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Environmental Stress: Approximate Entropy Approach Revisited

Tatjana Loncar-Turukalo*, Dragana Bajic, Olivera Sarenac, Nina Japundzic-Zigon, Aleksandar Boskovic

Abstract—**Radiotelemetred male Wistar outbrad rats and Borderline Hypertensive rats (BHR) were exposed to acute and chronic environmental stress. Approximate entropy (*ApEn*) approach is applied in order to investigate the pulse interval (PI) response to two different types of environmental stress: shaker and restrain stress. The performance of *ApEn* method was evaluated from the parameter selection point of view. The purpose of the study is to quantify the complexity of response to stress and period of recovery after the stress in order to gain an insight in consequences of chronic stress exposure.**

I. Introduction

Appoximate entropy (*ApEn*) can be defined as a "regularity statistic" that quantifies the unpredictability of fluctuations in a time series [1]. The presence of repetitive patterns of fluctuation in a time series renders it more predictable than a time series in which such patterns are absent. *ApEn* has been widely used for the analysis of heart rate variability (HRV) as a computational mean for assessing the predictability stemming from linear stochastic and/or nonlinear deterministic correlations. Furthermore, *ApEn* provides regularity measure for short and noisy experimental time series in absence of nonstationarities and trends in data that preclude statistical analysis [2].

Calculation of both of the statistics requires the selection of the three parameters: m, r and τ, referred to as pattern length (embedding dimension), normalized threshold (tolerance, filter) and time delay, respectively. The choice of m and r parameter is critical in proper application of these statistics. Based on the preliminary conclusion drown in the work of Pincus [1], majority of the applications use the parameter choices m=2, r=0.1-0.2 times standard deviation of the signal and τ=1 (one sample delay). Recently, several studies questioned the choice of m and r, as well as influence of data length and sample frequency [4,5,6,7]. Critically revealing the consequences of *a priori* specifications of m and r, Chen et al [5] implies that the most appropriate threshold value is the one that provides maximum *ApEn* value and Lu et al [6] gives the formulas for automatic calculation of the threshold value for m=2,3 and 4. As for the choice of the parameter m, these studies propose False nearest neighbors (FNN) approach.

The parameter τ, however, is an issue in a very few studies. Recently published study by Kaffashi et al [8] raise the question of suitability of unit delay for signals with long range linear correlation. Chen et al [5], indeed, mention the influence of the oversampling the data and showed that downsampling (i.e. increasing τ) resulted in higher signal complexity. Kaffashi et al [8] suggested that τ should be chosen as the first minimum of autocorrelation function.

Stress, on the other hand, has been subject to numerous physiological studies, due to its proven correlation to many of the contemporary causes of morbidity and mortality. This study estimates the entropy of pulse interval (PI) time series of rats exposed to different types of environmental stress. The purpose of study is to quantify the complexity of response to stress and recovery after the stress. Furthermore, it investigates the influence of the choice of the threshold value r and time delay τ to consistency of *ApEn* values in stressed subjects.

II. Materials and Methods

A. Experimental Protocol

All experimental procedures in this study confirmed to European Communities Council directive of 24 November 1986 (86/609/ECC) and the School of Medicine, University of Belgrade Guidelines on Animal Experimentation.

Animals- outbred male Wistar rats and Borderline Hypertensive rats (BHR - were F1 offspring of Wistar dams and SHR – Spontaneous Sypertensive -– sires) weighing 330 ± 20 g were used. Rats were housed individually in Plexiglas cages (25x25x25 cm) with food and water *ad libitum*, in controlled laboratory conditions. The number of animals per experimental group (n=6) was calculated according to the variability of the parameters in the control group rats, using statistical software "Power Sample Size Calculation".

Surgery - ten days before stress experiments rats were submitted to surgery in which radiotelemetric probes (TA11PA-C40, DSI, Transoma Medical) were implanted in abdominal aorta under combined ketamine and xylazine anesthesia, along with gentamicin and followed by metamizol injections for pain relief.

Experimental protocol - Shaker stress: Blood pressure (BP) of rats was recorded 20 minutes before stress (BASELINE), during exposure to shaking platform at 200

T. Loncar-Turukalo is with Faculty of Technical Sciences, University of Novi Sad, Serbia (phone:+381214852535; fax:+381214752997 e-mail: tatjana.turukalo@ktios.net).

D. Bajic is with Faculty of Technical Sciences, University of Novi Sad, Serbia (; e-mail: dragana.bajic@gmail.com).

O.Sarenac is with the Medical Schol, University of Belgrade, Serbia (e-mail: osarenac@med.ac.rs).

N. Japundzic-Zigon is with the Medical Schol, University of Belgrade, Serbia (e-mail: zigon@med.ac.rs).

A. Boskovic is with Elektrovojvodina, Power Distribution Company of Serbia (e-mail: Aleksandar.Boskovic@ev.co.rs)

cycles/min for 10 minutes (FIRST STRESS, FS), and 30 minutes after exposure to stress (POST FIRST STRESS, PFS). This was followed by chronic exposure to 5 minutes-long shaking period 18 times per day for 3 days. Each day BP was recorded during the last stress, as well as 30 minutes after the last exposure to stress. For this study, the after stress period of last day exposure (POST LAST STRESS, PLS) was recorded. Restraint stress: BP was recorded 20 minutes before exposure of rats to restraint stress (BASELINE), 60 minutes during restraint by placing rats in a Plexiglas restrainer tube (ID 5.5cm with pores) in the supine position (FS) and 40 minutes after stress (PFS). BP was recorded during the exposure to restraint stress, as well as 40 minutes after the stress, each day. After-stress period for the last day exposure (PLS) was recorded as well.

Such record lengths ensure that number of data points exceeds the recommended values 10^m-20^m. Two types of rats (BHR and Normotensive) were exposed to two types of stress, yielding 4 different experimental conditions: Shaker – Normotensive (SN), Shaker-BHR (SB), Restraint–normotensive (RN) and Restraint-BHR (RB).

B. *Signal Preprocessing and Methods*

The arterial blood pressure (BP) signal was digitized at 1000Hz and relayed to a PC equipped with Dataquest A.R.T. 4.0. software, DSI for acquisitions and analysis of cardiovascular signals. Pulse interval (PI) series were derived from the arterial BP as interval between maxima in the pulse wave signal. After careful manual visual examination and artifacts removal, very slow component from PI series was removed using approach proposed by Tarvainen [9], as shown in Fig. 1; all detrended series have passed the stationarity test (in a wide sense) [10].

ApEn- given a time series $[x(j)]$, j=1,…,N, where N is the length of the time series, the vectors of the length m $X_m(1)$ to $X_m(N\text{-}m+1)$ are defined by :

$$X_m(i)=[x(i),x(i+1),...,x(i+m-1)] \text{ for } i=1,..N\text{-}m+1 \quad (1)$$

In order to compare these vectors, the distance between any two vectors i and j, $d_m(X_m(i),X_m(j))$, is defined as the maximum of absolute difference between their respective scalar components:

$$d_m(X_m(i),X_m(j))=\max_{k=0,..,m-1}\left[\left|x(i+k)-x(j+k)\right|\right]. \quad (2)$$

For each of the vectors $X_m(i)$, $i=1,...N\text{-}m+1$, the number $B_m(i)$ has to be determined as the number of vectors $X(j)$ for which the distance $d_m(X(i),X(j))<r$, where r is some predefined threshold value. The function

$$C_i^m(r) = \frac{B_m(i)}{N-m+1} \quad (3)$$

estimates the probability that any vector $X_m(j)$ is within the distance r from the vector $X_m(i)$. Another function

$$\Phi_m(r) = \frac{1}{N-m+1}\cdot\sum_{i=1}^{N-m+1}\ln\left[C_i^m(r)\right] \quad (4)$$

is average of the natural logarithms of the previous functions. The procedure is then repeated for vectors of the length m+1, and the approximate entropy is defined as:

$$\text{ApEn}(m,r,N) = \Phi_m(r)-\Phi_{m+1}(r). \quad (5)$$

The time delay parameter, τ, was calculated as the first minimum of the sample autocorrelation function. Introducing the time delay, the pattern vectors become:

$$X_m(i)=[x(i),x(i+\tau),...,x(i+(m-1)\tau)] \text{ for } i=1,..N\text{-}(m\text{-}1)\tau \quad (6)$$

The conditional probability $C^i_m(r)$ and $\Phi_m(r)$ change to:

$$C_i^m(r) = \frac{B_m(i)}{N-(m-1)\tau} \quad (7)$$

$$\Phi_m(r) = \frac{1}{N-(m-1)\tau}\cdot\sum_{i=1}^{N-(m-1)\tau}\ln\left[C_i^m(r)\right] \quad (8)$$

The delay introduced by τ aims to remove the effects of long range linear correlation. The autocorrelation of HRV signal decays very quickly, which minimize the influence of correlation to computation. The first minimum of sample autocorrelation function obtained for these nonuniformly sampled signals of rats was $\tau=2$ samples. If the signals were sampled at 10Hz, the first minimum of autocorrelation function would have been $\tau=4*Ts$, or in the oversampled case, at 20Hz, the value reaches $\tau=6*Ts$. This explains the increasing of the entropy with downsampling the data and elimination of statistically dependant samples, the result obtained by Chen at al [5].

III. Results

ApEn was evaluated for range of threshold r multiplied by signal standard deviation, σ. Reliable standard deviation estimate was ensured using detrended data that has passed the stationarity test. During the experiments, as expected, all the animals exposed to stress have shown decrease of pulse interval values (equivalently, increase of heart rate) and increase of SBP.

Calculating *ApEn* for the different values of r (0-0.5*σ) gives an insight at the (re)positioning of the maxima of the *ApEn* at different stages of experiment. Furthermore, by computation of *ApEn*(r), it was possible to assess the accuracy of theoretically obtained values for r_{theor} proposed in [5]. Suitability of these values would improve the efficiency of *ApEn* calculations for the signals of rats which are common in experimental praxis.

The value m=3 of embedding parameter was chosen using FNN approach, as proposed in [6].

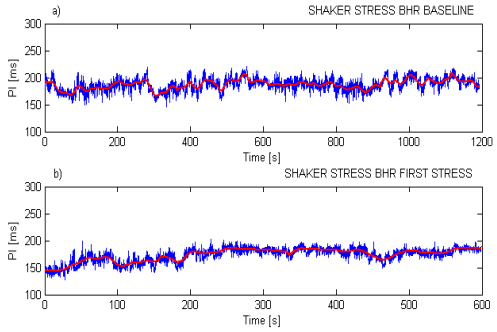

Fig. 1: Pulse interval (in [ms]) of a BHR rat in (a) BASELINE conditions and (b) during the exposure to the first shaker stress; thick line shows slow signal component

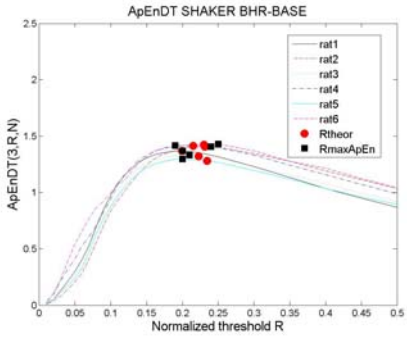

Fig. 2: *ApEn* for a set of BHR rats exposed to SHAKER stress; squares denote max *ApEn* and circles *ApEn* for theoretical threshold, for each rat

To explore the significance of the time delay parameter, all the calculations were done for τ=1, as originally proposed, and for τ=2 which is the first minima of the sample autocorrelation function.

Fig. 2 shows the *ApEn* plots for BHR rats, during the experiment with SHAKER stress. Black squares denote the maxima of *ApEn*. Red dots denote the *ApEn* evaluated for threshold obtained theoretically, according to the guidelines from [5], for m = 2 and 3:

$$m=2:\quad r_{theor}=(-0.02+0.23\sqrt{\mathrm{sd}_1/\mathrm{sd}_2})/\sqrt[4]{N}/1000 \tag{9}$$

$$m=3:\quad r_{theor}=(-0.06+0.43\sqrt{\mathrm{sd}_1/\mathrm{sd}_2})/\sqrt[4]{N}/1000 \tag{10}$$

Terms sd_1 and sd_2 in (9) and (10) can be regarded as short and long term variability of a (bounded) signal, where the first one is obtained as a standard deviation of differential series *PI(i)-PI(i-1),* while sd_2 is standard deviation of bounded PI time series [5]. When *ApEn* was calculated for τ=2, sd_1 was the standard deviation of series *PI(i)-PI(i-*τ*).*

Characteristic behavior of *ApEn* and threshold values is shown in Fig. 3. The first panels a) and b) show *ApEn* for a BASELINE condition, and when a SHAKER stress is applied for the first time for both m=2 and m=3. The remaining panels (c) and (d) are drown for the BASELINE condition and the first RESTRAINT stress.

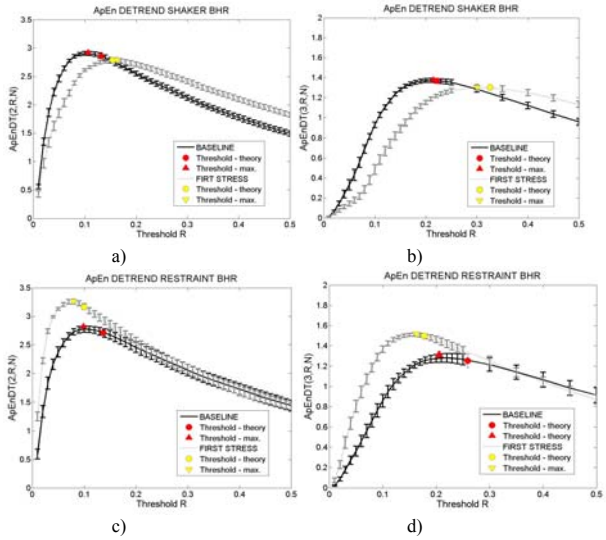

Fig. 3: Mean ApEn ± SE for different types of stress: a) shaker, m=2 and b) shaker, m=3, c) restraint m=2 and d) restraint m=3

The results of *ApEn(m=3,r,N,* τ*=1)* analysis are presented in Table1. The Table 1 shows the results of every parameter for four stages of experiment (BASELINE, FS, PFS, PLS) separated in columns and for two stress types applied to two types of rats (NRM, BHR). The trend of changes in PI and SBP through the course of experiment is shown in the first two rows.

The second row shows the values of experimental threshold r_{max}, for which *ApEn* gets the maximal value; the third row shows threshold values r_{teor} obtained using (10). A significant threshold increase during the first shaker stress and decrease in restraint stress remain regardless of the type of threshold choice.

The last three rows show *ApEn* values: experimentally found maximal *ApEn*, *ApEn* evaluated for theoretically obtained threshold; and *ApEn* evaluated for experimentally found threshold in BASELINE conditions This last case was an attempt to make an unbiased experiment: since the *ApEn* estimates a probability of "similar" patterns, the idea was that a choice of the same threshold for a whole set of experiments would ensure the same criterion of "similarity". However, it was found to be unnecessary. Although relative difference (in %) between the thresholds r_{max} and r_{theor} seems comparatively big (Fig.5 left), the difference between the entropies obtained for the two types of threshold is not (Fig.5 right). It does exceed 5% for a single experiment, but on average the difference between *ApEn(*r_{max}*)* and *ApEn(*r_{theor}*)* is less than 1.5%.

TABLE I
PI, SBP AND R_{MAX}, R_{THOR} AND APEN(3,r,N, τ=1)

		BASE	FS	PFS	PLS
PI [ms]	SN	167.81±5.27	157.97±4.15	167.80±3.92	175.76±9.55
	SB	186.56±9.25	175.82±7.10	181.95±9.67	189.00±7.69
	RN	179.29±5.58	131.46±2.33	141.91±2.26	169.48±4.77
	RB	183.45±3.93	153.21±5.95	143.93±5.81	150.54±2.62
SBP [mmHg]	SN	116.28±2.58	125.55±3.36	116.61±3.72	112.94±2.75
	SB	134.87±3.98	149.55±2.85	139.45±3.19	138.26±2.80
	RN	102.08±3.20	114.38±10.18	114.57±4.50	102.76±6.49
	RB	134.45±4.82	150.25±5.54	145.45±4.42	140.68±7.54
R max	SN	0.236±0.013	0.293±0.007	0.204±0.010	0.196±0.014
	SB	0.215±0.010	0.300±0.013	0.182±0.007	0.177±0.007
	RN	0.212±0.012	0.163±0.010	0.162±0.005	0.200±0.003
	RB	0.206±0.017	0.163±0.006	0.166±0.006	0.180±0.008
R Theor	SN	0.247±0.012	0.329±0.012	0.225±0.010	0.217±0.010
	SB	0.222±0.005	0.326±0.009	0.194±0.005	0.192±0.007
	RN	0.218±0.008	0.194±0.014	0.180±0.006	0.189±0.003
	RB	0.259±0.012	0.178±0.007	0.179±0.004	0.200±0.006
ApEn max	SN	1.417±0.022	1.359±0.009	1.513±0.015	1.369±0.019
	SB	1.376±0.021	1.306±0.025	1.427±0.021	1.374±0.025
	RN	1.373±0.036	1.497±0.032	1.557±0.018	1.544±0.025
	RB	1.310±0.030	1.520±0.019	1.540±0.016	1.467±0.030
ApEn Rtheor	SN	1.414±0.024	1.345±0.008	1.484±0.010	1.329±0.017
	SB	1.370±0.023	1.302±0.025	1.415±0.024	1.368±0.025
	RN	1.364±0.039	1.420±0.038	1.539±0.020	1.540±0.025
	RB	1.254±0.068	1.500±0.028	1.522±0.024	1.451±0.033
ApEn Rbase	SN	1.417±0.022	1.304±0.023	1.463±0.017	1.282±0.036
	SB	1.376±0.021	1.180±0.040	1.396±0.023	1.344±0.031
	RN	1.373±0.036	1.371±0.064	1.496±0.021	1.530±0.022
	RB	1.310±0.030	1.422±0.046	1.451±0.018	1.397±0.034

Results are given as mean+SE, the statistical significance was assessed using Repeated measures ANOVA test at levels p<0.05, p<0.01, p<0.005 indicated by the shades of gray, the stronger significance the darker the color.

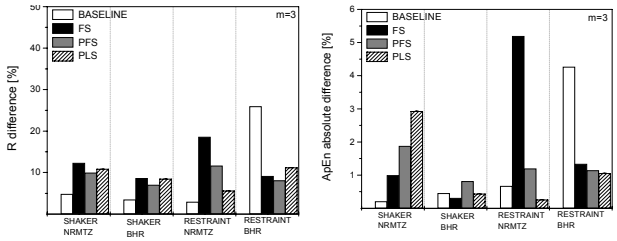

Fig.5 Relative absolute difference between thresholds r_{max} and r_{theor}, (left panel) and between maximal $ApEn(r_{max})$ and $ApEn(r_{theor})$ (right panel)

ApEn calculated in BASELINE conditions indicated significantly lower entropy values for BHR type of rats. During the exposure to shaker stress this relationship remains, which is in accordance with the freezing reaction of animal. On the contrary, defense reaction to restrain stress (FS) leads to higher maximum of *ApEn* in BHR rats then for NRM type of rats. When shaker stress is applied, *ApEn* decreases while threshold for which *ApEn* reaches its maxima is shifted towards higher values. When restraint stress is applied, the results are opposite: *ApEn* increases while threshold decreases.

ApEn values during the stress exposure and after stress periods indicate that *ApEn* returns to the basal value in the post stress period of shaker stress, but remains increased after the first and repeated exposures to restrain stress.

The *ApEn* was calculated for τ=2 as well. The results obtained for m=3 are shown in Table 2. Introducing time delay decreased the differences between the threshold values r_{max} and r_{theor}, which resulted in negligible differences between real maxima $ApEn(r_{max})$ and $ApEn(r_{theor})$. Otherwise, the trend of parameter changes during the course of experiment is preserved. The values of *ApEn* are increased, compared to the same values obtained for τ=1, reveling that modified method better reflexes system complexity.

IV. Discussion and Concusion

Restraint and shaker stress induce different patterns of cardiovascular response, subserving differences in behavioral response. The cardiovascular response to restraint is governed from dorsomedial hypothalamus, increasing blood pressure and heart rate. This is accompanied with increased entropy, showing that animal is adapting to new situation, preparing for defense. In contrast, the cardiovascular response to shaker stress is governed from dorsolateral hypothalamus. It does not change the blood pressure and heart rate significantly, but increases vigilance and freezing reactions. Animal is frozen, the reactions slowing down and the system is tending to state with the least energy consumption – the decreased entropy state.

Appropriate choice of parameters for *ApEn* calculation is crucial in obtaining meaningful results. The study shows that guidelines for automatic calculation of *r* given in [5] could be applied to signals of rats as well. Besides, the care should be taken of repositioning of *ApEn* maxima.

TABLE II
R_{MAX}, R_{THOR} AND APEN(3,r,N, τ=2)

		BASE	FS	PFS	PLS
R max	SN	0,235±0.008	0,297±0.009	0.210±0.007	0.192±0.013
	SB	0.223 ±0.007	0.306 ±0.006	0.193±0.005	0.183±0.008
	RN	0.216±0.007	0.168±0.007	0.173±0.005	0.198 ±0.005
	RB	0.220±0.015	0.164±0.007	0.168 ±0.006	0.191±0.006
R Theor	SN	0,234±0.008	0,297±0.009	0,215±0.006	0,205±0.009
	SB	0.218 ±0.004	0.306±0.007	0.197±0.005	0.198±0.005
	RN	0.213±0.007	0.176±0.008	0.177 ±0.005	0.185 ±0.003
	RB	0.246 ±0.005	0.164 ±0.006	0.175±0.002	0.196 ±0.008
ApEn max	SN	1,465±0.022	1,411±0.010	1,546±0.012	1,409±0.020
	SB	1.419±0.018	1.354 ±0.025	1.457±0.026	1.386±0.028
	RN	1.415±0.036	1.602±0.037	1.598±0.018	1.595±0.022
	RB	1.359±0.023	1.592±0.017	1.566 ±0.020	1.515 ±0.028
ApEn Rtheor	SN	1,463±0.021	1,405±0.011	1,534±0.011	1,393±0.016
	SB	1.415±0.017	1.353±0.025	1.451±0.027	1.384±0.029
	RN	1.411±0.036	1.585±0.039	1.591±0.017	1.588 ±0.021
	RB	1.318±0.023	1.581±0.022	1.558 ±0.025	1.503 ±0.033
ApEn Rbase	SN	1.465±0.022	1.339±0.018	1.522±0.013	1.352±0.026
	SB	1.419±0.018	1.231±0.033	1.438±0.026	1.364±0.066
	RN	1.415 ±0.036	1.494±0.064	1.552±0.016	1.584±0.020
	RB	1.318±0.023	1.503 ±0.039	1.497±0.022	1.466±0.034

Results are given as mean+SE, the statistical significance was in the same experimental group was assessed using Repeted measures ANOVA test. Significance levels $p<0.05$, $p<0.01$, $p<0.005$ indicated by the shades of gray, the stronger significance the darker the color.

Introducing the parameter τ leads to higher entropy values implying that modified method may better reflex underlying system dynamics. This parameter can overcome the problem of resampling which introduces interpolated, and therefore statistically dependant data, thus reducing the entropy.

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