Wavelet and fractal analysis of rat brain activity in seizures evoked by camphor essential oil and 1,8-cineole

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Abstract. We investigated the rat brain activity in acute seizures evoked by camphor essential oil or its main constituent 1,8-cineole by wavelet (primarily) and fractal analysis. Experiments were performed on anesthetized animals before and after intraperitoneal camphor oil or cineole administration. The properties of frequency bands in pre-ictal, ictal and inter-ictal stages have been determined by wavelet analysis. The domination of δ frequency band was confirmed in obtained brain activities, which participate with ≈45% of mean relative wavelet energy (MRWE) in control signals and arise up to ≈76% MRWE in energy spectrum during the ictal stage (after drug administration). Other frequency bands decreased during ictal stage and arised in inter-ictal stage. There was a dose-dependent response of cineole effect: increase in cineole concentration leaded to the higher values of relative wavelet energy (RWE) of δ frequency band while there were slight changes of the mean fractal dimension (FD) values as a measure of system complexity.

Key words: Electrocortical activity — Wavelet analysis — Fractal analysis — Camphor oil and 1,8-cineole — Epileptic seizures

Introduction

Neurotoxic effects could appear by wrong usage of essential oils and other plant preparations in alternative/traditional medicine, cosmetics and food preparations. Particularly, essential oils that have monoterpen constituents, such as camphor and 1,8-cineole, may induce epileptic seizures in humans and animals (Medvedev 1990; Grbić et al. 2006). As the wavelet transform was a powerful and suitable tool designed for analysis of non-stationary signals, a new scheme of optimum classification of epileptic seizures based on wavelet analysis of electroencephalograms has recently appeared (Ocak 2008). We already performed fractal analysis on cerebral electrocortical signals and found that during ictal stages the fractal dimension (FD) value was lower than before camphor essential oil administration as well as during interictal periods (Grbić et al. 2008). The aim of this study was to investigate the acute effect of 1,8-cineole as the main constituent of camphor essential oil and to develop a new technique to quantify the electrocortical changes by wavelet analysis. A preliminary account on this study has appeared recently (Culić and Keković 2008).

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Materials and Methods

Surgical procedure and camphor essential oil/cineole administration

The experiments were performed on adult male rats, as described in our recent study (Grbić et al. 2008), in accordance with the European Council Directive (86/609/EEC) and approved by our Institute's local Ethical Committee. The surgery was done under pentobarbitalsodium (Serva, Heidelberg) – initial dose of 35 mg/kg and subsequently ~8 mg/kg every 50–60 min when necessary, to obtain light anesthesia throughout the experiment. Each animal was mounted in a stereotaxic apparatus. Partial round-shaped craniotomies were made over the parietal cerebral cortex (P: 2.0–2.5 mm; L/R: 2.0–2.5, in respect to bregma). For inducing acute seizures, camphor essential oil (Institute for Medicinal Plant Research "Josif Pančić", Belgrade, Serbia) or 1,8-cineole (Sigma, USA) were used by intraperitoneal administration. The experimental animal was injected by camphor essential oil (at doses 400/500/600 µl/kg) dissolved in 1 ml of saline or by 1,8-cineole (at doses 300/400/500 µl/kg) – in 1 ml of saline. It should be pointed out that the main constituent of the used camphor essential oil was 1,8-cineole (73.01%) while the other constituents were as follows: camphor (9.18%); α-terpineol (2.14%); borneol (1.95%); p-cymene (1.65%) and terpinen-4-ol (1.05%). All the rats survived acute experimentation and did not show any behavior peculiarities in the following days.

Recording procedure and data acquisition

Local field potentials (LFPs) of the cerebral cortex were monopolarly recorded by epidurally positioned silver ball electrodes or, intracortically, superficially positioned tungsten micro-electrodes, with a ground electrode laid over the frontal bone and temporal muscles. Cortical activity was amplified and filtered by a multichannel processor (Alpha-Omega Eng, Nazareth) with band pass filter DC (direct current) to 1 kHz and a 50 Hz notch. We used the program package SIG VIEW (Jovanović 2004) for data acquisition. The biosignals were digitized at the sampling rate of 256 Hz, filtered to avoid artifacts which occasionally appeared at 61 and 106 Hz. The biosignals were recorded sequentialy during the period about 180 min, with ~5 min interuptions; each recorded sequence lasted ~2 min.

Fractal analysis

In brief, we calculated FD values of cerebral electrocortical activity in anesthetized rats before and after camphor oil/cineole administration using Higuchi's algorithm (Higuchi 1988; Klonowski et al. 2000; Spasić et al. 2005) with slight modifications. Parameter N = 200 was equivalent to an epoch (window) duration of 781 ms and parameter $k_{max} = 8$ was the optimum choice. Individual FD values were averaged across all epochs for particular experimental conditions before and at certain time exactly after camphor oil/cineole administration to obtain the mean FD.

Wavelet analysis

The original software of discontinual wavelet analysis was developed in this study. Therefore, the mathematical basis and certain details will be described more carefully.

In the wavelet analysis (Metin 1997; Latka et al. 2003) an arbitrary signal is analyzed by quickly vanishing oscillating functions called wavelet family $\psi_{a,b}(t)$ which are generated from mother wavelet $\psi(t)$:

$$\psi_{a,b}(t) = \frac{1}{\sqrt{|a|}}\psi\left(\frac{t-b}{a}\right) \tag{1}$$

where $a, b \in R, a \neq 0$ represent the scale and shifting parameters, and t is time. The scaling parameter a is inversely related to the frequency of the analyzed signal: by increasing this parameter lower frequencies can be analyzed. The shifting parameter b controls translation of wavelet until the whole signal is covered. As a result in continuous wavelet transform appear the wavelet coefficients defined by equation:

$$C_{a,b} = \frac{1}{\sqrt{a}}\int f(t)\psi\left(\frac{t-b}{a}\right)dt \tag{2}$$

where $f(t)$ signifies the analyzed signal. For the discrete set of parameters: $a_j = 2^{-j}$, $b_{j,k} = 2^{-j}k$ the wavelet family constitutes an orthonormal basis of the Hilbert space:

$$\int 2^{j_1}\psi(2^{j_1}t-k_1)\psi(2^{j_2}t-k_2)dt = \delta(j_1-j_2)\delta(k_1-k_2) \tag{3}$$

For simplicity, we choose sampling time $t_s = 1$ s then arbitrary signal $f(t)$ can be decomposed into a sum of wavelet coefficients and the appropriately constituent wavelets:

$$f(t) = \sum_{j=-N}^{-1}\sum_k C_j(k)\psi_{j,k}(t) \tag{4}$$

where N represents number of resolutions levels. Similar to the Fourier theory, there is a concept of total energy contained in the windows consisting of N_S sampling points of a signal. It is given by the expression:

$$E_{tot} = \sum_j\sum_k \left|C_j(k)\right|^2 \tag{5}$$

In expression given above terms: $E_j = \sum_k \left|C_j(k)\right|^2$ signify energy of signal at the resolution level j given by summing all over sampled time k. Now, we are in a position to define very useful quantifier for analyzing EEG signals (Daud et al. 2005; Magosso et al. 2007) and which is called relative wavelete ernergy (RWE):

$$\rho_j = \frac{E_j}{E_{tot}}; \qquad j = -N, -N+1, \ldots, -1 \tag{6}$$

From physical point of view this quantifier can be understood as a distribution of energy trough resolution levels and time. From the above formula it can be concluded that is always fulfilled:

$$\sum_j \rho_j = \left(\sum_j E_j\right)/E_{tot} = E_{tot}/E_{tot} = 1 \tag{7}$$

By continuing in this manner, we are expressing the mean relative wavelet energy (MRWE) which represents the mean value of RWE at some stage of a signal. In the purpose of investigating the frequency bands of a signal, it is necessary to establish connection between frequency bands and resolutions levels. This can be done *via* the formula:

$$\frac{f_C \cdot N_S}{2^{j+1}} \le \Delta f \le \frac{f_C \cdot N_S}{2^{j}} \tag{8}$$

where Δf is a frequency band of j-th resolution level, f_C is "central" frequency of the mother wavelet which is used for a particular choice of Haar wavelet as the simplest and the most important from Daubeshi family wavelet (f_C = 0.996 Hz), N_S is number of sampling points (N_S = 256). We marked the frequency bands as in our previous study (Ćulić et al. 2005).

Results

The increased amplitude of electrocortical activity with occasional single and multiple spiking of high amplitude (ictal activity) without behavior changes at parietal cortical level occurred about 2–10 min after camphor essential oil or its main constituent 1,8-cineole injection and lasted for 2–3 h. The behavioral signs of camphor essential oil/cineole evoked neurotoxicity – convulsions of forelimbs/hindlimbs, were presented only sometimes, because of the supression induced by anesthesia. We compared the cerebral electrocortical activity characterized by epileptic like seizures obtained in the typical C2D rat 45 min after camphor essential oil administration and control signals (before camphor oil administration), as shown on Fig. 1a,b.

By applying the discrete Wavelet transform to those signals, general characteristics of their frequency bands were obtained, where parameter RWE determined energy spectrum of the signal. Analysis of cerebral electrocortical activity 45 min after camphor oil administration (C2D13T1) presented on Fig. 2a,b, showed the trend of increasing RWE of δ frequency and decreasing RWE of θ, α, β, γ frequency bands during of epileptic seizures, with superposed peaks from α and β band. With dashed line end of epileptic seizures has been marked. After ending the seizure, the situation is completely different: there is increasing in parameter RWE of α, β and γ frequency bands with peaks from α and β bands. In both cases, the RWE

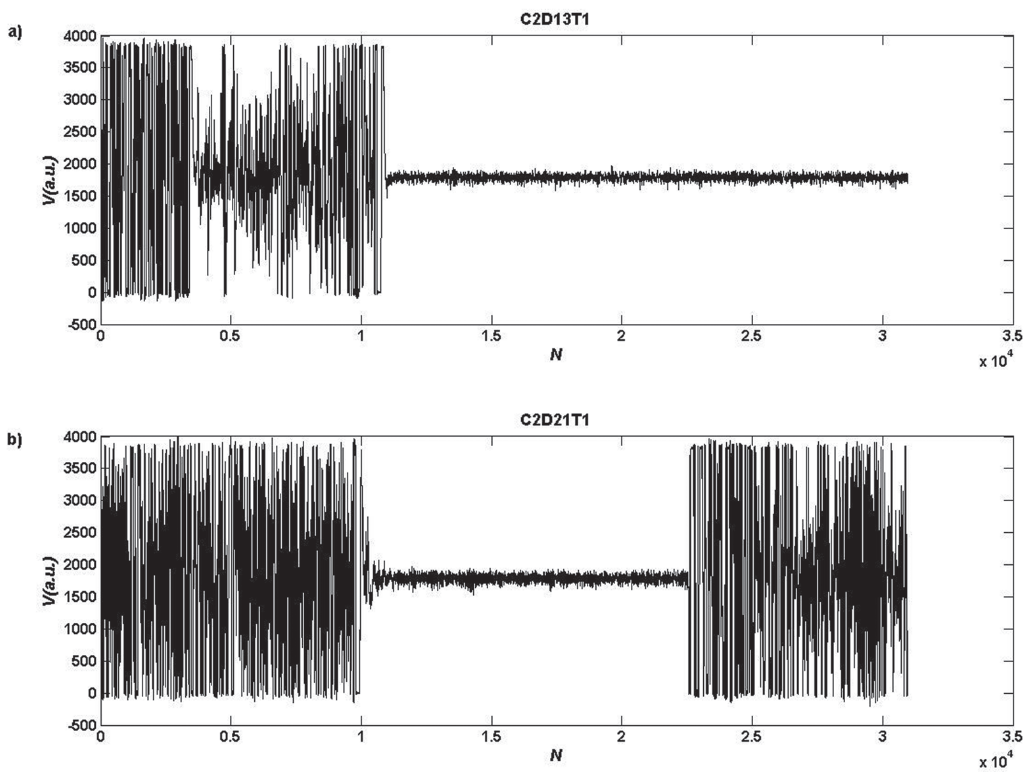

Figure 1. The characteristic signals with epileptic activity (ictal and inter-ictal): C2D13T1 – 45 min after (a) and C2D21T1 – 65 min after camphor oil administration (b). Signal intensity is expressed in arbitrary units (a.u.) of local field potential versus number of experimental points N.

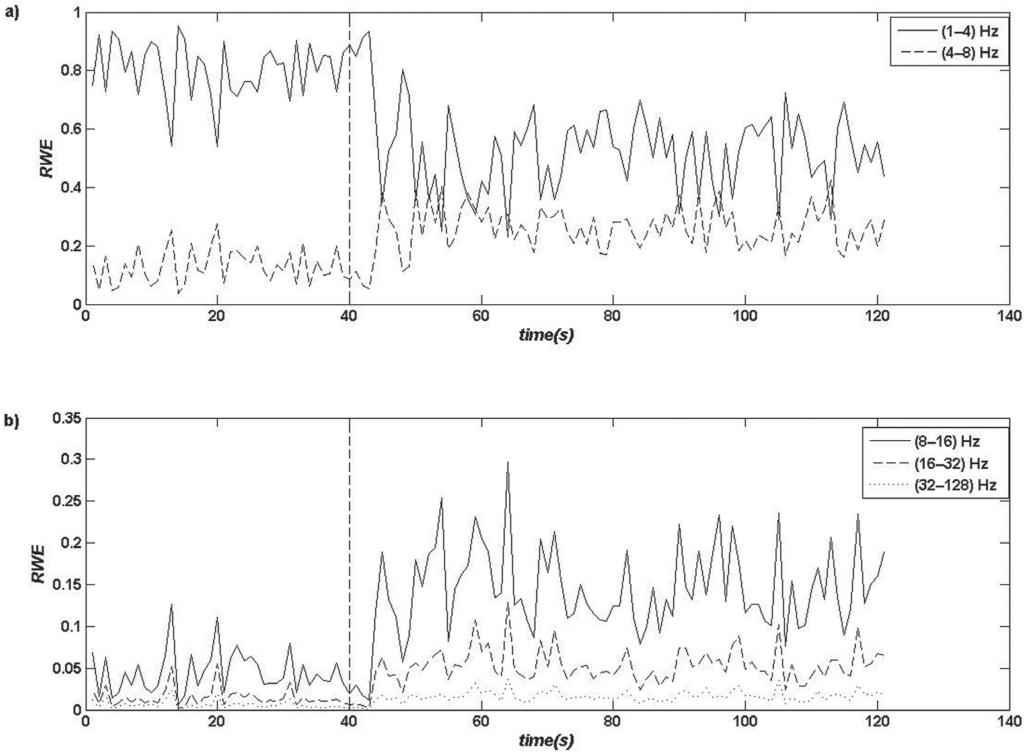

Figure 2. The distribution of parameter RWE of the signal C2D13T1: a) δ (1–4 Hz) and θ (4–8 Hz) b) α (16–32), low β (8–16 Hz) and γ (30–128 Hz) frequency bands, versus time (in seconds).

dominate in energy of spectrum of signal with characteristic values of MRWE = 0.81 during epileptic seizures and MRWE = 0.52 after epileptic seizures, which means that it is contained 52–81% in energy spectrum of the signal. Even 2 h after camphor oil administration, seizure activity has appeared and analysis of the signal C2D21T1 (Fig. 3a,b) could mark the start and end of inter-ictal stage. In this case, the value of parameter MRWE varies from 0.42 (inter-ictal stage) to 0.76 (ictal stage), which corresponds to 42–76% δ band in energy spectrum of RWE. The peaks from α and β bands could be observed in Fig. 3. However, in order to complete the analysis, it is necessary to define mean activity of all frequency bands and calculate standard devaitions. The features of various frequency bands of electrocortical activity 45 min after camphor oil administration (C2D13T1) could be observed from Table 1. During the ictal stage, δ frequency band (MRWE = 0.81 ± 0.10) increased, while θ (MRWE = 0.13 ± 0.06) slightly decreased, but activity of both bands were higher than activity of the control signal. Intensities and activities of other frequency bands decreased, except of γ frequency band where certain increase in parameter MRWE = 0.06 ± 0.05 could be noticed as compared to the control (MRWE = 0.03 ± 0.02).

Table 1. The values of parameter MRWE (±SD) of the control signal C2D02T1 and the signal C2D13T1 45 min after camphor oil administration

Frequency band (Hz)	Ictal stage MRWE		Post-ictal stage MRWE	
	Control	C2D13T1	Control	C2D13T1
1–4	0.64 ± 0.11	0.81 ± 0.10	0.71 ± 0.10	0.52 ± 0.13
4–8	0.19 ± 0.06	0.13 ± 0.06	0.16 ± 0.05	0.26 ± 0.07
8–16	0.09 ± 0.03	0.04 ± 0.03	0.08 ± 0.03	0.15 ± 0.05
16–32	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.02
32–128	0.03 ± 0.02	0.06 ± 0.05	0.02 ± 0.01	0.02 ± 0.01

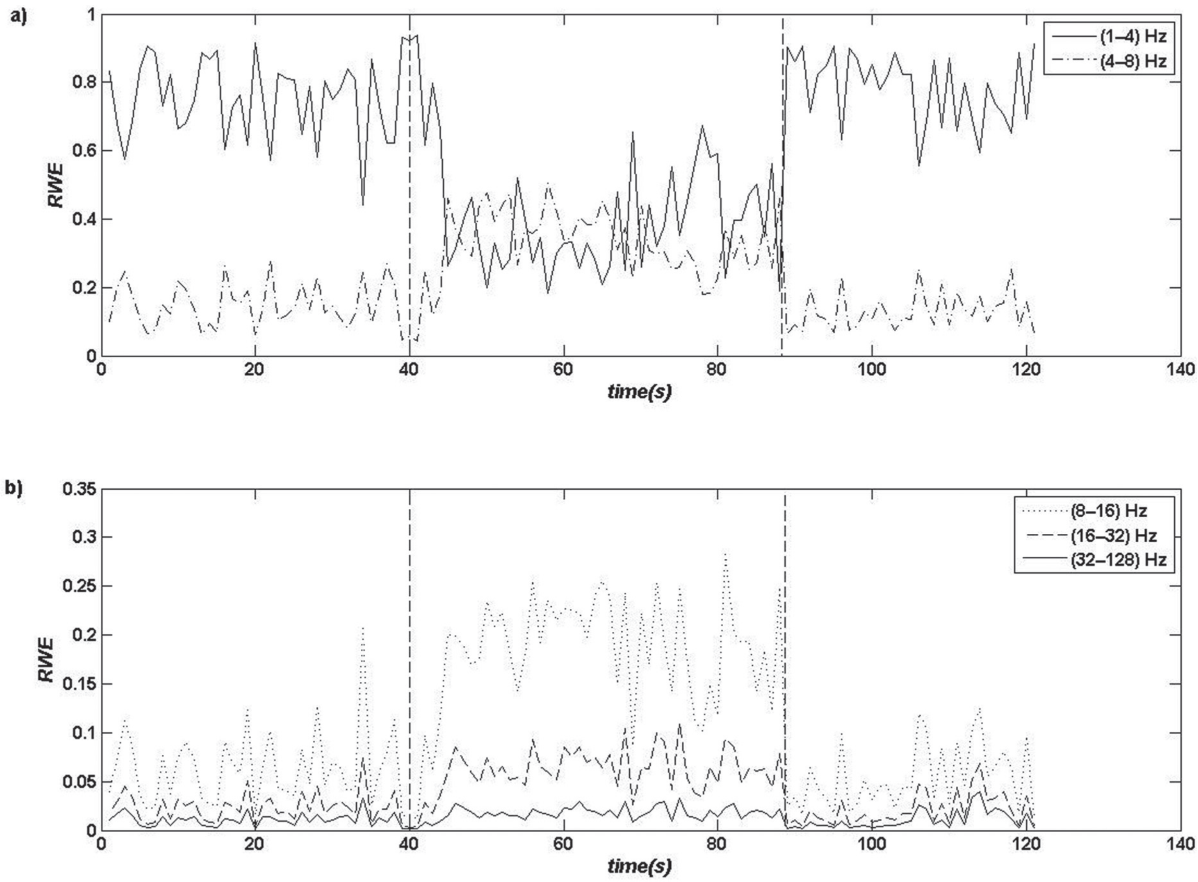

Figure 3. The distribution of parameter RWE of the signal C2D21T1: a) δ (1–4 Hz) and θ (4–8 Hz) b) α (16–32), low β (8–16 Hz) and γ (30–128 Hz) frequency bands, versus time (in seconds).

As shown in Table 2, in δ frequency band of the electrocortical activity, 65 min after cineole administration (C2D21T1), corresponding values of parameter MRWE = 0.76 ± 0.12 and its standard deviation during the ictal stage were obtained. In control signal (C2D02T1) before cineole administration, value of parameter MRWE = 0.64 ± 0.11 was lower. Likewise, parameter in θ band was MRWE = 0.15 ± 0.07 during ictal stage and MRWE = 0.19 ± 0.06 during control stage, which meant that this band was very active. The value of MRWE of higher frequency bands decreased during the ictal phase. In later post-ictal stages there was a tendency of equalizing those MRWE values with the control values, with exception of α and low β frequency band (8–16 Hz) which may be suggested as the possible indicators of inter-ictal stage. Of course, this position can not be taken as such before verification on more templates.

Table 2. The values of parameter MRWE (±SD) of the control signal C2D02T1and the signal C2D21T1 65 in after camphor oil administration

Frequency band (Hz)	Ictal stage MRWE		Inter-ictal stage MRWE	
	Control	C2D21T1	Control	C2D21T1
1–4	0.64 ± 0.11	0.76 ± 0.12	0.62 ± 0.14	0.42 ± 0.19
4–8	0.19 ± 0.06	0.15 ± 0.07	0.27 ± 0.11	0.3 ± 0.11
8–16	0.09 ± 0.03	0.06 ± 0.04	0.06 ± 0.03	0.18 ± 0.06
16–32	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.02
32–128	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

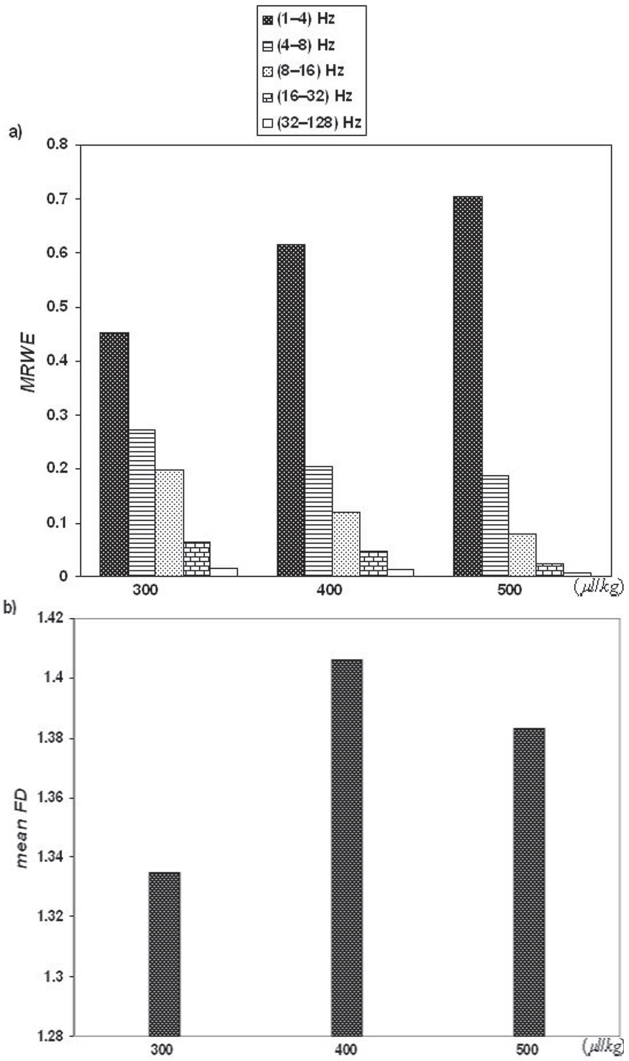

Figure 4. The influence of different doses of 1,8-cineole (300, 400, 500 μl/kg) on characteristic parameters of electrocortical activity after administration in three rats – E3, E4, E5: a) MRWE; b) mean FD.

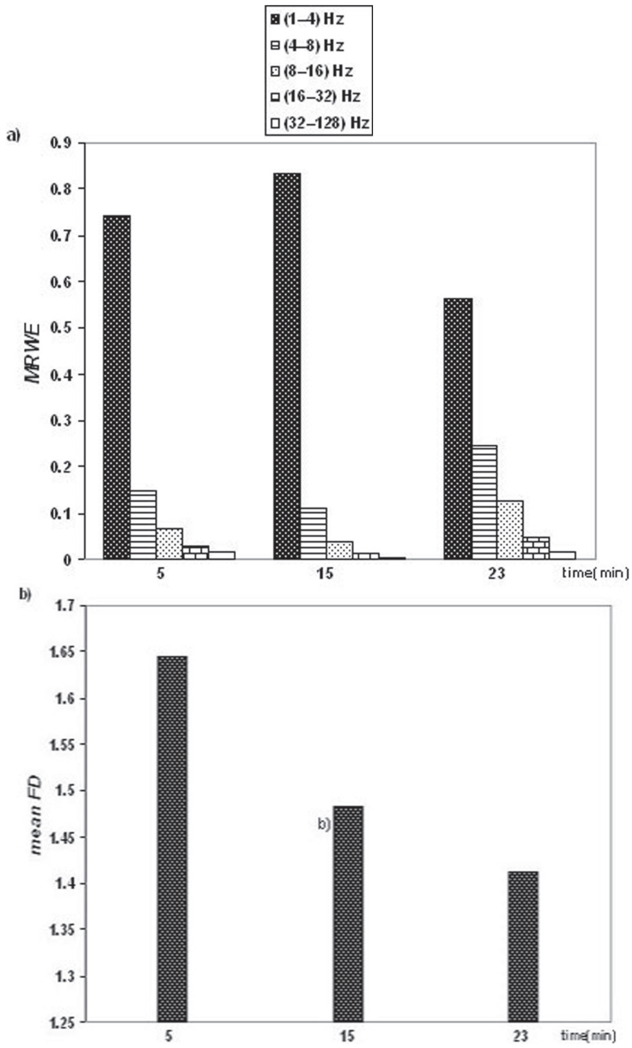

Figure 5. The distribution of characteristic parameters in terms of camphor oil induced effects: a) MRWE; b) mean FD, versus time (in minutes).

In order to complete the analytic approach to brain functioning we also calculated the FD of obtained biosignals which could be an indicator of uncontrolled electrical discharges, too. In that purpose, we compared changes of FD values with values RWE in relation to the concentration of administrated camphor essential oil/1,8-cineole. Let us first consider the distribution of RWE through energy spectrum and mean FD of signal in terms of concentration of 1,8-cineole (Fig. 4) and camphor oil (Fig. 5). There was a dose-response cineole effect: increment of cineole concentration leaded to higher value of RWE in δ frequency band. The mean FD values (as a measure of system complexity) also changed after camphor oil/cineole administration. The mean FD values followed changes in δ frequency band, but were very sensitive to changes in γ frequency band, too. Namely, the value of MRWE increased from 0.62 (after administration of 400 μl/kg of cineole) to 0.70 (after administration of 500 μl/kg of cineole), while at the same time, the FD values decreased from 1.41 to 1.38, due to changes in γ frequency band. We also monitored the changes of parameter MRWE values in time, after specified administration of camphor essential oil at the dose of 600 μl/kg (Fig. 5). The up-growth of parameter MRWE of δ band to values 0.74 indicated epileptic/like activit*y* 5 min after camphor oil administration, which was followed by decreased MRWE of other frequency bands. At the same time, the values of mean FD was 1.64 and lower relative to the control value. Furthermore, after 15 min we have recorded the higher values of MRWE of δ

(0.83) and degradation of MRWE of γ band from 0.02 to 0.01 while mean FD value decreased to 1.48. The reasonable explanation of this behavior may be that FD as a measure of chaos in brain is relatively simple: during ictal stages, because of reduced activity of higher frequencies bands, many degrees of freedom of system are removed. But, there is another point: mean FD has maximum values, while the values of MRWE of δ and γ frequency band are increasing, at the same time.

Discussion

The electrophysiologic effects of 1,8-cineole parenterally administrated at doses of 300–500 μl/kg completely resembled the effects of camphor essential oil injections at doses of 400–600 μl/kg. This work also showed that certain properties of acute epileptic like seizures induced by camphor essential oil or by its main constituent, 1,8-cineole could be described on satisfactory way in the frame of wavelet analysis. By applying this tool, the properties of frequency bands in preictal, ictal and inter-ictal stages have been determined. The domination of δ frequency band in cerebral eletrocortical activities has been confirmed and it participated with ≈45% MRWE in the control signal (before camphor oil/cineole admnistration) and arised up to ≈76% MRWE in energy spectrum during the ictal stage. Inversely, other frequency bands were decreasing during ictal stages and were rising during inter-ictal stages. This behavior could be explained by hypothesis on hierarchical organization of neural oscillations in the brain. Slow waves are trying to be in accord with higher frequency bands, but it could not happen, because there is a brain disorder in the form of uncontrolled electrical discharges or epileptic like seizures. In the post/inter-ictal stages the value of RWE of δ band is decreasing ≈32% relative to the control value but other frequency bands are increasing up to the values higher than the control values. It is especially interesting that α and low β (8–16 Hz) increased ≈173% relative to the control values and this nominates these frequency bands as possible indicators of inter-ictal stages. Also, the effects of electrocortical activity induced by different doses of 1,8-cineole or camphor essential oil were investigated. It has been shown that higher concentracions generated the higher values of parameter RWE of δ frequency band. This value is in correlation with the value of RWE of γ frequency band which has significant impact on the mean FD value as a measure of chaos in the brain.

There was a general trend of the relative increase in low frequency bands and decrease in high frequency bands during epileptic seizures in accordance with previous findings (Kharlamov 2003). This fact, once again, clearly confirms predominance of δ frequency band not only during anesthesia but the key role of slow waves in complex brain activity induced by neurotoxic drugs. There was an interesting report from Berkley (Canolty et al. 2006) who found … "the first experimental evidence that slow brain oscillations " tune in" the fast brain oscillations or γ waves. On that way different regions in the brain much easier transfer information among each other". This gives a new insight in brain functioning and introduces a very interesting hypothesis on hierarchy of oscillations in the brain. Our wavelet results in this study are in agreement with our recent studies on camphor essential oil effects on spectral changes in brain activity (Grbić et al. 2006) and fractal changes of cerebrocortical activity (Grbić et al. 2008), but we point out some advantages of wavelet analysis in possible prediction of epileptic seizures by defining the preictal stages. It has been suggested that a dimensional change in brain activity occurs before seizure onset and it is now thought to possess predictive powers (Babloyantz and Destexhe 1986). The application of nonlinear deterministic dynamics and powerful algorithms were devised to analyse the behavior of the seizure state (Schiff 1998). When principles of nonlinear dynamics are applied on the time series domain, they can yield measures of fundamental information about complex brain dynamics (Elger et al. 2000).

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