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Research article

Quantitative assessments of extracellular EEG to classify specific features of main phases of seizure acquisition based on kindling model in Rat

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HIGHLIGHTS

- Initial (ISSs), localized (LSS), and generalized seizure stages (GSSs) are studied.
- Delta in ISSs is higher than LSS and GSSs, but only significant with LSS.
- Delta decreases, while beta increases during kindling epileptogenesis.
- Theta/alpha and delta/beta ratios decrease during GSSs.
- Alpha significantly decreases in ISSs, but gradually increases during epileptogenesis.

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ABSTRACT

Objective: Quantitative assessments of extracellular EEG to identify specific features of three main phases of seizure acquisition based on kindling model in Rat.
Methods: Male rats were divided into 2 groups including kindled (n = 10) and sham (n = 7) and respectively underwent an amygdala rapid kindling and placebo kindling. EEG signals in stages 1–2 (initial seizure stages (ISSs)), 3 (localized seizure stage (LSS)), and 4–5 generalized seizure stages (GSSs) were divided into 5 bands: delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–28 Hz), and gamma (28–40 Hz). Spectral power of the sub bands and the ratios of theta/alpha and delta/beta were compared in the three phases and between the sham and kindling groups.
Results: Beta power significantly increased during kindling acquisition, though it was significantly lower than sham. Delta oscillation in ISSs was higher than LSS and GSSs, but the difference was significant only with LSS. Moreover, delta power was higher in all stages of kindling than sham. Gamma power in all stages of kindling was significantly lower than sham. Alpha power was significantly reduced in ISSs, compared with sham, but gradually increased during epileptogenesis. Theta/alpha and delta/beta increased in all stages, compared with sham ($p < 0.05$). Theta/alpha significantly decreased in LSS and GSSs, compared with ISSs ($p < 0.05$). Delta/beta decreased during kindling, but it was significantly different only between ISSs and LSS ($P < 0.05$).
Conclusion: Beta and alpha oscillations at ISSs significantly decreased, but gradually increased along with kindling progression. Furthermore, delta power significantly increased during kindling process.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common type of epilepsy with focal seizures originating from temporal lobe of brain which are quickly generalized to other brain regions [1]. Electrical kindling is a reliable experimental model used in the studies of TLE. Moreover, electrical kindling would benefit from the non-interference of chemical and pharmacological agents in experiments. Kindling can also help to identify different neuronal networks and circuits involved in the epileptogenesis process. In addition, it possesses the advantage of inducing no significant damage to different areas of brain. However, kindling has some disadvantages such as age and strain specificity which make it hard to generalize the results of a particular animal model [2,3]. Kindling is

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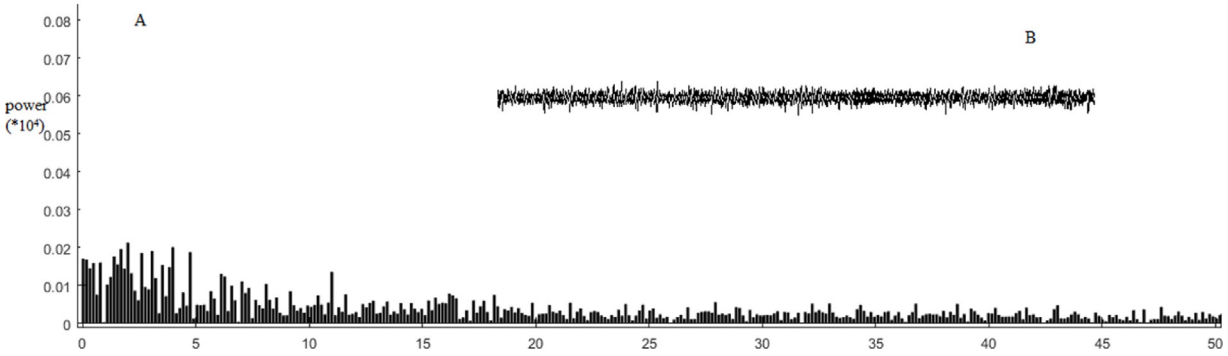

Fig. 1. A: An example of power spectrum of normal EEG signal in sham group, un-stimulated group. B: an example of EEG signal in sham group, baseline signal or normal EEG signal.

an expensive, time-consuming and highly demanding experimental procedure where animals should usually receive a large number of electrical stimulations to represent spontaneous seizures [4,5]. Furthermore, kindling stimulations may induce severe damage to some neuronal circuits [6].

In this model, repetitive electrical stimulations with specific intervals and afterdischarge (AD) threshold intensity are applied at particular regions of the brain to provoke AD in the electroencephalogram (EEG). By continuing these daily stimulations in the standard model, generalized seizures are induced similar to TLE through long term potentiation (LTP) process [7,8]. Several studies have reported the strong correlation between the increase of AD duration (ADD) and the behavioral progression of kindling [7,9–11]. In this regard, behavioral development of kindling was classified into 5 stages which have been known as Racine stages: stages 1 and 2 are shown with mouth and facial movement and head nodding, stage 3 emerges with fore-limb clonus, stage 4 with rearing, and stage 5 is characterized with loss of balance and falling [10]. Understanding the mechanisms involved in kindling-induced epileptogenesis has recently received considerable research attention. In this context, identifying alterations of EEG during kindling process can significantly help seizure prediction and understanding the epileptogenesis process. Dugladze et al. (2007) showed that suppressing theta oscillation in the epileptic murine hippocampus, while persisting gamma band power both in vivo and in vitro, confirmed the hypothesis that alteration in excitatory input and synaptic integration in the oriens—lucunosum-moleculare (O—LM) interneurons is the main cause of epileptogenesis development [12].

Recent studies have focused on finding appropriate EEG based indices such as frequency, energy, entropy, coherence, etc to predict epileptic seizures or adopt efficient treatment approaches [13,14]. EEG signal represents the synaptic potentials of pyramidal cortical neurons and records their electrophysiological alterations in different states.

EEG signal has a high temporal and spatial resolution to identify and localize sources of neural activities which are associated with different hemodynamical and physiological changes of brain [15–17]. Moreover, EEG signals related to different stages of kindling can be analyzed quantitatively to decrease the errors in behavioral assessments [17]. Transforming EEG signals into frequency domain is a common method to extract quantified variables associated with specific cognitive or even physiological functions. Therefore, using spectral analyses of EEG signals we can determine and extract quantified variables associated with behavioral characteristics of kindling stages [18].

The present study is a part of a large project conducted at Ahvaz Jundishapur University of Medical Sciences (AJUMS) to develop EEG based indices for categorizing kindling stages and ultimately developing efficient seizure prediction techniques. We have previously investigated features of stages 3, 4, and 5 of kindling [19]. Based on the findings of our previous study, this study is designed to quantitatively identify the main features of three main phases of kindling acquisition. In this regard, this study aims to analyze the process of kindling acquisition using Fast Fourier Transform (FFT) analysis of EEG signals in different stages where stages 1 and 2 were considered as initial seizure stages (ISSs), stage 3 as localized seizure stage (LSS) and stages 4 and 5 as generalized seizure stages (GSSs). In this study, we focus on quantitative assessments of extracellular EEG to identify and classify specific features of different seizure stages based on kindling model in Rat.

2. Methods

All of the experiments in this study were approved by local ethics committee of AJUMS which were in complete accordance with the guide for the care and use of laboratory animals by the National Institutes of Health (National Institutes of Health publication No. 86-23).

2.1. Animal and surgical procedure

Adult male rats weighing 200 ± 10 g at the time of surgery were maintained in individual cages with an ambient temperature (25 ± 2 °C) and 12-h dark: 12-h light cycle (Lights on from 7:00 to 19:00 h).

Rats were randomly divided into two groups (ten for the kindled group and 6 for sham). Under intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) mixture [20], all rats were anesthetized and fixed on a stereotaxic frame. One tripolar stainless steel electrode (bipolar for stimulating and monopole for recording) was implanted in the amygdala using Paxinos and Watson atlas coordinates. The stereotaxic coordinates used for the amygdala were anteroposterior: −2.5 mm; lateral: 4.8 mm; vertical: 7.2 and 0.2 mm below the pial surface [21]. In addition, three holes were drilled, one for positioning a monopolar electrode attached to a screw which was located near the frontal lobe as ground and reference, and also two others for anchor screws. Electrodes and screws were fixed using acrylic dental cement and attached to a socket.

2.2. Kindling process

Animals were recovered 7–10 days after surgery. After recovery period, at day 0 of experiment the threshold intensity was evaluated using a 3 s of monophasic square wave of 50 Hz initially applied at 30 μA and it was increased in increment of 15 μA at 15 min intervals until emerging at least 6 s of ADs. Animals that represented