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EFFECT OF A STATIC MAGNETIC FIELD ON THE FRACTAL COMPLEXITY OF BURSTING ACTIVITY OF THE BR NEURON IN THE SNAIL DETECTED BY FACTOR ANALYSIS

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Abstract – In the present work we report a new combination of fractal analysis and some advanced statistical methods and their application for the quantitative detection of the effects of a static magnetic field of 2.7 mT on fractal complexity changes of Br neuron activity in the subesophageal ganglia of the garden snail *Helix pomatia.* We used factor analysis (FA) in the analysis of the empirical distribution of fractal dimension (FD). FA showed that there are two factors in the empirical distribution of FD. Results indicated that the significant changes in the fractal complexity of Br neuron activity occurred during treatment with a magnetic field, were extended to the post exposure period.

Key words: Fractal dimension, factor analysis, PCA, Br neuron, magnetic field

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INTRODUCTION

Scientific interest in the magnetic field as an ecological and physiological factor and its effect on biological systems has increased over the past decades. Numerous studies (Balaban et al., 1990, McLean et al., 1990; Rosen, 1992, Prato et al., 1996, Ye et al., 2004) have dealt with the effects of magnetic fields on the nervous system.

On the basis of experimental data we have modified the fractal analysis method (Higuchi, 1987, Spasić et al., 2005, 2008; Spasić, 2010), usually applied on field potential neuronal activity which can be used for analysis of the bimodal pattern of single neuron activity. In the present work we report a new combination of fractal analysis and some advanced statistical methods and their use for the quantitative detection of the effects of a static magnetic field of 2.7 mT on the fractal complexity changes of Br neuron activity in the subesophageal ganglia of the garden snail *Helix pomatia*. Previous research (Nikolić et al., 2008) revealed changes in the bioelectrical properties of the Br neuron induced by 2.7 mT and 10 mT static magnetic fields. However, in this paper, we have applied a different methodology in order to reveal new information about the effect of a 2.7 mT magnetic field on the activity of the Br neuron.

MATERIALS AND METHODS

Experimental animals, recording procedure and data acquisition

The effect of a static magnetic field was tested on the Br neuron in the isolated subesophageal ganglion complex of *Helix pomatia*. The position of the identified Br neuron was established by binocular microscopy (Kerkut et al., 1975). The identified nerve cell was penetrated with 1 M potassium citrate-filled glass capillary microelectrodes with a resistance ranging from 8 to 18 MΩ. Magnets of 2.7 mT intensity (measured on the surface of the ganglion complex) were placed on a custom-made holder under the center of the recording chamber, with the North Pole up, approximately 3 mm distant from the bottom of the chamber. A single electrode voltage clamp (SEC-2 M, laboratory designed on Jozsef Atilla University, Szeged), two channel acquisition system (MiniDigi 1A, Axon Instruments) and AxoScope acquisition software (Axon Instruments) were used for current clamp recordings. A low-pass antialiasing filter, with a cutoff frequency of one fifth of the sampling rate, was used during real time recordings. Signals were sampled at 1 KHz and digitized using a 16 bit A/D converter. The registration of spontaneous bioelectrical activity was performed before (5 min), during (15 min) and after (20 min) exposure to the static magnetic field in six experimental animals.

Experimental data for estimating fractal complexity of signals were arranged as follows: each electrophysiological recording was fragmented into three units of 5 min duration at the beginning of each recording sequences. Therefore for each experiment, analysis was performed on the three files of the same duration: control, treatment - exposure to magnetic field (MF) and the post exposure period (AMF).

Fractal analysis

Fractal analysis was performed by estimating the fractal dimension of electrophysiological signals from Br neurons using Higuchi's algorithm (Higuchi, 1988; Klonowsky et al., 2003; Spasić et al., 2005, 2008; Spasić, 2010).

The fractal dimension is a nonlinear measure of signal complexity in the time domain. Briefly, signals were analyzed in time sequences $x(1), x(2),\ldots,x(N)$ and constructed in a new self-similar time series X_k^m as:

$$X_k^m : x(m), x(m+k), x(m+2k),..., x(m+\mathrm{int}[(N-m)/k]k),$$

for m=1, 2,…,k where m is initial time; k=2,…, k_{max}, where k is time interval, int(r) is integer part of the real number r. The length $L_m(k)$ was computed for each of the k time series or curves X_k^m.

$$L_m(k) = \frac{1}{k}\left[\left(\sum_{i=1}^{\mathrm{int}\left[\frac{N-m}{k}\right]} \left|x(m+ik) - x(m+(i-1)k)\right|\right)\frac{N-1}{\mathrm{int}\left[\frac{N-m}{k}\right]k}\right]$$

$L_m(k)$ was averaged for all m forming the mean value of the curve length $L(k)$ for each k=2,...,$k_{\max}$ as

$$L(k) = \frac{\sum_{m=1}^{k} L_m(k)}{k}$$

An array of mean values $L(k)$ was obtained and the FD was estimated as the slope of least squares linear best fit from the plot of ln($L(k)$) versus ln(1/k):

$$\mathrm{FD} = \ln(L(\mathrm{k})) / \ln(1/k).$$

After preliminary tests, we chose the parameter N = 25 equivalent to an epoch of 0.025 s duration to deal with non-stationary signals, and the parameter k_{max} was: k_{max} = 8. Signals were divided into 12000 epochs (windows). FD values were calculated for each epoch, without overlap. We formed empirical distribution of FD from the individual FD values. Therefore, we used, for further analysis, the empirical distribution of FD. Higuchi's algorithm is applied using MATLAB software.

In order to facilitate the interpretation of the FD values of the signals, the FD value of smooth curve (e.g. linear, a low frequency sine wave or less complex curve) was estimated to be ~1. The FD of random white noise or a more complex curve was estimated to be ~2. Therefore, we expected that the FD values of bursting periods should be ~1 et the average, and FD values of silent periods should be >1.5 et the average.

Factor analysis

Factor analysis (FA) is a classical technique in statistical data analysis, feature extraction and data reduction. It is a method for investigating whether a number of variables of interest $X_1, X_2, \ldots, X_p$ are linearly related to a smaller set of latent factors $F_1, F_2, \ldots, F_m$. These factors, also defined as sources, should explain a covariation structure of the original variables. Each observed signal is assumed to be a linear combination of statistically uncorrelated signals. So, for a data vector (matrix) **X**, where each row represents a different repetition of the experiment, the model of factor analysis is given by:

$$\underset{(p\times 1)}{\mathbf{X}-\boldsymbol{\mu}} = \underset{(p\times m)}{\mathbf{B}}\underset{(m\times 1)}{\mathbf{F}} + \underset{(p\times 1)}{\boldsymbol{\varepsilon}}$$

In this study, the number of variables was $p = 6$, which means that six experimental animals were used and that the number of factors was $m = 2$. Therefore, in practice, our model is given in matrix notation:

$$\mathbf{X}-\boldsymbol{\mu} = \mathbf{B}\,\mathbf{F} + \boldsymbol{\varepsilon}$$

where

$\mathbf{X}-\boldsymbol{\mu} = \left(X_1-\mu_1, X_2-\mu_2, \ldots, X_6-\mu_6\right)^T$, vector of mean-centering,

$\mathbf{F} = \left(F_1, F_2\right)$, vector of factors,

$\mathbf{B} = \begin{pmatrix} \beta_{11} & \beta_{12} \\ \beta_{21} & \beta_{22} \\ \vdots & \vdots \\ \beta_{61} & \beta_{62} \end{pmatrix}$, matrix of factor loadings, and

$\boldsymbol{\varepsilon} = \left(\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_6\right)^T$, matrix of specific factors.

It is assumed that each X variable is linearly related to the two components, as follows:

$$X_1-\mu_1 = \beta_{11}F_1 + \beta_{12}F_2 + \varepsilon_1$$

$$X_2-\mu_2 = \beta_{21}F_1 + \beta_{22}F_2 + \varepsilon_2$$

$$X_6-\mu_6 = \beta_{61}F_1 + \beta_{62}F_2 + \varepsilon_6$$

The error terms ε_i , $i = 1, \ldots, 6$ serve to indicate that the hypothesized relationships are not exact. We used principal components analysis (PCA) as a method (Bugli and Lambert, 2006; Jollife, 1986) of factor extraction in the factor analysis. PCA is a method used to find an orthogonal frame of reference direction with axes determined by the second order statistics of the original data vectors. PCA maximizes the variance of projected data along orthogonal directions. In PCA, the first principal component accounts for as much as possible variability in the data, and each successive orthogonal component accounts for as much for the residual as possible. The solution of the FA model is not unique, therefore it is necessary to rotate the factor axis. In this paper, the Promax method was used for factor rotation. The method of regression was used for the estimation of factor scores. Therefore, we used the PCA method to find factors in the empirical distribution of FD. In this case, the columns of the matrix **X** consist of the values of the empirical distribution of FD. So, we can say that isolated factors represent the sources of complexity in our experimental biological signals.

The differences between the factors of empirical distribution of FD in different experimental conditions (control, under exposure to magnetic field (MF) and post exposure magnetic field (AMF)) were tested by the nonparametric Kruskal-Wallis test for k independent samples, and the nonparametric Mann-Whitney test for two independent samples. We used SPSS Statistics software in statistical analysis.

RESULTS

We have calculated the Higuchi fractal dimension of the signals in the bursting activity of the Br neuron in the subesophageal ganglia of the snail in different experimental conditions: in control, under exposure, and after exposure to the magnetic field. The bimodal work pattern of molluscan bursting neurons is well described. Bursts of action potentials alternate with silent intervals. The burst consists of a train of action potentials whose frequency and duration changes during the train. The frequency increases until the midburst and then decreases. An example of Br neuron response during and after treatment with a magnetic field in one snail (P3) is presented in Fig.1. Higuchi fractal dimension values were calculated for all 18 signals in 6 snails. The corresponding FD values of the Br neuron activity of the P3 snail are also presented on Fig.1.

In addition, we formed the empirical distribution of FD for each signal. The shape of the empirical

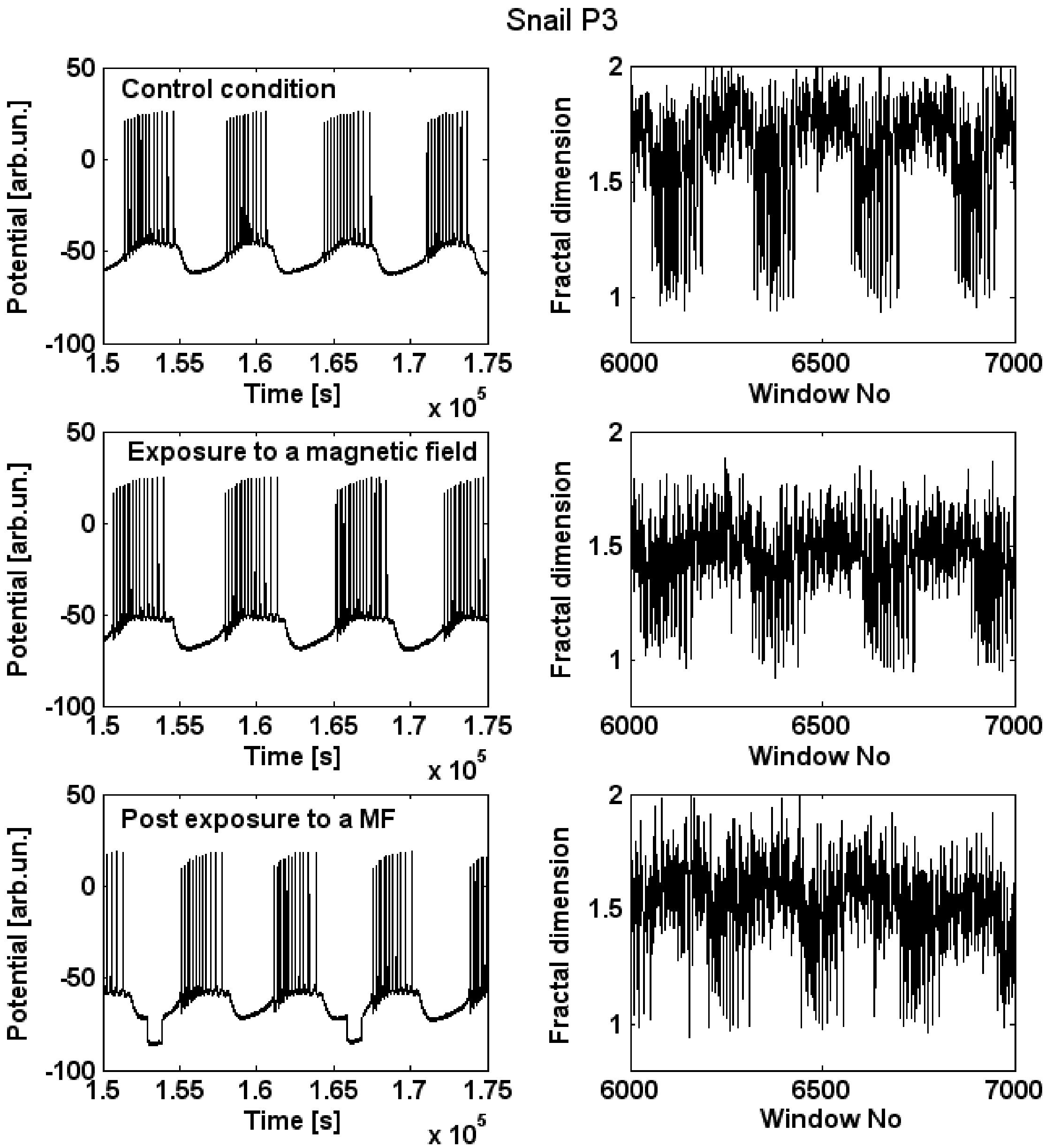

Fig. 1. Spontaneous activity of the Br neuron in the control condition, under exposure and after exposure to a static magnetic field of 2.7 mT (on the left side of figure), and corresponding fractal dimension values in time domain (on the right side).

distribution of FD pointed to a bimodal pattern in complexity of spontaneous Br neuron activity (Fig. 2). FD values in the interval of [0.9, 1.2] characterize the action potentials of tested neurons, whereas the FD values in the intervals of (1.2, 2.2] originate from the silent periods.

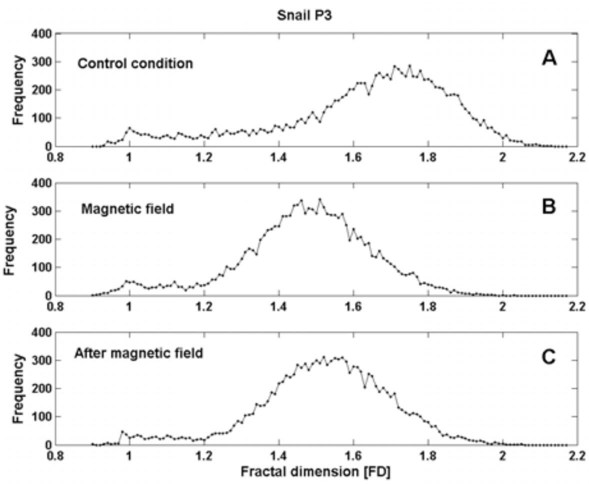

Fig. 2. Empirical distribution of FD for P3 snail in three experimental conditions: control (A), under exposure to a magnetic field (B), and after exposure to a magnetic field (C).

The main factors (F1 and F2) of the empirical distribution of FD in the control condition, under MF and after exposure to MF, are isolated by factor analysis (Figs. 3 & 4).

The statistical analysis with the Kruskal-Wallis Test (Fig. 3) showed there is no difference between factors F_1 in the control, MF and AMF conditions ($p=0.057$). However, the same statistical test (Fig. 4) showed there is a significant difference between factors F_2: in control, MF and AMF condition ($p<0.0001$). The Mann-Whitney test for the two independent samples showed that there were significant differences between the F_2 factors in the control vs. MF ($p<0.0001$), and control vs. AMF conditions ($p<0.0001$), but there was no difference between the F_2 factors in MF vs. AMF condition ($p=0.618$).

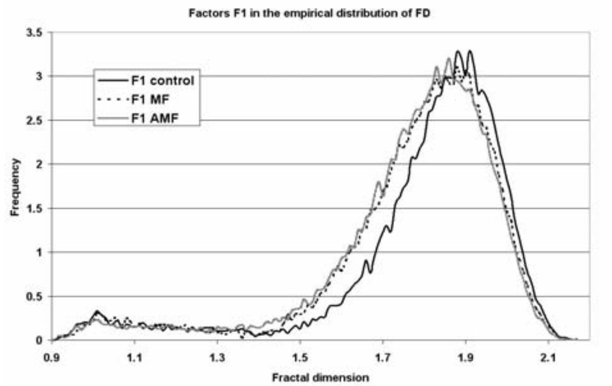

Fig. 3. Factors F_1 of the empirical distribution of FD in different experimental conditions are isolated by factor analysis. Statistical analysis with the Kruskal-Wallis Test showed there is no difference ($p=0.057$) between the F_1 factors in the control, MF and AMF conditions.

We have shown (Figs 3 and 4) that the signal complexity of the Br neuron spontaneous activity decreased both under exposure to the magnetic field and after exposure to the magnetic field compared to the control conditions.

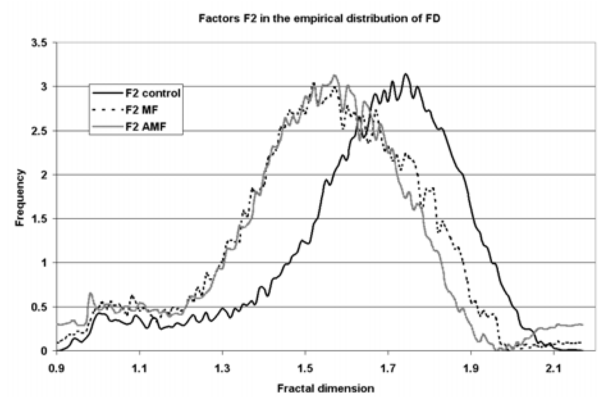

Fig. 4. Factors F_2 of the empirical distribution of FD in different experimental conditions (control condition, under MF and after exposure to MF) are isolated by factor analysis. The statistical analysis showed significant difference ($p<0.0001$) between the F_2 factors in the control condition, MF and AMF conditions. A Mann-Whitney test for two independent samples showed that there were significant differences between factors F_2 in the control vs. MF ($p<0.0001$), and control vs. AMF conditions ($p<0.0001$), but there was no difference between factors F_2 in MF vs. AMF condition ($p=0.618$).

DISCUSSION

In the present work we showed that a magnetic field of 2.7 mT intensity can induce persistent and prolonged changes in the fractal complexity of Br neuron activity. These findings are of interest because we uncovered changes in the fractal characteristics of these signals despite their being highly non-stationary. In our previous investigations (Spasić et al., 2005, 2008; Spasić, 2010) we used only the mean and standard deviation of FD to explain changes of EEG signal complexity in different experimental conditions such as brain injury, repeated brain injury or drug treatments. In this paper, we didn't use the mean of the FD values for the whole signal because this statistical measure could not be applied to explain changes in single neuron activity. However, we have analyzed the empirical distribution of FD to reveal changes in the spontaneous activity of a Br neuron exposed to a low static magnetic field. Generally, the high complexity of neuronal activity, EEG for example, is related to neuronal activation. We have shown that the signal complexity of Br neuron activity decreased both under exposure and after exposure to the static magnetic field compared to the control conditions. This study has proved the Higuchi fractal dimension to be valuable tool for measuring changes of neuronal signal complexity induced by a static magnetic field.

Namely, our recent results (Nikolić et al., 2008.) also demonstrated the response of the Br neuron to the 2.7 mT magnetic field, but with different methods of analysis. The experimental results showed that a magnetic field with intensity of 2.7 mT caused changes in the amplitude and duration of action potential of the Br neuron, while a 10 mT magnetic field changed the resting potential, amplitude spike, firing frequency and duration of action potential of the Br nerve cell. Furthermore, as observed during the course of our experiments, magnetic fields caused irreversible or reversible changes in the measured bioelectric properties of Br neuron.

Other authors have shown that a magnetic field can cause changes to neuron bioelectric parameters that are irreversible during the experiment, as was shown on the antennal lobe neuronal population of longhorn beetles *Morimus funereus* (Todorović et al., 2007).In this work, we have found that changes in the signal complexity of the Br neuron caused by a magnetic field are prolonged to the post exposure period. Having in mind our previous findings that a 2.7 mT magnetic field can cause irreversible and reversible changes in some bioelectric properties of the Br neuron, it may be reasonable to conclude that the method applied in this work has both applicability and sensibility.

In conclusion, we have used an efficient and data oriented method to extract information about single neuron activity in terms of signal complexity analysis, even those signals are non-stationary. Our results also indicate that the appropriately modified FD can be used for analysis of the bimodal pattern of neuronal activity, as well as for field potential neuronal activity such as EEG.

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