Olfactory short-term memory and related amygdala recordings in patients with temporal lobe epilepsy

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Summary
Olfactory short-term recognition memory was assessed with a delayed odour-matching task in 38 patients with unilateral temporal lobe epilepsy (TLE) and stereotactic electroencephalography (SEEG) recordings taken prior to surgical treatment. The amygdala SEEG activity associated with odorant stimulation was examined in 18 patients. Because the invasive SEEG procedure is only performed in a clinical framework, electrophysiological data obtained from these patients could not be analysed in comparison with data obtained from control subjects. Behavioural results (hits, false alarms, discrimination, bias scores) showed global impairment of odour recognition memory in patients when compared with controls. We also found lower discrimination and higher false alarm scores in left than in right TLE patients, and higher false alarm scores in male than in female patients. The hemisphere effect is discussed in terms of psychosocial trait differences between patients. Electrophysiological recordings collected from the amygdala demonstrated that odorant stimulation was associated with chemosensory evoked potentials (CSEPs). Analysis revealed that CSEPs obtained for target odorants had lower peak amplitudes and latencies than those obtained in response to sample odorants. The reduced peak amplitudes suggest a mechanism of repetition suppression—a process assumed to reflect neural activity related to high-level cognitive processes such as attention, memory and decision making. Latency modulations appear rather to be linked to early stages of information processing and may therefore reflect a facilitation process due to selective attention.

Keywords: olfaction; intracerebral recording; amygdala; TLE; short-term recognition memory

Abbreviations: CSEP = chemosensory evoked potential; N2 = negative peak of CSEP; P1 = positive peak of CSEP; SEEG = stereotactic electroencephalography; TLE = temporal lobe epilepsy

Introduction
The importance of temporal lobe structures in human olfactory function has been recognized since the 19th Century with the observation that olfactory auras could precede paroxystic seizures in epileptic patients (Jackson and Stewart, 1899). After post-mortem examination of a patient who had experienced a highly repulsive smell immediately before the seizures, Jackson and Beevor (1890) revealed a tumour in the right temporal lobe which affected most of the uncinate gyrus. Many investigators then tried to determine the influence of temporal lobe epilepsy (TLE) on olfactory function using behavioural studies. Olfactory sensitivity, assessed using detection or recognition threshold measurements, was usually reported to be in the normal range compared with healthy controls (Toulouse and Vaschide, 1899a, b; Santorelli and Marotta, 1964; De Michele et al., 1976; Campanella et al., 1978; Eskenazi et al., 1986; Martinez et al., 1993; Jones-Gotman et al., 1997; Savic et al., 1997). In contrast, a wide variety of tasks (e.g. odour-matching, discrimination, short and long-term recognition memory, identification and naming) highlighted consistent deficits in higher olfactory functions (Abraham and Mathai, 1983; Eskenazi et al., 1986; Carroll et al., 1993; Martinez et al., 1993; West and Doty, 1995; Jones-Gotman et al., 1997; Savic et al., 1997). Factors such as stimulation type (monorhinal or birhinal), stimulated nostril-side (ipsi- or contralaterally to the epileptogenic focus), and odorant ‘nameability’ appeared to be determining factors in patient performance. Due to the methodological differences
controls stimulation (Narabayashi et al., 2000; Harding et al., 2002). Investigators have also examined the electrophysiological activity of the human amygdala associated with odorant presentation when patients with epilepsy are undergoing an intracranial EEG procedure. The first recordings collected from electrodes acutely implanted in the human amygdala revealed that fast oscillatory activity (or spindles) was associated with odorant stimulation (Narabayashi et al., 1963; Hughes et al., 1972; Hughes and Andy, 1979). Similar spindles were obtained from chronically implanted electrodes, but these were thought to reflect rather an overall response linked to breathing and blood gas content than a uniquely olfactory response (Halgren et al., 1977a, b). We have recently demonstrated that, in addition to spindles, the human amygdala also produces chemosensory evoked potentials (CSEPs) (Hudry et al., 2001). Although studies have interpreted spindles differently, we found that the evoked potentials represent specific odor-induced responses. These CSEPs recorded from depth electrodes placed directly into the olfactory cerebral structures overcome the limitations of scalp-recorded CSEPs and functional MRI by combining their respective advantages of direct measurement of neural activity, real-time temporal resolution and fine-grain spatial resolution.

The purpose of this study was to assess short-term olfactory recognition memory in TLE patients using a delayed odour matching-to-sample task with unfamiliar odorants, and to examine the related electrophysiological activity recorded from the amygdala. Tests were performed pre-operatively by patients whose intracranial EEG activity was chronically recorded in either the right or left amygdala by stereotactic EEG (SEEG) using stereotactically implanted electrodes. Odorants were monorhinally administered in the nostril ipsilateral to the suspected epileptogenic focus side. This study allowed us to compare how the brain processes information during smell encoding and retrieval, and to compare the effects of matching- versus mismatching-stimuli upon the neural activity of the amygdala. Since most studies have implicated the right temporal lobe in odor processing, especially in odor recognition (Rausch et al., 1977; Abraham and Mathäi, 1983; Zucco and Tressoldi, 1989; Jones-Gotman and Zatorre, 1993), we hypothesized that odor recognition memory scores should be more altered in right than left TLE patients. From concepts based on electrophysiological findings obtained from behaving monkeys (Miller et al., 1993a, b), we supposed that the amygdala is sensitive to the repetition suppression process which is assumed to participate in recognition processes, and more specifically in stimulus novelty detection. Although we thus expected that presentation of a target stimulus similar to the sample would decrease the responsiveness of the amygdala neurons, resulting in CSEP amplitude decreases, such an effect was not expected in the mismatch condition with a new target stimulus. In addition, because selective attention is a phenomenon reported to modify sensory inputs at an early stage of processing and to preferentially select and process high priority information (Krauel et al., 1998), we expected that the match, but not the mismatch condition, would induce a reduction in CSEP latency. Since it is largely admitted that brain activity is affected by the behavioural response correctness (for a review, see Falkenstein et al., 2000), only electrophysiological data associated with correct responses (hits and correct rejections) were included in the analyses of the present study.

### Table 1
Number, age and smoking habits of right and left TLE patients, and control subjects included in the behavioural experiment, and right and left TLE patients included in the electrophysiological experiment

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Behavioural study</th>
<th>Electrophysiological study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Age (years)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Min</td>
</tr>
<tr>
<td>Rights TLE</td>
<td>Male 9</td>
<td>27.1 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>Female 11</td>
<td>35.2 ± 9.7</td>
</tr>
<tr>
<td>Left TLE</td>
<td>Male 9</td>
<td>37.0 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>Female 9</td>
<td>32.0 ± 7.6</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>32.8 ± 8.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>Right nostril</th>
<th>n</th>
<th>Age (years)</th>
<th>Smokers</th>
<th>Illness duration (years ± SD)</th>
<th>Right nostril n</th>
<th>Age (years)</th>
<th>Smokers</th>
<th>Illness duration (years ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male 11</td>
<td>24.9 ± 10.2</td>
<td>17</td>
<td>48</td>
<td>2</td>
<td>24.9 ± 6.4</td>
<td>2</td>
<td>44.0 ± 17.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Female 9</td>
<td>36.7 ± 8.3</td>
<td>22</td>
<td>50</td>
<td>2</td>
<td>24.1 ± 8.9</td>
<td>9</td>
<td>36.1 ± 105</td>
<td>23</td>
</tr>
<tr>
<td>Left nostril</td>
<td>Male 10</td>
<td>34.8 ± 12.7</td>
<td>21</td>
<td>60</td>
<td>1</td>
<td>24.9 ± 6.4</td>
<td>2</td>
<td>44.0 ± 17.0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Female 10</td>
<td>31.2 ± 9.5</td>
<td>20</td>
<td>47</td>
<td>1</td>
<td>24.1 ± 8.9</td>
<td>9</td>
<td>36.1 ± 105</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>31.6 ± 11.4</td>
<td>17</td>
<td>60</td>
<td>6</td>
<td>24.1 ± 8.9</td>
<td>18</td>
<td>35.8 ± 10.3</td>
<td>23</td>
</tr>
</tbody>
</table>

between the studies, it remains difficult to determine deficit patterns as a function of epileptogenic focus location and gender.

Numerous findings obtained in human beings and animals provide increasing evidence that the amygdala participates in aspects of odour processing, especially in relation to emotion (e.g. Slotnick, 1985; Zald and Pardo, 1997; Royet et al., 2000; Harding et al., 2002). Investigators have also examined the electrophysiological activity of the human amygdala associated with odorant presentation when patients with epilepsy are undergoing an intracranial EEG procedure. The first recordings collected from electrodes acutely implanted in the human amygdala revealed that fast oscillatory activity (or spindles) was associated with odorant stimulation (Narabayashi et al., 1963; Hughes et al., 1972; Hughes and Andy, 1979). Similar spindles were obtained from chronically implanted electrodes, but these were thought to reflect rather an overall response linked to breathing and blood gas content than a uniquely olfactory response (Halgren et al., 1977a, b). We have recently demonstrated that, in addition to spindles, the human amygdala also produces chemosensory evoked potentials (CSEPs) (Hudry et al., 2001). Although studies have interpreted spindles differently, we have found that the evoked potentials represent specific odor-induced responses. These CSEPs recorded from depth electrodes placed directly into the olfactory cerebral structures overcome the limitations of scalp-recorded CSEPs and functional MRI by combining their respective advantages of direct measurement of neural activity, real-time temporal resolution and fine-grain spatial resolution.

The purpose of this study was to assess short-term olfactory recognition memory in TLE patients using a delayed odour matching-to-sample task with unfamiliar odorants, and to examine the related electrophysiological activity recorded from the amygdala. Tests were performed
Material and methods

Subjects

Twenty right (nine males, 11 females) and 18 left (nine males, nine females) TLE patients who had undergone depth recordings before surgical treatment for the relief of intractable seizures participated in the study. The patients were subdivided according to the side of the epileptic focus and gender, resulting in four experimental groups. Their averaged performances were compared with those of four distinct control groups (Table 1). All the patients were right-handed except two who were ambidextrous. However, the preoperative intracarotid sodium amytal procedure used for assessing hemispheric dominance for language (Wada and Rasmussen, 1960) indicated that all the patients had a predominantly left-sided speech representation. None of the patients exhibited any nasal pathology, complained of any disturbance in their sense of smell, or had icat olfactory symptoms. Before the experiment began, patients had to have been seizure-free for at least 24 h. Individuals unable to understand the required task were not included in the study. The control subjects were 40 healthy right-handed volunteers with no history of chemosensory or cerebral disorders. They were matched to patients for gender, stimulated nostril and age.

The intracranial EEG procedure, independent of the present study, included the stereotactic implantation of between six and 15 depth electrodes in various intracerebral sites, most of which were ipsilateral to the suspected epileptogenic zone. This electrophysiological procedure was performed in order to ascertain that the onset of ictal discharges associated with clinical seizures was located in the temporal lobe. It is indeed possible that the pre-surgical data, obtained from the non-invasive video-scalp-EEG procedure or from neuroimaging, remained inconclusive and suggested a possible extra-temporal, fronto-temporal or temporoparieto-occipital epileptogenic zone. In a few cases, the SEEG was performed because of an uncertain lateralization of the epileptogenic zone. The choice of the anatomical targets varied between patients according to other pre-surgical data, which included video-scalp-EEG monitoring, MRI, fluorodesoxyglucose PET and ictal blood flow single photon emission computerized tomography. MRI demonstrated hippocampal atrophy in 17 patients (44.7%), malformation of the cortex in five patients (13.2%), and other types of lesion in six patients (15.8%). The MRI was normal in the remaining 10 patients (26.3%). Of the 38 patients included in the study, 15 had been implanted with a chronic SEEG electrode in the right (six males, nine females), 14 in the left amygdala (six males, eight females), whereas the nine remaining patients did not have an electrode implanted in the amygdala. Of the 29 patients who were implanted in either the right or left amygdala, 11 were excluded from the study because of: (i) epileptic discharges during the test and/or frequent interictal spikes in the amygdala (8 patients); (ii) unclear respiratory signals precluding a reliable CSEP average (two patients); or (iii) defective synchronization between video and SEEG recordings (one patient). Therefore, only the data collected from 12 right amygdalas (three males, nine females) and six left amygdalas (two males, four females) were ultimately retained for the electrophysiological study (Table 1). All patients gave their fully informed consent to participate in the study, which did not involve any additional invasive procedure to that of the intracranial EEG recordings routinely performed in our department.

Odorants

Twenty-four odorants were used in the present study (Table 2). As odorant familiarity appears to be a determinant variable in odour recognition memory (Cain, 1984; Zucco and Tressoldi, 1989; Jehl et al., 1997), these odorants were selected from those found to be unfamiliar in a previous study (Royet et al., 1999). They also presented similar subjective intensity rates and neutral hedonic rates so that they could not be discriminated between based solely on these characteristics. Indeed, mean values for these odorants evaluated with visual rating scales were homogeneous and close to the 5 out of 10 value for intensity, hedonicity and familiarity ratings. The odorants were contained in 20 ml yellow polyethylene bottles with screw lids (Fisher, Erlancourt, France). An odorant solution (10%) was obtained by diluting 0.5 ml of product in 4.5 ml of an odourless solvent (mineral oil). It was then placed in the bottle and absorbed by compressed filaments of polypropylene. Odorant preparations, which were kept in a refrigerator when not in use, were left to reach room temperature before the experiment began. They were changed every 3 months and were never used more than five times.

Electrophysiological methods

The electrode implantation procedure was the same as that described in our previous work (Hudry et al., 2001) and necessitated three-dimensional MRI and angiographic images as described by Talairach and Bancaud (1973). Briefly, the electrodes were implanted while the patient’s head was fixed in a stereotactic frame using a planar grid parallel to the midline vertical plane of the Talairach’s stereotactic atlas (Talairach and Tournoux, 1988). Thus, all the electrode tracks were perpendicular to the midline vertical plane. Each electrode had from 10 to 15 two mm long contacts with a 1.5 mm separation. The anatomical location allowed us to determine which contacts of an electrode were inside a given structure. It consisted of the superimposition of the frontal skull X-rays with the electrodes in place, and the frontal MRI slice corresponding to each set of electrode coordinates. The electrodes were left in place for up to 15 days where this was necessary for ictal recordings. Each contact on the MRI was localized in the Talairach space defined by the midline vertical plane, the anterior commissure –posterior commissure (AC–PC) horizontal plane, and the vertical frontal plane crossing the posterior margin of the anterior commissure.
SEEG activity was recorded continuously from up to 96 contacts with a 128 Hz sampling rate and with an analogue filter band-pass of 0.3–64 Hz (Micromed, Treviso, Italy). The CSEPs were recorded from electrodes stereotactically implanted in either the right or left amygdala, and were referenced to the contact of another depth electrode located extracortically near the skull. A low-pressure air flow sensor, based on a passive device comprising two Wheatstone bridges (Honeywell, Morristown, USA) and adapted for our purpose, simultaneously monitored the phase of the respiratory cycle, while a video synchronized with the recording of SEEG data monitored the procedure (see Hudry et al., 2001).

Experimental procedure

Sixteen pairs of odorants were presented in successive trials, 120 s apart. A trial consisted of a sample odorant (encoding condition) and a target odorant (retrieval condition) (Table 2). In eight trials, the sample odorant matched the target odorant (identical odorants) and in the other eight trials, the sample odorant mismatched the target odorant (different odorants). The trials were presented in a pseudo-random order that was identical for all the patients and control subjects.

To prevent subjects from sensory adaptation, the interval between a sample odorant and a target odorant was 30 s (Lawless et al., 1991; Jehl et al., 1994). Each stimulation was administered monorhinally in the nostril ipsilateral to the epileptogenic focus, while the patients held the contralateral nostril closed with one of their fingers. Half the control subjects, randomly chosen, were arbitrarily stimulated in the right nostril and the other half in the left nostril. The presentation time varied from 3 to 5 s, depending upon the duration of each subjects’ inspiration phase. Before testing, patients were instructed in how to smell the odorants in order to minimize the intra- and inter-subject breathing pattern variability. They first practised responding to the following instructions: (i) breathe out deeply through the mouth; (ii) block up the non-stimulated nostril with a finger (as a ‘ready’ signal); and (iii) breathe in evenly with the glass bottle containing the odorant under the stimulated nostril (in the training session, the glass bottle did not contain any odorants). This was to ensure that all the patients presented a steady respiratory rhythm during the stimulations so that the experimenter could synchronize stimulus presentation with breathing. Both the patients and control subjects, tested in ventilated rooms, were asked to remain attentive but relaxed during testing and were required to judge whether both odours of a pair matched or not. The patients were tested between the fifth and seventh day following electrode implantation in order to minimize the effects of the electrode implantation procedure.

Behavioural data analysis

Recognition memory performance was assessed using parameters issuing from the signal detection theory (Banks, 1970; Lockhart and Murdock, 1970). As a function of the experimental condition (match or mismatch) and the subject’s behavioural response (correct or incorrect), four variables were considered (see Jehl et al., 1994). If the two odours of a pair were identical (match condition) and declared so by a subject, a ‘hit’ was scored. If these two odours were

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>I</th>
<th>H</th>
<th>F</th>
<th>Target</th>
<th>I</th>
<th>H</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethyl acetate</td>
<td>5.97</td>
<td>3.04</td>
<td>5.45</td>
<td>Isopropyl acetate</td>
<td>5.85</td>
<td>3.21</td>
</tr>
<tr>
<td>2</td>
<td>Cherry</td>
<td>3.41</td>
<td>4.16</td>
<td>3.14</td>
<td>Cherry</td>
<td>3.41</td>
<td>4.16</td>
</tr>
<tr>
<td>3</td>
<td>Lilac</td>
<td>4.96</td>
<td>6.18</td>
<td>5.32</td>
<td>Violet</td>
<td>4.59</td>
<td>5.89</td>
</tr>
<tr>
<td>4</td>
<td>1-Octen-3-ol</td>
<td>6.41</td>
<td>2.97</td>
<td>4.39</td>
<td>Butan-1-ol</td>
<td>5.44</td>
<td>2.35</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl benzoylecetate</td>
<td>3.96</td>
<td>4.37</td>
<td>3.52</td>
<td>Ethyl benzoylecetate</td>
<td>3.96</td>
<td>4.37</td>
</tr>
<tr>
<td>6</td>
<td>Hexanal</td>
<td>6.23</td>
<td>2.86</td>
<td>3.56</td>
<td>Caprylic aldehyde</td>
<td>5.69</td>
<td>3.10</td>
</tr>
<tr>
<td>7</td>
<td>Ginger</td>
<td>4.37</td>
<td>4.44</td>
<td>4.32</td>
<td>Ginger</td>
<td>4.37</td>
<td>4.44</td>
</tr>
<tr>
<td>8</td>
<td>Acacia</td>
<td>4.82</td>
<td>5.65</td>
<td>5.17</td>
<td>Acacia</td>
<td>4.82</td>
<td>5.65</td>
</tr>
<tr>
<td>9</td>
<td>Plum</td>
<td>3.92</td>
<td>5.49</td>
<td>4.45</td>
<td>Muscat</td>
<td>4.27</td>
<td>5.06</td>
</tr>
<tr>
<td>10</td>
<td>Camphor</td>
<td>5.66</td>
<td>4.04</td>
<td>5.10</td>
<td>Camphor</td>
<td>5.66</td>
<td>4.04</td>
</tr>
<tr>
<td>11</td>
<td>Lime tea</td>
<td>5.86</td>
<td>6.24</td>
<td>5.83</td>
<td>Lime tea</td>
<td>5.86</td>
<td>6.24</td>
</tr>
<tr>
<td>12</td>
<td>Carrot</td>
<td>5.93</td>
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<td>4.00</td>
<td>Carrot</td>
<td>5.93</td>
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<tr>
<td>13</td>
<td>Orange</td>
<td>4.62</td>
<td>5.20</td>
<td>5.06</td>
<td>Tangerine</td>
<td>4.32</td>
<td>5.10</td>
</tr>
<tr>
<td>14</td>
<td>Ethyl nitrite</td>
<td>5.78</td>
<td>4.55</td>
<td>5.09</td>
<td>Acetophenone</td>
<td>6.63</td>
<td>4.03</td>
</tr>
<tr>
<td>15</td>
<td>Tobacco</td>
<td>4.25</td>
<td>4.51</td>
<td>4.09</td>
<td>Tobacco</td>
<td>4.25</td>
<td>4.51</td>
</tr>
<tr>
<td>16</td>
<td>Sage</td>
<td>5.61</td>
<td>4.69</td>
<td>5.16</td>
<td>Tarragon</td>
<td>5.20</td>
<td>4.44</td>
</tr>
</tbody>
</table>

Mean values | 5.11 | 4.51 | 4.60 | Mean values | 5.02 | 4.39 | 4.52 |

SD | 0.94 | 1.06 | 0.78 | SD | 0.89 | 1.04 | 0.86 |

Ratings given for intensity (I), hedonicity (H) and familiarity (F) were obtained from the experiment performed by Royet et al. (1999). Intensity, hedonicity and familiarity ratings were obtained from rating scales numbered from 1 to 10, with the number 10 indicating ‘highly familiar, intense or pleasant’ odorants, and number 1 ‘not at all familiar, intense and pleasant’ odorants.
incorrectly judged ‘different’, a ‘miss’ was scored. For pairs of unidentical odours (mismatch condition), a ‘different’ response was scored as a ‘correct rejection’ whereas an ‘identical’ response was recorded as a ‘false alarm’. From hit and false-alarm scores, four parameters were then calculated: hit rate (HR); false-alarm rate (FR); discrimination measurement $d’_L$; and bias response (C_L).

Corwin (1989) previously described these calculations as follows:

$$HR = (hits + 0.5)/N_1 + 1$$
$$FR = (false alarms + 0.5)/N_2 + 1$$
$$d’_L = \ln \left[HR(1- FR)/FR(1- HR)\right]$$
$$C_L = 0.5 \ln \left[(1- FR)(1- HR)\right]/(HR \times FR)$$

where $N_1$ and $N_2$ represent the number of match trials and no match trials, respectively.

To analyse recognition scores, general methods of three-way ANOVAs (analyses of variance) were performed (Winer, 1962). Differences between groups (control versus patient), genders (male versus female) and stimulated nostril sides (right versus left) were assessed by multiple orthogonal comparisons. In TLE patients, the stimulated nostril side also represents the epileptogenic focus side. Sample normalities and homogeneities of their variance were controlled with the Lilliefors test (Conover, 1971) and the Hartley test (Winer, 1962), respectively.

Electrophysiological data acquisition and analysis

CSEPs were recorded during the study and separated off-line for sample odorants of matching- and mismatching-pairs, and target odorants of matching- and mismatching-pairs. Electrophysiological analyses were performed with the Neuroscan software made by Neurosoft (Sterling, Virginia, USA). To average odorant-evoked responses, time onset of the stimulation was assigned to that of the respiratory signal, using the synchronized video-recording as an additional control (Hudry et al., 2001). Briefly, the breathing signal waveform contained a flat line corresponding to expiration through the mouth and to the block up nostril time, and a deflection corresponding to the inspiratory phase. The stimulus onset was determined by the end of the flat line, that is at the beginning of the first deflection. The referential averaged recordings revealed CSEPs composed of two main components that were consistent between subjects: a first positive peak (P1) followed by a negative peak (N2). Peak amplitudes and latencies were measured on the averaged recordings from the electrode contact where the response amplitudes were the largest. Averaged data were analysed according to the method described by Spelmann (1985).

Briefly, peaks were detected in the order of their appearance taking into account a minima/maxima in a latency-window (P1: 200–350 ms; N2: 350–600 ms). When peaks were biphasic or triphasic, the mean latency and the maximal amplitude of the ‘subpeaks’ were retained. The measured amplitude and latency peaks were then submitted to two-way ANOVAs with repeated measurements (Winer, 1962) as a function of the match–mismatch factor (different versus identical), and the sample–target factor (first versus second odorant). No correction for sphericity with the Greenhouse–Geisser test was applied, because only two conditions per factor were considered (Winer, 1962; Crowder and Hand, 1990). We also looked for polarity reversal of the CSEPs within the amygdala, using bipolar montages.

Results

Behavioural data

The arithmetic means of the scores obtained for hit rate, false alarm rate, discrimination measurement and bias response were computed as a function of subject group (control versus patient), gender (male versus female) and the stimulated nostril side (right versus left). Table 3 depicts the results of the three-way ANOVA obtained for hit rate, false alarm rate, discrimination and bias measurements. A significant effect of subject group for the four parameters was observed (Fig. 1A). Hit rates and discrimination measurements were lower, and false alarm rates and bias measurements higher in patients than in controls.

A significant effect of gender factor was also observed (Fig. 1B): discrimination measurements were lower and false
alarm rates higher in male than in female, irrespective of the
group of subjects (patients or controls) and the stimulated
nostril side (right or left). For false alarm rates, a significant
interaction between group and gender factors (AB inter-

Fig. 1 Mean scores obtained for hit rate, false alarm rate, discrimination measure and response bias revealing significant differences
\((P < 0.05 \text{ at least})\) as a function of \((A)\) group factor (TLE patient versus control subjects), \((B)\) gender factor (male versus female) and
\((C)\) the stimulated nostril factor (right versus left), and for \((D)\) group \(\times\) gender interaction and \((E)\) group \(\times\) nostril interaction. Vertical
bars, SEM.
action, Fig. 1D) could be explained by the false alarm rates being higher in male than in female patients \( F(1,70) = 13.97, P = 0.0004 \), and significantly higher in male and female patients than in their respective control groups \( F(1,70) = 46.03, P < 0.0001, \) and \( F(1,70) = 14.95, P = 0.0002 \), regardless of the stimulated nostril side, that is the epileptogenic focus side.

A significant effect of nostril factor was also noted (Fig. 1C): discrimination measurements were lower and false alarm rates were higher in the left than right nostril, irrespective of the group of subjects (patients or controls) and gender. Although the ANOVA did not reveal a significant effect of nostril factor for hit rates, a significant interaction between group and nostril side factors (AC interaction, Fig. 1E) could be explained by altered performances in left TLE patients, who scored significantly less hits than right TLE patients \( F(1,70) = 4.58, P = 0.0359 \). No significant difference was observed between controls stimulated either in the left or in the right nostril \( F(1,70) = 0.89, \) not significant.

**Electrophysiological data**

**Recordings from the amygdala**

The 32 olfactory stimulations performed during the test consistently induced CSEPs in the right and left amygdalas consisting of the succession of one positive and one negative peaks (P1, N2), as observed in the referential recordings (Fig. 2A). These components occurred with a respective mean latency of 279.8 ± 53.1 and 463.9 ± 83.9 ms, and had a respective mean amplitude of 101.3 ± 51.3 and –69.8 ± 37.8 μV. As described in our previous work (Hudry et al., 2001), the electrophysiological responses were exclusively collected from the contacts implanted within the amygdala (generally the four deepest ones) and not from the other electrode contacts. The CSEPs were large and observed in both the raw and averaged recording data, and their various components exhibited similar latencies on all the amygdala electrode contacts. In 14 patients, the referential recordings revealed that peak amplitudes progressively decreased from the deepest (A1) to the most external (A4) contact of the electrode. In the four other patients, the largest peak amplitude was observed on the second deepest contact (A2), and a polarity reversal of the positive and negative peaks was observed in bipolar recordings between the second and the third deepest contacts (A2–A3) of the electrode (Fig. 2B), suggesting that these contacts were the closest to the source of the CSEP. However, this finding does not give a clear indication of the orientation of a dipolar source in relation to the electrode track.

**Peak amplitudes and latencies of the CSEPs recorded in the amygdala**

Figure 3 illustrates typical examples of mean CSEPs obtained in one patient as a function of the match–mismatch factor (identical versus different) and the sample–target factor. It mainly shows that the CSEP amplitude was reduced in the match condition, and even more so in the mismatch condition, from the first to the second presentation of odors. The arithmetic means for amplitudes and latencies were analysed using a three-way ANOVA as a function of the hemispheric side, the match–mismatch and the sample–target factors. Analyses did not reveal any significant effect of the hemispheric side factor whatever the measurement considered (amplitude or latency of peaks P1 and N2). We therefore subsequently pooled the data obtained from the 12

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**Fig. 2** Referential and bipolar averaged CSEPs (filtered 0.3–64 Hz) obtained from the amygdala in response to the 16 sample odorant stimulations. (A) Enlargement of a response collected from the deepest electrode contact (A1) of Patient P68, and comprising the large positive peak (P1) and the negative one (N2). (B) Bipolar recordings obtained by digital subtraction of the referential data of Patient P49. Stimulus onset corresponds to the value 0 ms.
patients recorded in the right amygdala and those obtained from the six patients recorded in the left amygdala and performed two-way ANOVAs for amplitudes and latencies. Only that CSEP data associated with correct behavioural responses (hits or correct rejections) were analysed. Electrophysiological data related to incorrect behavioural responses (false alarms or misses) were not analysed separately because they only concerned 40% and 25% of the trials, respectively. Mean component amplitudes and latencies, in both cases associated only with correct behavioural responses, are represented in Fig. 4.

We found a significant effect of the sample-target factor for P1 and N2 peaks amplitudes computed from all the behavioural responses (Table 4). Orthogonal comparisons between pairs of means showed that P1 and N2 amplitudes were significantly higher for sample than for target odours in the match \(F(1,17) = 21.07, P < 0.0005\) and \(F(1,17) = 5.30, P < 0.05\), respectively] and mismatch \(F(1,17) = 6.10, P < 0.025\) and \(F(1,17) = 11.14, P < 0.005\), respectively] conditions. For amplitudes computed from the correct responses only (Fig. 4), the ANOVA also showed a significant effect of the sample-target factor for P1 and N2 peaks, but additionally showed a significant interaction between match–mismatch and sample–target factors for P1 peaks (Table 4). Orthogonal comparisons of means showed that P1 amplitudes were higher for sample than for target odours in the match condition \(F(1,16) = 11.71, P < 0.005\], and higher for target odours in the mismatch condition than in the match condition \(F(1,16) = 8.54, P < 0.01\]. The significant effect of the sample–target factor found for N2 peaks was due to amplitudes significantly higher for sample than for target odours in the match \(F(1,16) = 7.86, P < 0.025\) and in the mismatch \(F(1,16) = 4.43, P < 0.05\) conditions.

Fig. 3 Mean referential CSEPs (filtered 0.3–64 Hz) obtained from the right amygdala of Patient P76 for sample or target odorants in matching or mismatching conditions.

Fig. 4 Mean amplitudes of P1 (A) and N2 (B), and (C) mean latencies of P1 computed for 18 TLE patients and depicted for correct behavioural responses as a function of the match–mismatch factor (different versus identical), and the sample–target conditions (first versus second odorants). Vertical bars, SEM; *\(P < 0.05\).
We found a significant effect of the sample-target factor for P1 latency computed from either the totality of the behavioural responses or only the correct behavioural responses (Fig. 4C, Table 4). In both cases, multiple comparisons of means showed that P1 latencies were significantly shorter for target than sample odours in the match [all responses: $F(1,34) = 8.09, P < 0.025$; correct responses: $F(1,16) = 6.13, P < 0.025$] and in the mismatch [all responses: $F(1,17) = 5.81, P < 0.05$; correct responses: $F(1,16) = 5.02, P < 0.05$] conditions.

**Discussion**

The present study highlights the influence of TLE on the short-term memory recognition ability of unfamiliar odorants and assesses the related electrophysiological activity in the amygdala. First, the behavioural results show that TLE patients performed poorly in the delayed olfactory match-to-sample task with performances significantly impaired for hit, false alarm, discrimination and bias measurements when compared with normal controls. Specific effects of the gender factor were also observed with poorer performances in male than in female patients for false alarm rates, and of the nostril factor with lower scores in left than right TLE patients for hit rates. Secondly, the SEEG data mainly shows a significant effect of sample-target factor on the CSEPs recorded from the human amygdala, with P1 and N2 amplitudes and P1 latencies weaker for target than for sample odours. Electrophysiological results reveal that, when the behavioural response to odour memory recognition was correct, the P1 amplitude is modulated by perceptual match and mismatch effects.

**Influence of TLE on short-term memory recognition**

**Influence of hemispheric side**

Compared with our control subjects, the male and female TLE patients scored less hits and more false alarms (Fig. 1). This indicates that patients had difficulty in recognising two identical odours as being identical and often recognized two different odorants as being identical. In addition, results revealed various deficit patterns as a function of the stimulated nostril side (i.e. the epileptic focus location). Indeed, only the hit scores in left, but not right TLE patients were significantly lower than those of their respective control subjects. For false alarms, the scores were affected in both groups of patients, but a significant effect of the stimulated nostril side factor indicated that they were higher in left than in right TLE patients.

The primary measure (hits and false alarms) of performance on yes/no tasks reflects the ability of subjects to distinguish between the two criteria, that is discrimination and bias measurements. Discrimination scores in the present study were lower in patients than in control groups. This result highlights the difficulty for patients to discriminate between odorants. As olfactory discrimination ability includes the ability to store and remember the odorants to be compared, we can suppose that, in the interictal state, TLE alters the olfactory encoding and/or the olfactory short-term memory processes. As for hit and false alarm scores, various deficit patterns were found as a function of the stimulated nostril side, located on the epileptogenic focus side, with lower discrimination scores in left than in right TLE patients. Bias measurements, which assess the decision rule used when subjects are uncertain as to whether or not the odour pair matches, reveal that control subjects applied a criterion of neutral decision, whereas patients presented a criterion of more liberal decision. The neutral criterion used by the controls indicates that the task was not difficult enough to promote a bias in cognitive processing. However, the patients used a criterion of liberal decision demonstrating that, when uncertain, they tended to say ‘yes’ for mismatch odorants. No difference as a function of the TLE hemispheric side was, however, noted in patients for bias measurements.

All but one of the previous studies (Eskenazi et al., 1986) have reported recognition memory deficits in non-operated

<table>
<thead>
<tr>
<th>Behavioural responses</th>
<th>Wave</th>
<th>Match–mismatch factor</th>
<th>Sample–target factor</th>
<th>Interaction</th>
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<tbody>
<tr>
<td></td>
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<td>Amplitude</td>
<td></td>
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</tr>
<tr>
<td>All</td>
<td>P1</td>
<td>0.190</td>
<td>1.17 n.s.</td>
<td>24.54</td>
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<tr>
<td></td>
<td>N2</td>
<td>1.128</td>
<td>1.17 n.s.</td>
<td>21.139</td>
</tr>
<tr>
<td>Correct</td>
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<td>1.16 n.s.</td>
<td>5.50</td>
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<tr>
<td></td>
<td>N2</td>
<td>0.912</td>
<td>1.16 n.s.</td>
<td>19.478</td>
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<tr>
<td>Latency</td>
<td></td>
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<tr>
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<td>P1</td>
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<td>1.17 n.s.</td>
<td>14.693</td>
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<tr>
<td></td>
<td>N2</td>
<td>1.560</td>
<td>1.17 n.s.</td>
<td>3.694</td>
</tr>
<tr>
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<td>0.291</td>
<td>1.16 n.s.</td>
<td>13.947</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>2.702</td>
<td>1.16 n.s.</td>
<td>0.873</td>
</tr>
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</table>

n.s. = not significant
epileptic patients (Abraham and Mathaï, 1983; Carroll et al., 1993; Martinez et al., 1993; Savic et al., 1997). However, the authors of these studies did not find any clear difference between right and left TLE patients, or demonstrated an impairment only in right TLE patients. Data obtained from epileptic patients who had undergone a surgical excision of the epileptogenic focus also suggested that there might be some degree of specialization of the right temporal lobe for odour processing (Rausch and Serafetinides, 1975; Henkin et al., 1977; Rausch et al., 1977; Abraham and Mathaï, 1983; Eichenbaum et al., 1983; Eskenazi et al., 1983; Jones-Gotman and Zatorre, 1988, 1993; Zatorre and Jones-Gotman, 1991; Jones-Gotman et al., 1997). Conversely, we observed that left TLE patients were more impaired (hit scores) or tended to be more altered (false alarm and discrimination scores) than those suffering from a right-sided TLE. Zatorre and Jones-Gotman (1991) also observed decreased performance in odour discrimination which was apparently higher in patients with excision of the left temporal lobe stimulated via the left nostril than in patients with excision of the right temporal lobe stimulated via the right nostril, but this difference was not statistically tested.

The greater deficits observed in left than in right TLE patients can be related to differences in psycho-social traits between these two groups, such as differences in ‘self-ratings and the ratings of patients’ behaviour by observers’ (Bear and Fedio, 1977). These authors suggested that the right TLE patients tended to exaggerate their desired qualities and to deny undesirable ones, while left TLE patients tended to exaggerate their weaknesses and to minimize their strengths. In terms of response bias, which reflects the decision rule applied in situations of uncertainty, the left TLE patients would thus be less self-confident and would adopt a more liberal decision criterion. These considerations were used by Carroll et al. (1993) to explain why the left TLE patients were less likely to judge non-nameable odours to be familiar than the other groups of subjects. These findings can also be related to data from studies showing a relation between epileptogenic lesions in the left hemisphere and the affective content of verbal memory (Burton et al., 1999), the adoption of a more liberal decision criterion in recognition memory for verbal and visuo-spatial materials (Glosser et al., 1998) and depression (Septien et al., 1993a, b; Mendez et al., 1994).

They can finally be related to neuroimaging studies that indicate a strong involvement of left cerebral structures (amygdala, temporal pole, subcallosal, orbitofrontal and superior frontal gyri) in emotional processing (Royet et al., 2000).

**Influence of gender**

Results of the present study show higher false alarm and lower discrimination scores in male than in female TLE patients regardless of the epileptogenic hemisphere. The lower olfactory performances found in males than in females have been commonly observed in detection and identification tasks in healthy subjects (Toulouse and Vasiche, 1899c; Le Magnen, 1952; Cain, 1982; Doty et al., 1985; Murphy, 1985), but our study is the first one to report gender differences in olfaction in epileptic patients. Sex differences in spatial distribution of brain dysfunction (Savic and Engel, 1998) and depression occurrence (Septien et al., 1993a, b) have nevertheless been reported in epilepsy. Moreover, since depression has also been found to be more frequent when the epileptic focus is localized in the left hemisphere, a probable cumulative effect of hemispheric side and gender could explain the higher deficits found in our male patients with left TLE than in other groups. However, no measurements was taken in the present study to show that male patients were more depressed than female patients, and other investigations are needed to clarify this point.

**Recordings from the amygdala**

Previous SEEG recordings performed in patients with epilepsy allowed us to demonstrate that the amygdala was consistently responsive to odorant stimulations since it was found to produce large and reliable CSEPs (Hudry et al., 2001). These CSEPs were then found to be odor-related since they were obtained only in response to the odorant stimulations and not to the non-odorant stimulations consisting of mineral oil. The intracranial potentials obtained in the present study were also characterized by two main components: P1 peaking at 280 ms and N2 at 470 ms (Fig. 2A). Although no apparent effect of encoding and retrieval processes on the potential waveforms of sample and target odorants was evidenced, stimulus repetition (matching effect) and stimulus novelty (mismatching effect) were found to modulate peak latencies and amplitudes. Thus, our data suggest that the amygdala contributes to providing specific information to the cortex during the retrieval of recent memories.

**Repetition and novelty effects on CSEP amplitudes**

Olfactory sensory self- and cross-adaptations are temporary phenomena supposed to explain the reduction in sensitivity to an odour caused by the previous presentation of the same or another odour within the same modality (Köster and Wijk, 1991). When measured by electrophysiological scalp recordings, adaptation effects commonly reveal a progressive amplitude decrease of the evoked potential in response to olfactory stimuli presented successively (Kobal and Hummel, 1991; Pause and Krauel, 2000). Thus, adaptation could explain the amplitude reductions observed for the target odors for P1 and N2 (Fig. 4). However, since no amplitude reduction was observed for P1 in the mismatch condition when considering only the CSEPs associated with correct responses (Fig. 4, right), cross-adaptation phenomenon is unlikely to be the sole explanation of our results.
Electrophysiological studies of neural activity in behaving monkeys are in a position to provide a satisfactory explanation of our data. These studies have demonstrated that the act of holding a visual sample-stimulus in the memory decreased the responsiveness of a subpopulation of neurons to a re-occurrence of that stimulus (Miller et al., 1993a, b; Desimone, 1996). This effect, described as repetition suppression, appears to play a consequential role in discrimination and recognition processes by directing the subject’s (or the animal’s) behaviour and by triggering his decision to respond to the matching-stimuli. Thus, the repetition suppression hypothesis could explain amplitude reductions observed in the present study for matching-odorants for all responses, but also for correct responses (Fig. 4). In addition, Desimone (1996) noted that, when using pairs of stimuli with various degrees of similarity in a match-to-sample task, the more similar the target stimulus was to the sample, the more the neuronal response to the target was suppressed. Since we found a high false alarm rate in the mismatch condition, i.e. patients erroneously judging different odorants as being similar, the electrophysiological responses to the target odorants in the mismatch condition were consequently also affected and presented an abnormally reduced amplitude, as in a match condition. This phenomenon was no longer visible when only the correct responses were taken into account (Fig. 4A). The anterior medial temporal lobes (i.e. anterior to the hippocampus proper) have recently been found to be involved in a word continuous recognition memory task (Grunwald et al., 1998). In TLE epileptic patients without hippocampal sclerosis, amplitude reductions were observed for old (i.e. already presented) as well as new stimuli, suggesting that extrahippocampal epileptogenic lesions could interfere with the detection of both novelty and repetition. Since we found that the CSEP amplitude was more reduced in the mismatch than in the match condition, we are suggesting that the amygdala could also play a role in discrimination and recognition processes by participating in stimulus novelty detection and that this process is functionally altered by TLE.

**Attention and facilitation effects on CSEP latencies**

The present study shows that the amygdala neural activity is strongly and dynamically influenced by recent experience with an odour by producing CSEPs with shorter latencies for the target than for the sample odorants. This effect was observed for P1 in both match and mismatch conditions (Fig. 4C). Latency reductions are thus likely to be concerned with the earlier CSEP components rather than the later ones. We previously demonstrated that recent experience with an odour could improve the amygdala processing speed during stimulus repetition (Hudry et al., 2001). This improved perception was related to selective attention defined as an essential cognitive function enabling one to preferentially select and process high priority information (Egeth, 1967). Generally, the attention effect is assumed to modify sensory inputs at an early stage of processing, expressing a sensory gating of the neural generators which increase their level of activation for expected stimuli, and decrease it for unexpected ones (Näätänen and Michie, 1979; Hillyard and Mangun, 1987; Krauel et al., 1998). Considering this, we would expect specific P1 latency reductions in the match condition, but not in the mismatch one. However, olfactory processing enhancements are observed in both conditions (Fig. 4C). It thus appears that, in addition to this process of time-dependent facilitation generally observed for superficial processing such as simple detection tasks, another process of neural transmission enhancement might also be present for more complex cognitive tasks, such as olfactory recognition memory. Both expected and unexpected stimuli would then benefit from this facilitation effect.

**Conclusions**

Our behavioural study shows that TLE patients present impairments in olfactory recognition memory for several measurements (hits, false alarms, discrimination and bias). It further indicates more specific effects, with olfactory impairments higher in male than female patients for false alarms, and higher in left TLE patients stimulated in the left nostril than right TLE patients stimulated in the right nostril for hit rates. The SEEG evaluation also revealed that the human amygdala contributes to olfactory discrimination and recognition, and that the presence of an epileptogenic focus neighbouring on the amygdala functionally alters these processes by preventing TLE patients from correctly discriminating and/or recognising odorants.

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