Upregulation of opioid receptor binding following spontaneous epileptic seizures

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Animal and limited human data suggest an important anticonvulsant role for opioid peptides and their receptors. We aimed to provide direct human in vivo evidence for changes in opioid receptor availability following spontaneous seizures. We scanned nine patients within hours of spontaneous temporal lobe seizures and compared their postictal binding of the non-subtype selective opioid receptor PET radioligand [11C]diprenorphine (DPN), quantified as a volume-of-distribution (VD), with interictal binding and with binding changes in 14 healthy controls, controlling for a range of behavioural variables associated with opioid action. A regionally specific increase of opioid receptor availability was evident in the temporal pole and fusiform gyrus ipsilateral to the seizure focus following seizures (Z 5.01, P < 0.001, 16 432 mm³). Within this region, there was a negative correlation between VD and log10 time since last seizure (r = −0.53, P < 0.03), compatible with an early increase and gradual return to baseline. [11C]DPN VD did not undergo systematic changes between time points in controls. This study provides direct human in vivo evidence for changes in opioid receptor availability over a time course of hours following spontaneous seizures, emphasizing an important role of the opioid system in seizure control.

Keywords: temporal lobe epilepsy; opioids; neurotransmission; anterior temporal lobe; positron emission tomography

Abbreviations: DOP = δ opioid peptide; DPN = diprenorphine; KOP = κ opioid peptide; MOP = μ opioid peptide; TLE = temporal lobe epilepsy

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Introduction

Proconvulsant and anticonvulsant properties of opium have been described in clinical and experimental reports dating back to the 19th century (Tortella, 1988). While opioid peptides were originally considered to be endogenous convulsants, there is growing evidence for the existence of an endogenous anticonvulsant mechanism in humans modulated by an anticonvulsant substance with opioid characteristics. The effects of endogenous opioids are mediated through μ opioid peptide (MOP), κ opioid peptide (KOP) and δ opioid peptide (DOP) receptors. High frequency firing is required to release endogenous opioids (Wagner et al., 1990), implying a dynamic role of endogenous opioids in terminating seizures (Tortella and Long, 1985).

Previous interictal PET studies in temporal lobe epilepsy (TLE) have shown increased binding in the lateral temporal neocortex on the side of the epileptogenic focus with the MOP-receptor selective radioligand [11C]carfentanyl (Frost et al., 1988; Mayberg et al., 1991) as well as with the DOP-receptor selective radioligand [11C]methylinaltrindole (Madar et al., 1997) but no side-to-side differences of [11C]diprenorphine (DPN) binding (Mayberg et al., 1991; Bartenstein et al., 1994), which binds to all three opioid receptor subtypes (Lee et al., 1999). Only studies
quantifying receptor binding in the same subject over time can demonstrate the role of a given receptor–ligand interaction in a given event. PET studies have shown a decrease in $[11C]$DPN binding in association cortices during hyperventilation-induced absences (Bartenstein et al., 1993) and lower binding in reading-associated areas during reading-induced seizures in reading epilepsy compared to baseline scans (Koepp et al., 1998). Radioligand binding changes following spontaneous seizures have not been studied.

In the current study, we used $[11C]$DPN PET to measure changes in opioid receptor availability following spontaneous seizures in patients with TLE by means of a two-scan paradigm. The first scan was performed as soon as possible (within hours) after a spontaneous seizure; the second scan was acquired days to weeks later after as long a seizure free period as achievable in a given patient and served as an intrasubject control. In addition, healthy controls were scanned twice under the same conditions to establish the normal fluctuation of opioid receptor availability. Large increases of opioid receptor availability relative to the control condition in patients as well as relative to the controls were observed following seizures in a time dependent fashion specifically in the temporal pole ipsilateral to the epileptogenic focus.

**Material and methods**

**Subjects**

Inclusion criteria were age 25–65 years, and refractory TLE in patients. Exclusion criteria were concomitant neurological or psychiatric disorders or previous brain injury, use of opioid-containing medication within the two weeks prior to a scan, and a positive pregnancy test.

Patients from the Telemetry Unit at the National Hospital for Neurology and Neurosurgery (NHNN), Queen Square, London; the Sir William Gowers Assessment Centre at the National Society for Epilepsy (NSE), Chalfont St Peter, Buckinghamshire; a cohort of patients who had been evaluated for epilepsy surgery, and referrals from outpatient clinics at both the NHNN and NSE were screened for inclusion. Out of a screened population of 1200 patients, 60 patients agreed to participate in the study, had suitable spontaneous seizure frequencies and reliably recalled their seizures without suffering from unobserved nocturnal seizures or seizures which they did not recall, as verified during previous prolonged video-EEG monitoring and/or prolonged inpatient stays at the Chalfont Centre for Epilepsy. Low seizure frequencies were not an exclusion criterion but reduced our chances of obtaining a postictal scan. Frequent seizures did not exclude patients from the study as long as they occasionally had periods of two weeks without seizures in order to enable us to schedule an interictal scan. Patients were informed of scheduled scanning times but also encouraged to call the main investigator (A.H.) whenever there was any possibility of organizing a postictal scan within 24 h. Over four years, nine patients (four women; median age 47 years, range 32–60 years) were scanned twice, postictally within 24 h of a spontaneous seizure (median interval 8.5 h, range 1.5–21.33 h) and again interictally after a seizure-free interval (median 224 h/9.3 days, range 152–1344 h/6.3–56 days). Fourteen healthy control subjects (four women; median age 41.5 years, range 26–58 years) were recruited from acquaintances of the investigators and scanned twice under the same conditions. The median interval between scans was 121 (24–406) days for patients and 48 (1–101) days for controls.

Ethical permission from the Hammersmith, Queen Charlotte’s and Chelsea and Acton Research Ethics Committee, and the Joint Ethics Committee of The Institute of Neurology and the NHNN, and permission by the UK Administration of Radiation Substances Advisory Committee (ARSAC) were obtained, and all subjects gave informed written consent.

The diagnosis of TLE was based on history, seizure semiology, interictal EEG features and neuropsychological examination. The lateralization of TLE was additionally based on MRI findings and ictal EEG when available; for ‘MRI-negative’ patients ictal EEG was always obtained. In one case (Patient 5) where the ictal scalp EEG was inconclusive, lateralization was based on interictal EEG and the MRI finding of unilateral HS (Mintzer et al., 2004). Clinical characteristics are given in Table 1.

**PET data acquisition**

Scans were performed in three-dimensional acquisition mode on a Siemens/CTI ECAT EXACT3D PET camera, with a spatial resolution of reconstructed images of $\sim 5$ mm (Spinks et al., 2000). A median dose of 183 MBq (range, 125–200 MBq) of $[11C]$DPN with a median radiochemical purity of 98.4% (range, 94–100%) was injected. The median co-injected mass of unlabelled DPN was 3.1 µg (range, 1.2–21.0 µg), corresponding to an estimated median receptor occupancy of 0.24% (range, 0.08–1.19%), thereby fulfilling the tracer kinetics assumption.

Tomographic data acquisition was performed event-by-event, and data were rebinned into 32 time frames. A five-min transmission scan using a hydraulically driven $^{137}$Cs point source was acquired prior to each emission scan. Each subject’s head position was maintained with individualized foam holders and monitored throughout. Subjects were rested with lights dimmed and no external noise.

Image data were reconstructed using a reprojection algorithm (Kinahan and Rogers, 1989) with ramp and Colsher filters cutoff at Nyquist frequency. Measured attenuation maps were segmented (Bailey et al., 1998) and used for model-based scatter correction (Watson et al., 1996) and attenuation. Voxel sizes following reconstruction were 2.096 mm × 2.096 mm × 2.43 mm.

**Derivation of input functions**

Arterial blood was continuously withdrawn for 15 min through a 22-gauge cannula in a radial artery at a sampling rate of initially 5 ml/min and measured in a BGO detection system (Ranicar et al., 1991). Additional discrete blood samples were taken for cross-calibration, determination of the partition of radioactivity and determination of parent fraction (Luthra et al., 1993) for the creation of individual arterial plasma input functions corrected for metabolism.

**PET data analysis**

Standardized samples of high-contrast volumes-of-interest (VOIs) were defined directly on weighted activity images summed over the duration of the emission scans (ADD images). These VOIs
<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)/sex handedness</th>
<th>Onset/duration (years)</th>
<th>Seizures/year</th>
<th>Postictal/interictal interval (hours); interscan interval</th>
<th>Treatment</th>
<th>Seizures</th>
<th>EEG (interictal)</th>
<th>EEG (Ictal)</th>
<th>MRI/other imaging</th>
<th>Probable side of focus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41/M lh/ambidextrous</td>
<td>28/13</td>
<td>182</td>
<td>15/168; 172d</td>
<td>PHT, TPM, LEV, GBP, CLB</td>
<td>SPS, CPS</td>
<td>Spikes L:R 24:1</td>
<td>L frontotemporal</td>
<td>L HS</td>
<td>L</td>
</tr>
<tr>
<td>2</td>
<td>37/F lh</td>
<td>16/21</td>
<td>48</td>
<td>7/152; 125d</td>
<td>OXC, GBP, CLB, DZP prn, folic acid</td>
<td>CPS, 2 GTCS</td>
<td>L slow</td>
<td>No seizures</td>
<td>L HS</td>
<td>L</td>
</tr>
<tr>
<td>3</td>
<td>57/M rh</td>
<td>21/36</td>
<td>42</td>
<td>2/480; 24d</td>
<td>CBZ, LEV, acitretin, oxytetracycline</td>
<td>CPS</td>
<td>L mid TL</td>
<td>L TL</td>
<td>bil HS</td>
<td>L</td>
</tr>
<tr>
<td>4</td>
<td>32/F rh</td>
<td>3/29</td>
<td>144</td>
<td>1.5/192; 91d</td>
<td>CBZ, VPA, LEV</td>
<td>SPS, CPS</td>
<td>R TL</td>
<td>Only SPS recorded</td>
<td>R HS</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>49/M lh/ambidextrous</td>
<td>27/22</td>
<td>24</td>
<td>8.5/320; 70d</td>
<td>CBZ, LTG, LEV</td>
<td>SPS, CPS</td>
<td>R TL</td>
<td>L,TL/bilateral</td>
<td>R HS</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>60/F rh</td>
<td>1/59</td>
<td>12</td>
<td>13/1344; 195d</td>
<td>LEV, TPM, loperamide</td>
<td>SPS, CPS</td>
<td>L TL</td>
<td>L frontotemporal</td>
<td>L HS</td>
<td>L</td>
</tr>
<tr>
<td>7</td>
<td>57/M rh</td>
<td>5/52</td>
<td>108</td>
<td>19.5/192; 406d</td>
<td>LEV, diclofenac</td>
<td>SPS, CPS</td>
<td>R mid TL</td>
<td>R TL</td>
<td>MRIneg</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>44/F rh</td>
<td>14/30</td>
<td>44</td>
<td>21.33/224; 121d</td>
<td>PHT, LEV, CLB prn, clonidine, citalopram, insulin</td>
<td>SPS, CPS</td>
<td>Bitemporal R &gt; L</td>
<td>R TL + C4</td>
<td>MRIneg</td>
<td>R</td>
</tr>
<tr>
<td>9</td>
<td>47/M rh</td>
<td>11/37</td>
<td>42</td>
<td>6.33/264; 45d</td>
<td>PHT, CBZ</td>
<td>SPS, CPS</td>
<td>TL spikes L:R 4:1, ITL slow</td>
<td>R TL</td>
<td>MRIneg</td>
<td>R</td>
</tr>
</tbody>
</table>

CPS = complex partial seizure; EEG = electroencephalogram; M = male, F = female; lh/rh = left/right handed; d = days; PHT = phenytoin; TPM = topiramate; LEV = levetiracetam; GBP = gabapentin; CLB = clobazam; OXC = oxcarbazepine; DZP = diazepam; prn = pro re nata (if needed); CBZ = carbamazepine; LTG = lamotrigine; SPS = simple partial seizures; 2 GTCS = secondarily generalized seizures; L = left; R = right; TL = temporal lobe; HS = hippocampal sclerosis; HC = hippocampus; FDG = [18F]fluorodeoxyglucose.
were applied to the dynamic dataset and decay-corrected time-activity curves computed in order to assess movement during the scan. When movement was detected in a scan, both datasets of a pair were movement corrected with a frame-by-frame registration method using mutual information (Studholme et al., 1997). Dynamic PET images, input functions and spectral analysis (Tadokoro et al., 1993) were then used to create voxel-by-voxel parametric images of $^{[11]}$C DPN volume-of-distribution (VD).

**Other data acquisition**

Patients were monitored and questioned for possible seizures and in addition a ten channel EEG was recorded whenever possible starting about 1.5 h prior to each PET scan and recording throughout the PET scan; however technical and timing issues led to incomplete data, and EEG data were not further analysed. All subjects filled in a Beck Depression Inventory (BDI) (Beck et al., 1961) on every scanning day. In addition, we monitored the positive and negative affect score [PANAS (Watson et al., 1988)] and visual analogue scale ratings of interest, amusement, happiness, sadness and pain before and during each scan.

**Statistical analyses**

Statistical parametric mapping (SPM2; Wellcome Trust Centre for Neuroimaging, London) was used for spatial transformations and statistical analysis.

First, an in-house created template of $^{[11]}$C DPN 90 minute ADD images (von Spiczak et al., 2005) that occupies the standard stereotactic space defined by the Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 templates as supplied with SPM was right–left reversed, rigid-body coregistered onto itself and averaged using a soft mean, thus creating a symmetrical template approximating MNI/ICBM152 space. Datasets from the four patients with left TLE were then right–left reversed so the epileptogenic focus appeared on the same (right) side in all. A corresponding proportion of control datasets (6/14) was also right–left reversed. We have previously shown that right–left reversal prior to normalization to a symmetrical template did not alter results (Hammers et al., 2003). The approximate location of the anterior commissure was identified in all datasets and defined as the ‘origin’. All subsequent spatial operations were performed using the ADD images and applying the derived parameters to the parametric maps. Each subject’s second dataset was coregistered to the first dataset, but not resliced, using a normalized mutual information criterion, and then normalized to the symmetrical DPN template. The resulting parametric maps in stereotaxic space were finally filtered with a 12 mm $\times$ 12 mm $\times$ 12 mm Gaussian kernel.

Global VD values were computed as an overall mean over the entire matrix, thresholded at 1/8 of that value to create a brain mask, and averaged again within this mask. Global differences between postictal and interictal patient scans and first and second control scans were assessed with a univariate ANOVA.

To assess the effect of spontaneous seizures, compared to controls, we created a single design matrix with two groups, two scans and two conditions per subject, with global VD covaried out. This design keeps the maximum number of degrees of freedom by comparing all scans simultaneously, while simultaneously using the power of paired comparisons. This allows testing of both simple contrasts between the postictal and the interictal scan (e.g. $1 \, -1$), using the remainder of the control scans to derive variability, and to specifically test for interscan differences in the patients which were opposite to those seen in controls (e.g. $1 \, -1 \, -1 \, 1$).

The resulting statistical parametric maps were thresholded at $P<0.001$, without extent threshold. Resulting clusters were examined for significance at the cluster level at the conventional $P<0.05$ threshold.

To test for voxelwise correlations with clinical data, contrast images for the main contrast of interest ($1 \, -1 \, 1$ in the above model) were then taken to a second level analysis, again covarying out global values.

The area of statistical difference derived from the above model (i.e. the cluster in the temporal pole ipsilateral to the epileptogenic focus) was further used as a VOI, to extract scaled VDs using MarsBar (Brett et al., 2002) for comparison with seizure-to-scan intervals. Statistical testing on these extracted scaled VDs was performed using SPSS 12.0 for Microsoft Windows. Both scaled VDs and interscan differences were plotted against seizure-to-scan intervals, with control intervals set to zero.

Mean percentage changes ($\pm$ SD) of scaled $^{[11]}$C DPN VD in the ipsilateral VOI derived from the main SPM analysis were calculated as:

$$\frac{(VD1 - VD2)}{(VD1 + VD2)} \times 100$$

**Results**

There was no significant difference in global VD between patients’ postictal and interictal and healthy controls’ first and second scans (ANOVA, $P<0.90$), no correlation of global VD and age ($r=-0.13, P>0.3$), and no difference in global VD between genders ($r=0.08, P>0.5$). Global effects were removed in subsequent SPM analyses with an ANCOVA.

Neither patients nor controls showed a between-scan difference in depression ratings measured with the BDI; their PANAS scores; or visual analogue scale ratings of interest, amusement, happiness, sadness and pain, indicating that neither the test–retest situation nor seizures themselves changed these factors, which were therefore not included in the final statistical model.

**Patients versus controls**

After spontaneous seizures and compared to the interictal state, there was a highly significant postictal increase in $^{[11]}$C DPN VD encompassing the temporal pole ipsilateral to the electroclinical focus and extending posteriorly along the fusiform gyrus. Contrasted to show a postictal increase relative to the interictal scan, compared to the first versus the second control scan ($1 \, -1 \, -1 \, 1$ contrast), cluster values were $Z=4.53, P<0.002$ (13 888 mm$^3$). Inspection of the actual values showed minimal and unsystematic changes in the controls. Correspondingly, treating controls as background without enforcing a model ($1 \, -1$ contrast) yielded higher effect sizes for the postictal increase compared to interictal values ($Z=5.01, P<0.001$ (16 432 mm$^3$); $x/y/z$
co-ordinates for the cluster maximum in the MNI/ICBM152 coordinate system 40/16/−36 (Fig. 1). This cluster was used for subsequent VOI analyses. This latter analysis also showed some contralateral increases ($Z = 3.94$; n.s.) in a similar location (Fig. 1).

There were no significant postictal decreases in $[^{11}C]$DPN VD, either relative to the interictal scan or relative to changes in the control scans.

**Correlations of $[^{11}C]$DPN VD changes with clinical variables**

We first performed voxel-based searches for correlations across the entire brain. A negative correlation between the magnitude of the $[^{11}C]$DPN VD increase and the postictal interval (the time between the last seizure and the first, postictal scan) was observed in both posterior cerebellar hemispheres [contralaterally, $Z = 4.74$, $P < 0.001$ (7144 mm$^3$); ipsilaterally, $Z = 4.34$, $P < 0.001$ (4736 mm$^3$)], possibly suggesting a very early increase in receptor numbers in the cerebellum. There was no significant positive correlation between the postictal interval and the magnitude of the $[^{11}C]$DPN VD increase anywhere in the brain.

The absolute (scaled) VD values and their differences within the VOI from the main contrast were subjected to further analysis. Median percentage changes and interquartile ranges of scaled $[^{11}C]$DPN VD in the ipsilateral VOI derived from the main SPM analysis were $+7.8$ (+4.0 to +18.8)% for patients and $+1.0$ (−6.6 to +6.0)% for controls.

A rapid increase in opioid receptor number or affinity, as suggested by the main analysis, should be accompanied by a gradual return to baseline, i.e. a negative correlation between VD and time since last seizure, across all scans and intervals, within the VOI derived from the main SPM analysis. As the interval between last seizure and PET scan differed by an order of magnitude between postictal and interictal scans, all delays were subjected to a re-expression as the logarithm to the base 10. There was the expected negative correlation between VD and log$_{10}$ time since last seizure ($r = −0.53$, $P < 0.03$; Fig. 2). As there was no overall difference in this VOI’s VD between any of the conditions or groups, this indicates a gradual return to baseline.

![Fig. 1 Postictal increase of $[^{11}C]$DPN VD, relative to interictal values, in the ipsilateral (right of the image, crosshairs) anterior temporal lobe. Thresholded $t$ map overlaid onto MNI/ICBM152 average. Maximum intensity projection showing non-significant contralateral increases and absence of changes elsewhere. Colour bar, $t$ scores.](image-url)
Discussion

We demonstrate increases in opioid receptor availability following spontaneous seizures in humans. \[^{11}\text{C}]\text{DPN}\ binding did not undergo systematic changes in controls but increased by about 8% in the ipsilateral anterior temporal lobe in patients a median of 8.5 h after the last spontaneous seizure.

The finding of a postictal increase in \[^{11}\text{C}]\text{DPN}\ binding complements earlier findings of decreased \[^{11}\text{C}]\text{DPN}\ binding when scans were performed during reading-induced seizures and absences (Bartenstein et al., 1993; Koepp et al., 1998). Taking together the results of these previous acute seizure studies, and our present work, we suggest that synaptic opioid levels increase at the time of seizures, leading to a reduction in \[^{11}\text{C}]\text{DPN}\ binding, and that this is followed by a gradual recovery of available surface receptors with an overshoot over basal levels which is detected by PET about 8 h after seizures, with a gradual return to normal or low-normal levels during the interictal phase.

Cerebral blood flow changes cannot explain our findings. While regional blood flow increases by up to 70–80% after complex partial seizures (Theodore et al., 1996) it normalizes after about 30 min (Berkovic, 2000). The shortest postictal interval in our study was 90 min and none of our patients had a seizure during scanning. Further, the binding parameter \text{VD} does not vary with blood flow as long as this remains constant during the scanning period.

Acute release of endogenous opioids has resulted in reduced density of opioid receptors as assessed by \textit{in vivo} autoradiography with \[^{3}\text{H}]\text{diprenorphine in a rat model of drug addiction (Gerrits et al., 1999) and following two acute stress experiments in rats (Seeger et al., 1984), as well as in reduced binding potentials for the MOP receptor ligand \[^{11}\text{C}]\text{carfentanil in PET experiments in humans, in which acute deep tissue pain was generated through infusion of hypertonic saline into the masseter muscle (Zubieta et al., 2003, 2005). Agonist-driven desensitization may occur within minutes of agonist exposure and involves internalization and downregulation (Bohm et al., 1997). Until recently, restoration of receptor response and receptor density through, for example, recycling and resynthesis has received far less attention. Functional resensitization of the MOP-receptor after 4 h of exposure to the MOP-agonist DAMGO to induce desensitization, followed by various DAMGO-free intervals, occurred after as little as 10 min, and responses reached 100% of control after 60 min in HEK 293 cells (Pfeiffer et al., 2003). In other studies, MOP receptor proteins reached normal levels and were functional again after 6 h through recycling in enteric neurons of the guinea pig ileum (Minnis et al., 2003). Recycling to basal levels can occur in 60 min, with some agonists inducing increased receptor levels (at 110–120% of control) as measured with \[^{3}\text{H}]\text{diprenorphine in Sf9 insect cells transfected to express human MOP receptors (Chen et al., 2003}.)

Increased opioid receptor availability may also involve \textit{de novo} synthesis of receptors, which may occur within 8 h (Chaturvedi et al., 2000). While all of the preceding experiments were necessarily performed \textit{in vitro}, it can reasonably be assumed that the mechanisms are similar \textit{in vivo}.
Overall, transmitter peptide depletion is a less likely explanation for our findings: there is normally no basal opioidergic tone; and a decrease in transmission capacity would not be compatible with the increase in seizure threshold seen after seizures (Tortella, 1988). Indeed our results may provide an explanation for such increases in seizure threshold, as for example seen following repeated induction of seizures during a course of electroconvulsive therapy in humans (e.g. Sackeim et al., 1987). The KOP system may play a more important role in complex partial seizures of temporal lobe origin (Simonato and Romualdi, 1996). Dynorphin inhibits excitatory transmission and dynorphin depletion has been found to correlate with seizure propensity in rats with herpes simplex virus 1 infection (Solbrig et al., 2006b) or Borna disease virus infection (Solbrig et al., 2006a). It is possible that dynorphin release specifically may be responsible for a compensatory upregulation of mainly KOP receptors detected by the non selective DPN, consistent with their localization in humans (reviewed by Hammers and Lingford-Hughes, 2006).

DPN has approximately nanomolar affinity for all opioid receptor subtypes (Richards and Sadee, 1985), therefore we cannot infer which subtypes are involved. In addition, VD varies linearly with the ratio of receptor density over the dissociation constant, $K_d$ (which is inversely related to affinity), and a transient increase in affinity, although unlikely, cannot be ruled out as an alternative explanation. Receptor upregulation is not a universal response to seizures in humans. For example, the number of benzodiazepine binding sites associated with GABA$_A$ receptors (B'max) was more decreased in the ipsilateral hippocampus after a shorter interval after seizures, of the order of 4–5 days, compared with longer intervals (Bouvard et al., 2005). A limitation of our study design is that it was not practicable to obtain continuous video-EEG data throughout the 6.3–56 day seizure free intervals finally obtained prior to the interictal PET study. We, therefore, cannot categorically determine the exact duration of the seizure-free period as in previous EEG-telemetry-based studies (Bouvard et al., 2005). However, we took great care to only include patients in whom unrecognized seizures were highly unlikely. Similarly, the difference of two orders of magnitude in the interval between the seizure presumed to be preceding the first and second PET studies makes bias through possible unrecognized seizures highly unlikely.

The temporal pole has a central role in temporal lobe seizures. In a series of 48 patients with drug-refractory TLE investigated with depth electrode recordings (Chabardes et al., 2005), the temporal pole was involved in all, and in 48% at the onset of the seizure. Even in patients with clearly defined mesial temporal lobe epilepsy with hippocampal sclerosis, the temporal pole was the site of seizure onset in 9 out of 27 patients (Chabardes et al., 2005). While surgical removal of at least hippocampus, amygdala and parahippocampal gyrus has similar outcomes regardless of the extent of resection (Engel, 1996), removal of the anterior temporal lobe alone led to seizure freedom in 52% in a surgical series of 50 patients operated upon in the pre-MRI era (Hardiman et al., 1988).

Our findings are also compatible with data from animal experiments in which the hippocampus often functioned differently from the neocortex (Tortella, 1988), with opioids having proconvulsive rather than anticonvulsive effects. We did not find VD changes in the hippocampus itself. The finding of no overall difference in $[^{11}C]$DPN binding between patients and controls in the anterior temporal lobe is consistent with previous interictal studies in TLE (Mayberg et al., 1991; Bartenstein et al., 1994) and further supports the concept of a phasic rather than a tonic change. In a previous study of interictal MOP receptors measured with $[^{11}C]$carfentanil (Mayberg et al., 1991) there were increases in the ipsilateral temporal neocortex and decreases in the amygdala without reported changes in the hippocampus (which was not directly sampled). The interval after the last seizure was 8 ± 8 days; over this period, there was no correlation with $[^{11}C]$carfentanil binding. In addition, the authors did not observe a difference in $[^{11}C]$DPN binding measured in the same patients. This would suggest that after a mean of eight days, opioid receptor availability should have normalized.

In conclusion, our results provide evidence for an association of changes in endogenous opioid transmission with spontaneous seizures in temporal lobe epilepsy.

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**References**


