

# Effect of Selected Music Soundtracks on Cardiac Vagal Control and Complexity Assessed by Heart Rate Variability

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## Summary

Listening to music is experimentally associated with positive stress reduction effect on human organisms. However, the opinions of therapists about this complementary non-invasive therapy are still different. Purpose: The aim of our study was to investigate the effect of selected passive music therapy frequencies without vocals on selected cardio-vagal and complexity indices of short-term heart rate variability (HRV) in healthy youth, in terms of calming the human. Main methods: 30 probands (15 male, averaged age:  $19.7 \pm 1.4$  years, BMI:  $23.3 \pm 3.8$  kg/m<sup>2</sup>) were examined during protocol (Silence baseline, Music 1 (20-1000 Hz), Silence 1, Music 2 (250-2000 Hz), Silence 2, Music 3 (1000-16000 Hz), and Silence 3). Evaluated HRV parameters in time, spectral, and geometrical domains represent indices of cardio-vagal and emotional regulation. Additionally, HRV complexity was calculated by approximate entropy and sample entropy (SampEn) and subjective characteristics of each phase by Likert scale. Results: the distance between subsequent R-waves in the electrocardiogram (RR intervals [ms]) and SampEn were significantly higher during Music 3 compared to Silence 3 ( $p=0.015$ ,  $p=0.021$ , respectively). Geometrical cardio-vagal index was significantly higher during Music 2 than during Silence 2 ( $p=0.006$ ). In the subjective perception of the healthy youths evaluated statistically through a Likert scale, the phases of music were perceived significantly more pleasant than the silent phases ( $p<0.001$ ,  $p=0.008$ ,  $p=0.003$ , respectively). Conclusions: Our findings revealed a rise of cardio-vagal modulation and higher complexity assessed by short-term

HRV indices suggesting positive relaxing effect music especially of higher frequency on human organism.

## Key words

Cardiac vagal activity • Music therapy • Sound • Heart rate variability • Autonomic nervous system • Approximate entropy • Sample entropy

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## Introduction

Music art has been an integral part of human civilization since its beginning. The process of creation and perception of acoustic sound waves is a part of our everyday life [1]. The World Federation of Music Therapy (WFMT) defines music therapy (MT) as the professional use of music and its elements for interventions in health care, education, and the everyday life with an individual group, family or community that seeks to optimize the quality of their life and improve their psychological, social, communicative, emotional, and mental health and well-being [2]. Although current scientific knowledge on the influence of music on

humans and the human brain is constantly increasing, many MT mechanisms are still not exactly known.

The autonomic nervous system (ANS) regulates the activity of the heart, which is extremely sensitive to its regulatory inputs. The distance between subsequent R-waves (R-R intervals) on the electrocardiogram (ECG) is not constant, or otherwise, the time between two systoles is constantly variable. These oscillations represent the heart rate variability (HRV), which reflects the activity of the sympathetic and parasympathetic regulatory inputs on the sinoatrial node [3]. In addition, heart rate is also controlled by a higher neural system known as the central autonomic network influencing neuroendocrine, behavioural, and other responses [4]. Non-invasive techniques for assessing the cardiac autonomic control based on HRV analysis have emerged as the simple method to evaluate the sympathovagal balance at the sinoatrial node [3,5]. Scientific perspectives over the past decades have declared that HRV reflects self-regulatory capacity and can therefore be used as a certain biomarker of self-regulation and adaptability of the organism [6].

Further, it should be noted that, the spectral power in the high-frequency band of the heart rate variability (HF-HRV) is highlighted under physiological circumstances during parasympathetic activation maneuvers, together with its connection to the central autonomic network already mentioned above [3]. Notably, we selected standard used vagal-control indices: HF-HRV, root square of successive differences of consecutive RR intervals (rMSSD), and percentage of the number of successive heartbeats differing more than 50 ms (pNN50), which are considered essential indicators of cardio-vagal control also in other psychophysiological researches [7]. In addition, a novel geometrical parameter – cardiac vagal index (CVI) was used as index of parasympathetic regulation [8]. Furthermore, according to the mentioned neurovisceral integration model, the reduced cardio-vagal modulation can lead to a decreased ability of the organism to track the rapid changes in environmental demands and to organize an appropriate response [9]. That is why we consider it important to follow these HRV parameters in clinical practice reflecting the overall vitality of the ANS of the human body in a personalized approach to patients. Next, we monitor changes in the time domain parameters rMSSD and pNN50, frequency index HF-HRV which are also discussed in other studies as a possible reflection of the relaxing effect of music on the body, reducing stress and reducing anxiety [10,11]. Therefore, we suggest that

music can induce positive emotions, relaxation of the organism, and these indices would prove to be essential for shifting the dynamic balance of ANS. In addition, due to fact, that biological signals report non-linear features, the approximate entropy (ApEn) [12] and sample entropy (SampEn) [12,13] were used to detect complexity, randomness and uncertainty of the system during exposition of different music as well as silence after music [14]. Furthermore, the metric of HRV analysis and control is very sensitive to various stimuli (e.g. listening to different type of music), and therefore it is used as a non-invasive way for identification the imbalance of ANS. Subsequent HRV quantitative analysis in the frequency or time domain can timely reveal changes related to a variety of pathological conditions. Notably, reduced HRV is associated with higher risk of cardiovascular complications, as it was documented in several studies [9,10]. In general, with relation to allostasis, the healthier the ANS is, the more resilience, preparedness, and flexibility it exhibits when dealing with life's obstacles and situations [15]. Finn *et al.* reported in their systematic review that 13 out of 33 biomarkers tested showed changes in response to listening to music (e.g. HRV, stress hormones and others) [16]. The next study by Chuang *et al.* [17] concluded that MT could be clinically useful for promoting relax sensation and an increase of parasympathetic nervous system activity [17]. Moreover, comprehensive review of Kamioka *et al.* [18] demonstrated, that MT has improved global and social functioning in mental disorders. This study further noted that MT may have the ability to improve other diseases, but currently there is still not enough evidence for it, as individual approaches differ in treatment methods [18,19].

Individual MT frequencies in relation to HRV as a cognitive stimulus are studied rarely. Therefore, a new aspect of this study is to focus on selection and application of specific musical frequencies to the proband in the concept of passive MT. We assume that individually applied music frequencies in the form of a positive “stressor” could have a different effect on the ANS, especially on vagal regulation as one of indicators of calm state of human body. In this global aspect, the aim of our research is therefore to investigate, which of the selected MT frequencies (ranged: 20-1000 Hz, 250-2000 Hz, and 1000-16000 Hz) have the most positive effect on cardiac-vagal regulation indexed by time, spectral (rMSSD, pNN50, HF-HRV), and geometrical (CVI) HRV indices as well as HRV complexity (ApEn and SampEn) in

healthy probands, in terms of calming and relaxing the human body, which has not been exactly clarified in any study so far.

## Methods

### *Subjects*

In our study 30 healthy youths (15 of them male, age mean:  $19.7 \pm 1.4$  years, body mass index (BMI):  $23.3 \pm 3.8 \text{ kg/m}^2$ ) were selected for the study. The volunteers were medical students who were informed in detail about the course of the investigation. As a result of personal data protection, personal numbers were assigned to them for further data processing and analysis. Inclusion criteria were as follows: healthy normostenic students without any acute infections, never treatment for auditory, or neurological diseases, sleeping a minimum of 8 h and without higher physical activity two days before the examination, and abstaining from caffeine, alcohol and drugs potentially affecting HRV parameters at least 24 h before MT examinations. Exclusion criteria were as follows: auditory disorders, neurological, mental, cardiovascular, respiratory or other diseases affecting HRV parameters and perception of the sounds. Furthermore, they were not currently undergoing medical treatment, and did not show objective signs of obesity or malnutrition. For women, we did not perform an examination between the 10<sup>th</sup>-15<sup>th</sup> and 20<sup>th</sup>-25<sup>th</sup> day of menstrual cycle [20]. Each subject underwent a specific MT examination according to the protocol exactly once during one session.

### *Ethics statement*

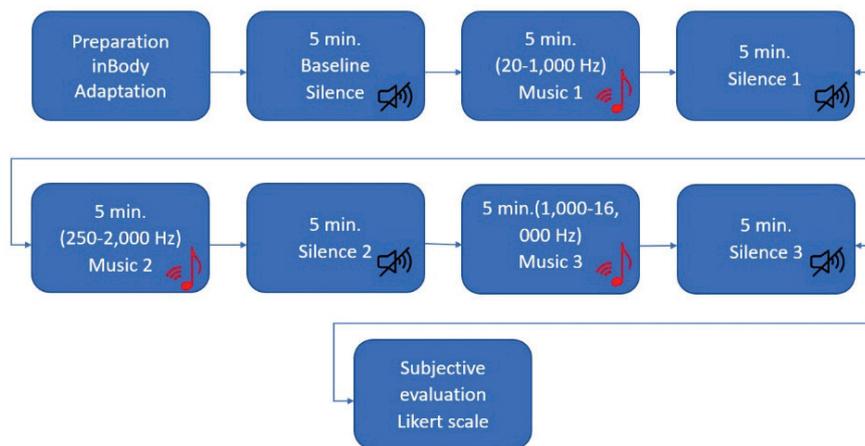
The study was approved in June 2021 by the Ethics Committee of the Jessenius Faculty of Medicine in Martin of the Comenius University in Bratislava, which is governed by the principles of the protection of human rights according to the Nuremberg Code, the Declaration of Helsinki and other related regulations and regulations (no. EK38/2021). All probands agreed to the study and provided informed consent as well as consent to the processing of personal data.

### *Measurement protocol*

The examination took place in the psychophysiological laboratory of Jessenius Faculty of Medicine and Biomed in Martin from 8:00 a.m. to 12:00 a.m. All investigated probands were exposed to musical exposure in individual interrupted intervals according to the measuring protocol. Music samples were

played by an audio player and applied through professional audio headphones Sennheiser 280 PRO (Sennheiser, GmbH & Co. KG, Germany) with neodymium magnets that faithfully reproduce the audio signal in the range of 8-25000 Hz. They are characterized by linear sound transmission, and the function of dampening ambient interference by 32 dB at an impedance of 64 Ohms. The examination took place in a quiet room with constant light intensity under standard conditions (temperature 22-23 °C, humidity 45-55 %) with minimal distractions, between 8:00-12:30 after the usual breakfast 2 h. before the examination. The examination itself was always preceded by a short anthropometric measurement (height, weight, body temperature) using the IN-BODY device (InBody J10, Biospace, Korea). After 10 min. of a resting period necessary to avoid potential effect of anticipatory stress, the subjects were asked to sit comfortably in a special chair and not to speak or move unless absolutely necessary [21,22]. Music samples used in clinical MT were played to volunteers. Music samples were adjusted using a band-pass filter and divided into 3 frequency bands as following: Music 1 – soundtrack in frequency range between 20 and 1000 Hz, Music 2 – soundtrack in frequency range from 250 to 2000 Hz, and Music 3 – sound track in frequency range from 1000 to 16000 Hz. The used MT soundtracks were played with a predefined volume ( $L_{Aeq} = 60.3 \pm 0.5 \text{ dB}$ ;  $L_{Aeq}$ , equivalent continuous sound level with A-frequency weighting). All the  $L_{Aeq}$  values were being measured for 1 min by integrating-averaging sound level meter Brüel & Kjær Type 2240 (Class 1 precision, Brüel & Kjær, Nærum, Denmark) [23]. Music samples with relaxing music style did not contain vocals and ranged from slow (Italian “*adagio*”) to moderate (Italian “*andante*”) according to the affective understanding of the speed of the musical compositions with a total duration of 5 min. The given music samples were played strictly in the same order for all probands, and they did not know which one they were listening to; each sample was played only once to proband. After each phase of music there was a phase of silence for 5 min. This measurement was performed in the following order: Silence baseline, Music 1, Silence 1, Music 2, Silence 2, Music 3, Silence 3 (Fig. 1).

At the end of each MT session, the examinee was asked to subjectively evaluate individual phases using a Likert scale questionnaire in the range of 1-5 (very unpleasant, unpleasant, neutral, pleasant, and very pleasant; respectively) [24].



**Fig. 1.** Music therapy protocol.

### HRV analysis

As an indicator of the effect of a musical stressor on the ANS, the recording of RR intervals was used for the analysis of short-term heart rate variability in the time, frequency, geometrical and complexity domains [8,12,25]. The ECG recording was continuously recorded by means of hardware and software equipment Medical DiANS PF8 (Dimea, Czech Republic) with a sampling frequency of 1000 Hz. A chest belt with two electrodes for sensing the electrical activity of the heart was placed in the area of the left part of the ribcage, medioclavicularly. All wireless recordings underwent a process of manual pre-processing in the form of filtering due to the elimination of artifacts and extrasystoles [25]. Subsequently, according to the recommendations for HRV 5-minute RR intervals were analyzed with the KUBIOS HRV Standard 3.5.0 software package [22,25,26]. It automatically detects R-R time intervals by applying an algorithm for detecting the QRS complex. The detector consists of a pre-processing part followed by decision rules. The pre-processing part includes band pass filtering of the ECG (to reduce power line noise, baseline deviations and other noise components), squaring of the data samples (to highlight peaks) and moving average filtering (to smooth close-by peaks). The decision rules include amplitude threshold, which we pre-set to the value “very low” [22,26].

From time and spectral-domain aspect, the following parameters of HRV were evaluated: RR intervals [ms], root square of successive differences of consecutive RR intervals (rMSSD [ms]), percentage of the number of successive heartbeats differing more than 50 ms (pNN50, [%]), and high frequency spectral power (HF-HRV, [ms<sup>2</sup>]) as the indices of cardio-vagal control [25,27,46,47]. Time indices rMSSD and pNN50 are considered as a gold standard parameters evaluating the

cardio-vagal activity [22,25,27]. Spectral analysis allows to evaluate the high frequency band (HF: 0.15-0.4 Hz) reflecting respiratory sinus arrhythmia, i.e. oscillations of the heart rate during breathing mediated through parasympathetic regulation [25,27].

Next, by geometric (nonlinear) analysis was assessed cardio-vagal index (CVI) [8]. The based principle consisted of the evaluation of two axes of Poincaré plot: standard deviation of the Poincaré cross-wise (SD1) and standard deviation of the Poincaré lengthwise (SD2) [9,28]. Consequently, the index CVI is determined as the logarithm of SD1 and SD2 product ( $\log(SD1 \times SD2)$ ) [8]. From physiological point of view, index CVI provides information about cardiac vagal control [8,29].

Further, in addition the complexity of system was determined by indices approximate entropy (ApEn) and sample entropy (SampEn), which describe the randomness, uncertainty, unpredictability, and regularity/irregularity of the system by evaluation of probabilities distribution of the expected value in the R-R time series [12,30]. The basic principle of ApEn and SampEn calculation is in Heaviside function with aim to detect the similarity between two patterns [31]. Further, ApEn and SampEn inform about the probabilities of similarity between vectors of length  $m$  (derived from  $N$  points long time series) and vector of length  $m+1$  within a given tolerance size  $r$ . The formulas for evaluation ApEn, and SampEn are following:

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

$$\text{SampEn}(m, r, N) = \log(\Phi_m(r) / \Phi_{m+1}(r)), \quad (2)$$

where  $N$  represents the data lengths (number of points from time series),  $m$  is selected the embedding dimension, and  $r$  informs about the tolerance value. In this study the  $N$ ,  $m$ , and  $r$  were selected as 300, 2, and 0.2 times the standard deviation of data, respectively,

according to standard for short-term time series [12,32]. Additionally, ApEn and SampEn values around zero detect more regular system, while the higher values of ApEn and SampEn indicate more complexity and randomness in clinical as well as experimental time series [12].

#### *Statistical analysis*

Statistical analysis was performed by jamovi version 1.6.9 (Sydney, Australia). First, the Shapiro-Wilk normality test was performed for evaluation of Gaussian/nonGaussian data distribution. The HF-HRV was logarithmically transformed because of inter-individual differences. All HRV data were nonGaussian distributed so nonparametric repeated ANOVA (Friedman test) was used to test effect of all periods each other of all HRV evaluated parameters with Durbin-Conover *post hoc* test. Differences in Likert scale during protocol was evaluated by Kendall's Tau-b test ( $\tau_b$ ). Spearman's Rho correlation coefficient ( $r_s$ ) was used for detection the relationships between subjective score evaluated by Likert scale and objective characteristics assessed by HRV parameters in each period of study protocol according to recommendation for this type of data [33]. Moreover, according to a priori power analysis the required sample size was evaluated to N=28 participants, where the CI=95 %, SD=0.5, and  $\alpha=5$  %. A value of  $p \leq 0.05$  was considered statistically significant. HRV data and Likert scale variables were expressed as median (interquartile range (IQR)).

## Results

#### *HRV parameters*

Non-parametric repeated ANOVA revealed significant effect of periods for indices: mean RR ( $F_{[6]}=12.9$ ,  $p=0.045$ ), CVI ( $F_{[6]}=19.6$ ,  $p=0.003$ ), and SampEn ( $F_{[6]}=15.4$ ,  $p=0.017$ ).

#### *Differences between Music and Silence*

Index CVI was significantly higher during Music 2 compared to Silence 2 ( $p=0.006$ ). Parameter mean RR was significantly prolonged during Music 3 compared to Silence 3 ( $p=0.015$ ). Index SampEn was significantly higher during Music 3 compared to Silence 3 ( $p=0.021$ ). Remain HRV parameters were without significant changes during different Music and Silence intervals. All HRV results were summarized in Table 1.

#### *Differences between Music 1, 2, and 3*

Index SampEn was significantly lower during

Music 1 compared to Music 3 ( $p=0.007$ ). Index CVI was significantly higher during Music 2 compared to Music 3 ( $p=0.050$ ). Remain HRV parameters were without significant changes during different used Music frequencies. All HRV results were summarized in Table 1.

#### *Differences between Silence baseline, 1, 2, and 3*

Index mean RR was significantly shortened during Silence baseline compared to Silence 2 ( $p=0.002$ ). Index CVI was significantly higher during Silence baseline compared to Silence 3 and during Silence 1 compared to Silence 3 ( $p=0.033$ ;  $p=0.012$ ; respectively). Remain HRV parameters were without significant changes during Silence intervals after individual Music intervals. All HRV results were summarized in Table 1.

#### *Likert scale variables*

##### *Differences between Music and Silence*

Subjective evaluation of music resp. silence pleasantness revealed significantly higher score of Likert scale during Music 1 compared to Silence 1, Music 2 compared to Silence 2, and Music 3 compared to Silence 3 ( $\tau_b=0.615$ ,  $p<0.001$ ;  $\tau_b=0.429$ ,  $p=0.008$ ;  $\tau_b=0.468$ ,  $p=0.003$ ; respectively). The responses were summarized in Figure 2.

##### *Differences between Music 1, 2, and 3*

Subjective evaluation of different music pleasantness revealed significantly higher score of Likert scale during Music 1 compared to Music 2, Music 1 compared to Music 3, and Music 2 compared to Music 3 ( $\tau_b=0.337$ ,  $p=0.042$ ;  $\tau_b=0.353$ ,  $p=0.030$ ;  $\tau_b=0.472$ ,  $p=0.003$ ; respectively). The responses were summarized in Figure 2.

##### *Differences between Silence 1, 2, and 3*

Subjective evaluation of silence pleasantness after different sounds revealed significantly higher score of Likert scale during Silence 1 compared to Silence 2, Silence 1 compared to Silence 3, and Silence 2 compared to Silence 3 ( $\tau_b=0.747$ ,  $p<0.001$ ;  $\tau_b=0.632$ ,  $p<0.001$ ;  $\tau_b=0.697$ ,  $p<0.001$ ; respectively). The responses were summarized in Figure 2.

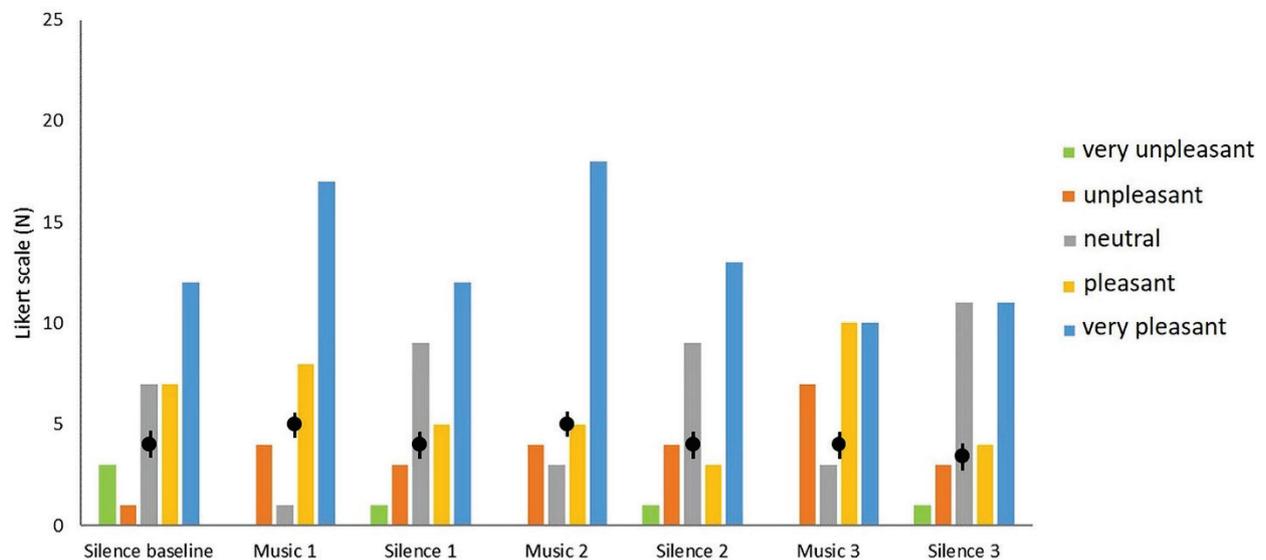
#### *Correlation analysis between HRV parameters and Likert scale variables*

Correlation analysis revealed significant positive correlation between HRV parameter mean RR and Likert scale during Silence 1, Silence 2, and Music 3 ( $r_s=0.501$ ,  $p=0.006$ ;  $r_s=0.442$ ,  $p=0.016$ ;  $r_s=0.390$ ,  $p=0.044$ ;

**Table 1.** Selected HRV parameters during protocol.

<i>Physiological parameter</i>	Silence Baseline	Music 1 (20-1000 Hz)	Silence 1	Music 2 (250-2000 Hz)	Silence 2	Music 3 (1000-16000 Hz)	Silence 3
<i>RR intervals [ms]</i>	804 (754, 920)	812 (746, 922)	801 (736, 998)	835 (748, 930)	812 (779, 941) <sup>EE</sup>	818 (765, 953)	805 (769, 948) <sup>B</sup>
<i>rMSSD [ms]</i>	40.2 (31.7, 50.1)	39.5 (30.5, 43.1)	38.3 (29.7, 47.2)	38.5 (28.6, 50.4)	41.6 (31.1, 50.5)	37.3 (30.5, 44.8)	41.1 (30.9, 49.8)
<i>pNN50 [%]</i>	21.6 (10.6, 33.5)	20.9 (12.1, 28.5)	22.1 (12.5, 42.3)	22.4 (14.5, 42.6)	24.8 (13.4, 39.1)	22.4 (13.9, 45.9)	19.2 (13.0, 42.9)
<i>lnHF-HRV [ms<sup>2</sup>]</i>	6.59 (6.03, 7.39)	6.49 (6.09, 7.19)	6.65 (6.04, 7.39)	6.83 (5.99, 7.50)	6.73 (5.97, 7.53)	6.42 (5.87, 7.84)	6.46 (6.01, 7.35)
<i>CVI</i>	0.50 (0.42, 0.63)	0.53 (0.44, 0.64)	0.49 (0.43, 0.63)	0.52 (0.46, 0.67)	0.49 (0.40, 0.63) <sup>AA</sup>	0.50 (0.44, 0.60) <sup>D</sup>	0.47 (0.39, 0.62) <sup>F,G</sup>
<i>ApEn</i>	1.10 (1.03, 1.18)	1.10 (1.05, 1.19)	1.13 (0.98, 1.17)	1.12 (1.03, 1.19)	1.11 (1.00, 1.19)	1.14 (1.04, 1.19)	1.11 (1.07, 1.17)
<i>SampEn</i>	1.51 (1.29, 1.75)	1.54 (1.30, 1.75)	1.59 (1.20, 1.73)	1.58 (1.40, 1.70)	1.59 (1.28, 1.74)	1.69 (1.39, 1.82) <sup>CC</sup>	1.5 (1.38, 1.77) <sup>B</sup>

rMSSD – root mean square of the successive differences of the R-R intervals duration, pNN50 – proportion of R-R50 divided by the total number of R-R, lnHF-HRV – spectral power in the high-frequency band of the heart rate variability, CVI – cardiac vagal index, ApEn – approximate entropy, and SampEn – sample entropy. Data are presented as median (interquartile range);  $p \leq 0.05$  is considered as statistically significant results. <sup>A</sup> represents significant differences between Music 2 and Silence 2; <sup>B</sup> represents significant differences between Music 3 and Silence 3; <sup>C</sup> represents significant differences between Music 1 and Music 3; <sup>D</sup> represents significant differences between Music 2 and Music 3; <sup>E</sup> represents significant differences between Silence baseline and Silence 2; <sup>F</sup> represents significant differences between Silence baseline and Silence 3 and <sup>G</sup> represents significant differences between Silence 1 and Silence 3. A marker letter represents  $p \leq 0.05$ ; two identical letter markers represent  $p < 0.01$ .



**Fig. 2.** The Likert scale evaluation during individual periods of protocol. The color bars represent the number of individual responses, black circles represents median and SEM for individual periods of protocol.

respectively) and positive correlation between index pNN50 and Likert scale during Music 1 ( $r_s=0.536$ ,  $p=0.002$ ). No significant correlations were found between other evaluated parameters.

## Discussion

Considering the effect of music on the human body, we evaluated the acute effects of three different music frequencies (20 to 1000 Hz (Music 1); 250 to

2000 Hz (Music 2); and 1000 to 16000 Hz (Music 3)) and three Silence periods after music applications on time, spectral, geometrical, and complexity domain indices of HRV as well as subjective characteristics of individual Music and Silence periods by Likert scale in the group of healthy youths. Our study revealed several important findings: 1) the significant increase of vagal modulation indexed by the geometrical index CVI during Music 2 and RR intervals during Music 3 indicating a possible calming effect of music on cardiac autonomic regulation; 2) the significant increase of the unpredictability and randomness indexed by SampEn during Music 3 compared to Music 1 as well as compared to Silence 3 reflecting higher complexity of organism during relax Music 3; 3) in the questionnaire, regardless of the sound frequency, each Music phase was subjectively evaluated with higher score on Likert scale (i.e. "pleasant to very pleasant") than Silence periods; 4) time HRV indices mean RR and pNN50 positively correlated with Music 3 and Music 1 respectively, which could pointed on higher cardio-vagal activity during exposition of Music. Due to our results, it seems that frequencies ranged from 250 to 16000 Hz of relaxed music were the most sensitive to evoke changes in cardio-vagal control which also reflects emotional regulation and well-being. Several mechanisms are supposed for later discussion.

Stress is in general state of disharmony or disturbed homeostasis inducing physiological and behavioural adaptive responses. The resulting stress response is conditioned by the integrated function of the central nervous system and ANS [34]. In connection with stress, MT is suitable for reducing stress as a non-pharmacological tool [2,10]. As part of our hypothesis, we assumed that HRV changes can be influenced by the specific interval frequency representation of the applied music sample. Our findings showed higher vagal activity during Music 2 indexed by CVI and bradycardic reaction evaluated by RR intervals during Music 3 which could represent efficient tool for decreasing stress and load as well as increasing calm effect of music on the organism. These results are in agreement with study Roy *et al.* [35] who observed prolonged mean RR, resp. decreased heart rate, higher rMSSD and CVI during exposition of binaural rotating auditory stimulus. Similarly, higher CVI found Roque *et al.* [36] during exposition of baroque Music compared to silence in healthy adult women. Next, more studies suggested that auditory stimulus/music may be a beneficial effect on cardio-vagal regulation [35,37]. In addition, Nakajima *et al.* [38] revealed that higher-

frequency music plays a bigger role in stress/calm response of organism than lower-frequency music. Moreover, our study confirmed this positive relationship between music and high parasympathetic activity by correlation analysis between vagal indices pNN50 and mean RR and subjective evaluation of pleasantness of music by Likert scale. Further, SampEn was significantly higher during Music 3 compared to Silence 3, and Silence 1. This result could reveal that music with higher-frequency band affect more agreeably to human organisms and that during the exposition of higher-frequency music occur an increasing of complexity, unpredictability, and randomness of system. The result is in agreement with the basic characteristics of SampEn, where higher values of entropy indicate a more complex thus more physiological system [39].

From physiological point of view, we suggest neurophysiological mechanisms underlying HRV and centers for music perception. More specifically, the vagal pathways of this system are influenced by psychophysiological processes, including emotions and hearing music mediated through the central autonomic network [40]. According to the neurovisceral theory, prefrontal cortex tonically inhibits subcortical sympatho-excitatory centers involved in stress-related emotional regulation [41]. It seems that higher-frequency music can reflect relax effect of MT associated with increased prefrontal cortex inhibitory functioning on subcortical sympathoexcitatory centers resulting in higher HRV [42]. Moreover, MT was associated with positive emotions, which was confirmed also by Likert scale in this study.

Next, from the biophysical aspect, music can be defined as a sequence of individual tones of different frequencies with an effect on the ANS [43]. A lot of surveys point to the fact that the patient does not need skills or talent in the field of music in order to benefit from music [44]. Many professional therapists also claim that a relax music can positively affect pain and anxiety resulting from stress and, thus to increase the quality of life of patients and healthy individuals [45]. From a physical viewpoint, the deeper sound is, the lower is its frequency, and the longer is the wavelength. A longer wavelength and greater acoustic energy mean a greater mechanical force of the wave and therefore a more intense perceived vibration. These stimuli in the inner ear create a receptor potential transmitted through the afferent nerve fibres to temporoparietal cortex. The receptor potential causes the synapse potentials in neurons in Corti ganglion, where it is further transmitted

to the brain *via* the auditory nerve in the form of neural excitation. Thus, sensory information about the pitch of sounds is basically spatial information from auditory receptors (hairs cells) located at different places of the organ of Corti [42]. Therefore, we subjectively perceive a variety of different frequencies by different ways. Along to this concept, we propose that musical tracks from the higher frequency range represent less mechanical force, lower vibrations and thus a more beneficial calming effect.

However, due to wide range of frequencies during individual Music phases and relative small group only with selected age, further research in this field of music-therapy frequency composition with HRV analysis may expand knowledge and improve medical practice for all regardless age and gender.

## Conclusions

Music of a specific frequency (Music 2, and Music 3) used in this study revealed a rise of cardio-vagal modulation and higher complexity assessed by short-term HRV indices, thus suggesting its positive relaxing effect on cardiac autonomic regulation. It seems that these findings could contribute to understanding effects of MT on the human organisms as a non-invasive complementary therapy. We suggest that understanding of “sound” autonomic regulation in the concept of passive MT as an important non-pharmacological treatment is crucial for MT recommendation to reduce stress “arousal” in clinical therapy.

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## Limitations of the study

The limitation of the study may be relatively small population sample of healthy volunteers. Another limitation is the relatively wide frequency interval in individual classes. One of the next limitation of this study is missing a complete audiometric examination in volunteers. Partly it was substituted by volunteer’s declaration about no auditory perception disorders. Next, another limitation could represent, that the respiration rate was not controlled in this study. The complementary respiratory rate could bring a novel view of the conventional used HF-HRV index (affected by respiration) during Music track exposition. Also, time is a question – will autonomic regulation change in upon repeated longer therapeutic time exposures? In order to assess the potential benefits of MT, we suggest for further long-term research to look for the long-term effects on patients with repeated therapies [3,18].

## Conflict of Interest

There is no conflict of interest.

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