5-HT$_{1A}$ receptor binding and intracerebral activity in temporal lobe epilepsy: an $[^{18}F]$MPPF-PET study

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Summary

The aim of our study was to assess abnormalities in 5-hydroxytryptamine-1A (5-HT$_{1A}$) receptor density in patients suffering from refractory temporal lobe epilepsy (TLE). Experimental data in animals show that 5-HT$_{1A}$ receptors are predominantly located in limbic areas, and that serotonin, via these receptors, mediates an antiepileptic and anticonvulsant effect. In TLE patients, we quantified 5-HT$_{1A}$ receptor density in epileptogenic and non-epileptogenic areas, as defined by intracranial recordings with stereo-electroencephalography (SEEG). Nine TLE patients and 53 control subjects were studied by PET using a 5-HT$_{1A}$ receptor antagonist ($[^{18}F]$MPPF). Anatomical regions of interest (ROIs) were drawn on patient and control MRIs coregistered with PET. PET data were quantified using a simplified model to assess binding potential (BP) values in each ROI, with cerebellum as reference. For each patient, a normalized percentage BP change was calculated as the relative variation of BP in each ROI compared with the corresponding ROI in control subjects. In patients, ROIs explored by SEEG were categorized according to their degree of epileptic activity (ictal onset, ictal spreading, interictal spikes, no epileptic activity) and according to their lesional aspect and volume (lesional with volume loss, lesional without volume loss, non-lesional). Compared with control values, the binding to 5-HT$_{1A}$ receptors in TLE patients was decreased in the epileptogenic temporal lobe. BP decrease was significantly greater in: (i) regions involved in the seizure onset than regions where only interictal paroxysms or no epileptic activity was recorded; and (ii) regions where the discharge propagated than regions where only interictal paroxysms or no epileptic activity was recorded. BP decrease was shown to be significantly influenced by the existence of a lesion on MRI. However, in the group of ROIs with normal quantitative and qualitative MRI aspect, BP decrease remained strongly correlated to the degree of epileptic activity. This study shows that in vivo availability of 5-HT$_{1A}$ receptors is decreased in epileptic patients compared with normal subjects. This decrease is highly correlated to the degree of epileptogenicity of cortical areas explored by intracerebral recordings, and does not reflect only pathological changes or neuronal loss in the epileptic focus.

Keywords: 5-HT$_{1A}$ receptors; intracerebral recordings; PET; serotonin; temporal lobe epilepsy

Abbreviations: AED = antiepileptic drug; AMT = α-[11C]methyl-L-tryptophan; BP = binding potential; 5-HT$_{1A}$ = 5-hydroxytryptamine-1A; IS = interictal spiking activity only; NE = no epileptic activity; NI = non-implanted; ROI = region of interest; SEEG = stereo-electroencephalography TLE = temporal lobe epilepsy


Introduction

Hyperexcitability, synapse re-organization and imbalance between inhibitory and excitatory synapses are three dysfunctions characterizing the epileptogenic zone. The neurotransmitters that influence the excitatory/inhibitory balance include the excitatory (glutamate and aspartate) and inhibitory (GABA) amino acids (Engelborghs et al., 2000). In
addition, monoaminergic systems, such as the serotoninergic ascending pathway, also play a role in the modulation of the cortical and subcortical activity in the mammalian nervous system (Barnes and Sharp, 1999). These ascending pathways may modify the cortical and subcortical excitatory/inhibitory balance, and therefore contribute to the control of epileptic activity. Among the 17 subtypes of serotonin receptors identified to date, the 5-hydroxytryptamine-1A (5-HT1A) receptor is the most widely studied (Peroutka, 1995). The possibility of an interaction between serotonin and epilepsy was first proposed in the late 1950s by Bonnycastle et al. (1957), who observed that brain serotonin concentration was increased by anticonvulsant agents. Since then, a number of studies have been performed on different types of experimental models of epilepsy. While some authors described a convulsant effect of 5-HT1A agonists in absence-type epilepsies (Gerber et al., 1998; Filakovszky et al., 1999), the majority of studies suggest that serotonin might have, on the contrary, an anticonvulsant and antiepileptic effect via 5-HT1A receptors. Serotonin, via the 5-HT1A receptor, was shown to delay the kindling process (Lerner-Natoli, 1987; Wada et al., 1992, 1997) to decrease the frequency of seizures induced by kainic acid (Gariboldi et al., 1996) or bicuculline (Salgado-Commissariat and Alkadhi, 1997), and to inhibit the epileptiform activity induced by an Mg2+-free medium on rat hippocampal slices (Tokarski et al., 2002). Moreover, agents that increase the concentration of endogenous serotonin, such as the inhibitors of serotonin re-uptake (fluoxetine), were evaluated as anticonvulsant in genetically epilepsy-prone rats (Dailey et al., 1992; Yan et al., 1994, 1995) in partial seizures generated by low-frequency electrical stimulation in rats (Watanabe et al., 1998), as well as in kindled rats (Wada et al., 1993). The anticonvulsant action of fluoxetine was recently described as being mediated by 5-HT1A receptors (Lu and Gean, 1998). Therefore, the predominant effect of serotonin in different experimental models of epilepsy (except in absence-type epilepsy) is a 5-HT1A-mediated inhibition of the epileptic activity.

In humans, immunohistochemical studies have revealed increased levels of serotonin in cortical dysplasia with focal epilepsy (Trottier et al., 1995). PET studies using [11C]methyl-L-tryptophan (AMT) suggest alterations in serotonin synthesis in lesional and cryptogenic epilepsy (Chugani et al., 1998; Fedi et al., 2001). Most recently, in a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Precipitating factors</th>
<th>Age at first seizure (years)</th>
<th>Duration of epilepsy (years)</th>
<th>Seizure frequency</th>
<th>Interval between last seizure and PET (days)</th>
<th>AED therapy (dose in mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>46</td>
<td>No</td>
<td>18</td>
<td>28</td>
<td>15/mo</td>
<td>11</td>
<td>Lamotrigine (450); clobazam (10)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>58</td>
<td>No</td>
<td>20</td>
<td>38</td>
<td>5–10/mo</td>
<td>1</td>
<td>Carbamazepine (1000); topiramate (400); phenobarbital (100); clobazam (10)</td>
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<tr>
<td>3</td>
<td>M</td>
<td>34</td>
<td>No</td>
<td>5</td>
<td>28</td>
<td>&lt;5/mo</td>
<td>1</td>
<td>Carbamazepine (600); lamotrigine (200); clobazam (20)</td>
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<tr>
<td>4</td>
<td>M</td>
<td>23</td>
<td>Febrile convulsion in childhood</td>
<td>2</td>
<td>21</td>
<td>5–20/mo</td>
<td>2</td>
<td>Levitiracetam (1500); sodium valproate (1000); carbamazepine (800); lamotrigine (300); topiramate (50); clobazam (20)</td>
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<td>5</td>
<td>M</td>
<td>31</td>
<td>Perinatal injury</td>
<td>10</td>
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<td>2</td>
<td>Carbamazepine (1200); topiramate (300); phenobarbital (200)</td>
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<td>6</td>
<td>F</td>
<td>29</td>
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<td>7</td>
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<td>5/mo</td>
<td>4</td>
<td>Phenytoin (200); clobazam (30)</td>
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<tr>
<td>7</td>
<td>M</td>
<td>24</td>
<td>No</td>
<td>19</td>
<td>4</td>
<td>10–15/mo</td>
<td>1</td>
<td>Carbamazepine (1200); alprazolam (1); clobazam (20)</td>
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<tr>
<td>8</td>
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<td>No</td>
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<td>Lamotrigine (600)</td>
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<tr>
<td>9</td>
<td>M</td>
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<td>Prolonged left hemiconvulsions in childhood</td>
<td>12</td>
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<td>15–30/mo</td>
<td>15</td>
<td>Gabapentin (2400); phenobarbital (100); hydroxyzine dichlorhydrate (40); clobazam (20)</td>
</tr>
</tbody>
</table>

M = male; F = female; mo = month.
PET study using [$^{18}$F]FCWAY in temporal lobe epilepsy (TLE), Toczek et al. (2003) reported that 5-HT$_{1A}$ receptor binding is reduced in TLE foci. In this paper, we explored the possible relationships between epilepsy and serotonin using PET and [$^{18}$F]MPPF, a 5-HT$_{1A}$ receptor antagonist, with patients suffering from refractory TLE who, as part of their pre-surgical evaluation, underwent stereotactic implantation of intracerebral electrodes [stereo-electroencephalography (SEEG)] and video-SEEG monitoring of their seizures. Our aim was to correlate changes in 5-HT$_{1A}$ receptor binding with the degree of epileptic activity in the explored regions, as assessed by the paroxysmal ictal and interictal activity recorded by intracerebral electrodes.

**Material and methods**

**Patients and controls**

Nine TLE patients, eight males and one female, aged 23–58 years (mean $\pm$ SD, 35.9 $\pm$ 11.8) were investigated at the Neurological Hospital in Lyon between February 2001 and January 2003. Patients, clinical features and depth electrode investigations are described in Tables 1 and 2.

All patients suffered from drug resistant temporal lobe epilepsy and all but one were receiving antiepileptic drug (AED) polytherapy at the time of PET investigation (see Table 1 for details). All patients underwent intracranial SEEG in the course of their presurgical investigation. The [$^{18}$F]MPPF-PET was performed either during the third week of the SEEG investigation with depth electrodes in place, or before or after (7 months) depth electrode implantation. The last seizure occurred 1–15 days before the PET study. Each patient had between seven and 15 depth electrodes implanted unilaterally (in five patients) or bilaterally (in four patients). The cortical structures to be explored were chosen according to interictal EEG, video-scalp-EEG ictal data, interictal [$^{18}$F]fluorodeoxyglucose PET, interictal and ictal single photon emission tomography (SPECT) and brain MRI data. The video-SEEG monitoring was carried out over 10–16 days.

Fifty-three healthy volunteers consisting of 27 women (age 19–70 years, mean $\pm$ SD, 42.6 $\pm$ 14.8) and 26 men (age 20–68 years, mean $\pm$ SD, 40.8 $\pm$ 13.4) also underwent a brain MRI and a [$^{18}$F]MPPF-PET and were used as a control population. None of the control subjects had psychiatric or neurological illnesses as assessed by medical examination and inspection of the anatomical T1 MRI. Similar to the patient group, control group investigation involved a cerebral MRI and a [$^{18}$F]MPPF-PET session.

Patients and controls were evaluated for depression using the General Health Questionnaire (GHQ-28) of Goldberg (Goldberg and Hillier, 1979). None of the patients or controls reached the threshold (i.e. score 7) for depression. GHQ-28 depression scores ranged between 0 and 5 in patients (mean $\pm$ SD, 2.75 $\pm$ 2.1) and between 0 and 4 in controls (0.42 $\pm$ 0.8).

All subjects gave their informed consent to the protocol, which was approved by the local ethical committee (CCPRPB, Centre Léon Berard, Lyon) in accordance with the Declaration of Helsinki.

**Data acquisition**

**MRI**

MRI acquisition consisted of a 3D anatomical T1-weighted sequence using a 1.5 T Siemens Magnetom scanner (Siemens AG, Erlangen, Germany). The anatomical volume covered the whole brain with mm$^3$ voxels.

**PET**

[$^{18}$F]MPPF was obtained by nucleophilic fluorination on a nitro precursor with a radiochemical yield of 20–25% at the end of synthesis and a specific activity of 32–76 GBq/µmol (Le Bars et al., 1998). PET sessions were performed on a CTI-SIEMENS HR+ (Siemens, Knoxville, TN, USA) during the afternoon. For tracer injections, an intravenous catheter was placed in the radial vein of the left arm. A thermoformable head holder was moulded for each
subject in order to limit head movement during acquisition. Prior to the emission acquisition, a 10-min transmission scan was performed using three $^{68}$Ge rod sources for the measurement of tissue and head support attenuation.

After intravenous injection of a bolus of $186 \pm 30$ MBq $[^{18}F]$MPPF, the dynamic PET scan of emission, consisting of 35 frames of increasing duration (20 s to 5 min) was acquired to evaluate the local radiotracer concentration during 50 min post-injection. The PET scanner was operated in 3D mode. Images were corrected for scatter and attenuation and reconstructed using a filtered back projection (Hamming filter of cut-off 0.5 cycles/pixels), in order to provide a 3D volume of 63 slices (2.42 mm thickness), with 128 × 128 voxels in plane (2.06 × 2.06 mm).

Data analysis
For each dynamic file, we visually controlled the head movements. In two cases where head movements occurred, the static images were taken as reference for an automatic linear spatial alignment (Automated Image Registration package; Woods et al., 1992) of the dynamic acquisition. From the realigned dynamic acquisition, we computed a new static image of equilibrium from 0 to 50 min post-injection. PET static images were re-oriented according to the bi-hippocampal plane. The same transformation matrix was applied to the dynamic data. Transaxial MRIs were co-registered with the static PET and re-sliced with the same sampling as the PET data. This pre-processing resulted in a complete data set (anatomic MRI, static and dynamic PET) with common orientation and size. Using a contour tool (CAPP; CTI/Siemens) we drew 400 regions of interest (ROIs) upon the registered MRI, grouped into 37 volumes of interest representing the actual anatomical regions (see Fig. 1). ROIs were outlined anatomically, following the grey matter ribbon. The identification of key sulci on individual MRI (central, precentral, postcentral, intraparietal, parieto-occipital, temporo-occipital) allowed for the anatomical delineation. Bilateral ROIs were drawn in temporal poles, parahippocampal gyri, amygdala, hippocampi and fronto-parietal opercula, as well as in occipital, inferior middle and superior temporal, inferior and superior parietal, orbito-frontal, inferior, middle and superior frontal, anterior and posterior cingulate, and insular gyri. As a reference for the simplified model, a large ROI was also drawn in the cerebellum. For raphe nuclei, which are difficult to delineate on MRI, contours were first

Fig. 1 ROI outlining. ROIs were outlined manually on each MRI slice and regrouped into volumes of interest. 1: cerebellum; 2: raphe dorsalis; 3: parahippocampal gyrus; 4: temporal pole; 5: inferior temporal gyrus (T3); 6: occipital gyrus; 7: hippocampus; 8: amygdala; 9: middle temporal gyrus (T2); 10: superior temporal gyrus; 11: insula; 12: orbitofrontal gyrus; 13: inferior parietal lobule; 14: thalamus; 15: superior parietal lobule; 16: post- and precentral operculum; 17: anterior cingulate gyrus; 18: posterior cingulate gyrus; 19: inferior, middle and superior frontal gyri.
drawn on the static PET image and then displayed on the subject MRI to verify their proper location in the peri-aqueductal grey matter of the brainstem.

All ROIs were applied to the dynamic image and, for each ROI, time–activity curves were extracted and used for data quantitation using a simplified reference tissue model developed for \([^{11}C]\text{WAY100635}\), another antagonist of 5-HT\(_{1A}\) receptors (Lammertsma and Hume, 1996; Gunn et al., 1998) and recently validated for \([^{18}F]\text{MPPF}\) studies (Costes et al., 2002). This model provides three parameters \(k_2, R_1 = k_1\text{ref}/k_{1\text{ROI}},\) and binding potential (BP) \(^{\ast}\) without the need for arterial sampling to define the input function: the free and non-specific ligand kinetic is based on the time–activity curve of a reference region (i.e. cerebellum) that is assumed to be devoid of 5-HT\(_{1A}\)-specific binding. Using this model, we previously confirmed that BP was a good index of local receptor concentration \((B_{\text{max}})\) in normal subjects (Costes et al., 2002). Of the three parameters extracted, only BP \((\text{BP} = B_{\text{max}}/K_d)\) values were further analysed. Reliability of cerebellum time–activity curves (TAC) was assessed by checking that each cerebellum individual TAC for controls and patients was included in the mean TAC obtained from all 53 controls \(\pm 2\) SD.

As gender differences in AMT uptake (Nishizawa et al., 1997; Okawaza et al., 2000) and gender-specific age differences in 5-HT\(_{1A}\) binding (Melzter et al., 2001) have previously been reported, BP values in male and female patients were respectively compared with gender-matched control groups (27 females and 26 males). For the ROI of each patient, the percentage BP variation compared with controls \((\Delta \text{BP})\) was calculated with the following formula:

\[
\Delta \text{BP} = 100 \times \frac{\text{BP}_{\text{patient}} - \text{BP}_{\text{control}}}{\text{BP}_{\text{control}}}
\]

**Influence of electrode implantation and other clinical parameters**

To test for a possible global effect of electrode implantation, we compared mean global \(\Delta \text{BP}\) and mean \(\text{BP}\) in raphé nuclei in the group of patients who underwent SEEG during PET versus the group of three patients who had intracerebral exploration either before (patients 1 and 4) or 7 months after the PET session (patient 9).

To test for a possible local effect of electrode implantation, we compared BP variations in: (i) explored areas without epileptic activity (NE); and (ii) in the homologous non-implanted regions on the contralateral side (NI-contra). This comparison was done in the group of patients who had implanted electrodes during PET. Patient 3 was excluded from this group as he had implanted electrodes in the homologous contralateral regions as well.

To test for a possible influence of the delay between last seizure and PET, the duration of epilepsy, the GHQ-28 scores, the carbamazepine, lamotrigine and benzodiazepine daily treatment dose on BP changes, all these factors considered independently were tested with a multiple regression analysis against: (i) global BP changes; (ii) BP changes in raphé; and (iii) BP changes in the hippocampus on the epileptogenic side.

**MPPF/SEEG data correlation**

In six of nine patients, SEEG was recorded during the PET session. No seizures occurred during the PET acquisition, and no discharge lasting more than 5 s was recorded in these six patients.

All data acquired during the video-SEEG session were reviewed \textit{a posteriori} and carefully analysed for categorization by three experienced reviewers blinded to PET results (K. Ostrowsky, J. Isnard and P. Ryvlin). The intracerebral activity was classified into:

(i) onset: ictal discharge onset (low voltage rhythmic activity or first ictal activity detected); (ii) spread: ictal discharge spreading; (iii) IS: interictal spiking activity only (spikes, polyspikes, spike and waves); and (iv) NE: no epileptic activity. Non-implanted regions (NI) were also categorized according to whether they were ipsilateral (NI-ipsi) or contralateral (NI-contra) to the epileptogenic zone. In one patient (No. 8), an isolated ictal discharge was recorded in the left hippocampus, whereas all other recorded seizures started in the right mesiotemporal structures. For this patient, the left hippocampus was categorized as an `onset' region along with the right mesiotemporal structures. In another patient (No. 9), an asymptomatic discharge was recorded in the right operculum outside the usual regions of seizure onset. Likewise, this region was categorized as an `onset'.

**MRI analysis**

In order to define the nature and extension of lesional areas (see Table 2), patient MRIs were visually analysed by the same observers used for the SEEG data (K. Ostrowsky, J. Isnard and P. Ryvlin). For this analysis, each ROI was categorized as either `lesional' or `non-lesional'. As described in Table 2, the visual analysis showed: (i) no abnormality in three patients (Nos 1, 2 and 8); (ii) a unilateral hippocampal sclerosis ipsilateral to the epileptic focus in four patients (Nos 3, 4, 6 and 9), associated, in two of them (patients 4 and 9), with a loss of grey–white matter differentiation in the temporo-polar region ipsilateral to the hippocampal sclerosis; (iii) a temporo-polar porencephalic lesion in one patient (No. 5); and (iv) a cavernous angioma of the left superior temporal gyrus, excised 1 year before SEEG investigation in the remaining patient (No. 7).

In addition, the volumes of ROIs were estimated quantitatively for all subjects (see Table 3). For patients, ROI volumes were considered as significantly reduced when below the mean corresponding ROI volume minus 2 SD in the gender-matched control subgroup. For lesional ROIs (visually defined), where a significant volume decrease was quantitatively measured, the ROI was re-labelled as ‘lesional with volume loss’. None of the non-lesional ROIs (as visually defined) showed significant volume reduction when compared with the corresponding control ROI.

**Statistical analysis**

After verifying that \(\Delta \text{BP}\) percentages were normally distributed, we performed an analysis of variance (ANOVA) statistical analysis (StatView\textsuperscript{\textregistered}; Abacus Concepts) on the full group of patients using \(\Delta \text{BP}\) as the dependent variable, and the type of intracerebral activity (onset, spread, IS, NE, NI-ipsi, NI-contra) as the factor. The same analysis was then performed separately on the group of non-lesional ROIs, lesional ROIs without volume loss and with volume loss.

**Results**

**SEEG results**

The results of the SEEG recording for each patient are reported in Table 4. Mesio-temporal seizure onsets were identified in seven patients (Nos 1–4, 6, 8 and 9), whereas in

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### Table 3  Mean ROI volumes (cm³) in controls and ROI volumes (cm³) in patients

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>Hipp</th>
<th>paraH</th>
<th>Pole</th>
<th>Ins</th>
<th>aCing</th>
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<th>Ofr</th>
<th>T3</th>
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<th>Oper</th>
<th>iPa</th>
<th>sPa</th>
<th>Raphe</th>
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<tr>
<td><strong>Mean (F)</strong></td>
<td>R</td>
<td>2.2</td>
<td>2.1</td>
<td>5.8</td>
<td>5.7</td>
<td>6.3</td>
<td>6.1</td>
<td>12.2</td>
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<tr>
<td><strong>(2 SD)</strong></td>
<td>L</td>
<td>0.8</td>
<td>0.9</td>
<td>1.7</td>
<td>1.6</td>
<td>3.1</td>
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<tr>
<td><strong>Mean (M)</strong></td>
<td>L</td>
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<tr>
<td><strong>(2 SD)</strong></td>
<td>L</td>
<td>0.6</td>
<td>0.5</td>
<td>1.8</td>
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<td>1.8</td>
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<td>3.1</td>
<td>5.2</td>
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</table>

A = amygdala; Hipp = hippocampus; paraH = parahippocampal gyrus; Pole = temporal pole; Ins = insula; aCing = anterior cingulate gyrus; pCing = posterior cingulate gyrus; Ofr = orbital-frontal; T3 = inferior temporal gyrus; T2 = middle temporal gyrus; T1 = superior temporal gyrus; Oper = operculum; iPa = inferior parietal lobule; sPa = superior parietal lobule; M = male; F = female. Italic type = ROIs where the visual analysis detected a lesion. *ROIs with a significant volume loss (= below the mean control values ± 2 SD). In patient 5, a significant volume loss was found in the right hippocampus. The visual analysis did not detect any asymmetry because the left hippocampus volume appeared smaller as well, although not significantly. In that case, the right hippocampus first labelled as non-lesional was then re-categorized into 'lesional with volume loss'.

### Table 4  Intracerebral data

<table>
<thead>
<tr>
<th></th>
<th>A</th>
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Empty cells = non-implanted structures. A = amygdala; Hipp = hippocampus; paraH = parahippocampal gyrus; Pole = temporal pole; Ins = insula; aCing = anterior cingulate gyrus; pCing = posterior cingulate gyrus; Ofr = orbital-frontal; T3 = inferior temporal gyrus; T2 = middle temporal gyrus; T1 = superior temporal gyrus; Oper = operculum; iPa = inferior parietal lobule; sPa = superior parietal lobule; ON = ictal onset; SP = discharge spreading; IS = interictal spikes only; NE = no epileptic activity. In all patients, interictal spikes were also always recorded in regions of ictal onsets and spreadings. Ictal onset was characterized by a fast low-voltage activity, except in case of patient 8 (2) (clonic activity) and patient 9 (mythemic spike activity). *Ictal discharge elicited only after electric stimulation of the posterior aspect of the superior temporal gyrus. **In patient 8, most ictal discharges started on the right [8 (1)], except one [8 (2)]. ***Asymptomatic ictal discharge.
the two remaining patients ictal discharges started outside the mesio-temporal structures. In patient 5, seizures started in the right temporal pole, where a major porencephalic lesion was clearly discernable and spread quickly to mesio-temporal structures as well as to neocortical temporal structures. In the second patient (No. 7), only post-discharges, but no spontaneous seizure, were recorded in the left superior temporal gyrus after electrical stimulation of the anterior or posterior superior temporal gyrus.

**PET data**

**Visual analysis**

*Controls.* As described previously, PET static images in controls showed a high uptake of $^{18}$F]MPPF in cortical regions recognized to be rich in 5-HT$_{1A}$ receptors, namely limbic (hippocampus, amygdala, parahippocampal gyrus) and paralimbic (temporal pole, insula, anterior and posterior cingulate gyri) regions, and a less intense concentration in neocortical regions. Owing to their high density in 5-HT$_{1A}$ auto-receptors, raphe nuclei were easily identifiable in the brainstem, despite their small volume. $^{18}$F]MPPF binding in a control subject is illustrated as an example at the top of Fig. 2.

*Patients.* In six of nine epileptic patients, a clear asymmetry could be visually identified before quantification on radioactivity PET images. Abnormalities were widespread in three patients (Nos 5, 6 and 9) and more focal in three other patients (Nos 1, 3 and 8). Two different examples (patients 3 and 9) are shown in Fig. 2 (bottom). Scans of the three remaining patients (Nos 2, 4 and 7) did not reveal any clear abnormality on visual inspection. These three patients were not different from the others in terms of history, AED therapy, seizure onset or brain MRI.

**ROI analysis**

The comparison of each patient with their gender-matched control group revealed significant BP changes in eight of nine patients. Results are presented in Table 5.

Significant BP decreases (i.e., inferior to the mean control values – 2 SD) were focal in three patients (Nos 1, 4 and 6) and located ipsilateral to the seizure onset, in the temporal pole (patient 1), in the hippocampus (patient 4) or in the mesiotemporal structures and temporal pole (patient 6). Two patients (Nos 5 and 9) showed a more widespread significant BP decrease ipsilateral to seizure onset in mesiotemporal regions, temporal pole and temporal neocortex, as well as in the ipsilateral insula for patient 5. In two patients (Nos 3 and 8), significant BP decreases were found in mesiotemporal structures and in the temporal pole bilaterally.

In addition, two patients (Nos 4 and 9) showed a significant BP increase in bilateral frontal and parietal regions (patient 4) and in the controlateral mesiotemporal regions, temporal pole, and inferior temporal gyrus (patient 9). Finally, one patient only showed a significant BP increase in raphe nuclei (patient 7), and another one showed no significant BP variation compared with controls (patient 2).

Compared with intracerebral data, the significant decrease of BP was concordant with the ictal onset region in five cases (patients 4–6, 8 and 9), occurred in a propagation area in another case (patient 1), and in the non-explored temporal pole in one patient (No. 3). In this latter case, however, the temporal pole was more than likely involved in the discharge spreading. In the last two patients (Nos 2 and 7), the maximal BP decrease was concordant with the onset zone but did not reach the significant threshold.

When considering BP changes in each patient with MRI abnormalities, the region of significant MPPF binding decrease matched the region of morphological change in one patient (No. 5). In most other cases, significant BP decreases could be found both in lesional and non-lesional regions. Finally, in one patient (No. 7), a BP decrease was found in the lesional region, but did not reach a significant level. In the three patients with no MRI abnormalities, one showed no abnormalities in MPPF binding (patient 2), one showed a focal decrease in the temporal pole ipsilateral to seizure onset (patient 1) and the third showed a bilateral decrease of BP in mesiotemporal and temporopolar regions (patient 8).

**Global BP and BP in raphe**

Global BP in patients ranged between 0.47 and 0.74 (mean ± SD, 0.60 ± 0.08) and were not significantly different for female (0.62 ± 0.11) or male (0.62 ± 0.10) controls.

BP in raphe ranged from 0.42 to 0.84 (0.59 ± 0.17) in patients, from 0.17 to 0.81 (0.48 ± 0.15) in male controls and from 0.23 to 0.99 (0.52 ± 0.17) in female controls. In patients 4 and 7, BP values in raphe exceeded mean control values +2 SD, respectively, by 76.4 and 70.8%.

There was no correlation between global BP and BP in raphe.

**Influence of electrode implantation and other clinical parameters**

Compared with controls, the mean (± SD) variation of global BP was –5.8 ± 12.9% in the group of six patients who had implanted electrodes during PET data acquisition, and 5.4 ± 12.1% in the group of three patients for whom the PET was not performed during the SEEG investigation. This difference was not significant. No significant difference was found in raphe nuclei between the mean BP variation in the group of implanted patients (24.4 ± 31.7%) compared with the group of non-implanted patients (18.3 ± 50.3%).

Similarly, there was no local influence of electrode implantation. Compared with controls, BP change was 3.4 ± 8.0% in implanted structures where no epileptic activity
was recorded (either interictal or ictal) and 2.5 ± 16.4% in the homologous regions of the non-implanted contralateral hemisphere. A within-subject paired t-test did not reveal any significant difference between BP changes in the two groups of regions.

The multiple regression analysis did not reveal any significant effect of epilepsy duration, delay between PET and last seizure, depression score, and AED treatment on global BP changes, BP changes in raphe or BP changes in the hippocampus on the epileptogenic side.

![Fig. 2 Co-registered MRI and static [18F]MPPF-PET data in a normal control subject (top) and in two epileptic patients (bottom). L = left; R = right. Whenever transaxial or coronal sections were passing through the raphe, this structure is indicated by a green arrow (control subject and patient 3). White arrows indicate asymmetries in [18F]MPPF binding in patients. Patient 9 shows a large asymmetry with a binding decrease in left mesiotemporal and lateral temporal structures, as well as in extratemporal regions (insula, operculum and frontal regions). Patient 3 shows a more focal asymmetry, with a binding decrease in the right hippocampus, part of the right inferior temporal gyrus, and in the right temporal pole.](image)
Correlation with intracerebral activity
Compared with the control group, BP in patients was decreased in 90, 74, 57 and 27% of ROIs, where, respectively, discharge onsets, spreading, interictal activity only and no epileptic activity had been recorded. These BP reductions encompassed the mean control values ± 2 SD in 45, 28 and 7%, and none of the ROIs belonging, respectively, to the onset, spread, IS and NE groups.

\[ \Delta BP \text{ versus type of intracerebral activity} \]
On average, BP values were decreased by 26.9 ± 20.7% in onset ROIs, by 17.3 ± 28.1% in spread ROIs, and by 2.5 ± 24.0% in regions where only interictal activity occurred (see Fig. 3). Conversely, BP was increased by 8.4 ± 11.1% in NE regions, 3.2 ± 26.4% in NI-ipsi regions and 10.2 ± 21.2% in NI-contra regions. ANOVA revealed a significant effect of the type of intracerebral activity on \( \Delta BP \) (\( F = 15.7; P < 0.0001 \)). The post hoc analysis revealed a significantly greater decrease of BP in regions of seizure onsets than in those where interictal activity (\( P < 0.004 \)) or no epileptic activity had been recorded (\( P < 0.0001 \)). Likewise, a significantly greater reduction of BP values was also observed in regions where discharges spread compared with regions where interictal activity (\( P < 0.04 \)) or no epileptic activity (\( P < 0.001 \)) had been recorded. No significant difference in mean BP variation was identified between onset and spread regions, or IS and NE regions.

\[ \Delta BP \text{ versus type of intracerebral activity in lesional regions} \]
Compared with controls, BP values were on average decreased by 58.2 ± 22.8% in lesional regions with volume loss and by 42.5 ± 15.6% in lesional regions without volume loss, and increased by 3.7 ± 24.0% in non-lesional regions. ANOVA revealed a significant effect of the ‘lesion’ factor on \( \Delta BP \) (\( F = 31.07; P < 0.0001 \)). The post hoc analysis revealed a significantly greater decrease of BP in lesional ROIs with volume loss than in non-lesional ROIs (\( P < 0.0001 \)), and in lesional ROIs without volume loss than in non-lesional ROIs (\( P < 0.0001 \)). No significant difference was identified between lesional regions with and without volume loss.

As illustrated in Fig. 4, for each type of intracerebral activity considered, the mean percentage of BP decreased gradually in non-lesional, lesional without volume loss and lesional with volume loss ROIs.

When considering only lesional regions with volume loss, no significant difference appeared between the BP decrease in onset and spread ROIs.

When considering only lesional regions without volume loss, BP was decreased by 41.2 ± 20.1% in onset ROIs, by 46 ± 4.1% in spread ROIs, by −61.5% (one value) in IS ROIs and by −38.4 ± 34% in NE regions. ANOVA did not reveal any difference of \( \Delta BP \) between these groups.

\[ \Delta BP \text{ versus type of intracerebral activity in non-lesional regions} \]
When considering only non-lesional ROIs, BP values were decreased by 19.1 ± 14.8% in onset ROIs and by 12.6 ± 26.1% in spread ROIs (see Fig. 4). BP was increased by 2.0 ± 17.6% in regions where only interictal activity occurred (IS), by 8.4 ± 11.1% in NE regions, by 4.3 ± 26.0% in NI-ipsi regions and by 10.2 ± 21.2% in NI-contra regions. ANOVA revealed a significant effect of the type of intracerebral activity on \( \Delta BP \) (\( F = 9.4; P < 0.0001 \)). The post hoc analysis showed a greater decrease of BP in regions of seizure onsets than in those where interictal activity (\( P < 0.02 \)) or no epileptic activity (\( P < 0.001 \)) had been recorded. Likewise, a significantly greater reduction of BP values was also observed in regions where discharges spread compared with regions where interictal activity (\( P < 0.05 \)) or no epileptic activity (\( P < 0.003 \)) was recorded. No significant difference in mean BP variation was identified between onset and spread regions, or IS and NE regions.

Discussion
By combining \(^{[18F]}\text{MPPF-PET}\) with intracerebral recordings, we show in this study that decreased 5-HT\(_{1A}\) receptor binding, recently reported by Toczek et al. (2003), is related to the paroxysmal activity in the epileptogenic temporal lobe, and does not just reflect lesion-related histological changes. Before discussing these conclusions, the influence of electrode implantation and clinical parameters on PET findings deserves some attention.

Parameters possibly influencing our results
Studies in a number of animal species have revealed that implantation of electrodes per se may induce neurochemical, histological or vascular alterations (Boast et al., 1976; Ben Attia et al., 1992; Loscher et al., 1995). However, we found no significant effect of electrode implantation on global brain or raphe nuclei BP. Moreover, regarding a possible local effect of electrode implantation, we found no BP differences between non-epileptic areas on the focus side and contralateral non-implanted homologous regions. This suggests that, in our group of patients, global or focal changes in 5-HT\(_{1A}\) binding due to electrode implantation are unlikely.

Symptoms of depression and anxiety are frequent in patients with epilepsy (Kanner and Palac, 2000), and might interact with our BP measures, as 5-HT\(_{1A}\) receptors have been shown to be involved in major depression (Cowen, 2000). Indeed a post mortem study on suicide victims with major depressive disorders showed reduced 5-HT\(_{1A}\) receptor mRNA in the hippocampus (Lopez et al., 1998). A PET study using \(^{[11C]}\text{WAY100635}\) showed an abnormal reduction in 5-HT\(_{1A}\) receptor BP in patients compared with controls located mainly in the raphe, and in mesiotemporal cortex, but extending also to occipital and postcentral regions (Drevets et al., 1992; Loscher et al., 1995). However, we found no significant effect of electrode implantation on global brain or raphe nuclei BP. Moreover, regarding a possible local effect of electrode implantation, we found no BP differences between non-epileptic areas on the focus side and contralateral non-implanted homologous regions. This suggests that, in our group of patients, global or focal changes in 5-HT\(_{1A}\) binding due to electrode implantation are unlikely.
et al., 1999). In our group of patients, although individual GHQ-28 did not reach the threshold for depression in any patient (or control), the mean depression score was different in patients from that in controls. However, no significant effect of this score could be identified on BP changes in raphe or in hippocampus on either side.

The delay between the occurrence of last seizure and the PET might also possibly influence $[^{18}F]MPPF$ binding.
Fig. 3 Degree of epileptic activity and BP changes. Mean BP variations relative to controls are provided for six subgroups of ROIs. Onset: regions where ictal discharges started; spread: regions where the ictal discharges propagated; IS: regions where only interictal spiking was recorded; NE: regions where no epileptic activity was recorded; NI-ipsi: non-implanted regions ipsilateral to the seizure onset; NI-contra: non-implanted regions contralateral to seizure onset. Error bars represent the SD.

Fig. 4 Effect of lesions on BP changes. Mean BP variations relative to controls for same six subgroups of ROIs as in Fig. 2. In each category, BP variations are averaged separately for lesional ROIs with volume loss, lesional ROIs without volume loss and non-lesional ROIs. In categories IS, NE, NI-ipsi and NI-contra, lesional regions had no significant volume loss. In category NI-contra, no ROI showed a lesional aspect on MRI. In category IS, only one ROI showed a lesional aspect and no significant volume loss. Error bars represent the SD.
Studies in animal models of epilepsy show a trend towards a binding increase to 5-HT$_{1A}$ receptors appearing 1 week after stage 5 in the kindling model (Cagnotto, et al., 1998), or at day 6 in the kainate model (Van Bogaert, et al., 2001). These changes were shown to be long-lasting. In our population of patients, we did not evidence any effect of the delay between last seizure and PET on BP changes in raphe or hippocampus, or on global BP modifications.

Finally, no significant effect of the AED treatment could be shown on BP modifications, although AED are known to interact with the serotonergic system and to be effective in depression disorders (Harden, 2002).

Nevertheless, given the very small number of measures, and the high SDs, this does not allow for definitely ruling out the influence of all these factors on our results.

**Individual data**

Given the small group of patients, individual results are difficult to discuss, especially in terms of relationships between the epileptic activity and the BP changes.

Individual results are compatible with a close relationship between the significant decrease in MPPF binding and the location of the area where seizures started and/or spread. Results are, however, very variable across patients, as some of them showed very focal areas of BP decrease, whereas in other cases the PET functional abnormality was more widespread. It is, however, interesting to note that in the case where bilateral discharges were recorded, significant bilateral changes in MPPF binding occurred.

Individual data also show a clear relationship between the existence of a lesion on MRI and a significant BP decrease. Indeed, a significant BP decrease was measured in 11 of 13 lesional regions (with or without volume loss). However, it is noteworthy that significant and major BP decreases also occurred in non-lesional regions.

Interestingly, in some patients, a significant increase in MPPF binding was measured in regions located on the contralateral side. In rats, an increase in binding to 5-HT$_{1A}$ receptors has been identified during the late chronic phase, bilaterally in the hippocampus, after kainic acid injection (Van Bogaert, et al., 2001) or kindling (Clark, et al., 1993; Cagnotto, et al., 1998). Contrary to these experimental results, the BP increase we observed in our group of TLE patients was never located within the epileptogenic zone, but involved contralateral regions. Given a possible antiepileptic effect of serotonin through 5-HT$_{1A}$ receptors, this increase could be interpreted as a regulation process in response to the epileptic discharges occurring, part of which could contribute to the modulation of neuronal hyperexcitability.

In two patients we observed a significant binding increase compared with controls in raphe nuclei. In these two patients, we failed to find a relationship between this increase and other clinical factors such as TLE syndrome (mesiotemporal versus neocortical seizure onsets), MRI, treatment, past history, or GQH-28 score for depression or anxiety. On the contrary, in their study, Toczek et al. (2003) described a decrease of [¹⁸F]FCWAY binding in raphe nuclei of epileptic patients. These contradictory results might be explained by the different binding properties of [¹⁸F]MPPF and [¹⁸F]FCWAY used in these two studies, as [¹⁸F]MPPF might be more sensitive to variation in the concentration of endogenous serotonin than [¹⁸F]FCWAY.

### Mechanisms of 5-HT$_{1A}$ receptor binding decrease in the epileptogenic temporal lobe

The reduction of 5-HT$_{1A}$ binding that we observe on the side of the epileptogenic zone is in agreement with data obtained in some epilepsy models. Indeed, in genetically epilepsy-prone rats, a decrease of binding of the agonist [³H]8-OH-DPAT [8-hydroxy-2-(di-n-propylamino) tetralin] to 5-HT$_{1A}$ receptors was observed in the hippocampus (Statnick, et al., 1996). It also is in agreement with results of a study using another antagonist of 5-HT$_{1A}$ receptors, [¹⁸F]FCWAY, showing reduced 5-HT$_{1A}$ binding in mesial and lateral temporal regions on the side of the epileptic focus (Toczek, et al., 2003).

[¹⁸F]MPPF has an affinity ($K_i = 3.3$ nM) close to that of serotonin ($K_i = 4.7$ nM) for the 5-HT$_{1A}$ receptor (Zhuang, et al., 1994), and was shown to be sensitive to endogenous variations of serotonin concentration (Zimmer, et al., 2002). Thus, a decrease of MPPF binding can be interpreted as reflecting either a decrease in receptor density or an increase of endogenous serotonin, resulting in a competition for receptor binding by the radioligand. In the first hypothesis, a causal process could be involved in which depletion in serotonin could lead to a down-regulation of 5-HT$_{1A}$ receptors and hyperexcitability. In the second hypothesis, a reactional process might be involved, in which epilepsy itself leads to an increase in serotonin concentration in order to modulate the neuronal hyperexcitability.

In the absence of a concomitant investigation of serotonin synthesis it is impossible to decide whether the binding decrease we observed in discharge onset and spreading zones reflects a decreased density in 5-HT$_{1A}$ receptors, or a higher occupancy of these receptors by endogenous serotonin. Studies in human epilepsy on resected tissues have indicated an increase of serotonin concentration and of its metabolite, 5-hydroxyindolacetic acid (5-HIAA), in the epileptogenic zone, this increase being greater in regions where frequent interictal spikes had been recorded (Louw, et al., 1989; Pintor, et al., 1990). These data suggest that the decrease in receptor binding observed in our patients could be explained by an increase of serotonin concentration resulting in a competition with the radioligand for receptor binding.

To that extent, the increase in [$^{11}$C]α-methyl-tryptophan ([¹¹C]AMT) uptake in tuberous sclerosis (Chugani, et al., 1998; Fedi, et al., 2003), cortical dysplasia and cryptogenic epilepsy (Fedi, et al., 2001), including TLE with no hippocampal volume loss (Natsume, et al., 2003), supports
this hypothesis, as far as [11C]AMT uptake is interpreted as reflecting 5-HT synthesis and not quinolinic acid synthesis, as recently suggested (Chugani and Muzik, 2000).

On the other hand, as in our study, Toczek et al. (2003) also reported a decrease in 5-HT$_{1A}$ binding in their patients, using a PET ligand with a much higher affinity for 5-HT$_{1A}$ receptors than MPPF, and being therefore potentially less displaceable. This therefore would be more in favour of a decrease in 5-HT$_{1A}$ receptor density than of a higher occupancy of these receptors by endogenous serotonin.

**Binding modifications in relation to intracerebral activity**

The analysis of our data using anatomical ROI allowed investigation of the relationship between regional BP decreases of 5-HT$_{1A}$ receptors and the type of epileptic activity recorded by intracerebrod electrodes. Independently of the existence of a lesion, BP variations in patients compared with controls gradually decreased from regions showing no epileptic activity to regions with interictal activity, seizure propagation and seizure onsets. Therefore, these data suggest that the reduction of BP is correlated to the degree of epileptic activity in the explored areas.

However, when considering only lesional regions, the BP decrease no longer correlated with the degree of epileptic activity, so that part of the above observed changes might reflect partial volume effect. Indeed, the limited spatial resolution of PET results in a partial volume effect, that in particular affects the quantification of signals in small structures or heterotopic nodules (Hoffman et al., 1979). Thus, when structural abnormalities are present, such as atrophy or inhomogeneity in grey matter content, the BP can appear smaller than it actually is, and a correction for partial volume effect is necessary in order to accurately quantify changes in the binding to receptors (Labbe et al., 1996; Koepp et al., 1998; Rousset et al., 1998; Hammers et al., 2001).

Accordingly, in our group of patients, the significantly greater BP decrease in lesional ROIs (either with or without volume loss) than in non-lesional regions can be attributed to partial volume effects. Therefore, to avoid any influence of partial volume effects, lesional regions were eliminated from the further statistical analysis.

Conversely, in non-lesional regions the differences in BP decrease for onset, spreading, interictal spiking and non-epileptic regions are unlikely to reflect partial volume effects, since the volume of homologous regions was not significantly different in patients and controls. In these non-lesional regions, we found that the BP decrease still correlated to the degree of epileptic activity. Therefore, these results show that MPPF binding not only reflects structural changes or neuronal loss, but it also can be considered as a marker of the epileptogenic zone.

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