Properties of *in vivo* interictal spike generation in the human subiculum

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A large proportion of hippocampal afferents and efferents are relayed through the subiculum. It is also thought to be a key structure in the generation and maintenance of epileptic activity; rhythmic interictal-like discharges were recorded in previous studies of subicular slices excised from temporal lobe epilepsy patients. In order to investigate if and how the subiculum is involved in the generation of epileptic discharges *in vivo*, subicular and lateral temporal lobe electrical activity were recorded under anesthesia in 11 drug-resistant epilepsy patients undergoing temporal lobectomy. Based on laminar field potential gradient, current source density, multiple unit activity (MUA) and spectral analyses, two types of interictal spikes were distinguished in the subiculum. The more frequently occurring spike started with an initial excitatory current (current source density sink) in the pyramidal cell layer associated with increased MUA in the same location, followed by later inhibitory currents (current source density source) and decreased MUA. In the other spike type, the initial excitation was confined to the apical dendritic region and it was associated with a less-prominent increase in MUA. Interictal spikes were highly synchronized at spatially distinct locations of the subiculum. Laminar data showed that the peak of the initial excitation occurred within 0–4 ms at subicular sites separated by 6 mm at the anterior–posterior axis. In addition, initial spike peak amplitudes were highly correlated in most recordings. A subset of subicular and temporal lobe spikes were also highly synchronous, in one case the subicular spikes reliably preceded the temporal lobe discharges. Our results indicate that multiple spike generator mechanisms exist in the human epileptic subiculum suggesting a complex network interplay between medial and lateral temporal structures during interictal epileptic activity. The observed widespread intra-subicular synchrony may reflect both of its intrinsic and extrinsically triggered activity supporting the hypothesis that subiculum may also play an active role in the distribution of epileptiform activity to other brain regions. Limited data suggest that subiculum might even play a pacemaker role in the generation of paroxysmal discharges.

**Keywords:** epilepsy; hippocampus; current source density; multiple unit activity; laminar recording

**Abbreviations:** CSD = current source density; EC = entorhinal cortex; FPG = field potential gradient; MUA = multiple unit activity


**Introduction**

Recently accumulating knowledge about the function and structure of the subiculum suggest its essential role in a number of normal and pathological processes. Besides its task in the formation and retrieval of short-term memory (Gabrieli *et al*., 1997; Hampson and Deadwyler, 2003) and
in the creation of cognitive maps (Sharp and Green, 1994), the subiculum is involved in Alzheimer’s disease (Davies et al., 1988), schizophrenia (Roberts and Greene, 2003) and temporal lobe epilepsy (Cohen et al., 2002; Arellano et al., 2004; Wozny et al., 2003; Huberfeld et al., 2007).

Animal studies show that the subiculum controls the input and output of the hippocampal formation by virtue of its position between the CA1 region and entorhinal cortex (EC) (Van Hoesen et al., 1979; Witter and Groenewegen, 1990; Witter et al., 1990; Nabers and Witter, 1998; Nabers et al., 2001; Menendez de la Prida, 2006). The local connections of subicular pyramidal cells form a characteristic pattern, hypothesized to facilitate the appearance of internal recurrent network activity (Harris and Stewart, 2001; Harris et al., 2001; Witter, 2006) that is under the control of local GABA-ergic inhibition (Seress et al., 1993; Arellano et al., 2004; Menendez de la Prida, 2006). The organization of both its extrinsic and intrinsic connections promotes multiple reentrant pathway formation, which might lead to synchronized reverberating circuits and under pathological conditions to epileptogenic plasticity (Kloosterman et al., 2004; Knopp et al., 2005).

Anatomical examination of human tissue derived from temporal lobe epilepsy patients has revealed varying levels of hippocampal size reduction and signs of tissue damage termed as hippocampal (or Ammon’s horn) sclerosis (Corsellis, 1957). This damage features pathological alteration and reorganization of both excitatory and inhibitory circuits of the Ammon’s horn (Cornu Ammonis) and the dentate gyrus manifesting in cell loss, axonal sprouting and gliosis (Sutula et al., 1989; Babb, 1999; Wittner et al., 2002, 2005; Magloczky and Freund, 2005). In contrast with the damaged Cornu Ammonis and dentate gyrus, the subiculum is relatively well preserved (Fisher et al., 1998; Cavazos et al., 2004). In vitro slice studies on excised human hippocampal tissue showed that spontaneous rhythmic synchronized network activity similar to interictal discharges is present in the subiculum, sometimes even in the absence of hippocampal sclerosis (Cohen et al., 2002; Wozny et al., 2003, 2005; Huberfeld et al., 2007). Recurrence rate and certain morphological analogies between in vitro subicular events and in vivo interictal spikes suggested that they may represent similar epileptic processes (Cohen et al., 2002; Wozny et al., 2003).

Subicular discharges can result from intrinsic (local subicular origin), extrinsic (extra-subicular input) or mixed network mechanisms. In non-epileptic animals, intrinsic generation of spontaneous, rhythmic, spatially synchronized subicular (Wu et al., 2005a, b, 2006) and hippocampal (Papatheodoropoulos and Kostopoulos, 2002; Kubota et al., 2003; Maier et al., 2003; Colgin et al., 2004) in vitro activity resembling interictal spikes share a number of similarities with that of the epileptic human (Cohen et al., 2002; Wozny et al., 2003, 2005; Huberfeld et al., 2007) in vitro studies, all using physiological incubation medium.

Subicular responses to hippocampal or cortical electrical stimulation also reproduce some basic features of subicular interictal spikes (Naber et al., 1999; Gigg et al., 2000; Nabers et al., 2001; Cappaert et al., 2007). These spikes show differential laminar and cellular patterns (Behr et al., 1998; Nabers et al., 1999; Gigg et al., 2000; Nabers et al., 2001; Cappaert et al., 2007) corresponding to known anatomical connections (Finch and Babb, 1981; Amaral et al., 1991; Witter et al., 2000; O’Mara, 2006; Witter, 2006).

While in vitro preparations have yielded valuable data on the epileptiform activity of the subiculum, there is a lack of microphysiological information concerning its in vivo behaviour in epileptic humans. In the present paper we investigate the electrophysiological events associated with interictal activity of the anatomically identified human subiculum and temporal lobe in vivo under general anaesthesia using laminar multielectrodes in patients with drug-resistant temporal lobe epilepsy. High spatial resolution laminar field potential gradient (FPG), multiple unit activity (MUA), current source density (CSD) from the subiculum and concurrently recorded temporal lobe electrocorticogram (ECOg) were analysed to elucidate the neuronal network mechanisms underlying interictal activity. The results suggest that multiple forms of interictal spike activity are generated in the subiculum, some of which may be projected to lateral temporal areas.

**Methods**

**Surgery and recording**

Eleven temporal lobe epilepsy patients undergoing standard anterior temporal lobectomy were included in this study. Intraoperative recordings were made under general anaesthesia (Propofol and N2O or Isofluran and N2O). All of the patients underwent MR imaging and video-EEG monitoring before the operation for localizing their seizure onset zone and diagnosed as having unilateral mesial temporal lobe seizure onset. Informed consent was obtained from the patients under the auspices of the local ethical committee according to the Declaration of Helsinki. For intrahippocampal recordings, one or two laminar multielectrodes were used. Each 10 cm long, 350 μm shaft diameter multielectrode had twenty-four 25 μm diameter Pt/Ir recording contacts separated by 100 μm (two cases) or 200 μm (nine cases). The multielectrode was mounted on a hydraulic micromanipulator together with the attached high impedance preamplifiers. In the case of dual laminar recordings, the electrodes were mounted in parallel, about 6 mm apart. All the equipment going into the surgical field was sterilized in ethylene-oxide. The manipulator with the mounted multielectrodes and preamplifiers was attached to a medical instrument holder, which allowed the surgeon to aim towards the hippocampus under visual control using an operating microscope. In order to conserve medial-lateral temporal pathways, the lateral ventricle was opened from a small incision involving the deep aspect of the superior temporal sulcus to reveal the head and body of the hippocampus. Under visual control, the multielectrode tip was positioned onto the ependymal surface of the hippocampus through the incision. After the initial positioning, the electrodes were advanced into the tissue.
with 2–4 mm increments using the hydraulic manipulator. At each step, the signal was recorded for 3–8 min continuously. One or two penetrations were made reaching the proximal and distal (with respect to CA1) or anterior and posterior part of the subiculum. Spatial FPG, the first spatial derivative of the local field potentials was digitized and stored (24 or 48 channels of data, depending on single or dual multielectrode recording) both in the low-frequency band (pass band: 0.1–300 Hz, each channel sampled at 2 kHz with 16-bit resolution) and in high-frequency band (150–5000 Hz, each channel sampled at 20 kHz with 12-bit resolution) simultaneously for off-line analysis. Details about the multielectrode, amplifier and recording system were published previously (Ulbert et al., 2001, 2004a, b). In addition to laminar multielectrode implantation, in two cases an 8-contact clinical strip electrode (Ad-Tech Medical Instrument Corporation, Racine, USA) was positioned over the temporo-basal cortical areas going around the pole of the temporal lobe. Based on the angle and visual inspection of the strip, it reached the temporo-polar (Brodmann, Br. 38), perirhinal (Br. 35–36) and inferotemporal (Br. 20) areas, but not the entorhinal (Br. 28) area. ECoG was filtered (0.1–1000 Hz), digitized at 5 kHz with 16-bit resolution and stored for off-line analysis (Brainvision Recorder, Brain Products GmbH., Gilching, Germany). Laminar and strip recordings were co-registered using a common trigger channel.

**Histology**

At the end of the session, the multielectrodes were pulled out, and the hippocampus was resected en bloc. The neurosurgeon and the histologist confirmed and registered the region and angle of the electrode insertion based on the surface vascularization of the hippocampus. Digital photographs were taken of the removed tissue, and then it was cut into smaller, 4–5 mm thick blocks in the operating room, parallel with the suspected electrode trajectory. Additional photographs were taken of the blocks, they were measured to allow subsequent estimation of shrinkage, and then immersed separately into fixative containing 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB, pH 7.4), as described earlier (Magloczky et al., 1997). The fixative was changed every hour to a fresh solution during constant agitation for 6 h, and then the blocks were post-fixed in the same fixative overnight. Vibratome sections (60 μm thick) were cut from the blocks, and photographs were taken from the electrode tracks (Fig. 1B). Following washing in PB, sections were immersed in 30% saccharose for 1–2 days then frozen three times over liquid nitrogen. Sections containing the electrode track were either stained with cresyl violet, or processed for immunostaining against glutamate receptor subunit 2 and 3 (GluR2/3), as follows. Sections were transferred to Tris-buffered saline (TBS, pH 7.4), then endogenous peroxidase was blocked by 1% H2O2 in TBS for 10 min. TBS was used for all the washes (3 x 3–10 min between each step) and dilution of the antisera. Non-specific immunostaining was blocked by 5% milk powder and 2% bovine serum albumin. A polyclonal rabbit antibody against GluR2/3 (1:100, Chemicon, Temecula) was used for 2 days at 4°C. The specificity of the antibody has been thoroughly tested by the manufacturer. For visualization of immunopositive elements, biotinylated anti-rabbit immunoglobulin G (1:300, Vector) was applied as secondary antiserum followed by avidin-biotinylated horseradish peroxidase complex (ABC; 1:300, Vector). The immunoperoxidase reaction was developed by 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma) dissolved in Tris buffer (TB, pH 7.6) as a chromogen. Sections were then osmicated (1% OsO4 in PB, 40 min) and dehydrated in ethanol (1% uranyl acetate was added at the 70% ethanol stage for 40 min) and mounted in Durcupan (ACM, Fluka).

The location of the electrode tracks were defined based on light microscopic examination. Only cases were included in this study, where the electrode passed through the subicular complex (including the subiculum, pre- and prosubiculum). In the cases without hippocampal sclerosis, the border between the CA1 region and the subiculum was determined by the increased extension of the pyramidal cell layer, with two sublaminae (Amaral and Insausti, 1990). In the sclerotic cases, the CA1-subiculum border...
was determined by the absence or presence of pyramidal cells, respectively. The entorhinal cortex was distinguished based on the presence of six cortical layers (including lamina dissecans) and islands of modified pyramidal cells in layer II (Amaral and Insausti, 1990). More details about the anatomy-electrophysiology co-registration were published earlier (Ulbert et al., 2004b).

### Data analysis

The second spatial derivative of the field potentials approximates the depth distribution of the extracellular sources and sinks of currents in laminated structures expressed in current source density units. Since we recorded the spatial gradient of the field potential, the CSD calculation involved only one spatial derivation of the FPG. Inhomogeneous conductivity and electrode distance were not taken into account. Hamming-window spatial smoothing, additional 0.1–100 Hz band pass filtering (zero phase shift, 12 dB/oct) and baseline correction (−500 to −100 ms) were applied if needed on the low-frequency band data. As shown on Fig. 1B and C, the directions of the penetrations were usually equal to or less than 30° compared to the perpendicular axis. Assuming a penetration angle of 30°, a pyramidal layer thickness of 2 mm, and an intra-subicular conduction velocity of 1.5 m/s (the lowest estimated value, see results), then linear estimation yields an expected timing error compared to the perpendicular insertion of about 0.75 ms between two contacts separated by 2 mm, and 0.075 ms between adjacent contacts. This would result in the steepest part of the CSD (initial sink) changing by an amount 5–10% compared to its entire swing during the estimated timing error. Based on the above assumptions we expect an approximately 0.75 ms timing error and about 5–10% amplitude inaccuracy in our oblique recordings between sites separated by 2 mm and negligible errors at adjacent contacts.

A continuous estimate of the multiple unit activity was derived by additional band pass filtering (zero phase shift, 500–5000 Hz, 48 dB/oct), full wave rectifying and finally low pass filtering (zero phase shift, 100 Hz, 12 dB/oct) of the high-frequency band data. NeuroScan (Compumedics, El Paso, TX, USA) and home written MatLab (MathWorks, Natick, MA, USA) tools were used for data analysis. Details about CSD and MUA calculations were published earlier (Ulbert et al., 2001, 2004a).

Subicular spikes were detected using amplitude criteria based on the polarity of FPG and CSD traces and their relationship to the local anatomy. An event was detected if the amplitude of the FPG exceeded the 2 standard deviation (SD) threshold calculated from a spike-free period. The earliest sharp CSD peak in the pyramidal layer was designated as time zero for further event triggered averaging. FPG is presented on line plots with positive deflections upwards, CSD data is presented on line plots and depth versus time maps, with colour-coded sink (red, positive values) amplitudes. MUA averages are presented on colour-coded depth versus time maps. Warm colours (red) depict MUA increase, cold colours (blue) depict MUA decrease compared to the pre-spike baseline period (−500 to −100 ms). T-test was used for CSD and MUA to reveal significant (P < 0.01) alterations compared to baseline activity and between event comparison (P < 0.01). For ECoG spike detection the 2 SD threshold criterion was used, time zero was defined by the earliest peak after threshold crossing. Expert neurologists classified ECoG events as interictal spikes when they had a short (<50 ms) initial peak width with a following slow wave (Gotman, 1980).

We also assessed the temporal synchrony and amplitude relationship of spikes recorded from two spatially distinct parts of the subiculum (n = 3) either on the same or on different electrodes. Temporal synchrony was expressed in percent; the number of coinciding FPG spikes within a ±10 ms window was divided by the total number of spikes. The correlation of peak FPG amplitudes between two spatially distinct subicular spikes and its significance was calculated using linear regression model.

Spectro-temporal analysis was performed using wavelet-based methods modified from EEGLAB (Delorme and Makeig, 2004). Spectral content of the spikes was calculated from single sweeps (FPG) followed by averaging of the individual time–frequency measures. Dividing the resultant values with the baseline (−500 to −100 ms) activation in each frequency band gives the relative change of spectral activity in time termed as the individual trial event-related spectral perturbation (iERSP) expressed in dB. Statistical significance (bootstrap, P < 0.01) of the iERSP against baseline was assessed using bootstrap analysis (Delorme and Makeig, 2004). Significant spectral activity increase and decrease is marked with warm (red) and cold (blue) colours, respectively, while non-significant values are shaded green. A non-parametric statistical test (Kruskal–Wallis ANOVA) was used to compare iERSP of different spike types with a P < 0.05 significance level.

### Results

We recorded subicular laminar electrical activity from 11 patients in 13 multielectrode penetrations under general anaesthesia. Hippocampus was removed en bloc after the implantation, containing the electrode track. In seven cases we found direct histological evidence that the electrodes reached the target and recorded subicular activity. In four cases the subiculum was damaged and lost during the removal. In the remaining four cases, reconstructions by expert morphologist based on the remnant tissue and the general anatomy revealed that it is highly likely that the electrodes also reached the target and recorded subicular activity. All available tissue was analysed with respect to cell loss and reorganization in the CA1 and subiculum with immunohistochemical methods (Wittner et al., 2005). In seven patients we observed severe cell loss in the CA1 region (severe hippocampal sclerosis, sHS); in four patients relatively mild cell loss was detected (mild hippocampal sclerosis, mHS, see Table 1). Subicular structure was well preserved and it appeared to be control-like in all but two cases. P4 showed patchy cell loss close to the electrode track, while in P25, one discrete patch of cell loss was detected at the border of the subiculum and CA1, remote from the electrode location (Table 1).

Nine out of the eleven patients (n = 6 with sHS, n = 3 with mHS) showed at least one spike exceeding the ±2 SD threshold for spike detection in the subiculum during the entire recording session (10–25 min). Overall spike frequency in six (P3, P10, P21, P22, P25 and P33) patients (n = 4 with sHS, n = 2 with mHS) exceeded the 1 spike/min value and yielded enough events to permit detailed analysis of their FPG, CSD, MUA and spectra. In five of the six patients the complete histology of the electrode track was
Table 1 | Summary of patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Included in detailed analysis</th>
<th>Gender</th>
<th>Impl. side</th>
<th>Duration of epilepsy (years)</th>
<th>MRI finding</th>
<th>Hippocampal damage</th>
<th>Subiculum damage</th>
<th>Elec. 1 loc.</th>
<th>Elec. 2 loc.</th>
<th>Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3</td>
<td>Yes</td>
<td>F</td>
<td>Left</td>
<td>36</td>
<td>Bilateral HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>NA</td>
<td>Propofol</td>
</tr>
<tr>
<td>P4</td>
<td>No</td>
<td>F</td>
<td>Left</td>
<td>30</td>
<td>Left HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Body</td>
<td>Propofol</td>
</tr>
<tr>
<td>P10</td>
<td>Yes</td>
<td>F</td>
<td>Left</td>
<td>20</td>
<td>Left HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Propofol</td>
<td>Isoflurane</td>
</tr>
<tr>
<td>P17</td>
<td>No</td>
<td>F</td>
<td>Right</td>
<td>22</td>
<td>Right HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Isoflurane</td>
<td></td>
</tr>
<tr>
<td>P20</td>
<td>Yes</td>
<td>F</td>
<td>Left</td>
<td>7</td>
<td>Tumour (left temporo-polar)</td>
<td>mHS</td>
<td>c-l.</td>
<td>Digit.</td>
<td>Propofol</td>
<td></td>
</tr>
<tr>
<td>P21</td>
<td>Yes</td>
<td>F</td>
<td>Right</td>
<td>7</td>
<td>Right HS</td>
<td>mHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Propofol</td>
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</tr>
<tr>
<td>P22</td>
<td>Yes</td>
<td>F</td>
<td>Left</td>
<td>10</td>
<td>Bilateral HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Head</td>
<td>Digit.</td>
<td>Propofol</td>
</tr>
<tr>
<td>P25</td>
<td>Yes</td>
<td>M</td>
<td>Left</td>
<td>6</td>
<td>Left HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Head</td>
<td>Propofol</td>
</tr>
<tr>
<td>P26</td>
<td>No</td>
<td>F</td>
<td>Right</td>
<td>26</td>
<td>Bilateral HS</td>
<td>sHS</td>
<td>c-l.</td>
<td>Body</td>
<td>Isoflurane</td>
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</tr>
<tr>
<td>P30</td>
<td>No</td>
<td>F</td>
<td>Right</td>
<td>15</td>
<td>Tumour (right temporo-lateral)</td>
<td>mHS</td>
<td>c-l.</td>
<td>Head</td>
<td>Propofol</td>
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</tr>
<tr>
<td>P33</td>
<td>Yes</td>
<td>F</td>
<td>Left</td>
<td>32</td>
<td>Tumour (left amygdala)</td>
<td>sHS</td>
<td>Head*</td>
<td>Head*</td>
<td>Propofol</td>
<td></td>
</tr>
</tbody>
</table>

Impl. Side = implantation and resection side; F = female; M = male; HS = hippocampal sclerosis; sHS = severe cell loss and reorganization of hippocampus (severe hippocampal sclerosis); mHS = mild cell loss and reorganization of hippocampus (mild hippocampal sclerosis); c-l. = control-like; Elec. loc. = electrode location; digit. = hippocampal digitations; according to (Duvernoy, 1998). NA = not applicable.

Dual electrode experiments, where both electrodes were implanted to and recorded successfully from the subiculum are marked by an asterisk.

available. In two of the six patients, ECoG was obtained concurrently from strip electrodes placed on the pial surface of the temporal lobe in addition to the subicular recordings. All of the six patients were anaesthetized by the combination of Propofol and N2O. Table 1 summarizes the patient data and Fig. 1 shows the reconstructed trajectories, block photos and typical histology of the electrode penetrations.

**Spike classification**

In general, the detected subicular events closely resembled the well-known interictal discharges frequently recorded from the temporal lobe of epileptic patients: the early sharp spike component was followed by a late slow wave. A minority of the spikes, the proximal part of the subiculum showed an initial sharp negative FPG peak around the somatic layer while the distal segment of the subiculum produced a positive FPG peak (Fig. 2A). A majority of the spikes were characterized by inverted polarity FPG peaks with more complex morphology (Fig. 2B). Note that FPG is a directional measurement, and thus will be inverted depending upon whether the electrode tip is pointed toward the soma versus apical dendrites. This orientation in each recording was deduced from careful co-registration of electrophysiology and anatomy, permitting consistent interpretation of FPG recordings.

Dual electrode recording experiments were made in two patients (P25, P33). Activity was simultaneously recorded...
from the anterior and posterior parts of the subiculum separated by 6 mm. Typical single-sweep FPG and the corresponding CSD traces are shown from a dual electrode experiment in Fig. 3. For simplicity, only 12 channels are shown.

**Complex CSD, MUA and spectral characterization**

Event triggered CSD, MUA and spectral averages for separate discharge classes were constructed for all the selected patients. Type 1 discharges revealed a brief initial CSD sink (25 ± 7 ms half amplitude duration) in the pyramidal layer (Fig. 4), while the late wave component was associated with a longer lasting (range: 50–300 ms) source current in the same location (Fig. 5). Both the early somatic sinks and late sources were complemented by early sources and late sinks, respectively in the apical dendritic region (Fig. 5). MUA showed a significant (t-test, \(P < 0.01\)) increase during the peak of the initial spike component, while it decreased below baseline during the late wave component in the somatic layer was significantly smaller than in Type 1 discharge (Fig. 4, t-test, \(P < 0.01\)), but still significantly greater than the baseline activity (Fig. 5, \(P < 0.01\)). The MUA decrease during the late wave component was detectable, but highly variable. In different subjects, this decrease could either be unchanged compared to Type 1 MUA or significantly smaller or larger. Type 1 event iERSP compared to Type 2 showed a significantly larger (Fig. 5, Kruskal–Wallis ANOVA, \(P < 0.05\)) initial spectral activation increase mostly in the higher frequency range (100–200 Hz). A decrease in spectral activity during the wave component of Type 2 events was also present, mostly in the higher frequencies (80–200 Hz) (Fig. 5).

**Intra-subicular and temporal lobe interactions**

In order to further characterize intra-subicular (\(n = 3\)) and subiculum-temporal lobe (\(n = 2\)) discharge relationships, FPG, CSD and ECoG were analysed. Spike time delay, synchrony index and spike amplitude correlations were computed using data obtained from spatially distinct subicular locations. In addition, spike onset and peak time delays and perievent time histograms (PETH) were
Fig. 3 Dual multielectrode, simultaneous FPG and CSD recordings from the anterior and posterior part of the subiculum indicating successively appearing Type 1 (triangle) and Type 2 (diamond) spikes in a somatically oriented penetration. Continuous, non-averaged data. As in the previous figure, the spikes appeared in a close temporal relationship to each other at both subicular locations. (A) FPG traces, 12 channels are shown from the anterior (channels I–12) and from the posterior (channels 25–36) multielectrodes, recorded simultaneously. Spatial separation between traces on a given multielectrode is 200 μm. Schematic neuron illustrates principal cell orientation and approximate location of the pyramidal and apical dendritic layers. Calibration: +40 μV, 100 ms, positivity upwards. (B) Continuous non-averaged CSD traces from the anterior and posterior multielectrodes computed from the corresponding FPG. Calibration: +10 μV, 100 ms. Positive deflections indicate sources (outward currents), negative deflections indicate sink (inward currents). Since the tissue conductivity and electrode spacing were not taken into account, the CSD measurement unit is expressed in μV. Type 1 spike initial component is associated with brief somatic sink, inward, presumably depolarizing current complemented by sources from the dendritic region. Type 2 spike initial component shows an inverted sink–source pattern indicating a possible initial apical dendritic depolarizing mechanism.

Fig. 4 Averaged CSD and MUA traces recorded from the pyramidal layer. Representative data from four patients indicating Type 1 (P10, P21, P25, P33: left two columns) and Type 2 (P10, P25: right column) spikes. In order to compare the time course of Type 1 spikes between patients, CSD and MUA averages (P10, P21, P25, P33: left two columns) were scaled to 1 using the peak values of the initial activation. In the right column, indicating Type 2 spike, only the CSD peak was scaled to 1, while MUA was scaled with the value derived from the Type 1 peak. This permits comparison of the MUA amplitude ratio between Type 1 and Type 2 spikes recorded from two patients (P10 and P25). For Type 1 spikes, the CSD and MUA time courses show remarkable similarities between patients. The shape and timing of the initial sink and MUA increase nearly overlap, while the late source and MUA decrease are also similar in appearance. The CSD and MUA time courses of Type 2 spikes show less correspondence between patients, but the main characteristics are similar: the initial source is followed by a developing sink and concludes with a slow source. MUA recordings revealed an initial small firing rate increase followed by a variable firing rate decrease during the late part of Type 2 spike. Comparison of Type 1 (middle column) and Type 2 (right column) spikes in the same patient (P10, P25) revealed inverted initial CSD activation pattern, while the late wave component appeared to be quite similar. MUA during the initial part of Type 1 spikes was significantly larger (indicated by the thick line above the plot) than in Type 2 spikes (t-test, P < 0.01) in both cases.
calculated using data obtained from simultaneous subicular and temporal lobe ECoG recordings.

In both single (Fig. 2) and dual (Figs 3 and 6) multi-electrode experiments reaching distinct subicular locations, a high degree of spike synchrony was observed. Detailed analysis revealed that synchronous subicular discharges (within a 10 ms window) were almost exclusively of the same type. Figure 7D (right column) PETH illustrates that P25 and P33 Type 1 discharges were not preceded or followed by a Type 2 spike within a 100 ms time window. Intra-subicular spike time delays in dual multielectrode experiments were estimated from the event triggered CSD to eliminate volume conduction effects. The peaks of the largest amplitude sink (in the case of Type 1 event) and source (in the case of Type 2 event) at a given multielectrode were chosen as triggering base for the averages. We found that a certain discharge type emerged at anterior and posterior subicular locations separated by 6 mm with a short, but variable sink or source peak time delay (Fig. 7A, values: −4, 0 and 2 ms) corresponding to a lowest estimate conduction velocity of 1.5–2 m/s. In cases when a single multielectrode covered both the proximal and distal parts of the subiculum in one track (Fig. 2), the initial sink and/or source were not concurrently available due to positioning problems, thus discharge timing was estimated from FPG threshold crossing. Absolute time delays between the proximal and distal subicular locations were in the range of 0–10 ms. Assuming an axonal path length of 15 mm between proximal and distal locations, the corresponding lowest estimate of conduction velocity would be around 1.5 m/s. Given the short distances (6–15 mm) between subiculum sites and the 0.5 ms sampling (corresponding to 2000 Hz digitization rate) accuracy in the low-frequency band, an upper conduction velocity estimate of 12–30 m/s can be reasonably measured between the subiculum sites investigated in here. Next we examined how often the spikes emerged in such a close temporal relationship at distinct subicular locations. We found that a large proportion (89–100%) of similar type spikes occurred concurrently (within a ±10 ms window) between

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**Fig. 5** CSD, MUA and iERSP maps of Type 1 and Type 2 spikes in different patients (P10, P25 and P33). X-axis indicates time: from −50 to 150 ms, Y-axis indicates depth: covering 2 mm of the subiculum. Approximate locations of somatic and apical dendritic layers are abbreviated on the figure. Small schematic pyramidal cell shows the orientation of the recording. Cold (blue) colours indicate activity decrease (dec.) compared to baseline, while warm (red) colours indicate activity increase (inc.) compared to baseline for MUA and iERSP. CSD sink is depicted in red, source in blue. Colour range was adjusted so that both increases and decreases were visible. iERSP is shown from one channel located in the pyramidal layer. Statistical comparison to baseline was performed using a t-test for CSD and MUA, and bootstrap analysis for iERSP. Significance level (bootstrap: \( P < 0.01 \), \( t \)-test: \( P < 0.01 \)) is indicated on the side colour bar by arrows. Statistically non-significant activity in iERSP is shaded green. Type 1 spike is initiated by an inward current and firing rate increase in the pyramidal layer, accompanied by a presumably passive source in the dendritic region. iERSP during this period revealed a significant wide band oscillatory power increase up to 200 Hz. The wave component was accompanied by a source and firing rate decrease in the pyramidal layer, accompanied by a presumably passive current sink in the apical dendritic region. iERSP during the wave component showed a significant oscillatory power decrease. Type 2 spikes showed an inverted initial CSD pattern with an MUA increase in the pyramidal layer and accompanying spectral activity increase up to 100 Hz. The wave component was associated with later CSD inversion, as well as firing rate and spectral activity decreases. Significant differences (Kruskal–Wallis ANOVA, \( P < 0.05 \)) between Type 1 and Type 2 spikes are indicated by the inclusion area of white (Type 1 activity is significantly higher than Type 2 activity) and black (Type 2 activity is significantly smaller than Type 1 activity) lines in the iERSP color map for the initial spike component.
anterior–posterior and proximal–distal sites, expressed by the synchrony index (Fig. 7B), suggesting strong functional coupling within the subiculum. Correlation between spike peak FPG amplitudes measured at different locations was moderate to high, and it was significant in all except one case (Fig. 7B), indicating a strong linear relationship between activities at distinct subicular locations.

In addition to intra-subicular discharge synchronization, we found that temporal lobe spiking was clearly associated in time with the subicular events (Figs 6 and 8). Long time scale PETH revealed temporal spike coupling between the subiculum and temporal lobe in P25 and P33 (Figs 6 and 8A and B). This association was strong for Type 1 events in P33, weaker and broader in P25 for the same type, and virtually absent in P25 for Type 2 discharges (Fig. 8C). We found significantly more spikes (two-tailed Fisher exact test, significance level of $P < 0.01$) in the range of ±200 ms around the zero point of the PETHs than expected from a random (uncorrelated) spike distribution both in P33 Type 1 ($P < 0.00001$) and P25 Type 1 ($P < 0.00001$) events, while P25 Type 2 ($P = 0.7432$) subiculum and cortical spikes appeared to be uncorrelated. We also quantified the statistical properties of lead or lag between subiculum and temporal cortex spikes occurring within ±200 ms of each other. Two-tailed Fisher exact test (significance level of $P < 0.01$) revealed that P33 Type 1 subicular events were significantly ($P < 0.00001$) more likely to occur before the cortical spikes than after them, indicating a highly reliable subicular lead over the cortex. Such preference was not seen in either P25 Type 1 events ($P = 0.3771$) or in P33 Type 2 events ($P = 0.6843$).

To investigate temporal directionality between subiculum and temporal lobe in P33, spike onset and peak timing were further analysed for Type 1 events. The timing of the initial sink peak and the earliest initial ECoG positive peak were correlated. Short time scale PETH showed that the earliest ECoG positivity follows the subicular sink peak with a $5.5 \pm 1$ ms delay (Fig. 8D). The delay was further confirmed by event triggered averaging based on the sink peak. Assuming a distance of about 30–60 mm between subiculum and temporal lobe recording sites, the estimated conduction velocity is in the range of 4.5–10 m/s. In Fig. 8B, $t$-scores (compared to baseline period) of CSD (multiplied by $\sqrt{1}$ for better visualization) from the anterior and posterior subiculum and the $t$-score of temporal lobe ECoG are shown. It is evident that both the onset and peak of activity are earlier in the subiculum than in the temporal lobe, suggesting a possible subicular driving role.

**Discussion**

Detailed FPG, CSD, MUA and spectral analysis of anatomically identified subicular electrical activity was performed on six mesial temporal lobe epilepsy patients during anterior temporal lobectomy under general anaesthesia. In two of the six cases, ECoG spikes were simultaneously obtained from the temporal lobe and were correlated with the subicular events. We found multiple and complex spike generation mechanisms. The general
appearance of the subicular spikes closely resembled that previously described for spike-wave discharges (Gotman, 1980; Altafullah et al., 1986; de Curtis and Avanzini, 2001). Based on waveform morphology, two distinct discharge types were classified.

In Type 1 events (comprising 73.5% of all discharges), the initial component started with a brief inward current (sink) accompanied by increased cell firing and broad-band spectral activity in the pyramidal layer, and a simultaneous outward current (source) in the apical dendrites. These characteristics strongly suggest active depolarizing perisomatic excitatory mechanisms with passive, electrotonic return currents drawn from the apical dendrites. Based on previous studies, the time course and localization of the early depolarizing current is at least partially compatible with the presence of fast glutamatergic excitation (Menendez de la Prida and Gal, 2004) in the pyramidal cell layer delivered through the local recurrent excitatory network (Harris et al., 2001; Witter, 2006). The wave component was characterized by longer lasting outward currents together with decreases in firing rate and spectral activity in the somatic layer, and simultaneous inward currents in the dendritic region. These characteristics strongly suggest active hyperpolarizing, inhibitory mechanisms at the soma of principal cells with passive, electrotonic return currents drawn from the dendrites. Membrane

Fig. 7 (A) Examples of event-triggered CSD averages at different subicular locations. Upper superimposed traces show Type 2 spike CSD derived from the pyramidal layer. Event triggering was based on the anterior subiculum electrode (dark trace). The resulting CSD peak at the posterior subiculum (light grey trace) preceded the anterior subiculum CSD peak by 4 ms. Middle superimposed traces show Type I spike CSD derived from the pyramidal layer of the same patient (P25). In this case event triggered CSD peaks were concurrent, with no delay. Lower superimposed traces depict spike Type I event (anterior subiculum peak) triggered CSD from patient 33. In this case, the posterior subiculum (light grey trace) follows the anterior (black trace) subiculum CSD peak by 2 ms delay. (B) FPG synchrony index and amplitude correlation. Correlation coefficient (Pearson’s $r^2$) and its significance is shown with light grey bars between the peak FPG amplitude at distinct subicular locations in three patients (P22, P25, P33) with proximal–distal and/or anterior–posterior penetrations for separate spike types (1 and 2). Synchrony index is shown with dark grey bars. (C) Instantaneous spike frequency of P33 Type I events calculated from the average time differences of three consecutively occurring spikes, expressed in Hz. (D) Left column shows the auto-correlograms (in 400 ms bins) of P33 (upper) and P25 (lower) Type I events indicating rhythmicity in spike occurrence. Right column shows PETHs of Type I and Type 2 events based on Type I events (in 200 ms bins) indicating sparse interaction between them.
conductances underlying the late hyperpolarizing current cannot be clearly identified from extracellular measurements. The rapid decay of the initial excitation is compatible with local circuit GABAergic inhibition (Cohen et al., 2002), while the later hyperpolarizing component is most likely to be a mixture of other synaptic (Cohen et al., 2006) and intrinsic (Alger and Nicoll, 1980) membrane currents, such as different kinds of potassium currents (Fernandez de Sevilla et al., 2006) or even disfacilitation (Gigg et al., 2000). Similar to the Type 1 spike in humans described here, electrical stimulation of CA1 in animals resulted in broad subicular excitation including the pyramidal layer (Cappaert et al., 2007) with initially increased pyramidal cell firing, followed by decreased firing (Gigg et al., 2000) and hyperpolarizing currents (Behr et al., 1998). A similar activation sequence was observed in human in vitro subicular slice preparations spontaneously or in response to electrical stimulation (Cohen et al., 2002; Huberfeld et al., 2007), suggesting that intrinsic activity can also be triggered.

Type 2 events (comprising 26.5% of all discharges) showed considerably more spatio-temporal variability than Type 1 events, suggesting more complex and/or less-stable generator mechanisms. The initial component of Type 2 events started as a brief outward current in the somatic layer and an inward current in the apical dendritic layer, with a relatively small increase in firing rate and spectral power. One plausible interpretation of this pattern is that the flow of active excitatory, depolarizing currents located in the apical dendritic region cause a passive return source linked by electrotonic conduction with the active dendritic sink (Uva and de Curtis, 2003; Wu and Leung, 2003). The following wave component emerged variably from the fading somatic source as a longer lasting outward current, accompanied by variable decreases in firing rate and spectral power. The most probable generator mechanism of this wave component is, as in the case of Type 1 events, a mixture of synaptic and intrinsic hyperpolarizing currents confined to the somatic layer. Similar to the Type 2 in vivo human spike activation sequence described here, cortical electrical stimulation in animals revealed an initial superficial molecular layer excitation in the subiculum (Cappaert et al., 2007) with increased pyramidal firing, and then a later action potential decrease (Gigg et al., 2000) accompanied by hyperpolarizing currents (Behr et al., 1998).

Opposing polarity spikes were observed in different phases of kindling in rats, dogs (Lopes da Silva et al., 1982; Wadman et al., 1983) and rabbits (Kogure, 1997).
In humans, opposing polarity spikes were shown in the entorhinal cortex (Bragin et al., 2002). Also in humans, interictal discharges in the neocortex were found to have different laminar characteristics depending upon whether they were de novo generated or propagated (Ulbert et al., 2004a). The seizure protective or promoting nature of interictal spikes is under debate (de Curtis and Avanzini, 2001), however it seems to be clear that some spikes are strongly linked to seizures. Recent data demonstrate that provoked spikes are good indicators of the epileptogenic area in the frontal and temporal lobes, including the hippocampus (Valentin et al., 2002, 2005a, b). Furthermore, high-frequency oscillations during spikes were associated with epileptogenesis in animal models of epilepsy (Bragin et al., 2004) and in humans (Staba et al., 2004, 2007). We have shown that a greater amount of high-frequency (100–200 Hz) activity and cellular firing was associated with Type 1 than with Type 2 spikes (Fig. 5), further suggesting that different spike types take different roles in epileptogenesis.

Measured over a relatively long time period, spike frequency was in the range of 9 and 1 spike/min for Type 1 and Type 2 events, respectively. Type 1 discharges showed occasional episodes of rhythmicity in four patients at 0.3–1 Hz. Interictal spike rate computed from long epochs in the human mesial temporal lobe under in vivo conditions show a large variability (Clemens et al., 2003). In general, however, spike frequencies in vivo, in human epilepsy are closer to our findings than has been observed in vitro (Cohen et al., 2002; Wozny et al., 2003, 2005), nonetheless bouts of rhythmic discharges at higher frequencies are not uncommon in vivo.

The effect of propofol anaesthesia on paroxysmal activity has been investigated by several authors. While there is an ongoing debate of its pro- or antiepileptic influence, a blood level controlled study demonstrated that interictal spiking was not significantly influenced by the infusion of the drug in a dose-dependent manner (Samra et al., 1995).

The anaesthetized state is characterized by large fluctuations in synaptic and action potential activity (Steriade, 2006). The slow oscillation (~1 Hz) detected in various species including humans (Massimini et al., 2004) during anaesthesia and natural slow wave sleep is composed of alternating phases of active (up-state) and inactive states (down-state). Human studies have revealed significant cortical excitability changes between up- and down-states (Massimini et al., 2003). In addition, the slow oscillation is modulated by the infraslow oscillation (~0.02 Hz), which was also shown to group interictal spikes in humans (Vanhatalo et al., 2004). Recent work of Clemens and co-workers (Clemens et al., 2007) pointed out the relationship of cortical slow oscillation, parahippocampal spiking and sharp wave-ripples in humans. The occasional rhythmicity (0.3–1 Hz) of subicular spiking observed by us, may be consistent with the coordinative role of the cortical slow oscillation (~1 Hz) in the cortico-hippocampal dialogue, not only in relatively mild (Clemens et al., 2007) but even in cases of more severe mesial temporal damage. The long-term subicular spiking frequency fluctuation may reflect the modulating role of the infraslow oscillation impinging on the cortical slow oscillation.

In addition to fluctuations in interictal spike frequency, variations in spike amplitude may also be related to the varying level of excitability stemming from the slow and infraslow oscillations. Furthermore, cortical excitability cycles may also differentially modulate spike thresholds in different temporal structures, and thus the transfer of interictal discharges between the subiculum and temporal neocortex. Further investigations are needed to test these speculations.

Locally generated interictal events were found to be synchronous within ±10 ms with high reliability at different locations of the subiculum. This was true for both Type 1 and Type 2 events. Presumably, in addition to de novo local generation or projected activity from the lateral temporal lobe, other limbic structures such as entorhinal cortex may also initiate widespread subicular and lateral temporal lobe synchrony. Subicular spike frequency did not correlate with the level of HS, but significantly more Type 2 discharges were detected in sHS than in mHS patients. Although the number of subjects is too small to formulate far-reaching conclusions, the reorganized input patterns of the subiculum due to the significant cell loss in the CA1 region might be related to this phenomenon. Besides the remarkable degree of intra-subicular synchrony, subicular spikes were also closely associated with temporal lobe spiking. We have shown that Type 1 discharges reliably preceded temporal lobe spikes with a short but accurate (5.5 ± 1 ms) delay.

One limitation of our acute intraoperative study stems from the unexplored effects of anaesthesia on the microphysiology of temporal lobe interictal spikes and seizures. Chronically implanted laminar multielectrodes would provide comparative data between different states of vigilance and anaesthesia, in addition to revealing more completely the relationship between spikes and seizures. Detailed evaluation of the spread of subicular activity requires implantation of more than one or two multielectrodes. Recent advances in silicone probe design may allow brain activity to be sampled in three-dimensions. The implantation of recording devices may itself have an unpredictable influence on brain activity. However, the size of our devices is a fraction of the conventionally used depth electrodes, probably inducing less damage, and thus improving the detection of pathological events.

Concerning for example the perisomatic inhibitory input of hippocampal principal cells, controversial results have been found in different rat models of epilepsy (Morin et al., 1999; Cossart et al., 2001) and in human studies (Wittner et al., 2005). These conflicting results require an extensive comparison and correlation of human and animal data to be able to extrapolate model predictions from animals to...
human. In addition to animal model validation, laminar microphysiology yields accurate templates for source localization procedures, which may further advance non-invasive diagnostic efforts. Elucidating the role of the subiculum in the generation and spread of epileptic activity within the temporal lobe may also result in a less invasive and more selective treatment of temporal lobe epilepsy.

Analysis of the ECoG, CSD, MUA and spectral fingerprints of subicular and temporal lobe events in our study revealed multiple spike generator mechanisms suggesting a complex network interplay between medial and lateral temporal lobe during epileptic activity. We have shown that spikes are generated locally and synchronously in the subiculum and temporal lobe during epileptic activity. We have shown that spikes are generated locally and synchronously in the subiculum and temporal lobe during epileptic activity.

This result supports the hypothesis that a subicular focus might also take an active role in the distribution of epileptiform activity to other brain regions (de la Prida et al., 2006).

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