Original article

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The epileptogenic zone in pharmaco-resistant temporal lobe epilepsy with amygdala enlargement

Hiroharu Suzuki [1], Hidenori Sugano [1], Madoka Nakajima [1], Takuma Higo [1], Yasushi Iimura [1], Takumi Mitsuhashi [1], Keiko Fusegi [1], Akiyoshi Kakita [2], Hiroshi Otsubo [3], Hajime Arai [1]

[1] Department of Neurosurgery, The Juntendo University, Tokyo, Japan
[2] Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan
[3] Division of Neurology, The Hospital for Sick Children, Toronto, Ontario, Canada

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ABSTRACT – *Aims*. Temporal lobe epilepsy with amygdala enlargement (TLE-AE) has been considered a subtype of TLE. We evaluated the epileptogenic zone in patients with TLE-AE, who underwent intracranial video-EEG (ivEEG) and/or intraoperative electrocorticography (ioECoG) as well as epilepsy surgery.

Methods. Eleven patients with TLE-AE were enrolled and investigated based on seizure profile, volumetric MRI, the Wechsler Memory Scale-Revised (WMS-R), the location of seizure onset zone (SOZ) and irritative zone (IZ) based on ivEEG (n=8), the location of interictal epileptiform discharges (IEDs) based on ioECoG (11), surgical procedure, and seizure outcome.

Results. The mean age at seizure onset was 34.9 years (range: 23-57). The mean duration of seizures was 5.0 years (range: 1-10). The number of AEDs was 2.3 (range: 1-5). The mean seizure frequency was nine per month (range: 1-30/month). All patients presented with focal impaired awareness seizures with (n=9) and without (2) secondary generalized convulsions. Volumetric MRI analysis showed unilateral enlarged amygdala with statistical significance ($p<0.01$). None of the patients' hippocampi had any abnormality based on MRI. Pre-operative mean verbal, visual, and delayed recall scores based on the WMS-R were over 100. The SOZ and IZ were identified in both the amygdala and hippocampus in seven patients and in only the amygdala in one patient based on ivEEG. IEDs were identified in the hippocampus in six patients and in both the amygdala and hippocampus in four patients based on ioECoG. All 11 patients underwent anterior temporal lobectomy, including amygdala resection, with multiple hippocampal transections (dominant hemisphere: seven patients) and resection (non-dominant hemisphere: three patients). Nine (81.8%) of 11 patients achieved seizure freedom with a mean follow-up of 26 months (range: 12-47). Post-operative WMS-R results did not show any significant deterioration, with a

Correspondence:
Hidenori Sugano
Division of Neurosurgery,
The Juntendo University,
2-1-1 Hongo, Bunkyo-ku,
Tokyo, 113-8421, Japan
<debo@juntendo.ac.jp>

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mean follow-up of 15 months (range: 12-24). The resected amygdala showed no histopathological abnormality.
Conclusion. The epileptogenic zone of TLE-AE involves both the amygdala and hippocampus. ivEEG may be needed to explore the SOZ in normal hippocampus in addition to enlarged amygdala. Amygdala resection and multiple hippocampal transections may control the epileptogenic limbic system and save memory function in patients with TLE-AE.

Key words: intracranial video-EEG, seizure onset zone, MRI negative hippocampus, multiple hippocampal transections, saving memory

Amygdala enlargement (AE) on MRI was first reported by Tebartz van Elst *et al*. in patients with temporal lobe epilepsy (TLE) and dysthymia (Tebartz van Elst *et al*., 1999). Asymmetrical volumes of amygdala related to comorbidity of psychiatric disorders. Because amygdala caused psychosis including aggressive behaviours, morphological changes of the amygdala were extensively studied using CT and MRI (Tebartz Van Elst *et al*., 2002).
In 2003, Bower *et al*. described seven patients with TLE-AE but without hippocampal sclerosis (Bower *et al*., 2003). Coan *et al*. reported that TLE-AE was found in 12% of MRI-negative TLE (Coan *et al*., 2013). The asymmetric enlarged amygdala became a valuable sign of temporal lobe seizures. Fourteen articles of TLE-AE revealed that 28 resected amygdala specimens from 107 patients with TLE-AE showed histopathological lesions consisting of dysplasia, hamartoma, and focal cortical dysplasia (Beh *et al*., 2016).

The epileptogenic zone in TLE-AE

The epileptic network between the amygdala and hippocampus is a key player in temporal lobe seizures. The common pattern of seizure onset is regional, involving both the hippocampus and amygdala simultaneously (Quesney, 1986; So *et al*., 1989). The amygdala kindles much faster than any other part of the brain, significantly faster than the hippocampus (Racine, 1986; Cain, 1992). The importance of the amygdala as a crucial structure in the pathogenic mechanism of temporal lobe seizures is underestimated.
Lv *et al*. reported that 22 out of 33 patients with TLE-AE demonstrated good seizure control and significantly reduced volume of the enlarged amygdala after taking AEDs (Lv *et al*., 2014). Beh *et al*. reported that 81.8%-100% of patients with TLE-AE responded well to AEDs but 26% of patients with pharmaco-resistant TLE-AE proceeded to surgical resection (Beh *et al*., 2016).

Multiple hippocampal transection (MHT)

Shimizu *et al*. reported hippocampal transections for 21 TLE patients with normal hippocampus, sparing verbal memory (Shimizu *et al*., 2006). Of 17 patients with more than one year of follow-up, 14 (82%) patients became seizure-free. Verbal memory functions of the dominant hemisphere remained the same as before surgery, at six months post-operation. MHT has been applied in TLE with MRI-negative hippocampus and intact memory functions but with extra-mesial temporal lesions (Usami *et al*., 2016; Girgis *et al*., 2017; Ishida *et al*., 2018). Minami *et al*. described 11 patients with pharmaco-resistant TLE-AE who underwent epilepsy surgery consisting of hippocampal resection in nine patients and MHT in two, in addition to amygdala resection (Minami *et al*., 2015). The intraoperative electrocorticography (ioECoG) showed that the sharp waves did not originate from the amygdala but from the hippocampus. There is no report of intracranical video-EEG (ivEEG) to localize the seizure onset zone (SOZ) or irritative zone (IZ) with active interictal epileptiform discharges (IEDs) in patients with pharmaco-resistant TLE-AE. This paper is the first preliminary report regarding ictal recording using ivEEG and epilepsy surgery in patients with pharmaco-resistant TLE-AE and MRI-negative hippocampus.

Hypothesis

We hypothesized that the epileptogenic zones in pharmaco-resistant TLE-AE involve both an enlarged amygdala and MRI-negative hippocampus. Surgical intervention for both the amygdala and hippocampus could control seizures. In patients with intact verbal memory functions and dominant-side temporal lobe seizures, MHT is an option to control seizures and save memory function.
The study was approved as #16-163 by the Research Ethics Committee of Juntendo University, Tokyo, Japan. We obtained written informed consent from all participants.

Materials and methods

Patients

Between 2013 and 2017, 96 patients with pharmaco-resistant TLE underwent epilepsy surgery at Juntendo University-Epilepsy Center in Tokyo, Japan.
We selected patients with pharmaco-resistant TLE-AE based on the following criteria:
– enlargement of amygdala compared to the contralateral side based on MRI;
– seizure semiology related to ipsilateral temporal lobe epilepsy;
– and seizures refractory to appropriate AEDs for one year.

Scalp video-EEG

All patients underwent long-term scalp video-EEG monitoring (EEG-1200, Nihon Kohden, Tokyo, Japan) using the 10-20 international system with 500-Hz sampling rate before surgery.

MRI data

We performed 3T MRI with T1 and T2-weighted spin-echo, three-dimensional fluid-attenuated inversion recovery (FLAIR) and double inversion recovery (Van Paesschen *et al.*, 1996; Mitsueda-Ono *et al.*, 2011; Wong-Kisiel *et al.*, 2016). The sections were oriented perpendicular to the long axis of the hippocampal body with section thickness of 1 mm. We defined the enlarged amygdala in axial and coronal sections. We excluded patients with additional hippocampal atrophy/sclerosis or any other signal changes. Patients with suspected tumours or vascular lesions were excluded.
To confirm visual diagnosis of TLE-AE in the included patients, amygdala and hippocampus volumes were quantified using fully automated volumetry using Free Surfer Software (Version 6.0.0; Martinos Center, Harvard University, Boston, MA, USA) with T1-weighted images (Pardoe *et al.*, 2009; Coan *et al.*, 2013). The MRI scans of 10 normal controls (20-29 years old; mean age: 23.2) were used for comparison.
Amygdala and hippocampus volumes were statistically analysed using paired t-tests between the epileptic and non-epileptic side of each patient with TLE-AE. The amygdala and hippocampus volumes of both the epileptic and non-epileptic sides of patients with TLE-AE were also compared to the normal controls using unpaired t-tests. These analyses were performed using R 3.4.4 statistical software (The R Development Core Team).

Wada test

The Edinburgh Handedness Inventory Test was conducted for all patients to estimate language dominancy (Oldfield, 1971). We performed the WADA test for three patients (Cases 2, 7 and 9) whose dominant hemisphere was ambiguous (Abou-Khalil, 2007).

Intracranial video-EEG

We performed intracranial video-EEG (ivEEG) to evaluate the IZ and SOZ in eight patients. One depth electrode (four contacts) was placed into the amygdala (*figure 1A, B*). Mesial temporal strip electrodes

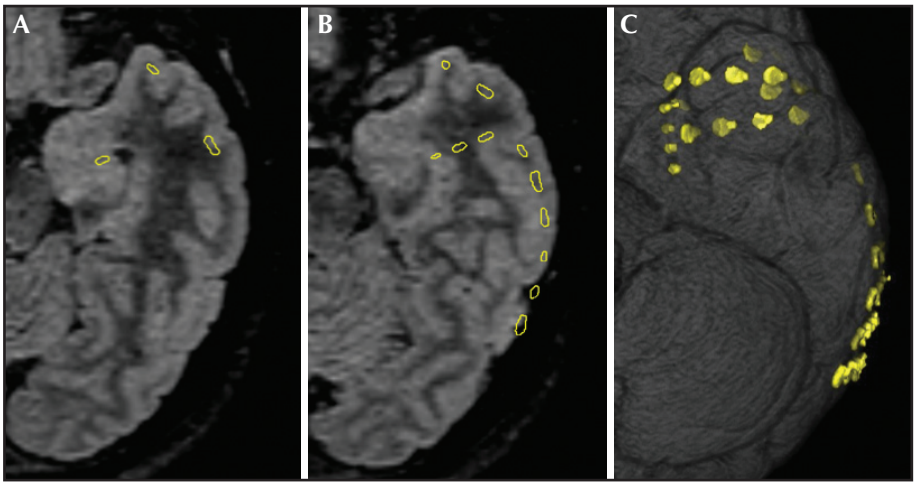

Figure 1. Fusion MRI and post-implantation CT. (A) Axial fluid-attenuated inversion recovery (FLAIR) shows the deepest contact (yellow circle) of the depth electrode in the enlarged amygdala (Case 3). (B) Axial FLAIR shows three consecutive contacts (yellow circles) of the depth electrode towards the enlarged amygdala (Case 3). (C) 3D-MRI shows the mesial temporal strip (T shape) that covers the hippocampus, the uncal strip, and a part of the grid that covers the lateral temporal region (Case 4).

were subtemporally inserted according to Shimizu's method (Shimizu *et al*., 1992). One T-shape strip electrode covered the hippocampus (four contacts) and subtemporal region (four contacts) (*figure 1C*). One uncal strip electrode (four contacts) was placed to cover the caudal end of the amygdaloid nuclear complex, continuing to the uncus and the parahippocampal gyrus (Carpenter, 1985). Other subdural grids were placed on the lateral temporal region (Unique Medical, Tokyo, Japan).
The ivEEG was recorded using EEG-1200 (Nihon Kohden, Tokyo, Japan) with a 2-kHz sampling rate. We defined the SOZ as:
– rhythmic spikes or sharp waves;
– paroxysmal fast activity;
– and attenuation of background activity.

Intraoperative ECoG

All patients underwent ioECoG recordings. Anaesthesia was maintained using 2.5% sevoflurane with an adequate muscle relaxant. End-tidal CO_2 levels were maintained at approximately 30 mmHg during ioECoG recordings (Sugano *et al*., 2007).
IoECoG was recorded on the surface of the hippocampus and amygdala using platinum electrodes (Unique Medical, Tokyo, Japan). IoECoG monitoring continued for 3-10 minutes before hippocampal resection or multiple transections.

Surgery

We applied the trans-sylvian approach to the inferior horn of the lateral ventricle (Yasargil *et al*., 1985). First, the amygdala was resected. Subsequently, standard anterior temporal lobectomy was performed 3 cm from the temporal tip. When ivEEG showed the SOZ in the hippocampus and/or ioEEG showed IEDs from the hippocampus, additional hippocampal multiple transections or resection were performed.

Seizure outcome

All patients were followed for at least one year after surgery. Postoperative seizure outcomes were assessed at the last visit according to Engel's classification (Engel *et al*., 1993).

Memory function

We performed Wechsler Memory Scale-Revised (WMS-R) to evaluate memory functions before surgery, six months after surgery, and between 12 and 24 months after surgery. We compared the scores at observation points in each subcategory of WMS-R. Changes in memory scores were analysed using repeated measures one-way ANOVA with SPSS statistics software version 22 (IBM Corp, Chicago, IL, USA). A level of $p < 0.05$ was considered statistically significant.

Histopathology

Amygdala and hippocampus specimens were fixed with phosphate-buffered 20% formalin, and embedded in paraffin for histological evaluation. The surgical specimens were sectioned with 4-μm thickness, and stained with haematoxylin-eosin and Klüver-Barrera myelin stain. Representative sections were immunostained with antibodies directed against neuronal nuclei antigen (NeuN) and glial fibrillary acidic protein (GFAP). Histopathological diagnosis was made by an independent neuropathologist (AK).

Results

Characteristics of patients

Eleven patients (five females) with TLE-AE were included in this study. The clinical features are described in *table 1*.
The mean age at surgery was 34.9 years (range: 23-57). The age at seizure onset was 29.9 years (range: 13-55). The mean duration of seizures was 5.0 years (range: 1-10). One patient had a history of febrile convulsions. No patient had any comorbidity of psychiatric disorders. The number of AEDs ranged from one to five with a mean of 2.3; multiple AEDs were taken by nine (81.8%), levetiracetam by nine (81.8%), carbamazepine and valproic acid were each taken by five (45.5%), lamotrigine by two, and clonazepam, gabapentin, lacosamide, zonisamide were each taken by one patient.

Characteristics of seizures

The frequency of seizures at the time of surgery ranged from one to 30 per month (with a mean of nine per month).
All 11 patients presented with seizures during scalp video-EEG. Five patients presented with preceding auras. None of them complained of fear. All 11 patients showed behavioural arrest of focal impaired awareness seizures (FIAS). Eight (72.7%) patients showed manual automatisms, one (9.1%) patient showed oral automatisms, and later generalized convulsions were seen in nine (81.8%) patients.

Table 1. Clinical profiles of our 11 patients included in the present study.

No.	Age at surgery (years)/gender	Duration of seizure (years)	Preoperative AEDs	Seizure frequency (per month)	Seizures during scalp video-EEG	
					Aura	Seizure type
1	27/M	10	CBZ / LEV / VPA	2	*Déjà vu* / Olfactory hallucination	FIAS, 2G
2	28/F	2	CBZ / LTG	10	-	FIAS, 2G
3	31/F	11	CBZ / LEV / LTG / VPA / ZNS	4	Unexplainable strange sensation	FIAS, 2G
4	32/F	12	LEV	4	-	FIAS, 2G
5	23/F	10	CBZ / GBP / VPA	8	Gastrointestinal sensation	FIAS, 2G
6	51/M	1	LEV	3	-	FIAS
7	57/M	2	LCM / LEV	30	-	FIAS
8	27/M	2	LEV / VPA	30	-	FIAS, 2G
9	39/M	1	CZP / LEV	8	-	FIAS, 2G
10	39/F	1	LEV / VPA	1	Palpitations	FIAS, 2G
11	30/M	3	CBZ / LEV	1	Gastrointestinal sensation	FIAS, 2G

CBZ: carbamazepine; CZP: clonazepam; FIAS: focal impaired awareness seizure; GBP: gabapentin; LCM: lacosamide; LEV: levetiracetam; LTG: lamotrigine; VPA: valproic acid; ZNS: zonisamide; 2G: secondary generalized seizure.

MRI results

MRI showed left amygdala enlargement in seven (63.6%) patients and right amygdala enlargement in four (36.4%) patients (*table 2*). There was no abnormal finding of hippocampus on MRI in all 11 patients. *Figure 2A, B* shows representative MRI of the enlarged amygdala and normal hippocampi. There was no other extratemporal abnormality on MRI. Enlarged amygdala of all 11 patients exhibited subtle increased signal changes on FLAIR.

The MRI data of 10 TLE-AE cases, except for Case 7, as well as 10 normal controls, were applicable to the automated volumetric MRI analysis. *Figure 3* shows the amygdala and hippocampus volumes on the epileptic and non-epileptic side of 10 patients with TLE-AE and 10 normal controls.

On the epileptic side of patients with TLE-AE, amygdala volume ranged from 1,768.6 mm^3 to 2,486.4 mm^3 (mean: 2,051.5 mm^3). On the non-epileptic side, amygdala volume ranged from 1,503.4 mm^3 to 2,471.3 mm^3 (mean: 1,789.8 mm^3). Amygdala volumes on the epileptic side were significantly greater than those on the non-epileptic side ($p<0.01$).

In the 10 normal controls, amygdala volume ranged from 1,441.5 mm^3 to 2,209.7 mm^3 (mean: 1,802.5 mm^3). Amygdala volumes on the epileptic side of patients with TLE-AE were significantly greater than those of normal controls ($p<0.01$).

On the epileptic side of patients with TLE-AE, hippocampus volume ranged from 4,061.2 mm^3 to 5,299.0 mm^3 (mean: 4,477.7 mm^3). On the non-epileptic side, hippocampus volume ranged from 3,625.5 mm^3 to 4,987.3 mm^3 (mean: 4,297.4 mm^3). Hippocampus volumes on the epileptic side of patients with TLE-AE were greater than those on the non-epileptic side ($p<0.05$).

In the 10 normal controls, hippocampus volume ranged from 3,591.1 mm^3 to 5,085.2 mm^3 (mean: 4,401.6 mm^3). Hippocampus volumes on the epileptic side of patients with TLE-AE were not smaller than those of normal controls.

Hemisphere dominance based on the WADA test

Ten patients were right-handed and left-hemispheric predominant based on the Edinburgh Handedness Inventory Test and WADA test. In the remaining patient (Case 9) who had poor verbal memory functions despite right amygdala enlargement and right-handedness, the WADA test showed language dominance in the right hemisphere.

Table 2. Surgery and seizure outcome.

No.	Lesion side		Intracranial video EEG		Intraoperative ECoG	Surgical procedures		Follow-up (months)	Decrease/ Increase of postoperative AEDs	Seizure Outcome (Engel's classification)
	Left / Right	Dominant/ Non-dominant	Irritative zone	Seizure onset zone	Interictal epileptiform discharges	Amygdala	Hippocampus			
1	R	Non-D	A+H	A+H	H	Resection	Resection	47	↓	Ia
2	L	D	A+H	A+H	H	Resection	Transection	18	↓	Ia
3	L	D	A+H	A+H	H	Resection	Transection	18	↓	IIa
4	L	D	A+H	A+H	H	Resection	Transection	23	↑	IIa
5	R	Non-D	A+H	A+H	A+H	Resection	Resection	19	↓	Ib
6	L	D	A+H	A+H	A+H	Resection	Transection	12	→	Ia
7	L	D	A+H	A+H	A+H	Resection	Transection	12	→	Ia
8	L	D	A	A (+H)*	-	Resection	-	40	↓	Ia
9	R	D	N/A		A+H	Resection	Transection	17	↓	Ia
10	L	D	N/A		H	Resection	Transection	42	↓	Ia
11	R	Non-D	N/A		(H)**	Resection	(Resection)**	12 (42)**	↓	IIIa (Ia)**

AEDs: antiepileptic drugs; A: amygdala; D: dominant hemisphere; ECoG: electrocorticography; H: hippocampus; L: left; N/A: not available; Non-D: non-dominant hemisphere; R: right; *subsequent intra-ictally involving hippocampus; **second surgery; ↓decrease in AEDs; ↑increase in AEDs; →no change.

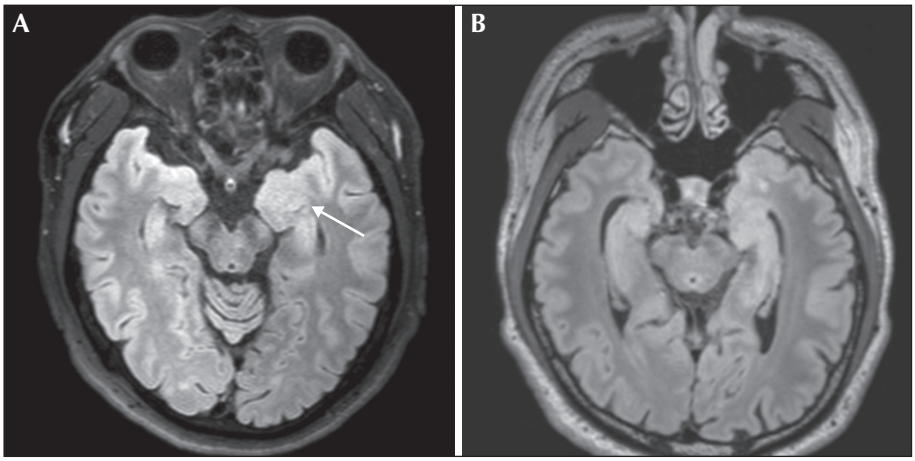

Figure 2. The enlarged amygdala and MRI-negative hippocampus (Case 6). (A) Axial fluid-attenuated inversion recovery (FLAIR) shows the left enlarged amygdala with slightly increased intensity (white arrow). (B) Axial FLAIR shows normal bilateral hippocampi.

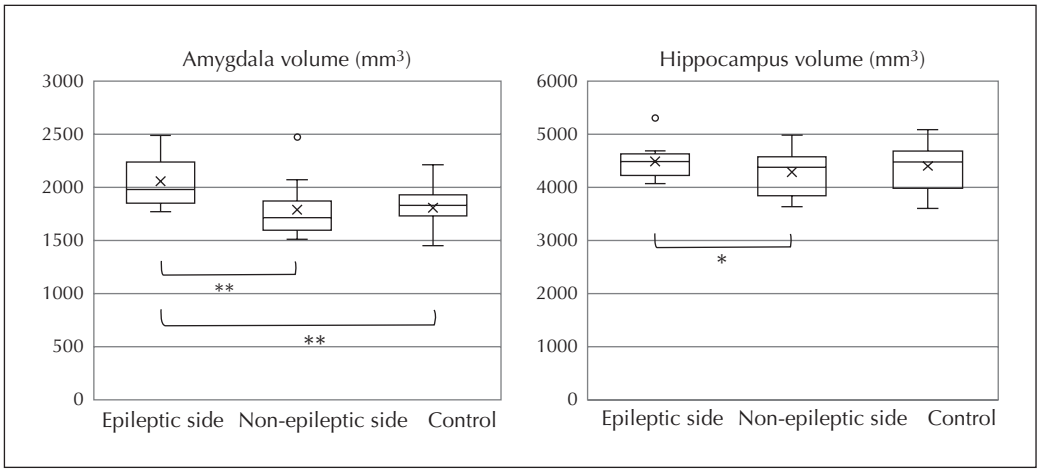

Figure 3. Comparison of amygdala and hippocampus volumes in patients with TLE-AE (epileptic side and non-epileptic side) and normal controls. Boxes signify the upper and lower quartiles of amygdala and hippocampus volumes. The black line within each box marks the median. The "x" in each box represents the mean. The whiskers extending above and below each box represent the largest and smallest data element; 1.5 times the interquartile range (IQR). Open circles demonstrate values outside this range. *$p<0.05$, **$p<0.01$. The amygdala volumes on the epileptic side were significantly larger than those on the non-epileptic side in patients with TLE-AE ($p<0.01$). The amygdala volumes on the epileptic side were significantly larger than those of the normal controls ($p<0.01$). The hippocampus volumes on the epileptic side were larger than those on the non-epileptic side in patients with TLE-AE ($p<0.05$). The hippocampus volumes on the epileptic side of patients with TLE-AE had no atrophic signs compared to those of the normal controls.

Intracranial video-EEG

We performed ivEEG in eight patients. The other three patients refused implantation of intracranial electrodes. The mean recording time was 68 hours (range: 37-202).

Seven patients had IEDs on both the amygdala and hippocampus. The other patient (Case 8) showed IEDs on only the amygdala (*table 2*).

We captured a total of 21 seizures (range: 1-6) with a mean of three seizures. The seizure onset was found in both the amygdala and hippocampus in seven patients (*figure 4*). The other patient (Case 8) showed seizures derived from only the amygdala. The seizures subsequently involved the hippocampus and manifested with FIAS and generalized convulsion.

Intraoperative electrocorticography

In all 11 patients, we performed the ioECoG on the amygdala and hippocampus before the resection/transections. The ioECoG showed IEDs on only hippocampus in five (45.5%) patients. Four (36.4%) patients had IEDs on both the amygdala and hippocampus. The remaining two patients did not show any IEDs.

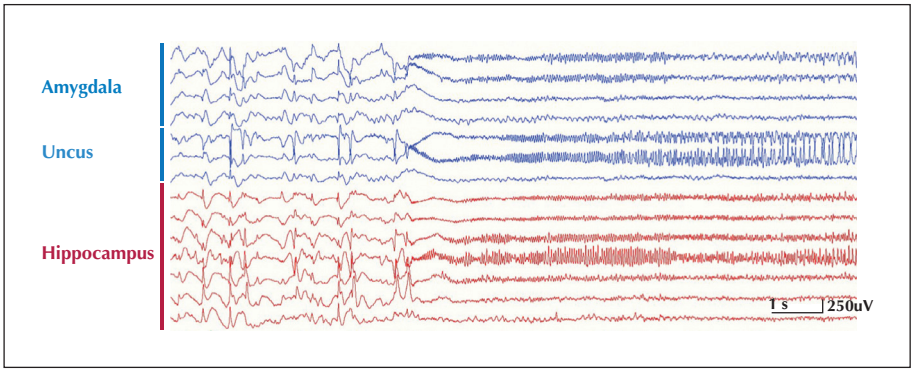

Figure 4. Intracranial video-EEG at the time of seizure onset (Case 6). Referential montage using an epidural electrode for reference. Blue: four amygdala depth electrodes (top) and three uncal strip electrodes (bottom). Red: four hippocampal electrodes (top) and three subtemporal electrodes (bottom) of the T-shape strip. Prior to seizure onset, high-amplitude rhythmic spike and slow waves are seen synchronously over the amygdala, uncus, and hippocampus. Paroxysmal low-amplitude fast activities started in the amygdala, uncus, and hippocampus at the seizure onset, with following evolution of amplitude and frequency. Two contacts (one from the uncal strip and the other from the subtemporal electrode) were eliminated due to artefacts.

Because of the refusal of ivEEG in three patients (Cases 9-11), only ioEEG was peformed. The ioECoG showed IEDs from both the hippocampus and amygdala in Patient 9 and from only the hippocampus in Patient 10. The ioECoG in Patient 11 showed no IEDs at initial surgery, however, he had recurrent seizures after the amygdala resection alone, and 12 months later, the epileptic-side hippocampus showed IEDs and was resected during the second surgery.

Surgical procedure

In all 11 patients, the amygdala was resected.
MHT was performed in seven (63.6%) patients. All seven hippocampi were located in the dominant hemispheres (six left and one right). The only patient (Case 9) with right-handedness and right-hemispheric dominance underwent multiple right-hippocampal transections.
Three (27.2%) patients underwent hippocampal resection in the non-dominant right hemisphere. Patient 11 underwent amygdala resection at initial surgery, which did not improve his seizures. We then performed right-hippocampal resection 12 months after initial surgery. Patient 8 underwent only amygdala resection because the ivEEG showed a seizure onset and IEDs in only the amygdala.

Seizure outcome

The mean follow-up period was 26 months (range: 12-47). Engel Class I outcome was achieved in nine (81.8%) patients, consisting of eight with Ia and one with Ib. Eight (72.7%) patients were able to reduce AEDs: six patients with Class Ia, one patient with Class Ib, and one patient with Class IIa.

Pre- and post-operative memory function

Data for pre- and post-operative verbal, visual, and general memory, attention/concentration, and delayed recall of serial WMS-R are presented in *figure 5*. Before surgery, mean ± standard deviation for verbal memory was 110 ± 14.6, visual memory 104 ± 12.5, general memory 110 ± 12.1, attention/concentration 102 ± 8.7, and delayed recall 101 ± 12.9. Pre-operative cognitive performance was not significantly different between left (seven patients) and right-sided (four patients) TLE-AE. At six months after surgery, mean ± standard deviation for verbal memory was 99 ± 18.7, visual memory 108 ± 8.5, general memory 100 ± 16.4, attention/concentration 109 ± 12.1, and delayed recall 102 ± 13.7. Verbal memory and general memory at six months after surgery showed a decline compared to before surgery without statistical significance, using one-way repeated ANOVA.
At one to two years after surgery, mean ± standard deviation for verbal memory was 102 ± 21.0, visual memory 108 ± 5.6, general memory 104 ± 15.7, attention/concentration 108 ± 8.7, and delayed recall 103 ± 13.2, with a mean follow-up of 15 months. There was no significant post-operative decline in any memory function.

Histopathology

Specimens from the amygdala in all 11 patients showed no histopathological abnormality. Non-specific mild gliosis in the amygdala was observed in three (27%) patients who underwent ivEEG.
Hippocampal specimens from three patients who underwent hippocampal resection did not show any sclerosis, gliosis, or inflammatory change. Subtle

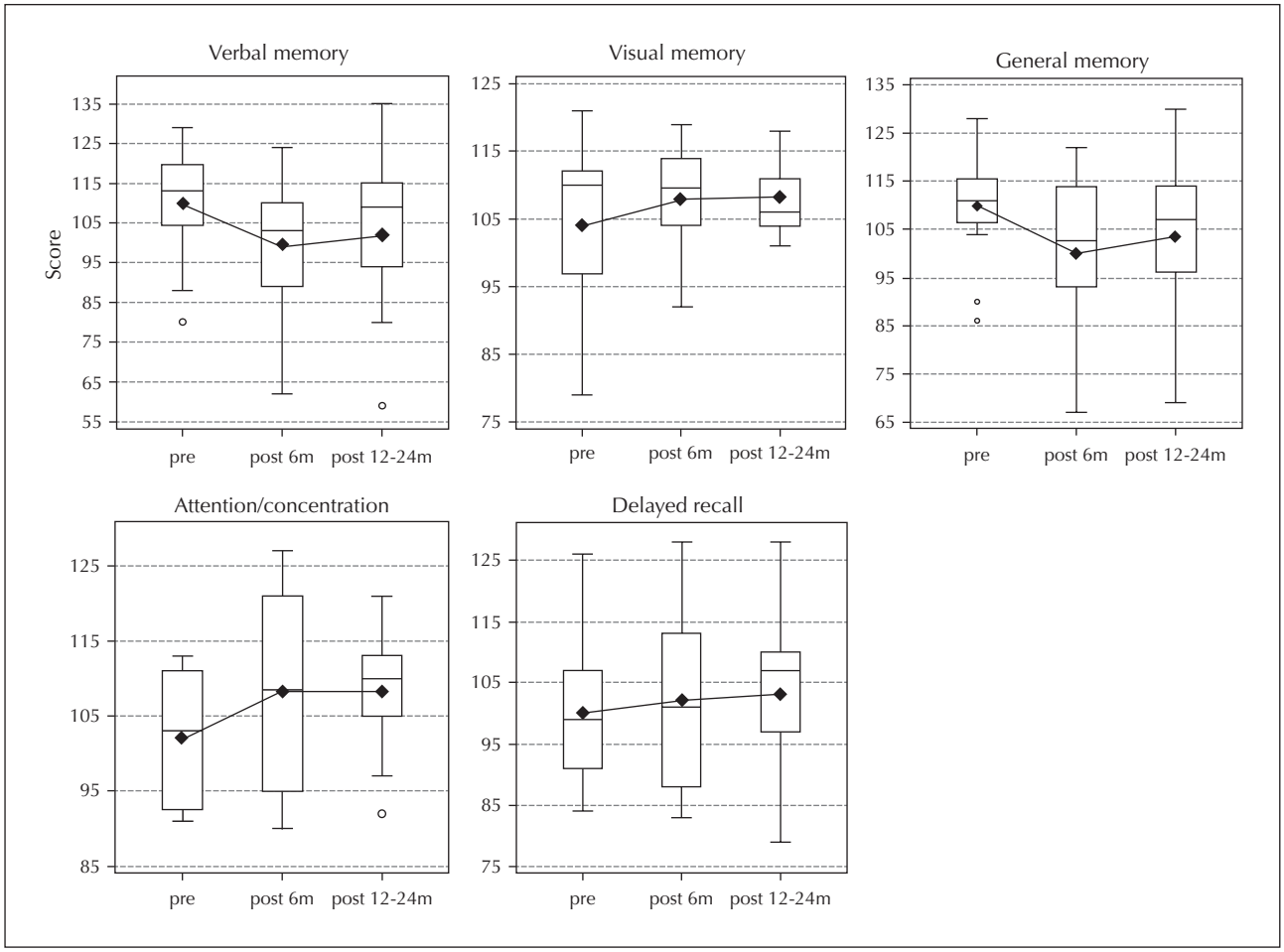

Figure 5. Pre- and post-operative memory functions of verbal, visual, and general memory, and attention/concentration and delayed recall (WMSR). The horizontal line inside each box indicates the median, and the length of each box indicates the interquartile range (IQR). The extremities of the whiskers indicate the data within 1.5 IQR from the upper or lower quartile. The open circles indicate outliers. The diamond plots indicate the mean score of memory functions before and after surgery (six months, and between 12 and 24 months). There is no significant difference between any of the pre- or post-operative memory functions.

granular cell dispersion was found in all three hippocampal specimens.

Discussion

Summary of findings

We investigated 11 patients with pharmaco-resistant TLE-AE. We confirmed unilateral enlarged amygdala and normal hippocampus without any atrophy by volumetric MRI analysis. The ivEEG in seven out of eight patients showed a SOZ involving the hippocampus in addition to the amygdala. The ioECoG revealed IEDs on the hippocampus in 10 out of 11 patients, including four patients with IEDs on the amygdala. All 11 patients underwent amygdala resection. Ten of them underwent additional hippocampal treatments: seven patients with MHT in the dominant hemisphere and three patients with hippocampal resection in the non-dominant hemisphere. Nine (81.9%) patients achieved seizure freedom, including eight patients in whom AEDs had been reduced after more than a year of follow-up. Postoperative memory functions were spared in all patients, even after MHT in the dominant hemisphere (seven patients) and hippocampal resection in the non-dominant hemisphere (three patients).

Epileptogenic enlarged amygdala

This paper is the first to report that the SOZ involves both the enlarged amygdala and MRI-negative hippocampus in TLE-AE patients. Amygdala-involved seizures were characterised by ictal fear, gastrointestinal sensations, and marked autonomic symptoms (Cendes *et al.*, 1994; Wieser, 2000; Biraben *et al.*, 2001). In our study, three (27.3%) patients reported

gastrointestinal and autonomic symptoms, but none reported ictal fear. The seizure semiology alone could not localize the SOZ in a subset of patients with TLE-AE. Minami *et al*. reported that, based on ioECoG in patients with TLE-AE, no sharp waves originated from the amygdala (Minami *et al*., 2015). Our ioECoG revealed the IEDs in the amygdala were less frequently seen than those in the hippocampus. Because we used a trans-sylvian approach to the inferior horn of the lateral ventricle, the amygdala may have been injured during the approach. IEDs based on ioECoG could not accurately localize the epileptogenic zone in a subset of patients with TLE-AE.

Our ivEEG revealed active interictal spikes in addition to SOZs localized in the amygdala and hippocampus. We treated both the amygdala and hippocampus with favourable seizure outcomes.

The hippocampus in TLE-AE

The ivEEG localized the SOZ and IZ in the hippocampus in seven out of eight patients with TLE-AE. In patients with pharmaco-resistant TLE-AE, the MRI-negative hippocampus could provoke seizures refractory to AEDs.

Seven (29.2%) out of 24 patients with pharmaco-resistant TLE had normal MRI (Cascino *et al*., 1991). Muhlhofer *et al*. reported that for 38-72% of patients with MRI-negative TLE, ivEEG revealed seizures arising from mesial temporal structures (Muhlhofer *et al*., 2017). An MRI-negative hippocampus was one of the high-risk factors for the epileptogenic network in pharmaco-resistant TLE. The SOZ of pharmaco-resistant TLE based on ivEEG demonstrated the existence of strong interactions between limbic networks (Bartolomei *et al*., 2004). Patient 11 did not achieve seizure control without hippocampal resection at initial surgery of the enlarged amygdala, and he subsequently required hippocampal resection to become seizure-free. The most common pattern of seizure onset was regional, involving both the hippocampus and amygdala simultaneously in temporal lobe seizures (Quesney, 1986; So *et al*., 1989). ivEEG consistently showed ictal onset and interictal discharges in both the amygdala and hippocampus, including Case 8 with intra-ictal hippocampal discharges. The epileptic network can be estabilised between the MRI-negative hippocampus and enlarged amygdala in patients with TLE-AE.

Our small series of patients with pharmaco-resistant TLE-AE did not show any memory problems, as patients presented with MRI-negative TLE. Compared to patients with mesial temporal sclerosis, TLE patients with MRI-negative hippocampus showed a lower rate of memory disturbance (Bell *et al*., 2011). The negative hippocampus pathology in our patients was similar to other reports of MRI-negative hippocampus (Immonen *et al*., 2010). The onset of seizures in our 11 patients showed late onset (mean: 29.9 years) and short duration (mean: 5.0 years). As amygdala kindling is faster than hippocampal kindling in provoking limbic seizures, hippocampal sclerosis and memory dysfunction may not appear at the time seizures become pharmaco-resistant, secondary to the enlarged amygdala.

MHT was performed in seven patients with a mean age of 39.5 (range: 28-57) and a mean verbal score of 102 for dominant hemispheres. The MHT succeeded in saving their verbal memory score with a mean of 93 at 12-24 months.

MHT was developed to terminate the epileptic network and seizure spread in the hippocampus but save the normal neural memory network (Shimizu *et al*., 2006). Usami *et al*. reported that MHT spared memory functions in 24 patients (14 dominant side and 10 non-dominant side) for up to five years (Usami *et al*., 2016). In patients with pharmaco-resistant TLE-AE, MRI-negative hippocampus, and normal memory functions, MHT and amygdala resection might be a surgical option after multiple AED trials fail to control seizures.

Minami *et al*. reported that pathological findings of the nine resected hippocampi showed mild gliosis and six of them were classified as Grade I according to Watson's pathological grading for hippocampal sclerosis (Minami *et al*., 2015). Our hippocampal specimens did not show any sclerosis, gliosis, or inflammatory change, but showed subtle granular cell dispersion. We could not find histopathological abnormalities in the epileptogenic hippocampus and amygdala. In pharmaco-resistant TLE-AE, both amygdala and hippocampal pathology play an important role, but the mechanism for the enlarged amygdala and normal hippocampus that leads to intractable TLE remains unclear. The combination of amygdala resection and MHT might disrupt the epileptogenic limbic system.

In the right/non-dominant hemisphere, hippocampal resection did not affect visual memory in our three cases, however, further studies of postoperative visual memory function following either multiple transections or resection of the non-dominant hippocampus are required to establish how visual memory may be affected.

Lack of histopathological abnormality in the enlarged amygdala

Different types of pathology have been found based on previous studies of TLE-AE: focal cortical dysplasia, glioneuronal tumours, low-grade gliomas, or neuro-

inflammatory processes (Kim *et al*., 2012; Beh *et al*., 2016; Malter *et al*., 2016). In this series, we excluded these abnormalities of the amygdala based on MRI. Despite the similarity of clinical features between patients with these different types of pathology and those from our series, we did not observe any histopathological changes in any of the resected amygdalas.

Recovery of amygdala enlargement based on MRI has been reported following good seizure control by AEDs (Lv *et al*., 2014). The reversible change in amygdala indicates that amygdala enlargement occurs as a secondary, reactive phenomenon and not actual neuronal damage or malformation. Our 11 patients suffered with seizures for more than one year with multiple AEDs, secondary to the enlarged amygdala. The size of the amygdala did not change before surgery. Their seizures never remitted during medical treatments. The epileptogenicity of the enlarged amygdala in our series probably provoked pharmaco-resistant temporal lobe seizures.

Amygdala volumetry

Volumetric MRI analysis of hippocampi has been established to detect even subtle degrees of hippocampal atrophy (Cascino *et al*., 1991; Cook *et al*., 1992). Lateralized hippocampus volume loss on the epileptic side was shown to predict the presence of histopathological hippocampal sclerosis (Cascino, 1995).

Bower *et al*. were the first to attempt to estimate amygdala volumes in "MRI-negative" TLE (Bower *et al*., 2003). The boundaries between the amygdala and the adjacent structures of the hippocampus, putamen, and parahippocampal gyrus are poorly demarcated (Watson *et al*., 1992; Cendes *et al*., 1993). Normal amygdala volumes were shown to span a wide range (Brabec *et al*., 2010). The level of volumetric MRI analysis with regard to TLE-AE has not yet reached the same level as that for hippocampus volumetry (Mitsueda-Ono *et al*., 2011; Kimura *et al*., 2015; Beh *et al*., 2016).

In our series of TLE-AE without mesial temporal sclerosis, the laterality of amygdala volume, confirmed by both visual inspection and amygdala volumetry, correlated with the epileptogenic mesial temporal network.

Limitations of intracranial recording

We applied depth electrodes for the amygdala and subdural electrodes for the hippocampus and the lateral temporal region.

Minami *et al*. reported that it was not possible to detect IEDs from the amygdala based on ioECoG using strip electrodes (Minami *et al*., 2015). We, however, recorded ictal and interictal epileptiform discharges from the amygdala in all eight patients who underwent ivEEG using depth electrodes into the amygdala. The depth electrode was inserted using a navigation system after the temporal lobe was exposed. Both depth and subdural electrodes were effective for presurgical assessment in temporal lobe epilepsy (Valentín *et al*., 2017). The epileptic discharges originating from the hippocampus were clearly indicated by the "T"-shape strip electrode which longitudinally covered the hippocampus (Shimizu *et al*., 1992). The combination of depth electrode for the amygdala and T-shape strip electrode for the hippocampus was efficient in revealing epileptic discharges during ivEEG in our patients with TLE-AE. We do not have stereotactic electroencephalography (SEEG) capability in our institution, and this may be accurate and less invasive for patients with TLE-AE.

Conclusions

When TLE-AE becomes pharmaco-resistant, the epileptogenic zone involves both the amygdala and hippocampus. ivEEG may be required to explore the SOZ in the normal hippocampus in addition to the enlarged amygdala. For patients with dominant-side TLE-AE, amygdala resection and MHT control the epileptogenic limbic system and save memory function in patients with TLE-AE. □

Supplementary data.
Summary didactic slides are available on the www.epilepticdisorders.com website.

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TEST YOURSELF

(1) Where is the seizure onset zone in patients with pharmaco-resistant temporal lobe epilepsy with amygdala enlargement?

(2) What is the surgical treatment for pharmaco-resistant temporal lobe epilepsy with amygdala enlargement and MRI-negative hippocampus?

(3) What is the histopathological finding of the enlarged amygdala in temporal lobe epilepsy with amygdala enlargement?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".