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Limbic P300s in temporal lobe epilepsy with and without Ammon's horn sclerosis

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Abstract

Limbic P300 potentials can be recorded within the mesial temporal lobes of patients with temporal lobe epilepsy (TLE). To delineate possible mechanisms of their generation and pathological alteration, we analysed limbic P300s in 55 TLE patients with and 29 without Ammon's horn sclerosis (AHS) and correlated their amplitudes with neuronal cell counts in 30 histopathological specimens. Limbic P300 amplitudes were reduced on the side of the epileptogenic focus only in patients with AHS. Moreover, in AHS patients, limbic P300 latencies were prolonged bilaterally; and in patients with left-sided AHS, amplitudes were reduced bilaterally. Both findings suggest bilateral functional deficits in TLE with unilateral AHS. Limbic P300 areas correlated significantly with neuronal densities of dentate gyrus granule cells but not hippocampal pyramidal cells in the CA1–4 (cornu ammonis) subfields. This finding points to a potential mechanism for the bilateral effects of unilateral AHS as both dentate gyri exhibit strong reciprocal contralateral connectivity.

Introduction

Rare target stimuli in auditory and visual 'oddball' paradigms elicit the P300 or P3b component of event-related potentials (ERP) recorded at the scalp (Sutton *et al*., 1965; Donchin *et al.,* 1975, 1978; Duncan-Johnson & Donchin, 1977). Intracranial recordings from depth electrodes situated in the medial temporal lobes (MTL) have revealed 'P300-like' potentials in limbic structures; these are termed 'MTL-P300' to indicate their local generation within the medial temporal lobe (Halgren *et al*., 1980; Smith *et al*., 1986; McCarthy *et al*., 1989). These potentials are absent or reduced on the side of the primary epileptogenic focus in temporal lobe epilepsy (TLE, Squires *et al*., 1983; Meador *et al*., 1987; Puce & Bladin, 1987; Grunwald *et al*., 1995). Puce *et al*. (1989) found that unilateral reductions in MTL-P300 amplitudes are typically associated with morphological alterations of the epileptogenic hippocampus, thus underscoring the added advantage of MTL-P300 analyses in patients in whom discordant data from non-invasive studies warranted the stereotactic implantation of depth electrodes. However, while they demonstrated high sensitivity of reduced limbic P300s for pathological hippocampal findings, specificity was rather low (44%). Furthermore, among these patients, some showed no significant difference between left and right MTL-P300 amplitudes, while others exhibited lower amplitudes on the side contralateral to the seizure origin. More importantly, it remains unclear whether abnormal limbic P300 potentials generally indicate lesional TLE or whether they are specific to TLE with Ammon's horn sclerosis (AHS).

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Both the amplitude and latency of the scalp P300 are sensitive to brain pathology (Knight, 1984; Pratap-Chand *et al*., 1988; Yamaguchi & Knight, 1991; Honig *et al*., 1992; Wirsen *et al*., 1992). For instance, scalp P300 latencies are prolonged in patients with complex partial seizures compared to those with primary generalized epilepsies or normal controls (Fukai *et al*., 1990; Triantafyllou *et al*., 1992; Konishi *et al*., 1995). However, despite the similarity in the functional and temporal characteristics of the scalp- and MTL-P300s (Stapleton & Halgren, 1987; McCarthy *et al*., 1989; Halgren *et al*., 1995a,b), it is difficult to relate the scalp P300 directly to MTL functions. The limbic component is significantly diminished by TLE, whereas scalp P300 amplitudes are not reduced by temporal lobectomies which include the hippocampal formation (Johnson, 1988). This could be explained by the suggestion that multiple neocortical generators contribute to the scalp P300 (Kiss *et al*., 1989; Smith *et al*., 1990; ; Neshige & Lüders, 1992; Baudena *et al*., 1995; Halgren *et al*., 1995a,b). However, it is more difficult to explain why no study to date has reported abnormal MTL-P300 latencies in temporal lobe epilepsy, when scalp P300 latencies are sensitive to brain pathology. Because of their multiple generators, it is likewise not easy to infer the brain structures involved from scalp P300s.

Because of their local generation and sensitivity to circumscribed MTL lesions, limbic P300s could help to further elucidate the functional correlates of the P300. To this end, we believe it is important to link MTL-P300 alterations to specific pathophysiological processes within the hippocampal formation. We thus analysed MTL-P300 potentials in 55 TLE patients with AHS and 29 without AHS. In all patients, specimens were available for histopathological analysis after epilepsy surgery. Hippocampal cell counts were obtained in 30 patients. These were correlated with

ERP-data to determine if changes in MTL-P300s are associated with hippocampal damage in general or with neuronal loss within specific subregions of the hippocampus.

Materials and methods

Subjects

Since 1994 we have employed intracranial ERP recordings as a routine component of depth electroencephalography (EEG) studies in those selected patients with medically intractable temporal lobe epilepsy in whom the presurgical evaluation necessitated invasive recordings (Grunwald *et al*., 1995, 1998; Elger *et al*., 1997). In all patients depth electrodes were implanted because non-invasive evaluations had remained non-conclusive with respect to the lateralization of the seizure onset zone and/or the functional integrity of the contralateral temporal lobe. This procedure has been shown to be associated with a low incidence of surgical or neurological complications (Spencer *et al*., 1993; Behrens *et al*., 1997; Van Roost *et al*., 1998). In addition, the finding that bilateral implantation of depth electrodes before right-sided amygdalo-hippocampectomy did not cause verbal memory deficits indicates a low risk of neuropsychologial sequelae (Fernández *et al*., 1997). For the present study, we considered only TLE patients in whom surgical specimens were available for histopathological diagnosis and in whom the ERP-signals were not contaminated by epilepsy-specific potentials, e.g. spikes or sharp waves according to visual inspection. Eight patients were excluded because of continuous medial temporal spike activity. Thus, for the present study we analysed stereo-EEGs from the medial temporal lobes of 84 patients with pharmacoresistant temporal lobe epilepsy (51 left TLE, 33 right; 38 females). Patients ranged in age from 13 to 51 years (mean, 31), and in the duration of their epilepsy from 2 to 48 years (mean, 20). All patients underwent subsequent epilepsy surgery and had a postoperative follow-up of at least 6 months (range, 6–55). Informed consent was obtained from all patients.

Classification into AHS and non-AHS groups

It has become widely accepted that TLE with AHS and TLE without AHS caused by extrahippocampal lesions, e.g. a tumor or neurodevelopmental malformation, represent distinct clinicopathological entities. We therefore divided our 84 patients into two groups according to the presence (AHS group) or absence of AHS (non-AHS group). AHS was confirmed by two neuropathologists, who independently evaluated paraffin-embedded coronal sections from different levels of the hippocampus along its longitudinal axis. Haematoxylin and eosin stains (H & E), Nissl stains, and combined haematoxylin-eosin-luxol-fast blue (H & E-LFB) stains were available for all the specimens. AHS was defined as cell loss of the CA1, CA3 and CA4 (cornus ammonis) segment of the Ammon's horn with relative sparing of the dentate granule cells, severe gliosis and axonal reorganization (Fig. 1). In the non-AHS group, MRI scans and seizure semiology suggested no dual pathology. Histological diagnosis of the extrahippocampal specimen was available in all cases.

ERP-paradigm and recording procedures

The study was conducted in a dimly lit special unit for simultaneous video and EEG monitoring, with the patient upright in an adjustable bed facing a monitor ≈ 80 cm distant. In a visual oddball paradigm, the letter 'x' was presented as a frequent stimulus with a probability of 80% and the letter 'o' as the rare stimulus with 20% probability. A total of 300 stimuli was presented, each for a duration of 100 ms; the interstimulus interval varied randomly from 1000 to 1400 ms (mean 1200 ± 200 ms). Patients were asked to press a button on a computer mouse to each rare stimulus.

In 84 patients, bilateral depth electrodes were implanted stereotactically along the longitudinal axis of the hippocampus from an occipital approach with the amygdala as target for the most anterior electrode. One patient had only one unilateral depth electrode. Each catheter-like, 1-mm-thick silastic electrode contained 10 cylindrical contacts of a nickel–chromium alloy (2.5 mm) every 4 mm. Electrode placements were verified by post-implant computertomography as well as magnetic resonance imaging. Their locations were determined by visual inspection of MRIs with reference to cross-sections published by Duvernoy (1988). Stereo-EEG recordings were referenced to extracranially linked mastoids and amplified with a bandpass of 0.03–85 Hz (12 dB/oct.). After 12-bit A-to-D conversion, signals were written continuously to a hard disk using a sampling rate of 173 Hz per channel. Digital information of stimulus-relevant parameters as well as the patients' reaction were sampled simultaneously. Selective averaging was performed on 1200 ms stimulus-related epochs containing a 200 ms pre-stimulus baseline. Epochs with no or incorrect reactions were excluded from further

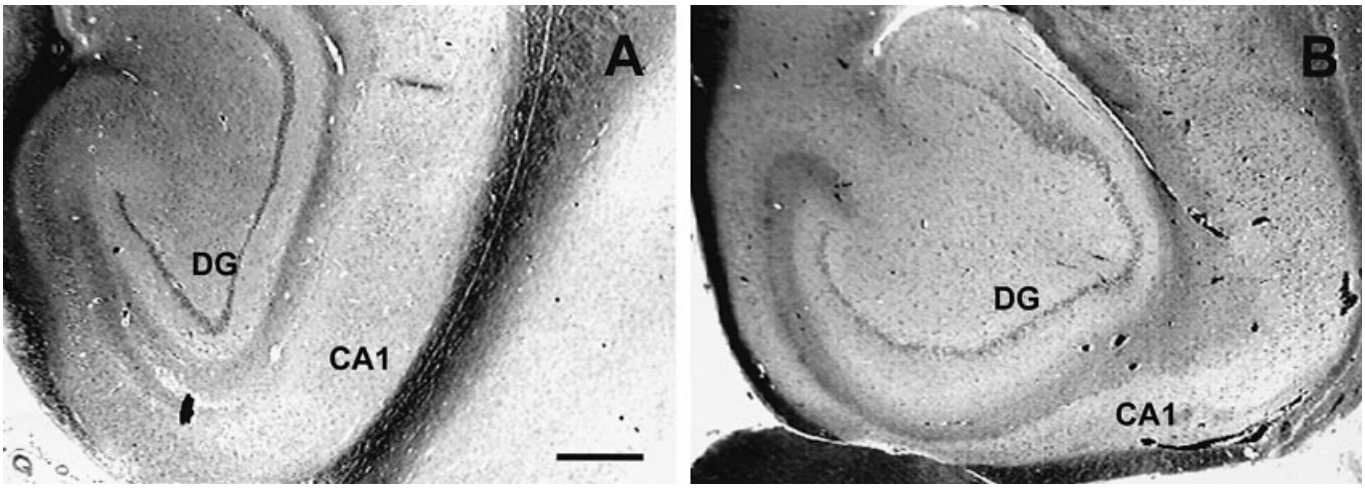

FIG. 1. Histopathological evaluation of hippocampal specimens. (A) No structural alterations were observed in the hippocampal formation of TLE patients with focal mass lesions in the adjacent mesial temporal structures. (B) AHS is characterized by segmental neuronal loss in area CA1 and CA4, whereas dentate gyrus granule cells are more resistant to epileptic damage. Haematoxylin-eosin-luxol-fast-blue stains. Scale bar, 300 μm.

analyses. At the time of ERP recordings, all patients received their usual anticonvulsant medication with serum levels within the so-called 'therapeutic range' (in most cases carbamazepine in monotherapy with blood levels between 8 and 12 μg/mL).When spike activity interfered with the ERP recordings, these were repeated at a later time on the same day or immediately before the explantation of depth electrodes.

ERP analysis and statistics

ERP-components were identified by visual inspection and quantified by computer-automated latency and amplitude measurements. Amplitudes and areas were determined relative to the mean amplitude of a 200 ms pre-stimulus baseline. For creating the grand averages as well as for the various statistical analyses, measurements were taken from the intrahippocampal site with the largest negativity from 300 to 700 ms in response to rare stimuli, for each hemisphere separately. Peak latency, amplitudes and areas were determined semi-automatically between 300 and 700 ms.

Multivariate analyses of variance were performed to examine the effect of AHS on ERP amplitudes and latencies. In addition, separate MANOVAs were conducted for patients with and without AHS, with the side of measurement with respect to the lateralization of the primary epileptogenic area as the independent parameter. In the case of significant effects, *post hoc* univariate analysis of variance and *t*-tests for independent samples were applied. Bivariate correlations of ERP amplitudes and areas with cell counts from the different hippocampal subfields were calculated using Pearson correlation coefficients with Bonferroni corrections because of multiple comparisons. Cell counts were also related to ERP parameters using stepwise multiple regression analysis.

Quantification of neuronal cell numbers

Neuronal cell densities were determined using the monoclonal antibody NeuN, which is directed against a neuronal nucleus-specific antigen, at a dilution of 1:500 according to Wolf *et al*. (1996). Paraffin sections were sliced at 4 μm, mounted on 3-amino-propyltriethoxysilane-coated slides (Dako, Glostrup, DK), air-dried overnight at 42 °C and stored for later use. All hippocampal specimens included in this study were stained under identical conditions using the gap slide holder technique. After deparaffination with xylene and several rinses in 100% and 95% ethanol, the slides were incubated in 2% hydrogen peroxide (Merck, Darmstadt, Germany) diluted in methanol for 15 min, rehydrated successively in 95%, 90%, 70% and 50% ethanol, and rinsed in phosphate-buffered saline (PBS). To improve the binding of the monoclonal antibodies, the sections were transferred into 0.01 M citrate buffer (Sigma, St. Louis, USA) and boiled twice for 5 min in a microwave oven according to the standard Dako microwaving protocol. The sections were transferred into PBS. Preincubation with 2% horse serum (Vector Laboratories, Burlingame, USA), 10% foetal calf serum (Seromed, Berlin, Germany), and 5% non-fat dry milk (Bio-Rad Laboratories, CA, USA) in PBS as a blocking reagent for unspecific immunoreactivity was performed for 3 h at 37 °C, followed by incubation with the primary antibody overnight at 4 °C in a humid chamber. Binding of primary antibody was detected by the Avidin-Biotin-Complex peroxidase method (ABC Elite, Vector Labs,), using 3,3′-diaminobenzidine (ICN, Cleveland, OH, USA) as a chromogen. All specimens were subjected to semiautomatical imaging using a Vanox microscope (Olympus, Japan) equipped with a CCD video camera (Sony, Japan) and the IP Lab imaging analysis software (Signal Analytics Corporation, Vienna, Austria) installed on a MacIntosh computer (7100/66; Apple, USA). To determine neuronal cell densities within the pyramidal cell layer of CA1, CA2, CA3 and CA4 and within the granule cell layer of the dentate gyrus, NeuN-labelled nuclei were tagged on the computer screen and the number of objects as well as the respective regions of interest were calculated by the IP Lab imaging analysis software. The results of five adjacent regions of interest per hippocampal subfield were recorded, averaged and expressed as mean cell number/mm^2 (neuronal cell density).

Results

Clinical data

Seventy-one patients (84.5%) were free of seizures following surgery, four (4.8%) experienced a reduction of seizure frequency of more than 90%. The outcome was less satisfactory in nine patients (10.7%, including reduction of seizure frequency of 75–90% in six patients (7.1%), and minor or no changes in three patients (3.6%).

In all 55 AHS patients (17 females; mean age 34 years, range 16–51; mean duration of epilepsy 24 years, range 7–48) only unilateral onsets of seizures were recorded. These were located in the left temporal lobe in 34 and the right temporal lobe in 21 patients. The surgical procedures consisted of 10 anterior temporal lobectomies including amygdala and hippocampus, and 45 selective amygdalo-hippocampectomies. Postoperative follow-up documented 47 patients (85.5%) to be seizure free. Two patients (3.6%) experienced a significant reduction of seizure frequency of more than 90%; the outcome was less satisfactory in six patients (10.9%).

The non-AHS group consisted of 29 patients (17 females; 17 left, 12 right TLE; mean age 26 years, range 13–49; mean duration of epilepsy 12, range 2–30; for pathological findings as well as the surgical procedures, see Table 1). In this group, 24 patients (80.8%) were seizure free postoperatively, two (6.9%) had a reduction of seizure frequency of more than 90%, and three patients (10.3%) were not significantly improved. Four of the patients without AHS had lesions involving the hippocampal formation (two astrocytomas WHO grade I and II, one oligodendroglioma WHO grade II and one dysembryoblastic neuroepithelial tumour). In all of these patients, extended lesionectomies included the hippocampus and resulted in complete seizure control.

General properties of MTL-P300

Rare target stimuli elicited a marked negative potential within the medial temporal lobe (MTL-P300) with maximum amplitudes confined to the hippocampus, and steep voltage gradients at the anterior and posterior hippocampal border (Fig. 2A). In the few patients in whom depth electrodes were located in a more posterior position and slightly steeper inclination, we found polarity inversions of the MTL-P300 with positive polarities posterior to the hippocampus proper. For all 84 patients, mean latencies (± SD) on the side of the epileptogenic focus were 499 ± 57 ms and 492 ± 60 ms on the non-focal side; their mean reaction times were 409 ± 65 ms. Mean MTL-P300 amplitudes were – 79 ± 63 μV on the focal and – 134 ± 77 μV on the non-focal side.

MTL-P300 latencies in AHS- and non-AHS patients

Because both groups represent distinct clinicopathological entities, with considerably more pronounced damage to the hippocampus proper in the AHS group, we predicted differences in MTL-P300 latencies between the two groups. MANOVA with presence or absence of AHS and side of the epileptogenic focus as independent factors revealed no influence of the side of seizure origin on ipsi- or contralateral MTL-P300 latencies, but significant effects of AHS ($F = 5.69$; $P = 0.005$). *Post hoc* univariate *F*-tests (d.f. = 1, 79)

TABLE 1. Patients without AHS, diagnosis, surgical procedure and outcome with respect to seizure control (Engel's classification)

Subject	Sex	Side	Histological diagnosis	Surgical procedure	Outcome
1	Female	Right	Hamartia	Extended lesionectomy	I
2	Female	Right	Necrosis	Extended lesionectomy	I
3	Female	Left	Ganglioglioma WHO grade I	Extended lesionectomy	I
4	*Male*	Right	*Oligodendroglioma WHO grade II*	*Mesial extended lesionectomy*	*I*
5	Male	Left	Ganglioglioma WHO grade I	Extended lesionectomy	II
6	Male	Left	None	Lobectomy	I
7	Female	Right	None	Lobectomy	I
8	Male	Left	Astrocytoma WHO grade I	Extended lesionectomy	I
9	Male	Left	Cavernoma	Extended lesionectomy	I
10	Male	Left	None	Topectomy	II
11	Female	Left	Heterotopia	Extended lesionectomy	I
12	Female	Right	Hamartia	Extended lesionectomy	I
13	Female	Left	Ganglioglioma WHO grade I	Extended lesionectomy	I
14	Male	Right	Cavernoma	Extended lesionectomy	I
15	Female	Right	Ganglioglioma WHO grade I	Extended lesionectomy	I
16	Female	Right	Hamartia	Extended lesionectomy	I
17	*Female*	*Left*	*DNT*	*Mesial extended lesionectomy*	*I*
18	Female	Right	Necrosis	Extended lesionectomy	I
19	Male	Right	Ectopia	Lobectomy	I
20	Female	Left	Hamartia	Extended lesionectomy	IV
21	Female	Left	Ganglioglioma WHO grade I	Extended lesionectomy	I
22	Male	Right	Ganglioglioma WHO grade I	Extended lesionectomy	I
23	Female	Left	Necrosis	Extended lesionectomy	IV
24	Female	Right	Oligodendroglioma WHO grade II	Extended lesionectomy	I
25	*Male*	*Left*	*Astrocytoma WHO grade I*	*Mesial extended lesionectomy*	*I*
26	Male	Left	Astrocytoma WHO grade I	Extended lesionectomy	IV
27	Male	Right	Encephalitis	Lobectomy	IV
28	Female	Left	Ganglioglioma WHO grade I	Extended lesionectomy	I
29	Male	Left	Hamartia	Extended lesionectomy	I
30	*Female*	*Left*	*Astrocytoma WHO grade II*	*Mesial extended lesionectomy*	*I*

Note that cases printed in italics had mesial temporal pathologies different from AHS.

demonstrated that this held not only for ipsilateral ($F = 7.08$; $P = 0.009$) but also for contralateral MTL-P300 latencies ($F = 9.70$; $P = 0.003$). Student's *t*-tests for paired samples showed that there were no significant differences between latencies (mean ± SD) on the focal and non-focal side in both the AHS (511 ± 57 versus 507 ± 62 ms; NS) and non-AHS group (476 ± 53 versus 464 ± 48 ms; NS). By contrast, *t*-tests for independent samples confirmed that in patients with AHS, MTL-P300 latencies were significantly prolonged on both the ipsilateral ($P = 0.009$) and contralateral side ($P = 0.001$).

MTL-P300 amplitudes in AHS- and non-AHS patients

As expected, the AHS- and non-AHS-groups also differed with respect to MTL-P300 amplitudes (Figs 2B and 3). MANOVA with ipsi- and contralateral MTL-P300 amplitudes as dependent variables showed significant effects of both AHS ($F = 26.73$; $P < 0.0005$) and the side of the epileptogenic focus ($F = 3.96$; $P = 0.02$). *Post hoc* univariate *F*-tests (d.f. = 1, 79) showed that AHS affected MTL-P300 amplitudes on the focal ($F = 52.59$; $P < 0.0005$) but not non-focal side ($F = 0.54$; NS). Student's *t*-tests for independent samples demonstrated that while there was no difference between amplitudes of AHS and non-AHS patients on the contralateral side (mean ± SD: −128 ± 77 versus −147 ± 77 μV; NS), patients with AHS had significantly smaller amplitudes on the focal side (−51 ± 32 versus −132 ± 72 μV; $P < 0.0005$).

MTL-P300 amplitudes in left and right TLE

To further analyse the unexpected finding that the right or left hemispheric location of the epileptogenic focus had significant effects on MTL-P300 amplitudes on both sides, we performed MANOVAs for left and right MTL-P300 amplitudes with the side of the epileptogenic focus as an independent factor for patients with and without AHS separately. In patients without AHS there was no significant effect of the hemisphere of seizure onset ($F = 0.82$; NS). By contrast, in patients with AHS, the epileptogenic focus had an effect on both left (d.f. = 1, 52; $F = 62.66$; $P < 0.0005$) and right MTL-P300 amplitudes (d.f. = 1, 52; $F = 8.64$; $P = 0.005$). The side of the epileptogenic focus also had an effect on the left minus right amplitude difference (d.f. = 1, 52; $F = 63.59$; $P < 0.0005$), which was previously shown to be a useful parameter for determining the laterality of the epileptogenic focus (Grunwald *et al.* 1995). Ipsilateral MTL-P300s were significantly reduced relative to the contralateral ones in AHS patients with right (mean ± SD: −58 ± 39 versus −169 ± 82 μV; $P < 0.0005$) and left TLE (−46 ± 28 versus −102 ± 60 μV; $P = 0.001$). However, only in patients with left but not right TLE were MTL-P300s also reduced contralaterally ($P = 0.001$). Finally, in patients without hippocampal sclerosis there were no intra- or interindividual differences between left and right or ipsi- and contralateral amplitudes (mean focal versus non-focal MTL-P300 amplitudes ± SD in left TLE without AHS: −117 ± 69 versus −141 ± 81 μV; NS; right TLE without AHS: −154 ± 72 versus −154 ± 74 μV; NS).

Medial temporal lobe lesions different from AHS

Four patients with hippocampal tumours in the absence of AHS showed pronounced MTL-P300s on the focal as well as non-focal side. Given their small number, *t*-tests were not applicable. Their ipsi- and contralateral amplitudes were similar to those in patients with extrahippocampal lesions, and their mean ipsilateral amplitudes (± SD) were markedly higher than those in patients with AHS (−123 ± 22 versus −51 ± 32 μV).

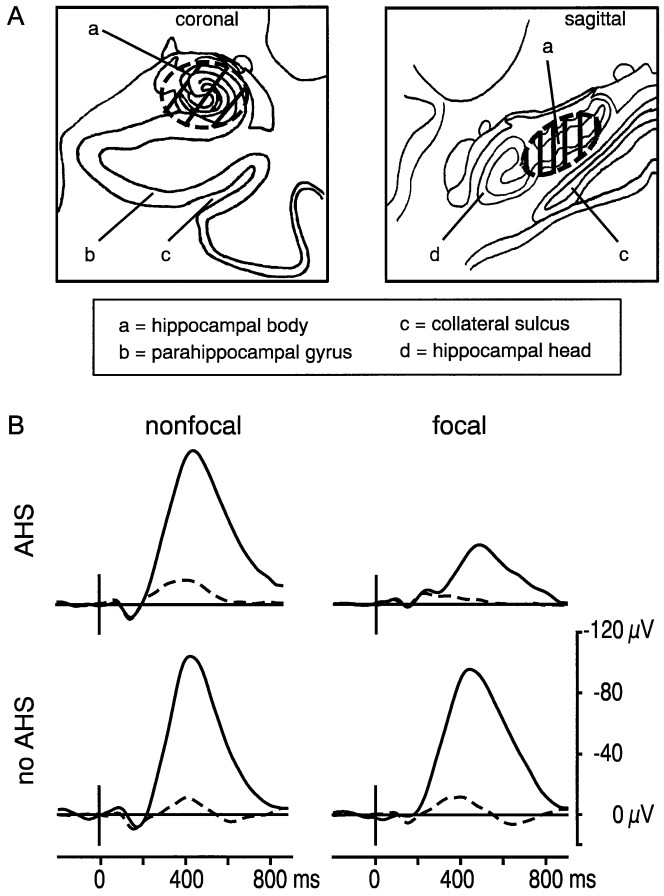

FIG. 2. Area of electrode locations at which maximal MTL-P300s were recorded and grand averages of MTL-P300s. (A) Schematics of recording sites of MTL-P300s. Hatched area indicates the area in which maximal MTL-P300 potentials were recorded in all patients. (B) Grand averages of MTL-P300s in the non-focal and focal temporal lobe in patients with AHS ($n = 55$) as well as in patients with extrahippocampal lesions and without AHS ($n = 29$). AHS, Ammon's horn sclerosis. Solid line: rare target stimuli; dashed line: frequent distractors.

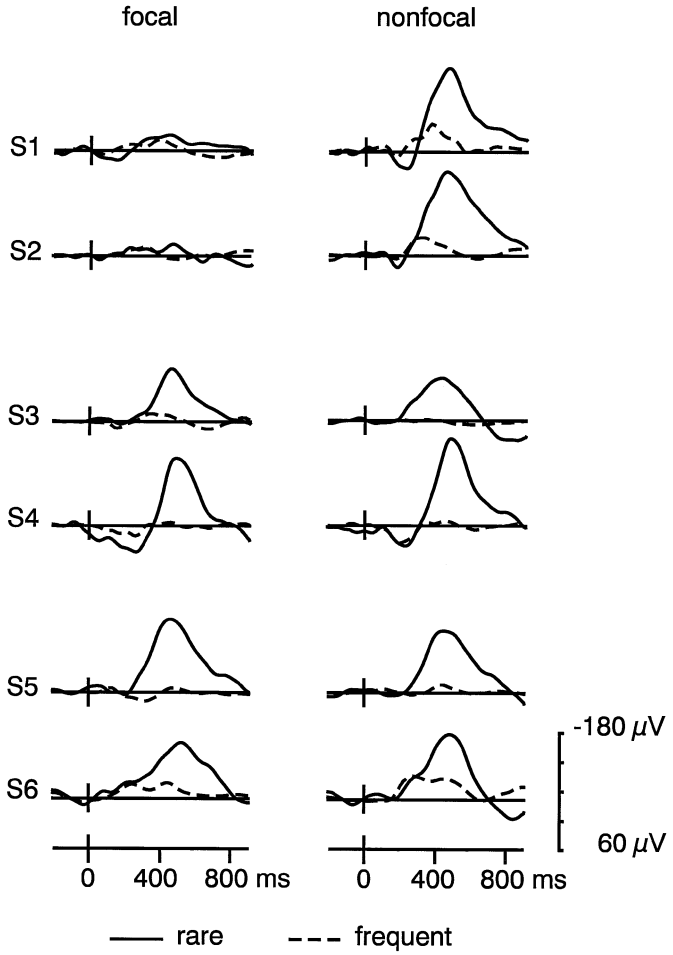

FIG. 3. Typical examples of MTL-P300s in patients with AHS (S1, S2), with hippocampal lesions different from AHS (S3, S4), and with extrahippocampal lesions (S5, S6).

Correlation with cell counts

Because MTL-P300 parameters were preferentially affected by AHS, we reasoned that the extent of hippocampal damage may correlate with MTL-P300 measures. In 28 out of 30 patients in whom hippocampal cell counts were available, histopathological analysis revealed astrogliosis and neuronal cell loss of a variable extent indicative of AHS. Two patients, both seizure free postoperatively, showed no pathological alterations of the hippocampus. One of these exhibited an extrahippocampal hamartia, while the other showed no morphological correlate of the epileptogenic focus. Because these two cases were qualitatively different, they were precluded from the correlational analyses.

MTL-P300 area (area under the ERP waveform between 300 and 700 ms after stimulus onset relative to the pre-stimulus baseline; mean: $13\,249 \pm 8647$ μV ms) correlated significantly only with the number of granule cells in the dentate gyrus but not with the densities of pyramidal cells within the hippocampal subfields CA1–4 (Table 2, Fig. 4). Stepwise multiple regression analysis with MTL-P300 areas as dependent variables was significant ($F = 19.83$; $P < 0.0005$) with only the density of dentate granule cells entering the regression equation ($\beta = 0.69$; $t = 4.5$; $p < 0.0005$), and thus accounted for 47% of the variance of MTL-P300 integrals.

Discussion

AHS and MTL-P300 amplitudes

Previous studies have shown that MTL-P300 potentials are locally generated within the hippocampus: the greatest amplitudes are observed within the hippocampus, and these potentials exhibit steep amplitude gradients and polarity inversions in regions immediately adjacent to the hippocampus both in orthogonal and longitudinal directions (Halgren *et al*., 1986; Stapleton & Halgren, 1987; McCarthy *et al*., 1989; Smith *et al*., 1990; Halgren *et al*., 1995a,b). Moreover, MTL-P300 generation is associated with hippocampal unit activity (Heit *et al*., 1990). Our patients show a typical distribution of MTL-P300s with polarity inversions posterior to the hippocampus. Previous studies have demonstrated that MTL-P300s are often reduced on the side of the primary epileptogenic area in temporal lobe epilepsy (Squires *et al*., 1983; Meador *et al*., 1987; Puce & Bladin, 1987; Grunwald *et al*., 1995). We replicated this observation and demonstrated in addition that this reduction in MTL-P300s is only present in patients with AHS. In other words, MTL-P300s were not attenuated simply by the presence of extrahippocampal epileptogenic lesions. This is consistent with Puce *et al*.'s observation that the MTL-P300 were absent in a higher percentage of AHS patients compared to a group with no significant hippocampal pathology (Puce *et al*., 1989). The fact that the four patients in our study with non-sclerotic hippocampal lesions were apparently not affected on the focal side

TABLE 2. Pearson correlation coefficients (r) for correlations between cell numbers in hippocampal subfields and ipsilateral MTL-P300 areas (area within the time window of 300–700 ms after onset of stimulus presentation)

MTL-P300	Dentate gyrus	CA1	CA2	CA3	CA4
Integral	0.651*	−0.310	0.192	0.128	0.250
Amplitude	0.303	−0.171	−0.084	0.019	0.076

*$P < 0.0005$; significant after Bonferroni correction.

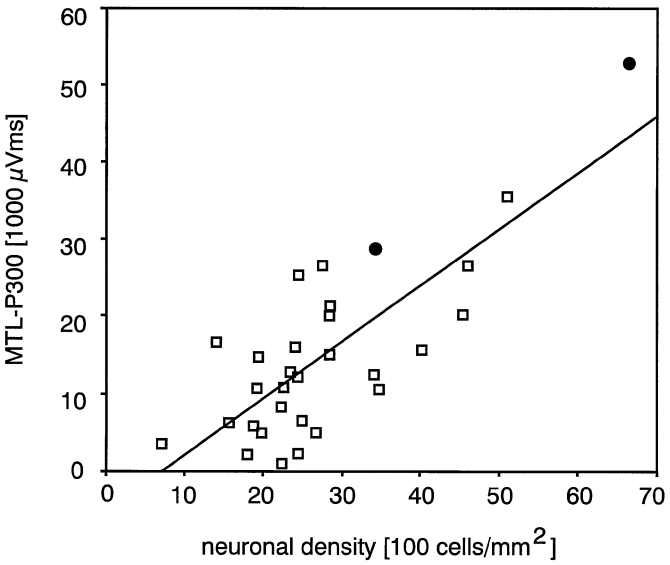

FIG. 4. Scatter diagram of focal MTL-P300s to rare target stimuli regressed onto ipsilateral neuronal density of dentate gyrus granule cells. Open squares: patients with AHS; filled circles: two patients without AHS who were precluded from correlational analyses.

suggests that morphological alterations of the hippocampus per se may not be sufficient to reduce MTL-P300s. Rather, MTL-P300 reductions appear to represent a specific consequence of AHS.

AHS and MTL-P300 latencies

To our knowledge, the question of whether the latencies of hippocampal MTL-P300s are altered by epileptogenic lesions has not been addressed previously. This may be because most studies focus on unilateral absence of MTL-P300s as an indicator of the epiletogenic temporal lobe, or because the marked asymmetry in MTL-P300 amplitudes rendered possible latency differences insignificant for the presurgical workup. In the present study, we found no differences in MTL-P300 latencies from the focal versus non-focal side. We did, however, observe that MTL-P300 latencies were significantly prolonged on both the focal and non-focal sides in patients with AHS compared to those without AHS. This effect of AHS may help to interpret the finding in the literature of prolonged surface P300 latencies in patients with TLE compared to normal control subjects as well as patients with idiopathic generalized epilepsy (Fukai *et al*., 1990; Triantafyllou *et al*., 1992; Konishi *et al*., 1995). AHS is likely to have been present in at least some of the TLE patients participating in those studies.

Left versus right AHS

MTL-P300 amplitudes on the side of the epileptogenic focus did not differ in patients with left and those with right AHS; both were reduced in amplitude. By contrast, only those with left AHS showed significantly reduced amplitudes on the side contralateral to the epileptogenic focus as well. This may be an effect of language dominance in the sense that left MTL-300s may normally be of greater amplitude than those from the right side. Alternatively, left but not right AHS could diminish hippocampal ERPs contralaterally. The fact that left and right MTL-P300 amplitudes did not differ for patients with left and right TLE without AHS tends to argue against an explanation based on language dominance. Thus, it may be the case that left but not right MTLE is associated with bilateral MTL-P300 amplitude reductions, possibly indicating more pronounced functional deficits induced by left as compared to right MTLE. Therefore, future studies should address the question of whether left TLE is more likely to lead to bilateral (i.e. verbal and figurative) memory deficits.

Bilateral effects of unilateral AHS

The fact that in the AHS group, MTL-P300 latencies were prolonged bilaterally indicates bilateral functional disturbances, even though imaging studies did not yield evidence of contralateral MTL pathology in those patients. Three interpretations appear possible: (i) AHS may be a bilateral disease (with unilateral predominance); (ii) contralateral functional disturbances may be induced by secondary epileptogenesis ('kindling'); or (iii) unilateral MTLE may cause contralateral functional impairments independent from contralateral epileptogenicity. Our data cannot adjudicate between these hypotheses. However, the finding that dentate granule cells seem to be associated with MTL-P300 generation points to a potential mechanism for contralateral changes in the MTL-P300. It is well known that both dentate gyri possess strong reciprocal contralateral connectivity via mossy cells (Zimmer *et al*., 1983; Goodman & Sloviter, 1992) or dentate basket cells (Seress & Ribak, 1984). Thus, generation of MTL-P300s may well be influenced by contralateral MTL pathology.

MTL-P300 and neuronal cell counts

We found that a large part of the MTL-P300 amplitude variance is accounted for by the density of granule cells in the dentate gyrus. In AHS, the neuronal cell density of dentate granule cells can be noticeably reduced (Margerison & Corsellis, 1966; Bruton, 1988; Wolf *et al*., 1993). This may explain why some patients with AHS have no ipsilateral MTL-P300 while others merely show reduced amplitudes. This may even account for cases in whom TLE is not accompanied by loss of ipsilateral MTL-P300s.

What conclusions can be drawn from the correlation between graded reduction in hippocampal neuronal cell counts with ERP areas? It has been suggested that MTL-P300s reflect dendritic excitation of hippocampal neurons (McCarthy *et al*., 1989; Halgren *et al*., 1986). Because the areas of MTL-P300s are significantly correlated with the density of dentate granule cells, it would appear possible that this component reflects EPSPs induced in or by this cell population. Of the cells receiving afferents from dentate granule cells, the CA3 pyramidal neurons are the most likely to contribute to MTL-P300 generation, as they are targets of granule cell excitation via the mossy fibre pathway. However, if CA3 neurons were critically involved in MTL-P300 generation, then we should have seen some correlation between their density and MTL-P300s; such a correlation was not observed. Thus, it seems more likely that the EPSPs in dentate granule cells contribute significantly to MTL-P300 generation.

Contribution of MTL-P300 to the presurgical evaluation of MTLE

Our findings indicate that unilateral reductions of MTL-P300 amplitudes reflect neuronal loss in hippocampal atrophy and

sclerosis. Advances in MRI technology, especially volumetry and relaxometry techniques, provide the presurgical evaluation of MTLE patients with very sensitive tools for the detection of AHS. When there is evidence of unilateral AHS, invasive procedures are warranted only when non-invasive studies are equivocal with respect of epileptogenicity of the sclerotic and/or functional integrity of the contralateral hippocampus. Therefore, in MTLE patients in whom MRI indicates unilateral AHS, and in whom neuropsychological and non-invasive electrophysiological studies yield concordant results, there is little need to record MTL-P300s invasively. However, whenever depth recordings are warranted, the analysis of MTL-P300s can provide additional information especially if MRI yields equivocal results with respect to AHS. Thus, recording MTL-P300s may be especially valuable in patients with bilateral small hippocampi or with possible dual temporal pathology. For patients without AHS it has been reported that hippocampal cell loss is greater when epileptogenic temporal lobe lesions are located close to the hippocampus (Spencer *et al*., 1993). Therefore, we cannot exclude the possibility that there are patients in whom neuronal cell loss in the dentate gyrus and the associated diminution in ipsilateral MTL-P300 amplitudes are consequent to a tumour or neurodevelopmental malformation.

Functional considerations

MTL-P300s depend on attention and are not elicited when rare target stimuli are ignored (Stapleton & Halgren, 1987; McCarthy *et al*., 1989). Because they are likely to be part of a larger 'N2b/P3b/slow wave'-system (Halgren *et al*., 1995a,b) and can be modulated by the brain stem (Kutas *et al*., 1990), a comprehensive theory of the neuropsychological correlate(s) of the MTL-P300 must take more than hippocampal physiology into account. Our data raise one interesting issue. Altafullah *et al*. (1986) drew attention to the similar topography of the MTL-P300 and the slow wave of mesial temporal spike-slow wave complexes. This finding together with the observation that on occasion MTL-P300s are accompanied by a decrease in firing rate of MTL neurons (Halgren *et al*., 1980) led Halgren to hypothesize that the P3 may be associated with an inhibitory modulation (Halgren, 1988). Along this line, our finding of a correlation between MTL-P300s and the density of dentate gyrus granule cells may support the hypothesis that this potential is linked to inhibitory processes. The dentate gyrus is likely to inhibit the CA1/CA3 system during spontaneous field events known as dentate spikes, which reflect anterograde activation of granule cells by the entorhinal pathway (Buszaki *et al*., 1994; Penttonen *et al*., 1997). Therefore, the MTL-P300 may indicate excitation of the dentate gyrus but inhibition within the hippocampus proper. Conversely, the diminution of MTL-P300 amplitudes in patients with AHS would indicate that the process of disinhibition contributes more to epileptogenesis in this form of TLE than in TLE with extrahippocampal lesions.

In summary, we demonstrate that hippocampal P300 potentials are selectively reduced by AHS compared to other epileptogenic lesions of the temporal lobe. Unilateral AHS is associated with bilaterally prolonged MTL-P300s and may thus lead to bilateral functional deficits. Furthermore, a striking difference was observed between patients with right AHS and left AHS. Those patients with left AHS exhibit reduced MTL-P300 amplitudes not only on the affected side but on the contralateral side as well. MTL-P300s were selectively correlated with the density of granule cells in the dentate gyrus, the activity of which has been shown to be primarily inhibitory for hippocampal pyramidal neurons. This correlation suggests a potential mechanism for bilateral effects of unilateral AHS because the dentate gyri exhibit strong reciprocal contralateral connectivity.

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Abbreviations

AHS, Ammon's horn sclerosis; CA, cornu ammonis (Ammon's horn); EEG, electroencephalography; ERP, event-related potential; MTL, medial temporal lobe; PBS, phosphate-buffered saline; TLE, temporal lobe epilepsy.

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