Brain Functional Localization Of Kidling Cats: Manganese Ion Enhances T1-Weighted MRI Study

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Citation

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Abstract

Purpose: Our previous studies have demonstrated that calcium overloading is involved in the epileptogenesis. Mn^{2+} is handled in a manner similar to Ca^{2+} in many biological systems and can act as an excellent MRI contrast agent, so our present study was performed to determine the encephalic region correlated to epileptogenesis by Mn^{2+}-fMRI and further to determine the correlation of epilepsy and calcium overloading.
Methods The cats were divided into two groups, the first group was divided into group A and B, A group was PTZ experimental group, B group was normal saline (NS) controlling group, The first group was used to EEG examination, the adult male cats were combined anesthetized with ketamine and chlorderazin, with intramuscular injected of PTZ (55mg/kg), the ethological and EEG changes were observed. The second group was divided into group A and B, group A was used to Mn^{2+}-fMRI of the acute epileptic seizures, group B was used to Mn^{2+}-fMRI at the time of 24 hours after the acute epileptic seizures were induced, group C was control group NS. The adult male cats were combined anesthesia with ketamine and chlorderazin; two femoral veins were taken one for $Mncl_2$ infusion, the other for mannitol infusion. The cats were injected with PTZ intramuscular, and the changes were observed under TI-Weighted MRI. Signal enhanced encephalic regions were dislodged to be done.
Results: The ratio of kindling cats was 80% with intramuscular injection of PTZ, the EEG were paroxysmal with spike-slow waves of high amplitude, the etiology and EEG of control group were normal. When the cats showed generalized tonic-clonic convulsive seizures, cerebral cortex showed diffusely signal enhancement, the enhancement rate of frontal-parietal-occipital lobe was 34.6; the enhancement rate of temporal lobe was 22.9, and they had significant difference compared to the control groups. The Mn^{2+}-fMRI at the time of 24 hours after epileptic seizures attacked also showed signal enhancement on frontal-parietal lobe. The neurons of enhanced encephalic regions show obvious degeneration and necrosis.
Conclusion Cats can be succeeded to kindling by intramuscular PTZ (55mg/kg). Frontal-parietal lobe was the correlated encephalic regions of epilepsy, Mn^{2+}-fMRI has important role on the localization and revealing pathogenesis of epileptic seizures.

INTRODUCTION

Felix et al [1] have demonstrated that enhancement of Ca^{2+} current in the neurons was observed in mouse model of absence epilepsy, our previous Ca^{2+} imaging studies also suggested that calcium overloading in the hippocampus CA3 neurons of kindling rats was correlated with the epileptogenesis[2]. Mn^{2+} has an ionic radius similar to that of Ca^{2+} , and it is handled in a similar to Ca^{2+} in many biological systems. Mn^{2+} is known to enter cells through calcium pathways such as voltage-gated calcium channels and microtubules transmission systems[3], Mn^{2+} is paramagnetic and can act as an excellent MRI contrast agent to direct imaging of brain function, it is a new promptly developing technique of brain imaging. Yi-Jen Lin[4] et al's studies using this technique have showed that activation of the brain with glutamate led to increase in MRI signal in the brain to 238±23% of the original. Different central manifestations in response to electro acupuncture at analgesic and no analgesic acupoints in rats were observed by manganese-enhanced functional MRI study[5]. Aoki [6] et al made middle cerebral artery embolism models, early ischemic region was observed on this model by use of Mn^{2+}-fMRI. But at present there is no report about the Mn^{2+}-fMRI of epilepsy.

Pathological foundation of epilepsy is the neuronic massive discharge, as for this discharge is now presumed to be briefly Volant Ca^{2+} influx and cellular depolarization caused by slow Ca^{2+} influx. Because PTZ can induce excitatory

amino acids(EAA) release, then EAA combines with its receptor, which causes calcium channels open, so calcium influx is enhanced, this event can influence the neurotransmitter release and then cause excitation-inhibition functional disorder, accordingly, epileptic seizure is induced. Here we used PTZ to make acute epileptic model of cats, a continuous infusion of 3.6μmol/min $Mncl_2$ combined with breaking the blood-brain barrier(BBB) with 25% hypertonic mannitol , and Mn^{2+}-fMRI was used to visibly image the brain functional activation, this method has important value on definition the correlated encephalic region and epileptic pathogenesis.

MATERIALS AND METHOD

ANIMALS

Forty-two cats (2.5kg-3.5kg, both female and male) were bought from experimental animal center of HuBei, China. The forty-two cats were divided to two groups; the first group was used to ethological observation and EEG tracings. Which was again divided to group A and B, group A was PTZ experimental group containing 10 cats, group B was control group (NS) containing 2 cats. The second group was divide to three groups, each group contains 10 cats, group A was used to Mn^{2+}-fMRI of the acute epileptic seizures, group B was used to Mn^{2+}-fMRI at the time of 24 hours after acute epileptic seizures were induced, group C was control group NS.

AGENT AND INSTRUMENT

Pentylenetetrazol (PTZ) made in Sigma Company, $Mncl_2$ (made in the factory of Tianjin chemical agents) was prepared to the concentration of 120mmol/l with NS. Magnetic resonance imaging scanner was Siemens 1.5T Magnetom Vision VB33G head coil.

ACUTE EPILEPTIC MODEL OF CAT'S ESTABLISHMENT

PTZ was prepared to the concentration of 2% diluted by 0.9% NS, cats were intramuscular injected with PTZ, they commonly showed epileptic seizures 5min later, and were succeeded to kindling about 3min after seizures appeared. After cats were combined anesthetized with ketamine (25mg/kg) and chlorpromazine (10mg/kg), they were fixed on the multifunctional fixing instrument, and intramuscular injected with PTZ, behavioral changes were observed. The degree of epileptic seizures were evaluated according to Wada grading[7]: I half face convulsion; II double face convulsion; III nodding attack; IV circumduction walking; V limbs overall stiffness; VI overall tonic clonic seizure. Above V is succeeded to kindling.

EEG RECORDING

Digit brain electrographic recording instrument Medlec: Profile was used to EEG tracings. Recording method was bipolar lead, needle electrode; recording spots were double frontal region, double parietals, and double occipital. The reference electrodes were placed on the middle of the two eyebrows, After cats were anesthetized with ketamine and chlorpromazine, they were fixed on the multifunctional fixing instrument, first the fundamentally electrical activity of brain was recorded, then cats was injected with PTZ intramuscular , electrical activity of brain was continuously recorded 30min. Electroencephalograph scaling: chart speed 2mm = 50 μV; sensitivity 50~100; Hi-filter 15; HZ L-filter 0.5; brain wave amplitude the height of 5mm was low amplitude, 6~10 mm was middle amplitude, 11~20 mm was high amplitude, when middle high amplitude sharp and spike wave appeared, eleptiform brain activity of brain was assessed.

METHOD OF MN- FUNCTIONAL IMAGING

After cats were anesthetized with ketamine and chlorpromazine, depilation was prepared in both inguinal areas and anterior chest region, femoral vein was cannulated bilaterally with a special needle to allow heavy solutions then 120mmol/l $Mncl_2$ was infused into the cats through one femoral IV-line at the speed of 3.6μmol/min, warm saline was also infused into the other femoral IV-line and 10mg/kg body weight of 20% mannitol solution through the IV-line in order to break the blood-brain barrier(BBB). Typically this was done in 15min before injection of PTZ. Electrodes were placed on the chest for monitoring EKG. Cats were fixed on the equipment and MRI was performed. Dynamic AIM-MRI method was referred and revised; four sequences of SE-T_1WI imaging were gained according to chronological order. First of all plain scan was done, the second sequence was gathered for 10 min $Mncl_2$(1.0mg/kg) was infused into the vein later, the third sequence was gathered for 10 min 20% mannitol was infused into the vein later , the fourth sequence was gathered for 10 min PTZ was injected later. PTZ intramuscular needle was not to be detained in the gluteus and the fourth sequence need not to be scanned of group B. Saline solution at the same dose with PTZ was infused for cats of group C before the fourth sequence, no other step was done for the group A. The parameters of SE-T_1WI-TR 500ms, TE 14ms, Flip angle 70, SL 3mm, Scan Time 2min 18sec.

PATHOLOGICAL SECTION OF ACUTE EPILEPTIC CATS

The cats were injected 30mg/kg of diazepam to terminate discharges after Mn^{2+}-- fMRI. Inter-cavities artery and abdominal aorta were ligated, cats was perfused through the ascending aorta to the heart with 0.9% NS (100-150ml) and fixative (4% Para formaldehyde, 250-400ml).The fixative was made in 0.1 M phosphate –buffered saline (PBS) at PH 7.4. After removal of the brain from the skull, brain tissue was fixed again with 4% paraform for twenty-fours, frontal lobe parietal lobe and hippocampus were paraffin imbedded and made to 30μm of thick abscissa axis slices, these slices were then dyed with hematoxylin and eosin (HE). The histopathological changes in frontal lobe parietal lobe and hippocampus were observed under Olympus optics microscope and survived neurons were counted under high power lens 40x10.

STATISTICS ANALYSIS

Signal intensity (SI) of the region of interest (ROI) in different time series image was measured, and Enhancement Rate was calculated, then time-intensity curve was drawed, Enhancement Rate $(SI_t-SI_1)/SI_1$. SI_1 was the signal intensity of plain scan, SIt was the SI measured at t time point. The comparison of Enhancement Rate was determined by t-test, and was assessed by SPSS12.0 package. Each value indicated the mean ±SEM. Neuronal loss in frontal lobe parietal lobe and hippocampus of control group and epileptic group was assessed also by t- test.

RESULTS

ETIOLOGY AND EEG CHANGES

ETHOLOGICAL CHANGES

A group Achievement ratio of epileptic seizures induced was 80%. Two cats achieved III grade, and their convulsion halted 20 min or so later. The rest of 8 cats began to show beard buffeting prosopotic at 5 min or so after injected with PTZ 55mg/kg and then showed tonic-clonic seizures within 3-10 min. Paroxysmal tonic-clonic seizures could continue 6 hours or so. Epileptic seizures continued 18 hours or so, the latency of epileptic seizures was 11.04±3.95 min. After cats of control group were injected with NS, their behaviors were the same as usual, and they did not exhibit paroxysmal behavior changes as that of group A.

EEG CHANGES

EEG of A and B group under anesthesia with ketamine (25mg/kg) the combination of chlordeazin (10mg/kg) was high amplitude 1.5-2.5cps of pantomorphic dentate slow wave including of middle slow wave of 14-17cps and slight 4-7cps of slow activity. (EEG Fig1). EEG of group A was from sharp-slow wave group to paroxysmal high amplitude of spike, spike rhythm, including of spine-slow combining wave when cats began showing epileptic seizures to VI grading attacks. (EEG Fig 2). When cats showed tonic-clonic convulsions EEG was continuous high amplitude of sharp wave, spike group and spine-slow combination. (EEG Fig 3).

Figure 1

Figure 1: EEG1: EEG manifestation of cats combined anesthesia with with ketamine (25mg/kg) chlordeazin (10mg/kg) was high amplitude 1.5-2.5cps of pantomorphic dentate slow wave including of middle slow wave of 14-17cps and slight 4-7cps of slow activity. EEG2: sharp-slow wave group turned to paroxysmal high amplitude of spike spike rhythm including of spine-slow combining wave. EEG3: continuous high amplitude of sharp wave, spike group and spine-slow combination.

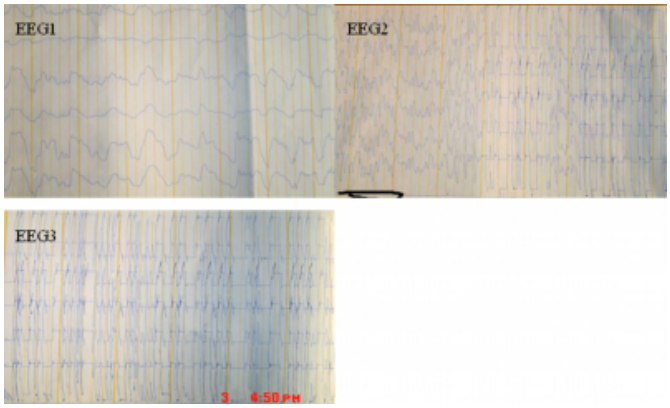


Figure 2

Figure 2: ECG manifestation of normal and epileptic cats. Respectively showed in ECG1 and ECG2.

Figure 3

Figure 3: Mn-fMRI at the time of epileptic seizures (1)Cerebral ventricular enhancing contrasting of T1 with T2WI with infusion of Mncl, there was significant enhancement in cerebral ventricles and but no engorgement in subarachnoid cavities (2) The same layer of brain tissue, s imaging at different time series. A was plain scan, B was scan at the time of 10 min after infusion of Mn , cerebral ventricular and facial muscles showed significant enhancement, brain substance showed diffusely slight enhancement. C was scan at the time of 10 min after infusion of mannitol, there was no obvious changes compared with B. D was scan at the time of 10 min after injection of PTZ, cerebral cortex showed significant enhancement. (3) At the time of10 min after injection of PTZ, A B C showed there was significant enhancement in the whole brain cortex, E was subtracting imaging, brain cortex showed linear enhancement, signal of remnant brain substance was subtracted. F was time-enhancing curve, it showed there was slight increase of signal intensity in brain cortex, after injection of PTZ; there was significant increase of signal intensity in brain cortex.

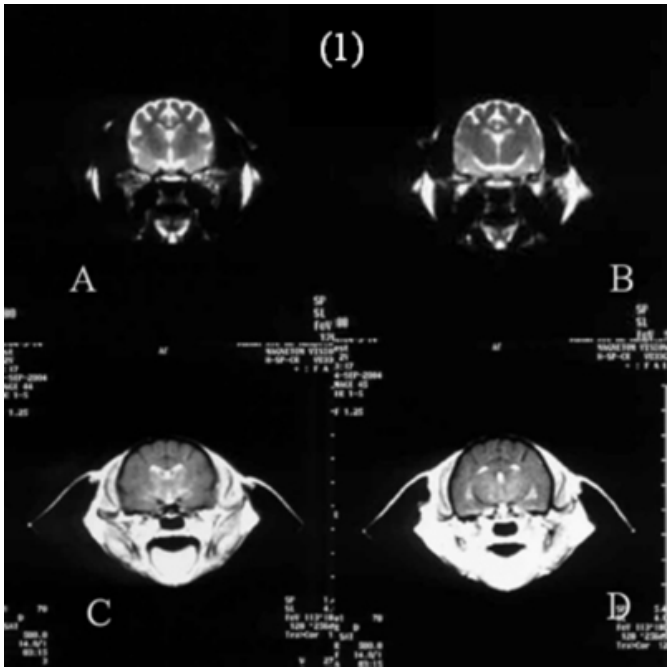


Figure 4

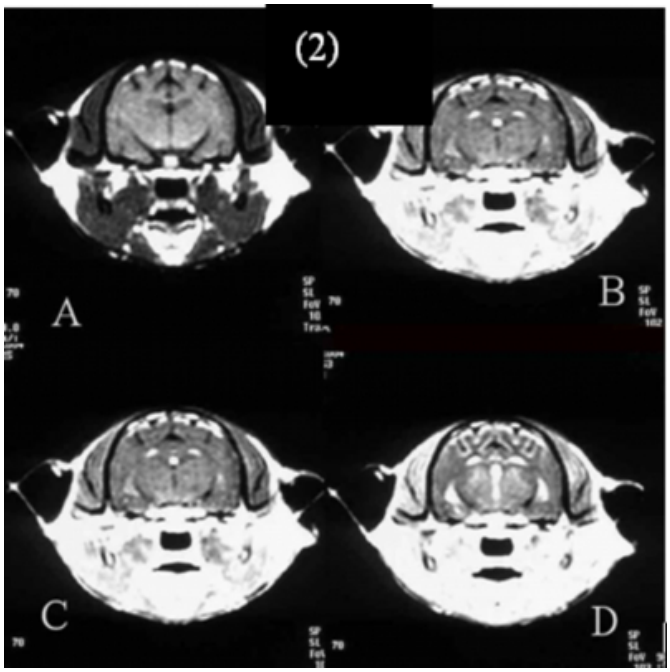
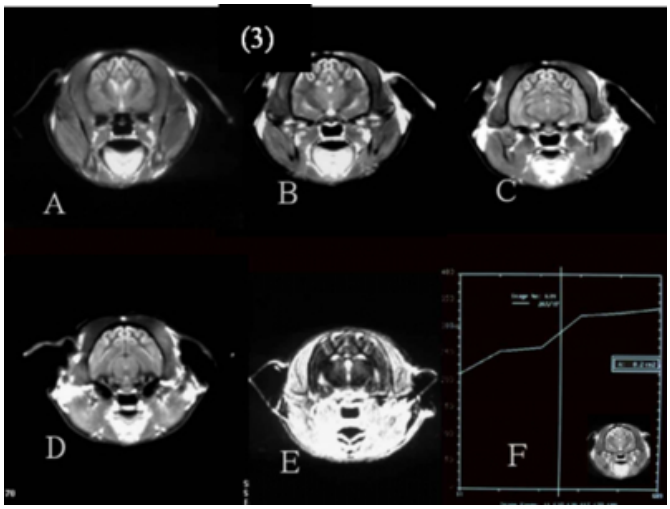


Figure 5



ELECTROCARDIOGRAPHY CHANGES (ECG) (ECG FIG 1-2).

Mn^{2+}- fMRI manifestation Mn^{2+}-- fMRI of cats with acute epileptic seizures

Infusion of Mn^{2+} into the anesthetized cats of epileptic group and control group leads to diffusely slight signal enhancement in brain tissue, the degree of enhancement in cerebral cortex hippocampus and basal nucleus was similar, Enhancement Rate achieved 10% or so. The enhancement in Alba was lighter, Enhancement Rate of it's was about 7%. The ventricular system and head muscle showed significant enhancement, Enhancement Rate of them was respectively

25%, 55%. There was no significant deviation between epileptic and control group. Following timing delaying, Enhancement Rate of above mentioned structure had increasing tendency, but it had no significant deviation compared with the previous data. (Fig 3). Frontal lobe parietal lobe and occipital lobe showed obviously macroscopic signal enhancement under T1-weighted MRI when cats exhibited tonic-clonic seizures after they were injected with PTZ (55 mg/kg), changes of signal intensity were quantification evaluation by calculating Enhancement Rate, of the total Enhancement Rate of frontal-parietal-occipital lobe was 34.6%, Enhancement Rate of cortex of temporal lobe was 22.9%. While the changes of Enhancement Rate of control group cats with intramuscular injection of tales doses of saline was not significant. The two group comparison had significant deviation ($p<0.01$). Enhancement Rate of hippocampus increased a little, but it had no significant deviation compared to the control group. (Listed in table 1).

Figure 6

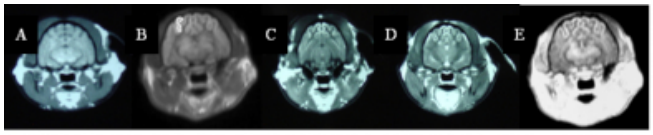
Attached table 1 Dynamic changing values and correlation of enhancement rate in ROI of epileptic A and control B group

ROI site	Enhancement Rate (%, $\bar{X}\pm S$)								
	infusion of Mn^{2+} later			infusion of mannitol later			intramuscular of PTZ later		
	A	C	comparison	A	C	comparison	A	C	comparison
frontal, parietal and occipital lobe	12.7±3.2	13.1±3.8	$p>0.05$	14.0±4.0	14.3±4.0	$p>0.05$	34.6±5.7	14.9±4.5	$p<0.01$
temporal lobe	9.8±2.0	10.8±2.6	$p>0.05$	9.0±2.2	10.8±2.6	$p>0.05$	22.9±6.5	11.6±3.2	$p<0.01$
hippocampus	11.6±4.2	12.0±5.0	$p>0.05$	10.9±3.8	12.3±5.2	$p>0.05$	13.8±5.8	12.6±5.3	$p<0.01$
cerebral ventricle	21.9±12.8	25.5±11.8	$p>0.05$	25.6±13.6	30.1±14.2	$p>0.05$	28.3±14.1	31.4±15.2	$p<0.01$
alba	6.7±3.2	7.3±3.8	$p>0.05$	7.7±4.2	7.5±4.6	$p>0.05$	8.0±4.6	8.3±5.1	$p<0.01$
basal nuclei	10.2±3.3	11.3±4.3	$p>0.05$	11.6±4.5	13.9±5.3	$p>0.05$	13.2±5.8	14.2±6.0	$p<0.01$
facial muscles	53.6±11.1	58.4±12	$p>0.05$	60.2±12.6	62.3±13.8	$p>0.05$	61.2±13.0	65.2±14.3	$p<0.05$

Mn^{2+}-- fMRI at the time of 24 hours after acute epileptic seizures of cats was induced Upon $Mncl_2$ infusion, significant signal enhancement could be seen in frontal lobe and parietal lobe of four cats, with perfusion of 20% mannitol (6mg/kg), diffusely signal enhancement was detected in cerebral cortex of three cats and signal enhancement in frontal lobe parietal lobe was most significant, with perfusion of mannitol (10mg/kg), diffusely signal enhancement could be seen in cerebral cortex of two cats, signal enhancement was not observed with naked eye in brain tissue of the else cat. (Showed in Fig 4.). The changes of signal intensity were evaluated by calculating Enhancement Rate, so it was discovered that Enhancement Rate in hippocampus increased, and it had variability compared with the control group. (Listed in table 2).

Figure 7

Figure 4: A was the normal imaging taken after anesthesia. B C D E were the Mn-fMRIs taken at the time of 24 hours after epileptic seizures?



Attached table 2 Dynamic changing values and correlation of Enhancement Rate in ROI of epileptic B and control C group

Enhancement Rate (%, $\bar{X}\pm S$)	ROI site						
	frontal, parietal lobe	temporal lobe	hippocampus	cerebral ventricle	alba	basal nuclei	facial muscles
B	33.2±3.6	24.7±4.2	19.6±3.2	27.9±9.7	8.2±6.4	13.1±4.8	63.2±11.2
C	14.9±4.5	11.6±3.2	12.6±5.3	31.4±5.2	8.3±5.1	14.2±6.0	65.2±14.3
Comparison of B and C	$P<0.01$	$P<0.01$	$P<0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

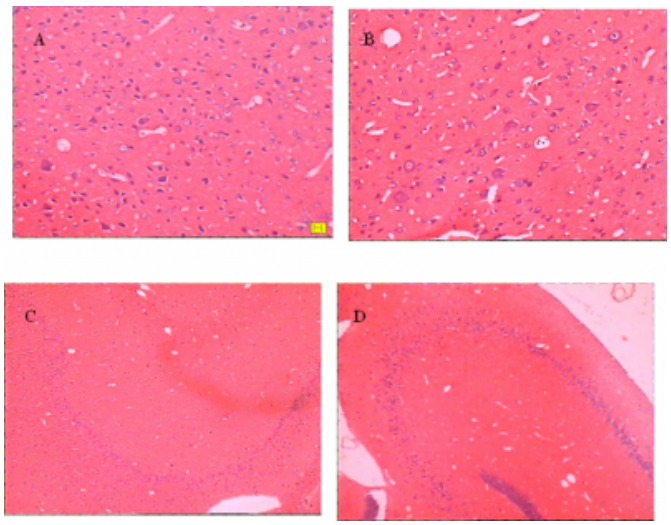
Note: $p>0.05$ means there is no significant variance between two group. $P<0.01$ means there is significant variance between two group.

HISTOPATHOLOGICAL CHANGES OF ACUTE EPILEPTIC MODEL

After Mn^{2+}-- fMRI of cats with acute epileptic seizures, All Layers of cortex of frontal lobe showed nerve cells edema degeneration with individually abnormal mega cell, the affection of surrounding cortical sulci was more severity, and individual neuron showed necrosis. Comparison with the frontal lobe, parietal lobe still showed glial cell and gelatinous fiber slightly hyperplasia. Hippocampus CA1, CA3, CA4 regions showed neuronal degeneration necrosis and effluvium and nerve cells of cortex degeneration necrosis. After Mn^{2+}-fMRI at the time of 24 hours acute epileptic seizures later, all layers of cortex showed nerve cells generally edema degeneration with individual cell necrosis, which was slightly more serious than that of group A The lost neurons in hippocampus were more serious than that of group A, too. (Showed in Fig5.). The number of survived neurons in frontal lobe and hippocampus of group A was 103.5±11.2/ mm and (81.7±9.2)/mm under high power lens (40x10). The number of survived neurons in frontal lobe and hippocampus of group B was 78.6±5.3/ mm and 47.3±7.2/mm under high power lens. There was significant variance between the two group ($p<0.05$).

Figure 8

Figure 5: A was histopathological changes of frontal lobe after Mn-- fMRI of cats with acute epileptic seizures: all Layers of cortex of frontal lobe showed nerve cells edema degeneration with individually abnormal mega cell. B was histopathological changes of frontal lobe after Mn-fMRI at the time of 24 hours acute epileptic seizures later, it showed slightly more serious than that of A. C: Hippocampus CA4 regions showed neuronal degeneration necrosis defluvium and nerve cells of cortex degeneration necrosis. D: Histopathological changes of hippocampus CA4 after Mn-fMRI at the time of 24 hours acute epileptic seizures later.



DISCUSSION

PTZ can produce animal's spasm immediately, reach peak in a short time, and auto stop after continuing a short time, so PTZ was known as the ideal drug duplicating epileptic model of acute or generalized seizures. Kindling model by PTZ has been generally applied to epileptic studies, but because the body of rat is small, it is not fit to the study of electrophysiology and imageology, while cats not only have large body but also their anatomical physiological biochemical aspects are more close to that of human, their bionergy is very powerful, recurrent attacks of them can not lead to death. Kai –Yan Wang[8] has succeeded in duplicating chronic epileptic model of cats used to the study of EEG and PET. With the purpose of temporal controllability, we duplicated acute epileptic model of cats, whether the model was succeeded was determined by observing ethological and EEG changes, the results showed that acute epileptic model could be succeeded to be induced by PTZ 55mg/kg, the acute epileptic seizures showed: beard buffeting prosopo-convulsion nutation limbs tic tonic- clonic seizures, which was similar with overall grand mal of human. EEG showed sharp-slow wave group, paroxysmal high amplitude of spike group, and spike rhythm including of spine-slow wave combination which were typically epileptic wave group.

Because cat is more close to human, its BBB is also very integrity, but Mn^{2+} is very difficult to cross the BBB, and the distribution of oxidation states of Mn^{2+} after acute infusion into blood and transport into brain is not known, nor is there a quantitative understanding of how much Mn^{2+} is free or complexes with proteins. For better exhibiting the calcium overloading of epileptic seizures using Mn^{2+}-fMRI, the BBB was disrupted by mannitol 10 min before epileptic seizures were induced. There has many literatures[9,10] that mentioned BBB was opened by mannitol, Rapport[10] et al discovered that BBB could be opened within 10 min after 25% mannitol 37, 0.12ml/s, 30s was infused and gradually shut off 10 min later. Now most scholars considered that opening processing of BBB could continue above 30 min after infusion of mannitol. In addition, someone else studied cervical arterial injection of mannitol caused brain vascular endothelial cell dehydration shrinkage, and tight junction of endothelial cell widen, so BBB was opened[11,12]. But cervical arterial cannula caused trauma bigger and stressing reflection of animals great, moreover, Mn^{2+}-fMRI of it could not reflect the changes of the whole brain tissue, only limited to half of brain tissue, so we selected infusion of mannitol and $Mncl_2$ through the femoral venous cannulation. Because infused Mn^{2+} redistributes to tissue with a half time of 4.7 min[13], infusion of Mn^{2+} through femoral vein into the brain leaded to diffusely slight signal enhancement in brain tissue, which was coincidence with the study of Yi-Jen Lin[4], and it may be the non-specific cerebration produced by non-specific and indifferent stimulus such as voices light rays or animals limited, accordingly, Mn^{2+} accumulated in the whole brain, moreover, following time delaying, this kind of nonspecific enhancement was more significant. Signal enhancement was not significant after infusion of mannitol; Enhancement Rate calculated was slightly increased.

After cats were kindled by PTZ, the cerebral cortex showed significantly contrasting signal enhancement, but cerebral cortex of control group showed no significant high signal, of the total frontal, parietal and occipital lobe showed the most significant signal enhancement, temporal lobe also showed significant enhancement, but its intensification degree was less than frontal, parietal, and occipital lobe. Cerebral cortex was considered as the obvious region of calcium overloading of epileptic cats. Excitable encephalic region of kindled cats by PTZ may be cerebral cortex. Mn^{2+}-- fMRI at the time of 24 hours after acute epileptic seizures showed that signal

enhancement could be seen in frontal lobe parietal lobe of four cats before no mannitol infused into the brain, signal enhancement could be seen with less doses of mannitol than that of group A, Enhancement Rate of hippocampus was high than that of group A, so it explained that frontal and parietal lobe were the damaged encephalic region after epileptic seizures, calcium overloading regions may be extended from frontal lobe and parietal lobe to the deep part of temporal lobe, calcium overloading in hippocampus aggravated gradually. Traditional methods have confirmed that the longer the time of epileptic seizures was, the more severe the damage of BBB was, therefore, signal enhancement could be seen with no mannitol infused into the brain of some cats. Pathological results after Mn^{2+}--fMRI demonstrated that histological changes of kindling 24 hours later were more obvious than that of kindling, and its loss of neurons was also more serious. Henshall[$_{14}$] et al discovered that discharge above IV could cause neurons die, the longer the time of discharges was, the more serious the neuronal death was, that matched to our previous study.

The localization of epileptogenic focus of most epileptic seizures with no obvious gross anatomical abnormality is constantly the hot spot paid attention to by epileptic surgeon. CT and MRI can not exhibit its abnormality, PET BOLD-fMRI EMG are powerful tools developed in recent years for its study. PET grounds for metabolic video picture and quantitative analysis, its whole locating system is the functional examination combined with structural localization, and its sensibility of detecting epileptogenic focus is very high, but its false-negative problem is present, receptor video picture and mechanism have no full-blown theory, and its reliability of test-retest is low. BOLD-fMRI reflects the changes of saturation of blood oxygen and blood flow, it does not directly exhibit neuronal functional activity, and the changes of blood oxygen levels can cause the deviation at the localization of brain transactivation domain. MEG detects the changes of magnetic field caused by neuronal postsynaptic potential, it is a noninvasive testing, and it has high time resolution and spatial resolution, but it is very difficult to detect the electromagnetic activity of cerebral deep part, its spatial resolution is about 1cm, short of that of MRI. Mn^{2+}-fMRI takes advantage of the dual roles of Mn^{2+} as a biological Ca^{2+} analog and a potent MRI contrast agent, it not only reflected neuronal physiological action, but also could locate the epileptogenic focus, moreover, it has very high spatial resolution , its contrast and signal-noise ratio are also high. But Mn^{2+} has a slow clearance rate after accumulation in brain tissue, so its time resolution is not high, if it combines with other functional imaging examination such as BOLD-fMRI MEG et al, its localization on the epileptogenic focus will be more accurate otherwise, because of Mn^{2+} toxic effect and limitations of crossing the BBB, if it is applied to the clinic, there will be some technologic problem to be overcome, but there are a number of excitable tissues that have no BBB in which Mn^{2+}-fMRI might be useful to investigate its functional changes, such as the heart. Skjold A[$_{16}$] et al have early determined the central zone of myocardial ischemia with the use of Mn^{2+}-fMRI. If we can find a way to break the BBB more simple and valid than mannitol or a kind of drug with less side effect which can take along with Mn^{2+} crossing the BBB, meanwhile, the concentration of Mn^{2+} should be lower than the level of the acute toxic reaction, and imaging contrast should be adequate, so Mn^{2+}-fMRI can be applied to the clinic.

Our experiment confirmed that Mn^{2+}-fMRI is very helpful to reveal the epileptic pathogenesis and exact localization of epileptogenic focus, the succeeding of breaking the BBB by infusion of mannitol through the femoral vein also provided a convenient feasible method and experimental evidence for the drug uneasy to permeate BBB under commen circumstances clinically crossing BBB easily to produce a marked effect. Simultaneously, following more deep going study and unceasing improvement of technologic method, its exact localization on the epilepsy will bring about big breakthrough to the epileptic surgical treatment.

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