increase cortical DA release, an effect potentially involved in the improvement of negative symptoms and cognitive dysfunction in schizophrenia (Rollema et al., 1997, 2000; Kuroki et al., 1999; Ichikawa et al., 2001a).

5-HT<sub>1A</sub> receptors are located in 5-HT neurons of the raphe nuclei, where they function as autoreceptors, and in cortical and limbic areas (Pazos and Palacios, 1985; Pompeiano et al., 1992). Their activation results in membrane hyperpolarization and reduction in neuronal activity (Sprouse and Aghajanian, 1986;

Received Feb. 24, 2005; revised Oct. 3, 2005; accepted Oct. 9, 2005.

This work was supported by Ministerio de Ciencia y Tecnología de España Grant SAF 2004-05525 and by Lilly, the Centro de Investigación de Enfermedades Neurológicas network [Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)-ISCIII RTIC C03/06], and Generalitat de Catalunya (2001-SGR00355). P.C. and A.B. were recipients of a Ramón y Cajal contract from the Ministry of Science and Technology. M.C.S. was a recipient of a postdoctoral fellowship from Fundación Carolina. L.D.-M. was a recipient of a predoctoral fellowship from IDIBAPS. We thank Leticia Campa and Judith Ballart for skillful technical assistance.

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DOI:10.1523/JNEUROSCI.2999-05.2005

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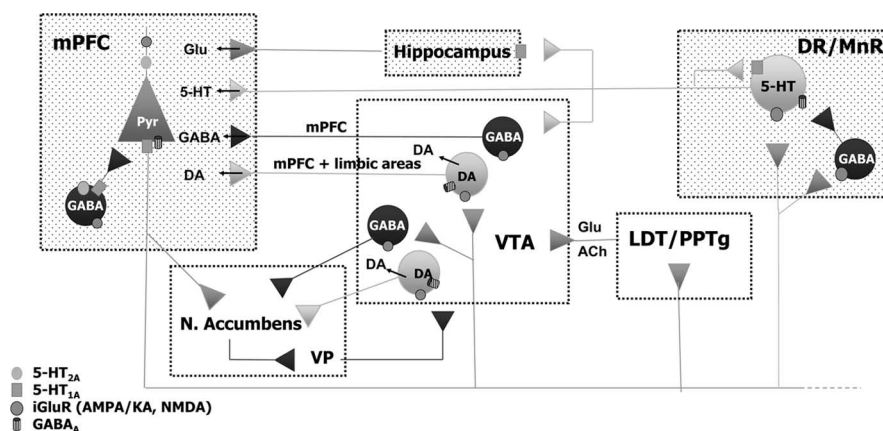
Araneda and Andrade, 1991; Amargós-Bosch et al., 2004). Likewise, 5-HT<sub>1A</sub> receptors modulate 5-HT release by presynaptic and postsynaptic mechanisms (Sharp et al., 1989; Adell et al., 1993; Celada et al., 2001). Interestingly, DA cell firing and DA release have been shown to be modulated by 5-HT<sub>1A</sub> receptor agonists (Arborelius et al., 1993a,b; Prisco et al., 1994; Lejeune and Millan, 1998; Sakaue et al., 2000). However, the mechanism(s) involved and the localization of the 5-HT<sub>1A</sub> receptors responsible for this effect have not been fully elucidated.

The activity of VTA DA neurons 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 tegmentum (LDT)/pedunculopontine tegmentum (PPTg) or the nucleus accumbens/ventral pallidum (VP) pathway (Tzschenke and Schmidt, 2000; Adell and Artigas, 2004; Omelchenko and Sesack, 2005) (Fig. 1). The PFC is highly enriched in pyramidal neurons expressing 5-HT<sub>1A</sub> receptors (also present in GABA interneurons) (Santana et al., 2004), in close overlap with projection neurons to the VTA (Thierry et al., 1979, 1983). Based on this anatomical and functional evidence, we conducted the present study under the working hypothesis that 5-HT<sub>1A</sub> receptors in the mPFC may modulate VTA DA neuron activity and DA release in the mesocortical pathway. Additionally, we examined whether atypical antipsychotics increase cortical DA release through this mechanism.

## Materials and Methods

**Animals and treatments.** Male albino Wistar rats weighing 250–320 g and C57BL/6 mice 10–12 weeks of age at the time of experiments were used (Iffa Credo, Lyon, France). 5-HT<sub>1A</sub> receptor knock-out KO (–/–) mice (referred onward as KO) had the same genetic background than their wild-type (WT) counterparts (C57BL/6) and were engendered as generated previously at Princeton University (Princeton, NJ) (Parks et al., 1998). From this initial source, a stable colony was grown in the animal facility of the University of Barcelona School of Medicine (Barcelona, Spain). Animals 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 (Official Journal of the European Communities L358/1, December 18, 1986) and was approved by the Institutional Animal Care and Use Committee. For the rat, stereotaxic coordinates (in millimeters) were taken from bregma and duramater according to the atlas of Paxinos and Watson (1998). For mice, coordinates were taken from bregma and top of skull according to the atlas of Franklin and Paxinos (1997).

Bicuculline, clozapine, haloperidol, apomorphine, nomifensine, and WAY-100635 were from Research Biochemicals (Natick, MA). R-(–)-2-(4-[(chroman-2-ylmethyl)-amino]-butyl)-1,1-dioxo-benzo[d] isothiazolone hydrochloride (BAY) (De Vry et al., 1998) was from Bayer (Wuppertal, Germany), olanzapine was from Eli Lilly (Indianapolis, IN), and ziprasidone was from Pfizer (Groton, CT). BAY was administered intravenously at 10–80 µg/kg (free base), and WAY-100635 was administered intravenously at the dose of 30–100 µg/kg. Except for ziprasidone, which

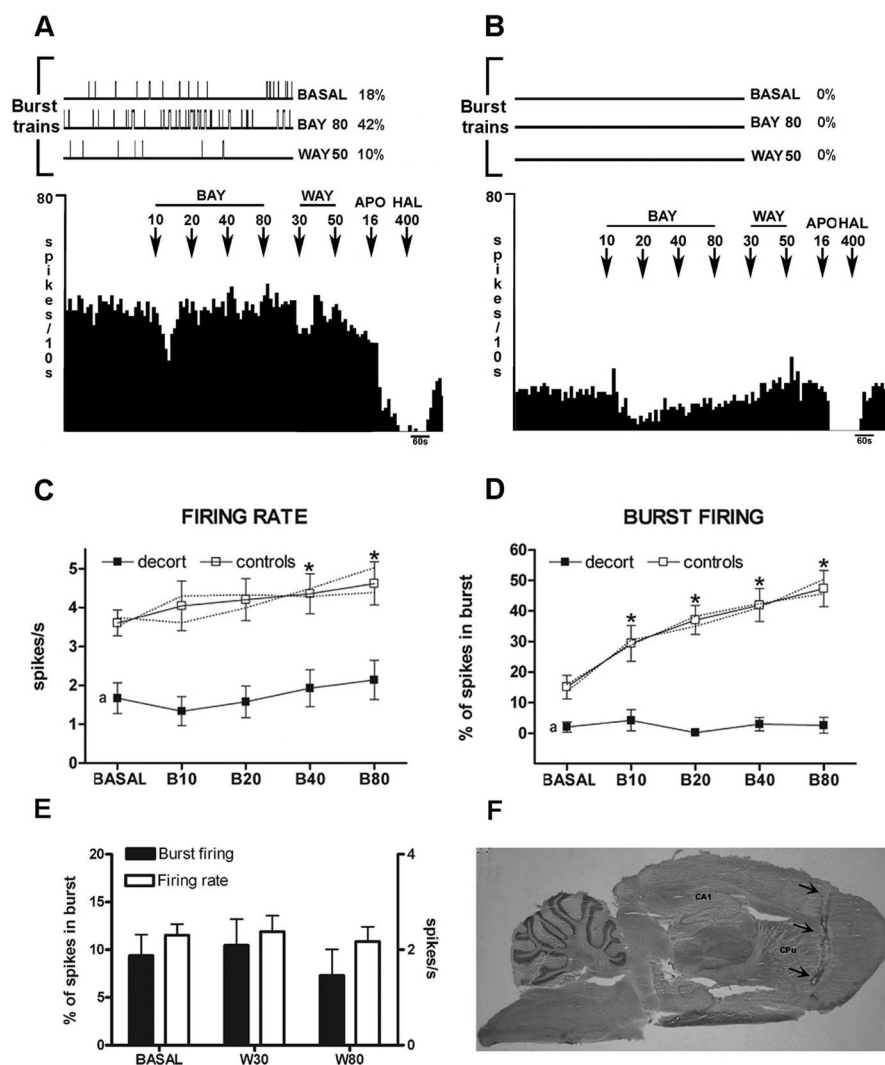


**Figure 1.** Schematic representation of the anatomical and functional relationships between the mPFC, the VTA, and the dorsal and median raphe nuclei (DR/MnR). Pyramidal neurons of the mPFC project directly to mesocortical (but not mesoaccumbal) DA neurons, closing a mPFC–VTA circuit (Carr and Sesack, 2000b). GABAergic neurons in the VTA project to mPFC and limbic areas as well. The mPFC may also modulate the activity of VTA neurons indirectly, including the basal ganglia circuit [e.g., mPFC→nucleus accumbens→VP pathway or through afferents to the LDT/PPTg, among other pathways (data not shown)] (Tzschenke and Schmidt, 2000; Floresco et al., 2003; Sesack et al., 2003; Adell and Artigas, 2004; Omelchenko and Sesack 2005). Likewise, the mPFC is reciprocally connected with the DR/MnR. Pyramidal neurons of the mPFC project to raphe 5-HT and GABA neurons and modulate their activity (Aghajanian and Wang, 1977; Hajós et al., 1998; Peyron et al., 1998; Celada et al., 2001). In turn, 5-HT neurons modulate the activity of pyramidal cells in the mPFC through various receptors, in particular 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, which are expressed by pyramidal and GABAergic neurons (Araneda and Andrade, 1991; Amargós-Bosch et al., 2004; Santana et al., 2004). The pharmacological activation of these receptors in the mPFC has been shown to modulate 5-HT neuron activity and terminal 5-HT release via descending afferents to the midbrain (Celada et al., 2001; Martín-Ruiz et al., 2001). In addition to the mPFC, 5-HT<sub>1A</sub> receptors are densely expressed in the DR/MnR (autoreceptors) and in areas projecting to the mPFC, such as the hippocampal formation (Pompeiano et al., 1992). The shaded boxes show 5-HT<sub>1A</sub> receptor-rich areas. Direct 5-HT afferents to the VTA may also be involved in the control of DA neurons by other 5-HT receptors, notably the 5-HT<sub>2C</sub> subtype, present in GABAergic neurons of the VTA (DiMatteo et al., 2001). Glu, Glutamate; Pyr, pyramidal neuron; ACh, acetylcholine; N. Accumbens, nucleus accumbens; iGluR, ionotropic glutamate receptor; KA, kainic acid. Data are expressed as means ± SEM.

was used in an injectable preparation (Zeldox), drugs were dissolved in saline at the appropriate concentrations and injected (up to 1 ml/kg) through the femoral vein. For the assessment of local or distal effects in microdialysis experiments, drugs were dissolved in the perfusion fluid or water [except clozapine (dissolved in acetic acid) and olanzapine (dissolved in HCl)] and diluted to appropriate concentrations in artificial CSF (aCSF). Concentrated solutions (pH adjusted to 6.5–7.4 with NaHCO<sub>3</sub> when necessary) were stored at –80°C, and working solutions were prepared daily by dilution in aCSF at the stated concentrations and applied by reverse dialysis (uncorrected for drug recovery). Control rats and mice were perfused with aCSF. The bars in the figures show the period of drug application (corrected for the void volume of the system).

**Single-unit recordings.** We examined the responses of VTA DA neurons to the systemic administration of drugs. Rats were anesthetized (chloral hydrate, 400 mg/kg, i.p.) and positioned in a David Kopf stereotaxic frame. Thereafter, chloral hydrate was continuously administered intraperitoneally at a dose of 50–70 mg/kg/h using a perfusion pump (Fa et al., 2003). Body temperature was maintained at 37°C with a heating pad. DA neurons were recorded extracellularly with glass micropipettes pulled from 2 mm capillary glass (World Precision Instruments, Sarasota, FL) on a Narishige (Tokyo, Japan) PE-2 pipette puller. 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, Delaware Water Gap, PA), postamplified and filtered with a Cibertec (Madrid, Spain) amplifier and computed on-line using a DAT 1401 plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Descents in the VTA were performed at anteroposterior (AP) –5.0 to –5.6, lateral (L) –0.5 to –1, and dorsoventral (DV) –7.5 to –9.0 to record DA neurons in both the parabrachial and paranigral subdivisions. The identification of DA neurons and burst-firing analysis was performed according to the criteria of Grace and Bunney (1984), as used previously (Celada et al., 1999). Briefly, neurons were considered dopaminergic if they possessed the following characteristics: 1) action poten-



**Figure 2.** *A, B*, Representative examples of the effect of the selective 5-HT<sub>1A</sub> agonist BAY (10–80  $\mu$ g/kg, i.v.; injections shown by vertical arrows) on the activity of VTA DA neurons in sham-operated (*A*) and cortically transected (*B*) rats. The integrated firing-rate histogram (abscissa, spikes per 10 s; ordinate, time in minutes) is shown. The top traces in these panels show representative burst trains corresponding to 1 min recordings obtained in baseline conditions after intravenous administration of 80  $\mu$ g/kg BAY and after intravenous administration of 50  $\mu$ g/kg WAY-100635. This representation of burst activity was generated using a modified Spike 2 software; each vertical trace corresponds to a single bursting episode, as defined in Materials and Methods. The percentage of spikes fired in bursts is also shown. The unit in *A* had firing rates of 4.2, 4.8, and 3.3 spikes/s and burst firing of 18, 42, and 10% (baseline, intravenous 80  $\mu$ g/kg BAY and WAY-100635, respectively). The unit in *B* had firing rates of 1.3, 1.1, and 1.6 spikes/s (baseline, intravenous 80  $\mu$ g/kg BAY and WAY-100635, respectively) with no burst during the entire recording. Note the dissimilar effect of BAY administration in the two units. *C, D*, The dose–response curves on firing rate (*C*) and burst firing (*D*) in controls (□;  $n = 11$ ) and in cortically transected rats (■;  $n = 7$ ). Dotted lines show the dose–response curves in naive and sham-operated rats, respectively. \* $p < 0.05$  versus baseline post-ANOVA;  $^{\circ}p < 0.05$  versus controls. BAY had the same effect in naive and sham-operated rats. See Results and Table 1 for statistical analysis. Although cortical transection did not significantly affect the baseline firing rate in the entire group of decorticated rats (see Results), this subgroup had a significant difference versus controls. *E*, The lack of effect of WAY-100635 (30–80  $\mu$ g/kg, i.v.) on the overall firing rate and burst firing of VTA DA neurons ( $n = 5$ ). *F*, A sagittal section of a rat brain at the approximate lateral coordinate 3.4 mm, taken from midline (Paxinos and Watson, 1998). The transection lesion is shown by arrows. WAY, WAY-100635; APO, apomorphine; HAL, haloperidol; decort, decorticated; CPu, caudate putamen. Data are expressed as means  $\pm$  SEM.

tial duration  $>2.5$  ms; 2) typical biphasic or triphasic waveform often with a notch in the initial rising phase; 3) slow firing rate (recorded neurons fired at 1–5 spikes/s in control rats); and 4) frequent presence 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  $\geq 160$  ms (Grace and Bunney, 1984). Recorded neurons in control rats had a  $14.1 \pm 2.7\%$  of spikes fired in bursts during baseline conditions. Additional pharmacological identification was performed with intravenous apomorphine, followed by haloperidol reversal whenever possible.

Groups of rats were subjected to transection of the prefrontal cortex. This was performed under chloral hydrate (400 mg/kg, i.p.) anesthesia using a fine metal needle (0.6 mm diameter), which was positioned at AP +1.0, DV  $-6.8$ , and L +0.8 and moved stereotactically to L +4.8. In the right hemisphere, the needle was placed with an angle of  $20^\circ$  to reach AP +1.0, DV  $-6.8$ , and L +1.2 to avoid damaging the sinus. The transection of the cortical afferents to the midbrain was performed by moving the needle between +1.2 and  $-4.8$  mm. The brain areas affected by the needle descent can be seen in plate 84 of the Paxinos and Watson (1998) atlas for rat brain (see Results). Recordings of VTA DA neurons were conducted 1 h after the transection.

**In vivo microdialysis.** Microdialysis procedures in rats and mice were conducted essentially as described previously by Bortolozzi et al. (2003) and Amargós-Bosch et al. (2004). Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and implanted with 4 mm concentric dialysis probes (Cuprophane) 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  $10^\circ$  that placed the probe tip at L  $-0.6$  and DV  $-8.7$ ). Microdialysis experiments in freely moving rats were performed  $>20$  h after surgery. Probes were perfused with aCSF pumped at  $1.5 \mu$ l/min. After an initial 100 min stabilization period, four baseline samples were collected (20 min each) before local (reverse dialysis) or systemic drug administration, and successive dialysate samples were collected. In anesthetized rats, the flow rate was set at  $3 \mu$ l/min, and fractions were collected every 10 min.

For mice, the manufacture of the probes was adapted from that described previously for rats. Surgical and microdialysis procedures were identical to those described for rats (freely moving), except for the dose of anesthesia (sodium pentobarbital, 40 mg/kg, i.p.), the length of dialysis membrane (2 mm), and the brain coordinates (in millimeters) of the mPFC: AP, +2.2; L,  $-0.2$ ; DV,  $-3.4$ .

The concentration of DA in dialysate samples was determined by HPLC, using a modification of a method described previously (Ferré et al., 1994). Brain dialysates were collected on microvials containing  $5 \mu$ l of 10 mM perchloric acid and 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. In one experiment, we also examined the effect of BAY on dialysate 5-HT concentration, which was determined following described

procedures (Amargós-Bosch et al., 2004). In this case, 5-HT was detected amperometrically at +0.6 V.

Microdialysis experiments were also conducted in rats subjected to cortical transection. These animals were subjected to the same procedure used in single-unit recordings, except that the surgical procedure was done 1 d before (e.g., at the time of probe implants, under pentobarbital anesthesia).

**Histology.** After experimental procedures were completed, animals were killed by an overdose of anesthetic. The brains were removed and frozen in



dry ice before being sectioned (70  $\mu$ m) with a cryostat in the sagittal or coronal planes, as appropriate. In some cases, recording electrodes were filled with Pontamine sky blue to verify the recording site. Brain sections were then stained with neutral red, according to standard procedures, to examine the correctness of the transections. In microdialysis experiments, sections were examined to verify the correct placement of probes in the VTA or mPFC.

**Data and statistical analysis.** Changes in the firing rate or the proportion of burst firing in DA neurons were assessed using repeated-measures ANOVA or paired Student's *t* test, as appropriate. These values were quantified by averaging the values during 3 min in basal conditions and 1–2 min after intravenous administration (omitting the first minute).

Microdialysis results are expressed as femtomoles per fraction (uncorrected for recovery) and are shown in figures as percentages of basal values (individual means of four predrug fractions). Statistical analysis was performed using ANOVA for repeated measures of the DA values during specified periods. Data are expressed as the mean  $\pm$  SEM. Statistical significance has been set at the 95% confidence level (two tailed).

## Results

### Effects of BAY on DA cell firing: dependence on cortical integrity

The intravenous administration of the selective 5-HT<sub>1A</sub> agonist BAY (10–80  $\mu$ g/kg, i.v.; cumulative doses) slightly enhanced the firing rate and markedly increased the burst firing of VTA DA neurons in naive, chloral hydrate-anesthetized rats ( $p < 0.0005$  for burst firing;  $n = 7$ ; one-way, repeated-measures ANOVA). A *post hoc t* test revealed a significant effect of all BAY doses (Fig. 2). Because 1) 5-HT<sub>1A</sub> receptors are highly expressed in the mPFC (Amargós-Bosch et al., 2004) and 2) BAY increased the firing rate of pyramidal neurons projecting to the VTA (Díaz-Mataix et al., 2005), we examined the possible involvement of 5-HT<sub>1A</sub> receptors in the mPFC on the effect of BAY on DA neuron activity.

For this purpose, a group of rats was subjected to frontocortical transection (see Materials and Methods). Sham-operated rats for these experiments ( $n = 4$ ) were subjected to the same surgical procedure, with the exception that the descent of the needle was omitted. The basal firing rate did not differ between sham and naive rats ( $3.7 \pm 0.4$  vs  $3.5 \pm 0.5$  spikes/s;  $n = 4$  and  $7$ , respectively). Likewise, burst firing did not differ between both groups ( $14 \pm 4$  vs  $16 \pm 6\%$  in sham-operated and naive rats;  $n = 4$  and  $7$ , respectively). The effect of the administration of BAY (10–80  $\mu$ g/kg, i.v.) on the firing rate and burst firing was comparable on both groups ( $p < 0.02$ , effect of the dose on firing rate, nonsignificant effects of group, and group-by-dose interaction;  $p < 0.0001$ , effect of the dose on burst firing, nonsignificant effects of group, and group-by-dose interaction) (Fig. 2C,D). Therefore, the data from both groups were pooled and used together as a single control group ( $n = 11$ ).

When considering the effect on the entire control group, BAY (10–80  $\mu$ g/kg, i.v.) significantly increased the firing rate ( $n = 11$ ;  $p < 0.03$ , dose effect; one-way ANOVA; significant differences of the intravenous doses of 40 and 80  $\mu$ g/kg vs baseline; *post hoc t* test). Likewise, BAY markedly increased the burst firing in control rats ( $n = 11$ ;  $p < 0.0001$ , dose effect; one-way ANOVA; significant dif-

**Table 1. Firing characteristics and effect of BAY on VTA DA neurons in control and cortically transected rats**

	Firing rate (spikes/s)	% of spikes fired in burst	Mean number of spikes per burst	Number of bursts in 1 min	Spikes in burst in 1 min
Basal					
Controls ( $n = 11$ )	$3.6 \pm 0.3$	$15 \pm 4$	$2.7 \pm 0.2$	$13 \pm 3$	$36 \pm 11$
Lesioned ( $n = 7$ )	$1.7 \pm 0.4^*$	$2 \pm 2^*$	2 (2)	$1 \pm 1^*$	$2 \pm 1^*$
BAY (80 $\mu$ g/kg)					
Controls ( $n = 11$ )	$4.6 \pm 0.5$	$49 \pm 6$	$3.4 \pm 0.3$	$41 \pm 6$	$149 \pm 28$
Lesioned ( $n = 7$ )	$2.1 \pm 0.5^*$	$3 \pm 3^*$	2 (1)	$2 \pm 2^*$	$4 \pm 4^*$

\* $p < 0.05$  versus the corresponding value in control rats. Numbers in parentheses are the number of neurons that showed burst.

**Table 2. Baseline dialysate DA values**

Species	Condition	mPFC	VTA
Rat	Awake, aCSF	$12.4 \pm 0.9$ ( $n = 31$ )	n.e.
	Awake, aCSF + BIC	$60.0 \pm 10.8^*$ ( $n = 26$ )	n.e.
	Anesthetized, aCSF	$5.4 \pm 1.1$ ( $n = 7$ )	$6.6 \pm 1.3$ ( $n = 11$ )
	Anesthetized, aCSF + NOM	$21.6 \pm 1.1^*$ ( $n = 11$ )	$17.9 \pm 5.0^*$ ( $n = 5$ )
	Anesthetized, aCSF + NOM, sham	n.e.	$12.1 \pm 1.4^*$ ( $n = 7$ )
	Anesthetized, aCSF + NOM, lesioned	n.e.	$19.9 \pm 4^*$ ( $n = 6$ )
Mice	Awake, aCSF, WT	$8.4 \pm 0.8$ ( $n = 65$ )	n.e.
	Awake, aCSF, KO	$9.0 \pm 1.0$ ( $n = 61$ )	n.e.

Data are expressed in femtomoles per 20 min fraction for awake animals and femtomoles per 10 min fraction for anesthetized animals (1.5 and 3  $\mu$ l/min flow rates, respectively; see Materials and Methods). \* $p < 0.05$  versus a CSF alone. n.e., Not examined; BIC, bicuculline (30  $\mu$ M); NOM, nomifensine (10  $\mu$ M).

ferences of all doses vs baseline; *post hoc t* test) (Fig. 2, Table 1). The administration of the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (30–100  $\mu$ g/kg, i.v.) significantly reduced the elevation in the firing rate ( $p < 0.05$ ) and burst firing produced by BAY ( $p < 0.0005$  vs 80  $\mu$ g/kg BAY, i.v.; nonsignificant difference between WAY-100635 and baseline periods; Student's paired *t* test). (Fig. 2). The administration of WAY-100635 alone did not significantly alter the activity of VTA DA neurons (Fig. 2E).

The administration of BAY (10–80  $\mu$ g/kg, i.v.; cumulative doses) to cortically transected rats did not elevate the firing rate or burst firing. Two-way ANOVA revealed a significant effect of treatment ( $p < 0.0001$ ) and group ( $p < 0.02$ ) on the firing rate compared with control rats ( $n = 7$  and  $11$ , respectively). The effect of BAY on DA burst firing in cortically transected rats was not statistically significant, whereas two-way ANOVA revealed a significant difference between controls and decorticated rats ( $p < 0.0001$ , treatment effect;  $p < 0.0001$ , treatment-by-group interaction) (Fig. 2).

In addition to the group used to assess the effect of BAY, other groups of decorticated rats were used to examine the effect of ziprasidone and haloperidol (see below). We assessed the effect of cortical transection on the spontaneous activity of VTA DA neurons using the data from all control and decorticated rats. When comparing naive and sham-operated rats, we found no difference in the overall firing rate (naive:  $2.9 \pm 0.3$  spikes/s,  $n = 20$ ; sham:  $3.3 \pm 0.4$  spikes/s,  $n = 10$ ) or in burst firing (naive:  $13.0 \pm 3.7\%$ ,  $n = 20$ ; sham:  $16.3 \pm 3.0\%$ ,  $n = 10$ ), and therefore the data were pooled and used together as a control group. Cortical transection had no significant effect on the overall firing rate ( $3.0 \pm 0.2$  spikes/s in controls,  $n = 30$ ;  $2.6 \pm 0.4$  spikes/s in decorticated rats,  $n = 20$ ). However, spontaneous burst firing was significantly reduced by cortical transection ( $14.1 \pm 2.7\%$  in controls,  $n = 30$ ;  $3.0 \pm 1.2\%$  in decorticated rats,  $n = 20$ ;  $p < 0.002$  Student's *t* test).

### Effect of BAY on extracellular DA concentration in the mesocortical pathway

We used *in vivo* microdialysis to examine the effect of local and systemic drug administration on the DA release in the mPFC and

VTA of rats. Baseline DA values in dialysates obtained in various experimental conditions are shown in Table 2.

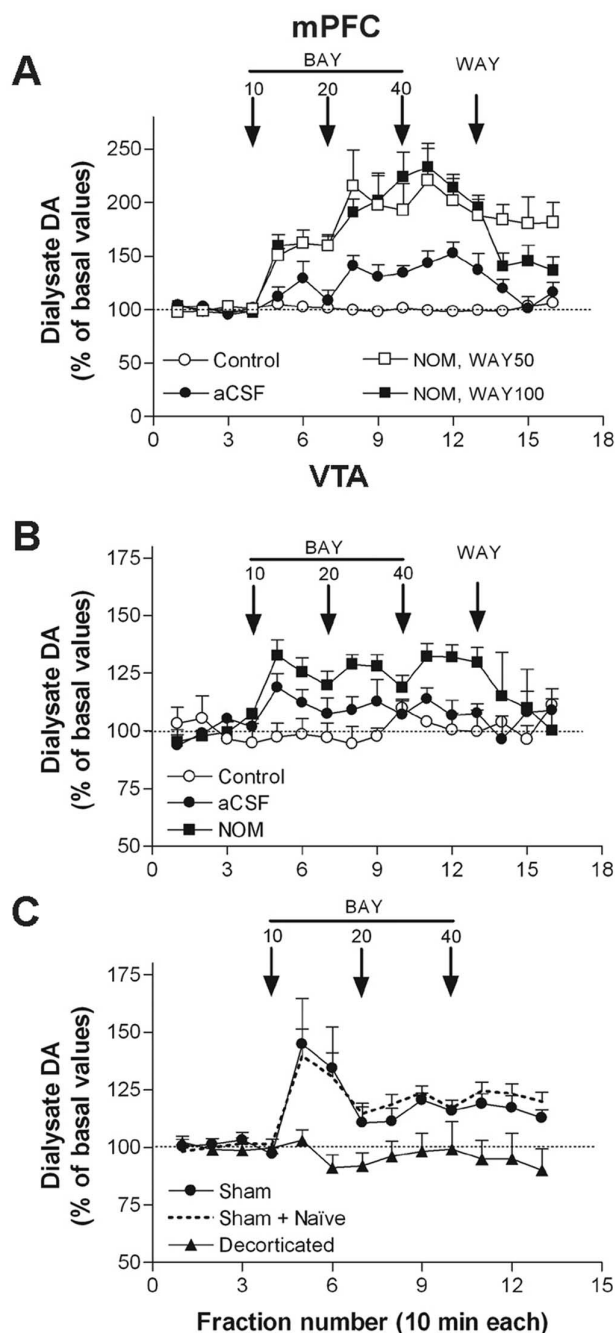
We examined the effect of BAY on DA release in the mesocortical pathway. In the first series of experiments, to mimic the conditions of electrophysiological recordings, rats were anesthetized with chloral hydrate, and the drug was administered intravenously through the femoral vein. Rats were implanted with two dialysis probes in the mPFC and VTA. In the first experiment, the administration of BAY (10–40  $\mu$ g/kg, i.v.) increased extracellular DA to  $153 \pm 11\%$  of baseline in the mPFC and to  $118 \pm 6\%$  in the VTA ( $p < 0.001$ , time effect for mPFC;  $p < 0.04$ , time effect for VTA; repeated-measures ANOVA;  $n = 7$  in each area) (Fig. 3). Given that 1) BAY increased burst firing of DA neurons and 2) changes in phasic DA release are strongly dependent on reuptake blockade (Floresco et al., 2003), we repeated these experiments in the presence of nomifensine (10  $\mu$ M) in the aCSF used to perfuse the dialysis probes.

The addition of nomifensine to the aCSF increased the baseline DA values 4-fold in the mPFC and 2.5-fold in the VTA (Table 2). In these conditions, the administration of BAY elevated extracellular DA to  $228 \pm 16\%$  in the mPFC and to  $132 \pm 6\%$  in the VTA ( $p < 0.0001$  for both regions; time effect; repeated-measures ANOVA;  $n = 8$  for mPFC;  $n = 5$  for VTA; VTA samples from three rats were lost during HPLC analysis). When considering the absolute DA values, the maximal DA elevation produced by BAY in the mPFC was  $7.9 \pm 1.4$  fmol/fraction in the standard dialysis fluid and  $48.0 \pm 2.9$  fmol/fraction in the dialysis fluid containing 10  $\mu$ M nomifensine. Two-way ANOVA revealed a significant effect of nomifensine on the elevation of DA release induced by BAY ( $p < 0.0001$ ; significant effect of time and of time-by-group interaction) (Fig. 3). WAY-100635 injected intravenously at the dose of 50  $\mu$ g/kg was unable to reverse the elevation of extracellular DA induced by BAY ( $n = 3$ ). Therefore, a higher dose was used in subsequent experiments. In a group of five rats, WAY-100635 (100  $\mu$ g/kg, i.v.) significantly reversed the effect of BAY in the mPFC ( $p < 0.0001$ ; time effect; repeated-measures ANOVA), although it did not reach statistical significance in the VTA, likely because of the small effect size of BAY in this region and the limited number of rats used in the VTA ( $n = 3$ ).

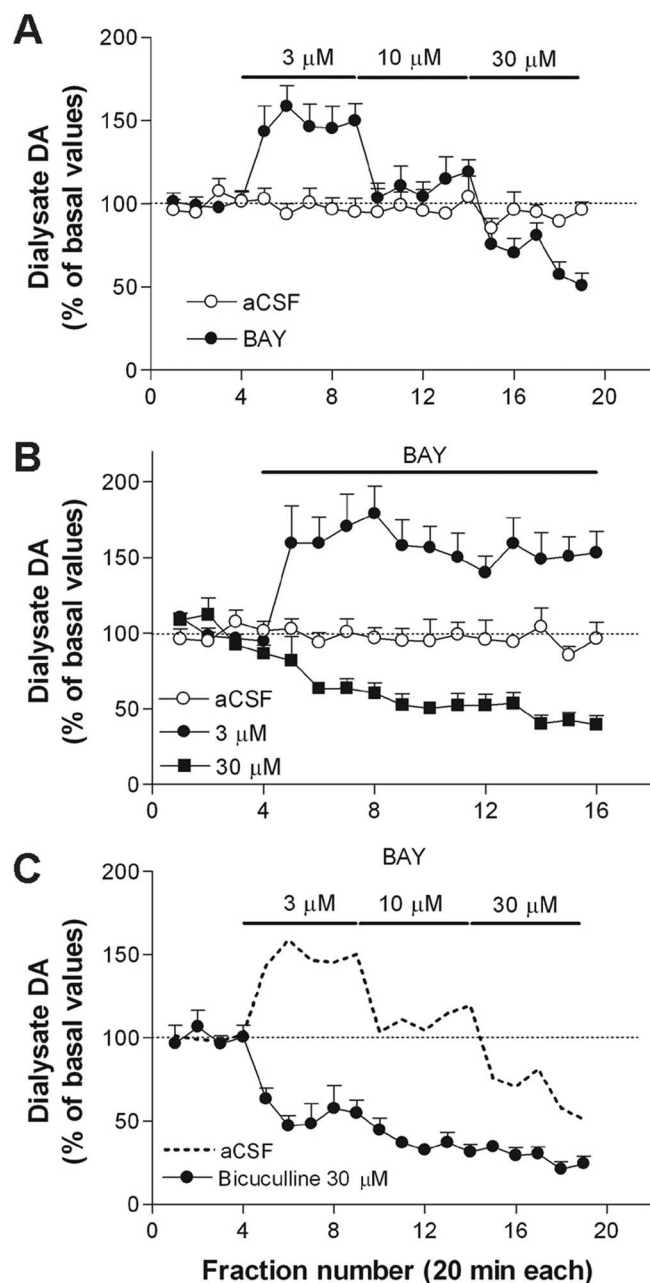
In additional experiments, we examined the effect of the cortical transection on the BAY-induced elevation of the extracellular DA concentration in the VTA of anesthetized rats. The intravenous administration of BAY (10, 20, and 40  $\mu$ g/kg, i.v.) elevated DA concentration to a maximal value of  $144 \pm 20\%$  of baseline in sham-operated rats ( $p < 0.001$ ; time effect; repeated-measures ANOVA). This effect was not statistically different from that seen in naive rats ( $n = 5$ ;  $p < 0.0001$ , time effect; nonsignificant effect of group or time-by-group interaction) (Fig. 3B,C). When considering all rats together, BAY elevated the DA concentration to a maximal value of  $139 \pm 12\%$  of baseline ( $n = 12$ ). This effect was totally abolished in rats subjected to cortical transection. Two-way ANOVA revealed a significant difference between the effect of BAY on cortically transected rats ( $n = 6$ ) compared with controls (sham and naive;  $n = 12$ ;  $p < 0.02$ , time effect;  $p < 0.001$ , group effect;  $p < 0.001$ , time-by-group interaction) (Fig. 3C).

#### Local effect of BAY on extracellular DA concentration in rat and mouse mPFC

Subsequent experiments in rats and mice were conducted in freely moving animals. The local application of BAY in rat mPFC at a low concentration (3  $\mu$ M; five fractions) significantly increased the extracellular DA concentration to  $158 \pm 12\%$  of base-



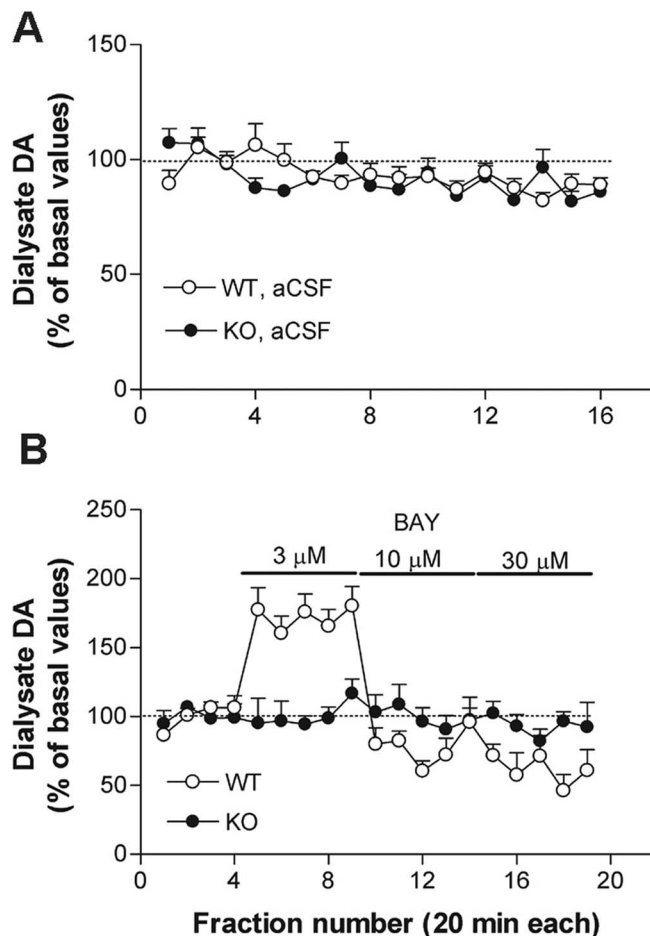
**Figure 3.** Effect of the administration of BAY (10–40  $\mu$ g/kg, i.v.) on the DA concentration in dialysates from the mPFC (**A**) and VTA (**B, C**) of anesthetized rats. **A**, The administration of BAY moderately elevated DA concentration in rats whose probes were perfused with normal aCSF ( $n = 7$ ; ●), an effect reversed by WAY-100635 (50  $\mu$ g/kg, i.v.). The DA elevation produced by BAY was more marked when probes were perfused with an aCSF containing 10  $\mu$ M nomifensine ( $n = 8$ ). Two groups of rats of three and five animals, respectively, were injected with BAY (□ and ■, respectively). BAY elevated DA similarly in both groups. The administration of intravenous 100  $\mu$ g/kg WAY-100635 (■;  $n = 5$ ) reversed the effect of BAY, whereas a lower dose (50  $\mu$ g/kg, i.v.; □;  $n = 3$ ) did not. **B**, Similarly, BAY elevated DA concentration in dialysates from the VTA when microdialysis probes were perfused with 10  $\mu$ M nomifensine (■;  $n = 5$ ). BAY administration induced only a very moderate but statistically significant elevation of DA when probes were perfused with an aCSF devoid of nomifensine (●;  $n = 7$ ). Control rats ( $n = 3$  in mPFC;  $n = 4$  in VTA) were given injections of saline (VTA probes of controls were perfused with normal aCSF). **C**, The intravenous administration of BAY (10, 20, and 40  $\mu$ g/kg) elevated the extracellular DA concentration in the VTA of sham-operated rats (●;  $n = 7$ ; aCSF containing 10  $\mu$ M nomifensine) as it did in naive rats (**B**). The dotted line shows the effect in naive and sham rats ( $n = 12$ ). This effect was totally abolished by cortical transection (▲;  $n = 6$ ). See Results for statistical analysis. WAY, WAY-100635; NOM, nomifensine. Data are expressed as means  $\pm$  SEM.



**Figure 4.** *A*, Effect of the local application of BAY by reverse dialysis in rat mPFC at 3, 10, and 30  $\mu$ M (100 min each) on the local extracellular DA concentration (●;  $n = 5$ ). The application of aCSF did not alter DA concentration (○;  $n = 5$ ). *B*, The perfusion of 3 and 30  $\mu$ M BAY for the entire experimental period also increased and decreased, respectively, dialysate DA concentration in different groups of rats ( $n = 6$  and 5, respectively). *C*, The local application of BAY (3, 10, and 30  $\mu$ M) using an aCSF containing bicuculline significantly reduced extracellular DA at all concentrations (●;  $n = 4$ ). The dotted line shows the effect of BAY at the same concentrations using standard aCSF, as shown in *A*. Note that the absolute values of DA in the presence of bicuculline were higher than with a normal aCSF (see Table 2). See Results for statistical analysis. Data are expressed as means  $\pm$  SEM.

line ( $p < 0.0001$ ; time effect; repeated-measures ANOVA;  $n = 5$ ). However, increasing the dose to 10  $\mu$ M abolished this effect (103–114% of baseline), and the perfusion of 30  $\mu$ M BAY (five fractions at each concentration) significantly reduced DA release to  $51 \pm 7\%$  of baseline ( $p < 0.0001$ ; time effect; repeated-measures ANOVA;  $n = 5$ ) (Fig. 4*A*).

Likewise, the perfusion of 3 and 30  $\mu$ M in the mPFC for the entire experimental period resulted in a sustained increase (max-

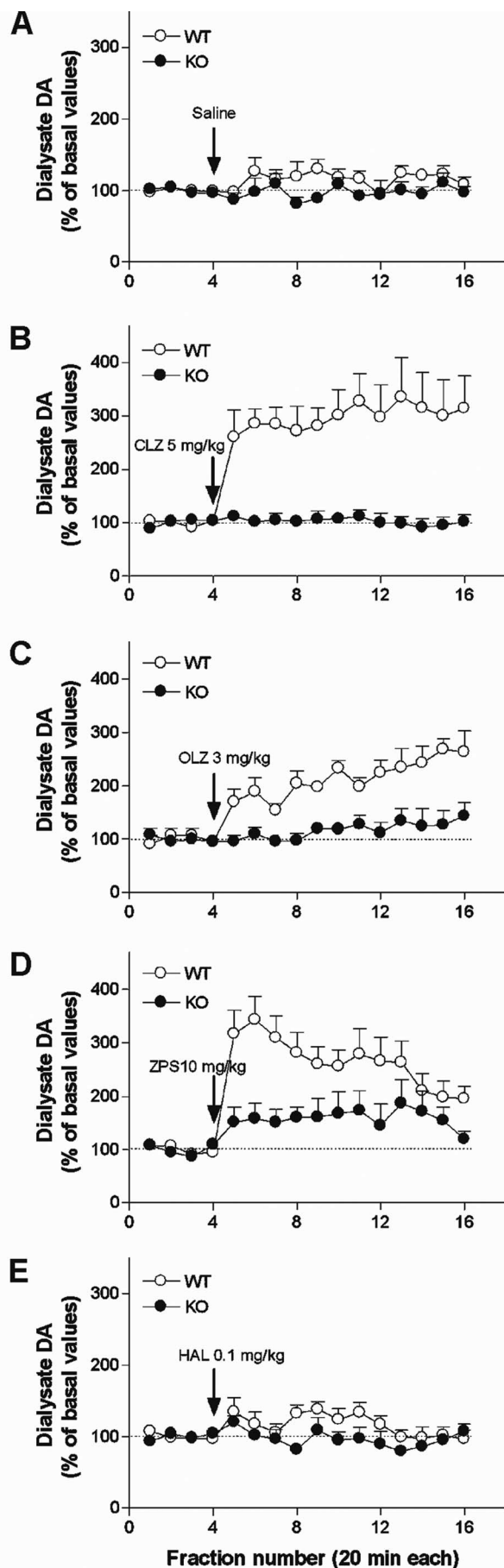


**Figure 5.** *A*, The perfusion of aCSF in the mPFC did not alter the extracellular DA concentration in the mPFC of WT (○;  $n = 6$ ) and 5-HT<sub>1A</sub> KO mice (●;  $n = 6$ ). *B*, Effect of the local application of BAY at 3, 10, and 30  $\mu$ M in the mPFC on the local DA release in WT (○;  $n = 4$ ) and 5-HT<sub>1A</sub> KO mice (●;  $n = 4$ ). As observed in rat mPFC, BAY exerted a biphasic effect on DA release, with an increase at 3  $\mu$ M and a reduction at 30  $\mu$ M. In mice, the 10  $\mu$ M concentration appeared to also reduce extracellular DA. Both effects were absent in 5-HT<sub>1A</sub> KO mice. See Results for statistical details. Data are expressed as means  $\pm$  SEM.

imal effect,  $178 \pm 18\%$ ) and decrease (maximal effect,  $39 \pm 6\%$ ), respectively, in the DA release in the mPFC ( $p < 0.0005$  for 3  $\mu$ M;  $p < 0.0001$  for 30  $\mu$ M; repeated-measures ANOVA;  $n = 6$  and 5, respectively). The perfusion of aCSF for the entire experimental period did not alter dialysate DA concentrations ( $n = 5$ ) (Fig. 4*B*). In contrast to DA, the local perfusion of 3  $\mu$ M BAY induced a sustained reduction in 5-HT release in the mPFC (maximal reduction to  $57 \pm 4\%$  of baseline;  $p < 0.001$ ; repeated-measures ANOVA;  $n = 5$ ; data not shown) as reported previously in the range 1–100  $\mu$ M (Casanovas et al., 1999).

The biphasic concentration–response curve suggested the involvement of different populations of 5-HT<sub>1A</sub> receptors at lower and higher concentrations of BAY that would control the mesocortical DA release in an opposite condition. Because 5-HT<sub>1A</sub> receptors have been reported to be expressed by GABAergic interneurons in the mPFC (Santana et al., 2004), we examined the effect of BAY in rat mPFC during the coproduction of bicuculline (30  $\mu$ M) to block local GABA<sub>A</sub>-mediated inputs onto pyramidal neurons. The perfusion of 30  $\mu$ M bicuculline resulted in a stable increase in DA release during the entire experimental period (Table 2). In rats whose probes were perfused with aCSF containing bicuculline, BAY significantly reduced DA release at all concen-





trations, reaching  $21 \pm 4\%$  of baseline at  $30 \mu\text{M}$  ( $p < 0.0001$ ; repeated-measures ANOVA) (Fig. 4C).

The perfusion of 3, 10, and  $30 \mu\text{M}$  BAY in the mPFC of WT mice affected DA release similarly to rats. At the lower concentration, BAY elevated extracellular DA to  $176 \pm 12\%$  of baseline, whereas at  $30 \mu\text{M}$ , it reduced DA release to a maximal effect of  $46 \pm 11\%$  of baseline ( $p < 0.0001$ ; repeated-measures ANOVA;  $n = 4$ ). Unlike in rats, the perfusion of  $10 \mu\text{M}$  appeared to slightly reduce DA release. Neither of these effects was observed when BAY was perfused in the mPFC of 5-HT<sub>1A</sub> KO mice, indicating that the effects of the lower and higher concentrations of BAY were attributable to the activation of 5-HT<sub>1A</sub> receptors in the mPFC (Fig. 5).

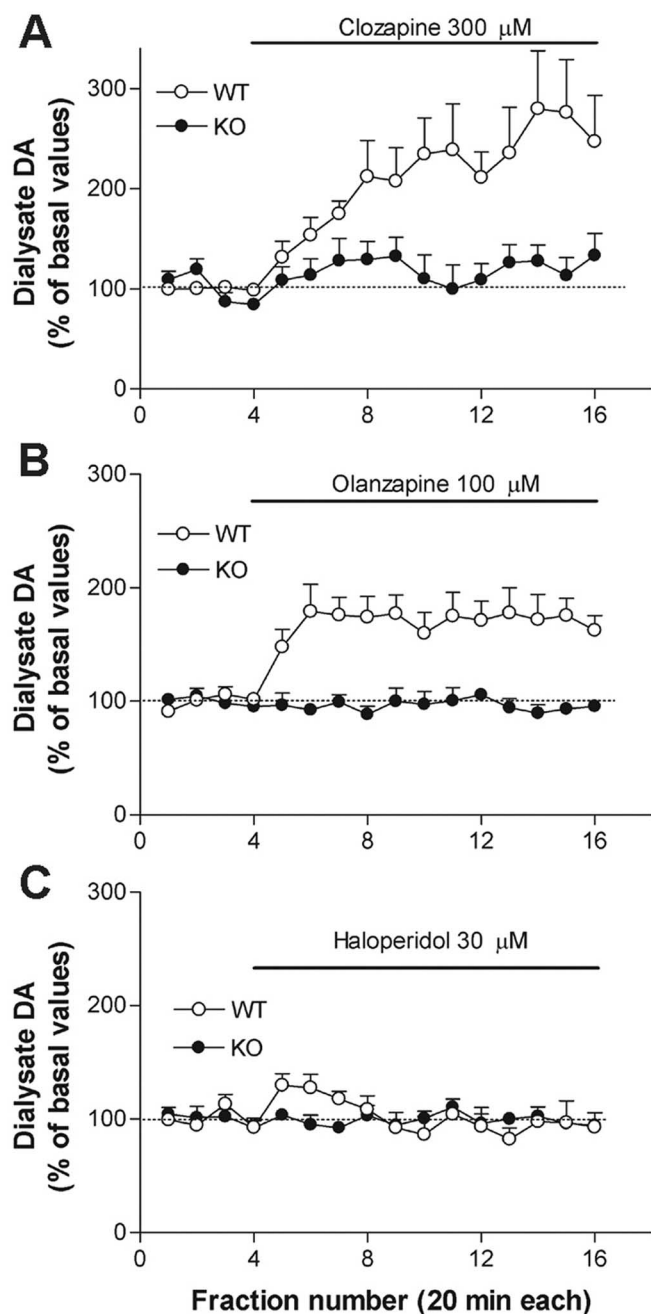
The local perfusion of WAY-100635 (3, 10, and  $30 \mu\text{M}$ ; four fractions each) produced a moderate reduction in the extracellular DA concentration at the higher dose ( $96 \pm 7$ ,  $100 \pm 8$ , and  $75 \pm 8\%$  at 3, 10, and  $30 \mu\text{M}$ ;  $n = 8$ ;  $p < 0.02$ ; repeated-measures ANOVA). However, as observed previously (Ichikawa et al., 2001a), the subcutaneous administration of WAY-100635 (0.3 mg/kg; a dose that fully blocks 5-HT<sub>1A</sub> receptors) (Forster et al., 1995) did not alter extracellular DA ( $105 \pm 2\%$  of baseline;  $n = 4$ ; data not shown).

#### Effects of antipsychotics on extracellular DA in the mPFC of WT and KO mice

The intraperitoneal administration of saline did not alter the extracellular DA concentration in the mPFC of WT and 5-HT<sub>1A</sub> KO mice (Fig. 6). Likewise, haloperidol administration (0.1 mg/kg, i.p.) failed to alter the extracellular DA concentration in the mPFC of WT ( $n = 9$ ) and 5-HT<sub>1A</sub> KO ( $n = 7$ ) mice. However, the intraperitoneal administration of clozapine (5 mg/kg;  $n = 5$  for WT and KO mice), olanzapine (3 mg/kg;  $n = 5$  for WT;  $n = 6$  for KO), and ziprasidone (10 mg/kg;  $n = 6$  for WT and KO mice) increased extracellular DA significantly more in the mPFC of WT than of 5-HT<sub>1A</sub> KO mice. Actually, these doses of clozapine and olanzapine did not significantly elevate extracellular DA in KO mice, whereas ziprasidone moderately increased the DA concentration in KO mice, but this effect was markedly lower than that observed in WT mice. Two-way ANOVA revealed a significant effect of the genotype on clozapine ( $p < 0.00001$ , time effect;  $p < 0.002$ , group effect;  $p < 0.00001$ , time-by-group interaction), olanzapine ( $p < 0.0001$ , time effect;  $p < 0.00001$ , group effect;  $p < 0.00001$ , time-by-group interaction), and ziprasidone ( $p < 0.00001$ , time effect;  $p < 0.03$ , group effect;  $p < 0.00001$ , time-by-group interaction) (Fig. 6). A lower dose of olanzapine (1 mg/kg) increased extracellular DA to  $138 \pm 12\%$  in WT mice ( $n = 7$ ) but not in KO mice (maximal effect,  $106 \pm 4\%$ ;  $n = 6$ ; data not shown).

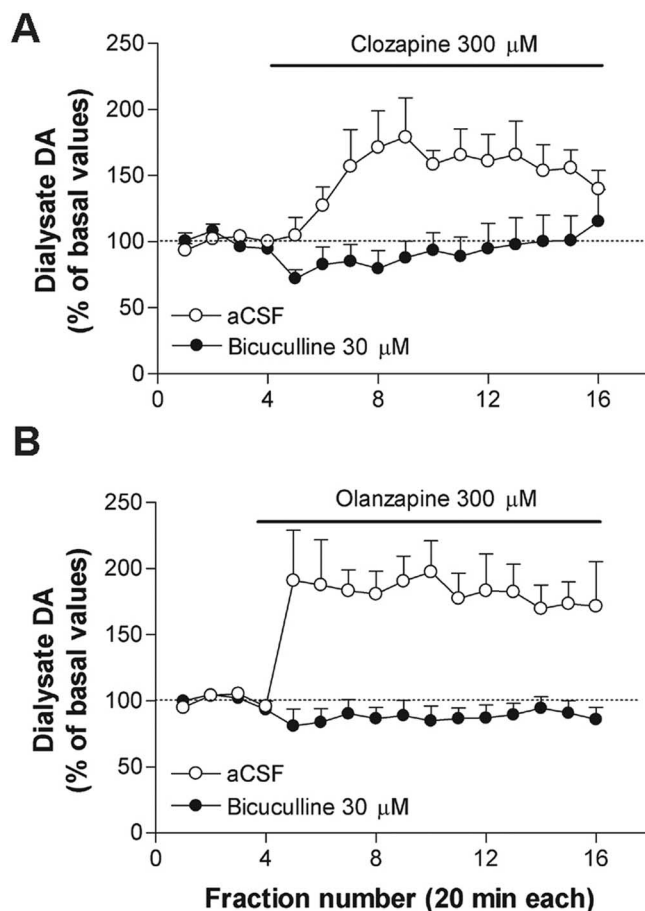
Likewise, the local application of clozapine ( $300 \mu\text{M}$ ) and olanzapine ( $100 \mu\text{M}$ ) in the mPFC of WT mice steadily increased in the local DA release (Fig. 7). Clozapine perfusion increased DA release to  $280 \pm 57\%$  and olanzapine to  $180 \pm 24\%$  of baseline ( $p < 0.0001$ ; time effect for both drugs; repeated-measures ANOVA;  $n = 6$  and 5, respectively). The perfusion of haloperidol ( $30 \mu\text{M}$ ) elevated DA release to  $129 \pm 10\%$  in the first fraction, but

**Figure 6.** Effects of the intraperitoneal administration of saline (A), clozapine (CLZ; 5 mg/kg; B), olanzapine (OLZ; 3 mg/kg; C), ziprasidone (ZPS; 10 mg/kg; D), and haloperidol (HAL; 0.1 mg/kg; E) on the extracellular DA concentration in the mPFC of WT (○) and 5-HT<sub>1A</sub> KO (●) mice. Data correspond to the following number of mice per group: saline (7 WT, 7 KO), clozapine (5 WT, 5 KO), olanzapine (5 WT, 6 KO), ziprasidone (6 WT, 6 KO), and haloperidol (9 WT, 7 KO). See Results for statistical analysis. Data are expressed as means  $\pm$  SEM.



**Figure 7.** The local application of clozapine (300  $\mu$ M;  $n = 6$ ; **A**) and olanzapine (100  $\mu$ M;  $n = 5$ ; **B**) in the mPFC of WT mice ( $\circ$ ) increased the local DA release for the entire application period. The application of haloperidol (30  $\mu$ M;  $n = 5$ ; **C**) induced a moderate and transient increase in WT mice ( $\circ$ ) shortly after the beginning of the application. However, none of these effects was observed when clozapine, olanzapine, or haloperidol was applied in the mPFC of 5-HT<sub>1A</sub> KO mice ( $n = 5$  for clozapine or olanzapine;  $n = 4$  for haloperidol). See Results for statistical analysis. Data are expressed as means  $\pm$  SEM.

the effect faded rapidly ( $p = 0.09$ ; repeated-measures ANOVA;  $n = 5$ ) (Fig. 9). The elevations in prefrontal DA release produced by clozapine or olanzapine were abolished in 5-HT<sub>1A</sub> KO mice ( $n = 5$  for each drug) (Fig. 7). Two-way ANOVA revealed a significant effect of the genotype on the effect of clozapine ( $p < 0.003$ , group effect;  $p < 0.0001$ , time effect;  $p < 0.0001$ , time-by-group interaction) and olanzapine ( $p < 0.0002$ , group effect;  $p < 0.0001$ , time effect;  $p < 0.0001$ , time-by-group interaction). In contrast, the effect of haloperidol did not differ between WT and KO mice (Fig. 7). We



**Figure 8.** **A**, As observed in WT mice, the local application of clozapine (300  $\mu$ M) in rat mPFC increased the local DA extracellular concentration when perfused with a standard aCSF ( $\circ$ ;  $n = 5$ ). However, this effect was attenuated when clozapine was perfused in the presence of 30  $\mu$ M bicuculline ( $\bullet$ ;  $n = 9$ ). **B**, Similarly, olanzapine application (300  $\mu$ M) in rat mPFC increased local DA release in rats perfused with standard dialysis fluid ( $\circ$ ;  $n = 5$ ). However, this effect completely disappeared in the presence of 30  $\mu$ M bicuculline ( $\bullet$ ;  $n = 7$ ). See Results for statistical details. Data are expressed as means  $\pm$  SEM.

could not test the local effects of ziprasidone on extracellular DA because the vehicle used (a cyclodextrin) resulted in the clogging of the microdialysis membranes.

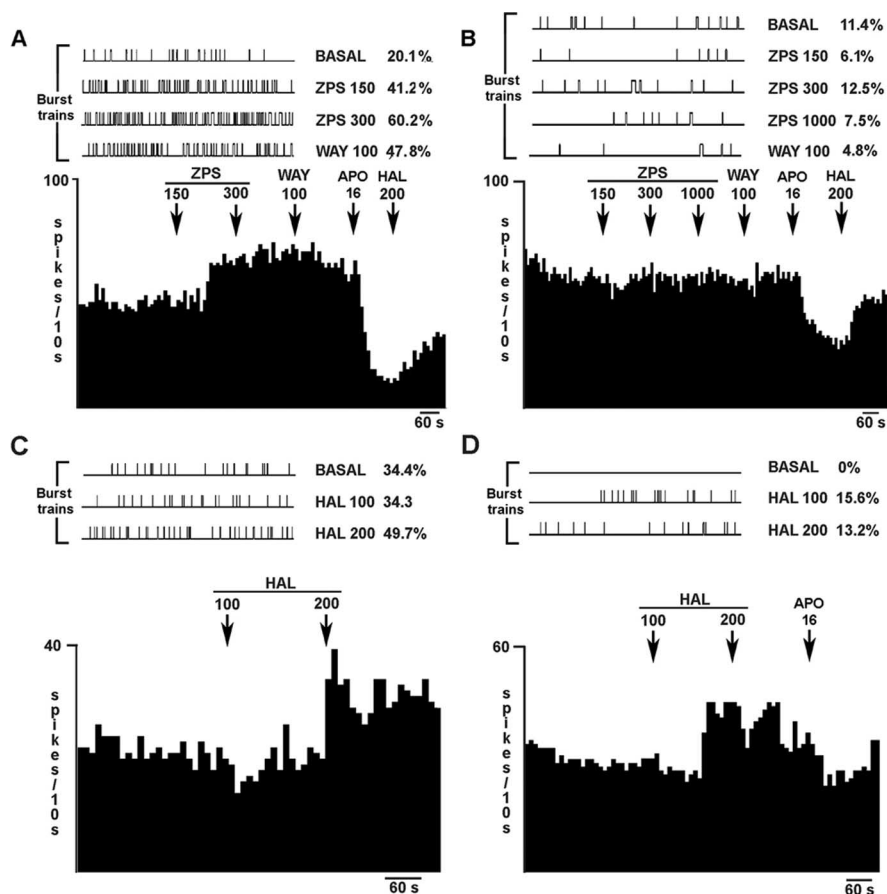
#### Effects of atypical antipsychotics on extracellular DA in rat mPFC

The local application of clozapine (300  $\mu$ M) in rat mPFC increased the local extracellular DA concentration (maximal effect,  $179 \pm 29\%$  of baseline;  $p < 0.0001$ , time effect; one-way, repeated-measures ANOVA;  $n = 5$ ). Likewise, the local application of olanzapine (300  $\mu$ M) elevated extracellular DA to  $197 \pm 23\%$  of baseline ( $p < 0.0001$ , time effect; one-way, repeated-measures ANOVA;  $n = 5$ ). The effect of clozapine was abolished in the presence of bicuculline ( $n = 9$ ;  $p < 0.03$ , group effect;  $p < 0.0001$ , time effect;  $p < 0.0001$ , time-by-group interaction; two-way, repeated-measures ANOVA). Likewise, the coproduction of bicuculline totally suppressed the elevation of extracellular DA produced by olanzapine ( $n = 7$ ;  $p < 0.0002$ , group effect;  $p < 0.0001$ , time effect;  $p < 0.0001$ , time-by-group interaction) (Fig. 8).

#### Effect of ziprasidone and haloperidol on VTA DA cell activity in the rat: dependence on cortical integrity

The above results suggested that the increase in the activity of mesocortical DA neurons by atypical antipsychotics involved the





**Figure 9.** Effect of the intravenous administration of ziprasidone (ZPS; **A, B**) and haloperidol (HAL; **C, D**) in sham-operated rats (**A, C**) and in rats subjected to cortical transection (**B, D**). Arrows show the administration of drugs. The integrated firing-rate histograms (abscissa, spikes per 10 s; ordinate, time in minutes) are shown. The top traces in each panel show representative burst trains corresponding to 1 min recordings obtained in baseline conditions and after drug administration, as in Figure 2. The percentage of spikes fired in bursts is also shown. Note the increase in VTA DA neuron activity in control rats and the lack of effect of ziprasidone (but not haloperidol) in cortically transected rats. See Table 3 for statistical analysis. ZPS, Ziprasidone; HAL, haloperidol; WAY, WAY-100635; APO, apomorphine.

**Table 3. Differential effect of the intravenous administration of ziprasidone (ZPS) and haloperidol (HAL) in control rats and in rats subjected to cortical transection**

Drug	Controls		Lesioned	
	Basal	Postdrug	Basal	Postdrug
<b>ZPS (0.15–0.30 mg/kg)</b>				
Firing rate (spikes/s)	3.4 ± 0.5	5.1 ± 0.6*	3.3 ± 1.1	3.3 ± 1.1
% of spikes fired in bursts	14.6 ± 4.6	40.8 ± 11.6*	2.3 ± 1.3 <sup>#</sup>	3.0 ± 2.0
n	6	6	6	6
<b>HAL (0.1–0.2 mg/kg)</b>				
Firing rate (spikes/s)	3.0 ± 0.4	4.4 ± 0.4*	2.8 ± 0.8	3.8 ± 0.6*
% of spikes fired in bursts	22.4 ± 8.0	40.9 ± 10.1*	4.7 ± 3.0 <sup>#</sup>	17.2 ± 8.0*
n	8	8	7	7

\* $p < 0.05$ , different from basal values (paired Student's *t* test); <sup>#</sup> $p < 0.05$ , different from control rats (*t* test).

activation of 5-HT<sub>1A</sub> receptors in the prefrontal cortex (see below for extended discussion). As a first test of this hypothesis, we examined the effect of the intravenous administration of haloperidol and ziprasidone on the activity of VTA DA cells in control rats and in rats subjected to cortical transection.

The effect of the administration of haloperidol (0.1–0.2 mg/kg, i.v.) on the firing rate and burst firing was comparable in control rats and in rats subjected to cortical transection ( $p < 0.005$ , effect of the treatment on firing rate, nonsignificant effects of group, and group-by-dose interaction;  $p < 0.005$ , effect of the

treatment on burst firing, nonsignificant effects of group, and group-by-dose interaction) (Fig. 9, Table 3). These results indicate that 1) VTA DA neurons are able to discharge in bursts in absence of cortical inputs and 2) the effect of haloperidol did not depend on such cortical inputs.

Ziprasidone (0.15–0.30 mg/kg, i.v.) also increased the overall firing rate and burst firing in control rats, but, unlike haloperidol, this ability was lost in decorticated rats (Fig. 9, Table 3). Two-way ANOVA revealed a significant difference between controls and decorticated rats ( $p < 0.002$ , treatment effect on firing rate;  $p < 0.002$ , treatment-by-group interaction;  $p < 0.006$ , treatment effect on burst firing;  $p < 0.02$ , effect of group;  $p < 0.007$ , group-by-dose interaction).

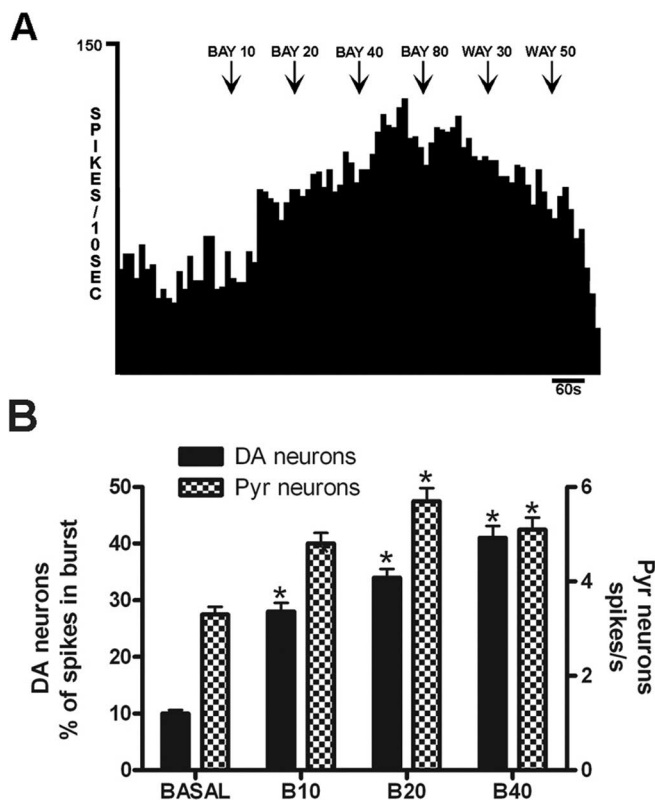
## Discussion

The present results suggest that the activity of DA neurons in the VTA and the mesocortical DA release are modulated by 5-HT<sub>1A</sub> receptors in the mPFC. The increase in mPFC DA release produced by the atypical antipsychotics clozapine, olanzapine, and ziprasidone, but not haloperidol, seems to involve 5-HT<sub>1A</sub> receptor activation. The action of a low BAY concentration and that of atypical antipsychotics seems to involve GABAergic interneurons, as judged from the bicuculline reversal.

### Modulation of DA neuron activity by 5-HT<sub>1A</sub> receptors

The mPFC projects to the VTA as assessed by electrophysiological and tracing methods (Thierry et al., 1979; Carr and Sesack, 2000a,b), and mPFC stimulation induced burst firing in VTA DA neurons (Gariano and Groves, 1988; Tong et al., 1996). Likewise, chemical and electrical stimulation of the mPFC enhanced DA neuron activity and DA release in the VTA (Murase et al., 1993; Bortolozzi et al., 2005). The reduction in spontaneous bursting activity of DA neurons by cortical transection is consistent with these findings and suggests a direct or indirect excitatory influence of mPFC on VTA DA neurons (Fig. 1).

Previous reports show a complex influence of 5-HT on midbrain DA pathways, mainly involving 5-HT<sub>1A</sub> (Arborelius et al., 1993a,b; Prisco et al., 1994; Ichikawa et al., 1995; Kuroki et al., 1996; Lejeune and Millan, 1998; Rollema et al., 2000; Sakaue et al., 2000) and 5-HT<sub>2A/2C</sub> (Ichikawa et al., 2001b; Lucas et al., 2001; Porras et al., 2002) receptors. Here, we show that 5-HT<sub>1A</sub> receptors in the mPFC are deeply involved in the modulation of dopaminergic activity. An additional role of raphe 5-HT<sub>1A</sub> autoreceptors was suggested, yet it appears controversial (Prisco et al., 1994; Sakaue et al., 2000). Our results clearly support the involvement of prefrontal



**Figure 10.** Effect of the systemic administration of BAY on the firing of pyramidal neurons in the mPFC projecting to the VTA (as assessed by antidromic stimulation) and on VTA DA neurons. **A**, Effect of the administration of BAY on the firing rate of a pyramidal neuron in the mPFC. **B**, Bar diagram showing the increase in the firing rate of pyramidal neurons ( $n = 19$ ) and the percentage of burst firing for DA neurons ( $n = 11$ ). Note the comparable effect of the various doses of BAY  $\times$  3702 on the activity of both neuronal groups. Data from pyramidal neurons were taken from the study by Díaz-Mataix et al. (2005). \* $p < 0.05$  versus the respective baseline ( $t$  test post-ANOVA). WAY, WAY-100635; Pyr, pyramidal neuron. Data are expressed as means  $\pm$  SEM.

(postsynaptic) 5-HT<sub>1A</sub> receptors, because BAY did not alter DA cell activity or DA release in the VTA in cortically transected rats. Indeed, 5-HT<sub>1A</sub> receptors are densely expressed in mPFC areas that project to the VTA (Thierry et al., 1979; Amargós-Bosch et al., 2004), which provides an anatomical substrate for the present observations. Moreover, BAY increased DA cell firing at doses higher than those suppressing 5-HT cell firing (Casanovas et al., 2000) in accordance with the lower sensitivity of postsynaptic versus presynaptic 5-HT<sub>1A</sub> receptors (Sprouse and Aghajanian, 1987).

5-HT<sub>1A</sub> receptor activation in the mPFC induced by local application of agonists or raphe stimulation results in cellular hyperpolarization and reduction in neuronal activity (Araneda and Andrade, 1991; Ashby et al., 1994; Puig et al., 2005). However, the systemic administration of 5-HT<sub>1A</sub> agonists, such as 8-OH-DPAT (Borsini et al., 1995) or BAY (Díaz-Mataix et al., 2005), increased the activity of prefrontal neurons. The latter study was conducted in pyramidal neurons activated antidromically from the VTA, which supports the notion that BAY enhances cortical excitatory inputs into DA neurons (Fig. 10). The reasons for such an increase in pyramidal cell activity after the systemic (but not local) administration of 5-HT<sub>1A</sub> agonists are unclear and may involve the activation of 5-HT<sub>1A</sub> receptors in local inhibitory neurons (Santana et al., 2004) or in areas (e.g., hippocampus) projecting to mPFC GABAergic neurons (Tierney et al., 2004).

Irrespective of the mechanism(s) involved, BAY increased

the activity of pyramidal neurons in mPFC and DA neurons in the VTA alike. This parallelism, together with the dramatic effect of cortical transection on the effects of BAY, supports the involvement of mPFC 5-HT<sub>1A</sub> receptors. It is yet unknown whether the increase in DA cell activity is mediated by direct mPFC→VTA afferents (Thierry et al., 1979; Carr and Sesack, 2000a) or whether a more complex circuitry is involved (Fig. 1). In particular, the inputs from the PPTg/LDT and VP have been shown to modulate, respectively, phasic and tonic inputs onto VTA DA neurons (Floresco et al., 2003). The increase in burst firing produced by BAY and the sensitivity of extracellular DA to nomifensine (see below) are consistent with an increase in phasic inputs. The inability of WAY-100635 to modulate baseline DA cell activity also agrees with the absence of tonic inputs involving 5-HT<sub>1A</sub> receptors.

#### Modulation of DA release by 5-HT<sub>1A</sub> receptors in the mPFC

BAY also increased DA release in the VTA and mPFC in the experimental conditions used for DA cell recordings (intravenous administration to anesthetized rats). The simultaneous increase in burst firing and DA release is consistent with previous reports (Chergui et al., 1994). Moreover, BAY increased extracellular DA more markedly in the presence of nomifensine. In agreement with a recent report (Floresco et al., 2003), this further suggests that BAY increases phasic inputs onto DA neurons. The effect of nomifensine seen in the mPFC was smaller than in the nucleus accumbens (Floresco et al., 2003), perhaps because of the lower density of DA fibers and DA transporter in the mPFC (Sesack et al., 1998) and/or the distinct stimuli used in both studies. Moreover, the smaller effect of nomifensine in the VTA possibly reflects differences between terminal and somatodendritic DA release.

BAY application in the mPFC affected local DA release in a bell-shaped manner, suggesting the involvement of more than one receptor population. 8-OH-DPAT also increased DA release in the mPFC when perfused at 10  $\mu$ M, but no additional doses were used (Sakaue et al., 2000). The use of 5-HT<sub>1A</sub> receptor KO mice allowed us to clearly establish that both the stimulatory and inhibitory effects of BAY were 5-HT<sub>1A</sub> receptor mediated. Prefrontal 5-HT<sub>1A</sub> receptors are involved in the long-loop modulation of the 5-HT system via descending afferents to the raphe nuclei (Ceci et al., 1994; Hajós et al., 1999; Celada et al., 2001). The fact that the 5-HT release in the mPFC is reduced by BAY at 1–100  $\mu$ M (Casanovas et al., 1999; this study) suggests a differential regulation of 5-HT and DA neurons by mPFC 5-HT<sub>1A</sub> receptors.

5-HT<sub>1A</sub> receptors are localized to cell bodies and/or axon hillocks of pyramidal neurons (Azmitia et al., 1996; Kia et al., 1996; Riad et al., 2000; De Felipe et al., 2001; Czyrak et al., 2003), excluding the possibility that changes in DA release are mediated by terminal receptors. The presence of 5-HT<sub>1A</sub> receptors in mPFC GABAergic interneurons (Santana et al., 2004), together with the reversal of the stimulatory effect of BAY by bicuculline, suggests that low BAY concentrations preferentially activate 5-HT<sub>1A</sub> receptors in GABA interneurons. This may eventually result in disinhibition of pyramidal neurons projecting to the VTA. A higher BAY concentration may overcome this effect, activating directly pyramidal 5-HT<sub>1A</sub> receptors and reducing the prefrontal excitatory output to DA neurons. Although such cellular difference in 5-HT<sub>1A</sub> receptor sensitivity remains to be established, previous reports are consistent with this possibility (Sprouse and Aghajanian, 1986, 1987; Beck et al., 1992), perhaps because of a different

expression level in different neuronal populations (Hoyer and Boddeke, 1993).

### Atypical antipsychotics and 5-HT<sub>1A</sub> receptors

Previous studies showed that the systemic administration of atypical antipsychotics (but not haloperidol) increased extracellular DA in the mPFC by a 5-HT<sub>1A</sub>-dependent mechanism (Rollema et al., 1997, 2000; Kuroki et al., 1999; Ichikawa et al., 2001a). Here, we show that this effect depends on the activation of 5-HT<sub>1A</sub> receptors in the mPFC. Interestingly, drugs displaying high (ziprasidone), very low (clozapine), or negligible (olanzapine) *in vitro* affinity for 5-HT<sub>1A</sub> receptors (Arnt and Skarsfeldt, 1998) share a common pattern of *in vivo* action to modulate prefrontal DA release. Although the effect of ziprasidone is likely attributable to the direct activation of 5-HT<sub>1A</sub> receptors, this is not the case for clozapine or olanzapine, although clozapine (6 mg/kg) displaced ~40% of the [<sup>11</sup>C]-WAY-100635 labeling in monkey brain (positron emission tomography scan) despite its ~700 nM *in vitro* affinity (Chou et al., 2003). In addition to a partial occupancy in the case of olanzapine, other mechanisms must be involved. Given the high coexpression of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the mPFC (~80%) (Amargós-Bosch et al., 2004), it might be thought that the concurrent blockade of 5-HT<sub>2A</sub> receptors could shift the physiological balance of 5-HT activation toward 5-HT<sub>1A</sub> receptors. However, this possibility seems unlikely because the selective 5-HT<sub>2A</sub> receptor antagonist M100907 did not increase DA release (Bortolozzi et al., 2005). Thus, the exact way in which clozapine and olanzapine interact with 5-HT<sub>1A</sub>-mediated neurotransmission remains to be determined. However, the effect of these drugs (and that of 3 μM BAY) was cancelled by bicuculline, which points toward 5-HT<sub>1A</sub> receptors in GABA interneurons. This action might eventually result in an increased excitatory cortical output to the VTA to enhance DA neuron activity, as observed previously with atypical antipsychotics (Gessa et al., 2000). The fact that the increase in DA neuron activity produced by ziprasidone (but not by haloperidol) was cancelled by cortical transection is consistent with this view. Hence, haloperidol (but not ziprasidone) can increase DA neuron activity in the absence of cortical inputs.

In summary, the present results show that 5-HT<sub>1A</sub> receptors in the mPFC are deeply involved in the modulation of DA neuron activity and of DA release in the PFC and VTA, an effect mediated via direct mPFC→VTA inputs or long loops. This adds to previously reported targets for antipsychotic drugs such as catecholamine autoreceptor blockade, which also modulate DA cell activity and DA release (Gessa et al., 2000). Altogether, these results may help to elucidate the mechanisms involved in the elevation of mesocortical DA release produced by atypical antipsychotics.

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