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EFFECT OF CAMPHOR ESSENTIAL OIL ON RAT CEREBRAL CORTEX ACTIVITY AS MANIFESTED BY FRACTAL DIMENSION CHANGES

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Abstract — The aim of our study was to investigate the effect of camphor essential oil on rat cerebral cortex activity by fractal analysis. Fractal dimension (FD) values of the parietal electrocortical activity were calculated before and after intraperitoneal administration of camphor essential oil (450-675 μl/kg) in anesthetized rats. Camphor oil induced seizure-like activity with single and multiple spiking of high amplitudes in the parietal electrocorticogram and occasional clonic limb convulsions. The FD values of cortical activity after camphor oil administration increased on the average. Only FD values of ictal ECoG sequences were lower than those before camphor oil administration.

Key words: Camphor oil, electrocortical activity, seizure, fractal dimension, rats

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INTRODUCTION

Plant-derived preparations, including those which influence brain function, are not inherently superior to synthetic substances with regard to efficacy and safety in matters related to human health (J o h n s t o n, 2003). Serious neurotoxic effects may result from misuse of essential oils and other herbal medicinals widely used in alternative and traditional medicine, as well as cosmetics. Essential oils containing large amounts of monoterpens like camphor and cineole could cause the epileptic activity in animals and humans (S t e i n m e t z et al., 1987; M e d v e d e v, 1990; R u h a et al., 2003).

Our recent results on brain activity after injury as studied by fractal analysis (S p a s i ć et al., 2005 a) indicate the possibility of using such analysis as a promising tool for recognition of some pathophysiological states. Chaotic behavior may be a typical response of biological systems (S a v i, 2005). Epileptogenic activity as a dangerous consequence of essential oil activity therefore represented a new challenge for our investigation. It is still unclear whether normal electroencephalographic signals have a higher complexity than signals related to pathological states (epileptic seizures, for example), which exhibit low dimensional complexity. In some episodes of epileptic activity, there is a suggestion of loss of brain signal complexity (L e h n e r t z and E l g e r, 1995). The ictal, interictal, and even preictal states can be detected from the brain dynamics of patients with neocortical partial epilepsy (N a v a r r o et al., 2002). However, the prediction of epileptic seizure from electroencephalograph recordings is an open issue and there is still no method that can reliably provide predictions despite the success of many methods from different fields, such as nonlinear dynamics and chaos, neural networks, statistical testing etc. (S t a m, 2005; K u g i u m i t z s et al., 2007).

Our recent study (S p a s i ć et al., 2007) on fractal analysis of brain signals in an animal model of acute epilepsy evoked by camphor essential oil showed that there was increased complexity of cerebral and cerebellar interictal activity during 60 min after camphor oil administration. In this study, the aim was to investigate in more detail the effect of camphor essential oil on rat parietal electrocortical activity described by fractal dimension changes.

MATERIALS AND METHODS

Experimental animals, surgical procedure and camphor essential oil administration

The experiments were performed on eight male rats at 3.5 months of age. All procedures were done in accordance with the European Council Directive (86/609/EEC) and rules for the care and use of laboratory animals practiced at the Institute for Biological Research in Belgrade. Surgery was performed under sodium pentobarbital (Serva, Heidelberg) in an initial dose of 35 mg/kg and subsequently ~ 8 mg/kg every 50-60 min as needed, to obtain light anesthesia throughout the experiment. Each animal was mounted in a stereotaxic apparatus. Partial round craniotomies were made over the parietal cerebral cortex (P: 2-2.5 mm; L/R: 2-2.5 mm). In the acute experiments, the rats were injected intraperitonealy with camphor essential oil at doses 450-675 µl/kg in 1 ml of saline. All rats survived acute experiments and did not show any behavioral peculiarities.

Components of camphor essential oil

For qualitative and quantitative analysis of components of the essential oil, gas chromatography-mass spectrometry (GC-MS) was performed with a Varian instrument, model 3400, using a capillary column (0.32 mm x 30 mm; DB-WAX) and the following program: carrier gas H_2, (3 ml/min); injector temperature, 250°C (SPLIT 1:1000); detector temperature, 300°C; and temperature program, 50-220°C, 10°C/min. The gas chromatograph was connected via an open split interface and a fused silica capillary (at 250°C) to the ion source of a Finnigan MAT 8230 mass spectrometer equipped with a PD.11/74 computer. The working conditions were as follows: carrier gas, 2 ml He/min; other GC conditions, as indicated above; and MS: ion source (electron impact), 170°C, 70 eV. Gas chromatography and mass spectrometric analysis showed that the main constituents of the applied camphor essential oil were: 1,8-cineole (73.01 %); camphor (9.18 %); α-terpineol (2.14 %); borneol (1.95 %); p-cymene (1.65 %); terpinene-4-ol (1.05 %).

Recording procedure and data acquisition

Local field potentials of the cerebral cortex were monopolarly recorded by epidurally positioned silver ball electrodes or intracortically by superficially positioned tungsten microelectrodes, 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 a low-pass filter (150 Hz) and a 50 Hz notch. Each recorded sequence lasted 121-241 s, and there were at least 20 recorded sequences during two experimental periods: before (for 30 min) and after (for 120 min) camphor oil administration. We analyzed digitized electrocortical signals at a sampling rate of 256 samples/s, filtered to avoid artifacts at 61, 107, and 121 Hz.

Fractal and statistical analysis

In the present work, we applied fractal analysis to study variability in complexity of the preictal, ictal, and interictal ECoG sequences. Fractal dimension (FD) values of cerebral electrocortical activity in anesthetized rats were calculated before and after camphor oil administration using Higuchi's algorithm (Higuchi, 1988; Klonowsky et al., 2003; Spasić et al., 2005a).

On the supposition that a series of cortical activity was a time sequence $x(1), x(2),\dots,x(N)$, we constructed k new self-similar time series $x(k,m)$ for $m=1, 2,\dots,k$ and $k=1,\dots,k_{max}$. The length $L(m,k)$ was computed for each of the k time series or curves $x(k,m)$. The $L(m,k)$ values were averaged for all m, forming the mean value of the curve length $L(k)$ for each k. An array of mean values $L(k)$ values was obtained, and the FD value was estimated as follows: $FD=\log(L(k))/\log(1/k)$. In this study, ECoG recordings were divided into non-overlapping epochs to deal with nonstationarity of ECoG signals. Parameter $N = 200$ was within the range used by other authors (Cizsewski et al., 1999) and was equivalent to an epoch (window) duration of 0.78125 s at a sampling rate of 256 s^{-1}. We have chosen $k_{max} = 8$ on the basis of our recent study (Spasić et al., 2005 b) on the optimum choice of k_{max} values. Individual FD values were averaged across all epochs for particular experimental conditions before and at the time immediately after camphor oil administration to obtain the mean and standard deviation of FDs.

We performed analysis of variance (one-way ANOVA) and post-hoc least significant difference (LSD) to test changes of FD values during the time after camphor oil administration.

RESULTS

The effect of intraperitoneal administration of camphor essential oil can be recognized within 3-15 min after drug administration from amplitude and frequency changes in parietal electrocortical activity of both hemispheres, but also at longer latency after camphor oil injection. The behavioral signs of camphor oil neurotoxicity – tonic-clonic convulsions of forelimbs/hindlimbs – were sometimes present, but usually they were suppressed by anesthetic administration. Ictal electrocortical activity with occasional single and multiple spiking of high amplitude and slow frequency without behavior changes can occur not only at the beginning of the camphor oil effect, but also during the next 1-2 hours with shorter or longer interictal episodes, as shown on Figs 1 and 2.

Fractal analysis of brain activity after camphor oil administration revealed interesting changes in the electrocortical signal. On the example of rat C7 (Fig. 1), we found an increase of the mean FD in the left cerebral cortex after intraperitoneal administration of 525 µl/kg of camphor oil. The mean FD of cerebral electrocortical activity increased from the control value of 1.26 (before camphor oil injection) to a preictal value of 1.31 soon after camphor oil administration. In the later phase, 55 min after camphor oil administration, there was an increase of the mean FD to 1.39.

In the case of rat K3, changes of FD values after 675 µl/kg camphor oil administration in the preictal, ictal, and restitution episodes are shown in Fig. 2. The mean FD of cerebral electrocortical activity in rat K3 was 1.37 (before camphor oil injection), after which the preictal mean FD value (10 min after camphor oil administration) was 1.45. In the ictal phase (45 min after camphor oil) the mean FD was 1.34 whereas in the restitution phase 95 min after camphor oil the mean FD was 1.51 (Fig. 2).

We would like to underline the notable changes

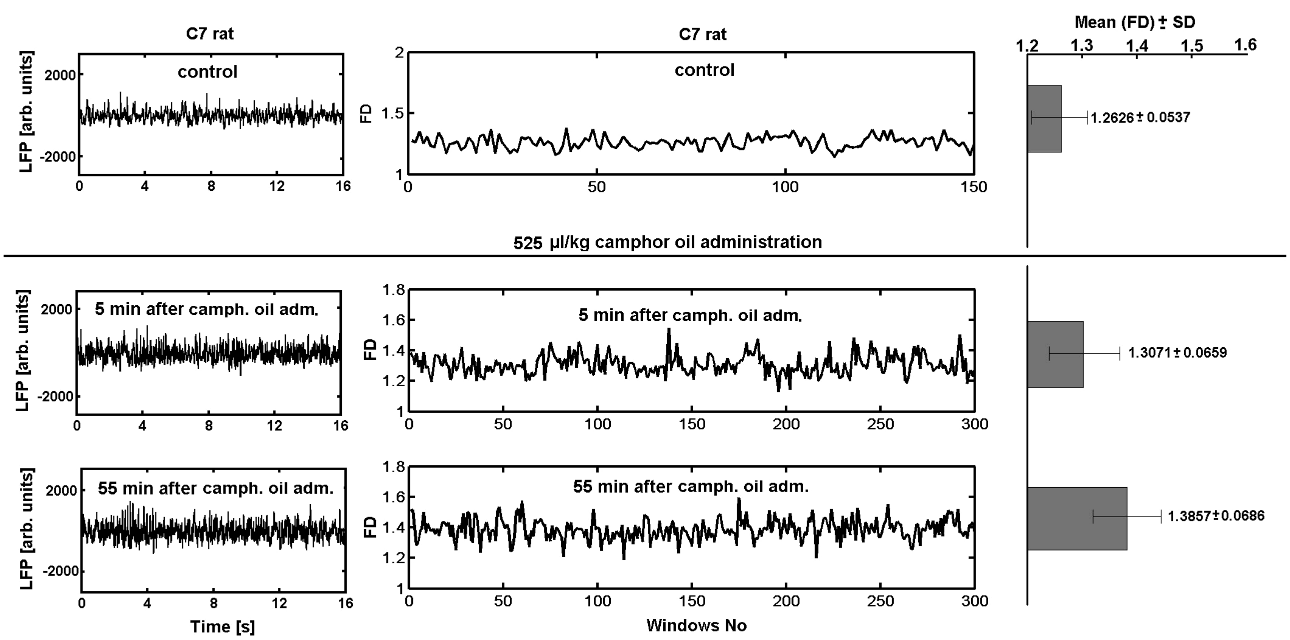

Fig. 1. Parietal electrocortical activity of rat C7 before and after intraperitoneal camphor oil (525 µl/kg) administration (left panel). Fractal dimension of ECoG in time domain (middle panel). Means and standard deviations of FD (right panel).

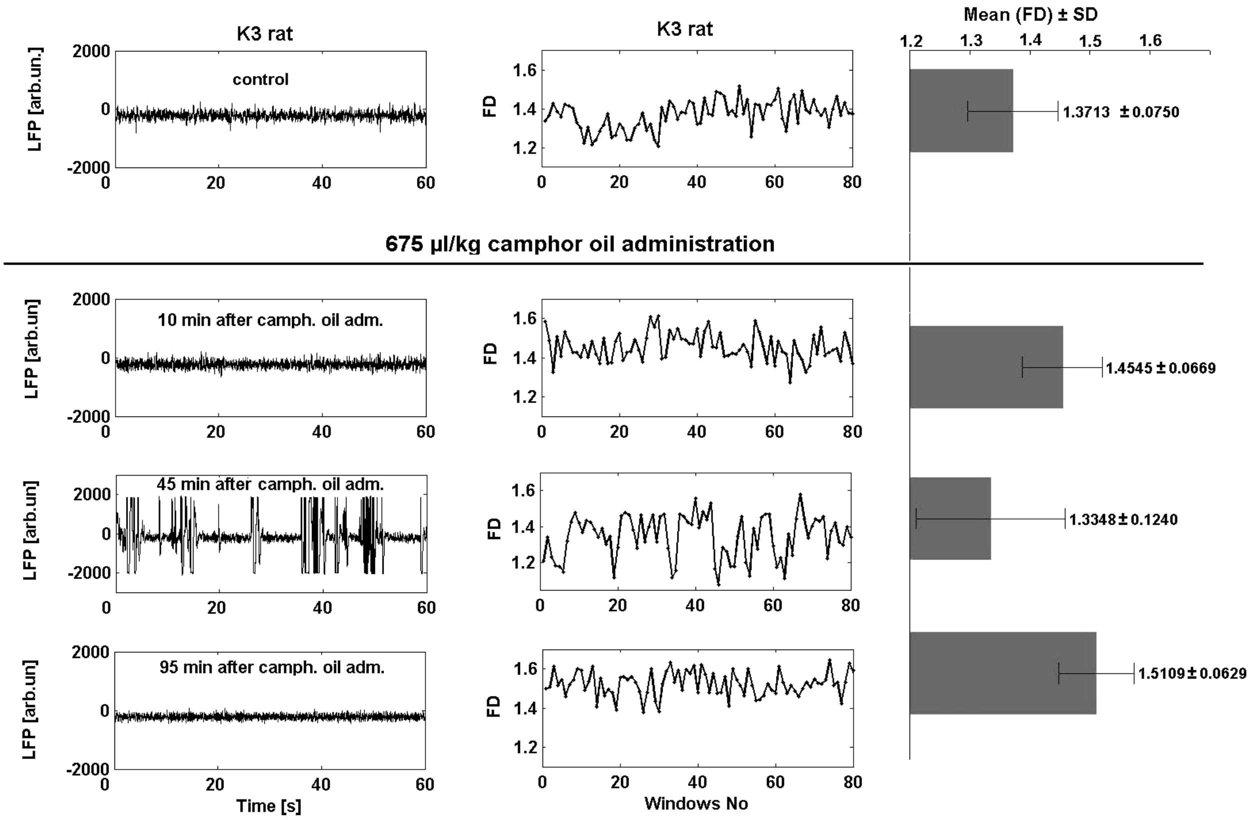

Fig. 2. Parietal electrocortical activity of rat K3 before and after intraperitoneal camphor oil (675 µl/kg) administration (left panel). Fractal dimension of ECoG in time domain (middle panel). Means and standard deviations of FD (right panel).

of FD values in the ictal phase - 45 min after camphor oil administration. It was observed that FD values ranged from a very low value (1.06) during ictal episodes to a very high value (1.58) during interictal segments with large standard deviation (0.12). In this case, the mean of FD is lower than the mean of FD in the control state due to the dominant presence of ictal phases.

The appearance of preictal and ictal phases during time varied in rats and evoked variation of FD changes after camphor essential oil administration in the group of eight animals in such way that particular changes of the preictal and ictal phases can overlap. However, our result clearly showed that FD increased after camphor oil administration. Means and standard deviations of FD values of parietal electrocortical signals from eight rats in the control phase and in the preictal phase, two ictal phases, and restitution phase are shown in Fig. 3. There was a significant increase of the mean FD in the ictal phases after camphor essential oil administration compared to the control values (* for $p<0.05$, ** for $p<0.01$). Also, there was a significant decrease of the mean FD in the restitution phase compared to the last ictal phase ($p<0.05$).

DISCUSSION

The camphor essential oil we used corresponds to the oil of *Cinnamomum camphora,* known also as ravensara camphor oil of the 1,8-cineole type (J u l i a n i et al., 2005). Our results confirm that camphor essential oil has convulsant properties even in anesthetized rats. There are changes in rat electrocortical activity after intraperitoneal administration of camphor oil, as we have already shown by spectral analysis (G r b i ć et al., 2006) and now with

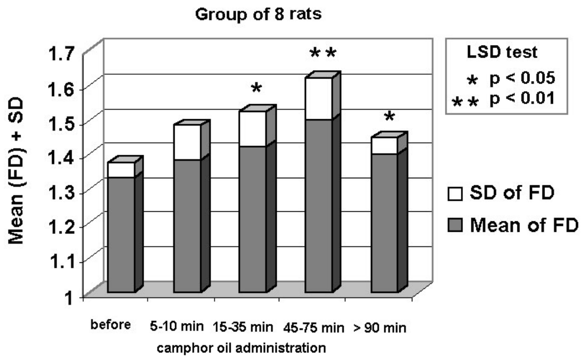

Fig. 3. Means and standard deviations of FD values of parietal electrocortical signals from eight rats before camphor oil administration and over four periods (5-10min, 15-35 min, 45-75 min, and more than 90 min) afterwards.

fractal analysis. However, the mechanisms of toxicity are not resolved. We believe that the effect is due to the highly reactive monoterpenes: camphor and cineole and their synergistic action, as previously suggested (B u r k h a r d et al., 1999). The effective doses of the essential oil used (with respect to these main constituents) were much lower than those of synthetic camphor used in awake epilepsy-prone rats (M e d v e d e v, 1990), but several hundred times greater than the doses of lavender essential oil used for plant therapy of dementia (P e r r y et al., 2003). Dose-related pharmacotoxic symptoms were noted (F a r h a t et al., 2001) in mice following injections of *Salvia libanotica* essential oil (with about 50% of 1,8-cineole and about 12% of camphor as its main constituents). Serious pediatric toxicity resulting from exposure to small amounts of camphor-containing products has long been a problem (L o v e et al., 2004; G u i l b e r t et al., 2007). There are reports of convulsions in a child exposed to a cosmetic balm containing terpenes (L a r i b i e r e et al., 2004). The mechanisms of terpenoid effects are not well-known, but drugs derived from natural plant extracts and used for cognitive improvements in Alzheimer's disease were based on inhibition of the enzyme acetylcholinesterase (M i y a z a w a et al., 2001). Camphor specifically blocks nicotinic acetylcholine receptors and increases catecholamine secretion (P a r k et al., 2001).

To the best of our knowledge, the only estimation, of neural networks within the motor cortex to imply efficacy of recurrent inhibition mechanisms and weakening of hippocampal excitability during long-term effects of camphor was given by Medvedev (1990). We are aware that in our model, considering that we used general anesthesia, there was barbiturate enhancement of the response of $GABA_A$ receptors to GABA (B e l e l l i et al., 1999).

We would also like to point out that fractal dimension analysis could be an appropriate tool in the prediction of convulsions. We detected electrocorticographic signs of seizures in parietal cortical areas within the first 15 min after camphor essential oil administration at low doses and described seizure development by fractal analysis. It seems, generally, that the complexity of brain signals was greater after than before camphor oil administration, but during excessive discharges with spiking the fractal dimensions were very low. Great variations of fractal dimension and consequent great standard deviation of FD were obtained. It would be interesting to compare the fractal analysis in our animal model with analysis in patients suffering from epilepsy. Fractal analysis of intracranial electroencephalograms in patients with temporal lobe epilepsy during evaluation for surgery provides an efficient method for electrocortical complexity characterization and a promising computational tool for determination of seizure onset (E s t e l l e r et al., 1999). Comparing our results in the animal model of seizure activity with similar parameters in diverse seizure types in humans, we see that the most notable aspects are: the value of FD is relatively low during the preictal period and increases during the initial stages of seizure activity, maintaining an increased level interictally but reaching the lowest complexity level at the maximum of high-voltage cortical spiking. S a c k e l l a r e s et al. (1999) argue that epileptic brains repeatedly make abrupt transitions into and out of the ictal state because the epileptogenic focus drives them into self-organizing phase transitions from chaos to order. Furthermore, the authors postulate that the seizure serves to reset the system. A fractal wavelet-based spectral method in electrocortical analysis in three types of rat seizures (X i a o l i et al., 2005) reveals the characteristic signs of an approaching seizure. Comparison of linear and nonlinear methods for identification and prediction of epileptic seizures (McSharry et al., 2003) provides little concrete evidence of deterministic chaos in electrocortical signals.

However, a collection of complex criteria indicates an approach to global instability in the pre-focal area of epileptic seizures induced by bicuculline (Eftaxias et al., 2006). We therefore plan to study specific effects of the main camphor essential oil constituents on rat neocortical and hippocampal activity with much longer sequences of recordings (up to 1800 s) immediately after camphor oil or cineole administration in order to find new analytical criteria which would generally improve ictal predictability.

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ЕФЕКАТ КАМФОРОВОГ ЕСЕНЦИЈАЛНОГ УЉА НА АКТИВНОСТ КОРЕ ВЕЛИКОГ МОЗГА ПАЦОВА У ФУНКЦИЈИ ПРОМЕНА ФРАКТАЛНЕ ДИМЕНЗИЈЕ

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Циљ наше студије је било истраживање ефекта камфоровог есенцијалног уља на активност коре великог мозга пацова помоћу фракталне анализе. Вредности фракталне димензије паријеталне електрокортикалне активности су израчунате пре и после давања камфоровог есенцијалног уља (450-675 μl/kg, i.p.) анестезираним пацовима. Камфорово есенцијално уље изазвало је мождану активност налик на напад са појединачним и вишеструким шиљак-таласима велике амплитуде у паријеталном електрокортикограму као и повремене клоничне конвулзије екстремитета. После давања камфоровог уља, средње вредности фракталне димензије кортикалне активности су се повећале у просеку. Једино вредности фракталне димензије икталних секвенци електрокортикограма су биле ниже него оне пре давања камфоровог уља.