

Complexity of electrodermal activity to mental stress is changed during adolescent age-period

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Abstract. Complexity characterizes behaviour of all physiological systems whose components interact in multiple ways usually quantified by entropy techniques. However, complexity analysis regarding electrodermal activity (EDA)-related sympathetic cholinergic nervous system is rare. Thus, we aimed to study EDA dynamics complexity changes from aspect of various embedding dimensions (m) and timescales (τ) (sample entropy (SampEn) with $m \in \langle 2, 7 \rangle$, and multiscale entropy (MSE) in $\tau \in \langle 1, 20 \rangle$) in association with traditionally used EDA indices (skin conductance level (SCL) and non-specific skin conductance responses (NS.SCRs)) to mental stress (mental arithmetic test – MAT) in healthy participants at critical adolescent age. The cohort (total group) consisted of 60 adolescents (17.5 ± 0.5 yrs) divided into three groups: Group-1: early (13.1 ± 0.3 yrs), Group-2: middle (16.6 ± 0.2 yrs) and Group-3: late (22.9 ± 0.1 yrs) adolescence. SampEn ($m > 2$) and MSE (for all τ) were significantly higher during MAT than baseline in total group and Group-2 ($p < 0.05$). Index MSE for all τ was significantly higher during MAT than baseline in total group, and Group-2; for $\tau \in \langle 2, 13 \rangle$ in Group-1 ($p < 0.05$). Additionally, while SCL was significantly higher during MAT than baseline in all groups, NS.SCRs was lower during stress only in Group-3 ($p < 0.05$). In conclusion, this study revealed distinct EDA complexity characteristics in individual examined groups indicating importance of complexity evaluation in stress-related sympathetic regulatory mechanisms within individual adolescent age ranges.

Key words: Electrodermal activity — Multiscale and sample entropy — Adolescent age — Sympathetic nervous system — Mental arithmetic test

Introduction

Complexity represents one of the intrinsic features characterizing all physiological systems, which can be visible and evaluable in the temporal course of the organism's variables (Faes et al. 2017). The complexity, randomness, and uncertainty of biological processes are usually quantified by entropy techniques characterizing the frequency of information creation, particularly sample entropy (SampEn),

and multiscale entropy (MSE), in various biological time series datasets including EEG (Jia et al. 2017; Kosciessa et al. 2020), R-R intervals (Byun et al. 2019; Richman and Moorman 2000; Costa et al. 2002, 2005), electromyogram (EMG) (Zhang and Zhou 2012; Tang et al. 2018), or electrodermal activity (EDA) (Hossain et al. 2022; Nardelli et al. 2022; Soni and Rawal 2022). While EEG, and ECG complexity represents well-established research tool in the evaluation of individual patterns under physiological as well as pathological conditions (Sabeti et al. 2009; Shaffer and Ginsberg 2017), complexity analysis from other biosignals including EDA is relatively novel. Additionally, while ECG biosignal is influenced by both autonomic nervous system (ANS) branches (i.e. parasympathetic and sympathetic nervous system) (Silva et al. 2017) and therefore complexity

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analysis can reflect mixed effect of both systems, the EDA is controlled solely by the sympathetic nervous system and therefore can provide detailed information just on the sympathetic regulatory network (Boucsein 2012). As the process of the sympathetically mediated sweat glands stimulation is not simply static, but relatively dynamic and nonlinear (Amin and Faghih 2022), the non-linear parameters could bring overall important information about complex sympathetic regulatory network (Dawson et al. 2007; Fowles 2007; Boucsein 2012; Boucsein et al. 2012). From this perspective, non-linear EDA analyses including entropy, deterministic chaos, recurrence plot, correlation dimension, detrended fluctuation analysis or the Lyapunov exponent have been adopted to evaluate sympathetic functioning in response to different stress stimuli (Eckmann and Ruelle 1992; Lippa et al. 2007; Lanatà et al. 2012; Bolea et al. 2014; Byun et al. 2019). For example, nonlinear EDA measures have been shown to be more sensitive to detect sympathetic nervous system (SNS) changes in response to affective visual stimuli (i.e. exposure to the sets of images from the International Affective Picture System; Lanatà et al. 2012) or mental stress (i.e. Stroop colour-word test; Visnovcova et al. 2016) in healthy adults. However, to the best of our knowledge there is no study so far regarding evaluation of EDA complexity in response to stress at adolescent age. Therefore, we aimed to study changes in the complexity of EDA dynamics from the aspect of various embedding dimensions and timescales (i.e. SampEn with embedding dimensions from 2 to 7, and MSE in timescales from 1 to 20) also in association with traditionally used EDA time domain tonic (skin conductance level (SCL)) and phasic (non-specific skin conductance responses (NS.SCRs)) indices in response to mental stress (i.e. mental arithmetic test (MAT)) in healthy adolescents.

In addition, adolescence represents a sensitive developmental period with a large amount of changes, the evaluation of complex features (at rest as well as in response to stress) in the sympathetic regulatory pathway in healthy adolescents can bring important knowledge for the early assessment of discrete abnormalities in SNS regulation. Moreover, regarding stress perception, the average adolescents' stress seems to decrease during late adolescence, but to not change during

early adolescence (Seiffge-Krenke et al. 2009). In light of these considerations, we further aimed to investigate potential differences in stress-induced sympathetic functioning using above-mentioned indices in healthy adolescents divided into three groups of different age categories, specifically, early adolescence corresponding to the age range from 11 to 14 years, middle adolescence corresponding to the age range from 15 to 17 years, and late adolescence corresponding to the age range from 18 to 24 years (Jaworska and MacQueen 2015). Thus, we hypothesized higher EDA complexity during cognitive stress compared to baseline conditions, and different EDA complexity patterns in individual adolescent age subgroups in response to stress probably due to more easily stress perception through more lived experiences and more matured coping mechanisms in older adolescents.

Materials and Methods

Participants

The studied cohort (total group) consisted of 60 adolescent healthy volunteers (41 females/19 males; aged 17.5 ± 0.5 yrs, $BMI = 22.0 \pm 0.4$ kg/m²), which were consequently divided into three groups according to age: Group-1: 20 volunteers in early adolescence (16 females/4 males; average age 13.1 ± 0.3 yrs, $BMI = 20.8 \pm 0.7$ kg/m²), Group-2: 20 volunteers in middle adolescence (12 females/8 males; average age 16.6 ± 0.2 yrs, $BMI = 22.6 \pm 0.7$ kg/m²), and Group-3: 20 volunteers in late adolescence (13 females/7 males; average age 22.9 ± 0.1 yrs, $BMI = 22.7 \pm 0.7$ kg/m²) (Table 1). The inclusion criteria were following: age from 12 to 24 years, normotension, right-handedness, minimum 8 hours of sleeping before the examination, cognitive regularity (without dyslexia, dyscalculia, etc.), avoidance of physical exercise at least 24 hours before the examination, females were included in proliferative phase (i.e. between 6th and 11th day of menstrual cycle). The exclusion criteria were following: acute or chronic disease, weight abnormalities (overweight, obesity, underweight), any skin disorders or problems, abuse of drugs, alcohol,

Table 1. Subject characteristics

	Total group	Group-1	Group-2	Group-3
N, Gender (F/M)	60, (41/19)	20, (16/4)	20, (12/8)	20, (13/7)
Age (years)	17.5 ± 0.5	13.1 ± 0.3	16.6 ± 0.2	22.9 ± 0.1
BMI (kg/m ²)	22.0 ± 0.4	20.8 ± 0.7	22.6 ± 0.7	22.7 ± 0.7
WHR	0.82 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	0.81 ± 0.01
PT (°C)	30.2 ± 0.4	30.5 ± 0.5	29.6 ± 0.7	30.6 ± 0.9

Total group, all examined adolescent volunteers; Group-1, early adolescent volunteers; Group-2, middle adolescent volunteers; Group-3, late adolescent volunteers; N, total number of volunteers; F, female; M, male; BMI, body mass index; WHR, waist to hip ratio; PT, peripheral temperature.

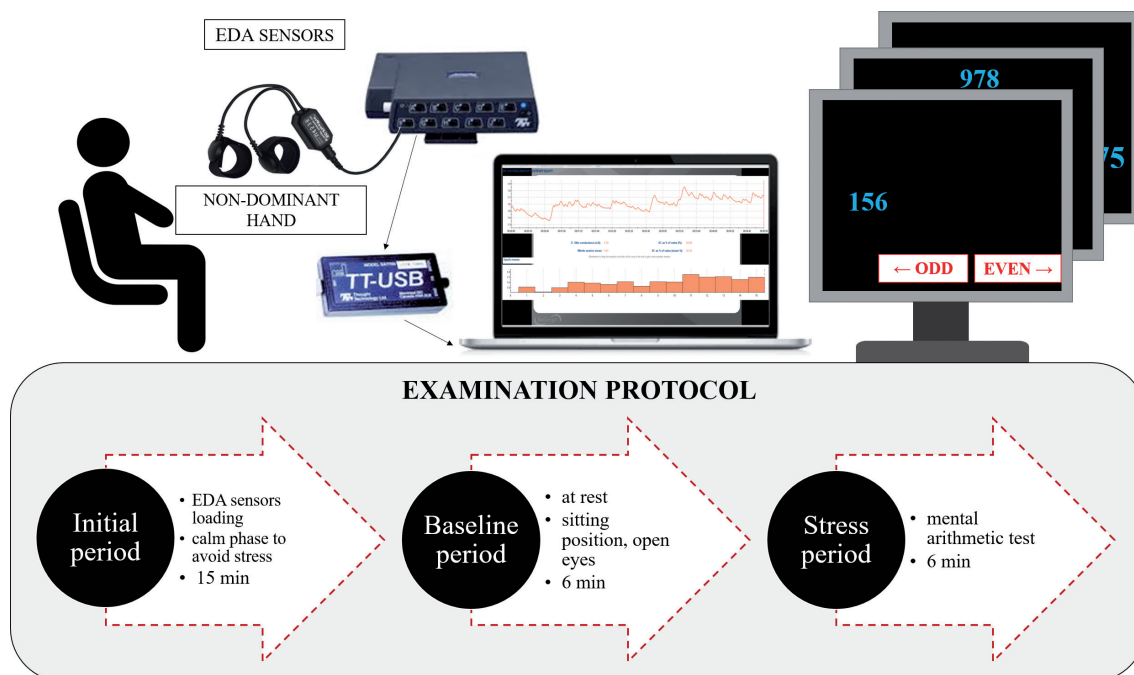


Figure 1. Examination protocol. The recording device, TT-USB, and electrodermal activity (EDA) sensors in picture are used from Thought Technology (thoughttechnology.com).

and caffeine, smoking, and medication or dietary supplementation potentially affecting the autonomic nervous system. Anthropometric parameters required to exclude the potential effect of weight abnormalities were examined by device InBody J120 (Biospace, Korea) with technology of DSM-BIA (direct segmental multi-frequency bioimpedance analysis). This method enables the body composition analysis in 5 segments (trunk, lower and upper extremities) together with the evaluation of fat distribution (Ling et al. 2011). Moreover, the blood pressure measurement to exclude a potential hypertension was performed according to standard recommendations before the examination (Frese et al. 2011).

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava in accordance with the 1964 Helsinki declaration and its later amendments (protocol code EK15/2019). All volunteers and their parents/legal representatives (for younger volunteers than 18 years) were thoroughly instructed about the study protocol and signed an informed consent to participate in the study prior to the examination.

Protocol

The volunteers were examined under standard conditions, i.e. room temperature of 22°C, humidity 45–55%, and illumination of 200 lux, between 8:00 and 10:30 a.m. after

light breakfast. After anthropometric measurement, for obesity/underweight exclusion, the volunteers were instructed to sit comfortable in a special armchair, where the examination was performed. Before the examination, the volunteers were in the resting sitting position for 15 min to avoid potential effects of stress. After resting period, a continuous recording of EDA was performed in following order: baseline period (lasted 6 min) and mental arithmetic test (MAT) period (lasted 6 min) using FlexComp Infinity Biofeedback (Thought Technology, Canada) with a sampling rate of 256 Hz (Fig. 1). The EDA was monitored by two dry Ag-AgCl bipolar electrodes localized in second phalanges of second and forth fingers on non-dominant hand according to recommendation of EDA biosignal measurements (Fowles 2007).

Mental arithmetic test (MAT)

MAT represents standard moderate intensity stressor used in psychophysiology for detection the variables in ANS regulation (especially in sympathetic branch of ANS) (Schneider et al. 2003). The basic principle was in counting of the three-digit numbers random displayed on the PC screen into one-digit number (e.g. the number 156 was displayed on screen. The participant calculated the sum of digits: $156 \rightarrow 1 + 5 + 6 = 12$ (i.e. 2-digits), so continued in calculation to 1-digit result $\rightarrow 1 + 2 = 3$). Then, the

participant's task was to decide if the one-digit results were even or odd by pushing the arrows with dominant hand on the computer keyboard (odd-left, even-right) (Fig. 1). Moreover, the metronome was used as a distracting sound.

Data analysis

First, EDA recordings were carefully checked and artifact-free 5-min recordings were used in the following analysis. The recording (technical) artifacts considered as high-frequency noise were removed by low-pass filter. A discrete Haar wavelet transform was used for physiological artifacts such as motion or recording value ≤ 0 (Swangnetr and Kaber 2013; Taylor et al. 2015). Consequently, the complexity features of EDA were evaluated by following nonlinear indices: sample entropy (SampEn) with embedding dimension m ranged from 2 to 7, and multiscale entropy (MSE) for timescales from 1 to 20.

The SampEn, representing a negative logarithm of conditional probability of a data vector sequences, is mathematically calculated as follows: First, a data vector (length N points (300 for this study)) $X_N = \{x_1, x_2, x_3, \dots, x_N\}$ is transformed into sequences of m consecutive points (ranged from 2 to 7, respectively) $X_m(i) = \{x_i, x_{i+1}, \dots, x_{i+m-1}\}$ and $X_m(j) = \{x_j, x_{j+1}, \dots, x_{j+m-1}\}$ ($i, j \in [1, N - m]$, $i \neq j$) are selected to evaluation the maximum distance and compared with given tolerance r ($0.2 \times SD$, SD represents the standard deviation of X_N) for repeated sequences calculating, according to Equation (1) (Richman and Moorman 2000):

$$\begin{aligned} [X_m(i), X_m(j)] &= \max [|x_{i+k}, x_{j+k}|] \leq \\ &\leq r (k \in [0, m - 1], r \geq 0) \end{aligned} \quad (1)$$

Then, $C^m(r)$ as the average amount of $C_i^m(r)$ for $i \in [1, N - m]$, and $C^{m+1}(r)$ as the average amount of $m+1$ consecutive points were counted, for consequence SampEn estimation according to Equation (2):

$$\begin{aligned} \text{SampEn}(N, m, r) &= -\ln \left[\frac{C^{m+1}(r)}{C^m(r)} \right] = \\ &-\ln \left[\frac{(N-m-1)^{-1} \sum_{i=1}^{N-m-1} C_i^{m+1}(r)}{(N-m)^{-1} \sum_{i=1}^{N-m} C_i^m(r)} \right] \end{aligned} \quad (2)$$

Additionally, the higher values of SampEn represents a low probability of repeated sequences, thus more irregularity, uncertainty, and complexity of system and *vice versa* lower values of SampEn indicates increased regularity, and predictability in the data (Wei et al. 2012; Hansen et al. 2017).

The MSE, representing a distribution of the complexity on multi-temporal scales, is computed as follows: First, before

the analysis the original time series are a coarse-graining by averaging the data points within non-overlapping windows with cumulative length (τ) to generate a new sequence from the data according to Costa et al. (2002, 2005). The Equation (3) represents the coarse-grained time sequences $y_i^{(\tau)}$ based on the scale factor τ :

$$y_i^{(\tau)} = \frac{1}{\tau} \sum_{j=(i-1)\tau+1}^{i\tau} x_j \quad (3)$$

Then, the MSE is computed according to Equation (4) as follows:

$$\text{MSE}(N, m, \tau, r) = -\ln \left[\frac{A^{m+1}(r)}{A^m(r)} \right] \quad (4)$$

where N is number of points into time series (300 for this study), m is the length of repeated mode in the data vector ($m = 2$), τ signifies scale factor ($\tau \in [1, 20]$), tolerance r ($0.2 \times SD$) characterizes the limitation condition of repeated mode, and both $A^m(r)$, and $A^{m+1}(r)$ represents the averages repeated amount of two sequences to calculate $C_i^m(r)$. Similarly to SampEn, higher value of MSE represents more complex, unpredictable, healthier system (Costa et al. 2005).

In addition, for SCL and NS.SCRs indices was used the tonic component of signal extracted by the 10th order low-pass finite impulse response filter (Posada-Quintero et al. 2016). Index SCL (in micro Siemens (μS)) indicating sympathetic sudomotor activity was evaluated as the average amplitude of the tonic EDA from 5 min-long artifact-free recordings (Venables et al. 1980; Fowles 2007; Benedek and Kaernbach 2010; Boucsein 2012; Society for Psychophysiological Research Ad Hoc Committee on Electrodermal Measures 2012; Thammasan et al. 2020). Index NS.SCRs representing momentary arousal (Braithwaite et al. 2013), was calculated as the rate of spontaneous skin conductance responses occurrences without external stimuli (Boucsein et al. 2012). Moreover, the magnitude of difference between baseline and MAT was determined as value during MAT minus value during baseline.

Statistical analysis

Statistical analysis was performed by Jamovi software version 1.6.9 (Sydney, Australia). The data distribution (parametric/non-parametric) were assessed by Shapiro-Wilk normality statistical test with the null hypothesis that the sampling distribution is Gaussian (Ghasemi and Zahediasl 2012). Data expressed subject characteristics (age, body mass index (BMI), waist to hip ratio (WHR), and peripheral temperature (PT)) were normally distributed. The effect of group of subject characteristics data was evaluated by one-way analysis of

Table 2. Evaluated EDA parameters during baseline period and MAT period within all examined adolescent groups

Indices	Baseline	MAT	<i>p</i>	Indices	Baseline	MAT	<i>p</i>
Total group				Group-2			
SampEn				SampEn			
m = 2	0.027 (0.010, 0.048)	0.042 (0.016, 0.086)	0.065	m = 2	0.017 (0.010, 0.040)	0.062 (0.031, 0.102)	0.020
m = 3	0.024 (0.009, 0.046)	0.042 (0.014, 0.084)	0.022	m = 3	0.017 (0.008, 0.039)	0.064 (0.031, 0.102)	0.018
m = 4	0.021 (0.009, 0.043)	0.042 (0.014, 0.082)	0.004	m = 4	0.017 (0.008, 0.038)	0.066 (0.032, 0.100)	0.012
m = 5	0.020 (0.008, 0.040)	0.041 (0.014, 0.079)	0.002	m = 5	0.017 (0.007, 0.035)	0.066 (0.032, 0.096)	0.012
m = 6	0.020 (0.008, 0.038)	0.041 (0.013, 0.076)	0.002	m = 6	0.017 (0.007, 0.033)	0.064 (0.032, 0.092)	0.011
m = 7	0.019 (0.008, 0.038)	0.039 (0.012, 0.074)	0.002	m = 7	0.019 (0.009, 0.036)	0.062 (0.030, 0.089)	0.009
MSE				MSE			
τ = 1	0.0047 (0.0018, 0.0095)	0.0088 (0.0055, 0.0199)	<0.001	τ = 1	0.0055 (0.0015, 0.0091)	0.0131 (0.0074, 0.0227)	0.012
τ = 2	0.0091 (0.0031, 0.0178)	0.0168 (0.0104, 0.0333)	<0.001	τ = 2	0.0107 (0.0024, 0.0167)	0.0261 (0.0144, 0.0433)	0.008
τ = 3	0.0134 (0.0037, 0.0240)	0.0243 (0.0144, 0.0476)	<0.001	τ = 3	0.0158 (0.0034, 0.0246)	0.0379 (0.0207, 0.0636)	0.005
τ = 4	0.0173 (0.0048, 0.0311)	0.0307 (0.0178, 0.0621)	<0.001	τ = 4	0.0205 (0.0044, 0.0321)	0.0466 (0.0263, 0.0797)	0.004
τ = 5	0.0198 (0.0057, 0.0376)	0.0360 (0.0198, 0.0747)	<0.001	τ = 5	0.0246 (0.0053, 0.0393)	0.0527 (0.0314, 0.0940)	0.004
τ = 6	0.0221 (0.0066, 0.0442)	0.0405 (0.0205, 0.0841)	<0.001	τ = 6	0.0285 (0.0061, 0.0461)	0.0595 (0.0352, 0.1110)	0.004
τ = 7	0.0239 (0.0074, 0.0504)	0.0449 (0.0227, 0.0918)	<0.001	τ = 7	0.0321 (0.0071, 0.0519)	0.0657 (0.0382, 0.1220)	0.004
τ = 8	0.0261 (0.0081, 0.0557)	0.0484 (0.0247, 0.0989)	<0.001	τ = 8	0.0358 (0.0078, 0.0574)	0.0698 (0.0406, 0.1360)	0.004
τ = 9	0.0287 (0.0079, 0.0609)	0.0504 (0.0267, 0.1030)	<0.001	τ = 9	0.0384 (0.0083, 0.0632)	0.0746 (0.0433, 0.1450)	0.004
τ = 10	0.0307 (0.0084, 0.0664)	0.0542 (0.0291, 0.1040)	<0.001	τ = 10	0.0417 (0.0089, 0.0685)	0.0773 (0.0430, 0.1490)	0.005
τ = 11	0.0335 (0.0081, 0.0721)	0.0548 (0.0307, 0.1090)	<0.001	τ = 11	0.0437 (0.0094, 0.0757)	0.0802 (0.0440, 0.1610)	0.006
τ = 12	0.0348 (0.0084, 0.0766)	0.0627 (0.0330, 0.1130)	0.001	τ = 12	0.0452 (0.0098, 0.0811)	0.0848 (0.0450, 0.1590)	0.006
τ = 13	0.0376 (0.0089, 0.0818)	0.0639 (0.0350, 0.1130)	0.002	τ = 13	0.0465 (0.0106, 0.0862)	0.0872 (0.0461, 0.1650)	0.009
τ = 14	0.0404 (0.0350, 0.1130)	0.0645 (0.0367, 0.1170)	0.003	τ = 14	0.0483 (0.0113, 0.0885)	0.0907 (0.0478, 0.1660)	0.008
τ = 15	0.0420 (0.0096, 0.0877)	0.0690 (0.0374, 0.1210)	0.004	τ = 15	0.0503 (0.0119, 0.0914)	0.0925 (0.0475, 0.1740)	0.006
τ = 16	0.0437 (0.0102, 0.0926)	0.0692 (0.0368, 0.1280)	0.005	τ = 16	0.0518 (0.0123, 0.0970)	0.1020 (0.0507, 0.1840)	0.011
τ = 17	0.0451 (0.0109, 0.0962)	0.0684 (0.0373, 0.1320)	0.007	τ = 17	0.0528 (0.0130, 0.1010)	0.1070 (0.0538, 0.1780)	0.011
τ = 18	0.0475 (0.0112, 0.0970)	0.0740 (0.0385, 0.1320)	0.009	τ = 18	0.0584 (0.0132, 0.1030)	0.1110 (0.0569, 0.1890)	0.014
τ = 19	0.0502 (0.0111, 0.0977)	0.0744 (0.0414, 0.1360)	0.010	τ = 19	0.0563 (0.0142, 0.1100)	0.1140 (0.0547, 0.1870)	0.017
τ = 20	0.0516 (0.0161, 0.1050)	0.0776 (0.0439, 0.1420)	0.008	τ = 20	0.0592 (0.0228, 0.1130)	0.1140 (0.0561, 0.1980)	0.012
SCL (μS)	2.60 (1.11, 4.91)	4.46(2.80, 7.89)	<0.001	SCL (μS)	4.52 (2.26, 7.52)	6.64 (3.78, 8.24)	<0.001
NS.SCRs	1.00 (0.00, 1.25)	1.00 (0.00, 2.00)	0.246	NS.SCRs	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.167
Group-1				Group-3			
SampEn				SampEn			
m = 2	0.056 (0.014, 0.087)	0.069 (0.041, 0.106)	0.571	m = 2	0.021 (0.006, 0.032)	0.015 (0.011, 0.025)	0.955
m = 3	0.037 (0.013, 0.079)	0.070 (0.040, 0.112)	0.216	m = 3	0.020 (0.005, 0.029)	0.014 (0.011, 0.024)	0.999
m = 4	0.026 (0.012, 0.065)	0.066 (0.040, 0.111)	0.090	m = 4	0.020 (0.004, 0.026)	0.014 (0.011, 0.024)	0.888
m = 5	0.026 (0.012, 0.063)	0.059 (0.041, 0.100)	0.083	m = 5	0.019 (0.004, 0.025)	0.014 (0.010, 0.024)	0.751
m = 6	0.026 (0.013, 0.057)	0.055 (0.041, 0.095)	0.070	m = 6	0.017 (0.004, 0.024)	0.013 (0.010, 0.024)	0.695
m = 7	0.024 (0.013, 0.053)	0.055 (0.041, 0.092)	0.070	m = 7	0.016 (0.004, 0.023)	0.012 (0.010, 0.023)	0.695
MSE				MSE			
τ = 1	0.0052 (0.0029, 0.0153)	0.0143 (0.0080, 0.0221)	0.076	τ = 1	0.0041 (0.0005, 0.0066)	0.0057 (0.0037, 0.0068)	0.225
τ = 2	0.0095 (0.0054, 0.0257)	0.0289 (0.0155, 0.0431)	0.006	τ = 2	0.0078 (0.0009, 0.0123)	0.0107 (0.0073, 0.0133)	0.185
τ = 3	0.0138 (0.0078, 0.0431)	0.0433 (0.0224, 0.0623)	0.002	τ = 3	0.0112 (0.0013, 0.0173)	0.0152 (0.0102, 0.0197)	0.140
τ = 4	0.0171 (0.0101, 0.0436)	0.0560 (0.0285, 0.0759)	0.002	τ = 4	0.0144 (0.0017, 0.0222)	0.0185 (0.0126, 0.0253)	0.112
τ = 5	0.0182 (0.0122, 0.0513)	0.0648 (0.0346, 0.0873)	0.002	τ = 5	0.0177 (0.0021, 0.0270)	0.0201 (0.0149, 0.0303)	0.140
τ = 6	0.0200 (0.0141, 0.0571)	0.0740 (0.0399, 0.0953)	0.003	τ = 6	0.0202 (0.0025, 0.0314)	0.0205 (0.0171, 0.0351)	0.191
τ = 7	0.0225 (0.0158, 0.0616)	0.0822 (0.0456, 0.1050)	0.008	τ = 7	0.0217 (0.0029, 0.0356)	0.0224 (0.0185, 0.0383)	0.225
τ = 8	0.0253 (0.0177, 0.0656)	0.0903 (0.0498, 0.1060)	0.006	τ = 8	0.0237 (0.0032, 0.0391)	0.0248 (0.0198, 0.0411)	0.255
τ = 9	0.0277 (0.0193, 0.0697)	0.0964 (0.0500, 0.1170)	0.012	τ = 9	0.0261 (0.0036, 0.0423)	0.0263 (0.0215, 0.0421)	0.287
τ = 10	0.0298 (0.0205, 0.0756)	0.0980 (0.0525, 0.1210)	0.017	τ = 10	0.0284 (0.0039, 0.0466)	0.0287 (0.0225, 0.0440)	0.287
τ = 11	0.0314 (0.0216, 0.0795)	0.1010 (0.0548, 0.1250)	0.024	τ = 11	0.0314 (0.0044, 0.0490)	0.0305 (0.0224, 0.0466)	0.401
τ = 12	0.0340 (0.0228, 0.0858)	0.1020 (0.0600, 0.1200)	0.036	τ = 12	0.0323 (0.0047, 0.0527)	0.0316 (0.0232, 0.0464)	0.422
τ = 13	0.0377 (0.0239, 0.0910)	0.1060 (0.0625, 0.1200)	0.036	τ = 13	0.0329 (0.0050, 0.0545)	0.0335 (0.0231, 0.0492)	0.513
τ = 14	0.04040 (0.0251, 0.0960)	0.1030 (0.0599, 0.1220)	0.070	τ = 14	0.0341 (0.0054, 0.0568)	0.0359 (0.0229, 0.0496)	0.723
τ = 15	0.0420 (0.0263, 0.1020)	0.0996 (0.0671, 0.1250)	0.123	τ = 15	0.0376 (0.0055, 0.0602)	0.0374 (0.0239, 0.0507)	0.723
τ = 16	0.0437 (0.0258, 0.1050)	0.0990 (0.0691, 0.1300)	0.114	τ = 16	0.0379 (0.0059, 0.0624)	0.0367 (0.0243, 0.0518)	0.808
τ = 17	0.0468 (0.0251, 0.1160)	0.096 (0.0665, 0.1330)	0.189	τ = 17	0.0397 (0.0065, 0.0655)	0.0372 (0.0249, 0.0536)	0.867
τ = 18	0.0485 (0.0264, 0.1150)	0.0990 (0.0728, 0.1320)	0.177	τ = 18	0.0413 (0.0065, 0.0669)	0.0379 (0.0262, 0.0508)	0.837
τ = 19	0.0536 (0.0259, 0.1170)	0.1040 (0.0724, 0.1360)	0.245	τ = 19	0.0445 (0.0063, 0.0670)	0.0403 (0.0262, 0.0574)	0.867
τ = 20	0.0532 (0.0262, 0.1280)	0.0998 (0.0760, 0.1420)	0.202	τ = 20	0.0433 (0.0069, 0.0698)	0.0425 (0.0281, 0.0581)	0.723
SCL (μS)	1.11 (0.71, 2.19)	2.76 (1.53, 4.94)	<0.001	SCL (μS)	3.13 (1.24, 4.49)	5.40 (3.64, 7.24)	<0.001
NS.SCRs	1.00 (0.00, 1.25)	1.00 (0.00, 1.00)	0.552	NS.SCRs	1.00 (1.00, 2.25)	2.50 (2.00, 5.00)	0.026

MAT, mental arithmetic test period; EDA, electrodermal activity; Total group, all examined adolescent volunteers; Group-1, early adolescent volunteers; Group-2, middle adolescent volunteers; Group-3, late adolescent volunteers; SampEn, sample entropy; m, embedding dimension; MSE, multiscale entropy; τ, timescale; SCL, skin conductance level; NS.SCRs, non-specific skin conductance responses. The data are expressed as median (IQR). The degrees of freedom (df) was equal 57 within all comparisons. A value of *p* < 0.05 is considered a statistically significant result.

In addition, the magnitudes of differences between baseline and MAT period in the nonlinear EDA parameters (SampEn for all m , MSE for all τ) as well as traditional indices (SCL and NS.SCRs) within all examined groups are summarized in Table 3.

Discussion

This study for the first time describes the changes of EDA complexity *via* employing SampEn (for m ranged from 2 to 7) and MSE (for τ ranged from 1 to 20) analysis in response to mental stress in healthy adolescents with consequent comparisons of stress-related differences in evaluated indices within individual adolescent subgroups. Moreover, the differences in traditionally used EDA indices – SCL and NS.SCR were also assessed. The major findings of this study are following: (1) indices SampEn for $m \geq 3$ were higher during MAT compared to baseline in the total group, and indices SampEn for all evaluated m in Group-2, (2) parameter MSE for all evaluated τ was higher in response to MAT compared to baseline in the total group and Group-2, and MSE for τ from 2 to 13 was higher during MAT compared to baseline in Group-1, (3) additionally, while SCL significantly increased during MAT stress in all evaluated groups, (4) NS.SCRs significantly decreased during MAT period only in Group-3. Several mechanisms are proposed.

From a methodological point of view, this study was focused to detect the sensitivity of SampEn for different embedding dimensions' m (ranging from 2 to 7) and MSE for different τ (ranging from 1 to 20) to detect stress-induced changes of complex sympathetic regulatory network in healthy adolescents. With respect to SampEn in individual embedding dimensions, SampEn for m from 3 to 7 were increased during MAT compared to baseline with the highest magnitudes of differences between baseline and MAT for m from 4 to 6 in the total group. On the other hand, SampEn for $m = 2$ which is generally taken in sample entropy calculations was without significant changes in the total group. Interestingly, in spite of significant differences regarding SampEn in the total group, individual groups divided according to age showed apparently different patterns of SampEn in individual embedding dimensions. Specifically, Group-1 representing early adolescents and Group-3 representing late adolescents showed no significant differences in SampEn all embedding dimensions (i.e. $m = 2-7$) in response to mental stress compared to baseline. In contrast, Group-2 representing the middle adolescent period showed significant differences in SampEn for all embedding dimensions between baseline and MAT with the highest magnitudes for $m = 3$ and $m = 4$.

With respect to MSE, MSE for all evaluated τ (i.e. τ from 1 to 20) within the total adolescent group were significantly

higher in response to MAT compared to baseline with the maximum magnitude for $\tau = 17$. Similarly to SampEn, there were different patterns within individual age groups. Specifically, MSE for τ from 2 to 13 was significantly higher during MAT compared to baseline with the maximum magnitude for $\tau = 13$ in Group-1, MSE for all evaluated τ was significantly higher during MAT compared to baseline with the maximum magnitude for $\tau = 19$ in Group-2, and no significant differences were found in MSE for all evaluated τ in Group-3. Taken together, as we hypothesized, the complexity of EDA evaluated by SampEn and MSE was significantly lower during baseline compared to stress period in adolescence. Similar findings were found in healthy adults (aged from 22 to 41 yrs.), i.e. EDA complexity evaluated by Rényi entropy was significantly higher during cognitive mental tests (arithmetic test and Stroop colour word stress) compared to baseline and SampEn of EDA was significantly increased during Stroop test compared to baseline period (Nardelli et al. 2022). Therefore, we assume that the determination of EDA complexity changes during cognitive stress using entropy indices seems to be crucial for understanding the developmental characteristics of sympathetic complex regulation already in vulnerable adolescent age.

From the physiological point of view, EDA complexity analysis pointed to the significant changes in all evaluated non-linear indices (i.e. SampEn for m ranged from 2 to 7 and MSE for τ ranged from 1 to 20) in response to mental stress in Group-2 (i.e. middle adolescence) group followed by Group-1 (i.e. early adolescence) with significant changes only in MSE τ ranged from 2 to 13. Contrary, Group-3 (i.e. late adolescence) showed no significant changes in all evaluated complexity indices. These results can indicate the middle adolescence as the most “malleability” period during which the modulation of the stress-induced complex sympathetic regulatory network functioning is highlighted. These findings are in accordance with the middle adolescence reported to be one of the most critical developmental switch points, according to Adaptive Calibration Model of stress responsivity, during which the stress-response systems including ANS temporarily become more “plastic” to the environmental influence (Diamond and Cribbet 2013). In addition, several studies assessing changes in stress perception during adolescence found decreased stress perception during late adolescence, but no changes during early adolescence (Petersen et al. 1991; Seiffge-Krenke et al. 2009). Therefore, as we assumed, the individual subgroups' differences may be due to the fact that older adolescents perceive stress more easily based on a greater number of lived experiences in association with more matured coping mechanisms. Next, the SNS activity indexed by EDA in response to stress can be modulated by complex regulatory mechanisms within the central nervous system, with two independent regulatory mechanisms:

Table 3. The magnitudes of differences between baseline and MAT period in EDA evaluated parameters within all analysed adolescent groups

Indices	Total group	Group-1	Group-2	Group-3
SampEn				
m = 2	0.008 (-0.015, 0.047)	0.024 (-0.044, 0.057)	0.036 (0.003, 0.074)	0.004 (-0.017, 0.008)
m = 3	0.009 (-0.010, 0.052)	0.026 (-0.016, 0.062)	0.038 (0.003, 0.080)	0.004 (-0.015, 0.009)
m = 4	0.011 (-0.007, 0.056)	0.031 (-0.009, 0.064)	0.038 (0.003, 0.077)	0.005 (-0.011, 0.011)
m = 5	0.011 (-0.004, 0.056)	0.033 (-0.008, 0.064)	0.037 (0.003, 0.075)	0.004 (-0.010, 0.011)
m = 6	0.011 (-0.004, 0.053)	0.030 (-0.008, 0.065)	0.034 (0.004, 0.073)	0.004 (-0.008, 0.011)
m = 7	0.010 (-0.004, 0.043)	0.032 (-0.007, 0.065)	0.026 (0.003, 0.066)	0.004 (-0.007, 0.010)
MSE				
$\tau = 1$	0.004 (-0.0002, 0.012)	0.006 (-0.002, 0.012)	0.008 (0.001, 0.016)	0.001 (-0.001, 0.004)
$\tau = 2$	0.007 (0.001, 0.024)	0.012 (0.001, 0.024)	0.016 (0.003, 0.034)	0.003 (-0.003, 0.007)
$\tau = 3$	0.010 (0.0007, 0.035)	0.017 (0.004, 0.036)	0.023 (0.004, 0.048)	0.005 (-0.003, 0.009)
$\tau = 4$	0.013 (-0.0004, 0.045)	0.022 (0.006, 0.047)	0.029 (0.005, 0.063)	0.006 (-0.004, 0.013)
$\tau = 5$	0.015 (-0.002, 0.050)	0.027 (0.006, 0.056)	0.033 (0.006, 0.078)	0.008 (-0.004, 0.015)
$\tau = 6$	0.018 (-0.002, 0.057)	0.030 (-0.001, 0.063)	0.038 (0.008, 0.091)	0.009 (-0.005, 0.018)
$\tau = 7$	0.019 (-0.003, 0.064)	0.035 (-0.003, 0.067)	0.042 (0.008, 0.109)	0.009 (-0.005, 0.019)
$\tau = 8$	0.020 (-0.004, 0.070)	0.040 (-0.005, 0.071)	0.046 (0.008, 0.112)	0.009 (-0.006, 0.021)
$\tau = 9$	0.023 (-0.004, 0.073)	0.042 (-0.007, 0.078)	0.050 (0.009, 0.119)	0.042 (-0.007, 0.078)
$\tau = 10$	0.024 (-0.009, 0.078)	0.041 (-0.015, 0.079)	0.054 (0.010, 0.123)	0.009 (-0.009, 0.024)
$\tau = 11$	0.024 (-0.010, 0.079)	0.044 (-0.021, 0.081)	0.057 (0.010, 0.124)	0.008 (-0.011, 0.024)
$\tau = 12$	0.023 (-0.011, 0.078)	0.043 (-0.024, 0.079)	0.061 (0.010, 0.123)	0.009 (-0.011, 0.018)
$\tau = 13$	0.022 (-0.014, 0.079)	0.046 (-0.030, 0.083)	0.063 (0.012, 0.115)	0.010 (-0.014, 0.020)
$\tau = 14$	0.024 (-0.016, 0.078)	0.042 (-0.032, 0.080)	0.063 (0.011, 0.119)	0.009 (-0.016, 0.018)
$\tau = 15$	0.023 (-0.017, 0.070)	0.043 (-0.034, 0.073)	0.066 (0.009, 0.126)	0.010 (-0.018, 0.020)
$\tau = 16$	0.022 (-0.019, 0.077)	0.040 (-0.032, 0.087)	0.070 (0.010, 0.125)	0.008 (-0.020, 0.020)
$\tau = 17$	0.025 (-0.020, 0.076)	0.042 (-0.042, 0.085)	0.064 (0.011, 0.125)	0.008 (-0.022, 0.024)
$\tau = 18$	0.023 (-0.020, 0.082)	0.044 (-0.043, 0.085)	0.067 (0.010, 0.129)	0.008 (-0.020, 0.022)
$\tau = 19$	0.023 (-0.021, 0.081)	0.044 (-0.045, 0.034)	0.072 (0.010, 0.125)	0.008 (-0.023, 0.022)
$\tau = 20$	0.024 (-0.021, 0.085)	0.043 (-0.046, 0.090)	0.062 (0.011, 0.129)	0.008 (-0.021, 0.025)
SCL (μ S)	1.65 (0.86, 2.77)	1.09 (0.70, 2.67)	1.48 (0.95, 2.28)	2.54 (1.44, 2.77)
NS.SCRs	0 (0, 1)	0 (0, 0)	0 (0, 0)	1 (0, 4)

For abbreviations, see Table 2.

cortical motor control *via* premotor cortex and pyramidal pathways (Sequeira-Martinho and Roy 1993; Blain et al. 2010; Figner and Murphy 2011), and the subcortical regulatory structures such as hypothalamus, limbic system, and reticular formation. In this context, several studies revealed direct correlations between electrostimulation of several brain areas (e.g. amygdala, hippocampus, cingulate cortex, and prefrontal cortex) and EDA responses (Mangina and Beuzeron-Mangina 1996; Critchley 2002). It is important to note that adolescence represents a critical period of ongoing maturational processes within the central nervous system. Specifically, the axons are dramatically more myelinated, the neuroplasticity of brain elevates, the dendrites increase their connectivity and receive and transmit more information. Thus, the individual cortical and subcortical brain areas work more efficiently with better integrating the experiences into decision making, cognition, and regulation of the emotion and social behaviours (Xi et al. 2011; Donoso et al. 2014; Griffin 2017). From this perspective, the maturational processes of the complex neurophysiological networks dependent on age can be implicated in

the differences in EDA complexity in individual adolescent age periods. In other words, EDA modulation reflected in signal complexity can be affected by many inhibitory and excitatory influences on SNS from different brain structures parts which undergoes important maturational processes during critical adolescent period.

Lastly, with respect to traditionally used EDA parameters, the tonic EDA index – SCL was higher in response to mental stress in all examined groups. This finding of stress-induced increase in EDA is generally known and is in agreement with other studies reflecting sympathetic EDA-related overactivity in response to various cognitive tests (Dawson et al. 2007; Setz et al. 2010; Svetlak et al. 2010; Reinhardt et al. 2012; Visnovcova et al. 2016; Lipovac et al. 2022). On the other hand, our study revealed that conventional phasic EDA index NS.SCRs was lower in response to mental stress in late adolescent group potentially indicating the presence of the anticipatory anxiety before the cognitive mathematical test with consequent calmness during the MAT course. To sum, our findings of distinct patterns in conventional and complexity EDA analysis could provide important and in-

dependent information concerning different stress-related neuro-psychophysiological processes in youth.

Limitations

In this study, the cohort consisted of a relatively small sample of volunteers; therefore, it needs to be validated in a larger sex-matched cohort. Despite the fact that the group consists of more females, we do not assume, that higher values of EDA SampEn and MSE could be influenced by sex, because from sex aspect, the males exhibit higher sweating rates than females, despite the fact that females have generally greater density of sweat glands than males (Buono and Sjöholm 1988; Smith and Havenith 2012; Notley et al. 2017; Baker 2019). From hormonal aspect, EDA is mediated by the sympathetic nervous system activity which can be influenced by sex hormones, mainly the hormonal background in girls during the menstrual cycle. More specifically, the sympathetic activity is increased *via* elevated levels of estrogen and progesterone during the luteal phase of the cycle compared to the follicular phase when estrogen and progesterone levels are low (Minson et al. 2000). It is also important to note that although the EDA seems to be higher in adult females than males (but without significant difference) (Bari 2020), the sex differences regarding EDA in adolescents is understudied. From this reasons, the effect of sex on EDA complexity in adolescents remains questionable and warrants further studies. Furthermore, only one type of cognitive stressor was used, and there was not recovery phase after stress period. In this aspect, further research considering the application of several types of stressors (emotional or physiological), as well as study of recovery periods, is needed.

Further, SNS regulation was assessed only by the EDA biosignal, therefore, other sympathetically mediated parameters such as blood pressure, pre-ejection period or peripheral temperature could be recorded and evaluated for complex assessment of sympathetic regulatory mechanisms in response to stress.

Conclusions

This study focused on adolescence revealed different EDA non-linear patterns in response to mental stress indicating the importance of the complexity analysis in the multiple embedding dimensions and timescales within distinct adolescent-linked age periods. Specifically, we found differences in the EDA complexity in response to stress in individual groups of adolescents with the greatest response of the sympathetic nervous system to stress observed in Group-2, i.e. in middle adolescence. Therefore, our findings should set a step of importance for the analysis

of non-linear EDA components in narrower adolescent subgroups in future studies. Moreover, we suggest that our findings can contribute to the understanding of maturation in complex sympathetic regulatory network indexed by EDA across the transient span adolescence that is important for clarifying the discrete sympathetic abnormalities associated with stress-related disorders and psychopathology in youth.

Conflicts of interest. The authors declare no conflict of interest.

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