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Focal cortical dysplasia: pathophysiological approach

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Focal Cortical Dysplasia: Pathophysiological approach

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Abstract

Clinical and experimental studies on focal cortical dysplasia (FCD) were carried out. For the experimental study, an experimental FCD model of rats was developed. Twenty Wistar rats at 0-2 days after birth were used for the study. Kainic acid (KA) solution was injected stereotaxically into medial and lateral sites of the sensori-motor cortex. Bipolar electrodes were inserted in 5 rats. Their behavior and EEG were recorded using a digital video-EEG monitoring system. After observation periods of 1, 2 and 6 months, rats were perfused for pathological study. FCD was observed adjacent to the site of KA

injection in all rats more than one month after the injection. EEG recording demonstrated focal spike discharges in and around the site of injection. However, clinical seizure was not observed. Pathological studies showed decrease in GABA-A receptors and increase in GABA-B receptors not only in the lesion but also in perilesional areas. Fifteen surgical cases of FCD with intractable epilepsy were subjected to the clinical study. Neuro-imaging studies including high-resolution MRI and SPECT were performed. Conventional EEG studies demonstrated focal EEG abnormalities with epileptic phenomena. At surgery, intraoperative electrocorticography (ECoG) was performed in order to localize epileptic foci under neuroleptoanalgesia. Thirteen patients showed epileptiform discharges on preresection ECoG. All foci in non-eloquent areas were resected. Pathological studies including immunohistochemical staining were performed, and characteristics of the FCD in relation to EEG findings were analyzed. Patients in whom total lesionectomy with complete focus resection was performed had favorable postoperative courses. Nine patients (64.3%) have been seizure-free with reduced medication, and significant improvement was achieved in two patients (14.3%). Electrophysiological examination revealed epileptogenecity not only in the lesions but also in perilesional areas. The immunohistochemical studies showed decrease in GABA-A receptors and increase in GABA-B receptors in both the lesions

and perilesional areas, but NMDA receptors were almost negative in both areas. Glutamate R-1 was decreased in both areas, but glutamate R-2 was increased in both areas. These findings support the results of electrophysiological study. In conclusion, not only the epileptic property of experimental focal cortical dysplasia but also perilesional epileptogenesis was demonstrated. These findings supported the results of surgery for patients with focal cortical dysplasia. In cases of FCD, total removal of the lesion and resection of the perilesional epileptic focus are needed for a good outcome.

Introduction

Cases of intractable epilepsy have been aggressively treated in recent years by surgical therapies, including focus resection. Moreover, due to recent developments in imaging techniques, it has been revealed that various types of cerebral dysplasia, such as focal cortical dysplasia (FCD) and dysembryoplastic neuroepithelial tumor (DNT), are causes of intractable epilepsy in children. Such lesions have shown abnormal neuroglial maturation and differentiation, and it has been shown that there is a close relationship between these dysplastic lesions and epileptogenecity. In epileptic focus resections, we have tried to precisely identify the epileptic focus by using intraoperative electrocorticography (ECoG) and to perform complete resection, but we have often

observed epileptiform discharges also in perilesional areas such as the site of FCD. We have carried out pathological studies on epileptic foci. In the present study, we carried out neuroradiological, electrophysiological and pathological examinations using specimens of resected epileptic foci and an FCD model that we developed in neonatal rats. In this paper, we will discuss about possible mechanism of epileptogenicity of the focal cortical dysplasia.

Materials and Methods:

1. Study in experimental focal cortical dysplasia (FCD) model of rats

Twenty Wistar rats at 0-2 days after birth were used for the study. Kainic acid (KA) solution was injected stereotaxically into the lateral sites on the sensori-motor cortex. Bipolar electrodes were inserted in 5 rats 60 days after KA injection. Their behavior and EEG were recorded using a digital video-EEG monitoring system. After observation periods of 1, 2 and 6 months, rats were perfused for pathological and autoradiographical studies. Immunohistochemical staining for GABA-A and GABA-B receptors and for NMDA-R1 and NMDA-R2 was performed in addition to conventional HE staining for pathological examination of resected specimens.

2. Study in FCD clinical cases

Fifteen cases of FCD with intractable epilepsy were analyzed. The 15 patients included 7 males and 8 females with ages ranging from 0.9 to 18 years (mean age: 8.5 years). The duration of disease ranged from 0.6 to 8 years (mean duration: 4.6 years). The types of seizures were GS in 12 patients, SPS in 8 patients, CPS in 4 patients and West syndrome in one patient. The mean follow-up period after surgery was 18 months. Lesions and epileptic foci were examined by high-resolution MRI, ictal and/or interictal SPECT and conventional EEG, and ECoG and cortical mapping were also performed after insertion of a subdural electrode for cases with lesions in eloquent areas. After the electrophysiological and radiological examinations, lesionectomy and/or resection of the epileptic focus were performed. At surgery, intraoperative ECoG was performed in order to localize epileptic foci under neuroleptoanalgesia. The resected specimens were subjected to pathological examination, including HE and immunohistochemical staining for GABA-A,B, NMDA receptors 1,2 and glutamate receptors 1,2.

Results

1. Study in experimental focal cortical dysplasia (FCD) model of rats

Epileptiform discharges at and around the site of injection were observed by digital video-EEG monitoring in the 5 Wistar rats in which kainic acid (KA) was injected. Sporadic spike discharges were also observed around the site of injection (Fig. 1, upper right). However, clinical seizure was not observed during observation period of 6 months. An autoradiogram obtained by using ^{125}I-iomazenil showed a reduction in uptake at the site of KA injection, suggesting a decrease in GABA－A receptors (Fig. 1, lower right). Specimens stained with HE showed very localized focal cortical dysplasia (FCD) as well as dyslamination and clusters of dysplastic neurons under the cortex, and the severity of dysplasia was diagnosed to be Palmini Grade **I** a ~ **II** a (Fig. 1, right). Immunohistochemical staining for GABA-A showed a reduction in staining intensity at and around the site of FCD and faint staining in some clusters of dysplatic neurons (Fig. 2, upper left). Staining for GABA-B showed staining at and in a large area around the site of FCD (Fig. 2, upper right). There was almost no staining for NMDA-R1 or NMDA-R2 (Fig. 2, lower). Results of staining are summarized in Table 1.

2. Examination in FCD clinical cases

Lesion sites could be identified in all 15 cases by MRI, in 12 cases (75%) by EEG and

in 9 cases (60%) by SPECT (Fig. 3). Intraoperative ECoG have been recorded in 14 cases, and sporadic spike discharges occurred in all cases not only at the lesion site but also around the lesion, indicating the presence of seizure-induced zones and irritable zones (Fig. 4). Lesionectomy plus focus resection was performed in 14 cases (93.3%), and multiple subpial transection (MST) was also performed in 5 of those cases (35.7%) because of incomplete focus resection especially in the eloquent areas. Following surgery, 10 patients (66.7%) were free of seizure (Engel class Ⅰ), 2 patients were Engel class Ⅲ and 3 patients were Engel class Ⅳ. Pathological examination of resected specimens stained with HE showed FCD of Palmini type Ⅰa～Ⅱc in all cases and angiogenesis suggesting vascular abnormality in one case (Figs. 5, 6). Immunohistochemical study showed a decrease in GABA-A receptors and an increase in GABA-B receptors and also showed an increase in glutamate-2 receptors but no or only a slight increase in NMDA receptors (Fig. 6). The results of immunohistochemical study, which are summarized in Table 2, showed decrease in GABA-A staining and positive GABA-B staining in both the lesions and perilesional areas. Moreover, NMDA R-1/R-2 staining and glutamate R-1 staining were negative and glutamate R-2 staining was positive.

Discussion

Various methods have been used to make FCD models[4-6,10]. We have made various epilepsy models using injection of KA[12-14,16]. In the model used in the present study, KA was injected stereotaxically in newborn rats, and the reproducibility of focal cortical dysplasia was therefore high with outerlayer of the cortex (I and II). Although epilepsy seizures were not seen by video-EEG monitoring, the fact that frequent intermittent slow spikes and waves and spike bursts in the sensori-motor cortex at the injection site were seen on EEG and the fact that the rats had FCD of Palmini Grade I a ～II a[9] indicate that the model is relatively stable and highly reproducible and is therefore a useful model. EEG showed sporadic spike discharges not only in the lesion but also around the lesion in the model. ^{125}I-iomazenil autoradiography showed decrease in accumulation of the tracer in the lesion, suggesting a decrease in GABA-A receptors. On the other hand, results of immunohistochemical staining showed reduction in GABA-A staining at the site of FCD and in normal brain tissue around the FCD site, but GABA-B staining was positive in both the lesion and perilesional areas. NMDA staining was negative. The results of our electrophysiological and pathological studies taken together with the results of a study indicating that GABA-A is involved in suppression of seizures and that GABA-B is involved in promotion of seizures[15]

suggest that there is epileptogenecity not only in FCD but also in the area around the lesion.

Results of electrophysiological study in the clinical FCD cases similarly showed the existence of seizure onset zones, seizure induced zones and irritable zones not only in the lesion but also around the lesion. Pathological study showed a decrease in GABA-A and an increase in GABA-B as were seen in the rat model. NMDA was almost negative, as was glutamate R-1. However, glutamate R-2 was positive in all cases. There have been a few recent reports on the use of pathological study to identify epileptogenecity in cases of intractable epilepsy accompanying dysplasia such as FCD. Spreafico et al.[11] carried out immunohistochemical studies on FCD resected specimens and reported that an increase in excitatory neurons and a decrease in GABAergic interneurons were involved in epileptogenecity. Benardete et al.[2] observed a dramatic increase in epileptiform discharge when GABA-A receptors were blocked in a CD model made by administration of BCNU to pregnant rats, and they proposed that GABA is involved in epileptogenecity. Mikuni et al.[7] and Najm et al.[8] also carried out immunohistochemical studies on FCD resected specimens and reported that NMDA R-1 and NMDA R-2A/B are involved in epileptogenecity. Aronica et al.[1] examined metabotropic glutamate receptor subtypes (mGluRs) in

FCD resected specimens and reported that group I mGluRs are involved in epileptogenecity. Hagemann et al.[3] produced freeze lesions in rats which mimic human polymicrogyria and performed immunohistochemical studies on NMDA, AMPA and KA receptors. They reported the lack of gross and/or widespread alterations of glutamate-receptor subunit distribution in the surround of focal cortical dysplasia, and suggested the presence of other or additional mechanisms underlying the increased excitatory neurotransmitter binding and excitability in cortical malformations.

The results of our studies in the experimental model and clinical cases suggest the involvement of GABA receptors in epileptogenecity. However, further study is needed in immunohistochemical study using other antibodies for various receptors. Further study using imaging techniques other than autoradiography and SPECT, such as PET, is also needed when these tools become more high resolution level.

Postoperative control of epilepsy in the clinical cases was good: 10 patients (66.7%) were free of seizure (Engel class I), 2 patients were Engel class III and 3 patients were Engel class IV. The outcome was better in patients in whom sufficient resection of the focus including the perilesional area was performed. This indicates the importance of sufficient focus resection after precise identification of the epileptic

focus by preoperative and intraoperative examinations with neuroradiological and electrophysiological examinations.

Conclusion

Electrophysiological and pathological examinations of the FCD model in rats and clinical cases of FCD revealed the presence of epileptogenecity not only in the lesion but also in the perilesional area. These findings were supported by the results of surgery: postoperative control of epilepsy was very good in patients in whom sufficient focus resection was performed in addition to lesionectomy. Precise identification of the epileptic focus by preoperative and intraoperative electrophysiological examinations is therefore important for a good outcome.

Figure legends

Fig.1: Left: Both low magnification (upper) and high magnification (lower) images of HE-stained specimens from Wistar rats in which kainic acid (KA) had been injected showed localized dysplasia (FCD) in and around the site of KA injection as well as dyslamination and clusters of dysplastic neurons under the cortex, and the dysplasia was diagnosed as Palmini Grade Ⅱa. Upper right: Digital video-EEG

monitoring showed the appearance of epileptiform discharges in and around the KA injection site and sporadic spike discharges in and around the injection site. Lower right: ^{125}I-iomazenil autoradiography revealed reduction in uptake at the site of KA injection, suggesting a decrease in GABA-A receptors.

Fig.2: Immunohistochemical staining in the rat focal cortical dysplasia (FCD) model showed a reduction in staining intensity for GABA-A at and around the site of FCD and faint staining in some clusters of dysplatic neurons (upper left). Staining for GABA-B showed staining at and in a large area around the site of FCD (upper right). Lower: There was almost no staining for NMDA-R1 (left) or NMDA-R2 (right).

Fig.3: Case 15 was an 18-year-old female with CPS (7 year-old onset). Brain MRI (upper images) revealed a cystic lesion in the left posterior temporal lobe. The FLAIR image on the left shows high signal intensity in the deep part of the lesion. The lesion was not enhanced by administration of Gd-DTPA on the right. EEG topography (lower images) showed epileptiform discharges corresponding to the lesion site. The ^{125}I-IMP ictal-SPECT image (middle) showed accumulation of tracer corresponding to the lesion site, but less accumulation at the same site was seen on a ^{125}I-iomazenil SPECT

image.

Fig.4: Since the lesion in case 15 was near Wernicke's area, a subdural electrode was inserted and video-EEG monitoring and cortical mapping were performed. Observation of the brain surface at surgery showed a dark-red lesion with small protrusions and slight cloudiness of the arachnoid membrane but no obvious abnormalities in the perilesional areas (upper left). ECoG at the time of seizure is shown in the upper right, and cortical mapping findings are shown in the lower images. A seizure onset zone, seizure induced zone and irritable zone were present in perilesional areas, and they overlay with Wernicke ' s area. Lesionectomy and as much focus resection as possible were performed in this patient. However, the epileptiform discharges did not disappear, and multiple subpial transection was also performed. The patient has been free of seizure (Engel class I) since surgery.

Fig.5: Case 4: 9-year-old boy with CPS (5 year-old onset). The upper images are brain MR images (left: FLAIR image, middle: T2W1 image, right: T1W1-Gd image), and the lower left image is a brain CT image. An enhanced mass was seen in the right medial temporal lobe. The lower right images are an interictal SPECT image (left)

and an ictal SPECT image (right). In the lesion, a decrease in blood flow was seen in the seizure interval stage and an increase in blood flow was seen in the paroxysmal period.

Fig.6: Case 4. HE staining of an epileptic focus resected specimen is shown in the upper left image. Angiogenesis can be clearly seen in a part of the FCD site. In the lower left image, reduced staining intensity for GABA-A can be seen in the lesion and perilesional area except for blood vessels. In the lower right image, GABA-B staining can be seen in the lesion and in a large area around the lesion. Glutamate R-1 staining (upper right) was mostly negative, whereas glutamate R-2 staining (lower) was positive in a large area.

Table 1. Summary of the results of immunohistochemical study of the rat focal cortical dysplasia model: GABA-A was negative and GABA-B was positive in both the lesion and perilesional area, and NMDA-R-1 and R-2 were negative in both areas.

Table 2. Summary of results of immunohistochemical study in FCD clinical cases. GABA-A was negative and GABA-B was positive in both the lesion and perilesional

area in all cases. NMDA R-1/R-2 and glutamate R-1 were negative and glutamate R-2 was positive in all cases.

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Fig. 1

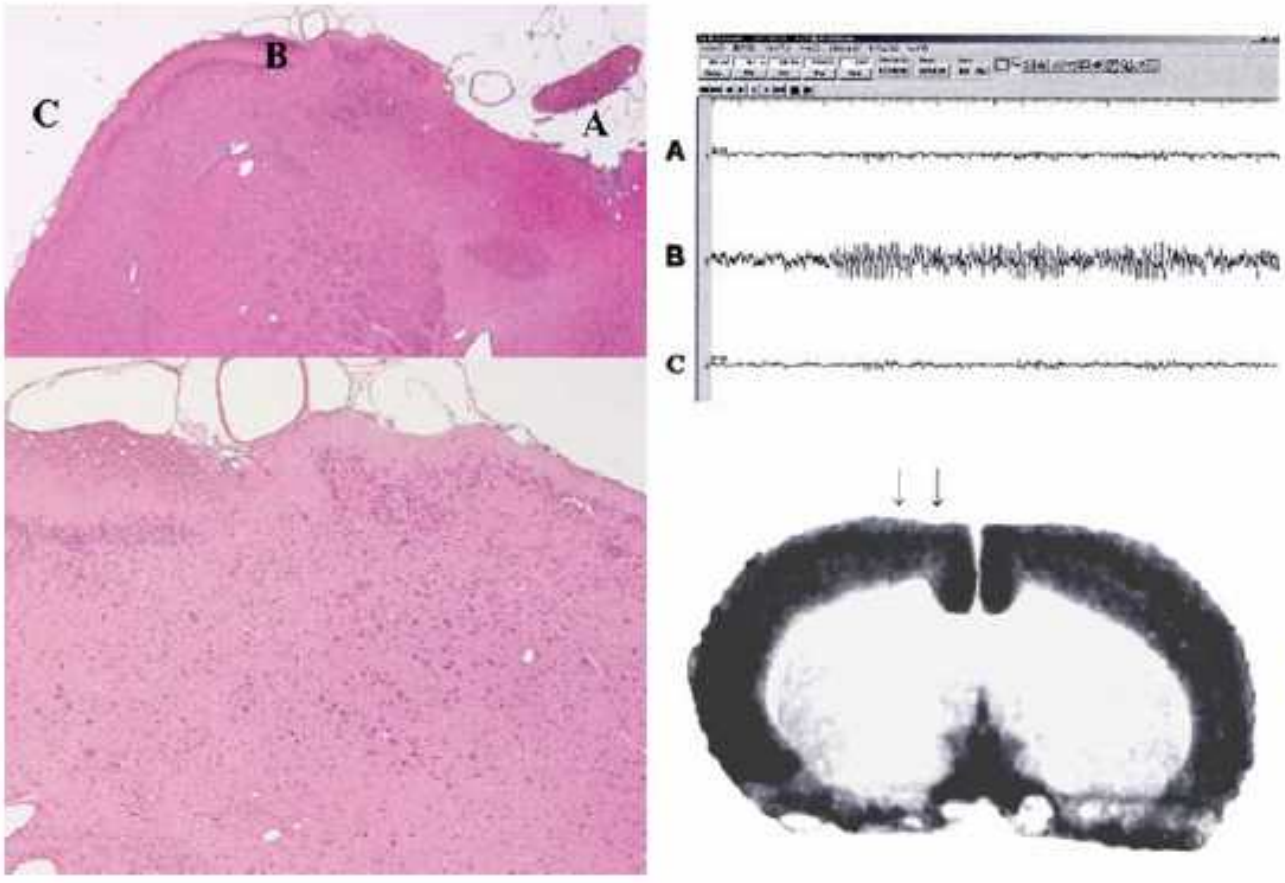

Fig. 2

Table 1

Area	GABA-A	GABAB-B	NMDA-R1	NMDA-R2
FCD	−	+	−	−
Perilesional	−	+	−	−

Fig. 3

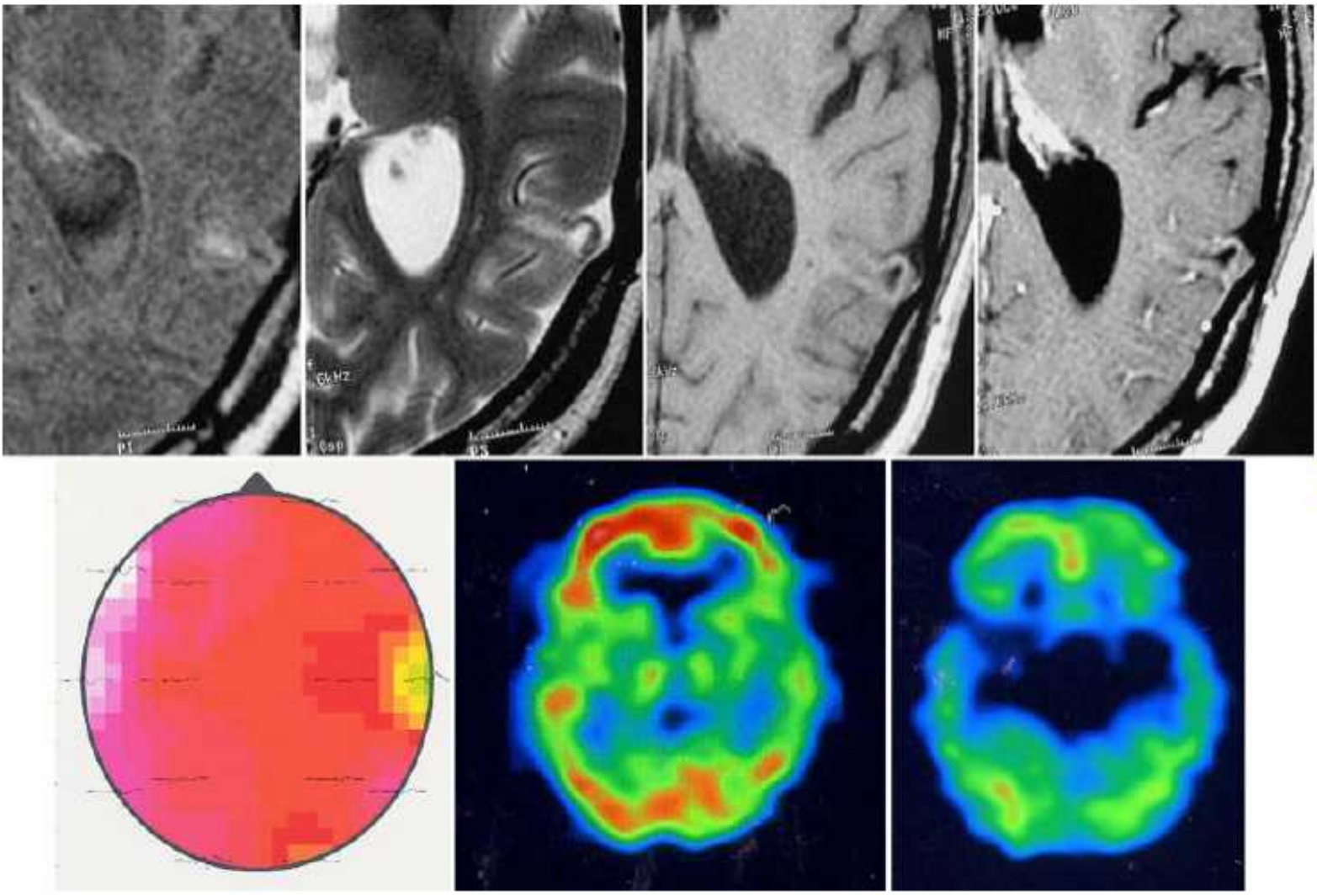

Fig. 4

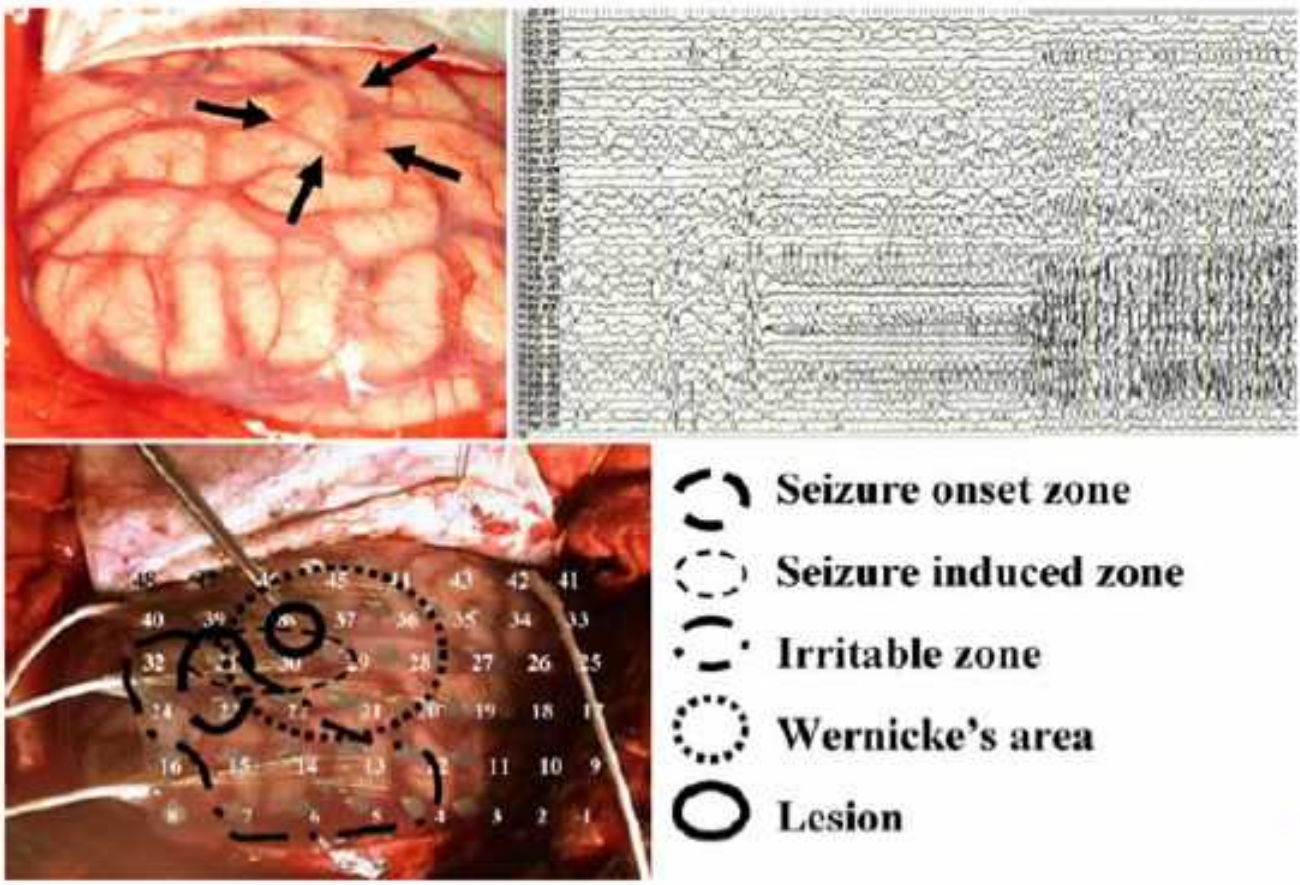
Seizure onset zone
Seizure induced zone
Irritable zone
Wernicke's area
Lesion

Fig. 5

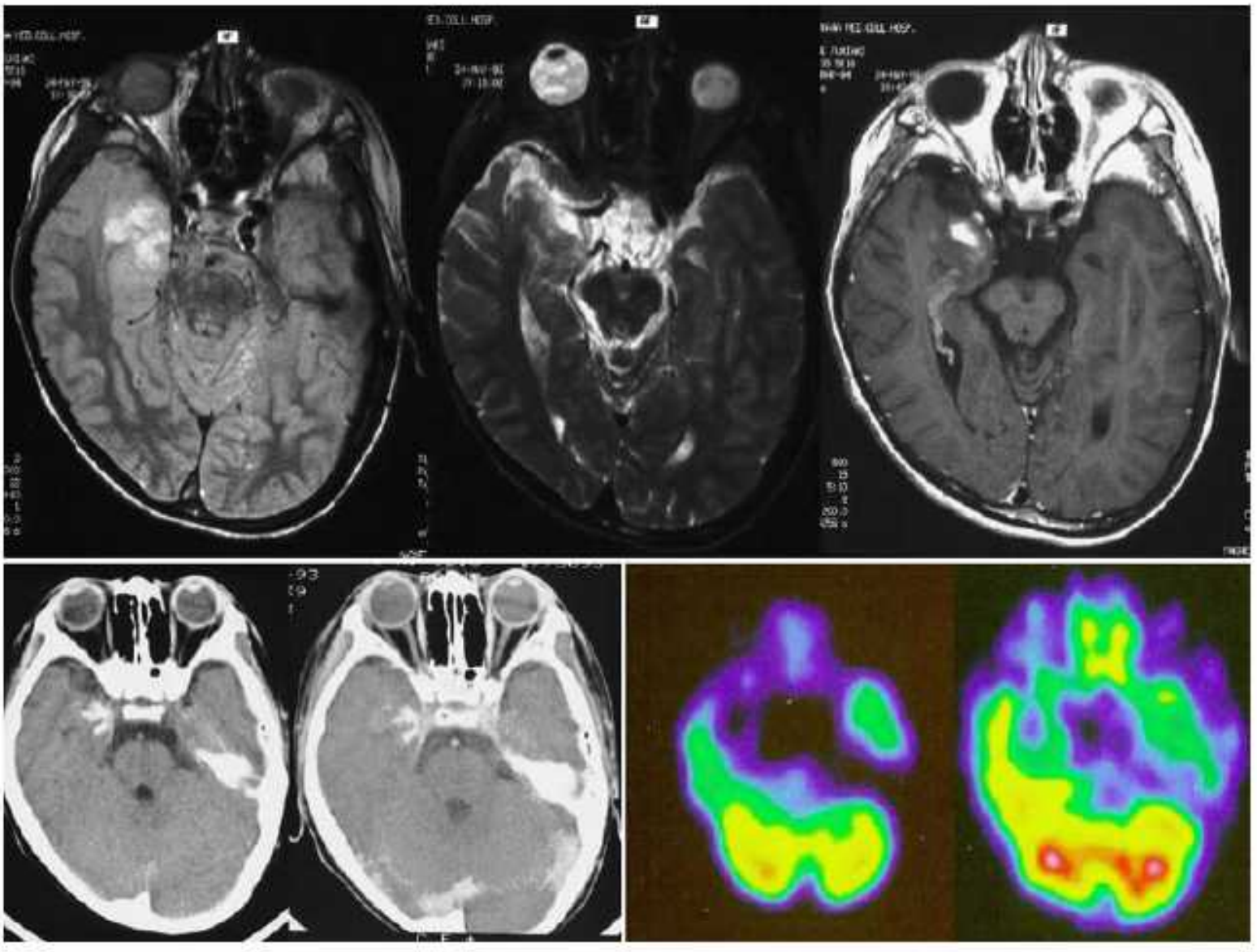

Fig. 6

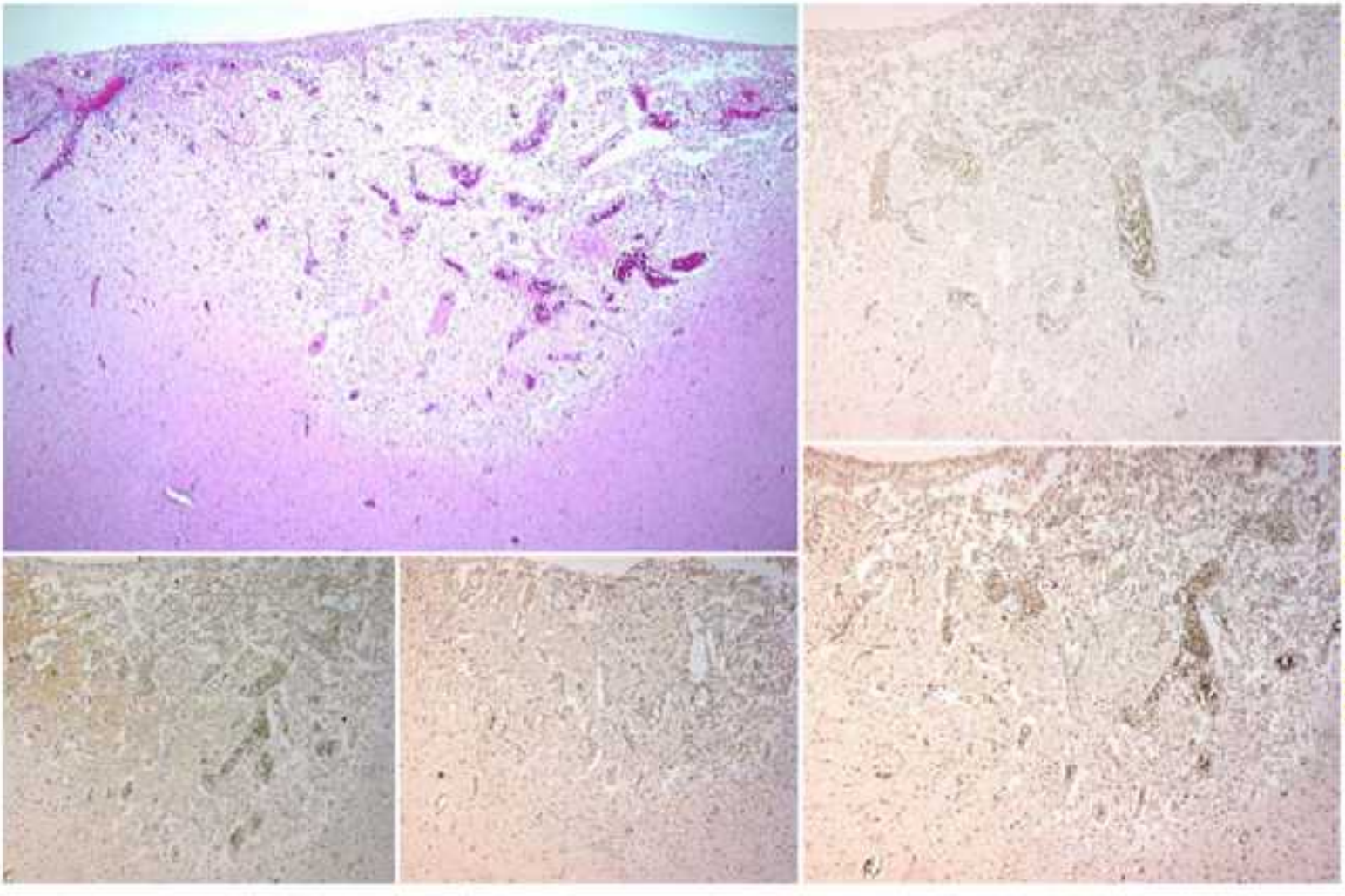

Table 2

Area	GABA-A	GABAB-B	NMDA-1	NMDA-2	Glu-R1	Glu-R2
LESION	−	+	−	−	−	+
Perilesional	−	+	−	−	−	+