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Hypoxic-ischemic brain injury in neonatal piglets with different histological outcomes: an amplitude-integrated EEG study

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***Abstract*—To test the reliability of amplitude-integrated electroencephalogram (aEEG) in cerebral hypoxic ischemia (HI), 12 neonatal piglets subjected to different levels of HI are divided into three groups based on the histological outcomes obtained 4 days after experiment. Results show that concomitant with the increased severity of brain injury, the upper and lower margins of aEEG decrease significantly ($p < 0.05$) during early recovery period after HI (about 2 hours post-resuscitation). We conclude that aEEG method reliably reflects hypoxic-ischemic cerebral injury and constitutes a valuable monitoring tool in neonatal intensive care unit (NICU).**

I. Introduction

ASPHYXIA during perinatal period (from 28th week of gestation to 1 week after birth) remains a major cause for neonatal mortality and morbidity involving multiple organ systems [1]. Particularly, the central nervous system is sensitive to damage, known as hypoxic-ischemic encephalopathy (HIE). The incidence of severe HIE is between 2-4 cases per 1000 births in most developed countries and even higher in developing countries [2]. It has been proved that only early (within 6 hours after injury) diagnosis and neuroprotective therapy could be effective to reduce delayed neuronal death or programmed cell death caused by hypoxic ischemia (HI) [3]. Therefore, timely estimation of cerebral injury in clinic, especially in neonatal intensive care unit (NICU) is essential. Further, there is a need to provide continuous feedback to pediatricians regarding the neurological status of newborns.

Cerebral cortex is very sensitive to generalized HI. Electroencephalogram (EEG) reflects the postsynaptic potentials generated from cortical neurons and is a direct method for evaluation of brain injury or dysfunction. However, conventional EEG has some difficulties in clinical cerebral monitoring [4]: 1) it is hard to maintain impedances of multiple electrodes for a long time; 2) it is difficult to discern trends of EEG during hours by inspecting a very long EEG record (usually 10-20 s per page); 3) interpretation of the waveform requires extensive experience. In order to make it practical in clinic, a simplified EEG, amplitude-integrated EEG (aEEG) was developed in 1960s [5], making use of amplitudes in single-channel EEG. aEEG has been used to diagnose and monitor cerebral function of neonates from 1980s [6]. It not only estimates the brain state directly, timely, objectively and non-invasively, but also greatly simplifies the process of electrode fixing and makes the bedside monitoring feasible.

This paper investigates the reliability of aEEG monitoring in a porcine model of perinatal asphyxia. Neonatal piglets are subjected to HI insult and classified into three levels of brain injury based on histological outcomes. It is expected that aEEG could accurately and consistently evaluate the injury severity and the level of histological outcome in HI survivors.

II. Materials and Methods

A. Animal Experiment

The Animal Care and Use Committee of the Johns Hopkins Medical Institutions approved the experimental protocol used in this study. 12 neonatal male piglets (7 to 10 days old) were subjected to hypoxic-asphyxia to induce HI brain injury. Asphyxia and resuscitation protocol was performed as previously reported [7].

In brief, each piglet was anesthetized and intubated for artificial ventilation. This was followed by a 10-min period where EEG recording was initiated as a baseline. The piglets were then assigned to hypoxia with 9% or 10% oxygen for a period of 30, 40 or 45 min in order to vary the injury induced. Hypoxia process was followed by a gas washout phase of 5 min preceding complete asphyxia for 7 min. The piglet was then resuscitated and allowed to recover. The animals were kept on a warming blanket to maintain their physiological temperature between 38.5-39.5 ℃ throughout the experiment. Among 12 animals, one was chosen as the control and underwent sham operation without hypoxia or asphyxia.

Two channels of hemispheric EEG recording (Fp1-O1 and Fp2-O2) were continued from baseline to post-resuscitation period for a total recording time of 200 min. One channel out of two was randomly chosen from each recording for further

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analysis. Sampling rate was 512 Hz.

At 4 days after resuscitation, the animals were euthanized and the brain was perfused with paraformaldehyde for further hematoxylin-eosin staining. For each animal, the total numbers of ischemic and normal neurons were counted in the primary sensorimotor area where cortical injury is most severe at this stage of development in piglets [8]. A histological measure called *histology index* is defined as the ratio of ischemic neurons to total number of neurons in cortical layer V. The cohort of animals is then subdivided into three groups (labeled as L1 to L3) according to the histological outcomes: L1 with histology index of 0-0.4; L2 with 0.4-0.8; and L3 with 0.8-1. The control piglet without hypoxic-asphyxia insult is labeled as C (i.e. control).

B. aEEG Analysis

Commercial aEEG monitor obtains EEG signal with bipolar scalp electrodes. Then the signal is filtered, rectified, smoothed and amplitude-integrated before it is written out at a slow speed (6 cm/h). In this paper, we obtain the aEEG waveform from raw EEG data (i.e. the data recorded by conventional EEG monitoring system) using digital signal processing method as follows [9]:

a) *Filtering* Band-pass filter (2-15 Hz) the raw EEG to minimize artifacts [5]. In order to give equal weight to the energy of non-rhythmic components at each frequency, an asymmetrical bass-pass filter is designed here, providing greater amplification for higher frequency range [10].

b) *Terminal point extraction* The upper and lower margins of aEEG waveform reflect maximum and minimum fluctuations of raw EEG. The maximal and the minimal peak-to-peak amplitudes in each 2-sec EEG epoch were extracted as upper and lower terminal points of associated aEEG line.

c) *Amplitude compression* Draw aEEG lines in a sheet with log-scale y-axis so to reduce the dynamical range of fluctuations in raw EEG. In order to give prominence to low amplitudes, terminal points of aEEG below 6 μV are plotted in linear y-axes [10].

d) *Time compression* In order to show long-time cerebral functions on the whole, aEEG lines should be drawn densely along time axis (x-axis).

With aforementioned processes, aEEG recording can reflect the changes of cerebral function in a narrow paper ribbon. In this study, a 20-min waveform during about 2 hours after resuscitation (181-200 min in the total recording) is selected for further statistical analysis since aEEG during this period develops into a relatively stationary status.

Based on the values of normal neonates, the aEEG activity is classified according to its upper and lower margin [11]: *normal amplitude* — the upper margin of aEEG band > 10 μV and the lower margin > 5μV; *moderately abnormal amplitude* — the upper margin of aEEG band > 10 μV and the lower margin ≤ 5μV; *suppressed amplitude* — the upper margin of aEEG band < 10 μV and lower margin < 5 μV. To check the significant differences in the amplitude of upper and lower margins among groups L1, L2, and L3, a nonparametric test (Kruskal-Wallis analysis of the variance) is used.

III. Results

The aEEG waveforms from 12 piglets are obtained and shown in Fig.1. Although the amplitude of aEEG apparently decreases as soon as complete asphyxia is induced, it appears that diverse waveforms present during hypoxic period even in animals with similar histology indexes: some of aEEGs are suppressed obviously after hypoxia followed by gradual restoration (refer to L1 a in Fig. 1 for instance); others rise to certain extent during hypoxia (e.g. L1 b), while still others decrease mildly soon after hypoxia but recover to normal level very quickly (e.g. L1 c). However, in all these cases, the aEEG develops into a relatively stationary waveform after about 100 min post-resuscitation. The aEEG epoch from 181 to 200 min in each recording is selected during this stationary period for further statistical analysis. Amplitudes of upper and lower margins in aEEG are reported in Table 1 [median (25th-75th percentile)] with other experimental details. The result of Kruskal-Wallis analysis of the variance (ANOVA) shows that there are statistically significant differences in both upper margin and lower margin of aEEG among three animal groups ($p < 0.05$). To show these differences obviously, the median amplitude of upper and lower margin in each 20-min aEEG epoch is plotted in Fig. 2. In this figure, the three horizontal lines in each box reflect the lower quartile, median and upper quartile. The whisker is equal to one interquartile range.

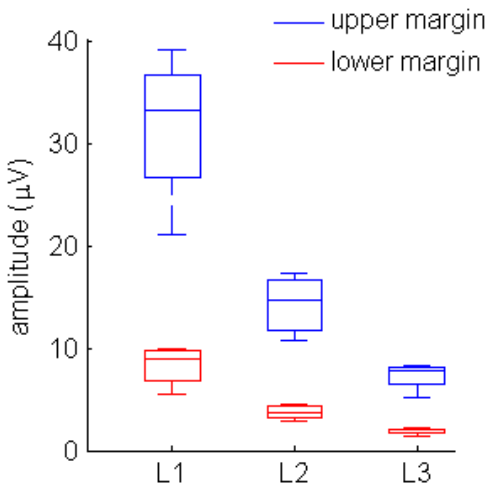

Fig. 2 The median amplitude of upper and lower margins in each 20-min aEEG epoch from 11 piglets with hypoxic-asphyxia insult.

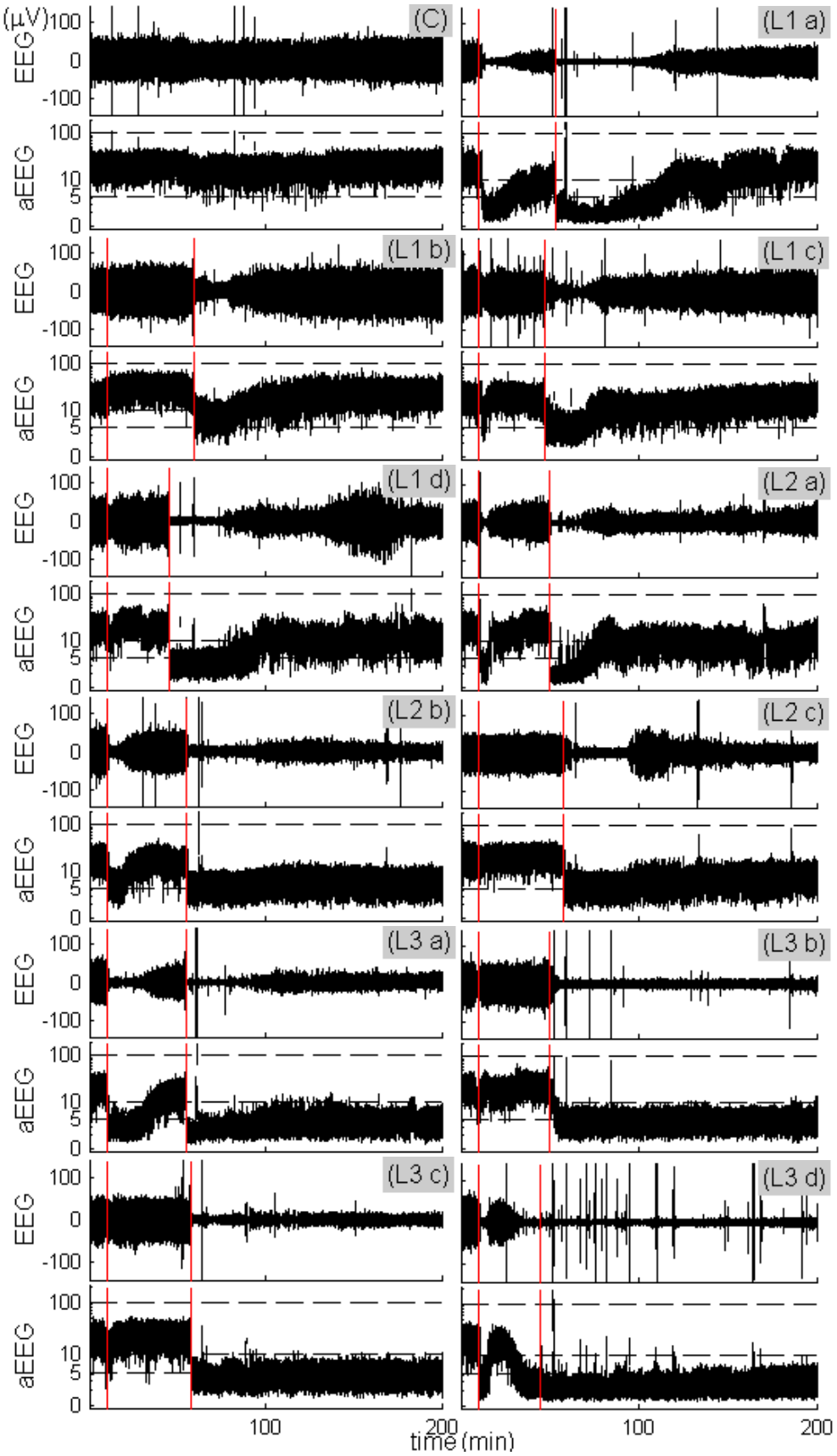

Fig. 1 EEG and aEEG recordings from 12 piglets with different histology indexes. EEG and aEEG during baseline (10 min), the entire hypoxic duration (30, 40 or 45 min), gas washout phase (5 min), complete asphyxia (7 min), resuscitation and

recovery periods spanning 200 min are compressed in these plots. In each panel, EEG (top) and aEEG (bottom) waveforms are plotted simultaneously. The two erect red lines denote the beginning time of hypoxia and the beginning time of asphyxia respectively.

TABLE I
EXPERIMENTAL DETAILS AND AEEG RESULTS

Pig ID	Hypoxic time (min)	Oxygen [b] (%)	Histology Index [c]	Upper margin (μV) [d]	Lower margin (μV)	aEEG result
C [a]	n/a	n/a	0	37.2 (33.0-41.4)	9.5 (7.6-11.9)	normal
L1 a	40	10	0	34.3 (29.4-40.0)	9.5 (7.4-12.8)	normal
L1 b	45	10	0.170	39.1 (34.2-44.8)	10.0 (8.0-12.4)	normal
L1 c	30	9	0.174	32.2 (28.5-35.9)	8.2 (6.5-10.0)	normal
L1 d	30	9	0.347	21.1 (18.2-24.7)	5.5 (4.4-6.8)	normal
L2 a	40	10	0.459	17.4 (15.0-20.7)	4.5 (3.5-5.7)	moderately abnormal
L2 b	40	10	0.600	10.8 (9.7-11.9)	2.9 (2.3-3.4)	moderately abnormal
L2 c	45	10	0.698	14.7 (12.9-16.4)	3.7 (3.0-4.5)	moderately abnormal
L3 a	40	10	0.854	7.8 (6.8-9.4)	2.1 (1.7-2.6)	suppressed
L3 b	40	10	0.940	8.3 (7.4-9.4)	2.2 (1.7-2.7)	suppressed
L3 c	45	10	0.983	7.7 (6.8-8.7)	2.0 (1.7-2.5)	suppressed
L3 d	30	9	1	5.2 (4.7-5.8)	1.3 (1.1-1.6)	suppressed

[a] the control piglet who underwent sham operation without injury.
[b] concentration of oxygen during hypoxia.
[c] the ratio of ischemic neurons to total number of neurons in cortical layer V at 4 days after resuscitation.
[d] the amplitude of margin is shown as median (25th–75th percentile).

IV. DISCUSSION AND CONCLUSION

aEEG is a simplified monitoring method which enhances the "readability" of EEG recordings spanning a long time. The classification of aEEG is based on the amplitude of its upper and lower margins. The results shown in Table 1 prove that aEEG accurately stratifies HI brain injury — with normal amplitude, moderately abnormal amplitude, and suppressed amplitude correlated with L1, L2, and L3 injury respectively. Thus, aEEG provides valuable diagnostic information on the neonatal asphyxial brain injury and may be beneficial for the real-time tracking of human neonatal HI recovery.

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