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

Running title

Temporal lobe epilepsy-amygdala enlargement

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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), and epilepsy surgery.

Methods:

We enrolled 11 patients with TLE-AE. We investigated seizure profiles, volumetric MRI analysis, the results of the Wechsler Memory Scale-Revised(WMS-R), the locations of seizure onset zone(SOZ) and irritative zone(IZ) on ivEEG(n=8), the locations of interictal epileptiform discharges(IEDs) on ioECoG(11), surgical procedures, and seizure outcomes.

Results:

The mean age at seizure onset was 34.9 (range, 23-57). The mean duration of seizure was 5.0 years (1-10 years). The number of AEDs was 2.3(1-5). The mean seizure frequency was nine per month(1-30 per month). All patients presented focal impaired awareness seizures with(n=9) and without(2) secondary generalized convulsions.

Volumetric MRI analysis showed unilateral enlarged amygdala with a statistical significance ($p<0.01$). No patient's hippocampus had any abnormality on MRI. Pre-operative mean verbal, visual, and delayed recall scores on WMS-R were over 100. IvEEG showed SOZ and IZ in both amygdala and hippocampus(7) and in only amygdala(1). IoECoG showed IEDs in hippocampus(6) and in both amygdala and hippocampus(4). All 11 patients underwent anterior temporal lobectomy, including amygdala resection, with multiple hippocampal transections(7, dominant hemisphere) and resection(3, non-dominant hemisphere). Nine(81.8%) of 11 patients achieved seizure freedom with a mean follow-up of 26 months(12-47 months). Post-operative WMS-R results did not show any significant deteriorations with a mean follow-up of 15 months(12-24 months). The resected amygdala showed no histopathological abnormality.

Conclusion:

The epileptogenic zone of TLE-AE involves both 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 could control the epileptogenic limbic system and save memory function in patients with TLE-AE.

1, Introduction

1.1, Temporal lobe epilepsy with amygdala enlargement

Amygdala enlargement (AE) on MRI was first reported by Tebartz et al. in patients with temporal lobe epilepsy (TLE) and dysthymia in 1999 (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 amygdala were well studied using CT and MRI (Tebartz Van Elst et al., 2002).

Bower et al. presented seven patients with temporal lobe epilepsy with amygdala enlargement (TLE-AE) but without hippocampal sclerosis in 2003 (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 seizure. Fourteen articles of TLE-AE summarized 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).

1.2, Epileptogenic zone in temporal lobe epilepsy with amygdala enlargement

The epileptic network between 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 (Cain, 1992, Racine, 1986). The importance of amygdala as 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 the surgical resection (Beh et al., 2016).

1.3, Multiple hippocampal transections

Shimizu et al. developed hippocampal transections for 21 TLE patients with normal hippocampus and spared verbal memory (Shimizu et al., 2006). Of 17 patients

with more than one-year follow-up, 14 (82%) patients became seizure free. Verbal memory functions of the dominant hemisphere remained the same as before surgery for postoperative six months. Multiple hippocampal transection (MHT) has been applied in TLE with MRI-negative hippocampus and intact memory functions but with extra-mesial temporal lesions (Usami et al., 2016, Ishida et al., 2018, Girgis et al., 2017). Minami et al. described that 11 patients with pharmaco-resistant TLE-AE 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 the sharp waves were not originated from the amygdala but from the hippocampus. There is no report of the intracranical video EEG (ivEEG) to localize the seizure onset zone and irritative zone with active interictal epileptiform discharges (IEDs) in patients with pharmaco-resistant TLE-AE. This paper is the first preliminary report about ictal recording using ivEEG and epilepsy surgery in patients with pharmaco-resistant TLE-AE and MRI-negative hippocampus.

1.4, Hypothesis

We hypothesize that the epileptogenic zones in the pharmaco-resistant TLE-AE are both enlarged amygdala and MRI-negative hippocampus. Surgical intervention for both 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.

2, Materials and Methods

2.1, 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: (1) enlargement of amygdala compared to the contralateral side on MRI, (2) seizure semiology related to ipsilateral temporal lobe epilepsy, and (3) seizures

refractory to appropriate AEDs for one year.

2.2, Scalp video-EEG

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

2.3, MRI

We performed 3T MRI with T1 and T2-weighted spin-echo, three-dimensional fluid-attenuated inversion recovery (FLAIR) image 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 tumors or vascular lesions were excluded.

To confirm visual diagnosis of TLE-AE in the included patients, amygdala

and hippocampus volumes were quantified by a fully automated volumetry using the Free Surfer Software (Version 6.0.0; Martinos Center, Harvard University, Boston, MA, U.S.A.) in T1-weighted images (Pardoe et al., 2009, Coan et al., 2013). The MRIs of 10 normal controls (20-29 years old; mean age, 23.2) were used for comparison.

Amygdala and hippocampus volumes were statistically analyzed by 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 in patients with TLE-AE were also compared to the normal controls by unpaired t-tests. These analyses were performed using R 3.4.4 statistical software (The R Development Core Team).

2.4, Wada test

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

2.5, Intracranial video EEG

We performed intracranial video EEG (ivEEG) to evaluate irritative zone and seizure onset zone in eight patients. One depth electrode (4 contacts) was placed into the amygdala (Figs. 1A and B). Mesial temporal strip electrodes were subtemporally inserted according to Shimizu's method (Shimizu et al., 1992). One T-shape strip electrode covered hippocampus (4 contacts) and subtemporal region (4 contacts) (Fig. 1C). One uncal strip electrode (4 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 2 kHz sampling rate. We defined the seizure onset zone as: (1) rhythmic spikes or sharp waves, (2) paroxysmal fast activity, and (3) attenuation of background activity.

2.6, Intraoperative ECoG

All patients underwent ioECoG recordings. Anesthesia 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 hippocampus and amygdala using platinum electrodes (Unique Medical, Tokyo, Japan). IoECoG monitoring continued for 3-10 minutes before hippocampal resection or multiple transections.

2.7, 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 seizure onset zone in hippocampus and/or ioEEG showed IEDs from hippocampus, additional hippocampal multiple transections or resection were performed.

2.8, Seizure outcome

All patients were followed up for at least one year after surgery. Postoperative seizure outcomes were assessed at the last visit according to Engel's classification

(Engel Jr, 1993).

2.9, Memory functions

We performed Wechsler Memory Scale-Revised (WMS-R) to evaluate memory functions before surgery, 6 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.

2.10, Histpathology

Amygdala and hippocampus specimens were fixed with phosphate-buffered 20% formalin, and embedded in paraffin for histologic evaluation. The surgical specimens were sectioned with 4 μm thickness. They were 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).

3. Results

3.1, Characteristics of patients

Eleven patients (five females) with TLE-AE were included in this study. Table 1 describes the clinical features.

The mean age at surgery was 34.9 (range, 23-57). The age of seizure onset was 29.9 (13-55). The mean duration of seizure was 5.0 years (1-10 years). One patient had a history of febrile convulsions. They had no comorbidity of psychiatric disorders. The number of AEDs ranged from one to five with a mean of 2.3. Nine (81.8%) patients had taken multiple AEDs. Levetiracetam was taken in nine (81.8%) patients. Carbamazepine and Valproic acid were each taken in five (45.5%) patients. Lamotrigine was taken in two patients. Clonazepam, Gabapentin, Lacosamide, Zonisamide were each taken in one patient.

3.2, Characteristics of seizures

The frequency of seizures at the time of surgery ranged from one to 30 per month (mean, nine per month).

All 11 patients presented seizures during scalp video EEG. Five patients presented preceding auras. None of them complained of fear. All 11 patients showed behavior arrest of focal impaired awareness seizures (FIAS). Eight (72.7%) patients showed manual automatism. One (9.1%) patient showed oral automatism. Following generalized convulsions were seen in nine (81.8%) patients.

3.3, MRI

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 in hippocampus on MRI in all 11 patients. Figures 2A and B show representative MRI images of the enlarged amygdala and normal hippocampi. There was no other extratemporal abnormality on MRI. Enlarged amygdala of all 11 patients had the 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 for 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 those of 10 normal controls.

On the epileptic side of patients with TLE-AE, amygdala volume ranged from 1768.6 mm^3 to 2486.4 mm^3 (mean, 2051.5 mm^3). On the non-epileptic side, amygdala volume ranged from 1503.4 mm^3 to 2471.3 mm^3 (mean, 1789.8 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 1441.5 mm^3 to 2209.7 mm^3 (mean, 1802.5 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 4061.2 mm^3 to 5299.0 mm^3 (mean, 4477.7 mm^3). On the non-epileptic side, hippocampus volume ranged from 3625.5 mm^3 to 4987.3 mm^3 (mean, 4297.4 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 3591.1 mm^3 to

5085.2 mm^3 (mean, 4401.6 mm^3). Hippocampus volumes on the epileptic side of patients with TLE-AE were not smaller than those of normal controls.

3.4, Dominant hemisphere by WADA test

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

3.5, 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 (37-202 hours).

Seven patients had IEDs on both 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 seizures) with a mean of three seizures. The seizure onset was found in both amygdala and hippocampus in seven

patients (Figure 4). The other patient (case #8) showed seizures coming from only the amygdala. The seizures subsequently involved the hippocampus to present FIAS and generalized convulsion.

3.6, Intraoperative electrocorticography

In all 11 patients, we performed the ioECoG on 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 amygdala and hippocampus. The remaining two patients did not show any IEDs.

Because of the refusal of ivEEG in the three patients (#9, #10, #11), we performed only ioEEG for them. The ioECoG showed IEDs from both hippocampus and amygdala in patient #9, from only hippocampus in patient #10. The ioECoG in patient #11 showed no IEDs at the first surgery. He had recurrent seizures after the amygdala resection alone. Twelve months later, the epileptic-side hippocampus showed IEDs and was resected at the second surgery.

3.7, Surgical procedures

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 (6 left; 1 right). The one 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 the first surgery, which did not improve his seizures. We then performed right-hippocampal resection 12 months after the first surgery.

Patient #8 underwent only amygdala resection because the ivEEG showed seizure onset and IEDs in only the amygdala.

3.8, Seizure outcomes

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

patients (Ia), one patient (Ib) and one patient (IIa).

3.9, Pre- and post-operative memory functions

Pre- and post-operative verbal, visual and general memory, note concentration, and delayed recall of serial WMS-R are presented in Figure 5.

Before surgery, mean ± standard deviation of these parameters was 110 ± 14.6 (verbal memory), 104 ± 12.5 (visual memory), 110 ± 12.1 (general memory), 102 ± 8.7 (note concentration) and 101 ± 12.9 (delayed recall). Pre-operative cognitive performance was not significantly different between left (7) and right (4) TLE-AE.

At six months after surgery, mean ± standard deviation of these parameters was 99 ± 18.7 (verbal memory), 108 ± 8.5 (visual memory), 100 ± 16.4 (general memory), 109 ± 12.1 (note concentration) and 102 ± 13.7 (delayed recall). Verbal memory and general memory at six months after surgery showed decline from those before surgery without statistical significance, using one-way repeated measures ANOVA.

At one to two years after surgery, mean ± standard deviation of these parameters was 102 ± 21.0 (verbal memory), 108 ± 5.6 (visual memory), 104 ± 15.7

(general memory), 108 ± 8.7 (note concentration) and 103 ± 13.2 (delayed recall) with a mean follow-up of 15 months. There was no significant post-operative decline in all memory functions.

3.10, 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 granular cell dispersions were found in all three hippocampal specimens.

4, Discussion

4.1, Summary of findings

We collected 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 seizure

onset zone involving hippocampus in addition to amygdala. The ioECoG presented IEDs on hippocampus in 10 out of 11 patients, including four patients showing IEDs on amygdala. All 11 patients underwent amygdala resection. Ten of them underwent additional hippocampal treatments: seven patients (MHT in the dominant hemisphere) and three patients (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 one-year follow-up. Postoperative memory functions were spared in all patients, even after MHT in the dominant hemisphere (7) and hippocampal resection in the non-dominant hemisphere (3).

4.2, Epileptogenic enlarged amygdala

This paper is the first to report that the seizure onset zone includes both enlarged amygdala and MRI-negative hippocampus in TLE-AE patients. Amygdala-involved seizures were characterised by ictal fear, gastrointestinal sensations, and marked autonomic symptoms (Wieser, 2000, Cendes et al., 1994, 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 seizure onset zone in a subset of patients with TLE-AE.

Minami et al. reported that ioECoG in TLE-AE showed no sharp waves originated from 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 approach. IEDs 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 seizure onset zones localized in the amygdala and hippocampus. We treated both amygdala and hippocampus with favourable seizure outcomes.

4.3, Hippocampus in temporal lobe epilepsy with amygdala enlargement

The ivEEG localized the seizure onset zone and irritative zone in hippocampus in seven out of eitht 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 38-72% of patients with MRI-negative TLE revealed seizures arising from mesial temporal structures on ivEEG (Muhlhofer et al., 2017). MRI-negative hippocampus was one of the high-risk structures for the epileptogenic network in pharmaco-resistant TLE. The seizure onset zone of pharmaco-resistant TLE 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 the first surgery of the enlarged amygdala resection. 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). All ivEEG showed ictal onset and interictal discharges in the both amygdala and hippocampus, including case #8 with intra-ictal hippocampal discharges. The epileptic network can be establised between 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 MRI-negative TLE. Compared to patients with

mesial temporal sclerosis, TLE patients with MRI-negative hippocamps 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 to provoke limbic seizures, hippocampal sclerosis and memory dysfunction may not appear at the time of seizures becoming pharmaco-resitant 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.

Multiple hippocampal transection 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 succeeded to spare 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 dispersions. 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 of the enlarged amygdala and normal hippocampus becoming 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. We need a further study of postoperative visual memory function by either multiple transections or resection for the non-dominant hippocampus.

4.4, Lack of histopathological abnormality in enlarged amygdala

Different types of pathology have been found in previous studies of TLE-AE: focal cortical dysplasia, glioneuronal tumors, low grade gliomas, or neuroinflammatory processes (Kim et al., 2012, Beh et al., 2016, Malter et al., 2016). We excluded these MRI abnormalities in the amygdala in this series. Despite their clinical features being similar to ours, we did not observe any histopathological changes in any of the resected amygdalas.

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

4.5, Amygdala volumetry

Volumetric MRI analysis of hippocampi has been established in detecting even subtle degrees of hippocampal atrophy (Cascino et al., 1991, Cook et al., 1992). The lateralized hippocampus volume loss on the epileptic side predicted 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). Amygdala has poorly demarcated the anatomical boundaries to adjacent structures of hippocampus, putamen, and parahippocampal gyrus (Watson et al., 1992, Cendes et al., 1993). The normal amygdala volumes spanned a wide range (Brabec et al., 2010). Volumetric MRI analysis with regard to TLE-AE has not reached the same level of hippocampus volumetry yet (Beh et al., 2016, Mitsueda-Ono et al., 2011, Kimura et al., 2015).

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.

4.6, Limitation of intracranial recordings

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

Minami et al. reported that ioECoG using strip electrodes could not detect IEDs from amygdala (Minami et al., 2015). We, however, recorded ictal and interictal epileptiform discharges from 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 well-reflected by the “T”-shape strip electrode which covered longitudinally on the hippocampus (Shimizu et al., 1992). The combination of depth electrode for amygdala and T-shape strip electrode for hippocampus were efficient in revealing epileptic discharges during ivEEG in our patients with TLE-AE. Meanwhile, we do not have Stereotactic Electroencephalography (SEEG) capability in our institution. SEEG can be accurate and less invasive for patients with TLE-AE in the future.

5. Conclusions

When TLE-AE becomes pharmaco-resistant, the epileptogenic zone involves both amygdala and hippocampus. The ivEEG may be needed to explore the seizure onset zone 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 TLE-AE.

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Disclosure

None of the authors have any conflict of interest to disclose.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure legends

Fig.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 toward the enlarged amygdala (case #3).

(C) 3D-MRI shows mesial temporal strip (T shape) to cover the hippocampus, uncal strip and a part of grid to cover the lateral temporal region (case #4).

Fig.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.

Fig.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 temporal lobe epilepsy with amygdala enlargement (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.

Fig.4. Intracranial video EEG at the time of seizure onset (case #6)

Referential montage using an epidural electrode for reference

Blue: 4 amygdala depth electrodes (top) and 3 uncal strip electrodes (bottom)

Red: 4 hippocampal electrodes (top) and 3 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 of uncal strip and the other of subtemporal electrode) were eliminated due to artifacts.

Fig. 5. Pre- and post-operative memory functions of verbal, visual, general memories, note concentrate 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 extremes of the whiskers contain 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 (6 months, between 12 and 24 months).

There is no significant difference between all pre- and post-operative memory functions.

Short questions and answers

Q-1）

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

A-1)

Amygdala and hippocampus.

Q-2)

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

A-2)

Amygdala resection and multiple hippocampal transections.

Q-3)

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

A-3)

No pathological change.

Figure 1

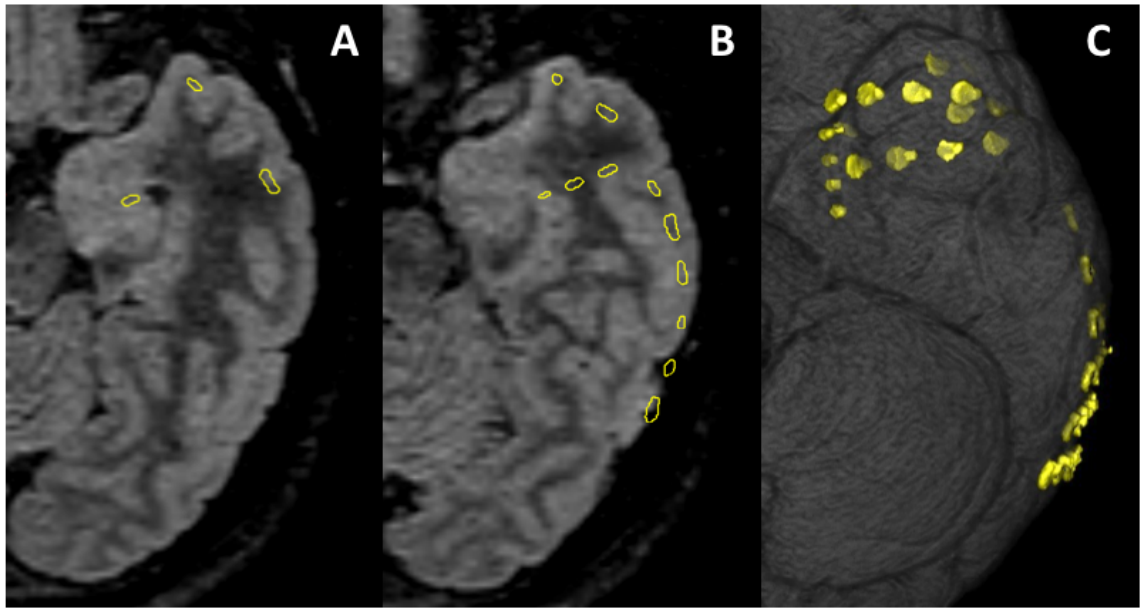


Figure 2

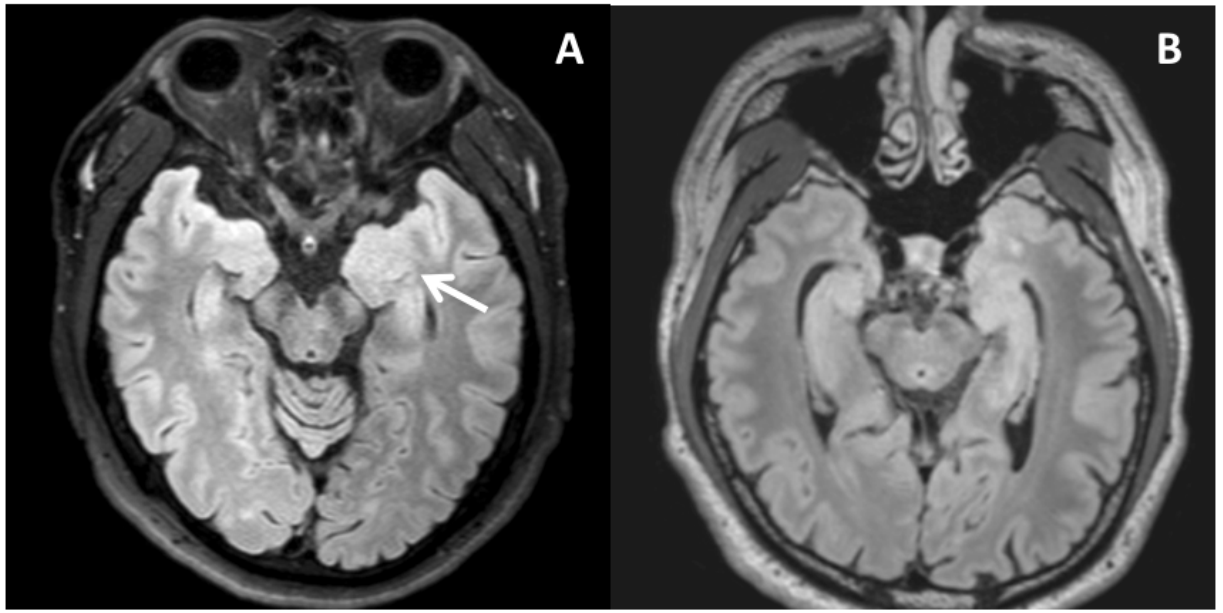
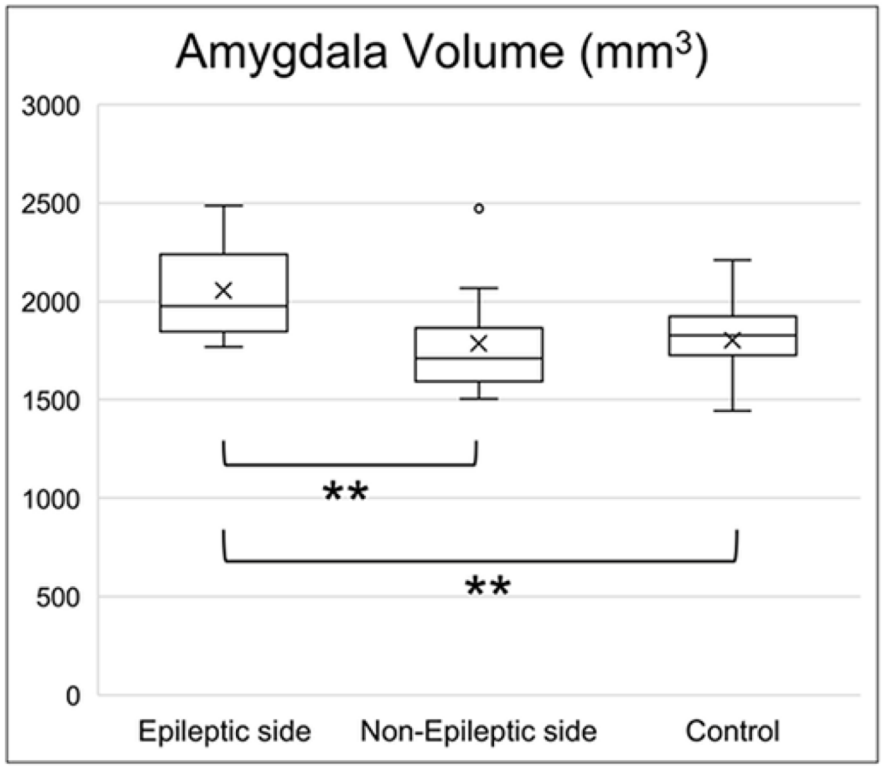


Figure 3


Amygdala Volume (mm3)
3000
2500
2000
1500
1000
500
0
**
**
Epileptic side
Non-Epileptic side
Control

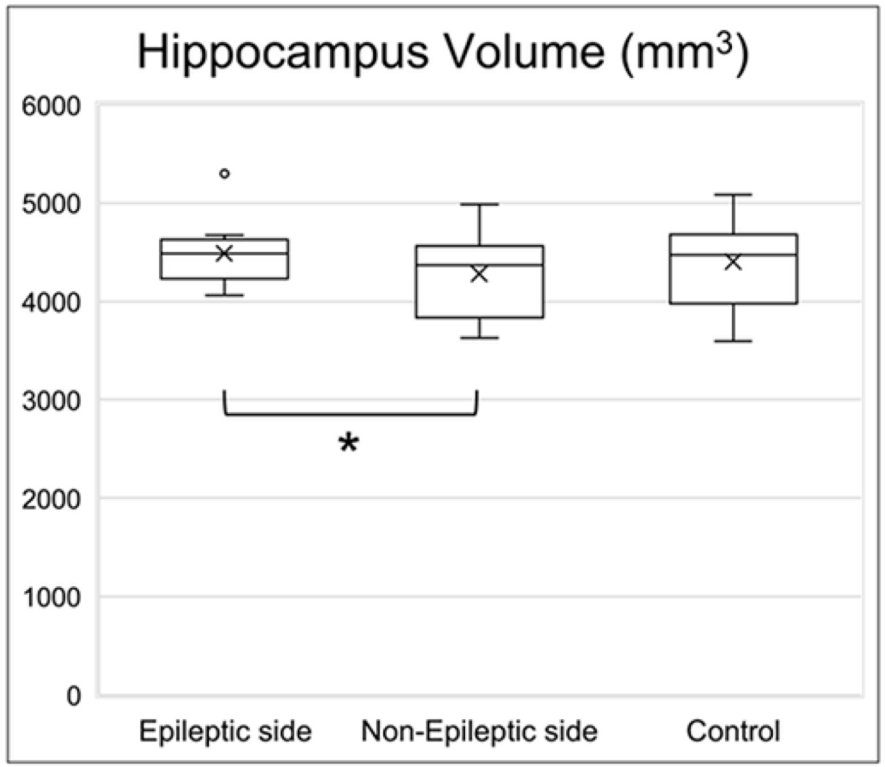

Hippocampus Volume (mm3)
6000
5000
4000
3000
2000
1000
0
*
Epileptic side
Non-Epileptic side
Control

Figure 4

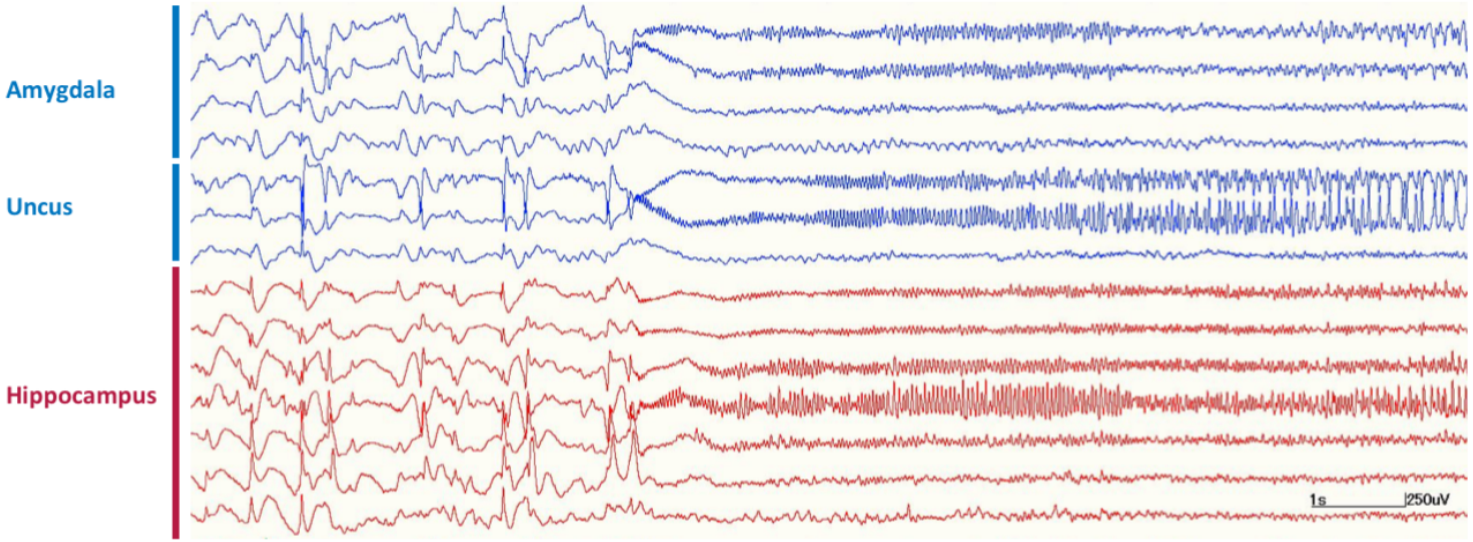


Figure 5

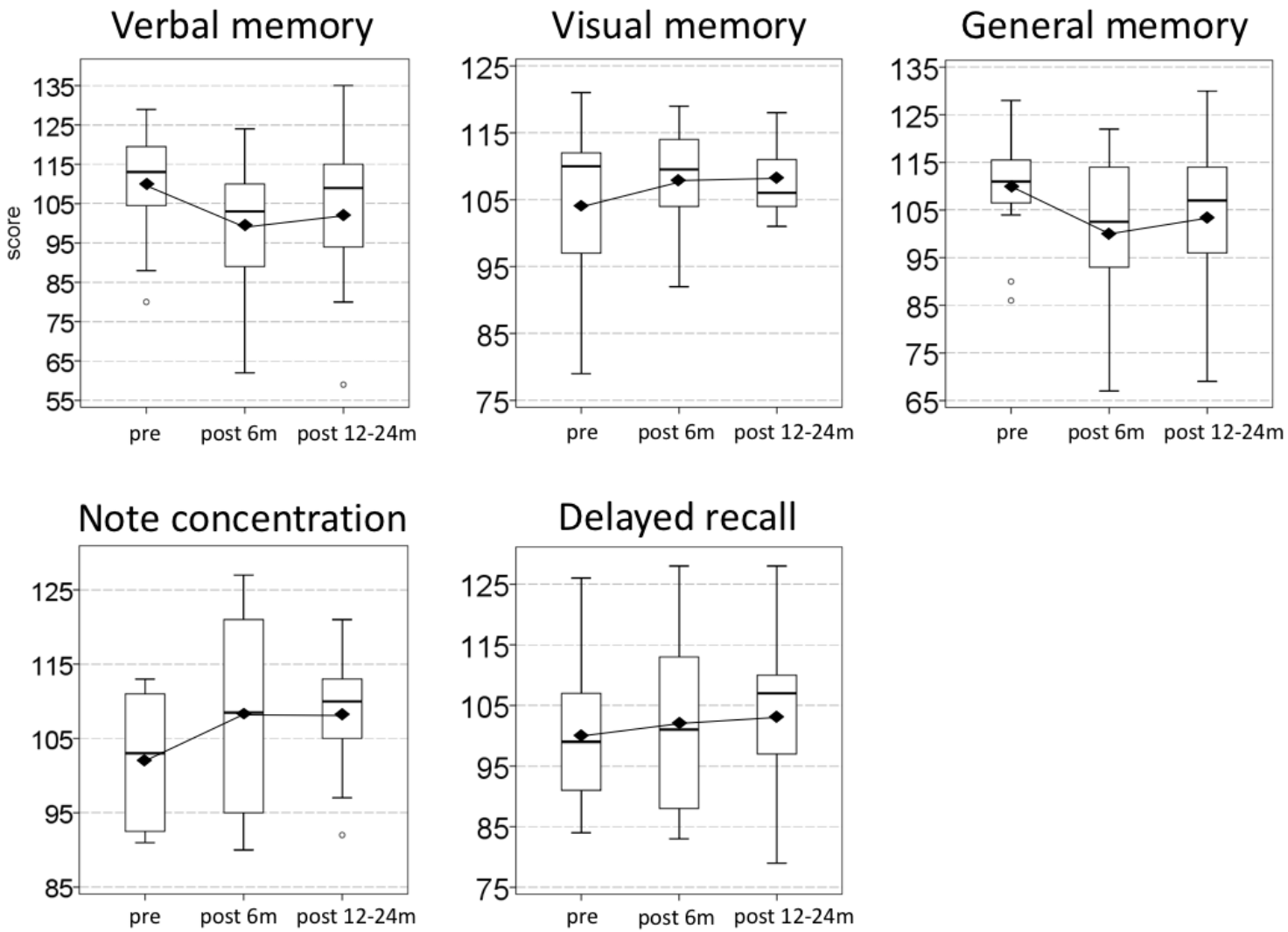

Verbal memory
Visual memory
General memory
Note concentration
Delayed recall
score
pre
post 6m
post 12-24m

Table1, Clinical profiles

No.	Age at surgery (years) Gender	Duration of seizure (years)	Preoperative AED	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,Lamotrigin; VPA,Valproic scid; ZNS,Zonisamide; 2G,Secondary Generalized Seizure

Table2, Surgery and seizure outcome

No. of case	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,Anti-epileptic drugs; A,Amygdala; D,Dominat 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 of AEDs; ↑,Increase of AEDs; →,No change