Brain Research Bulletin 127 (2016) 148–155

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Classifying amygdala kindling stages using quantitative assessments of extracellular recording of EEG in rats

Mostafa Jalilifar[a], Ali Yadollahpour[a,*], Ahmad Ali Moazedi[b], Zohreh Ghotbeddin[c]

[a] Department of Medical Physics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[b] Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[c] Department of Physiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form
13 September 2016
Accepted 19 September 2016
Available online 19 September 2016

Keywords:
Kindling
Electroencephalogram
Spectral power
Categorizing

ABSTRACT

Purpose: Determining different seizure stage specific features in a kindling model is a crucial step in developing efficient objective techniques for early prediction and treatment of seizures. This study identified and categorized kindling stages based on their electrophysiological features through processing extracellular field potentials of Amygdala rapid kindling.
Methods: Thirteen Wistar rats (200 ± 10 g) were divided into 2 groups including kindle (n = 7) and sham (n = 6) and respectively underwent an amygdala rapid kindling and placebo stimulation. EEG signals in each stage were classified into 7 bands: delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), low beta (12–16 Hz), mid beta (16–20 Hz), high beta (20–28 Hz) and gamma (28–40 Hz). Spectral power and power of sub bands of stage 3 (localized seizure stage (SS)) and stages 4 and 5 (generalized SSs) were compared between kindling and sham groups.
Result: Spectral analyses showed larger spikes in delta and theta subbands in the stages of 3, 4, and 5 of kindling, compared with sham animals. Generalized SSs contained more spikes than the localized SS in the kindling. Kindling process was accompanied by reduction in high beta and gamma oscillations and increase in delta sub band power which were significant in the generalized SSs. The theta/alpha ratio in the localized SS was higher than the generalized SSs and sham group, but the difference with the sham group was statistically significant.
Conclusion: Our results showed that reduced high beta and gamma and increased delta oscillations power are associated with behavioral seizure progression.

1. Introduction

Epilepsy is an important neurological disorder which affects more than 1.5% of world population. Temporal lobe epilepsy (TLE) is the most common form of epilepsy among adults (Sloviter, 2005). Various experimental approaches have been suggested to analyze TLE; however, kindling is likely to be the closest model in rodents for determining the development of human epileptic seizures and the evoked electrical discharge associated with these seizures (Bertram, 2007). Some scientists have invented different kindling models to determine the mechanism of kindling-induced epileptogenesis (Fisher, 1989) but electrical kindling has gained much attention due to non-interference of chemical and pharmacological agents in experiments. Furthermore, kindling provides the possibility for determining different neuronal networks and circuits which participate in the development of seizures with the advantage that this model causes no considerable injury to the brain in comparison with other approaches (Douglas and Goddard, 1975; Goddard et al., 1969; Morimoto et al., 2004).

Kindling is performed through applying repeated electrical focal stimulations with afterdischarge (AD) threshold intensity delivered at specific site of brain to evoke AD in the Electroencephalographic (EEG) (Bertram, 2007; Goddard et al., 1969; Morimoto et al., 2004; Racine, 1972a, 1972b; Wada and Sata, 1974; Wada et al., 1974). Racine categorized progressive behavioral changes of rodents into 5 stages including: stages 1 and 2 emerged with mouth and facial movement and head nodding, stage 3 is characterized by forelimb clonus, stage 4 with rearing, and stage 5 is characterized with falling and loss of balance (Racine, 1972a). During kindling process, the number of astrocytes increased which in turn enhances the excitability of neurons and afterward development of secondary generalized seizures from different parts of brain (Racine, 1972a).

Initial experiments in this field have shown considerable increase of AD with development of seizures (Goddard et al., 1969;

* Corresponding author.
E-mail address: yadollahpour.a@gmail.com (A. Yadollahpour).

http://dx.doi.org/10.1016/j.brainresbull.2016.09.012
0361-9230/

Racine, 1972b; Wada and Sata, 1974; Wada et al., 1974). In this regard, Wada et al. (1974) showed the growth of AD amplitude propagation of interictal discharge through the seizure generalization in amygdala kindling model (Wada and Sata, 1974).

During recent years using appropriate biomarkers in early or differential diagnosis of various disorders has drawn plenty of research interests (Norouzi et al., 2016; Yadollahpour and Jalilifar, 2014; Ali Yadollahpour, 2016). In this regard, extracting different electrophysiological and physical features of EEG such as frequency, energy, entropy, etc during kindling process have gained considerable attention because it can help scientists for prediction, detection, and also early treatment of epileptic patients (Bragin et al., 1999; Engel Jr et al., 2009; Musto et al., 2009). EEG shows the activity of synaptic potentials of millions of pyramidal cortical cells which their amplitude and frequency change in different conditions. Moreover, EEG is considered as an appropriate and sensitive criterion for evaluation of overall activity of the brain. EEG signal can be classified into different sub-bands which their powers are considered as a synchronization of neural activity index. In fact synchronization of neural discharge can be characterized by the power ratio of different sub-bands of EEG (Sebban et al., 1999) which are divided into 5 basic sub-bands including delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12), beta (12–28 Hz) and gamma (28–40 Hz). Delta waves are synchronized in deep sleep and they associated with seizure-like activity in the brain (Walter, 1936). Theta waves originate from Medial Septum Area of hippocampus and they are associated with voluntary movements of rats. Alpha oscillations are mainly recorded from occipital and other sensory areas. Studies have revealed that these waves are affected by thalamus and sensorimotor cortex in rats (Hughes and Crunelli, 2005; Shaker, 2006). Beta and gamma rhythms are recorded in the neocortex and hippocampus of awake and consciousness humans and animals (Haenschel et al., 2000). Furthermore, these waves are synchronized in consciousness states.

Classification of kindling stages based on Racine stages were completely performed behaviorally and based on the changes of behavior of animal. Therefore, detection of onset and end of each stage of kindling and its duration has been determined from the observation of behavior change which in turn produces a relatively major source of error in measurements and data analysis. Therefore, it would be required identification and development some biological biomarkers which can indicate different stages of kindling objectively and quantitatively. In other word, it is necessary to determine the physiological and hemodynamical parameters of different stages of kindling. Due to high temporal resolution of EEG and its high accuracy in indicating hemodynamical changes of different regions of brain, it can be a reliable index to determine the mentioned features in the kindling model. Therefore, using signal processing of EEG makes it possible to identify variables or indicators that have significant correlation with physiological and hemodynamical features of different stages of kindling. Recently, identifying and developing these biological indexes of EEG have gained much attention to determine and classifying different neurological disorders especially epilepsy.

The present study aims to identify the distinctive features of different stages of kindling through processing of EEG. The main goal of determining these features is to develop an objective method for identifying different stages of kindling with the minimum error.

2. Materials and methods

2.1. Animal

Adult male Wistar rats (200 ± 20 g at the time of surgery) were obtained from the animal house of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). They were kept in a colony room with a constant temperature (25 ± 2 °C) and artificial 12:12-h light-dark cycle. The lights were turned on at 7:00 AM. Animals were kept in individual cages and had free access to standard food and water. All experiments were designed to minimize the number of rats used and carried out via a protocol was in complete compliance to the guide for the care and use of laboratory animals by the national academy of sciences (National Institutes of Health publication No. 86-23).

2.2. Surgical procedure

Thirteen adult male Wistar rats (200 ± 10 g) were divided into two groups (seven for the kindle group and 6 for sham). All rats were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg) and Xylazine (10 mg/kg) mixture (Esmaeilpour, 2013) and fixed on stereotaxic instrument. One tripolar stainless steel electrode (bipolar for stimulating and monopole for recording) was implanted in amygdala using Paxinos and Waston atlas coordinates: anteroposterior: −2.5 mm; lateral: 4.8 mm; vertical: 7.2 and 0.2 mm below the skull (Paxinos et al., 2009). The stimulating electrode consisted of electrodes with a tip distance of 0.5 mm. Moreover, a monopolar electrode connected to a screw was positioned in the frontal lobe of skull as ground and reference and also two holes were drilled for anchor. Electrodes were fixed using acrylic dental cement and inserted to a socket.

2.3. Kindling procedure

After surgery and implantation of electrodes, we allowed animals to recover between 7 and 10 days. Before applying kindling procedure, first the threshold intensity was evaluated using a 3 s of monophasic square wave of 50 Hz. The stimulating current was initially applied at 30 μA and it was enhanced in steps of 15 μA at 15 min intervals until producing at least 6 s of ADs. Animals in the kindle group were stimulated using a 3 s train of 50 Hz monophasic pulses of 1 ms duration with threshold intensity which were applied 12 times daily with 5 min intervals (Shahpari et al., 2012). This procedure was performed until observing stage 5 of kindling. Animals in sham group did not receive stimulation (Fig. 1). In fact, after surgery and implantation of electrodes, they experienced stimulation condition but were not subjected to stimulation. Therefore, the EEG of sham animals can be considered as a baseline.

2.4. Signal analysis of EEG

The EEG data were recorded using the one pole of the tripolar electrode implanted in the amygdala using a data acquisition system (Sciencebeam Co., Tehran, Iran). Digitization of data was performed at a sampling rate of 10 KHz. During kindling acquisition, the time and duration of stage 3, 4, and 5 of kindling acquisition were saved as event files which could be considered in extracting each stage. In this study, stage 3 was considered as localized seizure stage (SS) while stages 4 and 5 represented generalized SSs. FFT was employed to convert EEG signals into frequency domain and evaluate power spectrum of each sub-band including delta (1–4 Hz), Theta (4–8 Hz), alpha (8–12 Hz), low beta (LB) (12–16 Hz), mid beta (MB) (16–20), high beta (HB) (20–28), and gamma (28–40 Hz) (Vetterling et al., 1992). All of these analyses were performed using MATLAB 2013b.

2.5. Statistical analysis

The values are represented as the Mean ± standard error of mean (SEM). The normality of the variables was analyzed using Shapiro-Wilk test. The statistical significance of the differences between