

# In Vivo Characterization of p-[<sup>18</sup>F]MPPF, a Fluoro Analog of WAY-100635 for Visualization of 5-HT<sub>1A</sub> Receptors

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**KEY WORDS** 5-HT<sub>1A</sub> receptors; autoradiography; positron emission tomography; cat

**ABSTRACT** The in vivo and ex vivo distributions and the pharmacological profile of the fluorinated phenylpiperazine derivative p-[<sup>18</sup>F]MPPF (4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-fluorobenzamido]-ethylpiperazine) were evaluated in the cat brain as a potential selective antagonist for 5-HT<sub>1A</sub> receptors using PET. After intravenous injection of p-[<sup>18</sup>F]MPPF in cats, there was a rapid accumulation of radioactivity in the brain, with 4% of the total radioactivity injected present in the brain at 4 minutes postinjection. The highest uptakes of radioactivity were observed in the hippocampus and cingulate cortex, regions known to be rich in 5-HT<sub>1A</sub> receptors, whereas lower levels of radioactivity were observed in the cerebellum. The mean ratio of radioactivity in the hippocampus to the cerebellum was 4.29 (SD = 0.21; n = 5) from 40 to 90 minutes postinjection of p-[<sup>18</sup>F]MPPF. The corresponding ratio for the cingulate cortex was 3.01 (SD = 0.16; n = 5). Specific binding in the hippocampus and the cingulate cortex was markedly reduced following injection of unlabeled WAY-100635 and pindolol but was unaffected by treatment with  $\alpha$ 1, 5-HT<sub>2</sub>, or reuptake inhibitor agents indicating reversibility and selectivity of p-[<sup>18</sup>F]MPPF binding to 5-HT<sub>1A</sub> receptors. Ex vivo autoradiographic study with p-[<sup>18</sup>F]MPPF in cat brain sections showed labeling of areas rich in 5-HT<sub>1A</sub> receptors with a regional brain distribution that closely matched that observed using PET. These results indicate that p-[<sup>18</sup>F]MPPF may be a useful candidate for noninvasive PET imaging of 5-HT<sub>1A</sub> receptors in the living human brain. **Synapse** 35:192–200, 2000. © 2000 Wiley-Liss, Inc.

## INTRODUCTION

The serotonin (5-hydroxytryptamine, 5-HT) system has been implicated in several psychiatric and neurological disorders (Cross, 1988, 1990), and is often a target for new approaches to the treatment of these disorders.

The diverse actions of 5-HT are mediated by a number of specific receptors. Pharmacological and molecular cloning techniques have identified at least 14 different subtypes of 5-HT receptors, which have been classified in seven families (5-HT<sub>1–7</sub>) on the basis of their structural homology, pharmacological profile, and predominant transduction system (Hoyer et al., 1994). Among these receptors, 5-HT<sub>1A</sub> receptors are of particular interest because of their potential role in anxiety and depression (Gozlan et al., 1983; Andrade and Nicoll, 1987). In various species, including man, 5-HT<sub>1A</sub> receptors are located predominantly in brain regions concerned with mood and anxiety (the limbic system, i.e., hippocampus, septum, amygdala) and in areas

governing temperature, feeding, and locomotion (dorsal and median raphe nuclei). Whereas 5-HT<sub>1A</sub> receptors on raphe neurons are predominantly somatodendritic (Vergé et al., 1985; Weissmann-Nanopoulos et al., 1985), those in the hippocampus and cerebral cortex are essentially postsynaptic (Vergé et al., 1986; Palacios et al., 1990).

Studies to investigate the function of 5-HT<sub>1A</sub> receptors in vivo have been hampered by the lack of selective antagonists for this receptor and have relied on the effects of selective agonists, such as 8-OH-DPAT (Gozlan et al., 1983). However, WAY-100635 [(N-(2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl)-N-(2-pyridyl)-cyclohexanecarboxamide)] was recently described as an antagonist with high affinity (K<sub>D</sub> approximately

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0.37 nM) and selectivity for the 5-HT<sub>1A</sub> receptor (Fletcher et al., 1994; Khawaja et al., 1995). In vivo (Hume et al., 1994; Laporte et al., 1994) and in vitro (Khawaja, 1995) distribution studies of [<sup>3</sup>H]WAY-100635 in rodents revealed a high level of binding in brain regions known to be rich in 5-HT<sub>1A</sub> receptors, such as the septum, gyrus dentatus, hippocampus, raphe nuclei, and the neocortex. WAY-100635 has been labeled with <sup>11</sup>C in the carbonyl group and was characterized to bind selectively to 5-HT<sub>1A</sub> receptors in the monkey brain (Farde et al., 1997) and thus provided the first potent radioligand for the delineation of 5-HT<sub>1A</sub> receptors in vivo in humans using positron emission tomography (PET) (Pike et al., 1996).

A series of arylpiperazine-benzamido analogs of WAY-100635 have recently been developed. p-MPPI (4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-iodobenzamido]-ethylpiperazine) has been described as a potent and selective 5-HT<sub>1A</sub> receptor antagonist both in vitro and in vivo in rats (Zhuang et al., 1994; Kung et al., 1995; Thielen and Frazer, 1995). [<sup>123</sup>I]p-MPPI has been shown to be a useful radioligand for single photon emission computed tomography (SPECT) imaging of 5-HT<sub>1A</sub> receptors in nonhuman primates (Kung et al., 1996a). However, initial SPECT studies failed to demonstrate specific binding of [<sup>123</sup>I]p-MPPI to 5-HT<sub>1A</sub> receptors in the human brain (Kung et al., 1996b). The related radiofluorinated analog, p-MPPF, is also a competitive antagonist with a high affinity for 5-HT<sub>1A</sub> receptors (Zhuang et al., 1994). p-MPPF, however, displays a 4-fold lower affinity ( $K_i = 3.3$  nM) for 5-HT<sub>1A</sub> receptors than the parent compound WAY-100635 ( $K_i = 0.8$  nM) (Zhuang et al., 1994). An initial PET study in nonhuman primates suggested that p-[<sup>18</sup>F]MPPF is a potential radioligand for visualization of 5-HT<sub>1A</sub> receptors in vivo (Shiue et al., 1997), and deserves further investigation.

We recently reported an optimization of p-[<sup>18</sup>F]MPPF radiosynthesis, with a 3-fold improvement of the radiochemical yield by using microwave heating (Le Bars et al., 1998). The development of this radiotracer was motivated because of its relatively lower affinity for 5-HT<sub>1A</sub> receptors as compared with WAY-100635, which could make it more sensitive to changes in endogenous serotonin concentration. The present study reports on the use of p-[<sup>18</sup>F]MPPF as a potent selective antagonist radioligand to further characterize the in vivo binding characteristics and regional distribution of the 5-HT<sub>1A</sub> receptor in cats using PET.

## MATERIALS AND METHODS

### Compounds

Pindolol, ketanserin, fluoxetine and prazosin were obtained from Research Biochemicals International (Natick, MA). WAY-100635 was synthesized as previously described (Zhuang et al., 1994).

### Radiochemistry

No carrier added p-[<sup>18</sup>F]MPPF was synthesized by nucleophilic substitution of the corresponding nitro compound by [<sup>18</sup>F]fluoride in the presence of kryptofix 222, K<sub>2</sub>CO<sub>3</sub>, and microwave heating (3 minutes, 500 W), using a remotely controlled radiosynthesis (Le Bars et al., 1998). Baseline separation of p-[<sup>18</sup>F]MPPF from the nitro derivative was performed on a semipreparative HPLC C18 column (Waters (Rochester, MN) SymmetryPrep C18 (7.8 × 300 mm); THF/MeOH/NaOAc pH 5 (15/25/60) at 3 ml/min). After C18 Sep Pak formulation, the radiopharmaceutical was obtained within 70 minutes in 25 ± 5% overall yield with a specific activity of 1.5–4 Ci/μmol at end of synthesis. Quality control was performed on an analytical HPLC column (Waters Symmetry C18 Analytical column (3.9 × 150 mm); THF/MeOH/NaOAc pH 5 (17/28/55)) at 1 ml/min and Diode Array detector.

### In vivo studies

#### PET system

PET studies were performed on a Siemens (South Iselin, NJ) ECAT Exact HR+ used in three-dimensional mode. The system covers an axial distance of 15.5 cm (Brix et al., 1997). The transaxial resolution of the reconstructed images is about 4.1 mm full width at half-maximum (FWHM) in the center. Transmission scans were acquired with three rotating <sup>68</sup>Ge-<sup>68</sup>Ga sources and used to correct the emission scans for the attenuation of 511 keV photon rays through tissue and head support.

#### PET studies

Animal studies were performed by licensed investigators in accordance with French (87–848, Ministère de l'Agriculture et de la Forêt) and European Economic Community (86–60, EEC) guidelines for care of laboratory animals and were approved by the regional ethical animal use committee.

Five European male cats weighing about 2.5 kg were obtained from Iffa-Credo, Lyon, France. Anesthesia was induced with fluothane (4%). As soon as deep anesthesia was obtained, an endotracheal intubation was performed and anesthesia was maintained by constant insufflation of 2.5% fluothane in air. Carbon dioxide concentration in expired gases, heart rhythm, and body temperature were continuously controlled in the PET experiments. A head fixation system was used to secure a fixed and reproducible position of the cat head during the PET measurements. The head fixation system consists of a stereotaxic holding device made with Plexiglas where ear-bars, orbital, and hard palate pieces allowed stereotaxic positioning of the head. The horizontal plane was defined as including the interaural axis and the infraorbital ridges. A cannula was inserted in the sural vein for radiotracer injection.

In each cat, a baseline PET measurement was performed after a bolus injection of 1.5 mCi (55.5 Mbq) of p-[<sup>18</sup>F]MPPF into a sural vein for 2 sec. The specific radioactivity of p-[<sup>18</sup>F]MPPF was 0.5–2.7 Ci/μmol at the time of i.v. administration. Radioactivity was measured in a series of sequential time frames of increasing duration (from 1 minutes to 6 minutes). The total time for measurement of radioactivity in the brain was 90 minutes.

In a test-retest analysis, two cats were reexamined under the same experimental conditions 8 weeks following the baseline PET experiment. Displacement experiments were then performed on the two cats used in the test-retest analysis. In the first cat, four additional experiments were performed in which the selective 5-HT<sub>1A</sub> receptor antagonist WAY-100635 (0.5 mg/kg), the 5-HT<sub>1A</sub> receptor antagonist pindolol (1 mg/kg), or the α1 adrenoreceptor antagonist prazosin (2 mg/kg) were injected intravenously 30 minutes after the injection of p-[<sup>18</sup>F]MPPF.

In the second cat, two additional experiments were performed in which the selective 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor blocker ketanserin (1.5 mg/kg) or the 5-HT reuptake inhibitor fluoxetine (1 mg/kg) were injected intravenously 30 minutes after the injection of p-[<sup>18</sup>F]MPPF. In both cats, the period between experiments ranged from 1 to 6 weeks.

### Data analysis

Regions of interest (ROIs) for the whole brain, the ventral hippocampus, the cingulate cortex, and the cerebellum were drawn on the reconstructed PET images according to an atlas of the cat brain (Jasper and Ajmone-Marsan, 1954). Regional radioactivity was determined for each frame, corrected for decay and plotted vs. time. The time curve for the radioactivity concentration (nCi/ml) was calculated for each ROI. Specific ligand binding (B) was defined as the difference between the total regional radioactivity and that of the cerebellum, a reference region containing negligible densities of 5-HT<sub>1A</sub> receptors. Radioactivity in the cerebellum was used as an estimate for the free radioligand concentration and nonspecific binding in brain (F). The time curves for B (B(t)) and F (F(t)) were integrated from 40–90 minutes. The specific binding ratio (B/F) was calculated according to the equation:

$$\frac{B}{F} = \frac{\int_{90}^{40} B(t)dt}{\int_{90}^{40} F(t)dt}$$

5-HT<sub>1A</sub> receptor occupancy in the hippocampus and cingulate cortex was defined as the percentage reduction of the ratio B/F obtained after drug treatment as compared to the ratio B/F obtained at baseline (Farde et al., 1992).

### Blood analysis

As a preliminary study, the metabolism of p-[<sup>18</sup>F]MPPF in plasma was examined by measuring the fraction of radioactivity representing unchanged radioligand using thin layer chromatography, as described elsewhere (Mazière et al., 1993). Blood samples (1.5 ml) were drawn manually at 4, 15, 30, and 45 minutes after injection of p-[<sup>18</sup>F]MPPF. After centrifugation (3,000 rpm, 5 minutes, 5°C), plasma fractions were deproteinized with 1.5 volume of acetonitrile and rotary evaporated. Twenty μl of a 1 mg/ml p-MPPF solution was then added as carrier and 5 μl of the solution were spotted on a silica plate, developed in dichloromethane 93:methanol 7:triethylamine 0.5 (Rf for p-MPPF is 0.5). Plates were then analyzed on a Berthold tracemaster to obtain radioactivity profiles.

### Ex vivo studies

#### Tissue distribution of p-[<sup>18</sup>F]MPPF

A male cat weighing 2.5 kg was anesthetized with fluothane (4%), maintained under anesthesia with fluothane 2.5%, and injected in the sural vein with 2.1 mCi of p-[<sup>18</sup>F]MPPF. The cat was sacrificed at 30 minutes postinjection by cardiac failure induced by i.v. injection of saturated KCl. The brain was quickly removed and frozen by immersion in isopentane (–30°C) for 90 sec.

To determine tissue distribution of p-[<sup>18</sup>F]MPPF, various tissues were dissected and the radioactivity of each weighted samples was measured using a gamma counter. The percent dose of injected radioactivity per gram of tissue was calculated for each sample.

At the same time, series of two coronal adjacent sections (40 μm thick) of the brain were cut with a cryomicrotome (MICROM). Sections were selected at different anatomical levels including either the hippocampus, the cingulate cortex, the raphe nuclei, or the striatum. The first sections were Nissl-stained with cresyl violet for histological reference. The second sections were apposed against X-ray-sensitive hyperfilms (Amersham, Cleveland, OH) and exposed in cassettes for p-[<sup>18</sup>F]MPPF binding sites autoradiography.

### RESULTS

After i.v. injection of p-[<sup>18</sup>F]MPPF in cat, there was a rapid accumulation of radioactivity in the brain. Four minutes after injection, 4% of the total radioactivity injected was present in the brain. The highest uptake of radioactivity was observed in the hippocampus and cingulate cortex, whereas lower levels of radioactivity were observed in the cerebellum, a region devoid of significant amounts of 5-HT<sub>1A</sub> receptors (Fig. 1). The striatum could not be delineated from the background.

The mean ratio of radioactivity in the hippocampus to the cerebellum was 4.29 (SD = 0.21; n = 5) from 40 to 90 minutes postinjection of p-[<sup>18</sup>F]MPPF. The corre-

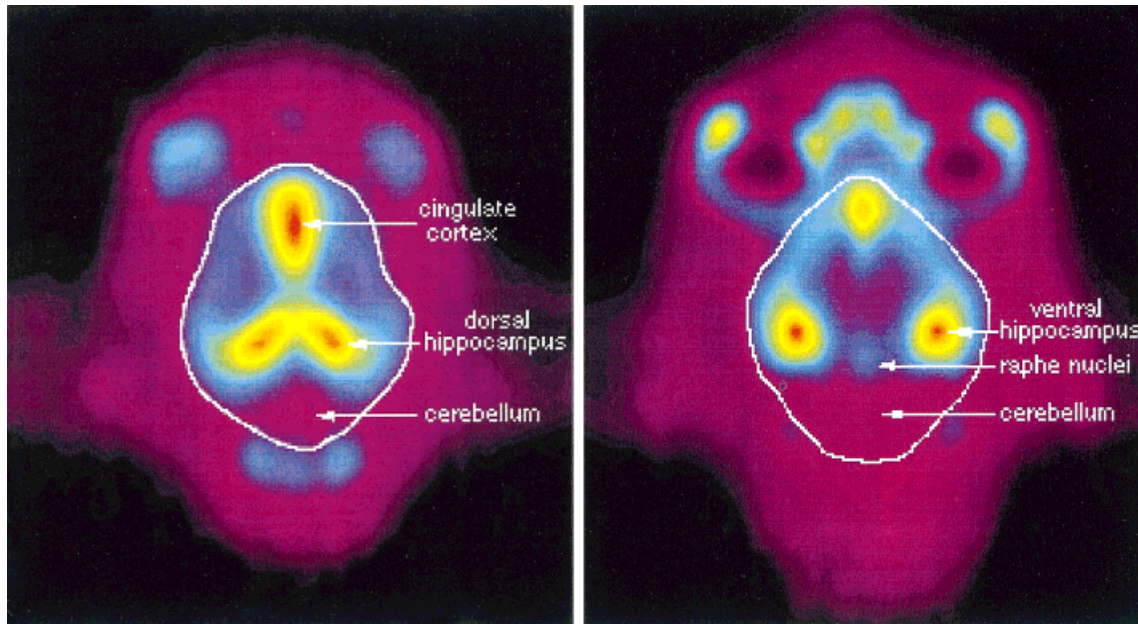


Fig. 1. Color-coded PET images showing the distribution of radioactivity in two horizontal sections of the cat brain after i.v. injection of p-[<sup>18</sup>F]MPPF at a higher (left) and a lower (right) level. The images represent the distribution of radioactivity from 9–90 minutes after

injection of the radioligand. High accumulation of radioactivity was observed in the cingulate cortex and hippocampus, together with intermediate accumulation in the raphe nuclei. Low accumulation was observed in the cerebellum.

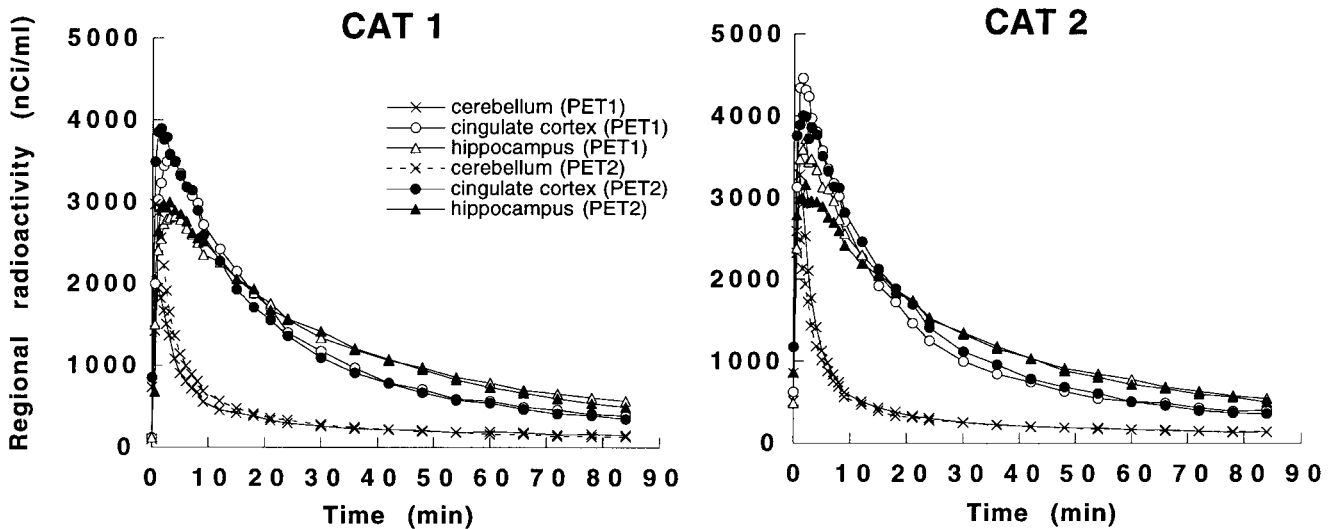


Fig. 2. Regional time-radioactivity curves obtained in two cats after injection of p-[<sup>18</sup>F]MPPF at baseline. In each cat two PET experiments (noted PET1 and PET2) were performed under the same experimental conditions at 8-week intervals.

sponding ratio for the cingulate cortex was 3.01 (SD = 0.16; n = 5).

In two cats, two PET experiments were performed at baseline at 8-week intervals. The corresponding time-activity curves are shown in Figure 2. In both cats, the quotient between the ratios of radioactivity in the hippocampus or the neocortex to the cerebellum calculated in the first and in the second PET experiment ranged from 0.96–1.03.

In displacement experiments, radioactivity in the hippocampus and cingulate cortex but not in the cerebellum was markedly reduced after injection of WAY-100635 and pindolol (Fig. 3). The 5-HT<sub>1A</sub> receptor occupancies calculated in the hippocampus and cingulate cortex were both 100% in the cat treated with unlabeled WAY-100635. In the cat treated with pindolol, the correspondent occupancies were 38% and 51%, respectively. There was no evident effect on hippocam-

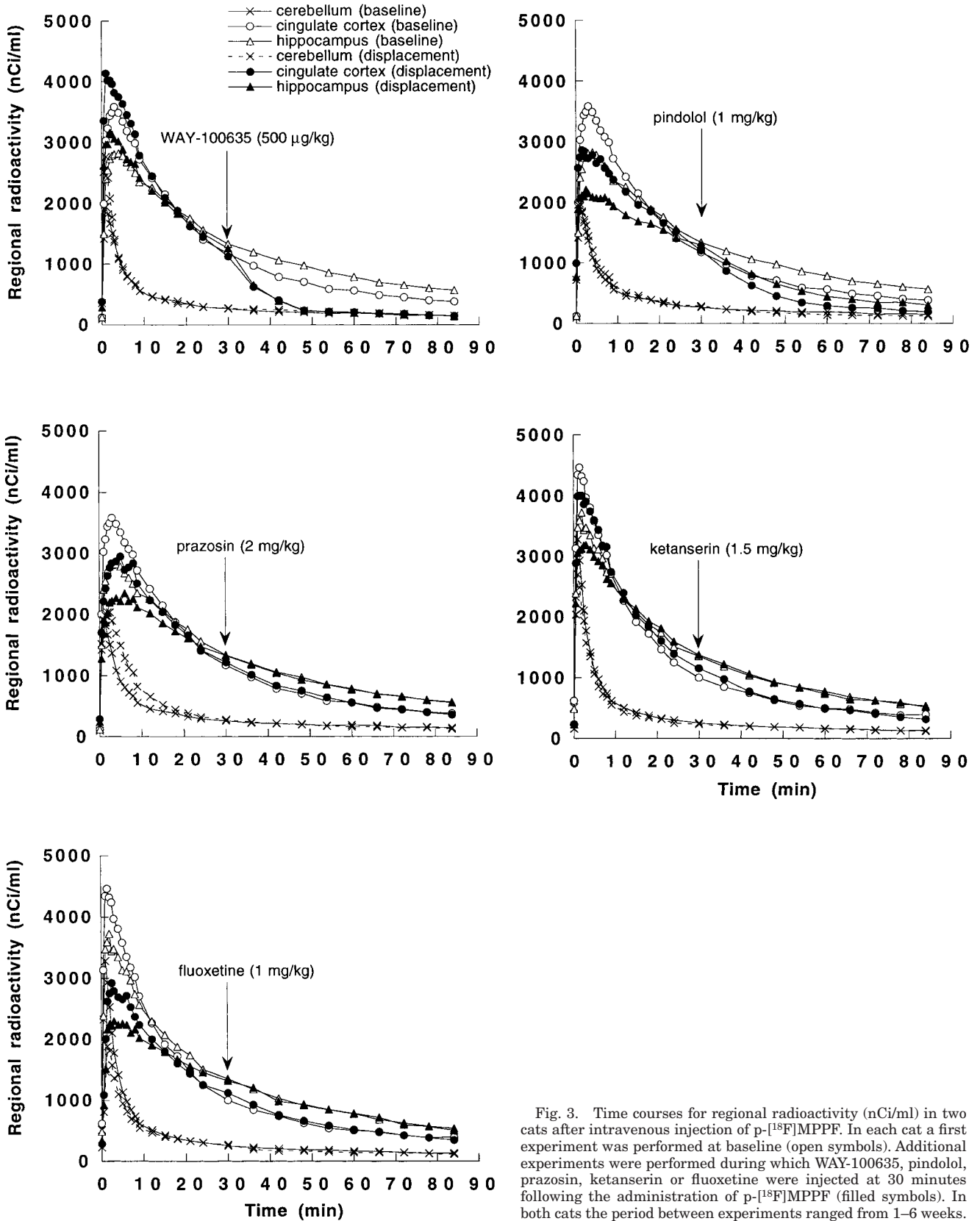


Fig. 3. Time courses for regional radioactivity (nCi/ml) in two cats after intravenous injection of p-[<sup>18</sup>F]MPPF. In each cat a first experiment was performed at baseline (open symbols). Additional experiments were performed during which WAY-100635, pindolol, prazosin, ketanserin or fluoxetine were injected at 30 minutes following the administration of p-[<sup>18</sup>F]MPPF (filled symbols). In both cats the period between experiments ranged from 1–6 weeks.

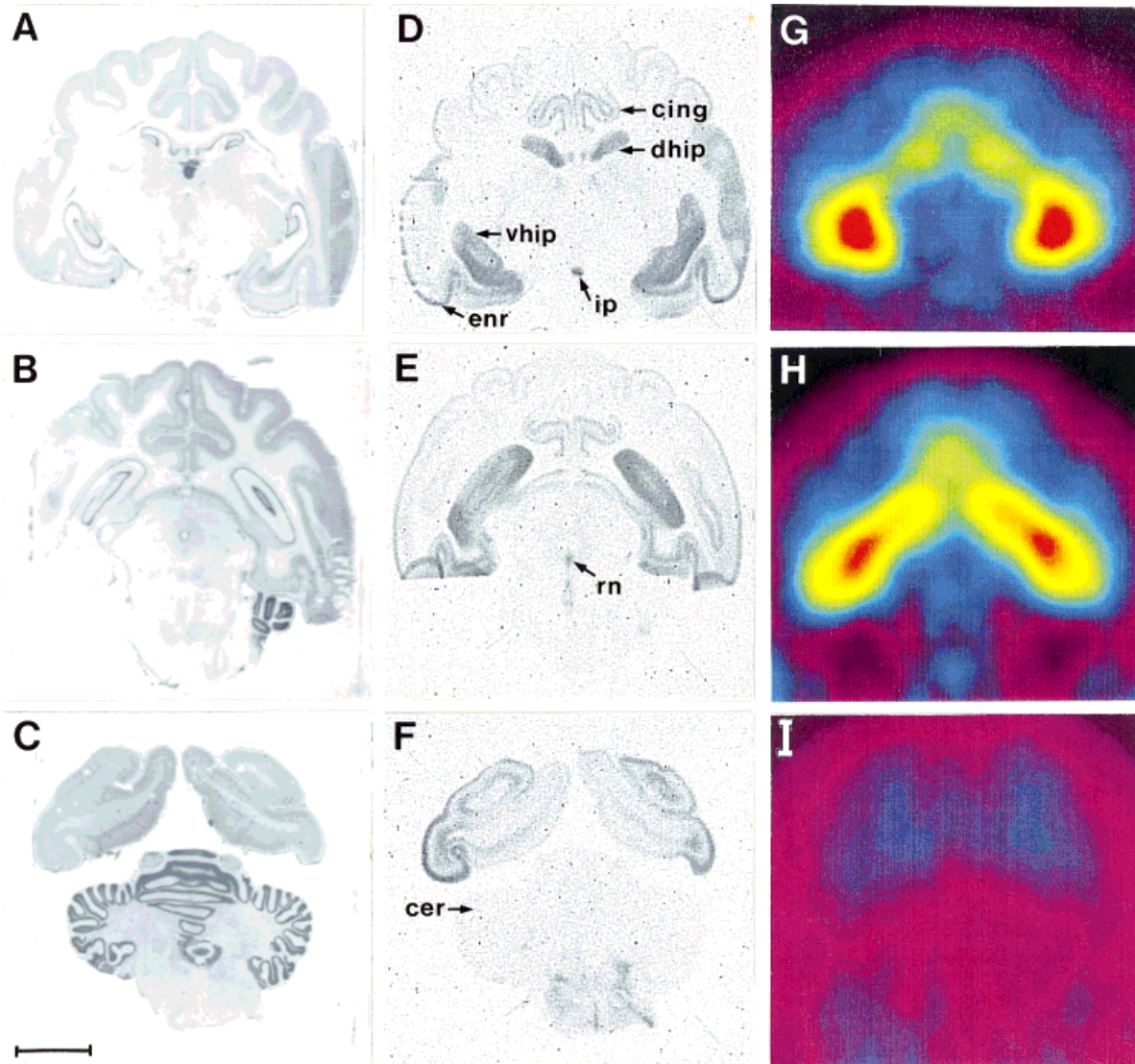


Fig. 4. Coronal sections of a cat brain at three caudo-rostral different levels. **A**, **B**, and **C** represent Nissl-stained sections. **D**, **E**, and **F** represent the ex vivo autoradiographic distribution of p-[<sup>18</sup>F]MPPF binding on corresponding adjacent sections. **G**, **H**, and **I** represent the in vivo distribution of p-[<sup>18</sup>F]MPPF binding as assessed using PET in the same cat on comparable but not strictly identical coronal levels. The autoradiograms show high levels of p-[<sup>18</sup>F]MPPF

binding in the cingulate cortex (cing), the dorsal (dhip) and ventral (vhip) hippocampus, the entorhinal cortex (enr), the interpeduncular nucleus (ip), and the raphe nuclei (rn). Binding in cerebellum (cer) was very low and similar to film background. The in vivo brain distribution of p-[<sup>18</sup>F]MPPF observed using PET matched well the ex vivo distribution of p-[<sup>18</sup>F]MPPF binding. Scale bar = 1 cm.

pal and cortical binding after injection of ketanserine, fluoxetine, or prazosin (Fig. 3).

In plasma, the fraction of unmetabolized parent compound decreased slowly after injection. Plasma metabolic profile showed the appearance of a polar metabolite increasing from 6% at 15 minutes to 21% at 30 minutes postinjection. At 60 minutes postinjection, 39% of radioactivity in plasma was still accounted for by p-[<sup>18</sup>F]MPPF.

The detailed distribution of p-[<sup>18</sup>F]MPPF binding was examined ex vivo in the cat brain. The autoradiograms showed after in vivo injection of p-[<sup>18</sup>F]MPPF very dense binding in the dorsal and ventral hippocam-

pus, the cingulate cortex, entorhinal cortex, dorsal raphe nucleus, and interpeduncular nucleus, regions known to have high density of 5-HT<sub>1A</sub> receptors (Fig. 4). By contrast, the striatum (not shown) and the cerebellum remained unlabeled. The ex vivo brain distribution of the radioligand observed using autoradiography paralleled the in vivo distribution of p-[<sup>18</sup>F]MPPF binding observed using PET.

The tissue distribution of p-[<sup>18</sup>F]MPPF in cat is shown in Table 1. Thirty minutes postinjection, the highest concentrations of radioactivity were found in liver and kidney.

TABLE 1. Tissue distribution of p-[<sup>18</sup>F]MPPF in the cat at 30 minutes post-injection

Organ	% injected dose/ gram of tissue
Blood	0.025
Brain	0.076
Heart	0.031
Lungs	0.091
Liver	0.226
Intestine	0.056
Kidneys	0.428
Adrenal glands	0.072

## DISCUSSION

The binding of p-[<sup>18</sup>F]MPPF was characterized in the cat brain *in vivo* using PET. The high uptakes of p-[<sup>18</sup>F]MPPF in the hippocampus and neocortex are consistent with the known regional distribution of 5-HT<sub>1A</sub> receptors in the brain *in vitro*. Very low binding was observed in the cerebellum, indicating that the cerebellar cortex is virtually devoid of 5-HT<sub>1A</sub> receptors. The ratios of radioactivity in hippocampus and neocortex to that in cerebellum integrated from 40–90 minutes after injection of p-[<sup>18</sup>F]MPPF were approximately 4 and 3, respectively. The binding in the hippocampus and the cingulate cortex was markedly reduced following injection of unlabeled WAY-100635, indicating that a major proportion of p-[<sup>18</sup>F]MPPF binding in brain represents specific binding to 5-HT<sub>1A</sub> receptors and that this binding is reversible. Radioligand binding in hippocampus and cortex was also displaced by pindolol but unaffected by treatment with  $\alpha$ 1, 5-HT<sub>2</sub>, or reuptake inhibitor agents indicating a high selectivity of p-[<sup>18</sup>F]MPPF for 5-HT<sub>1A</sub> sites. Regarding the effect of reuptake inhibitor agents on extracellular level of serotonin and the potential sensitivity of p-[<sup>18</sup>F]MPPF binding to change in extracellular serotonin, one could have expected a decrease in p-[<sup>18</sup>F]MPPF binding in hippocampus and cingulate cortex following fluoxetine administration. However, it has been shown from *in vivo* microdialysis studies that acute administration of fluoxetine at 1 mg/kg does not produce significant changes in extracellular serotonin levels in projection areas of the forebrain, such as in the hippocampus and frontal cortex (Malagié et al., 1995; Hervás and Artigas, 1998). Indeed, only high systemic doses of fluoxetine (10 mg/kg) consistently increase serotonin levels in these areas.

The initial raises of radioactivity in hippocampus, cingulate cortex and cerebellum were different in Figure 3, when comparing PET experimental data acquired at baseline condition and during displacement experiment in the same animal, but at several weeks intervals. The initial slope of the time–activity curves actually reflects the net transfer of p-[<sup>18</sup>F]MPPF across the blood–brain barrier. The uptake rate of radioligand from blood is a function of blood flow, capillary permeability, and plasma protein binding. Cat studies were

done under fluothane anesthesia. Sustained anesthesia with this drug can lead to cardiovascular depression (Green, 1979). It is thus possible that the intra-animal variability observed in the initial uptake of radioactivity may be due to physiological processes such as differences in blood flow between the two experiments. Owing to the difficulty in separating ligand–receptor binding effects from tracer delivery–flow effects in the initial part of the time–activity curves, quantification of p-[<sup>18</sup>F]MPPF binding was accordingly performed at a late time, *i.e.*, from 40–60 minutes following radioligand injection.

The results from the autoradiographic study confirm and extend those obtained by PET, showing that most of labeling was present in the dorsal and ventral hippocampus, the cingulate cortex, the entorhinal cortex, the dorsal raphe nucleus, and the interpeduncular nucleus. By contrast, the striatum (not shown) and the cerebellum remained unlabeled. The anatomical distribution of p-[<sup>18</sup>F]MPPF labeling was thus in general agreement with the reported distribution of 5-HT<sub>1A</sub> receptors in the rat (Pazos and Palacios, 1985; Kawaja, 1995), the cat (Waeber et al., 1989), and the human brain (Hoyer et al., 1986; Hall et al., 1997), using autoradiography and [<sup>3</sup>H]8-OH-DPAT, [<sup>3</sup>H]WAY-100635 or [<sup>14</sup>C]WAY-100635. Interestingly, the present autoradiographic results demonstrated the existence of differences in the anatomical distribution of 5-HT<sub>1A</sub> receptors in the cerebral cortex. Indeed, there was a marked difference in density between cortical regions. High density was observed in the cingulate and in the entorhinal cortices, whereas weak labeling was observed in other cortical regions. This regional disparity of 5-HT<sub>1A</sub> receptor density in the cerebral cortex of the adult cat has already been reported. Low density of 5-HT<sub>1A</sub> receptors were observed in the visual cortex, which distinguished them from the high density of 5-HT<sub>1A</sub> sites observed in the cingulate cortex (Dyck and Cynader, 1993).

The *ex vivo* brain distribution of the radioligand observed using autoradiography paralleled the *in vivo* distribution of p-[<sup>18</sup>F]MPPF binding observed using PET. However, owing to the lower resolution of PET (4.3 mm FWHM; Brix et al., 1997) as compared with autoradiography (approximately 50  $\mu$ m), p-[<sup>18</sup>F]MPPF binding could not be quantified in small cerebral regions such as the raphe nuclei or the interpeduncular nucleus when using PET. Moreover, it is likely that determination of radioligand concentration was underestimated in hippocampus and cingulate cortex due to partial volume effect. The cat brain has an average volume of 30 ml, which represents about 3% of the average human brain volume. Reports on the species differences in the anatomical distribution of 5-HT<sub>1A</sub> receptors sites showed that these sites exhibit a relative preserved regional distribution with the evolution of the vertebrate brain (Waeber et al., 1989). Studies of

p-[<sup>18</sup>F]MPPF binding in the larger human brain should thus allow more accurate quantitation of 5-HT<sub>1A</sub> receptors in these structures and optimize quantification in the raphe nuclei.

The anatomical distribution of 5-HT<sub>1A</sub> receptors has been reported to be in agreement with the in situ localization of 5-HT<sub>1A</sub> mRNA (Chalmers and Watson, 1991; Miquel et al., 1992). However, there are two distinct populations of 5-HT<sub>1A</sub> receptors in the brain, discriminated on the basis of their neuronal location and functional role. Somatodendritic 5-HT<sub>1A</sub> receptors in the raphe nuclei mediate inhibition of serotonin neuron activity, leading to a reduction in 5-HT synthesis and release in terminal areas, while postsynaptic 5-HT<sub>1A</sub> receptors in structures of the limbic system mediate the effect of synaptically released serotonin (de Montigny and Blier, 1992; Hutson et al., 1989; Sprouse and Aghajanian, 1988). The binding of p-[<sup>18</sup>F]MPPF to postsynaptic 5-HT<sub>1A</sub> receptors was clearly displaced by pindolol (1 mg/kg; i.v.). The calculated 5-HT<sub>1A</sub> receptor occupancies were 38% and 51% in the hippocampus and cingulate cortex, respectively. This result is in agreement with the 60% receptor occupancy induced by the same i.v. dose of pindolol on [carbonyl-<sup>11</sup>C]WAY-100635 binding in the neocortex of the primate brain (Farde et al., 1997). Interestingly, it has been shown in vivo that the combination of pindolol to selective serotonin reuptake inhibitors (SSRIs) treatment significantly accelerates the onset of therapeutic response in patients suffering from major depression (Artigas et al., 1994; Blier et al., 1997). It is assumed that this clinical benefit is due to the potentiation by pindolol of the ability of SSRIs to increase serotonin release in terminals by preventing the activation of somatodendritic 5-HT<sub>1A</sub> autoreceptors produced in the dorsal raphe nucleus by these drugs (Romero et al., 1996). At the postsynaptic level, our results showed that pindolol induced a substantial blockade of 5-HT<sub>1A</sub> receptors. Given the relevance of increased serotonergic neurotransmission in the treatment of depression, such a postsynaptic receptor blockade is paradoxical. However, experimental data demonstrated that even high doses of pindolol (15 mg/kg; i.p.) did not alter the responsiveness of hippocampal neurons to serotonin (Romero et al., 1996).

In conclusion, this study showed that p-[<sup>18</sup>F]MPPF binds specifically and reversibly to 5-HT<sub>1A</sub> receptors and demonstrated the feasibility of using this radioligand to visualize 5-HT<sub>1A</sub> receptors in the cat brain in vivo using PET. Studies are now in progress to evaluate the uptake and regional distribution of p-[<sup>18</sup>F]MPPF in the human brain using PET. Moreover, Van Wijnngaarden et al. (1990) reported the binding affinity (K<sub>i</sub> value) of 5-HT for the 5-HT<sub>1A</sub> receptor subtype to be 4.17 nM. The relatively lower affinity of p-MPPF (K<sub>i</sub> = 3.3 nM) for 5-HT<sub>1A</sub> receptors as compared with WAY-100635 (K<sub>i</sub> = 0.8 nM) (Zhuang et al., 1994) could make

it more sensitive to changes in endogenous serotonin concentration. Experimental studies in animals need to be performed to investigate the feasibility of using p-[<sup>18</sup>F]MPPF to evaluate changes in baseline serotonin concentration following pharmacological challenges.

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