Power Spectrum Density of EEGs of Sleeping Epilepsy-Prone El Mice and Their Non-epileptic Mother Strain

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Abstract: A power spectrum density (PSD) analysis was performed on electroencephalograms (EEG) of sleeping epileptic El mice and non-epileptic ddY mice. A respiratory peak at 3.13 Hz was detected in the EEGs of both categories. The PSD analysis revealed a peak at 8.59 Hz in the El mice, which was associated with interictal discharges. This peak may be helpful in diagnosing epilepsy.

Key words: mice, EEG, PSD, epilepsy.

Electroencephalograms (EEG) are helpful in the clinical diagnosis of epilepsy. EEGs are usually compound waves with component signals from various sources, including the heart beat, respiration, and body movement. In an earlier study, we developed an improved method to record EEGs in conscious and freely moving mice [1]. In the present study, to characterize the “pure” EEG component, we recorded monopolar EEGs of sleeping epileptic mice, El, and their non-epileptic mother strain, ddY, using epidural electrodes and a reference electrode placed subcutaneously near the nose, and we performed a power spectrum density (PSD) analysis of the EEGs.

Materials and method

Three El and three ddY mice were used in the study. The El mice, an established epileptic strain [2], were selected from a population that has been maintained at Nippon Veterinary and Life Science University [3] by sister-brother inbreeding since 1991 when the strain was donated by the National Institute of Health in Japan. The El mice and their non-epileptic mother strains, ddY, were kept in a conventional animal room at 23º–25ºC in 60% humidity, with lights on at 06:00 and off at 18:00. They were fed a commercial diet (EF, Oriental Yeast, Japan) and given water ad libitum. Electrodes were implanted in the El and ddY mice when they were 26 weeks old, as described previously [1, 4]. Briefly, the mice were anesthetized with ketamine and xylazine, and four burr holes were created symmetrically anterior to the coronal and lambdoid sutures for epidural electrodes consisting of 1-mm-diameter silver balls coated with epoxy resin. The dural attachment was 0.2 mm in diameter at the tip. A reference electrode was placed on the nasal bone, and a ground electrode was placed subcutaneously near the rump. An 8-pin header was used to connect the electrodes to an electroencephalograph (EEG-8310; Nihon Kohden, Japan) with a band-pass having a time constant of 0.1 s, a 120 Hz low-pass filter, and a 50 Hz band elimination filter. The EEG was sampled with an A-D converter at 200 Hz and recorded with a digital data recorder (DR-M3b; TEAC, Japan). The EEG waves were analyzed with Pc-Wave Form software (DEICY, Tokyo, Japan), which allows the user to perform fast Fourier transformations (FFT) for the PSD analysis. The EEG was recorded for more than 1 h once a day for 3 to 8 days. Sleep was confirmed by a visual system (CCD-TR55, SONY, Japan) to monitor mice behavior. During the recording, the mice went through several sleep and wake cycles; they were considered a sleep when conscious levels were substantially decreased, and they remained still in a lying-down position with their
eyes closed and with diminished response to environmental stimuli [5]. We picked up the periods when sleep was confirmed, and the EEGs synchronizing to the period were decided as sleeping EEGs. The PSD analysis used these representative sleeping EEGs. In the EEG of El mice, the areas selected for PSD analyses contained more than eight interictal discharges. The sampling period of each analysis was for 2 s.

The study was performed with permission from the Laboratory Animals Committee of Nippon Veterinary and Life Science University. All mice were treated according to NIH guidelines on the care and use of laboratory animals.

Results

All mice survived the surgery and remained in good health. The EEGs of the El mice repeated interictal discharges from several to around 20 times during one recording.

Figures 2 and 3 show typical monopolar EEGs obtained from the frontal left and their PSD (A) analytical results for ddY and El mice, respectively. A similar peak was detected at 3.13 Hz in both strains. Both figures (B) show the results of PSD analysis obtained from the frontal left-right bipolar recording EEG.

Figure 4 shows representative raw EEG signals containing interictal discharges recorded in a sleeping El mouse. These discharges were quite likely to occur synchronously at all four epidural electrodes, with different amplitudes and various fluctuations. Besides the respiratory peak at 3 Hz, there were peaks at 8.59 (I), 17.97 (II), and 26.56 Hz (III). There was no peak between the respiratory peak and peak I. For each strain, all three mice gave identical, reproducible results.

Discussion

Monopolar EEG waves were recorded in sleeping mice and analyzed by PSD by performing FFT on the raw data [6]. Periodic baseline fluctuations were observed in the monopolar EEGs, and PSD analyses identified a peak at 3 Hz in EEGs in both strains. In a previous study, we synchronously recorded monopolar EEGs of mice anesthetized with ether and, using a piezoelectric gauge, their abdominal movements due to respiration, and found that the abdominal movements corresponded with the regular baseline fluctuations in the EEGs at a frequency of about 3 Hz, suggesting that the peak is derived from respiratory movements [1].

In EEGs recorded in sleeping El mice, we often found interictal discharges, and the PSD analyses of the waves revealed three major peaks at frequencies higher than the respiratory peak. The three peaks are fairly evenly spaced apart, having frequencies of 8.59, 17.97, and 26.56 Hz, suggesting that the latter two could be harmonics of the former. Therefore, 17.97 and 26.56 Hz were multiple waves of the 8.59 Hz peak. The frequency multiplication wave of \( n \) times basic frequency coexists in an oscillation phenomenon of spike waves and is known physically. Therefore a frequency of these spectrums is emphasized in PSD. However, there was not a peak to the half frequency of 8.59 Hz. The 8.59 Hz peak always appeared in the PSD analytic results of the El mice EEG waves that contained interictal discharges. This peak is therefore proba-
bly a fundamental frequency associated with the interictal discharge. In humans, interictal discharges that do not accompany seizures have been recorded during non-REM sleep in the epileptics, and the frequencies of the interictal discharges were around 10 Hz [7], which is fairly close to that in the EI mice. Therefore the presence of interictal discharges at around 8–10 Hz might be an indicator of epilepsy in both humans and mice.

The present study indicates that more information can be extracted from the EEGs of EI mice by means of a PSD analysis. In a future study, we will perform PSD analysis on EEGs in EI mice during spontaneous ictal discharges.

REFERENCES
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