

A pilot studies in dynamic profile of multi parameters of EEG in a rat model of transient middle cerebral artery occlusion

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Abstract— The dynamic profile of multi parameters of electroencephalogram (EEG) pathology associated with middle cerebral ischemia occlusion in a rat model were measured. Both pronounced increase in delta activity and decrease in theta activity during ischemic injuries were observed accompanied with decrease in the complexity of EEG. Different characteristic dynamic profiles might imply different mechanism underline the specific pathological process.

I. INTRODUCTION

Stroke is the most common life-threatening neurological disease and the leading cause of serious long-term disability. Ischemia stroke is the most common type of stroke, accounting for 70-80 % of all strokes. Experimental models of focal ischemia may be produced by ligature or by intra-luminal occlusion of the middle cerebral artery (MCAO) in rats and mice. The knowledge about pathophysiology of cerebral ischemia is well enhanced in small-animal models combined with various techniques in different research level.

As a supplement to magnetic resonance imaging (MRI), positron emission tomography (PET) and computerized tomography (CT), electroencephalographs (EEG) is widely used in basis and clinical studies because of the high temporal resolution and easy accessibility. Normal brain function requires a complex integration of electrochemical signaling, which is altered in various pathological process. For example, EEG power is markedly reduced with delta activities accompanied by a decrease in beta activities producing a diffuse slow-wave EEG pattern.

Signal processing of EEG is to enhance and aid the recognition of some aspects of the EEG that correlate with the physiology and pharmacology of interest. A vast variety of approaches to the extraction of quantitative features from an EEG signal was introduced during more than 70 years of electroencephalography. The main object of these approaches has been proposed to detect the hidden important dynamical properties of the physiological or pathological phenomenon. The time domain methods and frequency domain methods

are two main classical methods used in studying EEG. Quantitative EEG (QEEG) based spectral analysis shows great advantages in its noninvasive nature and ability to rapidly analyze enormous amounts of electrophysiological data. The results of previous studies show that the EEG may actually be described by the basic stochastic concepts (in other words, by probability distributions), but only at rather short realizations, usually not longer than 10--20 s, because the EEG turned out to be an extremely non-stationary process. Autoregressive modeling obtained its success in describing the spectrum of short EEG segment [2]. Compared to classical fast Fourier transform (FFT) algorithm, this method is more reliable and may be used with high accuracy even in very short signals segments with its resistance to additive noise.

On another way, non-linear dynamics (or deterministic chaos) methods based upon the hypothesis that the brain's electrical activity can be described by stationary dynamic models were relatively successfully applied to the description of EEG. Investigators shown that complexity indices of the EEG are sensitive complementary measures of electrophysiological changes caused by local lesions such as subcortical stroke. The analysis of EEG based on complexity measure is also value of discrimination in different status of brain and stages of sleep [3].

In this work, EEG was recorded in three regions of rat brain throughout ischemia/reperfusion period on a MCAO model. The EEG signal was studied by evaluating the power spectra and nonlinear parameters. The histological experiments were performed to evaluate the neural damage.

II. MATERIALS AND METHODS

2.1 Animals:

All animals handling and surgery were performed in accordance with the Care Standard of the Laboratory Animal (China Ministry of Health publication, 1998). The study was carried out on three-month-old adult male Sprague-Dawley rats (n=6) weighing 283±19g. Before and after the surgical intervention, they were kept in a sound-attenuated room at 22°C, under 12h light/dark conditions (7:00 a.m.-7:00 p.m.), with free access to water and food.

2.2 Surgical procedure and EEG electrode placement

Animals were stereotaxically implanted with electrodes under chloral hydrate anaesthesia (360mg/kg i.p.). Two

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striatal depth electrodes (150 μ m coated stainless steel wire, uninsulated for the 60 μ m at the tip) were implanted on the both sides (coordinates: AP=2.0mm, ML= \pm 2.0mm D=3.5mm from Bregma). The hippocampal depth electrode was implanted on the right side (coordinates: AP=-5.8mm, ML=5.0mm D=5.0mm from Bregma). Reference electrode was positioned, epidurally on cerebellum (coordinates: AP=-10.0mm, ML=0.0mm D=0.0mm from Bregma). All of electrodes were fixed with dental cement. After surgery, all animals were allowed at least five days for recovery until they return to original weight. During that time, they were provide food and water ad libitum and allowed to acclimate to the recording chambers.

2.3 MCAo Procedure

All animals were subjected to MCAo as described by Zea-Longa et al. with modifications [1]. Briefly, a midline incision was made and the right common carotid artery (CCA), the external carotid artery (ECA) and the internal carotid artery (ICA) were exposed. The distal ECA was coagulated completely. The CCA and the ICA were temporarily clamped with microvascular clips. A monofilament nylon filament (O.D. = 0.234 mm) with a blunted tip (O.D.=0.38+0.02mm, length = 3.0mm) was introduced into the ECA lumen. The filament was then gently advanced to the distal ICA until it reached the clipped position. After removing the microvascular clip, the filament was inserted until resistance was felt, which ensured the occlusion of the origin of the middle cerebral artery. The distance between the CCA bifurcation and the resistive point was about 18.0+0.5mm. The filament was withdrawn from the ICA after 50 min to allow MCA reperfusion.

During the 50-min ischemia period and the initial 6-h reperfusion period, 75W warming lamp was positioned directly over the cage to maintain the rectal temperature being kept at approximately 37.0~37.5°C throughout the experiment. The chloral hydrate was administrated (360mg/kg i.p.) with hourly supplements (60mg/kg i.p.) to keep anaesthesia for four hours, then the animals was transfer to the cage six hours and allowed to recover from anaesthesia. Depth of anesthesia was monitored by testing motor responses to tail pinch.

2.4 EEG Experiments:

One day before the MCAo experiment, the rats were connected to EEG recording chambers to collect 0.5-h normal baseline signals. Behavioral responses for each subject, including sleep, sedation, ataxia, head weaving or circling movement, were also recorded on the EEG polygraph records as a correlate to their respective changes in EEG activity when EEG signals were collected from the animals in recording chambers. The animals were fasted and re-anaesthetized before 1-h before MCAo. During this period, the EEG recordings

were obtained in following 2-h MCAo surgical procedures and at least 1-h reperfusion period described above. Then animals were returned to their EEG recording chambers and allowed to recover from anaesthesia. 0.5-h EEG signals, with behavioral responses, were recorded again in 6, 12, 24, 48-h post-reperfusion.

2.5 EEG Recording:

The recording chambers (BAS U.S.A.) described above were equipped with self-made 4-channel mercury swivel commutator. The animals were allowed complete freedom of movement during recording sessions. The swivel commutator was interfaced with a preamplifier (FZG-8, Jialong, China), and the signals were stored digitally using a bioelectric recording system (Powerlab/4S, ADInstrument, U.S.A.) at a sample rate of 400Hz. Signals bandpass filters were set to cut-off at frequencies below 0.25 and above 30Hz.

2.6 Data Analysis:

1) MCAo injury analysis: After 3-d post reperfusion, the animals were sacrificed and their brains were removed for quantification of infarction. From each rat brain, seven coronal sections (2mm thick) were then taken from the region beginning 1 mm from the frontal pole and ending just rostral to the corticocerebellar junction. Analysis of ischemic cerebral damage, including total core infarct volume and hemispheric infarct size, was achieved using the 2, 3, 5-triphenyltetrazolium chloride (TTC) staining method and computer-assisted image analysis.

2) EEG data analysis: 23 recorded sessions were selected for offline EEG analysis and each of session contained three 10-sec epochs. The protocol of each session was shown in Fig.1

The EEG signals were examined visually for epileptic form activity including sharp waves, spike/wave complexes, large amplitude slow waves, and for depression of background activity. For power spectra analysis, the Burg method based on AR modeling was used to calculate spectrum of six bands: delta2 (0.25–2 Hz), delta (2–4 Hz), theta (4–7.5Hz), alpha (7.5–13.5 Hz) and beta1 (13.5–20 Hz), beta2 (20–30 Hz).

In this work, the EEG signals also were analyzed using six characteristic parameters: Kolmogorov complexity (KC), C1 complexity (C1), C2 complexity (C2), information Dimension (inD), correlation Dimension (coD) and Approximate Entropy (ApE). For complexity analysis these parameters were normalized by the baseline value obtained 1-d before MCAo surgery so that the animals can serve as their own control.

2.7 Statistics analysis:

Data are presented as the mean \pm S.E. The Bonferroni test was to determine the statistical significance of each parameter obtained in EEG analysis at each time point anaesthesia/ischemia/reperfusion in comparison with the prerecorded baseline value for each rat.

N1	N2	A1	A2	I1	I2	I3	I4	I5	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	
1d	1d	15min	30min	1min	15min	25min	35min	49min	1min	15min	30min	45min	60min	75min	90min	105min	120min	240min	6h	12h	24h	48h	
NORMAL		ISCHEMIA							REPERFUSION														
ANAESTHESIA																							

Figure 1. The protocol of EEG experiment

III. RESULTS

One rat died within 48-h of reperfusion with subarachnoid haemorrhage (SAH) verified at post-mortem. Another animal was excluded from more analysis because the seizure activities were observed during its reperfusion period. The other 4 rats which survived to 3 days were included in EEG analysis.

3.1 Ischemic Infarction:

The mean total infarct volume was $231 \pm 33 \text{ mm}^3$ (Fig. 2). The ipsilateral striatal electrode was confirmed at the site of the core infarct area whereas the ipsilateral hippocampal and contralateral striatal electrodes were located at non-infarct area.

3.2 Visual analysis of EEG waveform:

Severe voltage depression and diffused slowing of the EEG waveform developed as early as 1 min after MCAo in the ischemic core regions, producing a nearly isoelectric state in most animals. Induction of both MCAo and reperfusion caused an immediate drop in EEG amplitude in the ipsilateral stratum and hippocampus in all animals. The decrease of EEG amplitude in contralateral stratum was found in three of four animals. Representative EEG signals are presented in Figure 2. Each segment represents a 10-s synchronized EEG trace.

Power analysis: Pre-injury, normal distribution of EEG power among the 4 frequency bands was fairly consistent throughout the entire brain. On an average, striatal EEG power in the delta2, delta1, theta, alpha and beta1, beta2 bands constituted 18.50 %, 22.87%, 28.18%, 15.32%, 6.28% and 4.51% of the total EEG power in the global frequency band, respectively. Critically, the balance of power distribution in the brain was severely disrupted by anaesthesia and MCAo. The result of power spectrum and one-way ANOVA were showed in Figure 3 and Table 1.

As expected, anaesthesia and MCAo led to a 2.5-fold increase in delta2 power in all three regions. The results of Bonferroni test showed that great significant increases in both of striatal electrodes were only recorded as early as 1-min post MCAo when the maximum values were observed ($P < 0.001$).

Compared to normal baseline levels, significant decreases in theta power were obtained from administration of anaesthetic drugs to early reperfusion periods ($P < 0.001$), including the whole MCAo process. The significant decreases lasted to 30-min post reperfusion in ischemic striatum and 105-min post reperfusion in ipsilateral

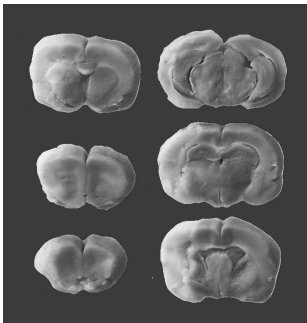


Figure 2. The photo images of TTC-stained coronal forebrain slice from representative rat subject to 50-min of transient MCAo.

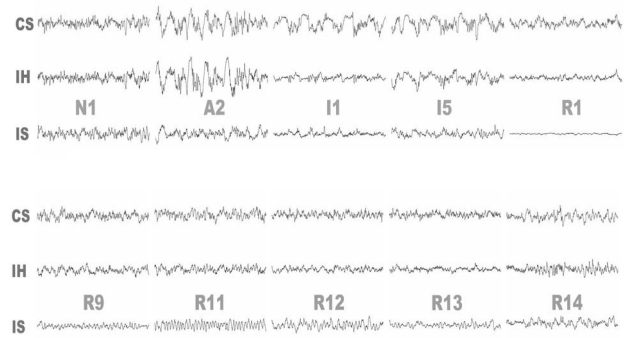


Figure 3. Ten synchronized segments (5-s) of EEG collected from 3 regions of a representative rat with MCAo. CS: contralateral striatum; IH: ipsilateral hippocampus; IS: ischemic striatum. The labels below each EEG segments indicate the sampling time-point described in Figure. 1.

hippocampus. No significant difference in theta power could be observed in contralateral striatum once the reperfusion began.

The enhanced delta power and compensated decrease in theta, alpha and beta power also resulted in the shift of central gravity frequency to lower frequency, which had been demonstrated by severe voltage depression and diffused slowing of EEG waveform. After onset of reperfusion, the disrupted power distribution showed a trend toward recovering. However, there was no significant difference.

Complexity analysis: Figure 4 depicts that the complexity of EEG immediately dropped during early anaesthesia phase compared to baseline in ipsilateral striatum. The

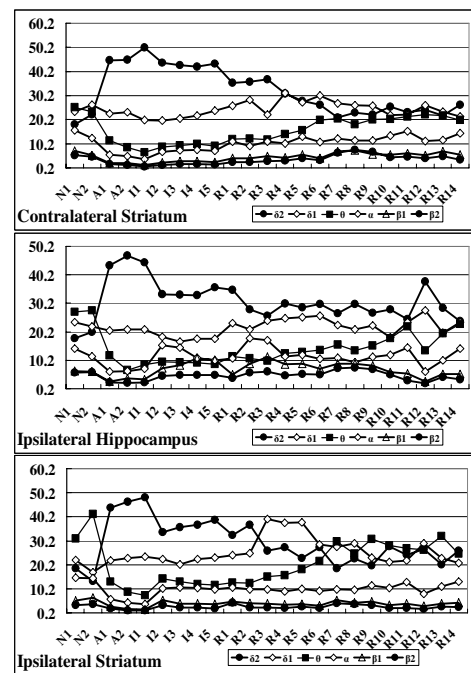


Fig. 4 Percentage of spectral power of delta2 ($\delta 2$), delta1 ($\delta 1$), theta (θ), alpha (α), beta1 ($\beta 1$) and beta2 ($\beta 2$) bands recorded in three regions of rat brain at different times.

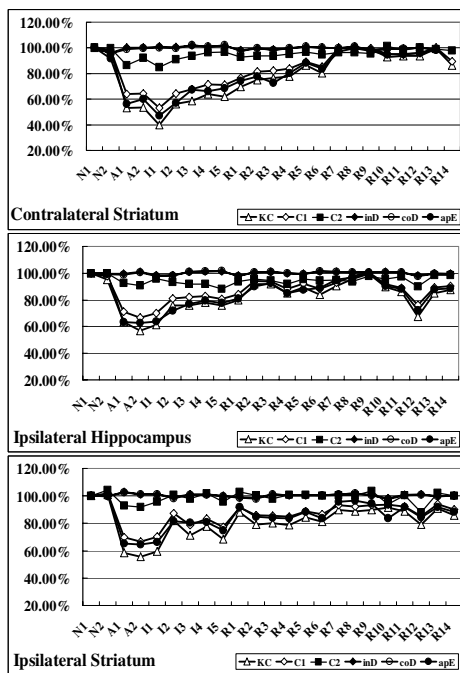


Fig. 5 The time course of mean complexity parameters obtained in three regions of rat brain; KC, C1, C2, inD, coD, ApE; minimum values were obtained 1-min post-occlusion and the complexity tended toward baseline. KC ApE and C1 complexity parameters exhibited similar tendencies throughout experiment. The results of one-way ANOVA test were summarized in Table 2. The complexity in early anaesthetic period (A1, A2) and acute phase of ischemia (I1) were significantly different from that in normal

TABLE. 1

Frequency Bands	delta2	delta1	thta	alpha	beta1	beta2
Site						
Contralateral Striatum	5.29†		4.89†	2.07†	2.96†	4.02†
Ipsilateral Hippocampus			6.21†			
Ipsilateral Striatum	3.28†	2.67†	3.63†			

†: Significance is at the 0.001 level;

TABLE. 2

Parameters	KC	C1	inD	coD	ApE
Site					
Contralateral Striatum	5.25†	5.26†	5.26†	3.86†	5.69†
Ipsilateral Hippocampus					1.79*
Ipsilateral Striatum	2.96†	3.06†	3.06†		2.02*

†: significance is at the 0.001 level;

* significance is at the 0.05 level

baseline level (N1, N2) and 2h post-reperfusion (P<0.01). Statistically outcome showed that the EEG complexity in contralateral striatum returned to baseline level after 90-min reperfusion. However the complexity of EEG recorded in infarct core were kept on lower level and did

not return baseline level even after 48-h reperfusion.

IV. DISCUSSION

In this study we measured the dynamic profiles of multi parameters of EEG in a model of transient focal ischemia in rat. The baseline EEG we obtained was similar to the previous studies. Both pronounced increase in delta activity and decrease in theta activity during ischemic injuries was confirmed in our experiments. Multi complexity parameters indicated that anaesthesia and ischemia led to the reduction of complexity of EEG. The more sampling points in this study allowed us to get more information about the changes of EEG pattern. With the help from other advanced techniques to measure the changes of biochemical reactions, a deep sight can be taken into mechanisms underlying them, which may be related to dynamic profiles of neurotransmitters, such as glutamate (GLU).

It is interesting to noted that both occlusion and reperfusion of MCA induced the decrease of amplitude of EEG in temporal domain, however characteristical measures, not only power spectrum in different frequency bands but also complexity parameters, did not show the similar changes in these periods. Further studies should be carried out to explain this interesting phenomenon. As the results shown, interference induced by anaesthesia in our studies partly overlapped the changes of EEG resulting from ischemic injuries. The inhibition of cerebral activities and breathing induced by chloral hydrate should be taken account into the further studies.

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REFERENCE

- [1]. Longa EZ, Weinstein PR, Carlson S, Cummins R, "Reversible middle cerebral artery occlusion without craniotomy in rats," Stroke, 1989, vol.20(1), pp.84-91.
- [2]. A. Schlögl. The electroencephalogram and the adaptive autoregressive model: theory and applications. PhD thesis, Medizinische Informatik and Bioinformatik, Graz, 2000.
- [3]. Zhang J, Zheng C. Using complexity measurement to study the EEG signal of focal ischemic cerebral injury. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 1999 Mar;16(1):37-41, 45