Comorbidity between temporal lobe epilepsy and depression: a $[^{18}\text{F}]$MPPF PET study

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Brain and brainstem changes of serotoninergic 5-hydroxytryptophan (5-HT)$_{1A}$ receptor density have been reported in patients with major depressive disorder as well as in patients with temporal lobe epilepsy (TLE), using PET and the selective antagonist radiotracers $[^{11}\text{C}]$WAY-100635 or $[^{18}\text{F}]$FC-WAY. We used a distinct 5-HT$_{1A}$ antagonist, $[^{18}\text{F}]$MPPF, whose binding potential depends on both receptor density and extracellular serotonin concentration, in 24 patients with drug-resistant TLE and MRI evidence of hippocampal sclerosis but without prior antidepressant exposure. Their Beck Depression Inventory (BDI-2) score ranged from 0 to 34, with nine patients having a score >11. We used a simplified reference tissue model, statistical parametric mapping and anatomical regions of interest (ROIs) to correlate parametric images of $[^{18}\text{F}]$MPPF BP with the total BDI score and its four subclasses. The total BDI score, as well as symptoms of psychomotor anhedonia and negative cognition, correlated positively with $[^{18}\text{F}]$MPPF BP in the raphe nuclei and in the insula contralateral to seizure onset, whereas somatic symptoms correlated positively with $[^{18}\text{F}]$MPPF binding potential in the hippocampal/parahippocampal region ipsilateral to seizure onset, the left mid-cingulate gyrus and the inferior dorsolateral frontal cortex, bilaterally. We confirm an association of depressive symptoms in TLE patients with changes of the central serotoninergic pathways, in particular within the raphe nuclei, insula, cingulate gyrus and epileptogenic hippocampus. These changes are likely to reflect lower extracellular serotonin concentration in more depressed patients, with an upregulation of receptors a less likely alternative.

**Keywords:** depressive symptoms; TLE; $[^{18}\text{F}]$MPPF

**Abbreviations:** 5-HT = 5-hydroxytryptophan; BDI = Beck Depression Inventory; BP = binding potential; MDD = major depressive disorder; TLE = temporal lobe epilepsy


**Introduction**

Mood disorders are the most frequent psychiatric comorbidity in patients with epilepsy (Mendez et al., 1986; Robertson, 1987), with a prevalence of depression estimated between 11% and 60% in patients with recurrent seizures (Kanner, 2003). Depression is reported most frequently in patients with temporal lobe epilepsy (TLE) (Jones et al., 2005a), particularly in those with left TLE (Robertson, 1987; Althshuler et al., 1990) and possibly hippocampal sclerosis (Quiske et al., 2000).

Serotonin (5-hydroxytryptophan, 5-HT) is known to be involved in the pathophysiology of major depressive disorder (MDD) (Dhaenen, 2001; Drevets, 2002), with numerous studies showing a reduction of serotonin plasma concentration in patients with MDD (Asberg et al., 1976; Meltzer, 1990) while pharmacological and post-mortem investigations suggest a reduction of serotoninergic neurotransmission in this condition (Lopez et al., 1998).

PET studies of 5-HT$_{1A}$ serotoninergic receptors in depressed patients, using the antagonist $[^{11}\text{C}]$WAY-100635,
have reported partly discordant results (Drevets et al., 1999, 2000, 2007; Sargent et al., 2000; Parsey et al., 2006). While the original investigations showed a reduction of binding potential (BP) in various limbic and neocortical brain regions, as well as in the raphe nuclei, of untreated and treated MDD patients (Drevets et al., 1999, 2000, 2007; Sargent et al., 2000; Bhagwagar et al., 2004), a recent study reported an increased BP in the same regions in MDD patients never exposed to antidepressants and no significant abnormality in those with previous exposure to these drugs (Parsey et al., 2006). Another $[\text{11C}]$WAY-100635 study using an inrasubject design to compare patients before and after antidepressant exposure showed no difference between the two conditions. However, at baseline, future non-responders demonstrated higher bilateral orbitofrontal BP than future responders (Moses-Kolko et al., 2007). These controversial findings might partly reflect a difference in the methods used to calculate BP (Innis et al., 2007), as well as in the selected patient samples.

Abnormalities of the 5-HT$_{1A}$ receptors in similar regions have also been reported in TLE using various antagonists, including $[\text{11C}]$WAY-100635, $[\text{18F}]$FCWAY and $[\text{18F}]$MPPF (4-(2’-methylxyphenyl)-1-[2’-(N-2-pirydynyl)]-p-fluorobenz-amido]-ethyl-piperazine). All showed a BP reduction that predominated over the epileptogenic temporo-limbic structures (Toczek et al., 2003; Merlet et al., 2004a, b; Savic et al., 2004; Giovacchini et al., 2005; Ito et al., 2007). Some of these studies also found a negative correlation between various depression scales and 5-HT$_{1A}$ BP, suggesting lower BP in more depressed patients (Savic et al., 2004; Giovacchini et al., 2005). This correlation was observed ipsilateral to the epileptogenic temporal lobe, either in the anterior cingulate gyrus (Savic et al., 2004) or in the hippocampus (Giovacchini et al., 2005; Theodore et al., 2007). Accordingly, it was recently reported that TLE patients with a current or past history of MDD showed lower $[\text{18F}]$FCWAY binding than did TLE patients with no such history in hippocampus, temporal neocortex, anterior insula, anterior cingulate and raphe nuclei (Hasler et al., 2007). All patients included in these studies were free from antidepressants but no information was provided regarding previous exposure to these drugs.

In the present study, we used another specific antagonist of 5-HT$_{1A}$ receptors, $[\text{18F}]$MPPF, in TLE patients with hippocampal sclerosis and no previous antidepressant exposure to investigate the correlation between 5-HT$_{1A}$ BP and various components of depressive symptoms, as measured with the Beck Depression Inventory (BDI-2) scale (Cathébras et al., 1994). $[\text{18F}]$MPPF has a lower affinity for 5-HT$_{1A}$ receptors than WAY-100635, close to that of 5-HT [Ki = 3.3 nM (Zhuang et al., 1994)], allowing its displacement by endogenous serotonin (Zimmer et al., 2002; Rabah et al., 2003). It thus offers the possibility to further investigate changes of the serotonergic system that would not solely reflect 5-HT$_{1A}$ receptor density but also the extracellular concentration of endogenous 5-HT.

**Material and Methods**

**Patients**

We prospectively included 24 consecutive patients who fulfilled the following criteria: (i) drug-resistant focal epilepsy, (ii) ongoing pre-surgical evaluation, (iii) temporal lobe origin of seizures highly suggested by all available electroclinical data (Wieser et al., 2004), (iv) MRI signs of hippocampal sclerosis with or without loss of grey-white matter demarcation in the anterior temporal lobe and no other significant MRI finding and (v) no previous or current exposure to antidepressants. All the selected patients also gave their informed consent to participate in this study, which was approved by the local Ethics committee (CCPPRB, Centre Léon Bérard, Lyon) in accordance with the Declaration of Helsinki. This series was independent from our previously published studies of $[\text{18F}]$MPPF-PET in seven patients with mesial temporal epilepsy, none of whom had benefited from a BDI assessment (Merlet et al., 2004a, b).

All patients gave a detailed description of their past history and ictal symptoms and underwent long-term video-scalp-EEG monitoring that provided informative ictal electroclinical data. They all benefited from an optimal MRI, as well as an FDG-PET. In addition, four patients underwent an intracerebral EEG investigation, primarily prompted by the presence of ictal signs or symptoms, suggesting the possibility of ‘temporal plus’ epilepsy (Barba et al., 2007). In these patients, the invasive investigation eventually demonstrated a temporo-limbic epileptogenic zone.

**Evaluation of depressive symptoms**

At the time of referral to our epilepsy centre, none of the selected patients was diagnosed as suffering from any psychiatric disorder, including MDD, but this partly reflects the fact that patients with previous exposure to antidepressants were excluded from the study as well as the current state of underassessment of psychiatric comorbidity in patients with epilepsy in France.

On the day of the PET scanning, depressive symptoms were evaluated using the BDI-2 scale (Katzmark et al., 2001). The BDI-2 is a validated self-rating questionnaire that consists of a 21-item scale, with four severity ratings for each item. Factor analysis has suggested that BDI-2 reflected one underlying second-order dimension of self-reported depression composed of two first-order factors representing cognitive and non-cognitive (somatic-affective) symptoms (Steer et al., 1999). The cognitive dimension includes the following eight items: #2 (pesimism), #3 (past failure), #5 (guilty feelings), #6 (punishment feelings), #7 (self-dislike), #8 (self-criticalness), #9 (suicidal thoughts) and #14 (worthlessness), whereas the somatic-affective dimension is represented by all remaining 13 items. More recently, the dimensions of the BDI-2 were further divided into four symptom classes (Dunn et al., 2002). One of these four dimensions, called negative cognition, includes six of the eight cognitive symptoms previously identified by Steer et al. (1999) (#3, #5, #6, #7, #8 and #14; see above). The other three components represent subscales of the somatic-affective dimension, as detailed below:

- Psychomotor anhedonia includes items #4 (anhedonia), #11 (irritation), #12 (social anhedonia), #13 (difficulty making decisions) and #15 (ability to work);
- Vegetative symptoms includes items #16 (late insomnia) and #18 (loss of appetite) and
somatic symptoms includes items #19 (loss of weight) and #20 (health worries).

In this exploratory study, we hypothesized that regional brain changes in \(5\text{-HT}_{1A}\) receptor BP underlying the cognitive, psychomotor anhedonic, vegetative and somatic symptoms of depression might differ from one another and chose to look at the four dimensions proposed by Dunn et al. (2002).

The ability of the BDI-2 to identify major depression in patients with epilepsy has also been validated in a series of 174 subjects, using the Mini International Neuropsychiatric Interview and Mood Disorders module of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders Axis I Disorders-Research Version (Jones et al., 2005b). ROC analysis showed that using a cutoff score of 11, the BDI-2 had a sensitivity of 0.962, a specificity of 0.8 and a negative predictive value above 0.99, indicating that any patients with a score \(\leq 11\) are very unlikely to suffer from MDD (Jones et al., 2005b).

Our patients did not benefit from a standardized psychiatric interview technique as part of this study. The primary aim of our study was to correlate \[^{18}\text{F}]\text{MPPF}\) PET data with various symptoms of depression and not with the presence or absence of MDD. However, the mental health of our patients was systematically assessed during their pre-surgical evaluation through a multi-level and individualized procedure. The latter always included a clinical assessment performed by the senior epileptologist in charge of the pre-surgical evaluation, in order to determine whether or not the patient was psychologically amenable to surgery and the risk of postoperative psychiatric complications. A second level of assessment, performed in the majority of patients, consisted of a global cognitive and psychological evaluation by a clinical neuropsychologist. A third step was performed by a psychiatrist in patients selected through the two former evaluations and consisted of a standardized psychiatric interview. The majority of our patients who showed a BDI-2 score \(>11\) (7 out of 9) underwent this third assessment. The delay between this psychiatric evaluation and the BDI-2 assessment varied from 1 week to 6 months. In contrast, the majority of those with a BDI-2 score \(<11\) only benefited from the two first steps. As noted above, the chance that any of these latter patients suffered from MDD is \(<1\%\).

**PET acquisition**

The methodology of tracer production, scan acquisition and data pre-processing has been previously described (Merlet et al., 2004a; Costes et al., 2005). Briefly, \[^{18}\text{F}]\text{MPPF}\) PET studies were performed using a CTI-SIEMENS HR+ camera (Knoxville, TN, USA) in the afternoon. Prior to the emission acquisition, a 10 min transmission scan was performed. After intravenous injection of a bolus of 181.4 ± 32.1 MBq (mean ± SD) of \[^{18}\text{F}]\text{MPPF}\) into a radial vein of the left arm, the dynamic PET emission scan, consisting of 35 frames of increasing duration, was acquired to evaluate the local radioactivity concentration during 60 min post-injection. Patients were closely supervised throughout the whole PET acquisition and kept awake by talking to them when their level of vigilance dropped. Images were corrected for scatter and attenuation and reconstructed by 3D filtered back projection (Hanning filter) to provide a volume of 63 slices (2.42 mm thickness) with 128 × 128 voxels in plane (2.06 × 2.06 mm).

**Pre-processing and visual analysis of PET data**

A frame-to-frame realignment was systematically performed in Activis 2.8 (http://www.isc.cnrs.fr/the/activis.html). A previously validated simplified reference tissue model (Gunn et al., 1998) was then used to generate parametric images of BP values [BP\(_{\text{ND}}\) (non-displaceable) in consensus nomenclature (Innis et al., 2007)]. Cerebellar white matter, which is assumed to be devoid of \(5\text{-HT}_{1A}\)-specific binding, was used as a reference region (Costes et al., 2002).

Two of the investigators (A.D. and P.R.) independently reviewed the parametric images of BP\(_{\text{ND}}\) of all patients and reported all visually detectable asymmetries thought to be clinically significant.

Using Statistical Parametrical Mapping (SPM2, Wellcome Trust Centre for Neuroimaging, UCL, London, UK; http://www.fil.ion.ucl.ac.uk/spm/), parametric images of BP\(_{\text{ND}}\) were transformed into a standard stereotaxic space (MNI/ICBM152) using a landmark-specific template produced in-house and resliced to voxels of dimensions \(2 \times 2 \times 2\) mm. Normalized BP\(_{\text{ND}}\) images were then smoothed using an \(8 \times 8 \times 8\) mm FWHM isotropic Gaussian kernel to take into account interindividual anatomical variability and to improve the sensitivity of the statistical analysis (Friston et al., 1991).

**Voxel-based analysis of PET data**

All analyses were performed using SPM2 and an explicit mask that includes the entire brain and brainstem. In order to restrict our analysis to grey matter areas that contain a significant amount of \(5\text{-HT}_{1A}\) receptors, we further used an implicit mask that excluded all voxels in which BP\(_{\text{ND}}\) was \(<20\%\) of the mean brain and brainstem activity. The resulting mask adequately delineated the cortex but failed to sample the raphe nuclei within the brainstem due to the important partial volume effect affecting this region. The latter was thus only evaluated using a region of interest [see Regions of interest analyses]. The threshold of 20% used for constructing the above mask adequately sampled the grey matter but might have excluded some voxels in epileptogenic brain regions showing a dramatic decrease in BP\(_{\text{ND}}\). To ensure that this did not affect our main findings, we reprocessed our analyses using an explicit mask of the grey matter without any additional implicit mask. This explicit mask was constructed from a segmented normal MRI template that included all voxels having a probability of 40% or more to belong to grey matter (Fig. 1).

We first extracted the global average BP\(_{\text{ND}}\) values of each individual and looked for correlations between this value and the total BDI score as well as the scores for each of the four symptom classes (negative cognition, psychomotor-anhedonia, vegetative symptoms and somatic symptoms), using the bivariate correlation and Pearson coefficient function of SPSS (version 12.0 for Windows), with a bilateral level of significance at \(P < 0.05\).

We then searched for correlations between BP\(_{\text{ND}}\) values and depressive symptoms using the analysis of covariance function of SPM, with a linear model at each and every voxel (Friston et al., 1995). This analysis was performed for the total BDI and the four symptom class scores. For each analysis, two contrasts were tested that looked for positive correlations (increasing BP\(_{\text{ND}}\) values with increasing BDI score) and negative correlations (decreasing BP\(_{\text{ND}}\) values with increasing BDI score). The global BP\(_{\text{ND}}\) was designated as a covariable of no interest. We processed all analyses using a visualization threshold of \(P < 0.01\) at the voxel level but only retained clusters that were significant at the conventional \(P < 0.05\) threshold at the cluster level after SPM standard correction for...
multiple comparisons. These results will be presented as $P_{corrected}$ values according to SPM nomenclature.

The above analyses were performed in a mixed population of patients with right and left TLE and thus searched for correlation between depressive symptoms and BPND values in brain regions regardless of their lateralization with respect to the epileptogenic temporal lobe. To further investigate this issue, we reprocessed the same analyses after flipping the PET volume of the eight patients with left TLE in order to get all epileptogenic temporal lobes lateralized to the right. Thus, left-sided correlations corresponded to brain regions contralateral to the epileptic temporal lobe and vice versa. Previous studies of $[^{11}C]$flumazenil PET, $[^{11}C]$diprenorphine PET and $[^{18}F]$MPPF PET have used the same methodology in populations of patients with right- and left-sided epileptogenic zones (Hammers et al., 2002, 2003, 2007; Merlet et al., 2004a). We will further refer to this analysis as the 'flipped PET data' analysis to facilitate its presentation in the following sections, although only one-third of all PET volumes (corresponding to the eight patients with left TLE) were actually flipped.

**Regions of interest analyses**

The major role played by the raphe nuclei in the regulation of the central serotonergic system and in the pathophysiology of MDD provided a strong rationale for specifically looking at this structure in our study. Since the raphe nuclei cannot be delineated on MRI but are the only detectable brainstem structure on $[^{18}F]$MPPF PET images, we traced a region of interest (ROI) encompassing this structure on an average $[^{18}F]$MPPF-PET image obtained from the 24 patients’ normalized datasets by thresholding the activity at 90% of the local maximum in the brainstem. This ROI extended on the 13 consecutive slices displaying the raphe, with a total volume of 400 mm$^3$.

We also used anatomical ROIs extracted from a brain atlas (Kabani et al., 1998) to measure the BPND in cortical regions where voxel-based analysis disclosed significant correlations with BDI, in order to confirm this finding at the level of pre-defined anatomical regions. As for SPM analyses, we evaluated left- and right-sided ROI values, regardless of the lateralization of the epileptic temporal lobe, as well as ROI data contralateral and ipsilateral to the side of seizure onset.

We searched for correlations between BPND values and depressive symptoms using the partial correlation and Pearson coefficient function of SPSS (version 12.0 for Windows), with the global BPND as a control variable. For the raphe nuclei, we used a bilateral level of significance at $P<0.05$, whereas we used a unilateral level of significance at $P<0.05$ for the other ROIs, since these analyses aimed at confirming correlations previously shown by SPM analysis, the sign of which was thus specified. For the same reason, we did not further correct these analyses for multiple comparisons.

**Post hoc ROI analyses**

The conventional and flipped PET data analyses disclosed ambiguous results by showing very comparable lateralized findings pointing to the left hemisphere and that contralateral to seizure onset, respectively. In order to further investigate this issue, we performed additional ROI analyses in the subgroups of right and left TLE patients, separately, using the same methodology as above. Again, because these post hoc analyses aimed at exploring the most likely interpretation of our previous findings, and did not intend to disclose new correlations, we did not apply further corrections for multiple comparisons.

**Results**

**Clinical data**

There were 12 males and 12 females with a mean age of 37.6 years (SD = 11.2 years; Table 1). Eight had left TLE and 16 had right TLE. The median duration of their epilepsy was 30.5 years (range, 10–55 years).

BDI scores ranged from 0 to 34 (median = 7), with nine patients reporting a score $>$11, eight of whom suffered from right TLE. Among these nine patients, seven had a psychiatric evaluation, including all six who were operated on. Among the seven evaluated patients, five fulfilled the Diagnostic and Statistical Manual of Mental Disorders criteria of MDD, whereas the two remaining patients were considered to suffer a milder form of depressive disorder. Among the two patients with BDI-2 score above 11 who were not evaluated by a psychiatrist, one was considered to suffer depression by the epileptologist in charge of the pre-surgical evaluation, but the patient declined to be referred to a psychiatrist for further evaluation, whereas the other was not diagnosed as being depressed during his pre-surgical evaluation. No other major psychiatric illness, including bipolar disease and schizophrenia, was noted in these 9 patients or in the 15 subjects with a BDI-2 score $<$11.
There was no difference in the proportion of patients with a BDI lower or greater than 11 receiving AEDs known to promote the release of serotonin. Indeed, 78% of patients with a BDI 11 were treated with carbamazepine, oxcarbazepine or lamotrigine at the time of the PET study, as compared with 73% of those with a BDI 11. Two patients in each group received a combination of carbamazepine and lamotrigine.

Visual analysis of PET data
The two investigators detected an asymmetry of regional BPND in all 24 patients. This abnormality always predominated over the mesial and polar aspects of the temporal lobes and pointed to lower BP on the side of the atrophic hippocampus and TLE.

Analysis of global BPND values
There was no correlation between the global BPND and either the total BDI score or that of the four symptom classes (Table 3). These correlations were observed within the same cluster as above for symptoms of negative cognition (Pcorrected < 0.001; Fig. 3) and psychomotor anhedonia (Pcorrected < 0.001). In contrast, somatic symptoms correlated positively with [18F]MPPF BPND in three other clusters corresponding to the left mid-cingulate gyrus (Pcorrected = 0.001), and the right (Pcorrected < 0.001) and left (Pcorrected < 0.001) middle and inferior frontal gyri (Fig. 4). Vegetative symptoms did not correlate with [18F]MPPF BPND in any region.

Voxel-based analysis of PET-data
We found a positive correlation between the total BDI score and [18F]MPPF BPND within a single cluster that primarily included the left insula and extended towards the adjacent left parietal operculum and inferior post-central cortex (Pcorrected = 0.006; Fig. 2). There was no negative correlation between [18F]MPPF BPND and BDI score. Similarly, positive but no negative correlations were demonstrated between [18F]MPPF BPND and three of the four symptom classes of the BDI (Table 3). These correlations were observed within the same cluster as above for symptoms of negative cognition (Pcorrected < 0.001; Fig. 3) and psychomotor anhedonia (Pcorrected < 0.001). In contrast, somatic symptoms correlated positively with [18F]MPPF BPND in three other clusters corresponding to the left mid-cingulate gyrus (Pcorrected = 0.001), and the right (Pcorrected < 0.001) and left (Pcorrected < 0.001) middle and inferior frontal gyri (Fig. 4). Vegetative symptoms did not correlate with [18F]MPPF BPND in any region.

These results were partly modified by flipping the PET volume of the 8 patients with a left TLE, resulting in the epileptogenic temporal lobe of all 24 patients being lateralized to the right (Table 3). Symptoms of negative cognition and psychomotor anhedonia still positively correlated with [18F]MPPF BPND in the insula and adjacent parietal operculum and post-central region contralateral to the epileptogenic temporal lobe (negative cognition: Pcorrected = 0.009; psychomotor-anhedonia: Pcorrected = 0.005; Fig. 5), but the total BDI score only showed a non-significant trend towards the same correlation with an

Table 1 Patients' clinical data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age (years)</th>
<th>BDI score</th>
<th>Pre-surgical Mental Health assessment</th>
<th>MRI findings (visual analysis)</th>
<th>Age at first seizure</th>
<th>Duration of epilepsy (years)</th>
<th>Antiepileptic drugs</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>28</td>
<td>20</td>
<td>Mild depression disorder*</td>
<td>L HS + ISWM</td>
<td>7 years</td>
<td>21</td>
<td>CBZ; TPM; LTG; VGB</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>46</td>
<td>1</td>
<td>–</td>
<td>LHS</td>
<td>28 years</td>
<td>18</td>
<td>CBZ; LEV</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>35</td>
<td>10</td>
<td>–</td>
<td>LHS</td>
<td>16 years</td>
<td>19</td>
<td>CBZ; LEV</td>
</tr>
<tr>
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<td>M</td>
<td>48</td>
<td>3</td>
<td>–</td>
<td>LHS</td>
<td>30 years</td>
<td>18</td>
<td>CBZ; LEV</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>55</td>
<td>2</td>
<td>–</td>
<td>LHS</td>
<td>9 years</td>
<td>46</td>
<td>PHT; LEV</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>50</td>
<td>7</td>
<td>–</td>
<td>LHS</td>
<td>40 years</td>
<td>10</td>
<td>OXC; LEV</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>41</td>
<td>7</td>
<td>–</td>
<td>LHS</td>
<td>6 years</td>
<td>35</td>
<td>CBZ; LEV; GBP</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>39</td>
<td>5</td>
<td>–</td>
<td>L HS + ISWM</td>
<td>6 months</td>
<td>39</td>
<td>OXC; VPA; LEV</td>
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<tr>
<td>9</td>
<td>M</td>
<td>29</td>
<td>2</td>
<td>–</td>
<td>RHS</td>
<td>3 years</td>
<td>26</td>
<td>CBZ; LTG</td>
</tr>
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<td>–</td>
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<td>30</td>
<td>CBZ; LTG</td>
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<tr>
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<td>F</td>
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<td>24</td>
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<td>8 months</td>
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<td>M</td>
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<td>4</td>
<td>–</td>
<td>RHS + ISWM</td>
<td>6 years</td>
<td>12</td>
<td>TPM</td>
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<tr>
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<td>F</td>
<td>50</td>
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<td>RHS</td>
<td>9 months</td>
<td>49</td>
<td>PHT; LEV; GBP</td>
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<td>55</td>
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<tr>
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<td>34</td>
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<td>–</td>
<td>RHS</td>
<td>2.5 years</td>
<td>32</td>
<td>PHB; TPM</td>
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<tr>
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<td>31</td>
<td>34</td>
<td>Major depression disorder*</td>
<td>RHS</td>
<td>at birth</td>
<td>31</td>
<td>CBZ; PHB; GBP</td>
</tr>
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<td>M</td>
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<td>18</td>
<td>–</td>
<td>RHS</td>
<td>15 months</td>
<td>34</td>
<td>VPA; LTG; LEV</td>
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<td>F</td>
<td>48</td>
<td>30</td>
<td>Major depression disorder*</td>
<td>R HS</td>
<td>9 months</td>
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<td>21</td>
<td>M</td>
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<td>R HS + ISWM</td>
<td>9 months</td>
<td>22</td>
<td>CBZ; LEV</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>32</td>
<td>4</td>
<td>–</td>
<td>RHS</td>
<td>15 years</td>
<td>17</td>
<td>CBZ</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>44</td>
<td>7</td>
<td>–</td>
<td>RHS</td>
<td>33 years</td>
<td>11</td>
<td>PHB; PHT; LEV</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>40</td>
<td>6</td>
<td>–</td>
<td>RHS</td>
<td>10 years</td>
<td>30</td>
<td>OXC</td>
</tr>
</tbody>
</table>

M = male; F = female; L = left; R = right; HS = hippocampal sclerosis; ISWM = increased T2-weighted signal in anterior temporal white matter; CBZ = carbamazepine; TPM = topiramate; LTG = lamotrigine; VGB = vigabatrin; CBZ = carbamazepine; LEV = levetiracetam; OXC = oxcarbazepine; GBP = gabapentin; PHB = phenobarbital; PHT = phenytoin; VPA = valproic acid; N/A = not applicable.

*Diagnosis based on a standardized psychiatric interview.
<table>
<thead>
<tr>
<th>Patient #</th>
<th>Side of TLE</th>
<th>Global BP\textsubscript{ND}</th>
<th>Raphe Nuclei</th>
<th>Insula</th>
<th>Post-central</th>
<th>Cingulate</th>
<th>Middle Frontal</th>
<th>Inferior Frontal</th>
<th>Hippocampus</th>
<th>Para-hippocampal</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Left</td>
<td>0.471</td>
<td>0.284</td>
<td>0.911</td>
<td>0.581</td>
<td>0.479</td>
<td>0.284</td>
<td>0.911</td>
<td>0.581</td>
<td>0.479</td>
</tr>
<tr>
<td>1</td>
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<td>0.435</td>
<td>0.098</td>
<td>0.642</td>
<td>0.436</td>
<td>0.273</td>
<td>0.243</td>
<td>0.422</td>
<td>0.441</td>
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<td>2</td>
<td>Left</td>
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<td>0.295</td>
<td>0.820</td>
<td>0.718</td>
<td>0.526</td>
<td>0.519</td>
<td>0.792</td>
<td>0.772</td>
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<tr>
<td>3</td>
<td>Left</td>
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<td>0.148</td>
<td>0.764</td>
<td>0.711</td>
<td>0.228</td>
<td>0.257</td>
<td>0.518</td>
<td>0.487</td>
<td>0.366</td>
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<td>4</td>
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<td>0.083</td>
<td>0.752</td>
<td>0.483</td>
<td>0.285</td>
<td>0.316</td>
<td>0.515</td>
<td>0.454</td>
<td>0.318</td>
</tr>
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<td>0.684</td>
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<td>0.254</td>
<td>0.419</td>
<td>1.409</td>
<td>0.361</td>
</tr>
<tr>
<td>6</td>
<td>Left</td>
<td>0.434</td>
<td>0.243</td>
<td>0.815</td>
<td>0.562</td>
<td>0.373</td>
<td>0.405</td>
<td>0.492</td>
<td>0.531</td>
<td>0.414</td>
</tr>
<tr>
<td>7</td>
<td>Left</td>
<td>0.487</td>
<td>0.220</td>
<td>0.793</td>
<td>0.613</td>
<td>0.418</td>
<td>0.458</td>
<td>0.500</td>
<td>0.497</td>
<td>0.495</td>
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<td>8</td>
<td>Right</td>
<td>0.500</td>
<td>0.088</td>
<td>0.693</td>
<td>0.738</td>
<td>0.458</td>
<td>0.454</td>
<td>0.575</td>
<td>0.561</td>
<td>0.534</td>
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<tr>
<td>9</td>
<td>Right</td>
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<td>0.178</td>
<td>0.489</td>
<td>0.731</td>
<td>0.284</td>
<td>0.353</td>
<td>0.591</td>
<td>0.543</td>
<td>0.452</td>
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<tr>
<td>10</td>
<td>Right</td>
<td>0.480</td>
<td>0.223</td>
<td>0.721</td>
<td>0.814</td>
<td>0.373</td>
<td>0.427</td>
<td>0.546</td>
<td>0.522</td>
<td>0.453</td>
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<td>11</td>
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<td>0.610</td>
<td>0.318</td>
<td>0.937</td>
<td>0.969</td>
<td>0.579</td>
<td>0.642</td>
<td>0.714</td>
<td>0.724</td>
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<tr>
<td>12</td>
<td>Right</td>
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<td>0.273</td>
<td>0.878</td>
<td>0.874</td>
<td>0.439</td>
<td>0.471</td>
<td>0.599</td>
<td>0.582</td>
<td>0.543</td>
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<tr>
<td>13</td>
<td>Right</td>
<td>0.532</td>
<td>0.168</td>
<td>0.653</td>
<td>0.813</td>
<td>0.510</td>
<td>0.576</td>
<td>0.616</td>
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<td>0.657</td>
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<tr>
<td>14</td>
<td>Right</td>
<td>0.446</td>
<td>0.231</td>
<td>0.613</td>
<td>0.714</td>
<td>0.356</td>
<td>0.392</td>
<td>0.570</td>
<td>0.561</td>
<td>0.387</td>
</tr>
<tr>
<td>15</td>
<td>Right</td>
<td>0.500</td>
<td>0.209</td>
<td>0.793</td>
<td>0.831</td>
<td>0.386</td>
<td>0.327</td>
<td>0.674</td>
<td>0.617</td>
<td>0.511</td>
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<tr>
<td>16</td>
<td>Right</td>
<td>0.400</td>
<td>0.006</td>
<td>0.468</td>
<td>0.627</td>
<td>0.387</td>
<td>0.345</td>
<td>0.529</td>
<td>0.499</td>
<td>0.533</td>
</tr>
<tr>
<td>17</td>
<td>Right</td>
<td>0.424</td>
<td>0.259</td>
<td>0.619</td>
<td>0.795</td>
<td>0.330</td>
<td>0.388</td>
<td>0.517</td>
<td>0.535</td>
<td>0.421</td>
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<tr>
<td>18</td>
<td>Right</td>
<td>0.460</td>
<td>0.133</td>
<td>0.642</td>
<td>0.758</td>
<td>0.376</td>
<td>0.525</td>
<td>0.645</td>
<td>0.592</td>
<td>0.457</td>
</tr>
<tr>
<td>19</td>
<td>Right</td>
<td>0.492</td>
<td>0.194</td>
<td>0.608</td>
<td>0.855</td>
<td>0.420</td>
<td>0.463</td>
<td>0.512</td>
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<td>20</td>
<td>Right</td>
<td>0.295</td>
<td>0.036</td>
<td>0.441</td>
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<td>0.157</td>
<td>0.262</td>
<td>0.385</td>
<td>0.231</td>
<td>0.344</td>
</tr>
<tr>
<td>21</td>
<td>Right</td>
<td>0.512</td>
<td>0.160</td>
<td>0.704</td>
<td>0.764</td>
<td>0.504</td>
<td>0.525</td>
<td>0.640</td>
<td>0.590</td>
<td>0.535</td>
</tr>
<tr>
<td>22</td>
<td>Right</td>
<td>0.420</td>
<td>0.215</td>
<td>0.554</td>
<td>0.677</td>
<td>0.403</td>
<td>0.368</td>
<td>0.448</td>
<td>0.381</td>
<td>0.268</td>
</tr>
<tr>
<td>23</td>
<td>Right</td>
<td>0.498</td>
<td>0.172</td>
<td>0.585</td>
<td>0.747</td>
<td>0.460</td>
<td>0.428</td>
<td>0.558</td>
<td>0.520</td>
<td>0.476</td>
</tr>
</tbody>
</table>

Table 2 \[^{18}\text{F}]\text{MPPF-PET} \text{BP}_{\text{ND}} \text{ values in regions showing correlations with depressive symptoms}
uncorrected $P$-value of 0.007 at the cluster level. $^{[18F]}$MPPF $B_{\text{PD}}$ also displayed positive correlation with somatic symptoms, comparable to those observed with non-flipped PET images, including the mid-cingulate gyrus contralateral to seizure onset ($P_{\text{corrected}} = 0.001$) and the middle and inferior frontal gyri bilaterally (ipsilateral: $P_{\text{corrected}} < 0.001$; contralateral: $P_{\text{corrected}} = 0.001$; Fig. 4). In addition, somatic symptoms positively correlated with $^{[18F]}$MPPF $B_{\text{PD}}$ in the mesial temporal region ipsilateral to seizure onset, within a cluster encompassing the parahippocampal gyrus and atrophic hippocampus ($P_{\text{corrected}} = 0.002$). This was the only significant finding not previously disclosed in the SPM analyses of non-flipped PET volumes.

The above results were obtained using a 20% threshold implicit mask but were all confirmed when replacing the latter mask by an explicit mask of the grey matter.

**ROI analyses**

ROI analysis of the raphe nucleus showed a positive correlation between $^{[18F]}$MPPF $B_{\text{PD}}$ and total BDI score ($r = 0.55; P = 0.006$), as well as with symptoms of psychomotor-anhedonia ($r = 0.56; P = 0.006$) and negative cognition ($r = 0.44; P = 0.032$) but not with somatic or vegetative symptoms (Table 3).

ROI analysis of the left insula confirmed the positive correlation between $^{[18F]}$MPPF $B_{\text{PD}}$ and total BDI score ($r = 0.49; P = 0.009$), symptoms of psychomotor-anhedonia ($r = 0.49; P = 0.009$) and negative cognition ($r = 0.48; P = 0.011$), previously disclosed by SPM analyses. Similarly, we confirmed a positive correlation between $^{[18F]}$MPPF $B_{\text{PD}}$ in the post-central region and negative symptoms ($r = 0.36, P = 0.047$) and a positive correlation between $^{[18F]}$MPPF $B_{\text{PD}}$ in the left cingulate gyrus and somatic symptoms ($r = 0.46; P = 0.013$), whereas the frontal regions did not show the correlation observed with voxel-based analysis.

ROIs analysis of flipped PET data confirmed positive correlations between symptoms of negative cognition and psychomotor anhedonia and $^{[18F]}$MPPF $B_{\text{PD}}$ in the insula contralateral to seizure onset ($r = 0.46; P = 0.014$ and $r = 0.49; P = 0.009$, respectively), as well as between somatic symptoms and $^{[18F]}$MPPF $B_{\text{PD}}$ in the ipsilateral hippocampus ($r = 0.44; P = 0.018$), parahippocampal gyrus...
In addition, [18F]MPPF BP ND in the insula contralateral to seizure onset significantly correlated with the total BDI score ($r = 0.51; P = 0.006$). We confirmed positive correlations between [18F]MPPF BP ND in the postcentral region contralateral to seizure onset and total BDI score ($r = 0.41; P = 0.027$) and symptoms of negative cognition ($r = 0.37; P = 0.043$). Conversely, correlation between depressive symptoms and [18F]MPPF BP ND delineated by SPM over the bilateral inferior and midfrontal gyri failed to be confirmed by ROI analyses.

### Table 3: Positive correlations between depressive symptoms and [18F]MPPF BP ND using SPM and ROI analyses

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>BDI total</th>
<th>Negative Cognition</th>
<th>Psychomotor anhedonia</th>
<th>Somatic</th>
<th>Vegetative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPM</td>
<td>ROI</td>
<td>SPM</td>
<td>ROI</td>
<td>SPM</td>
</tr>
<tr>
<td>Raphe nuclei</td>
<td>NA</td>
<td>$r = 0.55; P = 0.006$</td>
<td>NA</td>
<td>$r = 0.44; P = 0.034$</td>
<td>NA</td>
</tr>
<tr>
<td>Insula L</td>
<td>$Z_{max} = 3.6; k = 1991; P = 0.006$</td>
<td>$r = 0.49; P = 0.009$</td>
<td>$Z_{max} = 4.49; k = 3676; P &lt; 0.0001$</td>
<td>$r = 0.48; P = 0.011$</td>
<td>$Z_{max} = 4.43; k = 3839; P &lt; 0.0001$</td>
</tr>
<tr>
<td>Postcentral L</td>
<td></td>
<td></td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Cingulate L</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Frontal inf L</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Frontal mid L</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Frontal mid R</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Insula C</td>
<td>$Z_{max} = 3.7; k = 871; P = 0.161^*$</td>
<td>$r = 0.51; P = 0.006$</td>
<td>$Z_{max} = 4.05; k = 1732; P = 0.009$</td>
<td>$r = 0.46; P = 0.044$</td>
<td>$Z_{max} = 4.56; k = 1923; P = 0.005$</td>
</tr>
<tr>
<td>Postcentral C</td>
<td></td>
<td></td>
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<td></td>
<td>NS</td>
</tr>
<tr>
<td>Cingulate C</td>
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<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Frontal inf I</td>
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<td>Frontal mid I</td>
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<td>NA</td>
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<td>NA</td>
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<tr>
<td>Frontal mid C</td>
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<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Hippocampus I</td>
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<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Parahipp I</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>NA</td>
</tr>
</tbody>
</table>

SPM = Statistical parametric mapping; ROI = Regions of interest; L = left; R = right; C = contralateral to seizure onset; I = ipsilateral to seizure onset; inf = inferior; mid = middle; hipp = hippocampus; parahipp = parahippocampus. *: No significant cluster detected by SPM analysis; NA = Not applicable; NS = Not statistically significant.

This cluster did not reach statistical significance after correction for multiple comparisons, but was associated with an uncorrected $P$-value = 0.007 at the cluster level.

(r = 0.37; P = 0.042) and contralateral cingulate gyrus (r = 0.39; P = 0.033). In addition, [18F]MPPF BP ND in the insula contralateral to seizure onset significantly correlated with the total BDI score (r = 0.51; P = 0.006). We confirmed positive correlations between [18F]MPPF BP ND in the postcentral region contralateral to seizure onset and total BDI score (r = 0.41, P = 0.027) and symptoms of negative cognition (r = 0.37, P = 0.043). Conversely, correlation between depressive symptoms and [18F]MPPF BP ND delineated by SPM over the bilateral inferior and midfrontal gyri failed to be confirmed by ROI analyses.

### Discussion

The main finding of this study was the positive correlation between the total BDI score and [18F]MPPF BP ND in the insula contralateral to seizure onset and the raphe nuclei in 24 TLE patients with hippocampal sclerosis and no previous exposure to antidepressants. The same correlations were observed between the insula and raphe [18F]MPPF BP ND and BDI subscales, reflecting symptoms of negative cognition and psychomotor-anhedonia. Conversely somatic symptoms positively correlated with [18F]MPPF BP ND in the hippocampus ipsilateral to seizure onset as well as in
the left mid-cingulate gyrus. These results, which contrast with previously published data obtained with other radiotracers, suggest that depressive symptoms in TLE patients are associated with a complex alteration of the serotonergic system that might combine changes in the extracellular concentration of 5-HT and 5-HT$_{1A}$ receptor density.

Direction of changes as compared with previous studies
The positive correlations observed in this study between $[^{18}F]$MPPF BP$_{ND}$ and depressive symptoms are at odds with the negative correlations previously reported by others in TLE patients using $[^{11}C]$WAY-100635 and $[^{18}F]$FCWAY (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007). There are several possible explanations for these discrepancies including differences in the brain regional distribution of PET changes, in the population studied and in the affinity of the PET ligands for 5-HT$_{1A}$ receptors. Each of these issues is discussed below.

Differences in the brain regional distribution of PET changes
One possible explanation of these discordant results could be that brain regions displaying positive correlations with depressive symptoms in this study differed from those showing negative correlations in previous series. Indeed, positive correlations primarily involved the raphe nuclei, insulo-parietal cortex, mid-cingulate gyrus and frontal dorsolateral cortex, whereas previously reported negative correlations were observed in the hippocampus and anterior cingulate gyrus ipsilaterally (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007). However, we also found a positive correlation between somatic symptoms of depression and $[^{18}F]$MPPF BP$_{ND}$ in the hippocampus ipsilateral to seizure onset, the same region where negative correlations were reported by others (Giovacchini et al., 2005), including after correction for partial volume effect in the atrophic hippocampus (Theodore et al., 2007). Furthermore, a recent $[^{18}F]$FCWAY PET study showed that TLE patients with current or previous MDD had lower $[^{18}F]$FCWAY binding than those without a history of MDD in many brain regions, including the anterior insula and the raphe (Hasler et al., 2007). Overall, the opposite direction of the correlations observed between depressive symptoms and $[^{18}F]$MPPF BP$_{ND}$ versus $[^{11}C]$WAY-100635 or $[^{18}F]$FCWAY BP is unlikely to primarily reflect regional differences in the distribution of positive versus negative correlations.

Differences in the population studied
Differences in the population studied could represent another explanation for the discordant findings observed...
with the various 5-HT$_{1A}$ PET ligands, though all of our patients demonstrated the typical pattern of 5-HT$_{1A}$ receptor abnormality reported by others using [$^{11}$C]WAY-100635 or [$^{18}$F]FCWAY that is a decreased [$^{18}$F]MPPF BP$_{ND}$ over their epileptogenic temporo-limbic regions (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007).

However, our patient group was more homogenous than those selected in previous studies, with all patients presenting MRI signs of hippocampal sclerosis, whereas the proportion of patients with hippocampal sclerosis varied from 57% to 73% in other series (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007). The pathophysiology of epilepsy-associated depression might differ between TLE patients with and without hippocampal sclerosis (Quiske et al., 2000; Hečimović et al., 2003) and could result in different observations in a population mixing these two types of patients as compared with a homogeneous group of TLE patients with hippocampal sclerosis. In addition, all our patients underwent a comprehensive pre-surgical evaluation that led to surgery in 71% of cases, 94% of whom were free of disabling seizure postoperatively (class I of Engel; Engel et al., 1998). In one previous study, 11 out of 14 patients entered a pre-surgical evaluation, only two of whom were operated upon and were seizure-free (Savic et al., 2004). In another series of 22 TLE patients, 14 were operated (64%), including 10 seizure-free postoperatively (71%) (Giovacchini et al., 2005).

Differences in antiepileptic drug plasma concentration might also be taken into consideration since the latter was found to influence the plasma-free fraction of [$^{18}$F]FCWAY in patients with epilepsy (Theodore et al., 2006). However, this issue is unlikely to play a significant role in our study.
since our model for calculating parametric images of $[^{18}\text{F}]$MPPF BP$_{ND}$ does not depend on the plasma-free fraction of the PET tracer. Another important aspect of AED treatment is their potential to modify the intracerebral concentration of serotonin. The latter was found increased in rats treated with carbamazepine (Dailey et al., 1997; Ahmad et al., 2005), oxcarbazepine (Clinckers et al., 2005) and phenytoin (Ahmad et al., 2005). However, when used at the therapeutic level, neither phenytoin nor phenobarbital was found to be associated with change in cerebral 5-HT (Sudha et al., 1996; Okada et al., 1997). The impact of lamotrigine on brain serotonin appears more complex with an acute decreased release of 5-HT but lack of chronic changes (Ahmad et al., 2004; Ahmad et al., 2005). The other AEDs taken by our patients were not found to alter the intracerebral concentration of 5-HT (Takebayashi et al., 1995; Pugsley et al., 1998) or were not evaluated in this respect. Importantly, the same proportion of patients with and without a BDI $>11$ were treated with carbamazepine or oxcarbazepine (77 and 73%, respectively). Conversely, previous series that correlated depressive symptoms with 5-HT$_{1A}$ receptor density either ignored this issue (Giovacchini et al., 2005; Theodore et al., 2007) or reported differences in the proportion of patients with and without depressive symptoms receiving carbamazepine (Savic et al., 2004). Indeed, in this latter serie, 25% of patients with Montgomery Åsberg Depression Rating Scale (MADRS) score $>20$ were treated with carbamazepine as compared with 50% of those with a MADRS score $<20$.

More importantly, none of our patients has been ever exposed to antidepressant drugs. Previously published studies included TLE patients free of antidepressants at the time of PET scanning but did not specify whether their patients were previously exposed to such treatment (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007). A recent $[^{11}\text{C}]$WAY-100635 study of patients with MDD suggests that a past history of antidepressant exposure has a major impact on the brain distribution of 5-HT$_{1A}$ receptors (Parsey et al., 2006). Indeed, MDD patients naïve of antidepressant exposure showed an increased $[^{11}\text{C}]$WAY-100635 BP in most brain regions compared with controls, including the insula, the cingulate gyrus and the raphe nuclei, whereas MDD patients with previous antidepressant exposure failed to demonstrate such abnormalities (Parsey et al., 2006). It is noteworthy that the overexpression of 5-HT$_{1A}$ receptors observed in medication naïve MDD patients is consistent with the higher $[^{18}\text{F}]$MPPF BP$_{ND}$ found in the same brain regions in our TLE patients with higher BDI score and no previous exposure to antidepressants. Overall, differences in the population of TLE patients included in our study as compared with previous series might partly account for the discordant PET findings.

Fig. 5 (A) Positive correlation between symptoms of psychomotor-anhedonia symptoms and MPPF BP$_{ND}$ values of flipped PET data. Height threshold $P$ uncorrected at the voxel level $P < 0.01$, $P$ corrected at the cluster level after correction for multiple comparisons $<0.005$. Only clusters showing a significant correlation after correction for multiple comparison are displayed in this figure. The blue cross shows the significant cluster localized in the insula contralateral to seizure onset. Colour bar, t-scores. (B) Regression plot of the MPPF BP$_{ND}$ values in the peak voxel of the significant cluster in the insula contralateral to seizure onset plotted against the psychomotor-anhedonia symptoms.
Differences in the affinity of the 5-HT\textsubscript{1A} PET ligands

A third explanation for these discrepancies could be the difference in affinity that distinguishes \([^{18}\text{F}]\text{MPPF}\) from \([^{11}\text{C}]\text{WAY-100635}\) and \([^{18}\text{F}]\text{FCWAY}\). Unlike the two latter PET tracers whose affinity is much higher than that of 5-HT, \([^{18}\text{F}]\text{MPPF}\) is characterized by a lower affinity, close enough to that of serotonin to be sensitive to the concentration of endogenous 5-HT (Zimmer et al., 2002; Rbah et al., 2003). Accordingly, a reduction of extracellular serotonin concentration could result in an increased \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND}, whereas it would not directly influence \([^{11}\text{C}]\text{WAY-100635}\) or \([^{18}\text{F}]\text{FCWAY}\) binding. Theoretically, this mechanism could even mask and override an underlying decreased density of 5-HT\textsubscript{1A} receptors. This hypothesis would be of particular interest for the positive correlation observed between somatic symptoms and \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND} over the epileptogenic hippocampus since BP\textsubscript{ND} was dramatically reduced in that region in most patients. Accordingly, a PET study using alpha-\([^{11}\text{C}]\text{methyl-L-tryptophan}\), a precursor of 5-HT, has reported a reduction of this tracer uptake in the anterior cingulate gyrus and left mesial temporal cortex in depressed patients supporting the possibility of reduced extracellular serotonin concentration in depression (Rosa-Neto et al., 2004). However, it remains impossible to precisely predict the impact of such an abnormality on \([^{18}\text{F}]\text{MPPF}\) binding since we ignore the proportion of 5-HT\textsubscript{1A} receptors occupied by endogenous 5-HT in the living human brain and the magnitude of its reduction in depressive disorders.

Overall, our results of higher \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND} in TLE patients with higher BDI score could either reflect greater 5-HT\textsubscript{1A} receptor density or a lower extracellular concentration of 5-HT that could be associated with various changes in the number of 5-HT\textsubscript{1A} receptors. A combination of decreased extracellular concentration of 5-HT and number of 5-HT\textsubscript{1A} receptors in the most depressed patients would be consistent with previous \([^{11}\text{C}]\text{WAY-100635}\) or \([^{18}\text{F}]\text{FCWAY}\) PET studies in TLE patients (Savic et al., 2004; Giovacchini et al., 2005; Theodore et al., 2007). Conversely, greater 5-HT\textsubscript{1A} receptor density in the more depressed patients, which might also well be associated with reduced extracellular concentration of 5-HT, would be more in agreement with the \([^{11}\text{C}]\text{WAY-100635}\) PET findings reported in antidepressant-naive MDD patients (Parsey et al., 2006). In any event, both hypotheses provide new evidences regarding the pathophysiology of serotonergic changes associated with depressive symptoms in TLE and point to the possibility of an underlying reduction of the extracellular concentration of 5-HT.

Interestingly, discordant \([^{11}\text{C}]\text{WAY-100635}\) PET findings have also been reported in the field of depression. In contrast with the increased BP reported in antidepressant-naive MDD patients (Parsey et al., 2006), a majority of studies have demonstrated decreased BP in depressed patients as compared with controls in most brain regions, as well as in the raphe nuclei (Drevets et al., 1999, 2000, 2007; Sargent et al., 2000). In successfully treated MDD patients, the same direction of changes was observed, except in the raphe nuclei in which BP was found to be normal (Bhagwagar et al., 2004). Although the combination of the above findings might suggest that 5-HT\textsubscript{1A} receptor abnormalities in the raphe might resolve with antidepressant treatment, a recent paired scan study that directly looked at antidepressant-induced 5HT\textsubscript{1A} changes in MDD patients failed to confirm this hypothesis (Moses-Kolko et al., 2007).

Localization and lateralization of changes as a function of depressive symptoms

The primary aim of our study was to correlate cerebral \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND} with the intensity of depressive symptoms, as measured by the BDI-2 scale, and not with the presence of MDD proper. This strategy allowed us to investigate for the first time the biological substratum of different dimensions of depressive symptoms in patients with epilepsy. Indeed, depression is a multidimensional disorder comprising cognitive, somatic, behavioural and social symptoms (Costello et al., 1992). Factor analysis has suggested that BDI-2 reflects two primary dimensions of depression, corresponding to cognitive and non-cognitive (somato-affective) symptoms (Steer et al., 1999). A more recent study has proposed a new cognitive dimension, referred to as negative cognition and restricted to a subset of six of the original eight cognitive symptoms (excluding pessimism and suicidal thoughts) and has further divided the 13 non-cognitive symptoms into three classes, defined as psychomotor anhedonia, vegetative and somatic (Dunn et al., 2002). We chose to use this latter and more detailed subdivision of depressive symptoms with the hope to more precisely delineate the underlying changes in 5-HT\textsubscript{1A} receptor BP. Nevertheless, the correlations found with negative cognition should similarly apply to the entire cognitive dimension defined by Steer et al. (1999) since these two scales highly correlated with each other in our population \((r = 0.97)\). Furthermore, the distinction between psychomotor anhedonia and somatic symptoms proved relevant since these two symptom classes correlated with \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND} in strikingly different brain regions as detailed below.

Correlations of the total BDI score and symptoms of negative cognition and psychomotor anhedonia with \([^{18}\text{F}]\text{MPPF}\) BP\textsubscript{ND} in the raphe nuclei and the insula

Total BDI scores as well as symptoms of psychomotor-anhedonia and negative cognition correlated with
[18F]MPPF BP<sub>ND</sub> in the raphe nuclei. The raphe nuclei play a pivotal role in the serotonergic system. 5-HT<sub>1A</sub> autoreceptors, located on the soma and dendrites of 5-HT neurons, exert a pre-synaptic negative feedback on serotonergic neuron firing activity (Weissmann-Nanopoulos et al., 1985; Richer et al., 2002). However, the type and extent of dysfunction of the raphe nuclei in depression remain unclear. Post-mortem studies in suicide patients have reported both increased and decreased density of 5-HT neurons and 5-HT<sub>1A</sub> receptors (Stockmeier et al., 1998; Underwood et al., 1999; Arango et al., 2001) depending on their rostrocaudal localization within the raphe nuclei (Boldrini et al., 2007). Similarly, as previously discussed, [11C]WAY-100 635 PET studies have reported both increased and decreased BP in the raphe of MDD patients, although the latter did not correlate with the intensity of depressive or anxiety symptoms (Drevets et al., 1999, 2000; Sargent et al., 2000; Sullivan et al., 2005; Parsey et al., 2006). In addition, a PET study showed reduced [18F]FCWAY binding in the raphe in TLE patients with current or previous MDD compared with TLE patients without a history of MDD (Hasler et al., 2007). Finally, in unipolar MDD patients, FDG-PET demonstrated a positive correlation between symptoms of psychomotor-anhedonia and negative cognition and glucose metabolism in the dorsal raphe (Dunn et al., 2002), a result reminiscent of our [18F]MPPF findings in TLE patients. Overall, the strong correlation observed between depressive symptoms and [18F]MPPF BP<sub>ND</sub> in the raphe nuclei support the view that an epilepsy-related dysfunction of these nuclei could represent part of the biological basis of comorbid depression in TLE.

Symptoms of negative cognition and psychomotor-anhedonia, as well as the total BDI score, also correlated with [18F]MPPF BP<sub>ND</sub> in the insula of our patients. The insula is a site of multimodal integration that plays a pivotal role in limbic interactions (Shelley and Trimble, 2004; see Nagai et al., 2007 for review) and limbic-cortical pathways (Mayberg et al., 1997). Several FDG-PET and [11C]WAY-100635 studies have demonstrated insular abnormalities in MDD patients (Mayberg et al., 1999; Drevets, 2000; Drevets et al., 2000; Sargent et al., 2000; Kennedy et al., 2001; Kimbrell et al., 2002; Parsey et al., 2006; Fitzgerald et al., 2008), including negative correlation between insular cortex and the item ‘agitation’ of the Hamilton Depression Rating scale (Graf-Guerrero et al., 2004) and positive correlation between glucose metabolism and negative cognition symptoms of the BDI (Dunn et al., 2002). Acute intense sadness provoked by rehearsed autobiographical scripts was associated with an increased blood flow in the insula in normal subjects (Mayberg et al., 1999). It is also worth noting that the insula is one of the brain regions in which 5-HT<sub>1A</sub> BP is sensitive to previous antidepressant exposure (Sullivan et al., 2005). Finally, in patients with TLE, co-morbid depression was significantly associated with increased blood flow and reduced 5-HT<sub>1A</sub> receptor density in the insula (Ring et al., 1999; Hasler et al., 2007).

**Correlations of somatic symptoms of depression with [18F]MPPF BP<sub>ND</sub> in the hippocampus ipsilateral to seizure onset and in the frontal lobes**

Somatic symptoms correlated positively with [18F]MPPF BP<sub>ND</sub> in the mesial temporal region ipsilateral to seizure onset and more specifically over the hippocampus and parahippocampal gyrus. This finding is reminiscent of the correlation reported between the total BDI score and [18F]FCWAY BP over the epileptogenic hippocampus of TLE patients, although in the opposite direction for reasons already discussed (Giovacchini et al., 2005; Theodore et al., 2007). A bulk of evidence suggests that the hippocampus belongs to the limbic-frontal-subcortical network involved in the pathophysiology of MDD (Mayberg et al., 1997; Price, 1999; Sheline, 2003; Seminowicz et al., 2004). Indeed, decreased hippocampal volume, blood flow and glucose metabolism were reported in patients with MDD (Bremner et al., 2000; Kennedy et al., 2001; Saxena et al., 2001; Frodl et al., 2002; Videbech et al., 2002; Sheline et al., 2003b; Fitzgerald et al., 2008). Regarding hippocampal 5-HT<sub>1A</sub> receptors, decreased mRNA levels for receptor protein were observed in suicide victims as well as in rats exposed to acute stress, whereas [11C]WAY-100635 BP was found either decreased or increased in patients with MDD (Lopez et al., 1998, 1999; Drevets et al., 1999, 2000; Sargent et al., 2000; Lopez-Figueroa et al., 2004; Parsey et al., 2006). Like the insula, the hippocampus is one of the brain regions in which 5-HT<sub>1A</sub> receptor density appears to be sensitive to previous antidepressant exposure (Sullivan et al., 2004), a finding that might partly account for the above discordant results. Paradoxically, in patients with TLE, the role of hippocampal atrophy in the pathophysiology of comorbid depression remains elusive. Indeed, no association was found between depression and the presence or intensity of hippocampal atrophy (Lehrner et al., 1999; Baxendale et al., 2005; Briellmann et al., 2007; Richardson et al., 2007; Theodore et al., 2007), while a negative correlation was observed in the same region between depressive symptoms and creatine/N-acetylaspartate ratio, as measured by magnetic resonance spectroscopy (Giliam et al., 2007). According to this complex framework, it appears that depressive symptoms in TLE patients are more closely related to the serotonergic dysfunction than to the atrophy of the epileptic hippocampus. In this respect, it is worth noting that the epileptic hippocampus consistently shows a decreased BP of 5-HT<sub>1A</sub> receptor across studies and PET ligands, even in patients with no hippocampal atrophy or after correction for partial volume effect (Merlet et al., 2004a, b; Savic et al., 2004; Giovacchini et al., 2005; Ito et al., 2007). Most importantly, [18F]MPPF BP<sub>ND</sub> was found decreased over the epileptic hippocampus of TLE patients with a magnitude comparable to that observed
with $^{18}$F]FCWAY, despite the fact that these two PET ligands show a correlation with BDI scores of opposing directions (Merlet et al., 2004a, b; Giovacchini et al., 2005; Theodore et al., 2007). In other words, our positive correlation between somatic depressive symptoms and $^{18}$F]MPPF BP$_{ND}$ in the epileptic hippocampus is built upon an epilepsy-related major decrease of 5-HT$_{1A}$ receptor availability in that region. In our view, these abnormalities are likely to represent one of the biological links between TLE and the more global pre- and post-synaptic serotonergic dysfunction associated with comorbid depression.

Correlations between somatic symptoms of the BDI and $^{18}$F]MPPF BP$_{ND}$ were also observed in the mid-cingulate gyrus, and to a lesser degree, over the frontal dorsolateral cortex. All these regions are thought to be involved in the pathophysiology of depression (Drevets, 2000; Drevets et al., 2000; Kennedy et al., 2001; Anderson et al., 2004). Indeed, various symptoms of MDD were found to correlate with cerebral blood flow, glucose metabolism and $^{11}$C]WAY-100635 BP in the cingulate gyrus and frontal cortex in previous studies (Bench et al., 1993; Dunn et al., 2002; Videbech et al., 2002; Graff-Guerrero et al., 2004; Milak et al., 2005). Furthermore, negative correlations were observed between $^{11}$C]WAY-100635 BP in the anterior cingulate gyrus and anxiety symptoms, including somatic anxiety (Sullivan et al., 2004) and the anxiety facet of neuroticism in control subjects (Tauscher et al., 2001).

**Limitations of this study**

We did not perform a systematic standardized psychiatric interview technique during this study, a limitation that prevents us from drawing firm conclusions regarding the pathophysiology of comorbid MDD in epilepsy. We nevertheless feel very confident regarding the reliability of the psychiatric status of the majority of our patients. First, the risk that any of our 15 TLE patients with a BDI-2 cutoff scores $\leq$11 suffered current MDD (i.e. within the past 2 weeks) is below 1%, according to previous work in the field (Jones et al., 2005b). Second, the mental health of all our patients was thoroughly evaluated by an experienced senior epileptologist during their pre-surgical evaluation. Third, seven out of nine patients with a BDI-2 cutoff scores $>11$ were further evaluated by a psychiatrist, leading to a diagnosis of MDD in five and mild depressive disorder in the remaining two. Finally, no other major psychiatric illness was noted in our population. The relevance of our approach is further supported by our results, showing that the different classes of depressive symptoms correlated with $^{18}$F]MPPF BP$_{ND}$ in distinct brain regions.

Whether the correlation observed in our study primarily involved the left insula or that contralateral to seizure onset remains uncertain. Indeed, both the conventional and flipped PET data showed lateralized findings, pointing to the left hemisphere and that contralateral to seizure onset, respectively. This ambiguity partly reflected an imbalance between patients with right and left TLE (16 and 8, respectively, in our sample) that was even more marked among patients whose BDI score was $>11$ (8 right TLE versus 1 left TLE). Unfortunately, we could not control for this situation since our protocol required the inclusion of a specific number of consecutive patients fulfilling our inclusion criteria, regardless of the lateralization of their epileptic focus. This imbalance resulted in right TLE patients’ data having a major influence on the overall findings. Consequently, left hemispheric data largely corresponded to those contralateral to seizure onset and vice versa. The fact that SPM analysis of unflipped PET volumes disclosed a significant correlation with the total BDI score over the left insula, while the analysis of flipped PET data only showed a non-significant trend within the insula contralateral to seizure onset (although this correlation proved significant when re-evaluated with ROIs), could argue for the former hypothesis. In our view, the less significant results obtained with flipped PET data is more likely to reflect anatomical differences between the left and right insula not corrected by spatial normalization, resulting in a suboptimal anatomical matching between the right and flipped left insula within our $^{18}$F]MPPF BP$_{ND}$ template and in a less sensitive statistical analysis of this brain structure. Indeed, ROI analyses that do not suffer this anatomical limitation showed similar Pearson coefficients for the left insula and that contralateral to seizure onset. Other minor and indirect arguments in favour of a left-sided lateralization of depression-associated $^{18}$F]MPPF PET changes derive from the observation of predominantly left hemispheric FDG-PET abnormalities in depressed patients (Kennedy et al., 2001; Drevets et al., 2002; Anderson et al., 2004) and higher rates of depression in patients with left frontal strokes or post-traumatic scars than in those with the same type of right frontal lesions (Lipsey et al., 1983; Robinson et al., 1984, 1988; Jorge et al., 1993). Some studies have also reported a higher incidence of depression in patients with left rather than right TLE (Robertson, 1987; Altshuler et al., 1990; Victoroff et al., 1994) but this issue remains debated (Kohler et al., 1999). For instance, in our population, only 13% of patients with left TLE had a BDI score $>11$, as compared with 50% of right TLE patients.

The post hoc ROI analyses of the subgroup of patients with left TLE provided a more compelling argument towards the alternative view of a primary involvement of the insula contralateral to seizure onset rather than of the left insula proper. In this population, the BDI correlated with $^{18}$F]MPPF BP$_{ND}$ in the right (i.e. contralateral to seizure onset) but not in the left insula (Table 4). Although these findings could be partly influenced by the smaller number of patients with left rather than right TLE, they are strongly supported by the Pearson coefficients obtained in these two populations for left and right TLE, respectively.
Overall, we believe that the correlations observed between depressive symptoms and $[^{18}F]MPPF$ BP$_{ND}$ are more likely to involve the insula contralateral to the epileptogenic zone than the left insula. The question then arises of the pathophysiological significance of this finding. One possibility could be that the serotoninergic dysfunction associated with depressive symptoms would normally affect both insula but would be obscured on the side ipsilateral to the epileptic temporal lobe by 5-HT$_{1A}$ receptor changes related to the frequent propagation of epileptic discharges into that region (Isnard et al., 2000). Another possibility could be that the emergence of depressive symptoms in patients with unilateral TLE depends more on the bilateralization of serotoninergic dysfunction than on the intensity of ipsilateral abnormalities.

As for the previous correlations observed in the insula, we cannot firmly conclude as to whether those seen in the cingulate gyrus primarily involve the left side or that contralateral to seizure onset, although post hoc analyses in left TLE patients favoured the former possibility. Interestingly, glucose metabolism in the left cingulate gyrus was also found to correlate positively with depressive symptoms in MDD patients, although not specifically with somatic symptoms (Dunn et al., 2002). A prior $[^{11}C]$WAY-100635 PET study of TLE patients also reported correlation between depression and 5-HT$_{1A}$ receptors density in the cingulate gyrus (Savic et al., 2004). However, this correlation was negative rather than positive, ipsilateral to seizure onset rather than left-sided or contralateral and predominated in the anterior rather than the mid-portion of the cingulate gyrus.

Overall, our data suggest that complex changes of the central serotoninergic system are associated with the type and intensity of depressive symptoms in TLE patients. These changes appear to predominate over the raphe nuclei and the insula, and to a lesser extent the left mid-cingulate gyrus, the left and right frontal dorsolateral cortex, and the hippocampal region ipsilateral to seizure onset, with some regional specificity for the different symptom classes. At present, it remains difficult to firmly conclude on the molecular substratum underlying higher $[^{18}F]MPPF$-BP$_{ND}$ in the more depressed TLE patients (i.e. decreased serotonin concentration versus higher number of 5-HT$_{1A}$ receptors) in as much as previously published $[^{11}C]$WAY-100635 and $[^{18}F]$FCWAY studies in TLE and MDD provide conflicting results. However, it is our view that our findings most likely reflect a decreased extracellular concentration of serotonin at the pre- and post-synaptic levels of the major central serotoninergic pathways.

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**References**


