

# Displacement of the PET Ligand $^{18}\text{F}$ -MPPF by the Electrically Evoked Serotonin Release in the Rat Hippocampus

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**KEY WORDS** 5-HT<sub>1A</sub> receptor; MPPF; serotonin release; microdialysis;  $\beta$ -microprobe

**ABSTRACT** The effects of the electrically evoked serotonin release were evaluated on the binding of  $^{18}\text{F}$ -MPPF in the hippocampus of anesthetized rats. The specific binding of  $^{18}\text{F}$ -MPPF was measured by an implanted  $\beta$ -microprobe and the serotonin (5-HT) extracellular concentration was measured by microdialysis under the same conditions. Our results showed that the 10-, 20-, or 30-min electrical stimulation of the raphe nucleus elicited a significant increase in extracellular 5-HT, only detectable in the presence of a 5-HT reuptake inhibitor in the perfusate (5  $\mu\text{M}$  clomipramine). Interestingly, the raphe stimulations were associated with a 27–76% reversible decrease of the  $^{18}\text{F}$ -MPPF specific binding in the hippocampus, but an unchanged extracellular  $^{18}\text{F}$ -MPPF collected in dialysates. Considered together, these observations suggest that  $^{18}\text{F}$ -MPPF binding is sensitive to 5-HT released at a neuronal level. This compartment, explored by the  $\beta$ -microprobe, is probably distinct from the extracellular compartment, explored by microdialysis. **Synapse 49:239–245, 2003.** © 2003 Wiley-Liss, Inc.

## INTRODUCTION

Over the last decade, numerous studies have demonstrated that noninvasive neuroreceptor imaging with PET (positron emission tomography) could be used to measure changes in cerebral concentration of neurotransmitters. The principle underlying this technique is the competition between an endogenous neurotransmitter and an exogenous radioligand: changes in neurotransmitter concentration translate into changes in transmitter receptor occupancy that can be detected as changes in the binding of the radioligand (Seeman et al., 1989). This application of neuroreceptor imaging offers the unique possibility of measuring the neurotransmission in the living brain and of correlating the molecular imaging with behaviors and symptoms.

This approach was mainly applied to the measurement of changes in dopamine synaptic concentration, principally with the  $^{11}\text{C}$ -raclopride (see Laruelle, 2000; Laruelle and Huang, 2001, for reviews). The serotonin (5-HT) system was less studied due to the lack of a good radiotracer that is sensitive to 5-HT fluctuations. For example, initial results obtained with the widely used 5-HT ligand  $^{11}\text{C}$ -WAY-100635 were disappointing (Mathis et al., 1995; Hume et al., 2001). Recently, we demonstrated for the first time that the specific binding of  $^{18}\text{F}$ -MPPF (a selective 5-HT<sub>1A</sub> antagonist: 4-2'-(methoxyphenyl)-1-[2'-(N-2''-pyridinyl)-p-fluorobenz-

amido]ethylpiperazine labeled with  $^{18}\text{F}$ -fluorine) was decreased after a fenfluramine-induced 5-HT increase (Zimmer et al., 2002a). Fenfluramine is known to cause 5-HT release through a reverse transport mechanism, independently of any cell firing (Bonanno et al., 1994; Rowland et al., 1986). It was thus of interest to explore the  $^{18}\text{F}$ -MPPF binding during an electrically evoked 5-HT release more relevant to the physiological neurotransmission process. Previous studies showed that electrical stimulation of the dorsal raphe nucleus (DRN) produces a 5-HT overflow in the rat hippocampus *in vivo* and suggested that this could be due to the activation of the DRN-hippocampal 5-HT pathway (Sharp et al., 1989, 1990).

The aim of this study was to assess the effect of the evoked 5-HT release produced by an electrical stimulation of the DRN on the *in vivo* binding of  $^{18}\text{F}$ -MPPF in the hippocampus of anesthetized rats. A recently described radiosensitive cerebral probe, the  $\beta$ -micro-

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probe, was used, allowing local counting of radioactivity in the hippocampus. The  $^{18}\text{F}$ -MPPF binding measurements were paralleled with a microdialysis approach allowing measurement of the extracellular 5-HT and of free  $^{18}\text{F}$ -MPPF in the hippocampus during electrical stimulations. Finally, the confrontation of these radiopharmacological and neurochemical approaches suggests that  $\beta$ -microprobe is related to a neuronal compartment distinct from the extracellular compartment explored with microdialysis.

## MATERIALS AND METHODS

### Animal surgery

Male Sprague-Dawley rats (Elevage Dépré) weighing 300–400 g were housed under standard conditions of temperature and humidity in artificial light (light/dark cycles: 12/12). All experimental procedures were in accordance with EEC guidelines and directives (86/09/EEC). A total of 56 male rats were used in this study. During all experiments the rats were anesthetized by a single intraperitoneal injection of urethane (Sigma Aldrich, St. Louis, MO) at a dose of 1.7 g/kg body weight. The tail vein was catheterized to allow the injection of the radioactive tracer ( $^{18}\text{F}$ -MPPF). Body temperature was maintained at  $37 \pm 1^\circ\text{C}$  throughout the test period using a thermostatically controlled heating blanket (CMA/Microdialysis). The anesthetized rats were positioned on a stereotaxic apparatus. The skull was exposed and the bregma point visualized. A stimulating electrode 0.5 mm in diameter was implanted stereotaxically in the DRN (anterior–posterior  $-7.6$ ; lateral  $0$ ; dorsal–ventral  $-6.0$  mm, relative to bregma and dura) according to Paxinos and Watson (1986) (Fig. 1). Electrical stimulation consisted of constant-current 300  $\mu\text{A}$  monophasic pulses with a frequency of 20 Hz with each stimulus being 1 ms in duration.

### Determination of extracellular 5-HT in hippocampus

In a group of rats with the stimulating electrode, a microdialysis probe (1 mm length PES membrane, Microbiotech) was implanted in the hippocampus (anterior–posterior  $-5.0$ , lateral  $-5.0$ , and ventral–dorsal  $-8.0$ ) according to Paxinos and Watson (1986). The probe was immediately and continuously perfused with a saline perfusion buffer (PBS = Dulbecco's modified medium + 2.2 mmol/L  $\text{CaCl}_2$ ) at 0.5  $\mu\text{L}/\text{min}$  using a microsyringe pump. In a distinct group, 5  $\mu\text{M}$  clomipramine was added in the PBS and, in another group, PBS without clomipramine. After a 2–3-h equilibration period, successive 10-min dialysate samples were collected and the 5-HT content assayed using HPLC with an Antec Leyden electrochemical detector (glassy carbon, Ag/AgCl set at 600 mV). We used a C18 reverse phase column (Uptisphere ODB, 3  $\mu\text{m}$ ,  $100 \times 2$  mm); the mobile phase, delivered at a rate of 0.2 mL/min was composed of 0.15 M  $\text{NaH}_2\text{PO}_4$ , pH 3.8, 0.5 mM EDTA, 0.01 mM octanesulfonic acid, and 12.5% methanol (vol/

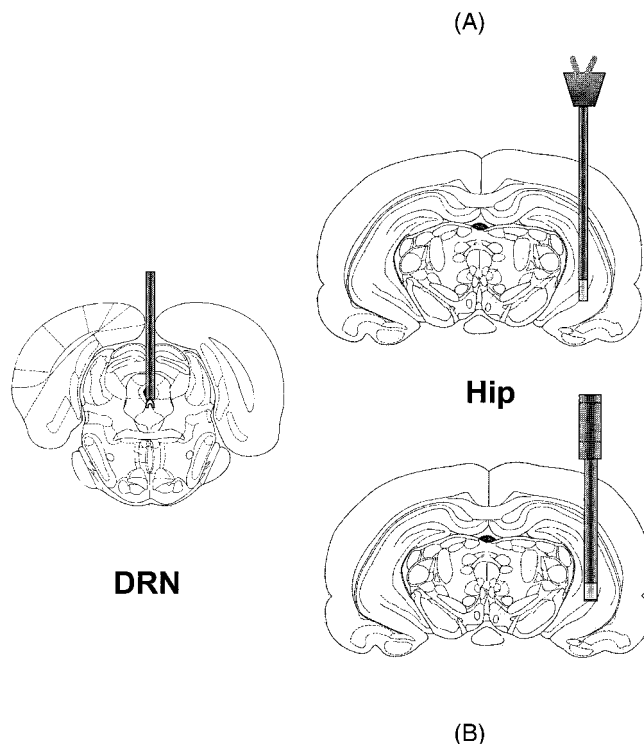


Fig. 1. Location of the stimulating probe in the dorsal raphe nucleus (DRN) and location of the microdialysis probe (A) and the  $\beta$ -sensitive probe (B) in the hippocampus (Hip) of the rat.

vol), according to Sharp et al. (1989). Samples were loaded onto the column using a Rheodyne 7125 (5- $\mu\text{L}$  loop). In our conditions, the retention time for 5-HT was 3.2 min and its detection limit was 0.5 pg/sample (10  $\mu\text{L}$ ).

Baseline was considered stable if 5-HT in dialysate did not differ by more than 20% over three consecutive samples. The experimental manipulation consisted of a tonic electrical stimulation (20 Hz) of the DRN which was applied for 10, 20, or 30 min in both groups (PBS and PBS + clomipramine). The dialysates were collected within 90 min after the stimulation.

### Synthesis of $^{18}\text{F}$ -MPPF

$^{18}\text{F}$ -MPPF (4-2'-(methoxyphenyl)-1-[2'-(N-2''-pyridinyl)-p-fluorobenzamido]ethylpiperazine) was synthesized with a radiochemical yield of 25% (decay corrected) in an automated synthesizer (Le Bars et al., 2001), using the chemical pathway previously described (Le Bars et al., 1998). Chemical and radiochemical purity were higher than 98% as determined by HPLC. Specific activity from the injected radiotracer ranged from  $74 \times 10^3$  MBq/mmol to  $148 \times 10^3$  MBq/mmol (2–4 Ci/mmol).

### $\beta$ -Microprobe acquisition in the hippocampus and in the cerebellum

Another group of rats was implanted with the stimulating electrode in the DRN and a  $\beta$ -microprobe in the

ventral hippocampus or in the cerebellum. The sensitive tip of the  $\beta$ -radiosensitive probe consisted of a 1-mm length, 1-mm diameter plastic scintillating fiber which takes advantage of the short range of  $\beta$  particles within biological tissue, allowing definition of the detection volume surrounding the probe (Zimmer et al., 2000b,c). The coordinates of hippocampus implantation were identical to the previous microdialysis experiment and the coordinates of the cerebellum were A/P -13.0, L/M 3.0, and V/D -4.0 from the bregma point and the dura, respectively.  $\beta$ -Microprobe acquisition was carried out 2 h after implantation of the probe as in our previous study (Zimmer et al., 2002a). During this time, corresponding to a stabilization period (Benveniste, 1989), the 5-HT extracellular levels decrease gradually to reach steady-state levels. For each acquisition, 37 MBq <sup>18</sup>F-MPPF (in a volume of 0.4 mL saline) was injected via the tail vein over a 40-sec period. This activity corresponded to a stable content from 250–500 pmol. The time course of radioactivity was studied for 90 min using 10-sec time integration acquisition. Electrical stimulation was applied to the DRN for a period of 10, 20, or 30 min, 20 min after the <sup>18</sup>F-MPPF injection, under equilibrium binding conditions. Control rats were not submitted to stimulations.

#### Determination of extracellular <sup>18</sup>F-MPPF in the hippocampus

A third group of rats implanted with the stimulating electrode in the DRN and a microdialysis probe (1 mm length) was implanted in the hippocampus according to the previously described coordinates. The probe was continuously perfused with perfusion buffer at 1.0  $\mu$ L/min (without clomipramine). Two hours after the beginning of perfusion, the rat received a 37 MBq <sup>18</sup>F-MPPF i.v. injection. Dialysates were collected every 2 min (2  $\mu$ L) and the hippocampus radioactivity of the dialysates was measured using an automated gamma-counter (Cobra II, Packard, Meriden, CT) calibrated in the fluorine-18 energy range. Under these experimental conditions the <sup>18</sup>F-MPPF probe recovery is 25%. Animals were stimulated or not 20 min after the <sup>18</sup>F-MPPF injection and following the previously described three protocols (10, 20, or 30 min DRN electrical stimulation).

#### Histology

After completion of the experiments the locations of the electrodes and dialyzing probes were verified for each animal. The anesthetized rats were sacrificed and their brains quickly removed and frozen at -80°C. Coronal 20  $\mu$ m-thick tissue sections were cut in a cryostat (Leica) at -20°C throughout the striatum and the cerebellum and thaw-mounted onto glass slides. The sections were stained with cresyl blue and the probe placements were atlas-matched. Data were included in the final analysis only if the final position of the stimulating electrode tip was well located in the DRN.

#### Data analysis

The  $\beta$ -microprobe data (expressed as mean of disintegration per 10 sec) were averaged every minute and converted in Becquerel. These data were corrected for radioactive decay and normalized with respect to the activity injected.

The specific radioligand binding to 5-HT<sub>1A</sub> receptors (B) was defined as the difference between the total regional radioactivity and that of the cerebellum. The cerebellum was used as reference tissue because this region is practically devoid of 5-HT<sub>1A</sub> receptors (Kia et al., 1996a,b). Therefore, radioactivity in the cerebellum was used as an estimate for the free radioligand concentration and nonspecific binding in the brain (F). The  $\beta$ -microprobe time curves for B (B(t)) and F (F(t)) were integrated from 20–30 min, 20–40 min, or 20–50 min after the <sup>18</sup>F-MPPF injection, respectively, for the 10-, 20-, or 30-min DRN electrical stimulation. According to a previous <sup>18</sup>F-MPPF study (Ginovart et al., 2000), the specific binding ratio (B/F) was calculated using the equation:

$$\frac{B}{F} = \frac{\int_{stim}^{20} B(t)dt}{\int_{stim}^{20} F(t)dt}$$

Statistical analysis was conducted by comparing the mean values obtained from both the control and treated animals for each time point (every minute) using one-way ANOVA on repeated measurements, followed by a post-hoc Student's *t*-test. A *P*-value < 0.05 was considered statistically significant.

## RESULTS

### Effect of electrical stimulation of the DRN on extracellular 5-HT in hippocampus

The amount of 5-HT in dialysates of the hippocampus was about 1.5  $\pm$  0.2 fmol/ $\mu$ L (without probe correction, in the absence of clomipramine) and following a 2–3-h baseline period was stable over time. As shown in the figures, a 10-min electrical stimulation (Fig. 2A), a 20-min electrical stimulation (Fig. 2B), or a 30-min electrical stimulation (Fig. 2C) produced no significant increase of the extracellular 5-HT in the hippocampus.

In the presence of clomipramine in the perfusate, the amount of 5-HT was about 5.0  $\pm$  0.2 fmol/ $\mu$ L and, following a 2–3-h baseline period, was stable over time. As shown in Figure 2A, a 10-min electrical stimulation of the DRN caused a clear-cut (~1.5-fold) but short-lived increase of 5-HT in hippocampal dialysates. In other experiments, a 20-min electrical stimulation of the DRN caused on average a 2-fold increase of extracellular 5-HT compared to baseline level, followed by a return to the baseline level within 30 min after the end

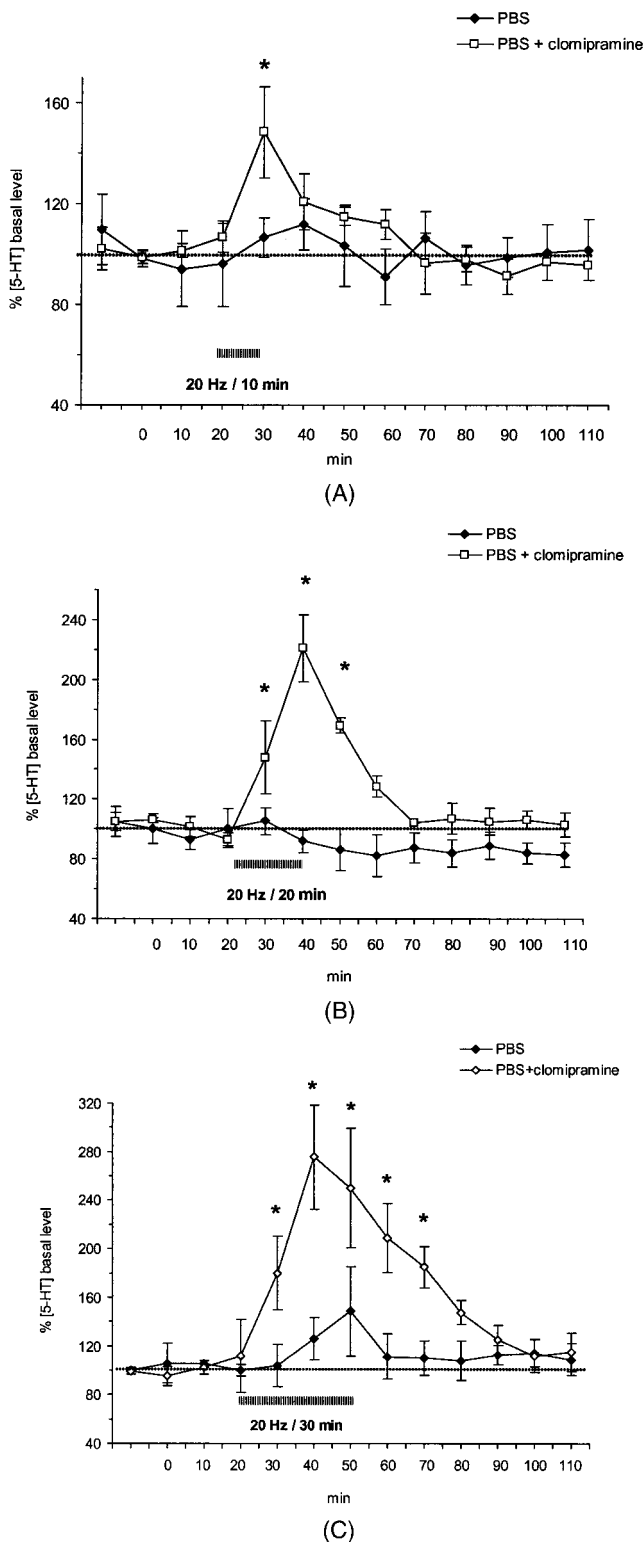


Fig. 2. Variation of extracellular serotonin (5-HT) in the hippocampus of rats with a 20 Hz DRN electrical stimulation for 10 min (A), 20 min (B), and 30 min (C). In a first group of rats a microdialysis probe was perfused with PBS ( $\blacklozenge$ ,  $n = 4$  for each stimulation duration  $\pm$  SEM) and in another group with PBS containing 5  $\mu$ M clomipramine ( $\square$ ,  $n = 4$  for each stimulation duration  $\pm$  SEM). \* $P < 0.05$ .

of the DRN stimulation (Fig. 2B). Finally, a 30-min electrical stimulation of the DRN induced in the hippocampus an average 2.8-fold increase of 5-HT in dialysates, followed by a return to the baseline level within 40 min after the end of the DRN stimulation (Fig. 2C).

Having established pharmacological conditions under which 5-HT output was detectable following DRN stimulations, experiments were carried out to determine whether this evoked 5-HT release modified the  $^{18}\text{F}$ -MPPF binding in the hippocampus.

### Effect of electrical stimulation of the DRN on the radioactivity kinetic curves of $^{18}\text{F}$ -MPPF in hippocampus and cerebellum

Figure 3A shows the radioactivity curves of  $^{18}\text{F}$ -MPPF in hippocampus and cerebellum of rats with, or not, a 10 min electrical stimulation of the DRN. For each 10-min stimulation the  $^{18}\text{F}$ -MPPF hippocampus curve decreased significantly as compared with the level measured in control rats ( $P < 0.05$ ) and, after the electrical stimulation, returned to control levels. The radioactivity kinetic curve of  $^{18}\text{F}$ -MPPF in cerebellum was similar between stimulated rats and control rats. The  $^{18}\text{F}$ -MPPF specific binding ratio (B/F), calculated from 20–30 min, was  $0.47 \pm 0.02$  for the control rats and was significantly lower ( $-27\%$ ) for the stimulated rats:  $0.34 \pm 0.03$  ( $P < 0.05$ ;  $\pm$  SEM).

Figure 3B shows the radioactivity curves of  $^{18}\text{F}$ -MPPF in hippocampus and cerebellum of rats with or without a 20-min DRN electrical stimulation. During and after the DRN stimulation the hippocampal curve was statistically different from that obtained in the control rats ( $P < 0.05$ ). The radioactivity kinetic curve of  $^{18}\text{F}$ -MPPF in cerebellum was similar between stimulated and control rats. The  $^{18}\text{F}$ -MPPF specific binding ratio (B/F), calculated from 20–40 min, was  $0.45 \pm 0.03$  for the control rats and  $0.21 \pm 0.04$  for the stimulated rats ( $-53\%$ ,  $P < 0.05$ ).

Figure 3C shows the radioactivity curves of  $^{18}\text{F}$ -MPPF in hippocampus and cerebellum of rat with or without a 30-min DRN electrical stimulation. During and after the DRN stimulation the  $^{18}\text{F}$ -MPPF curve decreased significantly in hippocampus as compared with the control rats ( $P < 0.05$ ), whereas the radioactivity kinetic curve in cerebellum was similar between both groups. The  $^{18}\text{F}$ -MPPF specific binding ratio (B/F), calculated from 20–50 min, was  $0.41 \pm 0.03$  for the control rats and  $0.10 \pm 0.04$  for the stimulated rats ( $-76\%$ ,  $P < 0.05$ ).

### Effect of electrical stimulation of the DRN on extracellular $^{18}\text{F}$ -MPPF in the hippocampus

Complementary experiments were carried out to determine if the extracellular  $^{18}\text{F}$ -MPPF collected by microdialysis was modified during DRN electrical stimulations.

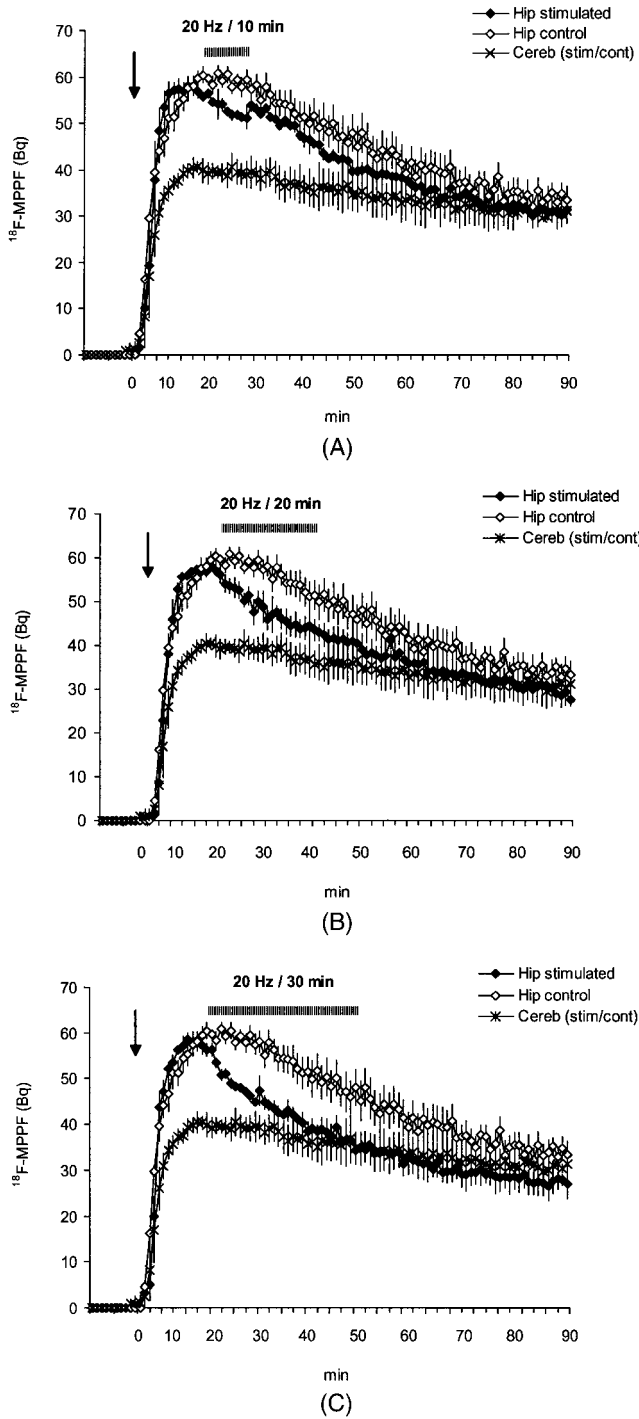


Fig. 3. Radioactivity curve measured by the hippocampal and cerebellar  $\beta$ -microprobes in rats after a 37 MBq <sup>18</sup>F-MPPF injection followed at 20 min by a DRN electrical stimulation for 10 min (A), 20 min (B), and 30 min (C). \**P* < 0.05. For each stimulation duration, four rats were stimulated (◆, stimulated  $\pm$  SEM) in comparison with four rats without stimulation (◇, control  $\pm$  SEM). The arrow figures the <sup>18</sup>F-MPPF injection. To clarify figures, the cerebellum curves (identical with or without electrical stimulation) were averaged (Cereb (stim/control)).

Figure 4 shows the radioactivity curves of <sup>18</sup>F-MPPF in the hippocampus of rat with electrical stimulation in the DRN or without electrical stimulation (control

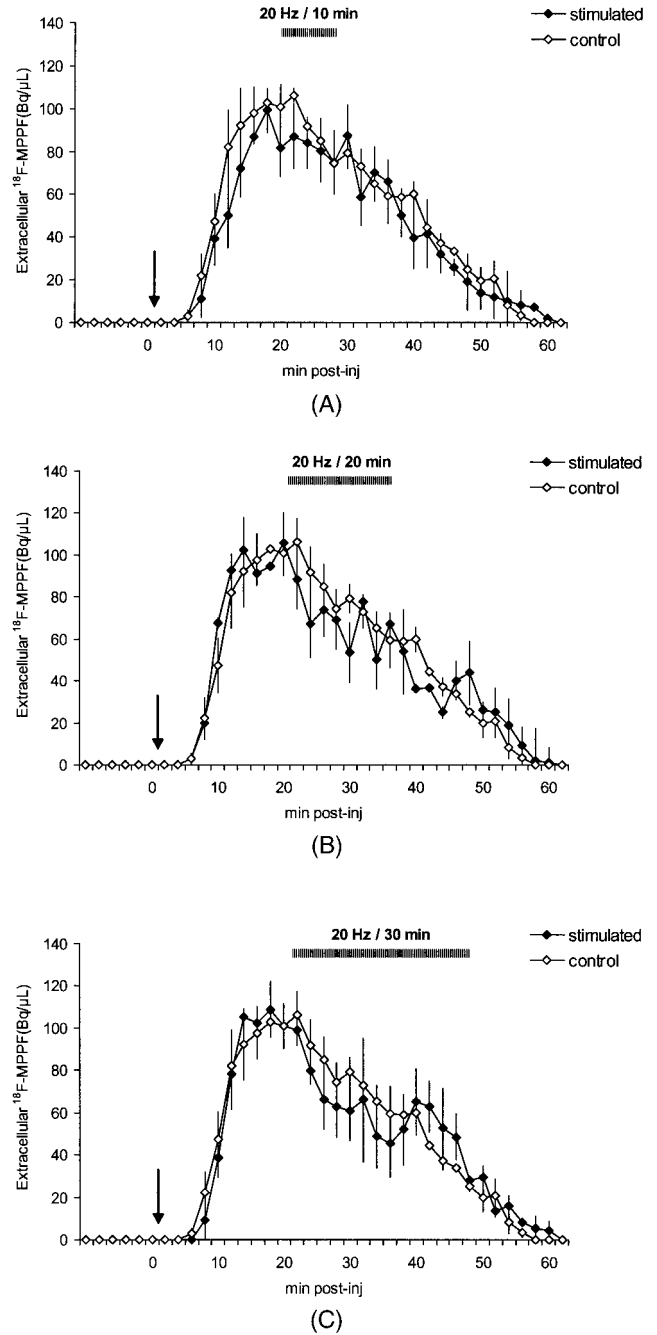


Fig. 4. Extracellular radioactivity, measured by an auto-gamma-counter, of the dialysates collected every 2 min from the hippocampus of anesthetized rats after a 37 MBq <sup>18</sup>F-MPPF i.v. injection followed at 20 min by a DRN electrical stimulation for 10 min (A), 20 min (B), and 30 min (C). Each group of stimulated rats (◆, *n* = 4 rats  $\pm$  SEM) was compared with a group of control rats without stimulation (◇, *n* = 4 rats  $\pm$  SEM).

rats). In control rats the maximal amount of radioactivity had already accumulated in the hippocampus 17 min following administration of <sup>18</sup>F-MPPF. Twenty minutes following injection, hippocampal radioactivity decreased slowly and became nil by 60 min postinjection. When a 20 Hz electrical stimulation was applied to the DRN, 20 min after the <sup>18</sup>F-MPPF injection, for 10

min (Fig. 4A), 20 min (Fig. 4B), or 30 min (Fig. 4C), the hippocampal extracellular radioactivity was unchanged in comparison with control rats.

## DISCUSSION

$^{18}\text{F}$ -MPPF is a PET radiopharmaceutical with a cerebral binding that is consistent with the distribution of 5-HT<sub>1A</sub> receptors, evidenced by a high uptake in the cat and rat hippocampus (Ginovart et al., 2000; Plenevaux et al., 2000). This property makes it suitable for use in clinical PET studies for the visualization of 5-HT<sub>1A</sub> receptors (Passchier and van Waarde, 2001; Passchier et al., 2000) and their quantification (Costes et al., 2002).  $^{18}\text{F}$ -MPPF has a moderate affinity for the 5-HT<sub>1A</sub> receptors,  $K_i = 3.3$  nM (Zhuang et al., 1994), which is comparable to the relative affinity of 5-HT for the 5-HT<sub>1A</sub> receptors,  $K_i = 4.12$  nM (van Wijngaarden et al., 1990). A recent study using six healthy volunteers showed that  $^{18}\text{F}$ -MPPF binding was not affected after a slow infusion of tryptophan, supposed to increase brain 5-HT levels (Udo de Haes, 2002). However, the methodological limit inherent in this clinical study is the lack of knowledge of the brain 5-HT increase after the tryptophan supplementation. In this context, we recently studied the binding of this radiotracer in the rat hippocampus and observed its displacement (reduction) after a fenfluramine injection (Zimmer et al., 2002a). Our aim in the present work was to study an alternative mode of release, electrically evoked, which is closer to physiological neurotransmission.

We therefore verified whether the phasic 5-HT release following electrical stimulation of the DRN influences the  $^{18}\text{F}$ -MPPF binding in the hippocampus. This release involves projections from the dorsal raphe nucleus and innervating largely the ventral hippocampal formation (Azmitia et al., 1978). It has been reported, using the microdialysis technique, that the output of 5-HT in the hippocampal formation increases in proportion with raphe stimulation at frequencies ranging from 5–20 Hz for 20 min (Sharp et al., 1989; Mokler et al., 1998). In our experiments, we applied a stimulus frequency of 20 Hz to the DRN, a high level of activity for serotonergic neurons (Aghajanian et al., 1978).

We first observed that extracellular 5-HT remained unchanged during the DRN electrical stimulation (up to 30 min). According to others (Sharp and Foster, 1989; McQuade et al., 1995), a detectable amount of 5-HT in the dialysates was only measured when a 5-HT reuptake inhibitor was added in the perfusate. An explanation is that the 5-HT reuptake mechanism is sufficiently able to prevent a significant spill-over of 5-HT from the release site into the extracellular fluid, since it is established that the concentration of 5-HT in the extracellular fluid is the result of an equilibrium between release and reuptake processes (Adell and Artigas, 1991; Adell et al., 2002; Di Chiara et al., 1996).

To determine whether the electrically evoked 5-HT release can affect  $^{18}\text{F}$ -MPPF specific binding in the hippocampus, we used a recent intracerebral  $\beta$ -sensitive detector (the  $\beta$ -microprobe) previously described and validated (Zimmer et al., 2002b,c). The  $^{18}\text{F}$ -MPPF radioactivity curves in the hippocampus and cerebellum of control rats were in accordance with our previous study (Zimmer et al., 2002a). A clear-cut finding of our present study was that the DRN electrical stimulation was associated with a significant decrease in  $^{18}\text{F}$ -MPPF specific binding in the hippocampus, showing that  $^{18}\text{F}$ -MPPF-specific binding is under control of endogenous 5-HT release. At the same time, the unchanged binding of  $^{18}\text{F}$ -MPPF in the cerebellum suggested that an eventual alteration of the blood flow during the electrical stimulation was without effect on the radioligand delivery.

With the aim of specifying the compartment occupied by  $^{18}\text{F}$ -MPPF during displacement challenges, we measured the extracellular radioactivity in hippocampus collected by a microdialysis probe. The collected radioactivity in dialysates can be attributed to the  $^{18}\text{F}$ -MPPF itself, since more than 90% of the radioactivity in the hippocampus is due to the unmetabolized compound (Plenevaux et al., 2000). Discrepancies between results from  $\beta$ -microprobe and microdialysis must be noted. Indeed, the decrease in  $^{18}\text{F}$ -MPPF binding during the 10-, 20-, or 30-min DRN stimulations evidenced with  $\beta$ -microprobe did not translate to an increase in free  $^{18}\text{F}$ -MPPF levels as measured with microdialysis and gamma-counting. Our interpretation is that the  $^{18}\text{F}$ -MPPF molecules, initially bound to 5-HT<sub>1A</sub> receptors, are displaced at a synaptic level by the 5-HT release. Thus, diffusing from the synaptic level, the tracer amount of the molecules is diluted in the extracellular level with free unbound species and cannot be detected with a microdialysis probe. Therefore, our hypothesis is that the  $^{18}\text{F}$ -MPPF binding reflects a displacement challenge within a functional synaptic level distinct from the extracellular level. Recent immunocytochemical studies in the rat hippocampus showed a synaptic location of 5-HT<sub>1A</sub> receptors at the edge of synaptic specialization (Kia et al., 1996b; Riad et al., 2000), but also distinct from the extracellular compartment.

To our knowledge, this is the first study to reveal the effect of an evoked release of 5-HT by DRN electrical stimulation on the *in vivo* binding of a serotonergic PET radiotracer. Our experiments showed that phasic 5-HT release induces a reversible  $^{18}\text{F}$ -MPPF displacement in the rat hippocampus. Moreover, this study suggests that  $^{18}\text{F}$ -MPPF is displaced from the 5-HT<sub>1A</sub> receptors at a neuronal level, measured by the  $\beta$ -microprobe, compartmentally distinct from the extracellular level explored by microdialysis. Complementary experimental studies need to be performed to investigate the mechanisms explaining  $^{18}\text{F}$ -MPPF binding vulnerability to changes in synaptic transmitter levels.

This work will be essential in interpreting the results of clinical PET studies.

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