

THE SPECTRAL ANALYSIS OF MOTION – AN „OPEN FIELD“ ACTIVITY TEST EXAMPLE

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In this work we have described the new mathematical approach, with spectral analysis of the data to evaluate position and motion in the „open field“ experiments. The aim of this work is to introduce several new parameters mathematically derived from experimental data by means of spectral analysis, and to quantitatively estimate the quality of the motion. Two original software packages (TRACKER and POSTPROC) were used for transforming a video data to a log file, suitable for further computational analysis, and to perform analysis from the log file. As an example, results obtained from the experiments with Wistar rats in the „open field“ test are included. The test group of animals was treated with diazepam. Our results demonstrate that all the calculated parameters, such as movement variability, acceleration and deceleration, were significantly lower in the test group compared to the control group. We believe that the application of parameters obtained by spectral analysis could be of great significance in assessing the locomotion impairment in any kind of motion.

Key words: motion variability, „open field“, spectral analysis, speed variability

INTRODUCTION

The analysis of motion is one of the main aspects of behavioral investigations. By observing and analyzing certain movements we can assess behavior, locomotion impairment, brain lesions or drug influence on the locomotion etc. Every motion consists of several parameters, such as speed, distance, acceleration,

and deceleration. These parameters were not assessed until now, in behavioral experiments in animal experimental models.

The „open field“ activity test (Giulian and Silverman 1975; Ericson *et al.*, 1991; Koob *et al.*, 2006) is the experimental design that is most frequently used in behavioral investigations in the animal experimental model. It represents a valid measure of both the basal locomotion and exploration activity in drug-treated, surgically, genetically or otherwise manipulated investigated subjects. The most commonly used systems for obtaining data and the data analysis in „open field“ tests are based on two main approaches: the IR beam-based (Koob *et al.*, 2006) and the video file-based systems (Lind *et al.*, 2005; Obradović *et al.*, 2009).

The validity and the accuracy of the results obtained in these kinds of investigations depend on the tools capable sensitive tracking, recording and analysis of the events in the experiments. In our previous work (Obradović *et al.*, 2009) we described a new video file-based experimental model for the behavioral investigations in animal studies. This model does not depend on specific light and arena color conditions; neither has limitation in the experimental time duration. It also allows sequential and retrospective analysis of the motion of investigated subjects on the retroactively applied virtual grid.

This video file-based experimental model is created by means of our two originally developed software packages named TRACKER and POST PROC. The TRACKER produces the log file from the captured video format containing comma separated values of: time, speed and estimated subject position. The POSTPROC is applied to analyze the log files obtained from TRACKER, and automatically calculates the following parameters: (1) average speed (V_a) in m/s, (2) maximal speed (V_{max}) in m/s, (3) total distance traveled by the animal, (S) in meters, (4) duration of the motion (D_m) in hours, (5) duration of the stillness (D_s) in hours.

All previously mentioned experimental data obtained by POSTPROC, as well as their derived parameters, quantitatively describe the motion. However, the quality of this motion, such as the variability and the acceleration has not yet been described. Therefore, in the mathematical analysis of the motion we included the spectral analysis of motion and several parameters that could quantitatively estimate the quality of motion.

Spectral analysis is the method of decomposition of the time-changing signal to harmonic components at different frequencies. Every frequency in the spectrum represents a particular variation rate of the signal in the time domain.

The aim of this work was to introduce several new parameters mathematically derived from experimental data by means of spectral analysis. We believe that the application of these parameters could be of great significance in assessing any kind of motion, which can be used for detection of motor impairments as early signs of various diseases.

MATERIAL AND METHODS

In all described experiments the investigated subjects were 3-month-old Wistar rats of both sexes (n=20, weighted 200-300 gr.), divided in the control group (n=10), and the test group (n=10) The test group was treated with diazepam (Bensedin[®], Galenika A.D., Beograd, Serbia), 2 mg/kg of body weight, *ip.*, 10 minutes before being placed in the arena. The control group was given saline in exactly the same manner as the test group was treated. Diazepam was used only to induce the locomotor impairment in the test group. 12h-12h dark-light conditions were achieved in the experiment. All experiments were performed according to the ethical standards recommended by EU (86/609/EEC) and Local Ethical Committee.

The arena used in this work was the „open field“ 50x70x30 cm. The camera was placed 230 cm above the arena. The rats were placed in a corner of the „open field“ and allowed to move freely.

The video format of the experiment was captured using the following equipment:

1. Standard or IR camera connected to a standard PC computer with 1 GHz CPU and 256MB of RAM memory.
2. PCI TV tuner card with Philips bt878 chipset for capturing the video input.
3. Operating system Windows XP.
4. Software DSCALER for video recording (open source software, freely available for downloading at the address: [http://deinterlace.sourceforge.net/.](http://deinterlace.sourceforge.net/))

The software TRACKER was used to produce the log file from the captured video format.

The software POSTPROC was applied to analyze the log files obtained from the TRACKER and to calculate automatically the following parameters: The average speed (V_a) in m/s, the maximal speed (V_{max}) in m/s, total distance traveled by the animal, (S) in meters, duration of the motion (D_m) in hours, duration of stillness (D_s) in hours. In addition, POSTPROC was used to automatically create the virtual grid, to determine the center and the periphery of the arena and to calculate the time spent in the center of the „open field“ (T_c in seconds) and time spent at the periphery of the arena (T_p in seconds). Using experimental data (T_c , T_p , D_m , D_s) obtained by POSTPROC, we were able to calculate two (derived) parameters we named the motion distribution (M_d) and the module of activity (A_m) by dividing adequate data as shown by the following equations:

$$M_d = T_c / T_p. \quad (Eq 1)$$

where T_c is the time that the investigated subject spent in the center of „open field“, and T_p is the time spent on the periphery of the „open field“.

$$A_m = D_m / D_s \quad (Eq 2)$$

where D_m is the time that the investigated subject spent in motion and D_s is the time spent in stillness.

For the purpose of describing the quality of motion we introduced spectral analysis of motion and parameters that are obtained by this method. As these quality describing parameters were not used in any previous work, we could not compare them with other results. The only way that we could estimate their liability is that they completely corresponded with the parameters obtained from filming the experiment and were calculated by computer in POSTPROC.

The spectral analysis was performed using Fourier transformation (Hubbard 1973; Hui *et al.*, 1996; Chirikjian and Wang 2003; Bhaduri *et al.*, 2010) over the signal (in this study it was the speed) in the time domain. Fourier transformation of the signal was given by the expression (Eq 3):

$$X(f) = \int_{-\infty}^{\infty} x(t)e^{-j2\pi ft} dt \quad (\text{Eq 3})$$

where $x(t)$ is signal, and $X(f)$ is the spectrum of the signal $x(t)$.

The energy of the signal components between the frequencies f_1 and f_2 could be calculated using the equation (Eq 4):

$$E = 2 \int_{f_1}^{f_2} |X(f)|^2 df \quad (\text{Eq 4})$$

where $X(f)$ is spectrum of the signal $x(t)$.

The median frequency (f_{median}) represents the frequency that divide the area under the curve of the amplitude spectrum density to two equal parts, and it can be calculated by the equation (Eq 5):

$$\int_0^{f_{\text{median}}} |X(f)| df = \int_{f_{\text{median}}}^{\infty} |X(f)| df \quad (\text{Eq 5})$$

where $X(f)$ is the spectrum of the signal $x(t)$.

The median frequency corresponds to the ratio of the energy contained in the high frequency components versus the energy contained in the low frequency components. The median frequency increase when the energy of the high speed components also increase, compared to the entire signal energy. Spectral analysis of the experimental data allowed us the possibility to calculate following parameters: MV (Index of Movement Variability), DM (Dynamics of Movement), SV (Index of Speed Variability), AV (Index of Acceleration Variability) and DV (Index of Deceleration Variability).

Parameter MV represents the motion variability. It could be calculated directly from the value of speed, as shown in the following equation (Eq 6):

$$MV = \frac{\Psi_v}{t} \quad (\text{Eq 6})$$

where Ψ_v is the number of the speed changes, and t is the time duration of the experiment.

Parameter DM was chosen to represent the total dynamic of the movement that was proportional to the total kinetic energy of the subject during the experiment. This parameter could be calculated according to the expression (Eq 7):

$$DM = 2 \int_0^{f_{max}} |V(f)|^2 df \quad (\text{Eq 7})$$

where $V(f)$ is the spectrum of the subject's speed and f_{max} is the width of the spectrum $V(f)$.

Another very important aspect of movement is the acceleration and the deceleration. We described these with parameters such as: SV, AV and DV. We suggested that the median frequency of the acceleration/deceleration spectrum represented SV.

Either the acceleration or the deceleration, $a(t)$ can be easily calculated from the speed $v(t)$ according to the equation (Eq 8):

$$a(t) = \frac{dv(t)}{dt} \quad (\text{Eq 8})$$

The negative values of $a(t)$ represent the deceleration and the positive values are considered as the acceleration.

Parameter SV corresponds to the ratio of the energy contained in the high frequency components versus the energy contained in the low frequency components and it could be calculated as the median frequency of the acceleration/deceleration's amplitude spectrum density as defined in the equation (Eq. 9).

$$\int_0^{SP} |A(f)| df = \int_{SP}^{\infty} |A(f)| df \quad (\text{Eq 9})$$

where $A(f)$ represents the spectrum of the subject's acceleration.

The AV parameter represents the ratio of the high and the low frequency variations of the positive part of the acceleration/deceleration-time relation, and the DV parameter represents the ratio of the high and the low frequency variations of the negative part of the acceleration/deceleration-time relation.

The above mentioned parameters could be calculated in the same manner as the SV, by using the spectrum of the positive part of the signal $a(t)$ for the AV, and the negative part of the signal $a(t)$ for the DV:

$$a_+(t) = \begin{cases} a(t) & \text{for } a(t) > 0 \\ 0 & \text{for } a(t) \leq 0 \end{cases} \quad (\text{Eq 10})$$

$$a_-(t) = \begin{cases} |a(t)| & \text{for } a(t) < 0 \\ 0 & \text{for } a(t) \geq 0 \end{cases} \quad (\text{Eq 11})$$

Both the AV and the DV can be calculated from the following equation:

$$\int_0^{AV} |A^+(f)| df = \int_{-DV}^0 |A^-(f)| df \quad (\text{Eq 12})$$

$$\int_0^{DV} |A^-(f)| df = \int_{-AV}^0 |A^+(f)| df \quad (\text{Eq 13})$$

where $A^+(f)$ is the spectrum of the subject's acceleration, and $A^-(f)$ is the spectrum of the subject's deceleration.

Statistical analysis of data were performed using t-test where $p < 0.05$ was considered as statistically significant. All data were analyzed using program SPSS 13.0.

RESULTS

As we stated before, POSTPROC is applied to analyze the log files obtained from TRACKER, and automatically calculates the following parameters:

- (1) The average speed (V_a) in m/s,
- (2) The maximal speed (V_{max}) in m/s,
- (3) The total distance travelled by the animal, (S) in meters,
- (4) The duration of the motion (D_m) in hours,
- (5) The duration of the stillness (D_s) in hours.

These parameters were obtained automatically by filming the actual event during the experiment and thus can be treated as data obtained from watching and measuring the actual motion of the test and control group of animals. In Table 1 the values of these parameters are shown in order to support the claim of locomotion impairment of the test group during the experiment.

To calculate the parameters including MV, DM, SV, AV and DV that could allow us to quantitatively estimate the quality of motion of both the control and the test groups of rats, we took the SV advantage of applying the spectral analysis.

Table 1. Values of parameters* obtained automatically by POSTPROC for the control and the test group

	Parameter	Control group	Test group
1	Va	2,3877	0,3523 †
2	Vmax	3,3467	1,2235 †
3	S	2446	221 †
4	Dm	0,2845	0,1742 †
5	Ds	23,7155	23,8258 †

*Va (the Average speed in m/s), Vmax (the Maximal speed in m/s), S (the total distance traveled by animal, in meters), Dm (the duration of the motion in hours), Ds (the duration of the stillness in hours).

† All results are analyzed after determination of normality of distribution with *t* – test where *P* value of <0.05 was considered statistically significant.

The derived parameters, obtained from spectral analysis are shown in the Table 2. Our results demonstrate that all the calculated parameters were significantly lower in the test group compared to the control group.

Table 2. Values of parameters* of the motion variability for the control and the test group

	Parameter	Control group	Test group
1	MV	1,0462	0,5115 †
2	DM	14,5131	6,1411 †
3	SV	13,4077	11,1795 †
4	AV	11,341	9,9026 †
5	DV	11,1564	10,0282 †

*MV (Index of Movement Variability), DM (Dynamics of Movement), SV (Index of Speed Variability), AV (Index of Acceleration Variability), DV (Index of Deceleration Variability).

† All results are analyzed after determination of normality of distribution with *t* – test where *P* value of <0.05 was considered statistically significant.

Parameter MV represented the motion variability, or more directly the number of the speed changes during the test time. As shown in Table 2, this parameter was nearly 50% lower in the test group compared to the control group. This result indicates that the investigated subjects that were under the influence of the test drug were 50% less likely to change the speed of the motion.

Parameter DM represented the total dynamic of the movement and it was proportional to the total kinetic energy of the subject during the experiment. From the results shown in Table 2, it was evident that the test group spent less than 50% of the kinetic energy in comparison to the control group.

The acceleration and the deceleration were described by the parameters AV and DV, respectively and SV. The median frequency of the acceleration/ deceleration spectrum represented SV.

The AV parameter represented the ratio of the high and low frequency variations of the positive part of the acceleration/deceleration-time relation, and

the DV represented the ratio of the high and low frequency variations of the negative part of the acceleration/deceleration-time relation. As shown in Table 2, the test group had lower accelerations as well as the decelerations.

Examples of spectral analysis of the motion from the experiments performed in this work are presented in Figure 1 and Figure 2.

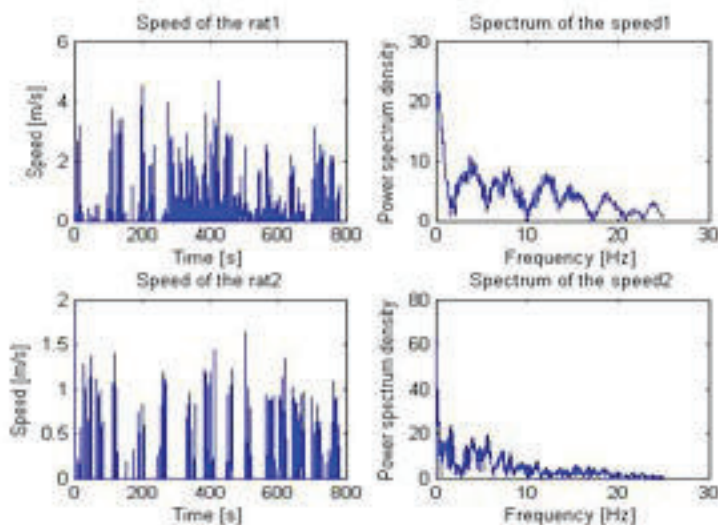


Figure 1. The speed and the spectral analysis of the speed, in one control and one test animal.

- A: The speed-time relation obtained by POSTPROC for rat 1 (control) and rat 2 (test) – MV parameter was calculated from this relation using Eq 6.
B: The spectral analysis of the motion for the rat 1 (control) and rat 2 (test) – DM parameter was calculated from this relation as the area under the curve by means of Eq 7.

On the left side of Fig. 1, the speed-time relation obtained by POSTPROC for the control rat 1 and the test rat 2 are presented. The MV parameter was calculated from this relation using equation 6 (Eq 6). The maximum speed of rat 1 was 4.81 m/s, and the maximum speed of rat 2 was 1.72 m/s. The average speed calculated by POSTPROC, for rat 1 was 0.81 m/s, and for rat 2 it was 0.32 m/s. On the right side of Fig. 1, spectral analysis of the motion for rat 1 (control) and rat 2 (test) was presented. The DM parameter was calculated from this relation as the area under the curve by means of (Eq 7).

Furthermore the acceleration/deceleration-time relation, AV and DV, obtained by (Eq 8) for rat 1 (control) and rat 2 (test) was shown. The positive values on the left side of Fig. 2 represent the acceleration and the negative values represent the deceleration relation. The SV parameter shown on the right side of

Fig. 2 was calculated from (Eq 9) as the median frequency of spectral analysis of acceleration/deceleration for rat 1 (control) and rat 2 (test).

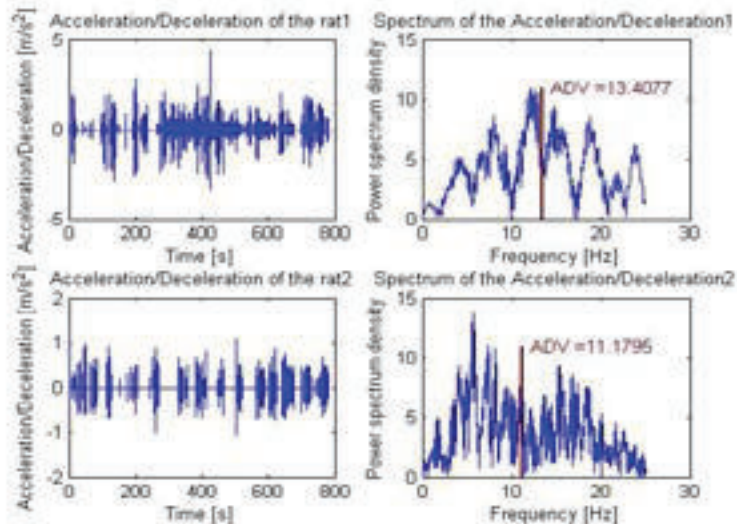


Figure 2. The acceleration/deceleration relation expressed as AV, DV and SV, and their spectral analysis, in one control and one test animal.

- A: The acceleration/deceleration-time relation obtained by (Eq 8) for rat 1 (control) and rat 2 (test). The positive values represent the acceleration and negative values represent deceleration.
- B: The spectral analysis of the acceleration/deceleration for rat 1 (control) and rat 2 (test) – SV parameter was calculated from (Eq 9) as the median frequency.

DISCUSSION

Spectral analysis has been used in medical investigations for the calculation of parameters in various medical procedures, such as ECG and EMG (Golden *et al.*, 1973; Riggs *et al.*, 1979; Lolov 1985; Lolov and Lolov 1985; Sesay *et al.*, 2003; Cardozo *et al.*, 2004; Liou *et al.*, 2011). Our study, for the first time, demonstrated that spectral analyses, could be applied to investigate locomotion.

The first parameter that we introduced in this work was MV. This parameter represents the number of speed changes in the function of time and quantitatively describes the motion variability. Some drugs may affect only the motion variability even if the two investigated subjects may have similar both the average and the maximal speed at the same time frame of the experiment. However if one of the subjects changes movement speed more frequently than the other, it is obvious

that its MV parameter would be significantly higher in comparison to the other subject. Therefore, this fact makes the MV parameter highly significant for the investigation of the motion activity of the subjects.

The DM parameter (the total movement dynamics or the total kinetic energy of movement) is proportional to the distance covered by motion for the same time duration. In addition, this parameter is proportional to the average speed (V_a) for the same distance covered by the motion. The main advantage and the main purpose of introducing the DM parameter is the possibility to demonstrate its changes in time. Therefore, by determination of the DM parameter, we can estimate not only movement dynamics, but also the chronology of this event.

The other important characteristics of motion are the acceleration and the deceleration. In our study we described these variables through SV, AV and DV. Our results showed that the subjects from the control group had longer periods of both the acceleration and the deceleration. Thus, these results suggest that the motion of the subjects from the control group was "smooth", without sudden changes either in speed or direction. On the other hand, our results indicate that the subjects from the test group displayed abrupt changes in both in the speed and direction of the movement. The parameter MV together with SV, AV and DV, described the motion of the subjects in the test group as the motion with few changes of speed. If these changes occurred, they happened suddenly. The speed in these subjects was mostly constant (nevertheless slower) with short periods of acceleration and deceleration. This kind of motion indicated that the mechanisms that control the locomotion of the subjects seem to be impaired.

In the test group treated with diazepam, all mathematical parameters obtained by means of spectral analysis, are significantly lower confirming locomotion impairment in this group of animals. A previous study (Segrt *et al.*, 2009) investigated spontaneous motor activity (SMA) of experimental animals using the SMA test. SMA test did not quantitatively estimate the quality of motion by mathematical parameters and the activity was evaluated discontinuously (180 seconds in every 30 minutes) (Segrt *et al.*, 2009). The advantage of our method is the continuous monitoring of the movement without time limitation and precise, mathematical quantification of the parameters of the quality of motion.

The described spectral analysis could be applied to analyze any kind of movement, not only the whole body movement, but the simple movements of one body part. For instance, the simple movement of one limb could be analyzed in order to estimate the velocity of the movements, its variability and precision.

The application of these parameters obtained by the spectral analysis could be of great significance in assessing the initial symptoms of various diseases associated with motor impairment, in the sense of the alternations in acceleration and deceleration (Multiple sclerosis, Alzheimer disease, Parkinsonism). The precision of any movement actually depends on adequate control and synchronization of the acceleration and the deceleration. Thus, the symptoms are sometimes difficult to detect, especially in the early stages of illness. Therefore,

by using these parameters we can analyze either the whole body movement or one limb movement. Also we could improve the detection of the initial motor impairment. Finally, since these parameters are quantitative, the symptoms could be classified according to their intensity.

Conclusion

The spectral analysis of motion during the „open field“ activity test allows us the estimation of the dynamics and variability of motion, speed variability, as well as the acceleration and/or the deceleration variability.

In the test group of animals, all mathematical parameters obtained by means of spectral analysis, were significantly lower confirming locomotion impairment in this group of animals.

The mathematical parameters obtain by the spectral analysis could be applied to analyze various kinds of motion (from the whole body movement to simple one limb movement), which has not only scientific but possible clinical importance, as well.

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Spektralna analiza kretanja – primer „open field“ testa

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SADRŽAJ

U ovom radu je opisan nov matematički pristup primenom spektralne analize podataka za procenu pozicije i kretanja u „open field“ testu. Cilj je bio da se uvedu novi parametri, dobijeni matematičkim putem spektralnom analizom iz eksperimentalnih podataka i da se kvantitativno proceni kvalitet kretanja. Dva originalna softvera (nazvani TRACKER i POSTPROC) su primenjeni za prenos video podataka u log fajl, koji se koristi za dalju kompjutersku analizu. Kao primer su navedeni rezultati dobijeni iz eksperimenata sa Wistar pacovima u „open field“ eksperimentima. Životinje iz test grupe su tretirane diazepamom. Naši rezultati su pokazali da su svi izračunati parametri, kao što su varijabilnost u kretanju, ubrzanje, usporavanje, bili značajno niži u test grupi u odnosu na kontrolnu grupu. Verujemo da bi primena parametara dobijenih spektralnom analizom bila od velikog značaja pri proceni poremećaja kretanja.