

## **The impact of sound exposure on heart rate variability in adolescent students – pilot study**

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### **Short title**

Heart rate variability under the impact of sound exposure

### **Summary**

Previous studies of physiological responses to music and noise showed the effect on the autonomic nervous system. The heart rate variability (HRV) has been used to assess the activation of the sympathetic and the parasympathetic nervous systems. The present study was aimed to examine HRV with exposure to four sine-wave pure tones (20 Hz, 50 Hz, 2 kHz and 15 kHz) in an environment where the sound intensity exceeded level 65 dB (A-weighted). The participants (20 adolescent girls) were lying in supine position during exposure protocol divided into 6 periods, the first time with generated sounds and the second time without sounds. In the protocol without sound exposure, the low frequency band of the HRV spectrum

was increased compared to the basal state before examination (period\_1:  $6.05 \pm 0.29 \text{ ms}^2$  compared to period\_5:  $6.56 \pm 0.20 \text{ ms}^2$ ,  $p < 0.05$ ). The significant increase of root Mean Square of the Successive Differences (rMSSD, period\_1:  $4.09 \pm 0.16 \text{ s}$  compared to period\_6:  $4.33 \pm 0.12 \text{ s}$ ,  $p < 0.05$ ) and prolongation of R to R peak (RR) interval (period\_1:  $889 \pm 30 \text{ ms}$  compared to period\_5:  $973 \pm 30 \text{ ms}$ ,  $p < 0.001$ ) were observed in the protocol without sound exposure comparing to the protocol with sound exposure where only bradycardia was observed. Contrary to rather polemical data in literature our findings suggest that sounds (under given frequencies) have no impact on the heart rate variability and cardiac autonomic regulation.

### **Keywords**

Autonomic nervous system, Heart rate variability, Cardiac regulation, Sound, Noise

### **Introduction**

Listening to music is usually a very pleasurable experience for the humans (Dube and Le Bel 2003). Music can be defined as an organization of the tones over the time with a strong impact on the allostatic regulatory mechanisms including autonomic nervous system (ANS) (Riganello et al. 2015). Several studies showed a correlation between listening to music, noise or pure sounds and activity of cardiac autonomic regulation (Perez-Lloret et al. 2014, Orini et al. 2010, Iwanaga et al. 2005).

The cardiac function is extremely sensitive to autonomic regulatory inputs. The heart rate variability (HRV) – perpetual heart rate oscillations around its mean value – reflects the activities of sympathetic and vagal components of the ANS on the cardiac sinus node (Sztajzel 2004). Notably, the heart rate is controlled by a complex regulatory network including a highly integrated neural system known as the central autonomic network (CAN) through which the brain controls visceromotor, neuroendocrine, and behavioral responses (Lane et al. 2009, Benarroch 1993). Furthermore, the Polyvagal Theory (Porges 2007, Porges

1995) describes the integration of neurocardiac regulation through n. vagus with the neural regulation of the middle ear muscles mediated by V and VII cranial nerves to extract human voice from background sounds as an important component of Social Engagement System that is crucial for physiological flexibility and adaptability (Porges 2009, Porges 2007, Porges 2003). Several studies reported cardiac-linked vagal dysregulation in clinical disorders characterized by problems in affective and social behavior such as depression or ADHD (Tonhajzerova et al. 2016, Tonhajzerova et al. 2010, Tonhajzerova et al. 2009). Importantly, the deficits in auditory processing can be manifested in expressive and receptive language skills leading to down-regulated Social Engagement System, such as in autism (Porges 2013a, Porges et al. 2013b). In this aspect, the effect of sounds with various frequencies on HRV was not sufficiently clarified in healthy humans. Sim et al. (2015) studied the impact of noise on HRV in men, with a focus on the noise type and noise intensity. They proved impact of noise on ANS concluding that the effect on the HRV activity might differ according to the noise type. For example, Iwanaga et al. (2005) examined the effect of repetitive exposure to sedative and excitative music on HRV. This study revealed a decrease of cardiac vagal regulation during excitative music; in contrast, another study (Da Silva et al. 2014) concluded that music with different tempos (no music, classical baroque and heavy metal music) did not influence the cardiac autonomic regulation indexed by HRV.

The question regarding the impact of the distinct tone frequency on HRV is still unresolved. The 20 Hz tone frequency is usually considered to be a threshold of humans hearing (Møller and Pedersen 2004) and also lower limit of spectrum analyzing or sound recording devices. The sound range of 2 - 5 kHz is the most sensitive frequency band for a human ear (Kanders et al. 2017, Javorka 2001) while the sounds with frequencies around 15 kHz are more difficult to hear, however, can still cause auditory perception (Bak and Rosniak-Bak 2013). Based on these studies, we hypothesized that exposure to low frequency sounds (20 Hz and 50 Hz)

could increase the parasympathetic nerve activity and decrease the sympathetic nerve activity and exposure to mid and high frequency sounds (2 kHz and 15 kHz) could result in increase of the sympathetic nerve activity and decreased the parasympathetic nerve activity. We aimed to identify the impact of four sine-wave pure tones on ANS by recording of HRV in the environment where the sound intensity exceeded level 65 dB (A-weighted), which is known to have a physiological impact on the human body (Žiaran 2008). To the best of our knowledge, this is the first study to examine the impact of repetitive pure tones on the cardiac autonomic regulation quantified by a short-term HRV analysis.

## **Methods**

### *Subjects*

Twenty adolescent girls in good health condition studying at Viliam Pauliny-Tóth grammar school (average age:  $17.4 \pm 0.2$  years, body mass index (BMI):  $20.9 \pm 0.3$  kg/m<sup>2</sup>) were enrolled in this study. Exclusion criteria were following: acute or chronic diseases potentially influencing HRV (flu, endocrinological or cardiovascular diseases), medications taking, obesity and underweight. Limits for BMI (weight (kg) / height<sup>2</sup> (m<sup>2</sup>)) were determined according to the international standards (centile range within 10<sup>th</sup> and 90<sup>th</sup> centile curves valid for girls at the age of 3 – 18 years) (Cole et al. 2007) and by BMI reference values adjusted for the Slovak population (Ševčíková et al. 2004). The same participants were also used for control measurements without sound exposure. The participants were asked to avoid smoking and drinking alcoholic and caffeinated beverages at least 24 h before the trial.

### *Ethics Statement*

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. All the participants and their parents

were carefully instructed about the study protocol. The written informed consent was admitted prior to the examination.

### *Sound exposure Protocol*

All 20 participants were exposed to four different pure tones generated by functional signal generator Agilent N9310A (Agilent Technologies, Santa Clara, CA). Subjects were examined in a quiet room with the same light intensity under standard conditions (temperature: 22-23 °C). Before examination, the body temperature, height, weight and blood pressure were measured (Misek et al. in press). After 10 min adaptation to the conditions in the examination room, the participants were instructed to lie down comfortably on a special bed and rest in the supine position. To avoid visual stimuli eye mask was fit on participant's head. The exposure protocol was performed in 6 successive periods (Period 1S, Period 2S, Period 3S, Period 4S, Period 5S, Period 6S), where each was at least 5 min long (required for the HRV analysis in time domain). 300 RR long interval was taken for short-term HRV analysis in the frequency domain (Camm et al. 1996).

During the first adaptation period Period\_1S the participants were exposed only to room noise without any generated sounds ( $L_{Aeq}=32.7\pm 0.7$  dB;  $L_{Aeq}$ , equivalent continuous sound level with A-frequency weighting), during Period\_2S, Period\_3S, Period\_4S and Period\_5S the participants were exposed to continuous sine wave tones with frequencies 20 Hz (Period\_2S,  $L_{Aeq}=65.5\pm 0.1$  dB as averaged through all participants), 50 Hz (Period\_3S,  $L_{Aeq}=65.7\pm 0.1$  dB), 2 kHz (Period\_4S,  $L_{Aeq}=65.7\pm 0.2$  dB) and 15 kHz (Period\_5S,  $L_{Aeq}=64.9\pm 0.5$  dB) during 5 min. During the last Period\_6S the participants were exposed only to room noise again without generated sounds ( $L_{Aeq}=32.3\pm 0.9$  dB) (Figure 1). The room noise (in our circumstances) represented sounds with  $L_{Aeq}\leq 35\pm 0.7$  dB which could be generated by any

activities or objects happening or placed outside the examining room but the sounds could be transferred through the walls or closed windows to our examining room.

All the  $L_{Aeq}$  values were being measured for 5 min by integrating-averaging sound level meter Brüel & Kjær Type 2240 (Class 1 precision, Brüel & Kjær, Nærum, Denmark). Soundmeter was mounted on a tripod and located at the distance of 10 cm from the right ear at the level of ears of participants. The minimal distance from the walls and large reflexive surfaces was 3 meters according to the recommendations for free field sound measurement and STN EN ISO 9612 standard. Sounds with the frequencies 20 Hz and 50 Hz were reproduced by 15” active subwoofer Ibiza Sound SUB15A (Lotronic s.a., Saintes, Belgium) and sounds with frequencies 2 kHz and 15 kHz were reproduced by loudspeakers Creative SBS A320 (Creative Technology Ltd, Singapore, Malaysia). For the verification of these reproduced sounds, they were recorded retrospectively by a capacitor microphone BLUE Yeti Blackout (Blue Microphones, Westlake Village, CA) connected to a soundcard in the personal computer. Subsequently, the recorded sounds were processed by fast Fourier transformation and the spectrum was checked for requested frequencies. Subwoofer and loudspeakers were placed 1m from the proband’s head. After each measurement period the sound exposure was interrupted for 2 mins. The body temperature and blood pressure were measured again after sixth period. Six-period measurement protocol was repeated (after 1 week) in all 20 participants during the second examination. However, the sound exposure (20 Hz, 50 Hz, 2 kHz, 15 kHz) was substituted by room noise without generated sounds marked as Period\_1N, Period\_2N, Period\_3N, Period\_4N, Period\_5N and Period\_6N ( $L_{Aeq}=35\pm0.7$  dB) that served as control measurements (Figure 2).

### *HRV measurement*

HRV is very sensitive to any excitement which can modify HRV parameters, heart rate and respiration rate. Therefore, no speaking was permitted during measurement periods (Bernardi et al. 2000). All students underwent one-lead electrocardiograph (ECG) recording during the sound exposure as well as without sound exposure periods. A chest belt with two electrodes was situated on the thoracic segment of midclavicular lines. ECG signal was recorded telemetrically using HRV system DiANS PF8 (Dimeia Group, Olomouc, the Czech Republic). All recordings were subjected to HRV manual filtration to identify and eliminate artifacts and extra-systoles of the heart electric activity according to the generally accepted recommendations (Camm et al. 1996). In ECG records lasting 300 RR intervals, a 256 RR intervals window was used for fast Fourier transformation spectral analysis to determine two distinct regions: the low frequency band (LF: 0.04-0.15 Hz), mainly reflecting the baroreflex activity mediated by both the sympathetic and parasympathetic regulation, and the high frequency band (HF: 0.15-0.5 Hz), reflecting predominantly the activity of the parasympathetic nervous system (Sim et al. 2015). The following HRV parameters were evaluated: RR intervals [ms], root Mean Square of the Successive Differences (rMSSD, [s]) indicating parasympathetic nerve activity, spectral power in low frequency (LF-HRV [ $\text{ms}^2$ ]) and high-frequency bands (HF-HRV [ $\text{ms}^2$ ]) reflecting ANS activity and respiratory rate (brpm, breaths per minute).

### *Statistical analysis*

Data processing was carried out using MS Office Excel and GraphPad InStat (La Jolla, CA) software. All data were analyzed for the Gaussian/non-Gaussian distribution with the Kolmogorov-Smirnov test. The parameters with the non-Gaussian distribution (rMSSD, LF, HF) were log-transformed to obtain the parameters with Gaussian distribution. Consecutively,

Student's paired test was used for comparison of the side differences. The probabilities  $p < 0.05$  were considered significant. The data were expressed as a mean  $\pm$  SEM.

## **Results**

### *Physiological parameters*

The significant decrease of the heart rate was observed in the protocol with sound exposure (before Period\_1S:  $74 \pm 3$  bpm vs. after Period\_6S:  $65 \pm 2$  bpm,  $p < 0.001$ ; bpm, beats per minute) and also during the protocol without sound exposure (before Period\_1N:  $75 \pm 3$  bpm vs. after Period\_6N:  $67 \pm 2$ ,  $p < 0.01$ ). Systolic blood pressure significantly decreased during the protocol without sound exposure (before Period\_1N:  $121 \pm 2$  vs. after Period\_6N:  $116 \pm 2$ ,  $p = 0.05$ ). The respiratory rate was not changed during all periods of protocol with sound exposure as well as during protocol without sound exposure.

### *HRV parameters*

The duration of RR intervals was significantly prolonged in Period\_2S, Period\_3S, Period\_4S, Period\_5S and Period\_6S during sound exposure protocol (compared to Period\_1S,  $p < 0.05$ ), as well as in Period\_3N, Period\_4N, Period\_5N and Period\_6N during protocol without sound exposure (compared to Period\_1N,  $p < 0.05$ ) (Table 1 and Table 2). rMSSD gradually increased from baseline in Period\_1N to Period\_5N towards the significant difference in Period\_6N (Period\_1N vs. Period\_6N,  $p < 0.05$ ).

## **Discussion**

Contrary to the former hypothesis, these findings revealed no significant effect of sounds under different given frequencies on the cardiac autonomic regulation indexed by the HRV parameters. In the protocol without sound exposure, the LF-HRV was increased in the Period\_5N compared to basal state in Period\_1N. The interpretation of the LF component is

controversial and it is considered as a marker of baroreflex activity determined by both sympathetic and parasympathetic regulation (Sim et al. 2015). Thus, we suggest dominant parasympathetic regulatory inputs on the heart rate control as physiological adaptive response to the supine position. Moreover, our results demonstrated the gradual RR intervals prolongation indicating a bradycardic reaction in both protocols – with sound and without sound exposure. In accordance to previous suggestion, it could reflect a shift of resting cardiac autonomic dynamic balance towards parasympathetic augmentation indexed also by the significant increase of the time domain HRV parameter rMSSD in the protocol without sound exposure. Several explanations are assumed.

The supine position represents physiological mechanism where the cardiac-linked vagal efferent inputs are maximal. In this aspect, the short-term HRV is mediated predominantly by the parasympathetic modulation, the sympathetic nervous system is slow to follow beat-to-beat heart rate oscillations (Javorka 2008). Thus, it could explain the increase of the cardiac parasympathetically-linked regulation represented by rMSSD observed in our study during the protocol without sound exposure.

On the contrary, the protocol with sound exposure did not show significant changes in HRV parasympathetic parameters, so we assume a modification of the cardiac autonomic regulation in the supine position as an effect of the sound exposure. Furthermore, the interaction between the cardiac autonomic regulation and auditory stimulation is based on the acoustic startle reflex evoked by loud sounds (around 110 dB) influencing the arterial blood pressure and heart rate as a response to abrupt loud auditory stimulus (Chikahisa et al. 2007). These stimuli are transferred through outer and middle ear on the hair cells in the inner ear where they create a receptor potential transferred on an afferent nerve fiber. The receptor potential causes synapse potentials in receptive terminals of neurons in Corti ganglion and they are transferred to the brain through auditory nerve in a form of the neural excitation (Javorka 2001).

Unlike the previous findings, this study was designed to assess the impact of distinct sound frequencies, rather than the intensity of generated sounds. The major studies investigated the impact of either various types of music or noise. Music is the organization of various pure tones over the time with the evidenced impact on autonomic nervous system (Riganello et al. 2015). The term noise refers to sound with multiple frequencies that is loud or unpleasant or that causes disturbance. The novelty of this study is the examination of pure tones with low and high frequencies and their impact on cardiac autonomic regulation indexed by the HRV parameters. Unlike our former expectations, the exposure to mid and high sound frequencies (2 kHz and 15 kHz) did not result in the increased sympathetic activity and decreased parasympathetic activity. However, significant increase of the rMSSD parameter and prolongation of RR intervals were observed as a physiological adaptive response to the supine position (lasting almost 1 hour) in the protocol without sound exposure contrary to the protocol with sound exposure where only bradycardia was observed. Furthermore, RR intervals parameter can be an indicator for both parasympathetic and sympathetic activity. To the best of our knowledge, this is the first study that examined the effect of sine wave pure tones on the autonomic nerve system in humans.

However, this study has several limitations. The first one is relatively small number of participants, therefore, these results should be validated in a large group. Furthermore, this study included only women, therefore, the gender differences should be investigated to elucidate this question. Regarding HRV parameters, we did not include the LF/HF ratio that is extensively discussed. Despite the fact that the previous studies indicated that the LF-HRV could be used as a possible marker of cardiac sympathetic regulation (Malliani et al. 1991, Pagani et al. 1986), recent evidence showed different results: 1) LF-HRV is substantially blocked by vagal, but not sympathetic blockade, 2) physiological and psychological manipulations increasing sympathetic outflow often do not raise the LF-HRV, but reduce it,

3) pharmacological interventions, e.g. isoprenaline, inducing sympathetic activation do not enhance LF-HRV, 4) there is a lack of associations between LF-HRV and valid indicators of sympathetic cardiac regulation, such as pre-ejection period (Reyes Del Paso 2013). Moreover, the mathematical expression of sympathovagal balance by LF/HF ratio includes the possibility that change of either the numerator or the denominator could result in interpretation that reduction of HF with no change in LF indicates a shift toward sympathetic predominance and reduction of LF with no change in HF express a shift toward vagal predominance (Billman 2013, Eckberg 1997). For these reasons, we did not include the LF/HF in this study. Nevertheless, our study suggests that sounds with frequencies 20 Hz, 50 Hz, 2 kHz and 15 kHz have no impact on the cardiac autonomic regulation analyzed by the heart rate variability. We suggest that this finding could contribute to better understanding of the “sound-autonomic regulation” in healthy subjects providing thus important information for clinical practice (e.g., musicotherapy). Further studies will be required to ascertain the role of various sound frequencies on ANS.

### **Conflict of Interest**

There is no conflict of interest.

### **Acknowledgements**

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**Tables with captions:**

**Table 1 Dynamics of HRV parameters in protocol with sound exposure**

Physiological parameter	Sound exposure					
	Period_1S	Period_2S (20 Hz)	Period_3S (50 Hz)	Period_4S (2 kHz)	Period_5S (15 kHz)	Period_6S
RR [ms]	888±30	909±32 *	937±35 **	944±30 ***	952±32 ***	938±24 ***
rMSSD [ln(s)]	4.20±0.11	4.18±0.11	4.22±0.12	4.17±0.13	4.21±0.12	4.23±0.12
LF-HRV [ln(ms <sup>2</sup> )]	5.91±0.17	5.82±0.16	6.20±0.16	6.26±0.11	6.14±0.20	6.29±0.17 *
HF-HRV [ln(ms <sup>2</sup> )]	7.42±0.2	7.30±0.22	7.37±0.23	7.26±0.24	7.36±0.22	7.40±0.22

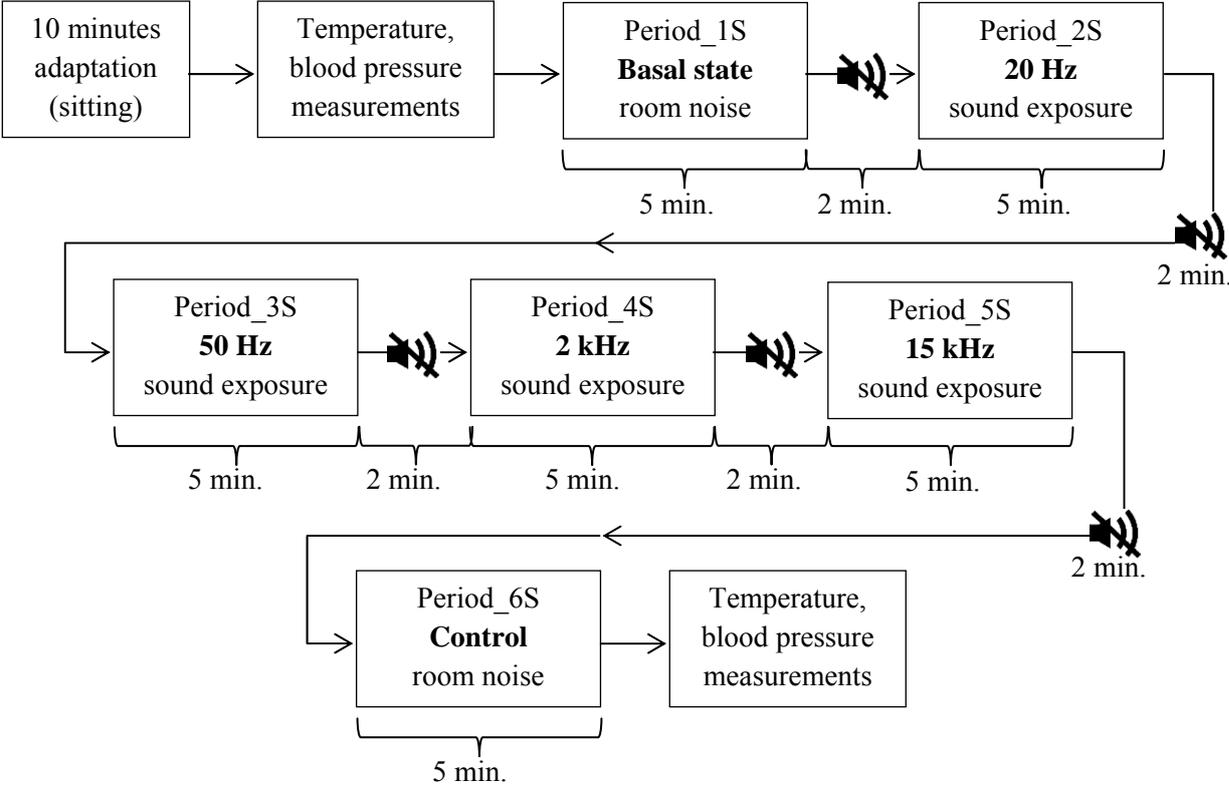
Data are presented as mean±SEM; RR, R to R peak interval [ms]; rMSSD, root Mean Square of the Successive Differences [s]; LF-HRV, low frequency band in spectral analysis [ms<sup>2</sup>]; HF-HRV, high frequency band in spectral analysis [ms<sup>2</sup>]; p values are compared to Period\_1S values; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

**Table 2 Dynamics of HRV parameters in protocol without sound exposure**

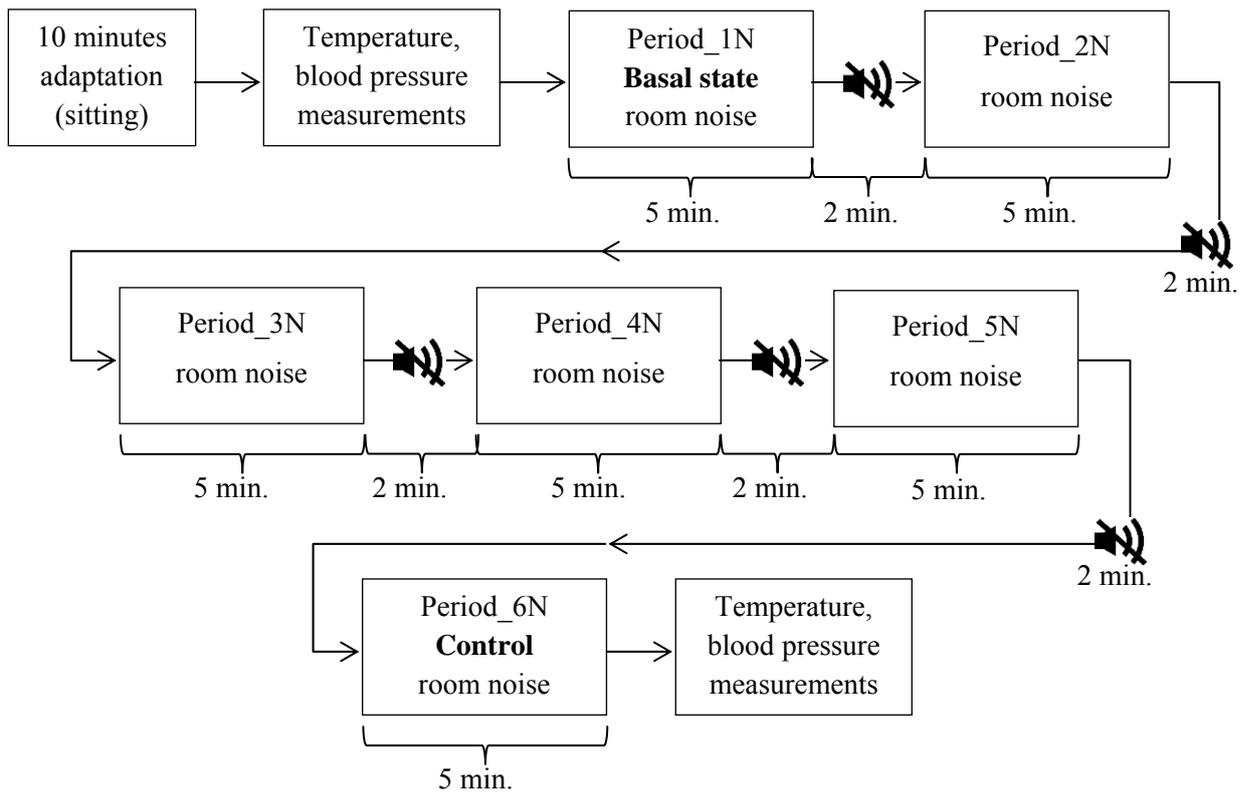
Physiological parameter	Without sound exposure					
	Period_1N	Period_2N	Period_3N	Period_4N	Period_5N	Period_6N
RR [ms]	889±30	919±34	947±32 *	951±31 **	973±30 ***	979±26 ***
rMSSD [ln(s)]	4.09±0.16	4.13±0.16	4.23±0.14	4.27±0.13	4.29±0.14	4.33±0.12 *
LF-HRV [ln(ms <sup>2</sup> )]	6.05±0.29	6.16±0.19	6.20±0.18	6.49±0.16	6.56±0.20 *	6.47±0.18
HF-HRV [ln(ms <sup>2</sup> )]	7.20±0.30	7.25±0.30	7.36±0.25	7.42±0.22	7.46±0.25	7.56±0.23

Data are presented as mean±SEM; RR, R to R peak interval [ms]; rMSSD, root Mean Square of the Successive Differences [s]; LF-HRV, low frequency band in spectral analysis [ms<sup>2</sup>]; HF-HRV, high frequency band in spectral analysis [ms<sup>2</sup>]; p values are compared to Period\_1N values; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

**Figures with captions:**



**Figure 1** Diagram of protocol with sound exposure



**Figure 2** Diagram of protocol without sound exposure