State Anxiety and Nonlinear Dynamics of Heart Rate Variability in Students

Dimitriy A. Dimitriev*, Elena V. Saperova*, Aleksey D. Dimitriev

Department of Biology, Chuvash State Pedagogical University, Chuvash Republic, Russia

* These authors contributed equally to this work.
* rothman68@mail.ru

Abstract

Objectives
Clinical and experimental research studies have demonstrated that the emotional experience of anxiety impairs heart rate variability (HRV) in humans. The present study investigated whether changes in state anxiety (SA) can also modulate nonlinear dynamics of heart rate.

Methods
A group of 96 students volunteered to participate in the study. For each student, two 5-minute recordings of beat intervals (RR) were performed: one during a rest period and one just before a university examination, which was assumed to be a real-life stressor. Nonlinear analysis of HRV was performed. The Spielberger’s State-Trait Anxiety Inventory was used to assess the level of SA.

Results
Before adjusting for heart rate, a Wilcoxon matched pairs test showed significant decreases in Poincaré plot measures, entropy, largest Lyapunov exponent (LLE), and pointwise correlation dimension (PD2), and an increase in the short-term fractal-like scaling exponent of detrended fluctuation analysis ($\alpha_1$) during the exam session, compared with the rest period. A Pearson analysis indicated significant negative correlations between the dynamics of SA and Poincaré plot axes ratio (SD1/SD2), and between changes in SA and changes in entropy measures. A strong negative correlation was found between the dynamics of SA and LLE. A significant positive correlation was found between the dynamics of SA and $\alpha_1$.

The decreases in Poincaré plot measures (SD1, complex correlation measure), entropy measures, and LLE were still significant after adjusting for heart rate. Corrected $\alpha_1$ was increased during the exam session. As before, the dynamics of adjusted LLE was significantly correlated with the dynamics of SA.
Conclusions
The qualitative increase in SA during academic examination was related to the decrease in the complexity and size of the Poincaré plot through a reduction of both the interbeat interval and its variation.

Introduction
Anxiety is a negative emotional response to threatening circumstances [1]. State anxiety (SA) can be conceptualized as “a state in which an individual is unable to instigate a clear pattern of behavior to remove or alter the event/object/interpretation that is threatening an existing goal” [2]. The neural organization of anxiety spans multiple levels of the brain, from the complex visceral and somatic integration of the limbic system, to the elementary adaptive activity of the brainstem [3]. Anxiety is associated with elevated high blood pressure [4], increased heart rate (HR) [5] and an enhanced respiratory rate [6]. A key system, involved in the generation of this physiological arousal is the autonomic nervous system (ANS) [7]. The ANS responds both to central stimuli and to activation of reflex sensory inputs [8]. The simple reciprocal concept of sympathovagal balance has been the keystone of ANS physiology for many years [9]. Reciprocity is true for many autonomic reflexes, such as the baroreflex [10] or orthostatic stress [11]. In contrast to homeostatic sensory inputs, however, descending influences from rostral brain structures can evoke different patterns of autonomic reactivity, such as reciprocal, independent or coactive changes in the parasympathetic and sympathetic branches of the ANS [7].

The principal property of the ANS is variability. Autonomic outflow has been well established as intrinsically periodic [12, 13]. Some researchers [14, 15] proposed that brainstem autonomic circuits generate this rhythm (the central oscillator theory). This theory is supported by the observation that different oscillations are present in the firing of sympathetic-related neurons of the medulla [16]. The alternative theory (the baroreflex feedback loop theory) postulates that a combination of time delays and feedback results in the oscillation of blood pressure and HR [17, 18]. Mathematical models of the ANS reveal nonlinear properties of these rhythms [19, 20].

Heart rate variability (HRV) is the difference between consecutive instantaneous beat intervals (RR) [21]. HRV may be an independent marker of cardiovascular health [22] and an indicator of ANS activity [23]. The HRV seems to show a beat-to-beat regulation to which the sympathetic and parasympathetic modulatory influences are probably opposite [24, 25]. The physiological background of HRV has been extensively described using statistical and linear spectral analysis methods [26].

A physiological system, that generates the RR time series data, has been conceptualized as a network of biological oscillators with non-linear proprieties [27]. Chaos is apparently a lawless behavior of a nonlinear system totally ruled by deterministic laws [28]. A healthy cardiovascular system is associated with HRV of a chaotic nature; this chaotic nature reflects adaptability, which can be defined as the capacity to respond to unpredictable stimuli [29]. Consequently, nonlinear behavior would indicate greater flexibility and smaller predictability than a linear behavior [30]. Complex temporal patterns of physiological signals can result from interaction between nonlinear oscillatory systems, including those demonstrating chaotic behavior [30].

Different nonlinear measures of HRV quantify different features of nonlinear dynamics of HR. Lyapunov exponents and entropy rates are measures of the dynamics on an attractor. The correlation dimension describes the complex structure of the attractor approximating the
fractal dimension. The Poincaré plot describes the evolution of a system. Detrended fluctuation analysis (DFA) quantifies the fractal correlation properties in physiological time series. By combining different nonlinear measures, different aspects of the underlying physiological patterns may be captured [19, 20, 27, 30].

The Poincaré plot is a scatterplot in which current R-R is plotted as a function of previous interval [31]. Poincaré plot analysis is based on a technique from nonlinear dynamics and provides detailed beat-to-beat information on the activity of the sinus node [31]. Analysis of the Poincaré plot can be used to not only to classify the signal into one of various classes (e.g. torpedo, butterfly, parabola, or comet) but also to fit an ellipse, which enables quantification of the Poincaré map [32]. Application of this method includes measurement of autonomic modulation, or randomness, of HR in physiological and clinical studies [32, 33, 34]. Anxiety is associated with a prominent reduction in the standard deviation of the Poincaré plot perpendicular to the line of identity (SD1) [35, 36]. Karmakar et al. [37] proposed a novel descriptor, the Complex Correlation measure (CCM), to quantify the temporal aspect of the Poincaré plot. In contrast to SD1 and dispersion along the line of identity (SD2), this measure incorporates point-to-point variation in the signal.

In time series analysis, time irreversibility refers to the lack of invariance of the statistical properties of a signal under the operation of time reversal [38]. Asymmetric patterns (i.e., those with the ascending side shorter than the descending side or vice versa) suggest irreversibility, but irreversibility might not imply the presence of asymmetrical patterns [39].

Asymmetry is present in physiological systems as it is an essential property of a non-equilibrium system [40]. A visible and statistically highly significant asymmetry has been shown in the Poincaré plot [41]. Porta et al. [39] examined the asymmetry of a Poincaré plot and showed an interrelationship between time irreversibility, pattern asymmetry, and nonlinear dynamics. Recent studies indicate that simple irreversibility indexes are sensitive to autonomic changes during active orthostasis [42] and head-up tilt [39]. Some studies utilized the Poincaré plot in the case of university examinations [43], mental effort [44], and anxiety disorders [35], but the utility of irreversibility indexes and complex correlation measure for anxiety research have not been well defined.

Fishman et al. [45] pioneered an innovative method of temporal Poincaré variability (TPV), which is a novel analysis to quantify the temporal distribution of points and to detect nonlinear sources responsible for physiological variability. Two measures of the Poincaré plot are proposed. The first, called time-delayed TPV (TPVTD) is the measure of the similarity of an interval to its successor. TPVTD is equivalent to SD1; and hence we excluded this method from consideration. The second measure is called long-term TPV (TPVA) and is calculated using the distance from the center of mass to the origin.

Another approach to the nonlinear analysis of HRV is quantification of complexity. The most commonly used non-linear complexity measures are fractal dimensions of various kinds, and measures based on entropy [46].

Entropy is the measure of system randomness and predictability, with greater entropy often associated with more randomness and less system order [46]. The concept of entropy, as it applies to signals such as RR intervals, is to quantify the repetition of patterns in that signal [47]. Pincus [48] developed approximate entropy (ApEn) as a measure of system complexity. ApEn \((m,r,N)\) is approximately the negative natural logarithm of the conditional probability that a dataset of length \(N\), having repeated itself within a tolerance \(r\) for \(m\) points, will also repeat itself for \(m + 1\) points. Reduced ApEn values, indicating large predictability and less complexity in HR dynamics, have been reported in patients with congestive heart failure [49] and schizophrenia [50]. In addition, ApEn increases during exercise have been reported [51]. Cholinergic blockade with atropine does not significantly impact ApEn [52].
To eliminate its limitation of dependency on the record length, Richmann and Moorman modified the ApEn and introduced Sample Entropy (SampEn) [53]. SampEn is precisely the negative natural logarithm of the conditional probability that two sequences similar for m points remain similar for m+1 points, within a tolerance r, excluding self-matches [54]. Thus, a higher value of SampEn also indicates less self-similarity in the time series [54]. Mateo et al. [55] found that pre-competitive SA was associated with low SampEn. However, SampEn has not yet been used as a measure of HRV in studies examining students’ SA.

The hallmark of physiological systems is their extraordinary complexity [56]. Experimental and theoretical evidence suggests that under healthy conditions physiological signals may have a fractal temporal structure [57]. Introduced by Peng and collaborators [58], DFA has become a widely used technique for the determination of (mono-) fractal scaling properties and the detection of long-range correlations in noisy, non-stationary time series. DFA is a scaling analysis method that involves the calculation of a simple quantitative parameter—the scaling exponent $\alpha$—to represent the correlation properties of a signal. The DFA method may be useful in identifying and quantifying different states of the same system according to its different scaling behaviors. For example, the scaling exponent $\alpha$ for heart interbeat intervals differs between normal and pathological conditions [39]. DFA was originally used to analyze 24-hr Holter recordings [58], but it is impractical for assessing HRV stress responses. Recent studies have reported the susceptibility of short-term HRV to DFA [60]; this was the basis for computation of DFA measures for a 5-min RR sequence. Unmedicated patients with major depressive disorder had a significantly increased DFA when compared with controls [35]. Pre-competitive anxiety is associated with an increased level of the short-term scaling exponent ($\alpha_1$) [54]. However, Mellilo et al. [43] found diminished $\alpha_1$ levels under academic stress.

In recent years, the interest in applying techniques that stem from the chaos theory in studies of electroencephalographic activity [61] and arterial pressure [62] has been increasing. The largest Lyapunov exponent (LLE) is a simple non-linear measure of how fast two initially nearby points on a trajectory will diverge or converge each other in a phase space; LLE quantifies the sensitivity of the system to initial conditions and provides a predictability [63]. As of yet, only a few studies have investigated the impact of acute stressors on HR measures of chaos in healthy individuals. Hagerman et al. [64] demonstrated that in healthy individuals (33–51 years of age), the LLE of HRV significantly decreased during exercise stress. Both chronic and acute stress experiences have been associated with a reduced LLE [43, 65].

In the presence of chaos, the complexity of HR dynamics can be quantified in terms of the properties of the attractor in phase-space, that is, its correlation dimension (D2) [66]. This measure is based on the presumption that dynamics is the output of a deterministic dynamical system, whereas time-domain measures assume that the variability is around a stationary mean and is noise [67]. D2 has been found to be greatly reduced by cholinergic blockade in both animal and human studies [68, 69]. Nahshoni et al. [67] found that patients with major depression had significantly lower mean correlation dimension than healthy subjects. Schubert et al. [65] showed that acute and chronic stresses are both associated with decreases in correlation dimension. The point D2 (PD2) estimate of the correlation dimension was developed by Skinner et al. [70]. Like D2, PD2 describes the complexity of a system (i.e. number of independent variables needed to describe a system). The advantage of PD2 over D2 is its robustness to non-stationarity (i.e., change over the measurement period).

The fact that high HR is associated with lower variability in RR-intervals is well-known [71]. Therefore, it is critical to correct HRV for the prevailing HR, as HR changes significantly in response to academic stress during examination. Sacha and co-workers [72] previously demonstrated that measures of HRV should be corrected by dividing or multiplying with the corresponding mean RR interval.
It is now generally accepted that nonlinear techniques are able to describe HRV in a more effective manner. However, the ability of nonlinear measures of HRV to enhance our understanding of anxiety has only been partly investigated. This paper is focused on the hypothesis that exam stress provokes changes in nonlinear parameters of HRV. Furthermore, we hypothesized that a decrease in HRV is the consequence of a concurrent increase in HR.

Materials and Methods

The study group consisted of 96 (15 men and 81 women) healthy, nonsmoking volunteers (students of Chuvash State Pedagogical University), whose ages ranged from 19 to 24 years (mean ± SE: 20.53 ± 0.11 years). All the volunteers underwent physical and neurological examinations, as well as routine laboratory tests, lung function test, a 12-channel electrocardiography (ECG) recording, and chest radiographic examination, before the study. No evidence of heart or pulmonary disease was found in any of the subjects. None of the subjects had been taking any medications for at least 2 weeks before the study. On the day of the study, the subjects were instructed to avoid alcohol and caffeinated beverages for the 12 preceding hours and to abstain from heavy physical activity since the day before. The study was approved by the local Ethical Committee for biomedical research of Chuvash State University named I. N. Ulyanov. Written informed consent was obtained from all the volunteers between 19 and 24 years of age (in Russia, the legal age of consent is 18 years).

The mean height and weight of the subjects were 165.25 ± 0.86 cm (range, 145.50–189.50 cm) and 57.06 ± 0.93 kg (range: 41.00–85.00 kg); their body mass index was 20.99 ± 0.28 (range, 16.63–28.58 kg/m2). ECG was recorded in the supine position for 5 min in two different days; the first recording was performed during the controlled resting condition (rest session), while the second one was conducted just before the university verbal examination (exam session). We chose the supine position for physiological and technical reasons. All time series were checked manually by careful visual inspection of the RR intervals, as described previously [73]. Two kinds of methods were used to avoid artifacts, such as false RR detection and ectopic beats. For each record, we first detected artifact and ectopic intervals by using three standard methods, namely percentage filter, standard deviation filter, and median filter [74, 75]. Next, we replaced abnormal RR intervals with the mean value of the neighboring RR intervals that were centered on the ectopic interval. Experiments were conducted at the same time of day (08.00–12.00 h) and in the same room, maintained at 22°C. The Russian version of Spielberger’s State-Trait Anxiety Inventory (STAI), was used to assess SA levels during the rest and exam sessions [76]. The reliability and validity of this version has been evaluated by many researchers [77]. The STAI State Anxiety Subscale evaluates the current state of anxiety by using items that measure subjective feelings of apprehension, tension, nervousness, worry, and associated with arousal of the autonomic nervous system [1]. The students’ STAI scores were classified as low (0–30), moderate (31–45), and high (≥46). Emotional reactivity refers to the tendency to experience frequent and intense emotional arousal. In this study, we examined the intensity of emotional experiences by using the STAI State Anxiety Subscale.

The following variables were used for the non-linear analysis: DFA (with the scaling components α1 and α2), ApEn, SampEn, and the Poincaré plots (SD1, SD2, SD1/SD2, SS, Guzik’s index of asymmetry (GI), and CCM). The Poincaré plots (return maps), correlating the observation n on the x-axis with observation n + 1 on the y-axis, were used to study HRV as a series of discrete events. The primary method for quantifying the Poincaré plot is an ellipse-fitting technique, although the ellipse serves only as a visual guide with no actual mathematical fit of the data to the equation of an ellipse [31]. Brennan et al. [31] developed a method for quantitative assessment of the ellipse, and we used this to estimate SD1, SD2, and SD1/SD2. The shapes
of the Poincaré plots being categorized were, according to the value of SD1/SD2: a normal, comet-shaped plot (SD1/SD2 > 0.15), and a torpedo-shaped plot (SD1/SD2 < 0.15) [32]. We assessed the asymmetry of Poincaré plots by computing GI as follows [41], according to the definitions of clouds proposed by Karmarkar et al. [78] Eq (1):

$$GI = \frac{\sum_{i=1}^{M} (D_i)^2}{\sum_{i=1}^{N} (D_i)^2} \times 100\%$$

where $D_i$ is the distance of the plotted points from the line of identity, and $M$ is the number of points in the increasing cloud. The numerator corresponds to the increasing cloud, and the denominator corresponds to the total number of points ($N$).

A novel extension of the Poincaré plot is the CCM, which measures beat-to-beat dynamics [37]. The CCM was computed in the windowed manner, in which the temporal information of the signal is embedded. The moving window of three consecutive points from the Poincaré plot is considered and the area of the triangle formed by these three points are computed. CCM is composed of all overlapping three-point windows and can be calculated as Eq (2):

$$CCM(m) = \frac{1}{C_n(N-2)} \sum_{i=1}^{N-2} ||A(i)||$$

where $m$ represents the lag of the Poincaré plot ($m = 1$), $A(i)$ represents the area of the triangle (formed with $i^{th}$, $i+1^{th}$ and $i+2^{th}$ points of the Poincaré plot) and $C_n$ is the normalizing constant, which is defined as: $C_n = \pi \times SD_1 \times SD_2$, representing the area of the fitted ellipse over the Poincaré plot.

ApEn ($m,r,N$) is the negative natural logarithm of the conditional probability that a dataset of length $N$, having repeated itself within a tolerance $r$ for $m$ points, will also repeat itself for $m+1$ points Eq (3). The function is:

$$ApEn = \ln \frac{Am(r)}{Bm(r)}$$

where $Am(r)$ is the probability that two sequences will match for $m$ points, and $Bm(r)$ is the probability that two sequences will match for $m+1$ points [79].

Computation of SampEn is similar to computation of ApEn, with only a small difference, which is SampEn does not count self-matches [54].

Elimination of self-matches makes SampEn more reliable over short data sequences than ApEn [54]. Previous works, in which measures of entropy were calculated for short sequences of RR, have indicated that sample entropy can be accurately estimated from a set of 100 to 5000 data points when the length of the sequences to be compared ($m$) is set at 1 or 2 and the tolerance level ($r$) for determining a difference between data points is set between 0.1 and 0.2 of the standard deviation of the total data set [80]. Based on this and other works [81, 82] we choose $m = 2$ and $r = 0.2 \times SD$.

DFA quantifies the presence or absence of fractal correlation properties of the RR intervals [58]. The DFA procedure [58] consists of four steps. First, the RR series obtained experimentally is integrated by using the expression Eq (4):

$$Y(k) = \sum_{k=1}^{N} [RR(i) - RRave]$$

where $Y(k)$ is the $k^{th}$ term of the integrated series ($k = 1, 2, \ldots, N$); $RR(i)$ is the $i^{th}$ value of the RR intervals, and $RRave$ is the mean of the RR intervals of the original series, with $N$ length. Second, $Y(k)$ is divided into $N(t)$ non-overlapping segments of equal length ($t$). Next, we
detrended the integrated time series, Y(k), by subtracting the local trend, Yn(k), in each box. In step 4 we averaged overall all segments and calculated the square root to obtain the fluctuation function Eq (5) as follows:

\[ F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} |Y(k) - Yn(k)|^2} \]  

This computation is repeated for all time scales, thereby obtaining a relationship between the mean of the fluctuations [F(n)] and the size of the intervals (n). As F(n) measures the average difference between two interbeat intervals separated by a time lag n, it quantifies the magnitude of the fluctuations over different time scales n [58]. Typically, F(n) will increase with box size n. A linear relationship on a log-log graph indicates a scale exponent law, that is, F(n) ≈ n×α. Under such conditions, the fluctuations can be characterized by a scaling exponent α, which can be calculated by linear regression on a log—log graph [83]. Note that α = 0.5 corresponds to a random walk (a Brownian motion), α = 1 represents 1/f noise and α = 1.5 indicates Brown noise, the integration of white noise [58].

Tan et al. [84] showed that a single exponent is inadequate to describe HR dynamics. Therefore, estimation of short- and long-term exponents, namely \(\alpha_1\) and \(\alpha_2\), has been proposed to better describe HR dynamics [58]. We calculated \(\alpha_1\) to a period of 4 to 11 beats and \(\alpha_2\) to periods longer than 11 beats [60].

We estimated LLE by using the algorithm proposed by Rosenstein et al. [85, 86], which has been shown to be particularly useful for small data series. It should be noted that before calculating the LLE, we estimated the number of embedded dimensions and time delay. Delay-time (\(\tau\)) was determined by using the first minimum of the auto mutual information function. The Cao method was used to estimate the minimal embedded dimension (m) for the present study. Finally, by using \(\tau\) and m, the phase-space trajectory was reconstructed [87].

Chaotic dimensions were calculated with the pointwise correlation dimension (PD2) algorithm [88] by using the Dataplore® software. TPVA was calculated on a beat-to-beat basis using a software package (http://engineering.case.edu/eecs/research).

In order to remove any mathematical bias from the HRV calculations, we used the HR correction methodology of Sacha et al. [72].

In the present investigation, we sought to address the following question: “Do nonlinear indexes of HRV quantify the autonomic expressions of anxiety, in addition to time- and frequency-domain indexes?” To address this question, we studied SDNN, the time-domain measure of HRV, and LF and HF, the spectral indexes of HRV.

Variables were not normally distributed; therefore, nonparametric statistical methods were applied. The Wilcoxon Matched Pairs Test was first used to detect significant differences in values obtained at different time points (during the rest period and before the examination). The Spearman’s rank-order correlation coefficient was calculated to assess the monotonic relationship between variables. Chi-square analysis was used to test proportions. The Mann-Whitney U test was used to analyze differences between the subject groups. Statistical significance was set at \(p < 0.05\). All data were expressed as mean ± SE.

**Results**

At rest, all the students had moderate to low STAI scores (mean, 25.98 ± 0.99). Fifty-nine students (61.5%) fell into the low STAI category while 37 (38.5%) had moderate scores. SA was indeed higher in the exam session (41.78 ± 1.05, \(z = 8.56\), \(p < 0.001\)). During the exam session,
11 students (11.46%) had low SA scores, 53 (55.21%) had moderate SA scores, and 32 students (33.33%) had high SA scores.

Individual SA results are summarized graphically in Fig 1.

Most participants (N = 90, 93.7%) showed an increase in SA during the exam session. The transition from the rest session to the exam evoked qualitative increases in SA among the 61 students, from low to moderate in 32 students, from low to high in 18 students, and from moderate to high in 11 students. We divided the participants into two groups based on their anxiety patterns as follows: first, with qualitative increases in state anxiety (N = 61) and second, with no increases (N = 35). The comparison between the two conditions, for the study of nonlinear parameters of HRV, is shown in Table 1.

Analysis of HR changes during the exam session yielded an overall increase in HR. The change in HR time from baseline did not differ between groups (first group, +11.95 ± 1.51 bpm; second group, +9.57 ± 1.9 bpm; p > 0.05). In our subjects, SDNN and HF were significantly higher during the rest period than during the exam session. The low-frequency component of RR variability decreased significantly during the exam session in the first group. SD1 decreased significantly before the examination in the first group but not in the second group. Increasing anxiety led to a significant decrease in SD1/SD2 value.

Fig 1. Individual data for examination-induced changes in state anxiety from rest session to the exam session. (A) Increasing from low to moderate anxiety levels. (B) Increasing from low to high anxiety levels. (C) Increasing from moderate to high anxiety levels. (D) Absence of qualitative changes in anxiety.

doi:10.1371/journal.pone.0146131.g001
The Poincaré plot at rest displayed a greater dispersion of points than the exam session. Fig 2 shows a significant reduction in area from low SA (rest session) to high SA (exam session), with a contraction of length and a shortening of width in the plots. Compared with a student with low anxiety, the center of the ellipse from a participant with high anxiety is shifted down and to the left. Although the increase in SA induced a significant reduction in the width of the Poincaré plots (Table 1), the correlation between the changes in SD1 and SA did not reach statistical significance (r = −0.08, p > 0.05). Our results did not show a relationship between SD2 dynamics and fluctuations of state anxiety (r = 0.05, p > 0.05). No significant (p > 0.05)

Table 1. Comparison between the two conditions of the study for heart rate variability analysis.

| HRV indexes | All participants | 1-st group | 2-nd group |
|-------------|-----------------|------------|------------|
|             | Rest            | Exam       | Rest       | Exam       | Rest         | Exam         |
| HR [bpm]    | 72.22±0.93      | 83.39±1.16#| 71.67±1.08 | 83.62±1.47#| 73.36±1.78   | 82.92±1.87#  |
| SDNN [ms]   | 53.55±1.80      | 44.78±1.56#| 53.75±2.14 | 45.10±1.77#| 53.14±3.32   | 44.12±3.13#  |
| LF [ms²]    | 800.16±64.15    | 650.33±51.45*| 809.86±76.34 | 640.96±55.74*| 780.25±118.08 | 669.26±108.76 |
| HF [ms²]    | 1221.42±109.40  | 673.73±71.60#| 1178.45±116.33 | 607.92±78.66#| 1309.62±235.45 | 808.82±145.58#|
| SD1         | 35.84±1.54      | 25.58±1.39#| 36.38±1.78 | 24.57±1.62#| 34.73±2.98   | 27.66±2.62#  |
| SD2         | 65.74±2.12      | 59.18±1.96#| 66.43±2.6  | 58.75±2.12#| 64.32±3.7    | 60.05±4.16   |
| SD1/SD2     | 0.55±0.02       | 0.42±0.01# | 0.55±0.02 | 0.41±0.02# | 0.54±0.03    | 0.45±0.02#   |
| G1          | 0.51±0.01       | 0.48±0.01# | 0.51±0.01 | 0.49±0.01# | 0.50±0.02    | 0.46±0.02    |
| CCM         | 0.26±0.01       | 0.19±0.01# | 0.27±0.01 | 0.18±0.01# | 0.25±0.02    | 0.21±0.01*   |
| TPVA        | 54.89±1.7       | 56.44±1.65 | 54.98±2.1 | 56.29±1.63 | 54.71±2.84   | 56.75±3.83   |
| ApEn        | 1.21±0.01       | 1.19±0.01  | 1.22±0.01 | 1.18±0.02* | 1.19±0.02    | 1.20±0.02    |
| SampEn      | 1.87±0.02       | 1.68±0.03# | 1.89±0.02 | 1.65±0.04# | 1.82±0.04    | 1.75±0.04    |
| α1          | 0.90±0.02       | 1.10±0.02# | 0.90±0.03 | 1.13±0.03# | 0.89±0.04    | 1.04±0.04    |
| α2          | 0.83±0.02       | 0.88±0.02* | 0.85±0.02 | 0.89±0.02  | 0.80±0.03    | 0.87±0.03    |
| LLE         | 0.30±0.01       | 0.25±0.01# | 0.31±0.02 | 0.23±0.01# | 0.27±0.02    | 0.29±0.02    |
| PD2         | 3.75±0.09       | 3.57±0.07# | 3.62±0.10 | 3.41±0.08# | 4.02±0.18    | 3.90±0.15    |

Exam vs rest:
* p<0.05;
#p<0.01.

doi:10.1371/journal.pone.0146131.t001

Fig 2. Poincaré plots during the rest (A) and exam sessions (B).

doi:10.1371/journal.pone.0146131.g002
differences in the changes in the quantitative measures of Poincaré plot shape were observed between the groups.

Statistical analysis of the ratio between width and length of Poincaré plots (SD1/SD2) revealed a significant decrease in this parameter in both groups. Increased SA was associated with decreased SD1/SD2 ($r = -0.20$, $p < 0.05$).

In this study, we used the GI range 0.49 to 0.51 as symmetrical [37]. Fig 3 shows the GIs for the rest and exam sessions.

During the rest and exam sessions, 93% and 85% of the subjects, respectively, were found to be asymmetrical. The chi-square analysis demonstrated a non-significant difference between the two sessions ($p > 0.05$). GIs, measured before examination, were slightly lower, but the difference did not reach statistical significance in either group. The results of the statistical analysis of GI showed no significant difference in response magnitude between the groups (mean GI change: $-0.03 \pm 0.01$ in the first group vs. $-0.04 \pm 0.02$ in the second group; $p > 0.05$).

In the assessment of whole range of SA from low to high levels measured at the rest and exam sessions, changes in GI did not show a consistent correlation with the dynamics of SA ($r = -0.04$, $p > 0.05$). CCM decreased significantly in both groups, but the changes were greater in the first group ($-0.09 \pm 0.01$ vs. $-0.04 \pm 0.02$; $p < 0.05$). This decrease was correlated with an increase in SA scores ($r = -0.21$, $p < 0.05$). The increment in TPVA between the rest and exam sessions was insignificant, and increases in SA scores showed a weak positive association with increases in TPVA ($r = 0.12$; $p > 0.05$).

Increases in SA scores were associated with significant changes in ApEn value in the group of students with high emotional reactivity (Table 1), and the dynamics of ApEn significantly correlated with changes in SA ($r = -0.29$, $p < 0.05$). The changes in ApEn score differed between the students who differed in SA scores (mean ApEn change: $-0.05 \pm 0.02$ in first group, vs. $0.014 \pm 0.03$ in second group; $p < 0.05$). As shown in Table 1, the mean SampEn values tended to decrease before the exam session in the first group. By contrast, we found that this decrease was significantly less prominent in the second group ($-0.25 \pm 0.04$ in first group; $p < 0.01$). The SampEn changes correlated significantly with changes in SA scores ($r = -0.26$, $p < 0.05$).

The effect of the quantitative increase in SA scores on short—term scaling exponent $\alpha_1$ was significant. The difference in magnitude of the $\alpha_1$ change between the groups was also significant (mean $\alpha_1$ change $-0.05 \pm 0.02$ in first group, vs. $0.014 \pm 0.03$; $p < 0.05$).
Our results show a positive correlation between increased SA scores and changes in $\alpha_1$ ($r = 0.22, p < 0.05$). No association was found between changes in SA scores and $\alpha_2$ ($r = 0.1, p > 0.05$).

We found significantly lower levels of LLE during the exam session. The changes in LLE differed between the groups ($p < 0.01$), with negative values found in the first group (mean LLE change $-0.08 \pm 0.02$ in the first group, vs. $0.013 \pm 0.02$ in second group). By plotting the dynamics of LLE as a function of the corresponding SA scores (Fig 4), we showed that LLE was significantly negatively correlated with SA ($r = -0.45; p < 0.05$).

In the second group of students, who did not have a qualitative change in anxiety, PD2 did not differ between the sessions (Table 1). However, PD2 was significantly decreased before the examination in the first group. The Pearson analysis demonstrated weak negative correlations between the changes in PD2 and the changes in SA ($r = -0.14; p > 0.05$).

We examined the correlations between measures of HRV and HR (Table 2). HR was significantly negatively correlated with the width (SD1) and length (SD2) of the long and short axes of the Poincaré plot images. The correlations for the exam session were slightly higher than the correlations for the rest session. Correlations between the Poincaré plot axes ratio (SD1/SD2) and HR indicated a strong, negative relationship between these two measures. The asymmetry index GI had a significant negative association with the ratio of HR. HR was negatively correlated with temporal dynamics of the Poincaré plot, estimated by the

![Graph showing correlation between dynamics of state anxiety and Largest Lyapunov exponent](Fig 4. Correlation between the dynamics of state anxiety (DYN SA = SA at exam − SA at rest) and Largest Lyapunov exponent (LLE; DIN LLE = LLE at exam − LLE at rest).)

doi:10.1371/journal.pone.0146131.g004
Complex correlation measure (CCM). Although Pearson correlation analysis showed that HR was significantly and negatively associated with all of the above-mentioned indicators of the Poincaré plot, the measure of long-term temporal Poincaré variability TPVA was not significantly correlated with HR. Approximate entropy showed a strong positive association with HR measured during the rest session, but did not have a significant correlation with HR measured before the exam session. Significant negative correlations were observed between HR and SampEn during both sessions, as shown in Table 2. We found that elevated HR was significantly associated with increases in the short-term scaling exponent $\alpha_1$. The long-term scaling exponent $\alpha_2$ showed a statistically significant positive correlation with HR during the exam session.

The formulas for correcting HRV are given in S1 Table. Table 3 shows nonlinear HRV indexes, adjusted for HR.

Table 3 presents the adjusted results of the SDNN, LF, HF and nonlinear HRV indexes for the resting and examination conditions. The Wilcoxon test for HR-corrected convenient HRV indexes showed no significant difference between the rest and exam sessions. After correction for HR, the corrected SD1 and SD1/SD2 were still decreased ($p < 0.01$), while SD2 was slightly increased ($p > 0.05$). The finding of decreased CCM in the second group (subjects with less pronounced emotional response) during the transition from the rest session to the exam session largely disappeared after adjustment for HR. The results of our analysis show that when HR is taken into account, the difference between the average GI during the rest and exam sessions was decreased to a non-significant level in the first group. HR correction did not influence the change in ApEn, SampEn, and LLE. The use of HR correction formulas led to a
significant reduction in PD2 dynamics in both groups. After adjusting for HR, the dynamics of LLE was still significantly negatively associated with the dynamics of anxiety ($r = -0.43; p < 0.05$). Differences in the changes in the HRV nonlinear parameters (with the exception of DFA) between the groups were not altered after correction for HR.

### Discussion

This study investigated how SA in academic conditions is related to the nonlinear dynamics of HRV. Nonlinear analysis methods are designed to assess the quality, scaling, and correlative properties of signals [29]. We confirmed the effect of increased anxiety on the HR fluctuation by comparing nonlinear HRV indexes between rest and exam sessions. The physiological meaning of Poincaré plot shape and indexes has been examined in different functional states [32, 89, 90]. The relationship between autonomic function and the shape of the Poincaré plot has been established; that is, the narrower the observed pattern, the larger the shift in sympathovagal balance toward an increase in sympathetic nervous system activity [91, 92]. Norepinephrine infusion in healthy volunteers has been shown to cause a sudden change in fixed RR interval dynamics, resulting in a torpedo-shaped Poincaré plot [32]. Meanwhile, atropine administration results in a reduction in the width of the Poincaré cloud [78]. SA is associated with a prominent change in sympathovagal balance [93]; nevertheless, normal comet-shaped scatter plots (and no torpedo-shaped plots) were observed for all subjects in both conditions.

Infusion of atropine induced a reduction in SD1 [87, 94], indicating that the “width” of the Poincaré plot is a measure of parasympathetic nervous system activity [95–97]. Our findings suggest that SA has a predominantly inhibitory effect on parasympathetic activity in exam situations, as the SD1 parameter is significantly lower in exam situations than in the rest situations. SD2 is a nonlinear index with uncertain physiological meaning and interpretation. It is thought to reflect the continuous long-term variability of the RR intervals [89, 98]. Guzik et al. [99]
interpreted the SD1/SD2 ratio as a measure of the balance between short- and long-term HRV. During the exam session, the SD1/SD2 ratio decreased significantly owing to the important reduction in SD1 compared with SD2. This highlights the parasympathetic withdrawal and sympathetic activation associated with the transition to a higher level of SA. Our results confirm the effect of anxiety and mental effort on the Poincaré plot [35, 44]. However, our research lends additional insight into the study of SA by using quantitative measures of Poincaré plot shape. In contrast to a previous work [43], we found a significant reduction in the SD2 measured before the examination.

Asymmetry of a Poincaré plot is associated with time irreversibility and nonlinear dynamics [39, 42]. Temporal irreversibility and asymmetry are prominent features of HRV, and differences between HR accelerations and decelerations are related to the physiological or pathological states of organisms [39, 42]. This property was confirmed by analysis of our scatterplots. Our results exhibit prominent asymmetry of the Poincaré plot, indicating that the heart period variability in most of the students was irreversible, regardless of SA levels. Considering that the detection of time irreversibility implies the presence of nonlinear dynamics, we can conclude that short-term heart period variability is nonlinear in a major portion of participants during both sessions [42]. Asymmetry in the GI slightly and non-significantly decreased during the exam session. According to Porta et al. [39], an important shift in sympathovagal balance toward vagal withdrawal is associated with an increase in the asymmetry of Poincaré plot. Tonhajzerova et al. [100] found prominent reductions in the resting HR time irreversibility indexes in adolescent female patients with major depressive disorder. The absence of the effect in our study may have to do with the difference between SA and depression [101].

From the theoretical definition of CCM, it is obvious that this measure quantifies variability in the temporal structure of Poincaré plots [37]. CCM quantifies underlying temporal dynamics in a Poincaré plot; the decrease in CCM indicates increased regularity and decreased variability [37]. The value of CCM decreased with the increase in parasympathetic activity during atropine infusion and 70° head-up tilt phase test [78]. This suggests that the low CCM during the exam session was caused by an increase in sympathovagal balance. The decrease in CCM indicates a reduction in RR variability and increasing regularity, associated with potential risk of cardiovascular events [102]. Our results can be compared with those from the work of Jellinek et al. [103]. Their study demonstrated a significant reduction in CCM in patients with depression, and they suggest that CCM is more sensitive to parasympathetic nervous system activity than SD1 and SD2. The increment in TPVA between the rest and exam sessions was insignificant, and the increases in SA level showed a weak positive association with the increases in TPVA (r = 0.12; p > 0.05). The authors of the temporal Poincaré variability methodology [45] theorized that this method assesses general patterns of temporal change in HR associated with nonlinear dynamics and complements other time-dependent methods. However, our results do not promote the utility of TPVA as a marker of anxiety-induced changes in HRV.

Entropy, as it relates to dynamical systems, is the rate of information production [46–48], and approximate entropy can be used to classify complex systems, such as physiological systems [53]. Reduction in entropy means greater regularity; this condition is associated with sickness and aging [47]. Previous studies reported discrepant results concerning the effect of negative emotions and stress on complexity measures based on entropy. Valenza et al. [104] found that the mean ApEn decreased significantly during arousal elicited by pictures. Mellilo et al. [43] showed decreased ApEn due to university examination. Anishchenko et al. [105] reported, in healthy young subjects, that short-term psychological stress was associated with both decreases and increases in HR complexity (i.e., approximated entropy). The present data show that ApEn and SampEn decrease significantly in the group of students with high
emotional reactivity. The decreases in indexes of HR complexity during the exam session reflects a shift of the sympathovagal balance toward sympathetic predominance [80] and a risk of cardiovascular events [106]. These effects of SA on entropy measures are in line with studies that examined the relationship between HR complexity and high-stress musical performance [107] or arithmetic stress [108].

Physiological investigations have shown that the heart and other physiological networks behave most chaotically when they are young and healthy [57, 59]. The chaotic behavior of healthy physiological networks should not be interpreted as transient perturbations produced by a fluctuating environment, but rather as a necessary component of normal functioning [57]. The output of healthy systems offers a type of complex variability associated with long-range, fractal-like correlations [57]. DFA has been suggested to be the most appropriate method to quantify the fractal properties of a time series of RR intervals. In the present study, the increased values of \( \alpha_1 \) observed during the shift to higher levels of SA revealed a strong positive association between STAI score and short-term correlations in the RR data. Norepinephrine spillover does not induce prominent changes in short-term scaling exponent [81], but tilt is associated with a significant increase in \( \alpha_1 \) [51]. Results on vagal blockade with atropine suggest that the increase in the short-range exponent during the exam session was due to cardiac parasympathetic withdrawal. The correlation between the dynamics of SA level and \( \alpha_1 \) was also related to changes in cardiac vagal activity.

Our results confirm the finding from the study of Valenza et al. [104] in which healthy volunteers were subjected to emotional visual elicitation, along with measurement of nonlinear indexes of HRV during the neutral and arousal sessions. Their research demonstrated that LLE decreased significantly during arousal elicitation. Using the PD2 algorithm of Skinner et al. [88], we showed that statistically significant changes in heartbeat PD2 as SA level increases.

Although the association between traditional measures of HRV and HR is well recognized, we have not found any studies concerning the correlation between HR and nonlinear measures of HRV. We demonstrated that all nonlinear indexes of HRV, except TPVA, are associated with HR. Monfredi et al. [109] proposed a biophysical model that explains this phenomenon. They postulated that “HRV is primarily dependent on HR and cannot be used in any simple way to assess autonomic nerve activity to the heart.” However, the decreases in SD1, SD1/SD2, CCM, SampEn and LLE in response to the stress from academic examination was not altered by correction for HR. By contrast, the association between anxiety and linear HRV measures was greatly attenuated by adjustment for HR. These data strongly suggests that even after adjusting for HR, anxiety induces reductions in the complexity of HRV.

Our findings resemble the Neurovisceral Integration Model [110]. Thayer and Friedman proposed a model that relates anxiety to vagal tone, autonomic flexibility, and adaptability [93, 110]. The model postulates that emotions may be characterized as a reaction to an environmental event that facilitates the rapid mobilization of cognitive, behavioral, and autonomic systems toward action. The efficient interaction between these systems allows for maximal organism flexibility in adapting to a changing environment. According to the neurovisceral integration model, flexibility is an important determinant of adaptation to threatening conditions and anxiety is associated with a systemic inflexibility grounded in poor inhibition [93]. Strong emotions, such fear or phobia, can induce loss of complexity and adaptability [93]. The observed decrease in sample entropy may suggest a shift toward simplification of cardiovascular regulation and reduction of flexibility [106]. Relatively low levels of SD1 and SD2 during the exam session indicate a decrease in HRV associated with an increase in SA; reduced HRV is common in a wide range of maladaptive conditions [93].

The present study is not without limitations. One limitation is that we did not complete the descriptive picture of the nonlinear dynamics of heart rate in anxiety through the use of fuzzy
measure entropy, the Hurst exponent, and multiscale entropy. Another limitation may be related to the small number of participants who had decreased SA levels during the exam session. However, these study limitations are balanced by strong points. The strength of this research is that we examined cardiac autonomic functions in a younger cohort of healthy participants without anxiety and depression disorders by using a battery of comprehensive Poincaré plot methods, including computation of complex correlation measures and Guzik’s index of asymmetry.

Most of the studies that investigated academic stress, tended to neglect interindividual differences in intraindividual changes in SA under exam stress. To our knowledge, only a handful of studies considered individual differences in anxious arousal. Although we do not claim absolute originality, the present study differs from previous studies in several ways. First, in keeping with the literature that suggests the existence of distinct types of anxiety style [88, 111, 112, 113], we included two groups of students divided according their patterns of anxiety arousal.

Second, compared with previous studies on exam stress [43, 114, 115, 116], we assessed the correlation between changes in HRV indexes and changes in SA scores. Finally, whereas previous studies only demonstrated the effects of stress on unadjusted nonlinear measures of HRV [43, 65, 108], the present study also provides analyses of HR-corrected HRV indicators. In sum, our approach thus signifies an important step toward understanding how stress influences nonlinear dynamics of HRV.

In conclusion, this study shows that SA is associated with alterations in the complexity of HRV. Our results also suggest that the decrease in HRV and the increase in short-term fractal exponent differed among subjects, and that the prominent loss in the complexity of heart rate variability is associated with a qualitative change in state anxiety.

Supporting Information

S1 Table. Formulae to adjust nonlinear HRV indexes for mean RR (avRR).
(DOCX)

S2 Table. Heart rate variability and anxiety data.
(XLSX)

Author Contributions

Conceived and designed the experiments: DAD EVS ADD. Performed the experiments: DAD EVS ADD. Analyzed the data: DAD. Contributed reagents/materials/analysis tools: DAD EVS. Wrote the paper: DAD EVS ADD.

References

1. Eysenck MW, Derakshan N, Santos R, Calvo MG. Anxiety and cognitive performance: attentional control theory. Emotion (Washington, DC). 2007; 7(2):336–353. doi:10.1037/1528-3542.7.2.336 PMID: 17516812

2. Power MJ, Dalgleish T. Cognition and emotion: From order to disorder. Hove, England: Psychology Press. 1997.

3. Bishop SJ. Neurocognitive mechanisms of anxiety: an integrative account. Trends in cognitive sciences. 2007; 11(7):307–316. doi:10.1016/j.tics.2007.05.008 PMID: 17553730

4. Paterniti S, Alperovitch A, Ducimetiere P, Dealberto MJ, Lepine JP, Bisserbe JC. Anxiety but not depression is associated with elevated blood pressure in a community group of French elderly. Psychosomatic medicine. 1999; 61(1):77–83. PMID: 10024070

5. Noteboom JT, Barnholt KR, Enoka RM. Activation of the arousal response and impairment of performance increase with anxiety and stressor intensity. Journal of applied physiology. 2001; 91(5):2093–2101. PMID: 11641349
6. Homma I, Masaoka Y. Breathing rhythms and emotions. Experimental physiology. 2008; 93(9):1011–1021. doi: 10.1113/exphysiol.2008.042424 PMID: 18487316

7. Cacioppo JT, Berntson GG, Larsen JT, Poehlmann KM, Ito TA. The psychophysiology of emotion. In: Lewis R, Haviland-Jones JM, editors. The Handbook of Emotion (2-nd ed). New York: Guilford Press; 2000. pp. 173–191.

8. Bolis L, Licinio J, Govoni S. Handbook of the Autonomic Nervous System in Health and Disease. New York: Marcel Dekker; 2002.

9. Berntson GG, Cacioppo JT, Quigley KS, Fabro VT. Autonomic space and psychophysiological response. Psychophysiology. 1994; 31(1):44–61. doi: 10.1111/j.1469-8986.1994.tb01024.x PMID: 8146254

10. Diedrich A, Crossman AA, Beighton LA, Tahvanainen K, Kuusela TA, Ertl AC, et al. Baroreflex physiology studied in healthy subjects with very infrequent muscle sympathetic bursts. Journal of applied physiology. 2013; 114(2):203–210. PMID: 23195626

11. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of HRV to assess the changes in sympathovagal balance during graded orthostatic tilt. Circulation. 1994; 90(4):1826–1831. PMID: 7923668

12. Huang WX, Yu Q, Cohen MI. Fast (3 Hz and 10 Hz) and slow (respiratory) rhythms in cervical sympathetic nerve and unit discharges of the cat. The Journal of physiology. 2000; 523 Pt 2:459–477. doi: 10.1111/j.1469-7793.2000.00459.x PMID: 10699089

13. Hayano J, Yasuma F. Hypothesis: respiratory sinus arrhythmia is an intrinsic resting function of cardiopulmonary system. Cardiovascular research. 2003; 58(1):1–9. doi: 10.1016/S0008-6363(02)00851-9 PMID: 12667941

14. Preiss G, Polosa C. Patterns of sympathetic neuron activity associated with Mayer waves. The American journal of physiology. 1974; 226(3):724–730. PMID: 4817426

15. Perlitz V, Lambertz M, Cotuk B, Grebe R, Vandenhouten R, Flattten G, et al. Cardiovascular rhythms in the 0.15-Hz band: common origin of identical phenomena in man and dog in the reticular formation of the brain stem? Pfügers Archiv. 2004; 448(6):579–591. PMID: 15138824

16. Montano N, Gnecci-Ruscone T, Porta A, Lombardi F, Malliani A, Barman SM. Presence of vasomotor and respiratory rhythms in the discharge of single medullary neurons involved in the regulation of cardiovascular system. Journal of the autonomic nervous system. 1996; 57(1–2):116–122. PMID: 8867094

17. deBoer RW, Karemaker JM, Strackee J. Hemodynamic fluctuations and baroreflex sensitivity in humans: a beat-to-beat model. The American journal of physiology. 1987; 253(3 Pt 2):H680–709. PMID: 3631301

18. Malpas SC. Neural influences on cardiovascular variability: possibilities and pitfalls. American journal of physiology Heart and circulatory physiology. 2002; 282(1):H6–H20. PMID: 11748042

19. Ringwood JV, Malpas SC. Slow oscillations in blood pressure via a nonlinear feedback model. American journal of physiology Regulatory, integrative and comparative physiology. 2001; 280(4):R1105–1115. PMID: 11247833

20. Chen Z, Brown EN, Barbieri R. Characterizing nonlinear heartbeat dynamics within a point process framework. IEEE transactions on bio-medical engineering. 2010; 57(6):1335–1347. doi: 10.1109/ IEMBS.2008.4649779 PMID: 20172783

21. Stauss HM. Heart rate variability. American journal of physiology Regulatory, integrative and comparative physiology. 2003; 285(5):R927–931. doi: 10.1152/ajpregu.00452.2003 PMID: 14557228

22. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. International journal of cardiology. 2010; 141(2):122–131. doi: 10.1016/j.ijcard.2009.09.543 PMID: 19910061

23. Saul JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ. Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. The American journal of physiology. 1990; 258(3 Pt 2):H713–721. PMID: 2316686

24. Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fujinami T, et al. Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. The American journal of cardiology. 1991; 67(2):199–204. doi: 10.1016/0002-9149(91)90445-Q PMID: 1987723

25. Furlan R, Porta A, Costa F, Tank J, Baker L, Schiavi R, et al. Oscillatory patterns in sympathetic neural discharge and cardiovascular variables during orthostatic stimulus. Circulation. 2000; 101(8):886–892. doi: 10.1161/01.CIR.101.8.886 PMID: 10694528

26. Malliani A, Montano N, Pagani M. Physiological background of heart rate variability. Cardiac Electrophysiology Review. 1997; 1(3):343–346.
27. Porta A, Guzzetti S, Furian R, Gneccchi-Rusicone T, Montano N, Malliani A. Complexity and nonlinearity in short-term heart variability: comparison of methods based on local nonlinear prediction. IEEE transactions on bio-medical engineering. 2007; 54(1):94–106. doi: 10.1109/TBME.2006.883789 PMID: 17260860
28. Elbert T, Ray WJ, Kowalk JZ, Skinner JE, Graf KE., Birbaumer N. Chaos and physiology: deterministic chaos in excitable cell assemblies. Physiological Reviews. 1994; 74(1):41–48. PMID: 8295931
29. Beckers F, Verheyden B, Aubert AE. Aging and nonlinear heart rate control in a healthy population. American journal of physiology Heart and circulatory physiology. 2006; 290(6):H2560–2570. doi: 10.1152/ajpheart.00903.2005 PMID: 16373585
30. Eke A, Herman P, Kocsis L, Kozak LR. Fractal characterization of complexity in temporal physiological signals. Physiological measurement. 2002; 23(1):R1–38. doi: 10.1088/0967-3334/23/1/201 PMID: 11876246
31. Brennan M, Palaniswami M, Kamen P. Do existing measures of Poincare plot geometry reflect nonlinearity of heart rate variability? IEEE transactions on bio-medical engineering. 2001; 48(11):1342–1347. doi: 10.1109/10.959330 PMID: 11686633
32. Tulppo MP, Makikallio TH, Seppanen T, Airaksinen JK, Huikuri HV. Heart rate dynamics during accentuated sympathovagal interaction. The American journal of physiology. 1998; 274(3 Pt 2):H810–816. PMID: 9530192
33. Manzano BM, Vanderlei LC, Ramos EM, Ramos D. Acute effects of smoking on autonomic modulation: analysis by Poincare plot. Arquivos brasileiros de cardiologia. 2011; 96(2):154–160. doi: 10.1590/S0066-782X2011005000013 PMID: 21271176
34. Huikuri HV, Stein PK. Heart rate variability in risk stratification of cardiac patients. Progress in cardiovascular diseases. 2013; 56(2):153–159. doi: 10.1016/j.pcad.2013.07.003 PMID: 24215747
35. Kemp AH, Quintana DS, Felmingham KL, Matthews S, Jelink HF. Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. PloS one. 2012; 7(2):e30777. PMID: 22355326
36. Alvares GA, Quintana DS, Kemp AH, Van Zwieten A, Balleine BW, Hickie IB, et al. Reduced heart rate variability in social anxiety disorder: associations with gender and symptom severity. PloS one. 2013; 8(7):e70468. doi: 10.1371/journal.pone.0070468 PMID: 23936207
37. Karmakar CK, Khandoker AH, Gubbi J, Palaniswami M. Complex correlation measure: a novel descriptor for Poincare plot. Biomedical engineering online. 2009; 8:17. doi: 10.1186/1475-925X-8-17 PMID: 19674482
38. Weiss G. Time-reversibility of linear stochastic processes. J Appl Probab. 1975; 12:831–836.
39. Porta A, Casali KR, Casali AG, Gneccchi-Rusicone T, Tobaldini E, Montano N, et al. Temporal asymmetries of short-term heart period variability are linked to autonomic regulation. American journal of physiology Regulatory, integrative and comparative physiology. 2008; 295(2):R550–557. doi: 10.1152/ajpregu.00129.2008 PMID: 18384029
40. Costa M, Goldberger AL, Peng CK. Broken asymmetry of the human heartbeat: loss of time irreversibility in aging and disease. Physical review letters. 2005; 95(19):198102. doi: 10.1103/PhysRevLett.95.198102 PMID: 16373585
41. Piskorski J, Guzik P. Geometry of the Poincare plot of RR intervals and its asymmetry in healthy adults. Physiological measurement. 2007; 28(3):287–300. doi: 10.1088/0967-3334/28/3/005 PMID: 17232593
42. Chladkova L, Turianikova Z, Tonhajzerova I, Calkovska A, Javorka M (2011) Time irreversibility of heart rate oscillations during orthostasis. Acta Medica Martiniana 11: 41
43. Melillo P, Bracale M, Pecchia L. Nonlinear heart rate variability features for real-life stress detection. Case study: students under stress due to university examination. Biomedical engineering online. 2011; 10:96. doi: 10.1186/1475-925X-10-96 PMID: 22059697
44. Mukherjee S, Yadav R, Yung I, Zajdel DP, Oken BS. Sensitivity to mental effort and test—aesthetics of heart rate variability measures in healthy seniors. Clinical Neurophysiology. 2011; 122(10):2059–2066. PMID: 21456665
45. Fishman M, Jacono FJ, Park S, Jamasebi R, Thungtong A, Loparo KA, et al. A method for analyzing temporal patterns of variability of a time series from Poincare plots. Journal of Applied Physiology. 2012; 113(2):297–306. doi: 10.1152/japplphysiol.01377.2010 PMID: 22556398
46. Pincus SM, Goldberger AL. Physiological time-series analysis: what does regularity quantify? The American journal of physiology. 1994; 266(4 Pt 2):H1643–1656. PMID: 8184944
47. Pincus SM, Viscarello RR. Approximate entropy: a regularity measure for fetal heart rate analysis. Obstetrics and gynecology. 1992; 79(2):249–255. PMID: 1731294
48. Pincus SM. Approximate entropy as a measure of system complexity. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88(6):2297–2301. PMID: 11607165

49. Wu GQ, Arzeno NM, Shen LL, Tang DK, Zheng DA, Zhao NQ, et al. Chaotic signatures of heart rate variability and its power spectrum in health, aging and heart failure. PloS one. 2009; 4(2):e4323. doi: 10.1371/journal.pone.0004323

50. Bár KJ, Boettger MK, Koschke M, Schulz S, Chokka P, Yeragani VK, et al. Non-linear complexity measures of heart rate variability in acute schizophrenia. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2007; 118(9):2009–2015. doi: 10.1016/j.clinph.2007.06.012 PMID: 17646130

51. Tulppo MP, Hughson RL, Makikallio TH, Seppanen T, Huikuri HV. Effects of exercise and passive head-up tilt on fractal and complexity properties of heart rate dynamics. American journal of physiology Heart and circulatory physiology. 2001; 280(3):H1081–H1087. PMID: 11179050

52. Perkiomaki JS, Zareba W, Badilini F, Moss AJ. Influence of atropine on fractal and complexity measures of heart rate variability. Annals of noninvasive electrocardiology. 2002; 7(4):326–331. doi: 10.1111/j.1542-474X.2002.tb00181.x PMID: 12431310

53. Richman JS, Moorman JR. Physiological time-series analysis using approximate entropy and sample entropy. American journal of physiology Heart and circulatory physiology. 2000; 278(6):H2039–H2049. PMID: 10843903

54. Lake DE, Richman JS, Griffin MP, Moorman JR. Sample entropy analysis of neonatal heart rate variability. American journal of physiology Regulatory, integrative and comparative physiology. 2002; 283(3):R789–R797. doi: 10.1152/ajpregu.00068.2002 PMID: 12185014

55. Mateo M, Blasco-Lafarga C, Martinez-Navarro I, Guzman JF, Zabala M. Heart rate variability and pre-competitive anxiety in BMX discipline. European journal of applied physiology. 2012; 112(1):113–123. doi: 10.1007/s00421-011-1962-8 PMID: 21503698

56. Goldberger AL, Peng CK, Lipsitz LA. What is physiologic complexity and how does it change with aging and disease? Neurobiology of aging. 2002; 23(1):23–26. doi: 10.1016/S0197-4580(01)00266-4 PMID: 11755014

57. West BJ. Fractal physiology and the fractional calculus: a perspective. Frontiers in physiology. 2010; 1:12. doi: 10.3389/fphys.2010.00012 PMID: 21423355

58. Peng CK, Havlin S, Stanley HE, Goldberger AL. Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos: An Interdisciplinary Journal of Nonlinear Science. 1995; 5(1):82–87. doi: 10.1063/1.166141 PMID: 11538314

59. Goldberger AL, Amaral LA, Hausdorff JM, Ivanov P, Peng CK, Stanley HE. Fractal dynamics in physiology: alterations with disease and aging. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99 Suppl 1:2466–2472. doi: 10.1073/pnas.012579499 PMID: 11875196

60. Yeh R-G, Chen G-Y, Shieh J-S, Kuo C-D. Parameter investigation of detrended fluctuation analysis for short-term human heart rate variability. Journal of Medical and Biological Engineering. 2010; 30(5):277–282.

61. van Straaten EC, Stam CJ. Structure out of chaos: functional brain network analysis with EEG, MEG, and functional MRI. Eur Neuropsychopharmacol. 2013 Jan; 23(1):7–18. doi: 10.1016/j.euroneuro.2012.10.010 PMID: 23158686

62. Eck VG, Feinberg J, Langtangen HP, Hellevik LR. Stochastic sensitivity analysis for timing and amplitude of pressure waves in the arterial system. Int J Numer Method Biomed Eng. 2015 Feb 16. doi: 10.1002/cnm.2711 PMID: 25684213

63. Acharya R, Kumar A, Bhat PS, Lim CM, Iyengar SS, Kannathal N, Krishnan SM. Classification of cardiac abnormalities using heart rate signals. Med Biol Eng Comput. 2004 May; 42(5):288–293. PMID: 15191072

64. Hagerman I, Berglund M, Lorin M, Nowak J, Sylvén C. Chaos-related deterministic regulation of heart rate variability in time- and frequency domains: effects of autonomic blockade and exercise. Cardiovasc Res. 1996 Mar; 31(3):410–418. doi: 10.1016/S0008-6363(95)00084-4 PMID: 8681328

65. Schubert C, Lambertz M, Nelesen RA, Bardwell W, Choi JB, Dimsdale JE. Effects of stress on heart rate complexity—a comparison between short-term and chronic stress. Biol Psychol 2009, 80:325–332.

66. Grassberger P, Procaccia I. Measuring the strangeness of strange attractors. Physica. 1983; D9:189–208. doi: 10.1007/978-0-387-21830-4_12

67. Nahshoni E, Aravot D, Aizenberg D, Sigler M, Zalsman G, Strasberg B, et al. Heart rate variability in patients with major depression. Psychosomatics. 2004; 45(2):129–134. doi: 10.1176/appi.psy.45.2.129 PMID: 15016926
68. Beckers F, Verheyden B, Ramaekers D, Swynghedauw B, Aubert AE. Effects of autonomic blockade on non-linear cardiovascular variability indices in rats. Clin Exp Pharmacol Physiol. 2006 May-Jun; 33(5–6):431–439. doi: 10.1111/j.1440-1681.2006.04384.x PMID: 16700875.

69. Osaka M, Saitoh H, Atarashi H, Hayakawa H. Correlation dimension of heart rate variability: a new index of human autonomic function. Front Med Biol Eng. 1993; 5(4):289–300. PMID: 8136314.

70. Skinner N, Brewer N. The dynamics of threat and challenge appraisals prior to stressful achievement events. Journal of personality and social psychology. 2002; 83(3): 678–692. doi: 10.1037//0022-3514.83.3.678

71. Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fujinami T, Yokoyama K, Watanabe Y, Takata K. Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects. Am J Cardiol. 1991 Jan 15; 67(2):199–204. PMID: 1987723.

72. Sacha J, Barabach S, Statkiewicz-Barabach G, Sacha K, Müller A, Piskorski J, Barthel P, Schmidt G. How to strengthen or weaken the HRV dependence on heart rate—description of the method and its perspectives. Int J Cardiol. 2013; 68(2):1660–1663. doi: 10.1016/j.ijcard.2013.03.038 PMID: 23578892

73. Huikuri HV, Valkama JO, Airaksinen KE, Seppanen T, Kessleri KM, Takkenen JT, et al. Frequency domain measures of heart rate variability before the onset of nonsustained and sustained ventricular tachycardia in patients with coronary artery disease. Circulation. 1993; 87(4):1220–1228. doi: 10.1161/01.CIR.87.4.1220 PMID: 8462148

74. Aubert AE, Ramaekers D, Beckers F, Breem R, Denef C, Van de Werf F, Ector H. The analysis of heart rate variability in untrained rats. Validation of method and results. Comput Methods Programs Biomed. 1999; 60(3):197–213. doi: 10.1016/S0169-2607(99)00017-6 PMID: 10579513

75. de Chazal P, Heneghan C, Sheridan E, Reilly R, Nolan P, O’Malley M. Analysis of scaling behaviour of ECG signals during atrial fibrillation. Computers in Cardiology. 2005; 32:627

76. Hanin YL. Quick Guide to the application of the scale of reactive and personal anxiety BH Spielberger. L.: LLNIK; 1976.

77. Ilyin EP. Emotions and feelings. StPb.: The Publishing House "Peter"; 2001.

78. Karmakar C, Khandoker A, Palaniswami M. Heart rate asymmetry in altered parasympathetic nervous system activity. Computers in Cardiology. 2010; 37: 601–606.

79. Ho KK, Moody GB, Peng CK, Mietus JE, Larson MG, Levy D, et al. Predicting survival in heart failure case and control subjects by use of fully automated methods for deriving nonlinear and conventional indices of heart rate dynamics. Circulation. 1997; 96(3):842–848. doi: 10.1161/01.CIR.96.3.842 PMID: 9264491

80. Porta A, Gnecci-Ruscone T, Tobaldini E, Guzzetti S, Furlan R, Montano N. Progressive decrease of heart period variability entropy-based complexity during graded head-up tilt. Journal of applied physiology. 2007; 103(4):1143–1149. doi: 10.1152/japplphysiol.00293.2007 PMID: 17569773

81. Baumert M, Lambert GW, Dawood T, Lambert EA, Esler MD, McGrane M, et al. Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder. American journal of physiology-Heart and circulatory physiology. 2009; 297(2):H674–679. doi: 10.1152/ajpheart.00236.2009 PMID: 19502559

82. Millar PJ, Rakobowchuk M, Adams MM, Hicks AL, McCartney N, MacDonald MJ. Effects of short-term training on heart rate dynamics in individuals with spinal cord injury. Autonomic neuroscience: basic & clinical. 2009; 150(1):116–121. doi: 10.1016/j.autneu.2009.03.012 PMID: 19406691

83. Mainardi LT, Sassi R. Analysis of scaling behaviour of ECG sig-nal during atrial fibrillation. Computers in Cardiology. 2005; 32:627–630

84. Tan CO, Cohen MA, Eckberg DL, Taylor JA. Fractal properties of human heart period variability: physiological and methodological implications. The Journal of physiology. 2009; 587(Pt 15):3929–3941. doi: 10.1113/jphysiol.2009.169219 PMID: 19528254

85. Rosenstein MT, Collins JJ, De Luca CJ. A practical method for calculating largest Lyapunov exponents from small data sets. Physica D: Nonlinear Phenomena. 1993; 65(1):117–134. doi: 10.1016/0167-2789(93)90009-P

86. Hegger R, Kantz H, Schreiber T. Practical implementation of nonlinear time series methods: The TISEAN package. Chaos: An Interdisciplinary Journal of Nonlinear Science. 1999; 9(2):413–435. doi: 10.1063/1.166424

87. Parlitz U. Nonlinear time-series analysis. Nonlinear Modeling: Springer; 1998. p. 209–239.

88. Skinner JE, Nester BA, Dalsey WC. Nonlinear dynamics of heart rate variability during experimental hemorrhage in ketamine-anesthetized rats. American Journal of Physiology-Heart and Circulatory Physiology. 2000; 279(4):H1669–H1678. PMID: 11009454
89. Tulppo MP, Makikallio TH, Takaia TE, Seppanen T, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. The American journal of physiology. 1996; 271(1 Pt 2):H244–252. PMID: 8760181

90. Toichi M, Sugiuira T, Murai T, Sengoku A. A new method of assessing cardiac autonomic function and its comparison with spectral analysis and coefficient of variation of R-R interval. Journal of the autonomic nervous system. 1997; 62(1–2):79–84. PMID: 9021653

91. Woo MA, Stevenson WG, Moser DK, Middlekauff HR. Complex heart rate variability and serum nor-epinephrine levels in patients with advanced heart failure. Journal of the American College of Cardiology. 1994; 23(3):565–569. doi: 10.1016/0735-1097(94)90737-4 PMID: 8113535

92. Carrasco S, Gaitan MJ, Gonzalez R, Yanez O. Correlation among Poincare plot indexes and time and frequency domain measures of heart rate variability. Journal of medical engineering & technology. 2001; 25(6):240–248. doi: 10.1080/030919001100866651 PMID: 11780765

93. Friedman BH. An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. Biological psychology. 2007; 74(2):185–199. PMID: 17069959

94. Karmakar CK, Khandoker AH, Voss A, Palaniswami M. Sensitivity of temporal heart rate variability in Poincare plot to changes in parasympathetic nervous system activity. Biomedical engineering online. 2011; 10:17. doi: 10.1186/1475-925X-10-17 PMID: 21366929

95. Kamen PW, Krum H, Tonkin AM. Poincare plot of heart rate variability allows quantitative display of parasympathetic nervous activity in humans. Clinical science. 1996; 91(2):201–208. PMID: 8795444

96. Brennan M, Palaniswami M, Kamen P. Poincare plot interpretation using a physiological model of HRV based on a network of oscillators. American journal of physiology Heart and circulatory physiology. 2002; 283(5):H1873–1886. doi: 10.1152/ajpheart.00405.2000 PMID: 12384465

97. Mourtot L, Bouhaddi M, Perrey S, Cappelle S, Henriet MT, Wolf JP, et al. Decrease in heart rate variability with overtraining: assessment by the Poincare plot analysis. Clinical physiology and functional imaging. 2004; 24(1):10–8. doi: 10.1046/j.1475-0961.2003.00523.x PMID: 14717743

98. De Vito G, Galloway SD, Nimmo MA, Maas P, McMurray JJ. Effects of central sympathetic inhibition on heart rate variability during steady-state exercise in healthy humans. Clinical physiology and functional imaging. 2002; 22(1):32–38. doi: 10.1046/j.1475-097X.2002.00395.x PMID: 12003097

99. Guzik P, Piskorski J, Krauze T, Schneider R, Wesseling KH, Wykretowicz A, et al. Correlations between the Poincare plot and conventional heart rate variability parameters assessed during paced breathing. The journal of physiological sciences: JPS. 2007; 57(1):63–71. doi: 10.2170/physiolsci.RP005506 PMID: 17266795

100. Tonhajzerova I, Ondrejka I, Chladekova L, Farsky I, Visonovcova Z, Caiokvskas A, et al. Heart rate time irreversibility is impaired in adolescent major depression. Progress in neuro-psychopharmacology & biological psychiatry. 2012; 39(1):212–217. doi: 10.1016/j.pnpbp.2012.06.023 PMID: 22771778

101. Lovibond PF, Lovibond SH. The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. Behaviour research and therapy. 1995; 33(3):335–343. PMID: 7726811

102. Jelinek HF, Md Imam H, Al-Aubaidy H, Khandoker AH. Association of cardiovascular risk using non-linear heart rate variability measures with the Framingham risk score in a rural population. Front Physiol. 2013 4:186. doi: 10.3389/fphys.2013.00186 PMID: 23898302

103. Jelinek HF, Khandoker AH, Quintana D, Imam MH, Kemp A. Complex correlation measure as a sensitive indicator of risk for sudden cardiac death in patients with depression. Computing in Cardiology. 2011; 38:809–812.

104. Valenza G, Allegrini P, Lanata A, Scilipio EP. Dominant Lyapunov exponent and approximate entropy in heart rate variability during emotional visual elicitation. Frontiers in neuroscience. 2012; 5:3. doi: 10.3389/fneng.2012.00003 pmcid: PMC3289832 PMID: 22393320

105. Anischenko VS, Igosheva NB, Pavlov AN, Khovanov A, Yakusheva TA. Comparative analysis of methods for classifying the cardiovascular system’s states under stress. Critical reviews in biomedical engineering. 2001; 29(3):462

106. Tulppo MP, Makikallio TH, Takaia TE, Seppanen T, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. The American journal of physiology. 1996; 271(1 Pt 2):H244–252. PMID: 8760181

107. Toichi M, Sugiuira T, Murai T, Sengoku A. A new method of assessing cardiac autonomic function and its comparison with spectral analysis and coefficient of variation of R-R interval. Journal of the autonomic nervous system. 1997; 62(1–2):79–84. PMID: 9021653

108. Vuksanovic V, Gal V. Heart rate variability in mental stress aloud. Medical engineering & physics. 2007; 29(3):344–349. doi: 10.1016/j.medengphy.2006.05.011 PMID: 16807051
109. Monfredi O, Lyashkov AE, Johnsen A-B, Inada S, Schneider H, Wang R, et al. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. Hypertension. 2014; 64(6):1334–1343. doi: 10.1161/hypertensionaha.114.03782 PMID: 25225208

110. Thayer JF, Lane RD. A model of neurovisceral integration in emotion regulation and dysregulation. Journal of affective disorders. 2000; 61(3):201–216. doi: 10.1016/S0165-0327(00)00338-4 PMID: 11163422

111. Raffety BD, Smith RE, Ptacek JT. Facilitating and debilitating trait anxiety, situational anxiety, and coping with an anticipated stressor: a process analysis. Journal of personality and social psychology. 1997; 72(4):892–906. PMID: 9108702

112. Karademas EC, Kalantzi-Azizi A. The stress process, self-efficacy expectations, and psychological health. Personality and individual differences. 2004; 37(5):1033–43. doi: 10.1016/j.paid.2003.11.012

113. Cassady JC, Finch WH. Using factor mixture modeling to identify dimensions of cognitive test anxiety. Learning and Individual Differences. 2015; 41:14–20. doi: 10.1016/j.lindif.2015.06.002

114. Lucini D, Norbiato G, Clerici M, Pagani M. Hemodynamic and autonomic adjustments to real life stress conditions in humans. Hypertension. 2002; 39(1):184–8. PMID: 11799100

115. Zhang Z, Su H, Peng Q, Yang Q, Cheng X. Exam anxiety induces significant blood pressure and heart rate increase in college students. Clinical and experimental hypertension. 2011; 33(5):281–6. doi: 10.3109/10641963.2010.531850 PMID: 21787237

116. Wegner M, Muller-Alcazar A, Jager A, Machado S, Arias-Carrión O, Budde H. Psychosocial stress but not exercise increases cortisol and reduces state anxiety levels in school classes-Results from a stressor applicable in large group settings. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders). 2014; 13(6):1015–20. PMID: 24923345