

## TREBALL 6

### ***In vivo* actions of aripiprazole on serotonergic and dopaminergic systems in rodent brain**

A. Bortolozzi\*, L. Díaz-Mataix\*, M. Toth, P. Celada, F. Artigas

En preparació (\*amb igual contribució)

En aquest treball estudiem l'efecte d'un nou antipsicòtic atípic: l'aripiprazol. Aquest fàrmac té unes característiques que el diferencien de la resta d'AAT: és agonista parcial dels receptors 5-HT<sub>1A</sub> i dels receptors de DA D<sub>2</sub>. L'administració sistèmica de l'aripiprazol incrementa l'alliberament de DA a l'EPFm mentre que disminueix l'alliberament de 5-HT a aquesta mateixa àrea. En quant a l'activitat de les neurones DA de l'ATV aquest fàrmac reverteix de forma parcial l'inhibició causada per l'apomorfina posant de manifest el caracter d'agonista parcial D<sub>2</sub>.



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***In vivo* actions of aripiprazole on serotonergic and  
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**Abbreviated title:** Aripiprazole actions on 5-HT and DA system

*Keywords:* antipsychotic, dopamine, prefrontal cortex, schizophrenia, serotonin receptors, ventral tegmental area

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## Abstract

The atypical antipsychotic drug aripiprazole (ARI) shows high *in vitro* affinity for 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and dopamine (DA) D2 receptors. In the present study we examined the *in vivo* actions of ARI in the rat and mouse brain using single unit recordings and microdialysis. The systemic administration of ARI reduced 5-HT release in the medial prefrontal cortex (mPFC) and dorsal raphe nucleus of the rat. Aripiprazole also reduced 5-HT release in the mPFC of wild-type (WT) but not 5-HT<sub>1A</sub> (-/-) knockout (KO) mice. As previously observed for other antipsychotic drugs, ARI reversed the elevation in 5-HT output produced by the local application of the 5-HT<sub>2A/2C</sub> receptor agonist DOI. Likewise, ARI increased the DA output in mPFC of WT but not 5-HT<sub>1A</sub> KO mice.

Contrary to haloperidol, which markedly increases the firing rate of DA neurons in the ventral tegmental area (VTA), ARI did not evoke consistent changes of dopaminergic activity. When administered after apomorphine, haloperidol fully reversed the inhibition in firing rate, whereas ARI evoked a moderate reversal which was significantly different from that evoked by haloperidol and from the spontaneous reversal of dopaminergic activity in rats treated with apomorphine and saline.

Overall, the present results indicate that ARI modulates the *in vivo* 5-HT and DA release in mPFC through the activation of 5-HT<sub>1A</sub> receptors. Likewise, ARI behaves as a partial agonist at DA D2 receptors *in vivo*, an action which clearly distinguishes it from conventional antipsychotics.

## Introduction

Aripiprazole is a novel antipsychotic that differs from other classical and atypical antipsychotics, improving both positive and negative symptoms of psychosis without producing extrapyramidal side effects or increases in serum prolactin (Tamminga, 2002, DeLeon et al., 2004). Aripiprazole shows high affinity for a large number of aminergic receptors, including dopamine (DA) D2, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (Shapiro et al., 2003; Green, 2004).

Concerning the dopaminergic system, ARI suppresses apomorphine-induced stereotypy in mice, and reduces DOPA synthesis in the forebrain of reserpine treated mice, suggesting that it exhibits antagonistic activity at post-synaptic D2 receptors, and agonistic activity at pre-synaptic dopamine (DA) autoreceptors (Kikuchi et al., 1995). *In vitro*, ARI potently activates D2 receptor-mediated inhibition of cAMP accumulation stimulated by forskolin in Chinese hamster ovary (CHO) cells transfected with the human D2L receptor gene (Burriss et al., 2002; Tadori et al., 2005). Consistent with these observations, ARI occupies more than 80% of striatal DA D2 receptors at therapeutic doses but does not produce extrapyramidal side effects (Yukoi et al., 2002).

In addition to its affinity for DA D2 receptors, ARI shows affinity for several 5-HT receptors (Lawler et al., 1999; Shapiro et al., 2003). Hence, it behaves as a partial agonist at 5-HT<sub>1A</sub> receptors and as an antagonist at 5-HT<sub>2A</sub> receptors, as assessed *in vitro* (Jordan et al., 2002; Tamminga, 2002, Shapiro et al., 2003; Green, 2004). These activities may also participate in the therapeutic action of ARI. Hence, in addition to the affinity for 5-HT<sub>2A</sub> receptors displayed by atypical antipsychotics (Meltzer, 1999) ARI may activate 5-HT<sub>1A</sub> receptors, which have been the focus of recent interest in the field of antipsychotics (Millan, 2000). Hence, atypical (but not classical) antipsychotics increase DA release in mPFC (Rollema et al., 1997; Ichikawa et al., 2001; Assié et al., 2005), an effect that depends on the activation of 5-HT<sub>1A</sub> receptors localized in mPFC (Díaz-Mataix et al., 2005). Given the crucial role of dopamine in cognitive function (Williams and Goldman-Rakic, 1995), the increase in cortical DA release may underlie the beneficial actions of clozapine and other atypical antipsychotic drugs on the spectrum of cognitive and negative/affective

symptoms by normalizing an impaired dopaminergic transmission in mPFC (Weinberger et al., 1994).

In the present study we examined the *in vivo* actions of ARI on the serotonergic and dopaminergic systems in rodent brain, with an special emphasis on the possible actions on 5-HT<sub>1A</sub> receptors and on its putative partial agonist activity on dopamine D2 receptors.

## Materials and methods

### Animals and treatments.

Male albino Wistar rats weighing 250-320 g and C57BL/6 mice 10-12 week old at the time of experiments were used (Iffa Credo, Lyon, France). 5-HT<sub>1A</sub> receptor knockout KO(-/-) mice (referred onwards as KO) had the same genetic background than their wild-type (WT) counterparts (C57BL/6) and were engendered as previously generated at Princenton University (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. 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 (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 according to the atlas of Paxinos and Watson (1998) for rat, and Franklin and Paxinos (1997) for mouse.

8-OH-DPAT, apomorphine, DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]), haloperidol and WAY-100635 were from RBI (Natick, MA). BAY x 3702 was from BAYER and ARI was from Bristol Myers Squibb. Concentrated stock solutions were prepared and aliquots were stored at -80 °C, except for apomorphine, which was freshly prepared each working day. Working solutions were prepared daily by dilution. Drugs were dissolved in saline at the appropriate concentrations and injected (1 ml/kg) through the femoral vein or subcutaneously, as indicated in Results. For the assessment of local effects in microdialysis experiments, drugs were dissolved in the perfusion fluid or water and diluted to appropriate concentrations in artificial cerebrospinal

fluid (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 were applied by reverse dialysis (uncorrected for drug recovery). For systemic administrations, ARI was dissolved in a vehicle consisting of hydroxypropyl-β-cyclodextrin (1.4 g dissolved in 15 ml distilled water). Control rats in experiments involving local drug administration were perfused with aCSF for the whole period. The bars in the figures show the period of drug application (corrected for the void volume of the system).

After experimental procedures were completed, animals were killed by an overdose of anesthetic and a careful histological verification of the correctness of the implants was carried out.

### **Single unit recordings**

We examined the responses of VTA DA neurons to the systemic administration of apomorphine. The suppressant action of apomorphine was then counteracted with haloperidol and aripiprazole. Rats were anesthetized (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 (Fa et al., 2003). Body temperature was maintained at 37 °C with a heating pad. In order to minimize pulsation, the atlanto-occipital membrane was punctured to release some CSF. DA neurons were recorded extracellularly with glass micropipettes pulled from 2.0-mm capillary glass (WPI, Sarasota, FL) on a Narishige PE-2 pipette puller (Narishige Sci. Inst., 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), postamplified and filtered with a Cibertec amplifier (Madrid, Spain) and computed on-line using a DAT 1401 plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Descents in VTA were carried out at AP -5.0 to -5.6, L -0.5 to -1, DV -7.5 to -9.0. The identification of DA neurons and burst firing analysis was carried out according to the criteria of Grace and Bunney (1984), as previously used (Celada et al., 1999). Briefly, neurons 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, and 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 a interspike interval of 160 ms or greater (Grace and Bunney, 1984).

We also examined the responses elicited by ARI in pyramidal neurons of the mPFC in anesthetized rats. Recordings were made essentially as described in Puig et al. (2003). Rats were administered chloral hydrate (400 mg/kg i.p.) and positioned in a David Kopf stereotaxic frame. Additional doses of chloral hydrate (80 mg/kg) were administered i.v. through the femoral vein. Typically, recordings were made between 10 and ~45 min after additional doses of anesthetic to avoid the effects of peak concentrations of chloral hydrate during recordings.

Bipolar stimulating electrodes consisted of two stainless steel enamel-coated wires (California Fine Wire, Grover Beach, CA) with a diameter of 150  $\mu\text{m}$  and a tip separation of ~100  $\mu\text{m}$  and *in vitro* impedances of 10-30 K $\Omega$ . Stimulating electrodes were stereotaxically implanted in the VTA (AP -6.0, L -0.5, DV -8.2). After implant, the electrodes were 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. Stimulating current was typically between 0.1-1.7 mA, 0.2 ms square pulses at 0.9 Hz.

Pyramidal neurons were recorded extracellularly with glass micropipettes pulled from 2.0-mm capillary glass, as described above for DA neurons. Descents in mPFC were carried out at AP +3.2-3.4, L -0.5 to -1.0, DV -1.0 to -4.0 below the brain surface. We systematically confirmed that only a single pyramidal neuron was recorded by a) identification by antidromic activation from VTA and b) collision extinction with spontaneously occurring spikes (Fuller and Schlag, 1976). Neurons without antidromic activation or without spontaneous firing activity were not considered. After the identification of a pyramidal neuron antidromically activated from the VTA, basal firing activity was recorded for 5 min before the i.v. administration of ARI.



## Microdialysis

Microdialysis procedures in rats and mice were conducted essentially as previously described in Bortolozzi et al., (2003) and Amargós-Boch et al. (2004). Rats were anesthetized with sodium pentobarbital (60 mg/Kg i.p.) and implanted with 4-mm concentric dialysis probes (Cuprophan) in mPFC at AP +3.2, L -0.8, DV -6.0. Groups of rats were also implanted with probes in the dorsal raphe nucleus (DR) at -7.4, L -3.1, DV -7.5 with a lateral angle of 30° (probe tip 1.5 mm). Microdialysis experiments were performed in freely moving rats >20 h after surgery. 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.

For mice, the manufacture of the probes was adapted from that previously described for rats (Bortolozzi et al., 2003). Surgical and microdialysis procedures were identical to those described for rats 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 mm) of the mPFC: AP + 2.2, L -0.2, DV -3.4.

The concentration of 5-HT and DA in dialysate samples was determined by HPLC with amperometric detection, using slight modifications of previously described methods. Updated procedures can be found in Bortolozzi et al., (2003) and Díaz-Mataix et al. (2005). Brain dialysates were collected on microvials and were injected into the HPLC. For DA analysis, microvials contained 5 µl of 10 mM perchloric acid. The amperometric detection (Hewlett Packard 1049 detector) of 5-HT and DA was carried out, respectively, at +0.6 and +0.75 V. Detection limits were typically 1-1.5 fmol for 5-HT and 3 fmol for DA.

## Data analysis

Changes in firing rate of DA neurons were assessed using repeated measures ANOVA and post-hoc *t*-tests. These values were quantified by averaging the values each minute 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). Data are expressed as the mean ± SEM. The statistical

analysis of microdialysis data will be carried out upon completion of the experiments.

## Results

### ***Effects of aripiprazole on spontaneous 5-HT release in rat and mice brain***

The administration of ARI (3, 10, 30 mg/kg i.p.) reduced the 5-HT output in the mPFC and the dorsal raphe (DR) of freely-moving rats, compared with rats treated with vehicle (hydroxypropyl- $\beta$ -cyclodextrin solution). Successive vehicle injections elicited moderate (~20%) increases of extracellular 5-HT, likely due to the injection associated stress (Adell et al., 1997) whereas ARI induced a significant reduction of 5-HT in DR ( $p < 0.005$  group effect,  $p < 0.01$  time effect,  $p < 0.005$  time x group interaction) and mPFC ( $p < 0.05$  group effect,  $p < 0.02$  time effect) (Figure 1). The maximal reduction in 5-HT release was attained at 3 mg/kg and subsequent injections of 10 and 30 mg/kg ARI did not seem to reduce 5-HT further.

The administration of vehicle did not alter the 5-HT release in mPFC of wild-type (WT) and 5-HT<sub>1A</sub> knockout, (KO) mice, except for a transient increase shortly after the injection, which, as that observed in rats, can be attributed to the stress produced by handling and injection (Bortolozzi et al., 2004) (Figure 2A). The i.p. administration of 3 mg/kg ARI induced a moderate but significant reduction of the 5-HT output which was not different between WT and KO mice ( $n = 6$  each;  $p < 0.0001$  significant effect of time but not of group or time x group interaction; Figure 2B). However, the subsequent injection of 0.5 mg/kg of the selective 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT markedly reduced the 5-HT output in a subgroup of WT but not KO mice ( $p < 0.03$  group effect;  $p = 0.06$  time effect;  $p < 0.001$  time x group interaction;  $n = 3$  each; Fig. 2B).

In contrast, the administration of 30 mg/kg ARI elicited a more marked reduction of the 5-HT output in WT than in WT mice ( $p < 0.00001$  time effect,  $p < 0.0005$  time x group interaction;  $n = 6$  each; Fig. 2C). As also observed previously, the subsequent i.p. administration of 0.5 mg/kg 8-OH-DPAT reduced the 5-HT output further in WT but not in 5-HT<sub>1A</sub> receptor KO mice ( $p < 0.0001$  group effect,  $p < 0.0001$  time effect,  $p < 0.0001$  time x group interaction;  $n = 6$

each; Figure 2C). Likewise, the i.p. administration of the same 8-OH-DPAT dose alone reduced 5-HT release in WT but not KO mice ( $p < 0.02$  group effect,  $p < 0.00002$  time effect,  $p < 0.00001$  time x group interaction;  $n = 3$ ; Fig. 3).

### ***Effects of aripiprazole on DOI-stimulated 5-HT release in rat and mice mPFC***

Previous reports indicate that the local application of both classical (chlorpromazine, haloperidol) and atypical (clozapine, olanzapine) antipsychotic drugs reverse the increase in mPFC evoked by the hallucinogen DOI, a 5-HT<sub>2A/2C</sub> receptor agonist (Bortolozzi et al., 2003). We therefore examined the ability of ARI to reverse DOI-stimulated 5-HT release in mPFC.

As previously observed in rat and mice mPFC (Martín-Ruiz et al., 2001; Bortolozzi et al., 2003), the application of 100  $\mu$ M DOI by reverse dialysis doubled the local 5-HT output ( $p < 0.0001$ ; Fig. 4). The co-perfusion of 100  $\mu$ M ARI did not counteract the effect of DOI whereas a higher concentration (300  $\mu$ M) significantly antagonized DOI's effect on 5-HT output ( $p = 0.057$  group effect,  $p < 0.00001$  time effect,  $p < 0.00001$  time x group interaction; Fig. 4). However, the same ARI concentration was ineffective in reducing the elevated 5-HT output evoked by DOI in WT and 5-HT<sub>1A</sub> KO mice (Figure 5). If anything, ARI seemed to induced a further elevation of 5-HT in KO mice ( $n = 7$  mice/group).

### ***Comparison of the effect of haloperidol and aripiprazole on the apomorphine-induced suppression of DA cell firing in rat VTA***

The systemic administration of ARI at various doses (max. 3.2 mg/kg) to chloral hydrate anesthetized rats ( $n = 5$ ) did not evoke any consistent effect on the firing activity of VTA DA neurons, whereas haloperidol (100-200  $\mu$ g/kg i.v.) increased cell firing (Figure 6) (data on haloperidol are from Díaz-Mataix, et al., 2005). Since the lack of effect of ARI on spontaneous DA cell firing could be attributed to a partial agonist effect on DA D2 receptors, we explored a different strategy. Rats were injected with a single dose of apomorphine (APO; 50  $\mu$ g/kg i.v.) which induced a marked suppression of the firing of DA cells in the VTA. Two minutes later, rats were given either haloperidol (HAL, 200  $\mu$ g/kg i.v.) or aripiprazole (ARI, 400 or 800  $\mu$ g/kg i.v.). These doses were established in

previous pilot experiments (not shown). As expected, haloperidol administration completely reversed the suppressant effect of apomorphine on the overall firing rate. The administration of both doses of ARI significantly reversed the suppressant effect of apomorphine on firing rate but the reversal was less marked than that produced by haloperidol. Figure 7 shows representative examples of the suppressant effect of apomorphine on DA cell firing followed by spontaneous reversal (7A) and by the effects of haloperidol (7B) and ARI (7C). Figure 8 shows the average effects in all units examined. Analysis of the data with two-way ANOVA showed a significant effect of the group ( $p < 0.01$ ), time ( $p < 0.00001$ ) and time x group interaction ( $p < 0.00001$ ). Post-hoc analysis revealed a significant difference between haloperidol and ARI vs. saline as well as a significant difference between haloperidol and ARI, with no significant differences between the two aripiprazole doses. Likewise, burst firing was significantly different among rats treated with apomorphine alone and with apomorphine + haloperidol and apomorphine + ARI ( $0.8 \pm 0.6$  %,  $18.6 \pm 11.7$  % and  $3.0 \pm 2.1$  %, respectively;  $p < 0.05$  one-way ANOVA; burst firing was calculated from the last three minutes of experiment).

#### ***Modulation of the mesocortical DA release by aripiprazole in mouse PFC***

The perfusion of artificial CSF and the i.p. administration of vehicle did not alter DA release in the mPFC of WT and 5-HT<sub>1A</sub> KO mice. A short-lasting increase was observed after the injection of vehicle, which, as that seen for 5-HT can be attributed to stress. (Figure 9A). The administration of the selective 5-HT<sub>1A</sub> agonist 8-OH-DPAT (0.5 mg/kg i.p.) increased the DA output in WT but not in 5-HT<sub>1A</sub> mice ( $p < 0.01$  group effect,  $p < 0.0001$  time effect,  $p < 0.02$  time x group interaction) (Figure 9B). In WT mice, the maximal increase in DA output was to  $181 \pm 24$ % of baseline. DA values remained stable for approximately 4 dialysate fractions (80 min). Thereafter, DA values ranged between 140-160% of baseline. In contrast, 8-OH-DPAT did not alter DA output in 5-HT<sub>1A</sub> KO mice.

Similarly, another selective 5-HT<sub>1A</sub> agonist, BAY x 3702 (0.5 mg/kg i.p.) also increased DA output in WT, but not in 5-HT<sub>1A</sub> mice ( $p < 0.0001$  group effect,  $p < 0.05$  time effect,  $p < 0.00001$  time x group interaction). The maximal increase of the DA output produced by BAY x 3702 was comparable to that

elicited by 8-OH-DPAT, although it was reached later, possibly due to differences in half-life of both 5-HT<sub>1A</sub> agonists (Fig. 9 C).

The systemic administration of 3 mg/kg i.p. ARI markedly elevated DA release in the mPFC of WT mice. However, the same treatment failed to elevate cortical DA in the 5-HT<sub>1A</sub> KO mice, except for a sharp increase just after the injection of ARI, which was also observed in WT mice ( $p < 0.02$ , group effect;  $p < 0.0001$  time effect; Fig. 10). The administration of a subsequent challenge dose of 8-OH-DPAT increased DA release in WT but not 5-HT<sub>1A</sub> mice, as expected (inset in Figure 10A).

The administration of 30 mg/kg i.p. ARI induced a DA increase in WT mice which was not statistically different to that produced by the dose of 3 mg/kg ( $p < 0.0001$  time effect, but not significant group or time x group interactions; Fig. 10B). As for the dose of 3 mg/kg, ARI did not increase the DA output in 5-HT<sub>1A</sub> KO mice ( $p < 0.04$  group effect).

In local perfusion experiments, the application of artificial CSF for the whole experimental period did not modify DA release in WT and KO mice (Figure 11). The local application of ARI reduced the DA output in the mPFC of WT mice but did not affect that in 5-HT<sub>1A</sub> mice ( $p < 0.02$  group effect,  $p < 0.04$  time effect,  $p = 0.056$  time x group interaction).

## Discussion

The present study examined the *in vivo* actions of ARI on the serotonergic and dopaminergic systems in rat and mouse brain using microdialysis in awake animals and single unit recordings of identified neurons in anesthetized rats. The data obtained support and extend previous *in vitro* and *in vivo* observations on a dual action of ARI on both neurotransmitter systems.

### *Electrophysiology*

The data obtained indicates that ARI has no major effects by itself on DA neurons of the VTA. In contrast, ARI partly reversed the suppressant action of APO on DA cell firing. This reversal is far from that obtained with the classical D2 receptor antagonist HAL (Gessa et al., 2000, this study). Consistent with

previous studies (Chiodo, 1988), the dose of APO chosen (50 µg/kg i.v. ) fully suppressed the firing rate of the majority of DA neurons recorded. The subsequent administration of HAL increased the firing rate above baseline levels, most likely by removing an endogenous tone on DA D2 autoreceptors. However, ARI administration significantly increased the firing rate of DA neurons above that produced by the spontaneous reversal (controls were injected with vehicle) yet this effect was far from that achieved with HAL. Two different doses of ARI were used, with no significant differences between them, indicating that 400 and 800 µg/kg i.v. were maximal and equally effective. The partial recovery of DA cell firing produced by ARI is consistent with its partial DA D2 antagonist character previously observed *in vitro* and *in vivo* (Amano et al., 1995; Green, 2004). However, unlike previous *in vivo* evidence, the present results are the first demonstration of a partial DA D2 antagonist effect on DA neurons, using a direct, unequivocal measure of dopaminergic activity, such is the electric activity of DA neurons in the VTA.

Given that ARI has also a partial agonist *in vitro* effect on 5-HT<sub>1A</sub> receptors and that selective 5-HT<sub>1A</sub> agonists modulate the activity of pyramidal neurons of the medial prefrontal cortex (Díaz-Mataix et al., in press), we also examined the effect of ARI on pyramidal cell activity. In pilot experiments, however, ARI did not alter the firing rate of pyramidal neurons. This difference may be due to the partial agonist character of ARI on 5-HT<sub>1A</sub> receptors, since only highly efficacious agonists, such 8-OH-DPAT or BAY x 3702 are capable to modulate pyramidal activity. Likewise, the affinities of ARI for various other receptors may counterbalance an effect on 5-HT<sub>1A</sub> receptors.

#### *Microdialysis experiments*

In the rat, ARI reduced the 5-HT output in a terminal area (mPFC) and in the cell body area (DR) at doses of 3- 30 mg/kg s.c. This decrease was moderate and was not dose-dependent since similar reductions were noted at 3, 10 and 30 mg/kg. In mice mPFC, the dose of 3 mg/kg produced also a moderate decline in the 5-HT output. However, this effect does not appear to be due to the activation of 5-HT<sub>1A</sub> receptors, since it was common to WT and 5-HT<sub>1A</sub> KO mice. Indeed, 8-OH-DPAT administration following that of ARI reduced 5-HT release in WT but not KO mice, further supporting the view that the 5-HT

decrease was due to other activities of ARI. It is unlikely that a partial agonist action of ARI on DA D2 receptors may contribute to the 5-HT decrease in mPFC and DR since DA D2 receptor agonists increase 5-HT release in the DR and the activity of ascending 5-HT neurons (Ferré et al., 1994; Martín-Ruiz et al., 2001a)., 5-HT<sub>2A</sub> receptor blockade is also unlikely to be involved given that the 5-HT<sub>2A/2C</sub> receptor agonist DOI reduced the firing of 5-HT cells and the 5-HT output in the mPFC of anesthetized rats after systemic administration (Martín-Ruiz et al., 2001b). Among the various pharmacological activities of ARI, blockade of  $\alpha_1$ -adrenoceptors may possibly be accountable since the  $\alpha_1$ -adrenoceptor antagonist prazosin reduces 5-HT cell firing (VanderMaelen and Aghajanian, 1983.) and 5-HT release in the DR and terminal areas (Bortolozzi and Artigas, 2003). Moreover, the atypical antipsychotics clozapine and olanzapine reduce 5-HT cell firing in the DR by blocking  $\alpha_1$ -adrenoceptors (Sprouse et al., 1999). At 30 mg/kg, ARI reduced 5-HT significantly more in WT than in KO mice, which suggests that this higher dose produced a direct activation of 5-HT<sub>1A</sub> receptors controlling 5-HT release in mouse mPFC. However, the *in vivo* activation of 5-HT<sub>1A</sub> autoreceptors at this dose is lower than that produced by a maximal dose of 8-OH-DPAT (typically 0.5 mg/kg i.p. in the mice), which is consistent with a partial agonist action.

As previously observed for other atypical antipsychotic drugs, such as clozapine or olanzapine, the local application of ARI in mPFC reversed the increase in 5-HT release produced by the 5-HT<sub>2A/2C</sub> agonist DOI (Bortolozzi et al., 2003). This effect may involve various activities, such as 5-HT<sub>2A</sub> receptor antagonism, 5-HT<sub>1A</sub> receptor agonism or  $\alpha_1$ -adrenoceptor antagonism, since antagonists of both excitatory receptors (e.g., M100907 for 5-HT<sub>2A</sub> receptors, prazosin for  $\alpha_1$ -adrenoceptors) or agonists of inhibitory receptors (e.g., 8-OH-DPAT or BAY x 3702 for 5-HT<sub>1A</sub> receptors) suppress the increase in 5-HT release produced by DOI (Martín-Ruiz et al., 2001; Bortolozzi et al., 2003). Indeed, these “functional” antagonisms are not due to a lack of specificity of the agents used but to the fact that these receptors are co-expressed in pyramidal neurons of the mPFC (Amargós-Bosch et al., 2004; Santana et al., unpublished observations) and play opposing roles on pyramidal cell activity (which subsequently results in a change in 5-HT release). An attempt to examine the

putative involvement of 5-HT<sub>1A</sub> receptors proved ineffective, since ARI 300 µM did not reverse the increase in 5-HT release produced by DOI in WT and 5-HT<sub>1A</sub> KO mice. Similar experiments with the selective, highly efficacious agonists 8-OH-DPAT and BAY x 3702 revealed that these agents completely reversed DOI's effect in WT but not in 5-HT<sub>1A</sub> KO mice (Amargós-Bosch et al., 2004). The failure of ARI to reverse the effect of DOI in mice, while eliciting an almost complete reversal in rat mPFC, suggests the existence of species differences in relation to the action of ARI in mPFC. Hence, the present data cannot clarify whether activation of postsynaptic 5-HT<sub>1A</sub> receptors in mPFC can be involved in the reversal of DOI's effect observed in rats.

Recent observations indicate that atypical antipsychotics (clozapine, olanzapine, ziprasidone), but not haloperidol increased the DA output in the mPFC of WT but not 5-HT<sub>1A</sub> KO mice (Díaz-Mataix et al., 2005). In the present study, we extend these observations to the case of aripiprazole. The administration of 3 mg/kg i.p. ARI enhanced DA release in the mPFC of WT, but not KO mice (except for a short-lasting increase in both groups, likely due to injection and handling stress). The effect size was comparable to that produced by doses of the selective 5-HT<sub>1A</sub> agonists 8-OH-DPAT and BAY x 3702 that are maximal or nearly maximal in terms of 5-HT<sub>1A</sub> receptor activation. However, when 8-OH-DPAT (0.5 mg/kg i.p.) was administered after ARI increased the DA output to nearly 300% of baseline in WT (but not 5-HT<sub>1A</sub> KO) mice. The additional increase produced by 8-OH-DPAT suggests the involvement of more than one 5-HT<sub>1A</sub> receptor population, given that both effects appear to be additive. A higher ARI dose (30 mg/kg i.p.) also produced a significantly different effect in WT and KO mice, although the effect size was comparable or slightly smaller than that produced by the 3 mg/kg dose. Similar results have been reported in rat brain at low ARI doses (Li et al., 2004) although higher doses appear to reduce DA release in rat PFC (Semba et al., 1995).

Recent observations indicate that local application of clozapine or olanzapine (300 µM) –but not haloperidol– in mPFC increased DA release in WT, but not KO mice (Díaz-Mataix et al., 2005). This suggests that the increase in mPFC DA release produced by atypical antipsychotics (Rollema et al., 1997, 2000; Ichikawa et al., 2001) involves the local activation of 5-HT<sub>1A</sub> receptors in mPFC. However, when ARI was locally applied in mPFC, it did not increase DA



output, possibly due to other pharmacological activities of the compound, such as for instance, blockade of 5-HT<sub>2A</sub> receptors or partial activation of DA D2 autoreceptors (Shapiro et al., 2003). Additionally, it is unclear why ARI reduced the DA output in 5-HT<sub>1A</sub> KO mice but not in WT mice an opposite profile was observed after systemic administration.

It seems paradoxical that 3 mg/kg ARI increased cortical DA release through an interaction with postsynaptic 5-HT<sub>1A</sub> receptors but could not reduce 5-HT release in mice mPFC at the same dose by a 5-HT<sub>1A</sub>-dependent mechanism –involving presynaptic 5-HT<sub>1A</sub> autoreceptors--, given the greater sensitivity of presynaptic (raphe) 5-HT<sub>1A</sub> receptors to selective agonists (Sprouse and Aghajanian, 1987). This would suggest that different receptor populations are involved in the suppression of 5-HT output and increase in DA output. One possible explanation to these discrepant findings is that atypical antipsychotics activate 5-HT<sub>1A</sub> receptors modulating cortical DA release in an indirect manner. Hence, clozapine and olanzapine, with low and negligible *in vitro* affinity for 5-HT<sub>1A</sub> receptors, increase the DA output in mPFC after local and systemic administration in WT mice but not in 5-HT<sub>1A</sub> KO mice (Díaz-Mataix et al., 2005). Moreover, the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 attenuated the DA elevations in mPFC produced by clozapine, olanzapine and risperidone, an antipsychotic drug which also lacks significant *in vitro* affinity for 5-HT<sub>1A</sub> receptors (Ichikawa et al., 2001). The cellular and molecular basis for such an effect remain unknown but may involve the direct or indirect activation of 5-HT<sub>1A</sub> receptors on cortical GABAergic interneurons, since the elevation in cortical DA output produced by the local application of the 5-HT<sub>1A</sub> receptor agonist BAY x 3702 and by the atypical antipsychotics clozapine and olanzapine were abolished by the concurrent perfusion of the GABA<sub>A</sub> blocker bicuculline (Díaz-Mataix et al., 2005).

In summary, the present results fully support the notion that ARI acts *in vivo* as a partial antagonist at DA D2 receptors, with a low intrinsic efficacy (otherwise, it would have reduced DA cell firing by itself, such as apomorphine) and it is capable to exert a partial recovery of dopaminergic activity following apomorphine administration. On the other hand, in common with other atypical antipsychotic drugs such as clozapine, olanzapine and ziprasidone (but not haloperidol), ARI increases DA release in mPFC at a low dose (3 mg/kg). This

effect may be related to the superior effects on cognition of atypical antipsychotics compared with classical DA D2 blockers. This, together with a lower blockade of postsynaptic DA D2 receptors, may be the basis for the unique clinical profile of ARI.

### **Acknowledgements**

Work supported by grants from the Spanish Ministry of Education and Science (SAF 2004-05525) and Bristol Myers Squibb. PC and AB are recipients of a Ramón y Cajal contract from the Ministry of Science and Technology. LDM is recipient of a predoctoral fellowship from IDIBAPS. Support from the Generalitat de Catalunya (2005) is also acknowledged. We thank Leticia Campa and Judith Ballart for skilful technical assistance

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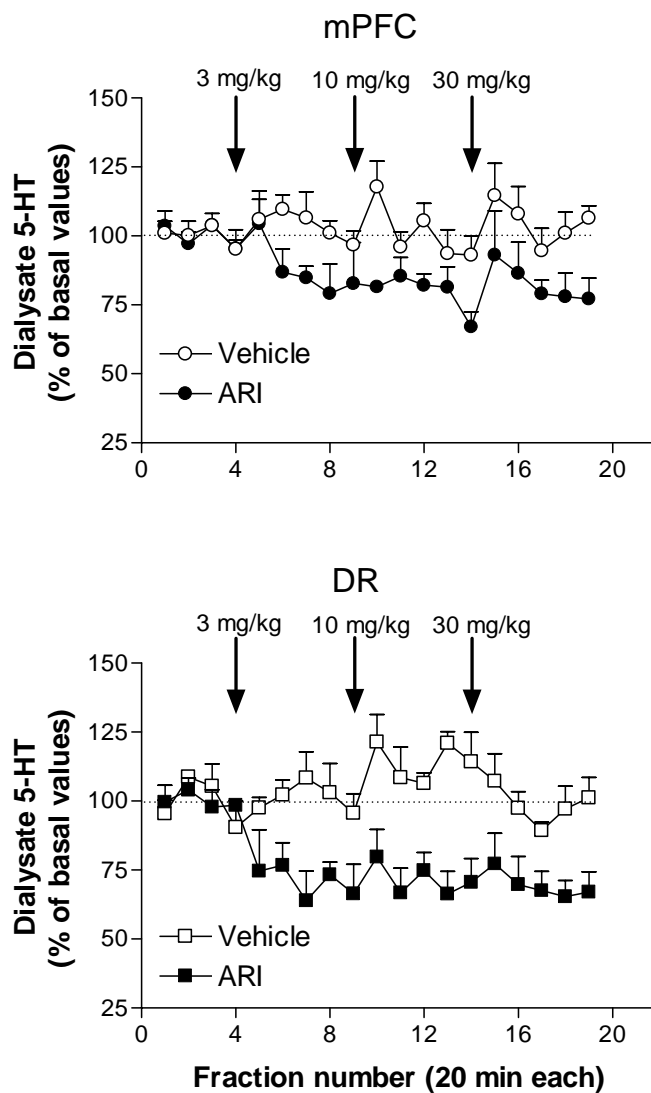
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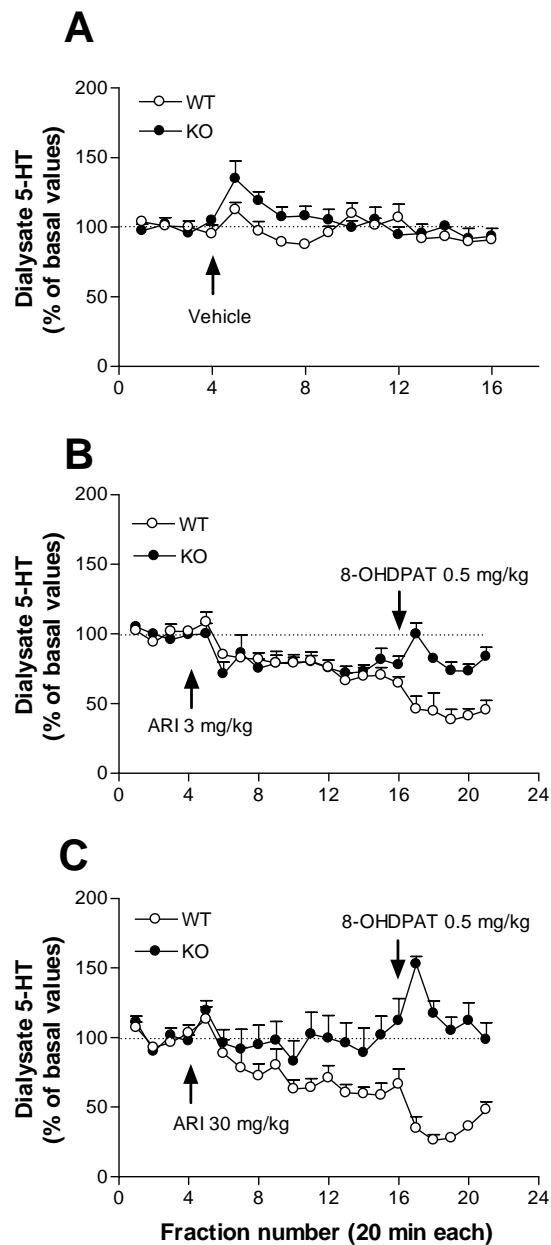
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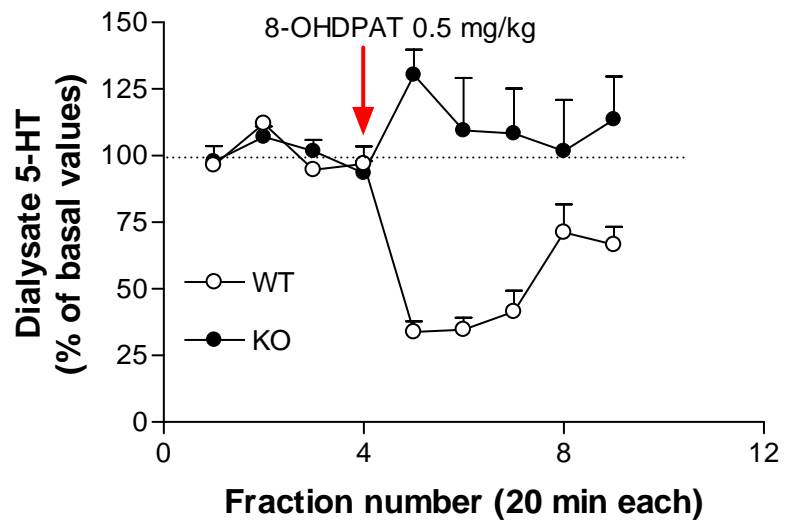


**Figure 1.** Effect of the i.p. administration of vehicle and aripiprazole (ARI, 3, 10 and 30 mg/kg) on the 5-HT output in the medial prefrontal cortex (mPFC) and dorsal raphe (DR) of rats. Data are means  $\pm$  SEM of 4 rats per group. See text for statistical details.

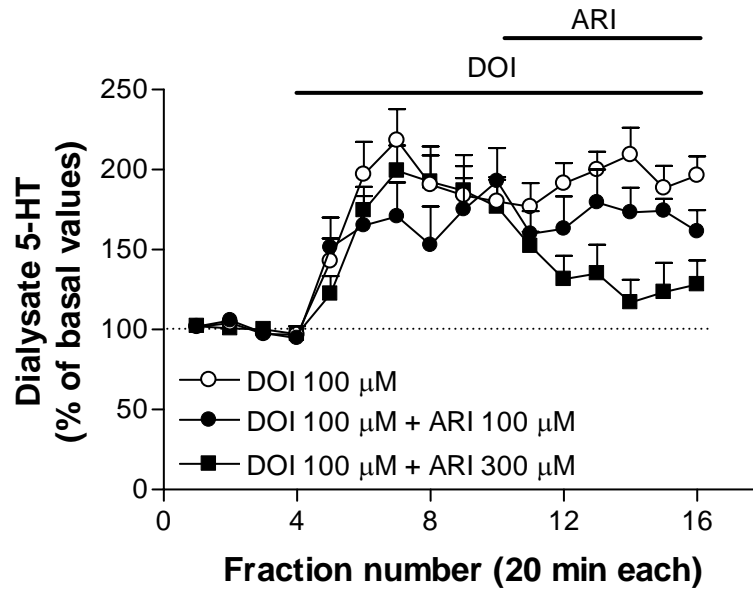




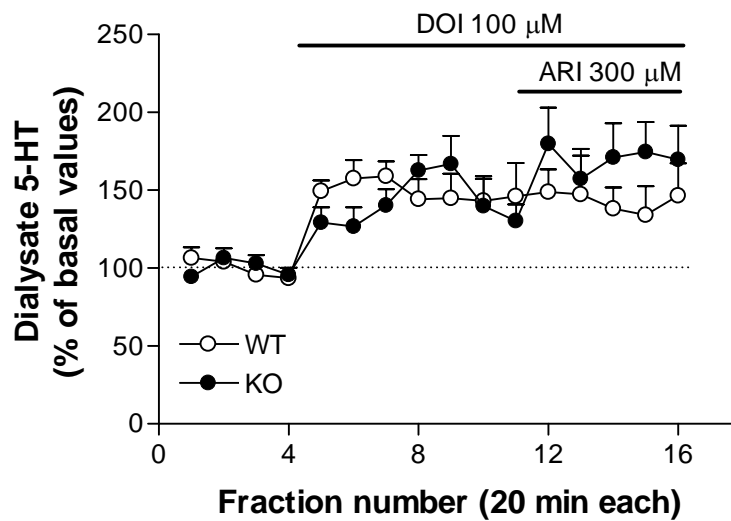
**Figure 2.** Effect of the i.p. administration of vehicle (panel A), 3 mg/kg aripiprazole (ARI) (panel B) and 30 mg/kg ARI (panel C) on the 5-HT output in the mPFC of wild type (WT) and 5-HT<sub>1A</sub> receptor knockout (KO) mice (n = 6 each except the vehicle/WT group, n = 5). The subsequent administration of the selective 5-HT<sub>1A</sub> agonist 8-OH-DPAT (0.5 mg/kg i.p.) reduced the 5-HT output in WT but not 5-HT<sub>1A</sub> receptor KO mice (n = 3 for the groups treated with 3 mg/kg ARI; n = 6 for the groups treated with 30 mg/kg ARI). See text for statistical details.



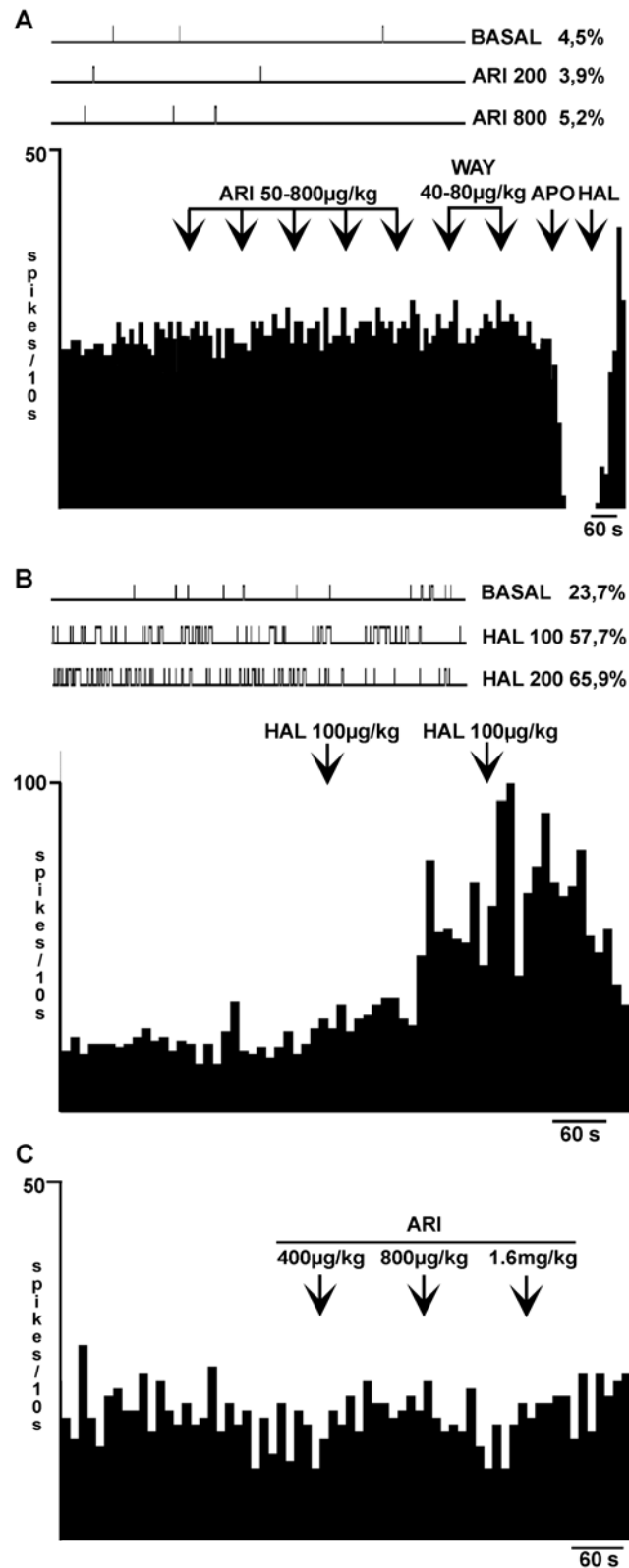
**Figure 3.** The i.p. administration of 0.5 mg/kg 8-OH-DPAT induced a marked reduction of the 5-HT output in the medial prefrontal cortex of wild-type (WT) but not of 5-HT<sub>1A</sub> receptor knockout (KO) mice (n = 3 rats/group). See text for statistical analysis.



**Figure 4.** The application of of the 5-HT<sub>2A/2C</sub> receptor agonist DOI by reverse dialysis enhanced the local 5-HT output in rat mPFC (n = 6). The co-perfusion of 300 μM (n = 7) but not 100 μM (n = 5) aripiprazole (ARI) significantly reversed the 5-HT elevation evoked by the application of DOI. Horizontal bars show the period of drug application. See text for statistical analysis.

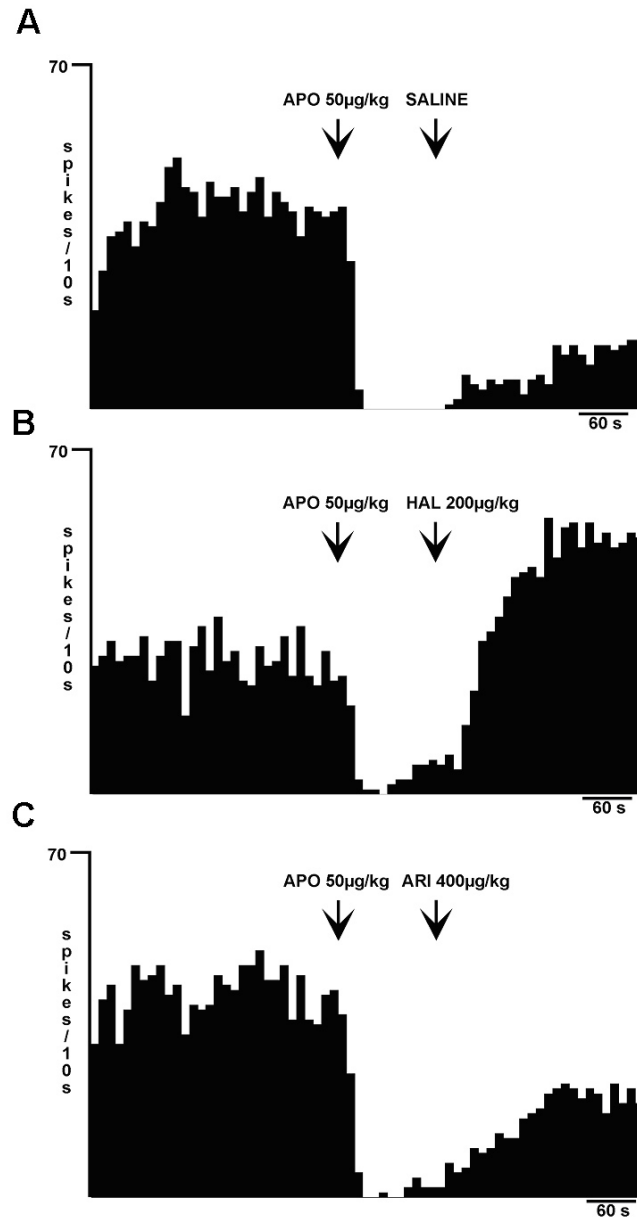


**Figure 5.** The application of DOI by reverse dialysis increased the local 5-HT output in the mPFC of WT and 5-HT<sub>1A</sub> KO mice (n = 7 each). However, the co-perfusion of 300 μM aripiprazole could not reverse the 5-HT elevation induced by DOI in neither group. Horizontal bars show the period of drug application.

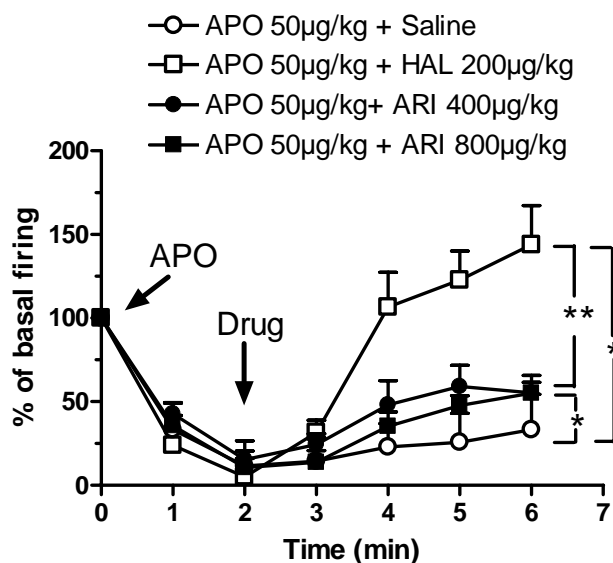


**Figure 6** Integrated firing rate histograms showing the effects of i.v. injections (arrows) of aripiprazole (ARI) and haloperidol (HAL) on the firing activity of dopaminergic neurons in the VTA (panels A and B, respectively; ordinate:

spikes/10 s; see bar time in abscissa). Note the increase in overall firing rate produced by haloperidol (from 2.14 in baseline conditions to 4.85 and 6.24 spikes/s after the first and second dose of haloperidol) and lack of effect of aripiprazole (firing rate in baseline conditions and after maximal aripiprazole dose: 2.6 and 2.5 spikes/s). The selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 was injected after aripiprazole to unmask any possible effect through 5-HT<sub>1A</sub> receptors (e.g., suppression of firing due to partial D2 antagonism + increase of firing due to 5-HT<sub>1A</sub> receptor agonism) but did not produce any effect. Note the dopaminergic nature of the recorded neuron by the suppression produced by apomorphine and subsequent reversal by haloperidol. The upper traces in panels A and B show burst trains corresponding to recordings obtained in baseline conditions and after administration of aripiprazole and haloperidol. The latter drug (but not aripiprazole) also increased the percentage of spikes fired in bursts. Panel C shows the integrated firing rate histogram of a pyramidal neuron in the rat medial prefrontal cortex in baseline conditions and after the administration of aripiprazole. As in a few more neurons examined, aripiprazole did not alter the firing rate of pyramidal neurons.

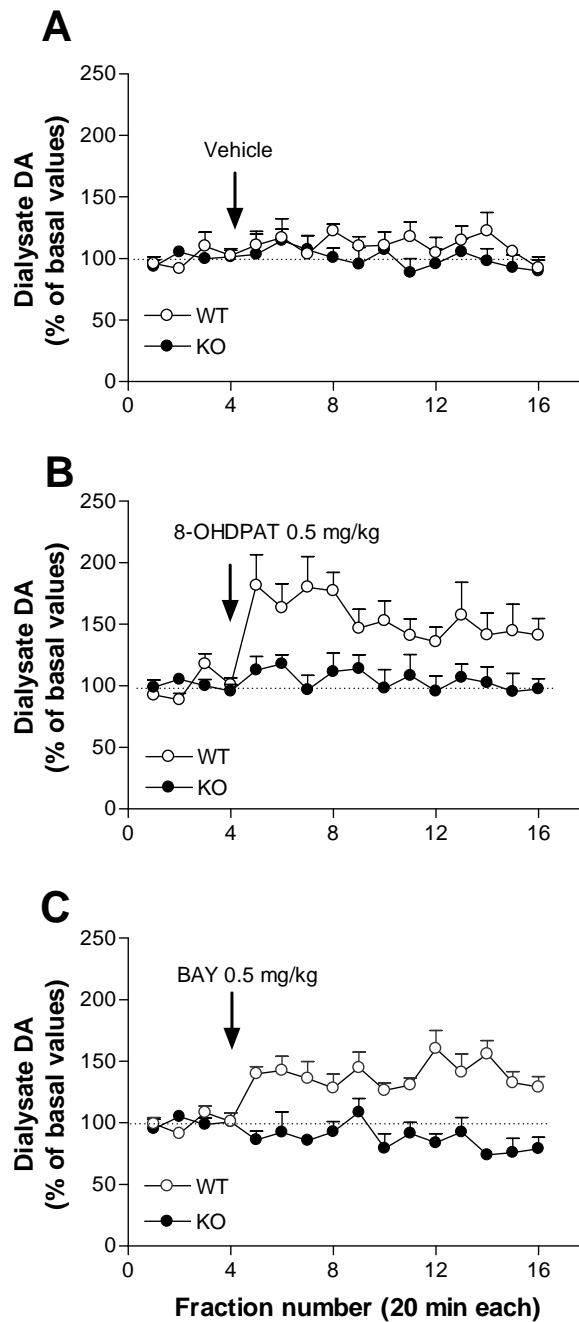


**Figure 7.** Integrated firing rate histograms of VTA dopaminergic neurons showing representative examples of the inhibitory effect of apomorphine (50 µg/kg i.v., first arrow) followed by injections (second arrow) of saline (panel A), haloperidol 200 µg/kg i.v. (HAL, panel B) and aripiprazole 400 µg/kg i.v. (ARI, panel C). Note the suppression of cell firing induced by apomorphine in all units. As expected, haloperidol produced a full reversal of the inhibition followed by a rebound increase that is likely due to the full blockade of self-inhibitory mechanisms mediated by D2 receptors at cell body level. In contrast, aripiprazole induced a partial reversal of the apomorphine-induced inhibition.

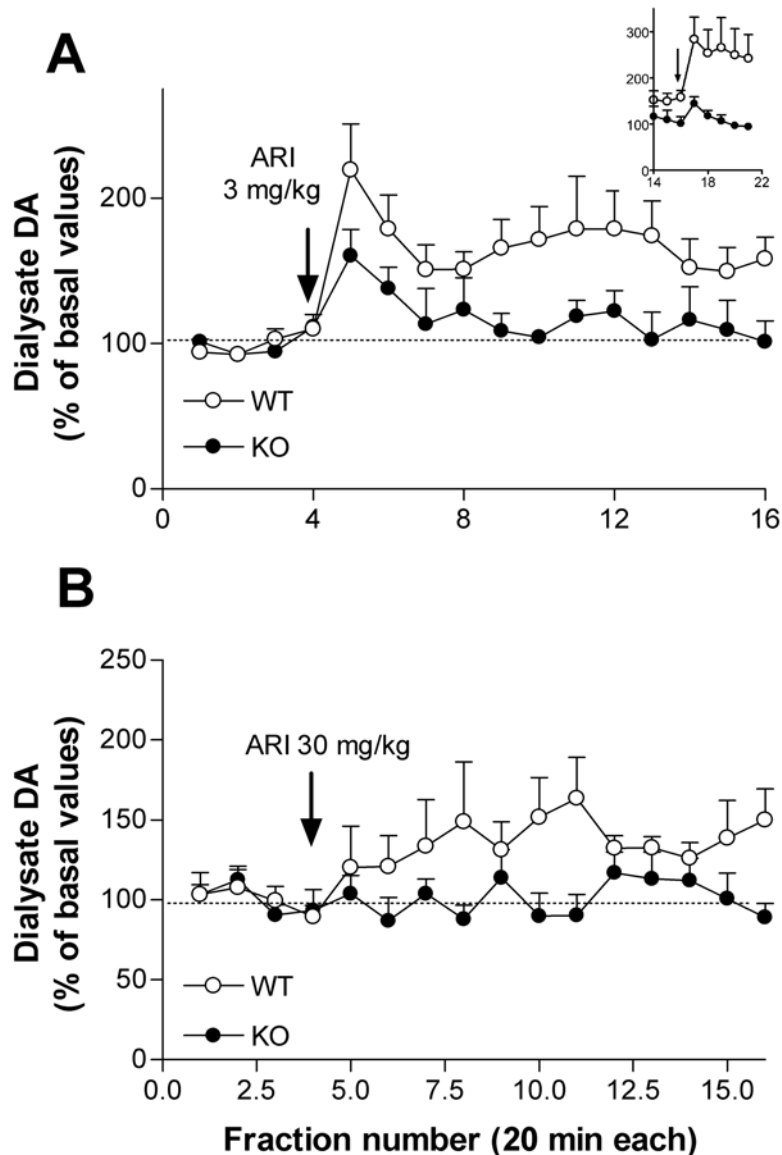


**Figure 8.** Average effects of the administration of apomorphine followed by saline (open circles,  $n = 9$ ), haloperidol 200 µg/kg i.v. (open squares,  $n = 6$ ), aripiprazole 400 µg/kg i.v. (filled circles,  $n = 15$ ) and aripiprazole 800 µg/kg i.v. (filled squares,  $n = 4$ ). \* $p < 0.05$  vs. apomorphine + saline, \*\* $p < 0.05$  vs. apomorphine + haloperidol.

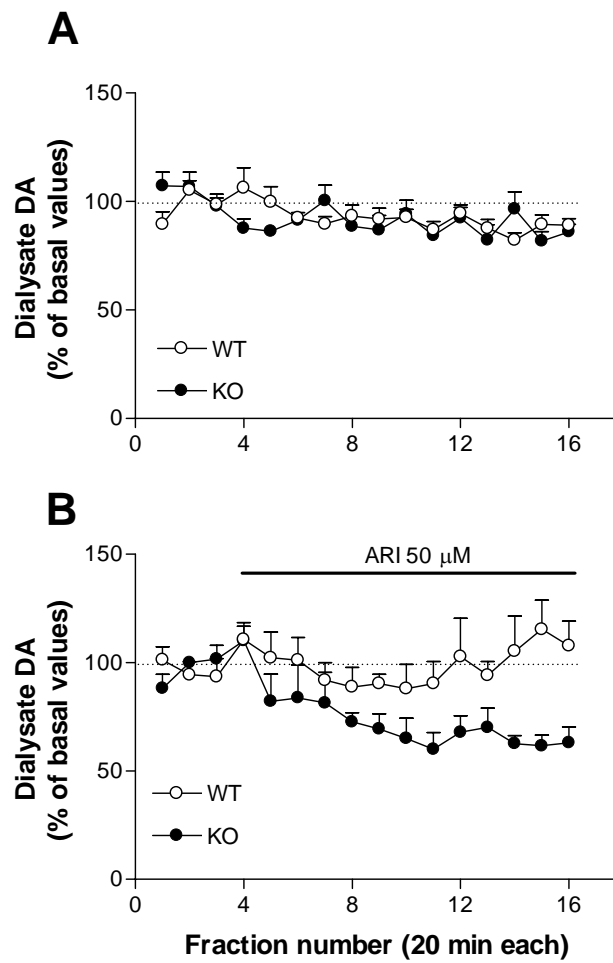




**Figure 9.** The administration of the selective 5-HT<sub>1A</sub> agonists 8-OH-DPAT (panel B) and BAY x 3702 (panel C) significantly increased the DA output in the mPFC of WT but not 5-HT<sub>1A</sub> KO mice (n = 6-8 rats/group). The injection of vehicle did not alter the DA output in WT or KO mice (n = 3 each). See text for statistical analysis.



**Figure 10.** A) The i.p. administration of aripiprazole at 3 mg/kg increased the DA output in the mPFC of WT but not 5-HT<sub>1A</sub> mice, except for a transient increase which was probably related to the injection stress (n = 6 mice/group). The injection of 30 mg/kg aripiprazole did not increase DA output further (panel B). The inset in A shows the effect of 0.5 mg/kg 8-OH-DPAT (arrow) injected at the end of the experiment (fraction 16). For comparative purposes, the effects of both doses of aripiprazole are shown with the same temporal scale. See text for statistical analysis.



**Figure 11.** A) The local application of the artificial CSF used to perfuse the probes did not alter the DA output in the mPFC of WT and 5-HT<sub>1A</sub> KO mice (n = 6). B) Aripiprazole application at 50  $\mu\text{M}$  reduced the DA output in 5-HT<sub>1A</sub> KO mice but left it unaltered in WT mice (n = 4 and 6, respectively). Horizontal bars show the period of drug application. See text for statistical analysis.