

RESEARCH PAPER

Preferential *in vivo* action of F15599, a novel 5-HT_{1A} receptor agonist, at postsynaptic 5-HT_{1A} receptorsL Lladó-Pelfort^{1,2}, M-B Assié³, A Newman-Tancredi³, F Artigas^{1,2} and P Celada^{1,2,4}

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Background and purpose: F15599, a novel 5-hydroxytryptamine (5-HT)_{1A} receptor agonist with 1000-fold selectivity for 5-HT compared with other monoamine receptors, shows antidepressant and procognitive activity at very low doses in animal models. We examined the *in vivo* activity of F15599 at somatodendritic autoreceptors and postsynaptic 5-HT_{1A} heteroreceptors.

Experimental approach: *In vivo* single unit and local field potential recordings and microdialysis in the rat.

Key results: F15599 increased the discharge rate of pyramidal neurones in medial prefrontal cortex (mPFC) from 0.2 µg·kg⁻¹ i.v. and reduced that of dorsal raphe 5-hydroxytryptaminergic neurones at doses >10-fold higher (minimal effective dose 8.2 µg·kg⁻¹ i.v.). Both effects were reversed by the 5-HT_{1A} antagonist (±)WAY100635. F15599 did not alter low frequency oscillations (~1 Hz) in mPFC. In microdialysis studies, F15599 increased dopamine output in mPFC (an effect dependent on the activation of postsynaptic 5-HT_{1A} receptors) with an ED₅₀ of 30 µg·kg⁻¹ i.p., whereas it reduced hippocampal 5-HT release (an effect dependent exclusively on 5-HT_{1A} autoreceptor activation) with an ED₅₀ of 240 µg·kg⁻¹ i.p. Likewise, application of F15599 by reverse dialysis in mPFC increased dopamine output in a concentration-dependent manner. All neurochemical responses to F15599 were prevented by administration of (±)WAY100635.

Conclusions and implications: These results indicate that systemic administration of F15599 preferentially activates postsynaptic 5-HT_{1A} receptors in PFC rather than somatodendritic 5-HT_{1A} autoreceptors. This regional selectivity distinguishes F15599 from previously developed 5-HT_{1A} receptor agonists, which preferentially activate somatodendritic 5-HT_{1A} autoreceptors, suggesting that F15599 may be particularly useful in the treatment of depression and of cognitive deficits in schizophrenia. *British Journal of Pharmacology* (2010) **160**, 1929–1940; doi:10.1111/j.1476-5381.2010.00738.x

Keywords: 5-HT_{1A} receptors; dopamine; pyramidal neurones; depression; prefrontal cortex; schizophrenia; microdialysis, local field potential

Abbreviations: DR, dorsal raphe nucleus; F15599, 3-chloro-4-fluorophenyl-(4-fluoro-4-[(5-methyl-pyrimidin-2-ylmethyl)-amino]-methyl)-piperidin-1-yl)-methanone; LFP, local field potential; mPFC, medial prefrontal cortex

Introduction

5-hydroxytryptamine (5-HT)_{1A} receptors are widely expressed in mammalian brain. They are located on the soma and dendrites of 5-hydroxytryptaminergic neurones, where they have an autoreceptor role, and are also located in pyramidal and GABAergic neurones of the neocortex and limbic system (Pazos and Palacios, 1985; Pompeiano *et al.*, 1992; Kia *et al.*, 1996; Riad *et al.*, 2000; Martinez *et al.*, 2001; Cruz *et al.*, 2004; Santana *et al.*, 2004), where they mediate inhibitory actions

of 5-HT (Andrade and Nicoll, 1987; Blier and de Montigny, 1987; Innis and Aghajanian, 1987; Araneda and Andrade, 1991; Puig *et al.*, 2005).

5-HT_{1A} receptors play a major role in the antidepressant action of selective 5-HT re-uptake inhibitors and other 5-HT-enhancing drugs. 5-HT_{1A} autoreceptor activation by the increased extracellular 5-HT induced by re-uptake or MAO inhibition in the median and dorsal raphe nuclei (DR) limits the neurochemical and clinical actions of these antidepressant drugs (Artigas *et al.*, 1996; 2001). On the other hand, the activation of postsynaptic 5-HT_{1A} receptors is a characteristic of several types of antidepressant drugs (Haddjeri *et al.*, 1998; Blier and Ward, 2003).

Although no 5-HT_{1A} receptor subtypes have been identified, 5-HT_{1A} receptor subpopulations exhibit contrasting properties. Thus, 5-HT_{1A} agonists such as 8-OH-DPAT and

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azapirones, preferentially activate raphe somatodendritic 5-HT_{1A} autoreceptors (Sprouse and Aghajanian, 1987; see Blier and Ward, 2003 for review). This regional selectivity has been interpreted as resulting from the existence of a receptor reserve in the raphe (Meller *et al.*, 1990; Cox *et al.*, 1993), which limits the potential antidepressant usefulness of existing 5-HT_{1A} agonists. Indeed, their administration first elicits a loss of 5-hydroxytryptaminergic tone on all other postsynaptic receptors, due to activation of somatodendritic 5-HT_{1A} autoreceptors, which also results in insufficient activation of postsynaptic 5-HT_{1A} receptors. However, 5-HT_{1A} agonists may be useful as adjuvant therapies for the treatment of negative symptoms and cognitive deficits in schizophrenia as they increase dopamine (DA) release in prefrontal cortex (PFC) as a result of the activation of postsynaptic 5-HT_{1A} receptors (Millan, 2000; Rollema *et al.*, 1997; 2000; Ichikawa *et al.*, 2001; Díaz-Mataix *et al.*, 2005). Clinical support for this assertion has been generated using the partial agonist tandospirone to improve cognitive function in schizophrenia patients treated with antipsychotics (Sumiyoshi *et al.*, 2001a, b; see Meltzer and Sumiyoshi, 2008 for review).

F15599 (3-chloro-4-fluorophenyl-(4-fluoro-4-[(5-methylpyrimidin-2-ylmethyl)-amino]-methyl)-piperidin-1-yl)-methanone) is a novel 5-HT_{1A} receptor agonist with >1000-fold selectivity for this receptor compared with other known monoamine receptors, transporters and enzymes (Newman-Tancredi *et al.*, 2009). F15599 is highly effective in the forced swim test, a measure of antidepressant-like properties (ED₅₀-100 µg·kg⁻¹ p.o.) (Assié *et al.*, 2010) suggesting a strong postsynaptic action. Moreover, it shows procognitive activity [attenuation of phencyclidine (PCP)-induced deficits in working memory] at 160 µg·kg⁻¹ i.p. (Auclair *et al.*, 2007; Pierre Fabre, unpublished observations). However, while the *in vitro* and *ex vivo* actions of F15599 reveal an agonist profile at 5-HT_{1A} receptors located in different brain regions that distinguish it from other agonists and chemical congeners (Buritova *et al.*, 2009; Newman-Tancredi *et al.*, 2009), nothing is known about its auto/heteroreceptor selectivity *in vivo*. Hence, in the present study the *in vivo* effect of F15599 on pre- and postsynaptic 5-HT_{1A} receptors was examined using single unit extracellular recordings and microdialysis in rat brain.

Methods

Electrophysiological studies

Animals. Male albino Wistar rats (230–300 g; Iffa Credo, Lyon, France) were kept in a controlled environment (12 h light–dark cycle and 22 ± 2°C room temperature) with food and water provided *ad libitum*. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee. Stereotaxic co-ordinates (in mm) were taken from bregma and duramater according to the atlas of Paxinos and Watson (1998). The total number of rats used in the electrophysiological experiments was 50.

Single unit recordings. We examined the responses elicited by F15599 on 5-hydroxytryptaminergic neurones of the DR and on pyramidal neurones of the medial PFC (mPFC). For

5-hydroxytryptaminergic neurones, recordings were made as described in Celada *et al.* (2001) and Romero *et al.* (2003). Pyramidal neurones were recorded as described in Puig *et al.* (2003) and Kargieman *et al.* (2007). Rats were administered chloral hydrate (400 mg·kg⁻¹ i.p.) and positioned in a David Kopf stereotaxic frame. Thereafter, chloral hydrate was continuously administered i.p. at 50–70 mg·kg⁻¹·h⁻¹ using a perfusion pump.

Neurones were recorded extracellularly with glass micropipettes filled with 2 M NaCl. Impedance was between 6 and 12 MΩ. Single unit recordings were amplified with a Neurodata IR283 (Cygnus Technology Inc., Delaware Water Gap, PA), postamplified and filtered with a Cibertec amplifier and computed on-line using a DAT 1401plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK). Local field potentials (LFP) were obtained by on-line band-pass filtering the signal from the recording electrode, as described (Kargieman *et al.*, 2007).

For recordings in the DR, a burr hole was drilled over lambda and the sagittal sinus was ligated, cut and deflected. Descents were carried out along the midline. 5-HT neurones were typically recorded 4.7–6.6 mm below the brain surface and were identified according to previously described electrophysiological criteria (Wang and Aghajanian, 1977).

Pyramidal neurones in the mPFC were recorded as follows. Bipolar stimulating electrodes were implanted in the VTA (AP –5.8, L –0.4, DV –8.2) for the antidromic stimulation of pyramidal neurones projecting to midbrain. All recorded units were identified as pyramidal neurones by antidromic activation from VTA and collision extinction with spontaneously occurring spikes as described previously (Puig *et al.*, 2003). 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. Basal firing activity was recorded for at least 5 min and then, one or increasing drug doses were administered i.v. every 3 min, followed by the 5-HT_{1A} receptor antagonist (±)WAY100635 when appropriate.

At the end of the experiments, rats were killed by an anaesthetic overdose. The placement of the stimulating electrodes was verified histologically. After being perfused with 10% formalin solution (Sigma), the brains were post-fixed, coronally sectioned (80 µm) and stained with Neutral Red. The data from rats with stimulating electrodes outside VTA were not included in the analysis.

Data analysis. Changes in discharge rate were quantified by averaging the values in the third minute after each drug injection. Neurones were considered to respond to drugs if firing rate was altered ±30% from baseline. Burst analysis was carried out using the method of Laviolette *et al.* (2005).

Power spectra were constructed using Fast Fourier Transformations (FFT) of 1 min signal intervals (same as discharge rate) with a resolution of 0.3 Hz (FFT size of 8192). Data are given as area under the curve (AUC) of the power spectrum between 0.3 and 4 Hz, expressed as percentage of pre-drug values.

Drug effects were assessed using Student's *t*-test or one-way ANOVA for independent or repeated measures, as appropriate. Data are expressed as the mean ± SEM. Statistical significance has been set at the 95% confidence level.

Microdialysis studies

Animals. Male Sprague-Dawley rats (OFA, Charles River, France), weighing 240–260 g upon arrival were group housed (three rats per cage), under controlled conditions (12/12 light/dark cycle: lights on 07h00min; ambient temperature $21 \pm 1^\circ\text{C}$; humidity $55 \pm 5\%$), with rat food (A04, SAFE, Augy, France) and filtered (0.2 μm pore diameter) tap water freely available. Animals were housed and tested in an Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC Int.)-accredited facility in strict compliance with all applicable regulations and the protocols were carried out in compliance with French regulations and with local Ethical Committee guidelines for animal research. The total number of rats used in the microdialysis experiments was 145.

Microdialysis procedure. Rats were anaesthetized with chloral hydrate (400–500 $\text{mg}\cdot\text{kg}^{-1}$, i.p.) or isoflurane. A guide cannula with a dummy probe was stereotaxically implanted into the mPFC, stereotaxic coordinates: AP +3.0 mm, L +0.8 mm, DV –1.7 mm or the hippocampus: AP –4.8 mm, L +4.6 mm, DV –4.6 mm, from bregma and skull surface. Following surgery and recovery from anaesthesia, animals were returned to their home cages. At the end of the day, each rat was placed in a microdialysis cage. On the following day, the dummy probe was replaced by a microdialysis probe (3 mm length, 0.5 mm diameter, CMA, Microdialysis AB). The probe was continuously perfused (1.1 $\mu\text{L}\cdot\text{min}^{-1}$) with artificial CSF (aCSF) containing 1 μM citalopram for the measurement of 5-HT. Samples were collected every 20 min with the first four samples used for baseline. For the experiments involving systemic administration of the compounds, saline or (\pm)WAY100635 was injected s.c., followed by i.p. administration of saline or F15599 40 min later. For the experiments involving local perfusion, saline was injected s.c and 40 min later, F15599 was added to the perfusion medium for the concentration-response experiment. For the antagonism (\pm)WAY100635 (or aCSF) was delivered through the dialysis probe and 40 min later, F15599 was added to the perfusion medium. Samples were collected for 140 min after administration or beginning of the perfusion of the agonist. At the end of the experiment, rats were killed by anaesthetic overdose (pentobarbital 160 $\text{mg}\cdot\text{kg}^{-1}$, i.p.) and the brain was removed, frozen and cut in a cryomicrotome (Jung Frigocut 2800) to verify the placement of the probe.

Analytical procedure. Analysis of 5-HT and DA was performed by HPLC, as described by Assié *et al.* (2005). The HPLC column was a reverse phase (Merck, Lichrocart 125-2, Superspher 100 RP-18). The mobile phase was pumped at 0.2 $\text{mL}\cdot\text{min}^{-1}$ (HPLC-118 solvent module, Beckman Coulter Inc., Fullerton, CA, USA). DA or 5-HT was electrochemically detected with a glassy carbon working electrode kept at +0.64 V versus Ag/AgCl reference electrode (DECADE detector, ANTEC Leyden BV, Leiden, the Netherlands). Data were acquired using a Beckman 32 Karats system. Concentrations of DA or 5-HT in the brain dialysates were estimated by comparing peak areas with those of external standards of known concentration of each neurotransmitter. The limit of

detection (three times baseline noise) was approximately 1 $\text{fmol}\cdot 20\ \mu\text{L}^{-1}$ sample.

Data analysis. Dialysate 5-HT or DA concentrations are given as $\text{fmol}\cdot 20\ \text{min}^{-1}$ fraction and expressed in the figures as percentages of baseline. Statistical analysis was carried out using repeated-measures ANOVA using treatment and time as variables. The mean % AUC values for the 140 min period after administration of F15599 were analysed by one-way ANOVA followed by Dunnett's test (GraphPad Prism, San Diego, CA, USA). Mean % AUC values were used to estimate the ED_{50} value of F15599 by linear interpolation between the two doses that increased DA or decreased 5-HT levels with amounts bordering 50% (vehicle control as 0% and maximal effect of the compound as 100%). The antagonist effect was analysed by comparing the overall effect of the agonist with its effect at the same dose in the presence of (\pm)WAY100635 using Student's *t*-test.

Drugs. F15599 (3-chloro-4-fluorophenyl-(4-fluoro-4-[(5-methyl-pyrimidin-2-yl)methyl]-amino)-methyl)-piperidin-1-yl)-methanone) tosylate salt was from Pierre Fabre Medicament. (\pm)WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide) hydrochloride was from RBI (Natick, MA). Stock solutions were prepared and aliquots were stored at -20°C . Working solutions were prepared daily by dilution in saline at the appropriate concentrations and injected i.v. (up to 1 $\text{mL}\cdot\text{kg}^{-1}$) through the femoral vein. Doses are expressed as weight of free bases.

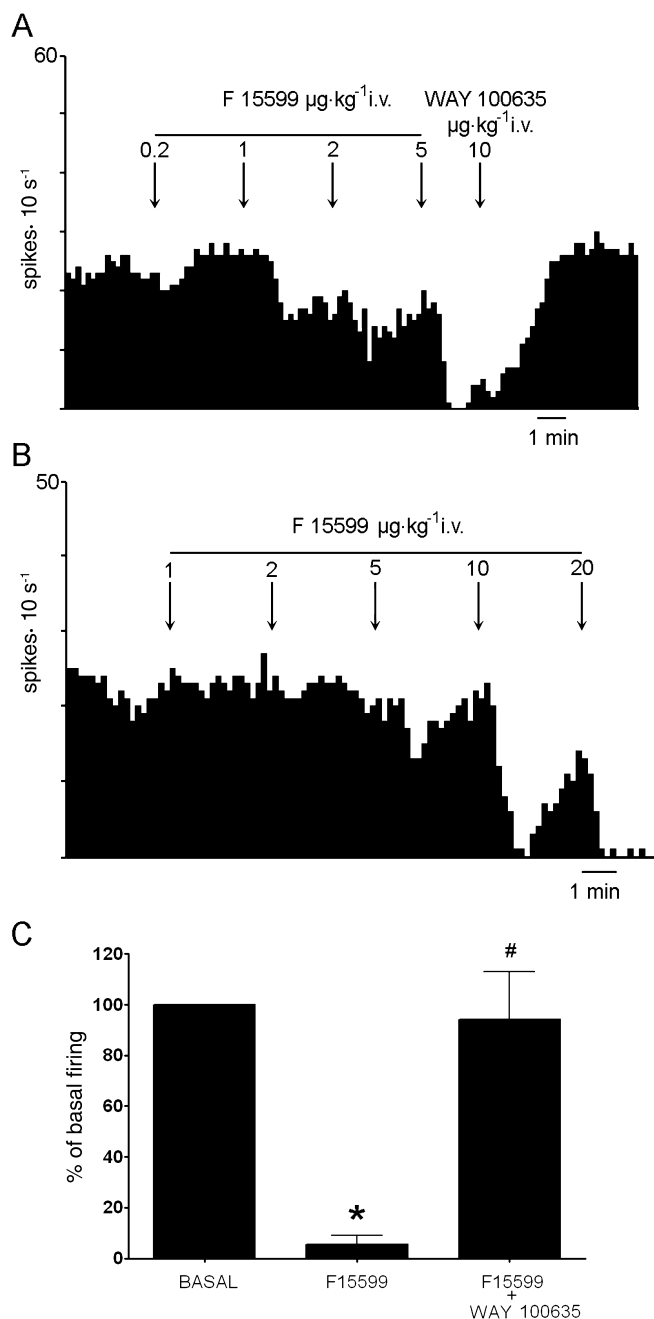
Chloral hydrate was purchased from Acros (Geel, Belgium), pentobarbital sodium from Ceva Santé Animale (Libourne, France), isoflurane from Baxter SA (Paurenas, France). Citalopram hydrobromide was kindly donated by Lundbeck (Copenhagen, Denmark). (\pm)WAY100635 and F15599 (fumarate salt) were synthesized at the Centre de Recherche Pierre Fabre. The doses of compounds are expressed as the weight of the free base. (\pm)WAY100635 and F15599 were dissolved in distilled water; the injection volume was 0.1 $\text{mL}\cdot\text{kg}^{-1}$.

All drug and molecular target nomenclature conforms to *The British Journal of Pharmacology's* 'Guide to Receptors and Channels' (Alexander *et al.*, 2008).

Results

Effect of F15599 on the activity of DR 5-hydroxytryptaminergic neurones

We examined the effect of F15599 on 5-hydroxytryptaminergic neurones ($n = 11$; one per rat) of the DR in the range 0.2–20 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. At the higher doses, F15599 decreased the activity of all 5-HT neurones. Most neurones were fully inhibited after the administration of 5–10 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. (corresponding to 8.2–18.2 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. cumulative dose). Figure 1 shows examples of the suppressant effect of F15599 on DR 5-HT neurones. Figure 2A shows the dose–response relationship for all neurones examined. One-way ANOVA indicated a significant effect of F15599 ($F_{5,46} = 13.5$; $P < 0.00001$) with significant post-hoc differences (Newman–Keuls test) at the doses of 8.2 and 18.2 $\mu\text{g}\cdot\text{kg}^{-1}$ vs. baseline. One-way



Figures 1 (A, B) Integrated firing rate histogram showing the effect of the i.v. administration of F15599 on the activity of DR 5-HT neurones recorded extracellularly in chloral hydrate anaesthetized rats. The activity of these neurones was completely suppressed at 8.2 and 38 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. (cumulative doses) respectively. (A) Shows the reversal of the inhibition induced by F15599 by the subsequent administration of the 5-HT_{1A} receptor antagonist (\pm)WAY100635. (C) Histogram showing the reversal by (\pm)WAY100635 (5–50 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v.) of the maximal effect on DR 5-HT cell firing induced by F15599. Data are means \pm SEM of five neurones. * $P < 0.05$ vs. baseline; # $P < 0.05$ vs. maximal inhibition; Newman–Keuls test post-ANOVA.

repeated measures ANOVA of the subset of neurones that received all F15599 doses ($n = 7$) also revealed a significant effect of treatment ($F_{5,30} = 19.1$; $P < 0.00001$) with significant post-hoc differences (Newman–Keuls test) at the doses of 8.2 and 18.2 $\mu\text{g}\cdot\text{kg}^{-1}$ vs. baseline. The minimal effective dose for the inhibition of DR 5-HT neurones was 8.2 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v.

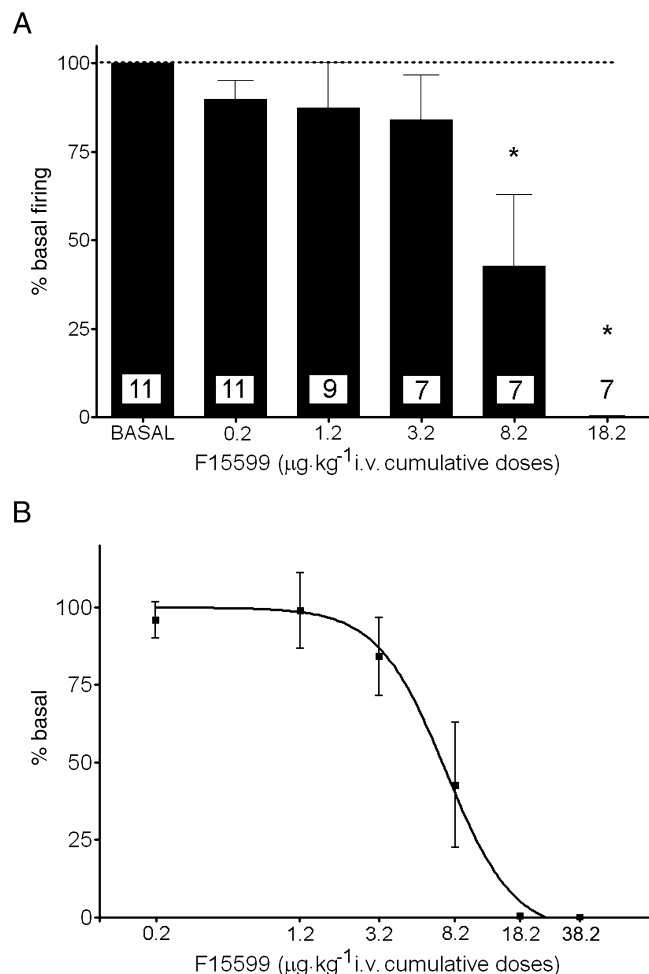


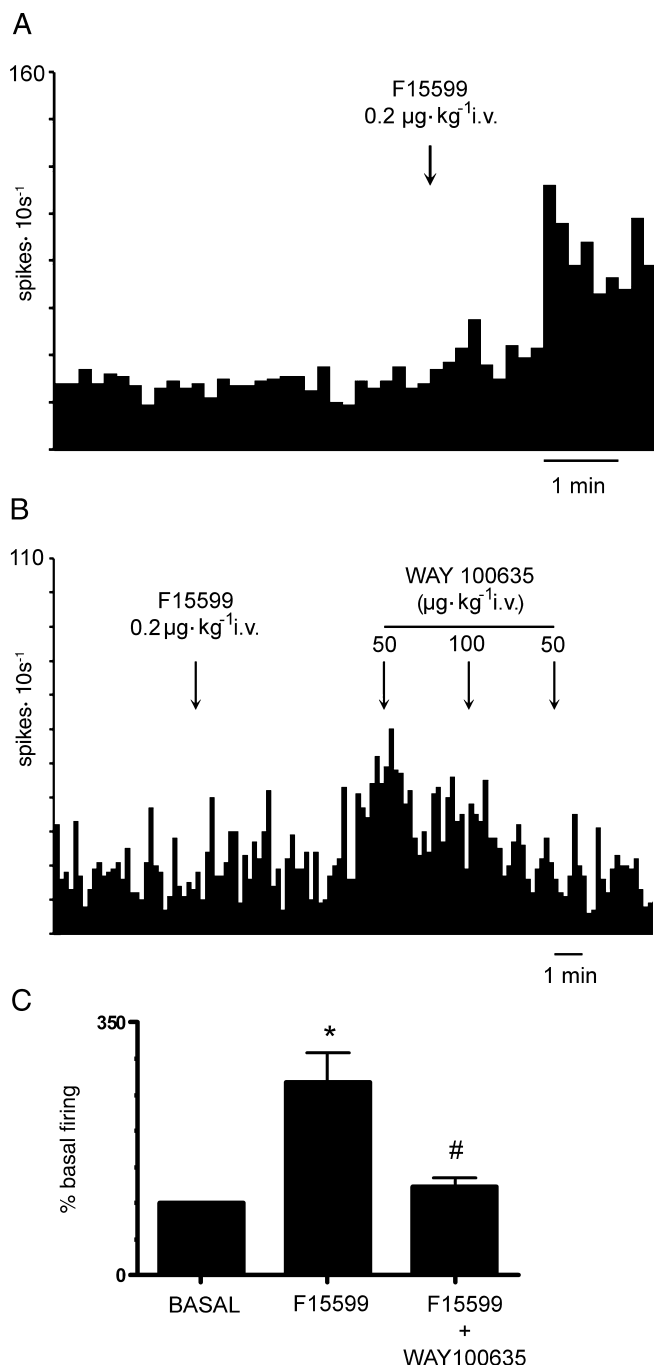
Figure 2 (A) Dose–response relationship of the effect of F15599 (0.2–38.2 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v., cumulative doses) on the discharge rate of 5-HT neurones in the DR. Data (means \pm SEM) are expressed as percentages of baseline. The number of neurones in each group is shown in the columns. The complete suppression of firing at the higher dose (38.2 $\mu\text{g}\cdot\text{kg}^{-1}$) is not shown. (B) Semi-log plot between cumulative F15599 dose (0.2–38.2 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v.) and the suppressant effect on DR 5-HT neurones receiving all F15599 doses ($n = 7$). The ED_{50} was 7.3 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. * $P < 0.05$ vs. baseline, Newman–Keuls test post-ANOVA.

ED_{50} values for the suppression of 5-HT cell firing have been calculated for the neurones of rats receiving all F15599 doses ($n = 7$) and for all rats receiving one or more doses of F15599 ($n = 11$). The corresponding values (given as cumulative doses) are 7.3 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. (all doses) and 7.4 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v. (all rats) (Figure 2B).

The inhibition produced by F15599 was reversed in all cases examined ($n = 5$) by the subsequent administration of the selective 5-HT_{1A} receptor antagonist (\pm)WAY100635 (5–50 $\mu\text{g}\cdot\text{kg}^{-1}$ i.v.) ($F_{2,8} = 24.1$; $P < 0.0005$) (Figure 1A and C).

Effect of F15599 on the activity of pyramidal neurones of the mPFC

After pilot experiments had revealed a marked enhancement of the firing rate of pyramidal neurones in the mPFC at the lowest doses used for DR recordings (0.2 $\mu\text{g}\cdot\text{kg}^{-1}$), two sets of



Figures 3 (A, B) Examples of the increase in discharge rate produced in mPFC pyramidal neurones by the i.v. administration of 0.2 µg·kg⁻¹ F15599. Note the reversal of F15599 effect by the subsequent administration of (±)WAY100635 (50–200 µg·kg⁻¹) in (B). (C) Shows the average effect produced by 0.2 µg·kg⁻¹ F15599 and its reversal by the subsequent administration of (±)WAY100635 (*n* = 5). **P* < 0.01 vs. basal; #*P* < 0.01 vs. F15599.

experiments were carried out. In the first experiment we examined the effect of this dose of F15599 and its reversal by (±)WAY-100635. A total of 15 neurones were recorded (1 neurone per rat) of which 11 were excited, two were inhibited and two remained unaltered by F15599 (Figure 3). The overall effect of this dose was a significant increase in the discharge

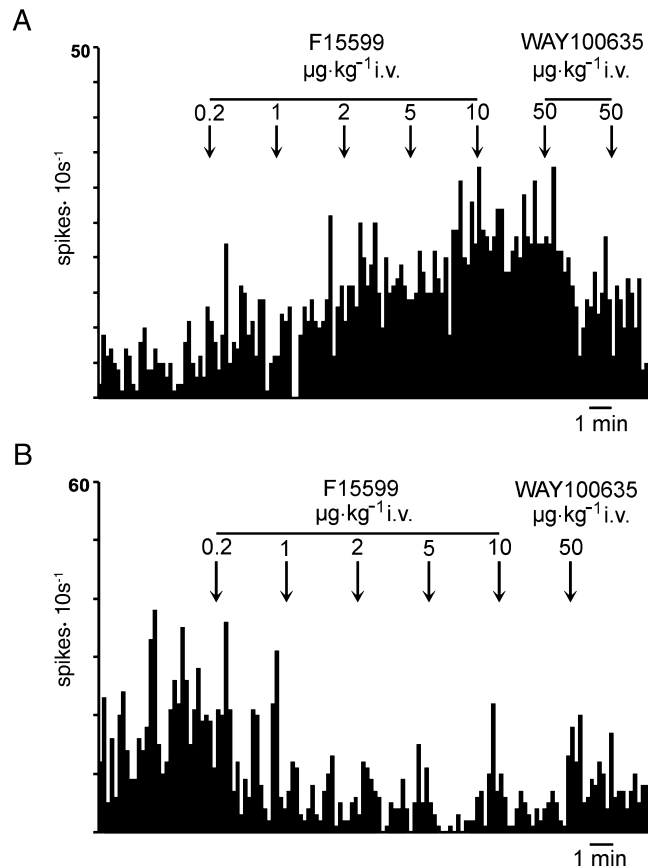


Figure 4 Examples of the effect of cumulative doses of F15599 on the discharge rate of pyramidal neurones of the mPFC. (A) This neurone was excited by F15599 at all doses examined. The maximal effect was attained after the administration of 10 µg·kg⁻¹ (18.2 µg·kg⁻¹ i.v., cumulative dose) and was antagonized by the subsequent administration of (±)WAY100635 (50–100 µg·kg⁻¹ i.v.). (B) A pyramidal neurone inhibited by low doses (0.2–1 µg·kg⁻¹ i.v.) of F15599 with an apparent maximal inhibition at 8.2 µg·kg⁻¹ i.v.; this inhibition was also reversed by (±)WAY100635.

rate to $210 \pm 33\%$ of baseline (*P* < 0.005, Student's paired *t*-test; *n* = 15). We attempted to reverse the action of 0.2 µg·kg⁻¹ F15599 in a subset of five neurones. One-way ANOVA indicated a significant effect of the treatment ($F_{2,8} = 11.45$; *P* < 0.005) with significant post-hoc differences (Newman–Keuls) between F15599 and baseline and between F15599 and F15599+(±)WAY100635 (Figure 3C).

In a second set of experiments we examined the response of pyramidal neurones to increasing doses of F15599 (0.2–18.2 µg·kg⁻¹ i.v., cumulative doses). From the pyramidal neurones examined (*n* = 16), 11 were excited and five were inhibited by F15599, with an overall excitatory effect of F15599. Figure 4 shows representative examples of neurones excited and inhibited by increasing doses of F15599. The minimal effective dose for the activation of mPFC pyramidal neurones was 0.2 µg·kg⁻¹ i.v.

Data from the neurones used in both experiments were pooled and analysed together. For neurones excited by F15599, ANOVA indicated a significant effect of the treatment ($F_{5,63} = 9.79$, *P* < 0.00001) with significant post-hoc (Newman–Keuls) differences between the doses (i.v.) of 0.2 µg·kg⁻¹, 2 µg·kg⁻¹, 8.2 µg·kg⁻¹ and 18.2 µg·kg⁻¹ vs. baseline (Figure 5A).

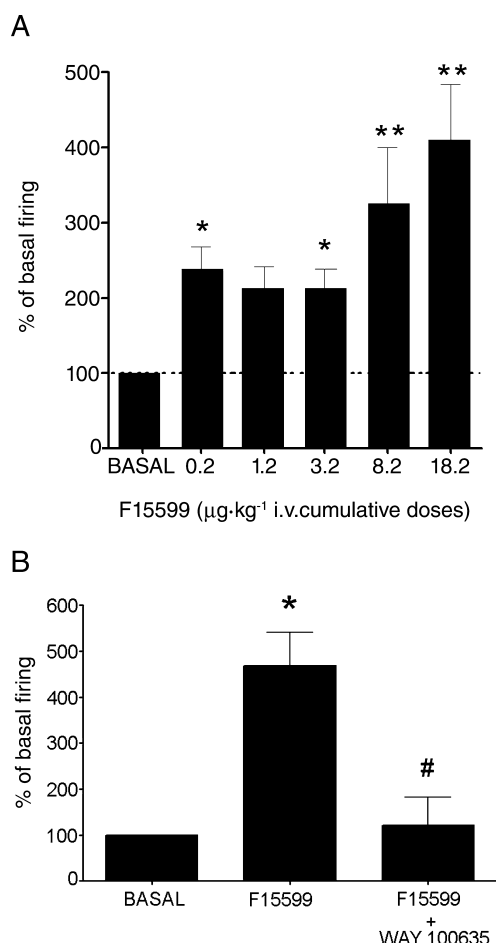


Figure 5 (A) Histogram showing the excitatory effect produced by F15599 on the firing rate of mPFC pyramidal neurones. Data correspond to neurones in the two experiments conducted (see text) which are 22, 20, 10, 7, 6 and 5 neurones for baseline, 0.2, 1.2, 3.2, 8.2 and 18.2 μg·kg⁻¹ i.v. F15599 (cumulative doses) respectively. (B) Histogram showing the reversal by the 5-HT_{1A} receptor antagonist WAY-100635 of the maximal excitatory effect of F15599 on mPFC pyramidal neurones ($n = 4$). * $P < 0.05$ vs. baseline; ** $P < 0.001$ vs. baseline; # $P < 0.02$ vs. maximal effect of F15599; Newman–Keuls test post-ANOVA.

As observed for the 0.2 μg·kg⁻¹ dose, F15599-induced excitations at higher doses (average 15.7 μg·kg⁻¹ i.v.) were also reversed by the subsequent administration of (±)WAY100635 (50–100 μg·kg⁻¹ i.v.) in all cases examined ($F_{2,6} = 12.14$, $P < 0.01$, $n = 4$) (Figure 5B).

The maximal inhibitory effect of F15599 was noted at a dose of 18.2 μg·kg⁻¹ ($50 \pm 26\%$ of baseline). ANOVA revealed a non-significant effect of the treatment ($F_{5,19} = 1.55$; $P = 0.23$), possibly due to the lower number of neurones inhibited compared with those excited by F15599.

F15599 had a minor impact on the burst firing of activated mPFC pyramidal neurones. From all the variables examined, only the number of spikes per burst was significantly affected, but only with a small effect size ($c. 10\%$).

Effect of F15599 on slow cortical oscillations

The non-competitive NMDA receptor antagonist PCP and the preferential 5-HT_{2A} receptor agonist 1-[2,5-dimethoxy-4-

iodophenyl-2-aminopropane] (DOI) increase PFC pyramidal cell discharge and reduce low frequency (delta) cortical oscillations (0.3–4 Hz) (Kargieman *et al.*, 2007; Celada *et al.*, 2008). Given the increase in pyramidal discharge induced by F15599, we examined whether this effect was accompanied by an alteration of low frequency oscillations.

Spikes were discharged in synchrony with active phases of LFP under baseline conditions and after F15599 administration. This occurred when spikes were fired as single events or as spike trains (not shown). However, the administration of F15599 did not alter the power of low frequency cortical oscillations (0.3–4 Hz) in the whole dose range examined (n.s., one-way ANOVA).

Extracellular concentrations of 5-HT in the ventral hippocampus

The mean basal extracellular level of 5-HT in ventral hippocampus was 41.6 ± 2.7 fmol·20 μL⁻¹ ($n = 36$). None of the treatment groups differed from controls (34.5 ± 5.9 fmol·20 μL⁻¹) although the F15599 160 μg·kg⁻¹ group (57.6 ± 9.2 fmol·20 μL⁻¹) was significantly greater than the WAY+F15599 630 μg·kg⁻¹ group (27.7 ± 6.6 fmol·20 μL⁻¹; $P < 0.05$) (post-hoc analysis).

Administration of F15599 (40–2500 μg·kg⁻¹) dose-dependently decreased extracellular 5-HT during the 140 min period after injection (Figure 6, top left panel). Two-way ANOVA for repeated measures indicated a significant effect of treatment ($F_{6,29} = 23.3$, $P < 0.0001$), time ($F_{6,165} = 6.24$, $P < 0.0001$) and treatment-time interaction ($F_{36,165} = 2.02$, $P = 0.02$). Post-hoc analysis by the contrasts method indicated a significant global effect at 160 ($P = 0.0016$), 630 and 2500 μg·kg⁻¹ ($P < 0.0001$). (±)WAY100635 (160 μg·kg⁻¹), when given alone, did not significantly alter extracellular 5-HT (Figure 6 left middle panel). The effects of 630 μg·kg⁻¹ F15599 were significantly reversed by (±)WAY100635 ($P < 0.0001$).

The effect of F15599, analysed by the mean AUC data for the 140 min period after administration, was significant at the doses of 160, 630, 2500 μg·kg⁻¹, compared with controls ($P < 0.05$, $F_{4,25} = 24.48$, one-way ANOVA followed by Dunnett's test; Figure 6, bottom panel). The ED₅₀ value of F15599, calculated by linear interpolation, was 240 μg·kg⁻¹. (±)WAY100635 significantly reversed the effects of F15599 630 μg·kg⁻¹ (Student's *t*-test).

Extracellular concentration of DA in the mPFC

Systemic administration of F15599. The mean basal extracellular level of DA in mPFC was 6.00 ± 0.36 fmol·20 μL⁻¹ ($n = 45$). One-way ANOVA indicated no significant difference between groups $F_{8,44} = 1.15$, $P = 0.36$.

Systemic administration of F15599 (10–2500 μg·kg⁻¹, i.p.) dose-dependently increased extracellular DA during the 140 min period after injection with a maximal increase of approximately 200% of basal level at the dose of 40 μg·kg⁻¹ (Figure 6 top right panel). Two-way ANOVA for repeated measures indicated a significant effect of treatment ($F_{8,36} = 13.2$, $P < 0.0001$), time ($F_{6,216} = 2.97$, $P = 0.0084$) and treatment × time interaction ($F_{48,216} = 1.70$, $P = 0.006$). The method of contrasts indicated a significant global effect at doses 40–2500 μg·kg⁻¹ ($P < 0.0001$). (±)WAY100635, when given alone, did not significantly alter extracellular DA (Figure 6 right middle panel).

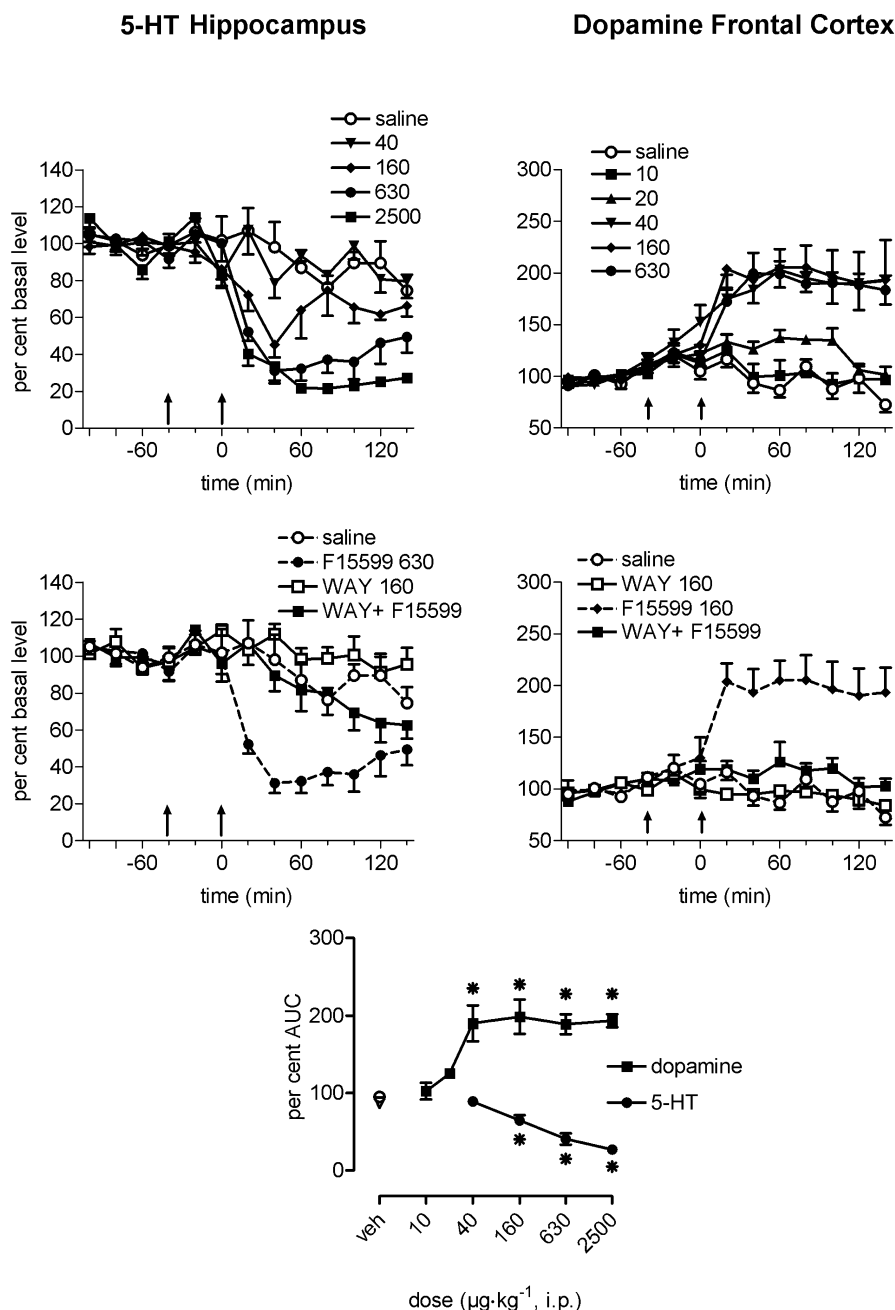


Figure 6 Effects of i.p. administration of F15599 on extracellular 5-HT in the ventral hippocampus (left panels) and on dopamine levels in the medial prefrontal cortex (right panels) of freely moving rats. Data are expressed as a percentage of the mean absolute amount of neurotransmitter in the four samples collected before treatment. The first arrow indicates injection of saline or (\pm)WAY100635 ($160 \mu\text{g}\cdot\text{kg}^{-1}$ s.c., middle panels), the second arrow indicates administration of saline or F15599. The bottom panel represents mean area under the curve (AUC) for 140 min after administration of the compound. Results are mean \pm SEM of five to seven animals per group. Note: to avoid overloading the top-right panel, the dose of $2500 \mu\text{g}\cdot\text{kg}^{-1}$ is not shown. AUC dose-response data were analysed using one-way ANOVA followed by Dunnett's test (* $P < 0.05$ compared with corresponding control animals).

The effects of F15599 ($160 \mu\text{g}\cdot\text{kg}^{-1}$) were significantly reversed by (\pm)WAY100635 ($160 \mu\text{g}\cdot\text{kg}^{-1}$) ($P < 0.0001$).

The overall increase in DA, analysed by the AUC data for the 140 min period after drug injection, was significant from 40 – $2500 \mu\text{g}\cdot\text{kg}^{-1}$ F15599 ($P < 0.05$, $F_{7,37} = 10.35$, one-way ANOVA followed by Dunnett's test; Figure 6 bottom panel). The ED_{50} value of F15599, calculated by linear interpolation was $30 \mu\text{g}\cdot\text{kg}^{-1}$. (\pm)WAY100635 significantly reversed the effects of F15599 $160 \mu\text{g}\cdot\text{kg}^{-1}$ (Student's t -test).

Local perfusion of F15599 through the dialysis probe. The mean basal extracellular level of DA was $7.19 \pm 0.35 \text{ fmol}\cdot 20 \mu\text{L}^{-1}$ ($n = 57$). One-way ANOVA indicated no significant differences between groups for the concentration-response data ($F_{5,22} = 1.29$, $P = 0.32$) nor for the antagonism experiments ($F_{5,33} = 0.89$, $P = 0.50$).

Local perfusion of F15599 through the dialysis probe in the mPFC (1 – $1000 \mu\text{M}$) increased extracellular DA in a concentration-dependent manner during the 140 min period

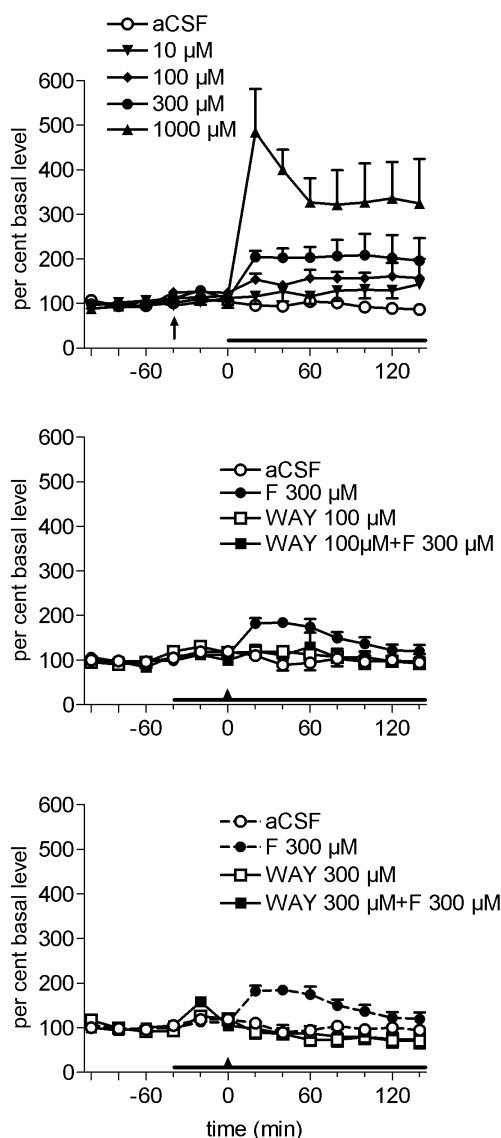


Figure 7 Effects of local perfusion of F15599 in the medial prefrontal cortex on extracellular dopamine levels and antagonism by (±)WAY100635. Data are expressed as a percentage of the mean absolute amount of dopamine in the four samples collected before treatment. Top panel: the effects of different concentrations of F15599 administered through the dialysis probe from time 0 (bar), the arrow indicates saline administration (s.c.). Middle and bottom panels: the antagonist effects of (±)WAY100635 100 and 300 µM, administered through the dialysis probe, on the increase in extracellular dopamine induced by 300 µM F15599. The bar indicates the presence of compounds in the perfusion medium, the arrow indicates the addition of F15599 to the perfusion medium. Results are mean ± SEM of three to eight animals per group.

examined. The maximal increase was approximately 470% of basal level at 1000 µM (Figure 7, top panel). The two-way ANOVA for repeated measures indicated a significant effect of treatment ($F_{5,17} = 6.02$, $P = 0.0022$) but no significant effect of time ($F_{6,102} = 0.87$, $P = 0.52$) nor treatment-time interaction ($F_{30,102} = 1.10$, $P = 0.36$). Post-hoc analysis by the contrasts method indicated a significant global effect at the concentrations 300 µM ($P = 0.048$) and 1000 µM ($P = 0.0001$).

(±)WAY100635 (100 and 300 µM), perfused through the dialysis probe 40 min before and together with F15599 300 µM prevented the increase in extracellular DA induced by F15599 (Figure 7, middle and bottom panels). The two-way ANOVA for repeated measures indicated a significant effect of treatment ($F_{5,28} = 5.64$, $P = 0.001$) and time ($F_{6,168} = 3.48$, $P = 0.0029$) but not a significant interaction ($F_{30,168} = 1.34$, $P = 0.12$). Post-hoc analysis by the contrasts method indicated a significant global effect at 300 µM F15599 ($P = 0.0036$) and a significant antagonist effect of (±)WAY100635 100 µM ($P = 0.018$) and 300 µM ($P = 0.0003$).

Discussion

The electrophysiological results from the present study indicate that the selective 5-HT_{1A} receptor agonist F15599 has a preferential action on cortical pyramidal compared with 5-hydroxytryptaminergic neurones in the rat brain. This regional selectivity is also supported by microdialysis experiments showing a greater sensitivity of cortical DA vs. hippocampal 5-HT output to the effects of F15599. Based on current knowledge on 5-HT_{1A} receptor function, this regional selectivity may be attributed to the preferential activation by F15599 of postsynaptic 5-HT_{1A} receptors. Overall, F15599 appears to be about one order of magnitude more active at postsynaptic than at somatodendritic 5-HT_{1A} autoreceptors.

To the best of our knowledge, F15599 is the first selective 5-HT_{1A} agonist shown to have a preferential postsynaptic action. F15599 increased pyramidal discharge in mPFC to ~200% of baseline at the lowest dose assayed, 0.2 µg·kg⁻¹ i.v., whereas most 5-hydroxytryptaminergic neurones were only inhibited by the administration of 8.2–18.2 µg·kg⁻¹ i.v. (cumulative doses). Both F15599-induced responses were due to the exclusive activation of 5-HT_{1A} receptors, as indicated by the *in vitro* selectivity of the compound and by the reversal of its actions by (±)WAY100635.

Because mPFC 5-HT_{1A} receptors control DA neurones in the VTA and are involved in the mechanism of action of atypical antipsychotic drugs (Rollema *et al.*, 1997; Millan, 2000; Bantick *et al.*, 2001; Díaz-Mataix *et al.*, 2005), we examined the actions of F15599 on the activity of pyramidal neurones in the mPFC projecting to VTA.

Given the inhibitory nature of 5-HT_{1A} receptors on neuronal activity (Andrade and Nicoll, 1987; Innis and Aghajanian, 1987; Araneda and Andrade, 1991; Puig *et al.*, 2005), the increase in mPFC pyramidal discharge elicited by the systemic administration of 5-HT_{1A} receptor agonists (Borsini *et al.*, 1995; Hajós *et al.*, 1999; Díaz-Mataix *et al.*, 2006; this work) may seem paradoxical, as (i) microiontophoretic application of these compounds at large concentrations in mPFC inhibits the discharge of putative pyramidal neurones (Ashby *et al.*, 1994; Rueter and Blier, 1999); (ii) high doses of 8-OH-DPAT inhibit mPFC pyramidal neurones (Hajós *et al.*, 1999); (iii) physiological release of 5-HT inhibits the activity of mPFC pyramidal neurones through 5-HT_{1A} receptors (Hajós *et al.*, 2003; Amargós-Bosch *et al.*, 2004; Puig *et al.*, 2005); and (iv) there is *in vitro* evidence of inhibition of mPFC pyramidal cells by 5-HT_{1A} agonists (Tanaka and North, 1993). Conceivably, this excitatory effect may result from an action on cortical

networks involving the activation of 5-HT_{1A} receptors in local GABAergic interneurons (Santana *et al.*, 2004) and the subsequent disinhibition of pyramidal neurones. Although this possibility requires further testing, it is supported by the existence of molecular and functional differences between neurotransmitter receptors expressed by GABAergic and glutamatergic cells (Jonas *et al.*, 1994). Likewise, differences in the composition and response of NMDA receptor subtypes have been shown (Hollmann and Heinemann, 1994; Plant *et al.*, 1997) and a differential response of pyramidal and GABAergic neurones in mPFC to MK-801 have been reported (Homayoun and Moghaddam, 2007). Although there are no molecular differences in the structure of 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors, functional differences may exist in the coupling to different transduction mechanisms, to the cellular receptor reserve or in cellular sensitivity to the opening of K⁺ channels, among others. Hence, the response of pyramidal neurones to 8-OH-DPAT in rat mPFC is changed by local blockade of GABA_A receptors (Lladó-Pelfort *et al.*, 2009). Further work is required to determine whether this also occurs for F15599.

Electrophysiological studies comparing the sensitivity of auto- (raphe) and postsynaptic (hippocampus) 5-HT_{1A} receptors have previously indicated a greater sensitivity of the former receptors to the inhibitory actions of 5-HT and exogenous agonists (Sprouse and Aghajanian, 1987, 1988; Dong *et al.*, 1997; Blier and Ward, 2003). Using similar experimental approaches, the selective 5-HT_{1A} agonists 8-OH-DPAT and BAY × 3702 (repinotan), were found to have ED₅₀ values around 1 µg·kg⁻¹ i.v. for suppression of 5-HT cell firing (Blier and de Montigny, 1987; Dong *et al.*, 1998; Hajós *et al.*, 1999; Casanovas *et al.*, 2000; Romero *et al.*, 2003). However, similar or higher doses are required to increase the discharge of cortical neurones (Borsini *et al.*, 1995; Hajós *et al.*, 1999; Díaz-Mataix *et al.*, 2006).

This preferential selectivity for 5-HT_{1A} autoreceptors was attributed to a receptor reserve in the raphe (Meller *et al.*, 1990; Cox *et al.*, 1993). This concept was partly supported by *in vitro* observations indicating that the agonist/antagonist profile of 5-HT_{1A} receptor agents varied depending on the cellular density of 5-HT_{1A} receptors (Hoyer and Boddeke, 1993).

Delta (0.3–4 Hz) oscillations recorded extracellularly in anaesthetized animals are similar to the physiological changes in cortical activity during slow-wave sleep (Steriade *et al.*, 1993). These oscillations play an important role in the disconnection of the brain from external afferent sources, information processing and memory consolidation during sleep (Marshall *et al.*, 2006). Particular attention has been paid in recent years to slow oscillations (≤1 Hz), which appear to reflect “up” and “down” states of cortical networks (Sanchez-Vives and McCormick, 2000). This slow oscillation is a major component of delta waves. Unlike the NMDA receptor antagonist, PCP and the 5-HT_{2A} receptor agonist, DOI, which markedly suppress slow oscillatory activity in the mPFC (Kargieman *et al.*, 2007; Celada *et al.*, 2008), F15599, did not alter slow cortical oscillations despite inducing similar changes in pyramidal discharge. Thus, F15599 does not disrupt the strict temporal pattern of slow oscillatory activity seen in basal conditions. Although we did not determine whether F15599 could normalize the loss of slow oscillatory activity induced

by PCP, F15599 has been shown to attenuate the deficits in working memory induced by this drug (Auclair *et al.* 2007 and unpublished observations), a property that may be related to the divergent influence of F15599 and PCP on PFC networks. This property suggests that F15599 might be also useful as add-on therapy in the treatment of cognitive deficits in schizophrenia.

Microdialysis data also support a preferential postsynaptic action of F15599. The 5-HT and DA output in hippocampus and mPFC are used as neurochemical indicators of the activation of somatodendritic autoreceptors and postsynaptic 5-HT_{1A} receptors respectively. Some studies support the view that 5-HT release in ventral hippocampus depends on the activation of somatodendritic 5-HT_{1A} autoreceptors (Hutson *et al.*, 1989; Adell *et al.*, 1993; McQuade and Sharp, 1997). However, as the activity of DR 5-HT neurones is also dependent on mPFC 5-HT_{1A} receptor activation (Ceci *et al.*, 1994; Hajós *et al.*, 1999; Celada *et al.*, 2001), it is uncertain whether hippocampal 5-HT release may be influenced to some extent by postsynaptic 5-HT_{1A} receptor activation, as happens with that in mPFC (Casanovas *et al.*, 1999; Celada *et al.*, 2001).

On the other hand, DA output in mPFC is dependent on the activation of cortical postsynaptic 5-HT_{1A} receptors, possibly located on or close to pyramidal neurones projecting to the ventral tegmental area (Díaz-Mataix *et al.*, 2005; Bor-tolozzi *et al.*, 2007). This assertion is further supported by the increase in DA output produced by the local application of F15599 in mPFC.

F15599 was more potent at promoting DA release in mPFC (postsynaptic 5-HT_{1A} receptor-mediated effect) than at suppressing hippocampal 5-HT release (5-HT_{1A} autoreceptor effect), with calculated ED₅₀ values of 30 and 240 µg·kg⁻¹ i.p. respectively. Interestingly, despite the different strain of rats and the routes of administration, the ratio between the suppression of 5-HT release and cell firing (autoreceptor effect) was similar to that between activation of pyramidal cell firing and DA release (postsynaptic 5-HT_{1A} receptor effect).

The cellular bases responsible for this regional *in vivo* selectivity of F15599 are not fully understood. In cell lines stably expressing 5-HT_{1A} receptors, this agent preferentially stimulated the phosphorylation of extracellular signal-regulated kinase (ERK1/2) compared with other signalling pathways linked to 5-HT_{1A} receptors, such as G-protein activation, receptor internalization or inhibition of cAMP accumulation. 5-HT and (+)8-OH-DPAT displayed a different rank order of potency for these responses (Newman-Tancredi *et al.*, 2009). In addition, F15599 stimulated [³⁵S]-GTPγS binding, ERK1/2 phosphorylation and *c-fos* expression more potently in frontal cortex than in the midbrain raphe, compared with other agonists (Buritova *et al.*, 2009; Newman-Tancredi *et al.*, 2009). Thus, a distinctive ‘signalling signature’ for specific intracellular transduction responses may underlie the brain region specificity of F15599. Furthermore, in microPET experiments, [¹⁸F]-F15599 preferentially labelled frontal cortex 5-HT_{1A} receptors in rat and cat (Lemoine *et al.*, 2008; 2010), which may add to the preferential postsynaptic action of F15599.

Thus, F15599 shows a preferential postsynaptic activity in cortical vs. raphe 5-HT_{1A} receptors, a property that may underlie the effects of very low doses of this drug in animal models

of depression and cognitive function, suggesting that F15599 may overcome the existing limitations of 5-HT_{1A} receptor agonists in the treatment of psychiatric disorders.

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Conflicts of interest

None.

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