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Associations of Brain Electrical Activity with Heart Rate Regulation and Body Awareness

SUMMARY OF DOCTORAL DISSERTATION

Biomedical sciences,
Biophysics 02 B

Vilnius 2018

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The summary of the dissertation was distributed on 8 October 2018.

The text of this doctoral dissertation can be accessed at the library of Vilnius University, as well as on the website of Vilnius University:
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Galvos smegenų elektrinio aktyvumo sąsajos su širdies ritmo reguliavimu ir kūno pojūčių įsisąmoninimu

DAKTARO DISERTACIJOS SANTRAUKA

Biomedicinos mokslai,
Biofizika 02 B

Vilnius 2018

Disertacija rengta 2014–2018 metais Vilniaus universitete

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Disertacija ginama viešame gynimo tarybos posėdyje 2018 m. lapkričio 8 d. 15 val. Vilniaus universiteto Gyvybės mokslų centro R202 auditorijoje.
Adresas: Saulėtekio al. 7, LT-10257 Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2018 m. spalio 8 d.

Disertaciją galima peržiūrėti Vilniaus universiteto bibliotekoje ir VU interneto svetainėje adresu:
https://www.vu.lt/naujienos/ivykiu-kalendorius

Abbreviations

ACC	anterior cingulate cortex.
aIC	anterior insular cortex.
ANS	autonomic nervous system.
EEG	1) electroencephalogram; 2) electroencephalography.
EKG	1) electrocardiogram; 2) electrocardiography.
ERP	event-related potential.
ERQ	*Emotion Regulation Questionnaire*.
fMRI	functional magnetic resonance imaging.
HCT	heartbeat counting task.
HEP	heartbeat-evoked potential.
HF	high frequency (from 0.15 Hz to 0.4 Hz) power component in heart rate variability spectral analysis.
HRV	heart rate variability.
LF	low frequency (from 0.04 Hz to 0.15 Hz) power component in heart rate variability spectral analysis.
MAIA	*Multidimensional Assessment of Interoceptive Awareness*.
PET	positron emission tomography.
pNN50	proportion derived by dividing the number of interval differences of successive NN intervals greater than 50 ms by the total number of RR intervals.
RMSSD	root mean square of successive differences: a square root of the mean of the sum of the squares of differences between adjacent NN intervals.
RRI	R-R interval.
SDNN	standard deviation of all NN (RR) intervals.
TMS	transcranial magnetic stimulation.

1. INTRODUCTION

Interactions between the heart and the brain are reciprocal: the brain receives interoceptive information (e.g., baroreceptive and other afferent information), the cardiac electromagnetic field and the mechanic blood pressure wave also reach the brain (Dirlich et al., 1997; Kern et al., 2013; Palma and Benarroch, 2014; Suzuki et al., 2012), and the brain sends efferent information through the autonomic nervous system to regulate the heart rate (Palma and Benarroch, 2014; Smith et al., 2017). The current knowledge about the pathways supporting brain-heart interactions is based primarily on studies in the anesthetized animal, while much is unknown about these interactions in the human body (Chang et al., 2016).

The human afferent fibers greatly outnumber efferent fibers in the ANS. This may indicate that the transmission of afferent information is given far greater priority than the efferent information transmission for regulating the inner organs (Vaitl, 1996). In the recent years, studies about brain-heart interactions became more and more popular in the field of *interoception* (e.g., Garfinkel et al., 2015; Khalsa and Lapidus, 2016). To note, the concept of interoception now is different from the concept that had dominated until the end of the 20th century. Previously, interoception was a synonym of visceroception (Ceunen et al., 2016; Vaitl, 1996). In the 21st century, interoception became an umbrella concept for multi-sensory sensations and a multimodal integrated perception of the physiological condition of the body (Ceunen et al., 2016); this broader view into interoception was initiated by Craig (2002). The modified concept of interoception includes visceral sensations (such as the heartbeat), vasomotor flush, hunger, thirst, air hunger, temperature, pain, itch, tickle, sensual/slow touch (not accurate touch), immune, hormonal, metabolic muscular activity (not movement related muscular activity) sensations and other sensations related to the body's state; it, however, does not include proprioception (Craig, 2002; 2003; 2009; Jänig, 2006, p. 36; Grossi et al., 2014). The broadened concept of interoception relies on discoveries about afferent somatosensory pathways (e.g., the lamina I spinothalamocortical pathway), the functions of the insular cortex and the exclusive properties of the insular cortex in humans as compared to animals (Allman et al., 2011; Bauernfeind et al., 2013; Craig, 2002; 2009; Evrard and Craig, 2015). Also, interoceptive awareness is linked with the anterior part of the insular cortex (aIC) (Craig, 2010; 2009). The range of interoception includes areas from reception on the receptor level to a conscious perception of a body's state. For those who are interested in the significance and history of interoception, it is recommended to read and compare works of Brener (1977), Lacey and Lacey (1978), Vaitl (1996), Cameron (2001), Dalgleish (2004), Craig (2002; 2010; 2009), Khalsa and Lapidus (2016), Khalsa et al. (2018), Johnson and Wilson (2018). In this dissertation, *interoception* will refer to the concept proposed by Craig, as it includes and reasonably expands the classic concept of visceroception, interoceptive awareness, unconscious interoceptive sensations and neural processes.

Only a fraction of interoceptive information is perceived consciously – this process is called *interoceptive awareness* (Mehling et al., 2009). The broader concept of *body awareness* (or *somatic awareness*) includes both interoceptive and proprioceptive awareness. There is growing empirical evidence that interoception is a multidimensional construct and different aspects of interoception are needed to be measured simultaneously: behavioral *quantitative* indices about whether or how much a person is aware about body sensations are not necessarily related to *qualitative* self-reports upon experiences of interoceptive states (Garfinkel et al., 2015; Grossi et al., 2014; Mehling et al., 2012). However, until now, there was no Lithuanian questionnaire designed to assess the qualitative aspects of body awareness.

The brain's responses to heartbeats can be detected without direct attention or perception (Shao et al., 2011). Neural brain-heart interactions are reflected in the heartbeat-evoked potentials (HEP) – an electrical activity of the brain that is locked to the heartbeat, usually to the R peak of EKG. HEP were analyzed in various time windows. HEP amplitudes at around 200–350 ms after the EKG R wave (i.e., at around systole) were mostly investigated, found as related to cardiac interoceptive accuracy and interpreted as the central nervous system's representation of sensory information processing (Jones et al., 1988; Leopold and Schandry, 2001; Pollatos et al., 2005; Pollatos and Schandry, 2004; Schandry et al., 1986; Yuan et al., 2007). Meanwhile, the later periods of HEP amplitudes (i.e. at diastole) are not related to cardiac interoceptive accuracy (Pollatos et al. 2016; Schulz et al. 2015). The HEP amplitudes at diastole were associated with other aspects related to interoception, for example, emotions (Couto et al., 2015), the dysregulation of emotions (Müller et al., 2015) and the self (Babo-Rebelo et al., 2016a; 2016b). However, the interpretation of the HEP amplitudes at diastole remains ambiguous, and the associations of these HEP amplitudes with the qualitative aspects of interoceptive awareness were not investigated. The early components of event-related potentials (ERP) may be sensory (related to the physical parameters of the stimulus), while later components may be related to the evaluation of stimuli (Sur and Sinha, 2009). HEP is suggested to follow this assumptions about ERP: an "event" is a heartbeat, and the "evaluation" could be related to individual qualitative differences in the awareness of body sensations.

The insular cortex and the anterior cingulate cortex (ACC) are among the major neural sources of HEP at both systole and diastole (Müller et al., 2015; Pollatos et al., 2005). The significance of aIC and ACC is visible from the phylogenetic point of view: these two neural structures together form a functional network that is one of two or three human resting-state networks having no functional correspondents in the brains of monkeys (Mantini et al., 2013). Besides, aIC and ACC together form the core of the human salience network (Goulden et al., 2014; Guo et al., 2016; Menon, 2015;

Uddin, 2015), which is crucial for maintaining the ANS basal parasympathetic outflow (Guo et al., 2016).

Knowledge about the influence that the brain areas higher than the brainstem have on heart regulation are primarily based on correlations between heart rate variability (HRV) and brain activity measured by functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) methods as well as on the cardiac manifestations of neurologic disorders (Chang et al., 2016; Nagai et al., 2010; Palma and Benarroch, 2014; Silvani et al., 2016; Smith et al., 2017; Thayer et al., 2012). The temporal relationship between natural changes in activity within the higher brain areas and changes in the *resting state* heart rate were found in several fMRI studies (Valenza et al., 2017; Wu and Marinazzo, 2016; Ziegler et al., 2009). However, the understanding of the temporal involvement of brain areas higher than the brainstem in the regulation of the heart rate timing is still limited, as both fMRI and PET methods have poor temporal resolution; meanwhile, EEG and EEG-based methods offer the advantage of monitoring brain activity with high temporal resolution. Nevertheless, it remains unclear whether the control of the heart rate by brain areas higher than the brainstem changes noticeably across the cardiac cycle during the resting states. Associations between HRV parameters and HEP amplitudes during systole were already investigated in two studies (Huang et al., 2017; MacKinnon et al., 2013); however, there are no published studies about the associations between HRV parameters and HEP amplitudes during diastole.

Aim:

To investigate the associations that the brain's electrical activity has with heart rate regulation and body awareness.

Tasks:

1. To prepare a Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* questionnaire and to assess its psychometric characteristics.

2. To investigate the associations between the heartbeat-evoked potentials' amplitudes at diastole and subjectively evaluated body awareness.
3. To investigate the associations between the heart rate and the electrical activity of the brain:
    a) To compare the heartbeat-evoked potentials' amplitudes before the deceleration of the heart rate with the amplitudes before the acceleration of the heart rate;
    b) To evaluate the associations between heartbeat-evoked potentials' amplitudes at diastole and heart rate variability.

Statements to be defended:

1. The psychometric characteristics of the Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* questionnaire are acceptable for scientific purposes.
2. Subjectively evaluated body awareness is differently/diversely associated with heartbeat-evoked potentials.
3. Heartbeat-evoked potentials are associated with heart rate regulation:
    a) heartbeat-evoked potentials' amplitudes differ before the deceleration and acceleration of the heart rate;
    b) heartbeat-evoked potentials' amplitudes at diastole are associated with heart rate variability parameters.

Scientific novelty:

1. *The Lithuanian version of the Multidimensional Assessment of Interoceptive Awareness* questionnaire was prepared to evaluate the tendencies and abilities of body awareness.
2. The relationship between heartbeat-evoked potentials at diastole and subjectively evaluated body awareness was investigated for the first time.
3. Heartbeat-evoked potentials were compared before the deceleration and acceleration of the heart rate for the first time.
4. Heartbeat-evoked potentials' amplitudes at diastole were associated with heart rate variability for the first time.

2. METHODS

2.1. Translating the *Multidimensional Assessment of Interoceptive Awareness* and assessing its psychometric characteristics

The *Multidimensional Assessment of Interoceptive Awareness* (MAIA) questionnaire consists of 32 items that are grouped into 8 scales. The scales are created to identify the following aspects: 1) *Noticing* – an awareness of uncomfortable, comfortable and neutral body sensations; 2) *Not-distracting* – a tendency not to ignore or distract oneself from the sensations of pain or discomfort; 3) *Not-worrying* – a tendency not to worry or experience emotional distress with the sensations of pain or discomfort; 4) *Attention regulation* – an ability to sustain and control one's attention to body sensations; 5) *Emotional awareness* – an awareness of the connection between body sensations and emotional states; 6) *Self-regulation* – an ability to regulate distress by attention to body sensations; 7) *Body listening* – an active listening to the body for insight; 8) *Trusting* – an experience of one's body as safe and trustworthy.

The MAIA was translated from English into Lithuanian independently by the author of dissertation, Aida Grabauskaitė and with the help of a hired translator from a translation agency. Both researchers had discussed the translations to choose the most proper forms of items. Consultations with the authors of the MAIA via email concerning the proper translation of some items were arranged. A pretest was performed in a sample of 32 students of kinesiotherapy at Vilnius University. The Lithuanian MAIA version was translated back into English by an independent translator and was sent to the original authors of the MAIA. The final Lithuanian MAIA version (MAIA^{LT}), approved by the original authors, was published online at www.osher.ucsf.edu/maia/.

To assess the psychometric characteristics of MAIA^{LT}, 376 respondents (184 females and 192 males), aged 18–30 years, filled in the questionnaire. Few respondents later participated in the study of interoception and filled this questionnaire in again – in this case, we used the data we gathered on the second time. Cronbach's alpha was calculated to evaluate the reliability of internal consistency. A confirmatory analysis was performed using *cfa* and *sem* extensions for *R* statistics package. The fitness of the confirmatory factor analysis was evaluated in accordance with four fit indices: RMSEA, SRMR, IFI and CFI.[1]

2.2. The main study of interoception

2.2.1. Participants

The study sample consisted of 39 volunteers (19 females and 20 males), aged 18–30 years. The volunteers had no history of heart activity-related disorders and were not taking any medication that might affect their mental or physiological processes.

2.2.2. Psychophysiological measurements

The *WaveGuard* EEG cap were placed and signals from sixteen EEG electrodes were registered according to the International 10–20 system (see Figure 2.1). The recording reference was Cz, later computationally re-referenced with the average of mastoids (M1 and M2). The ground electrode was placed at AFz. EKG electrodes were placed on the forearms near the wrists. EOG electrodes were used for the detection of horizontal and vertical eye movements. Electrical signals were recorded using the *ASA-Lab* (*ANT Neuro*, the Netherlands) system. The signals were recorded using a 1024 Hz sampling rate.

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[1] RMSEA – Root Mean Square Error of Approximation,
SRMR – Standardized Root Mean square Residual,
IFI – Incremental Fit Index,
CFI – Compar ative Fit Index.

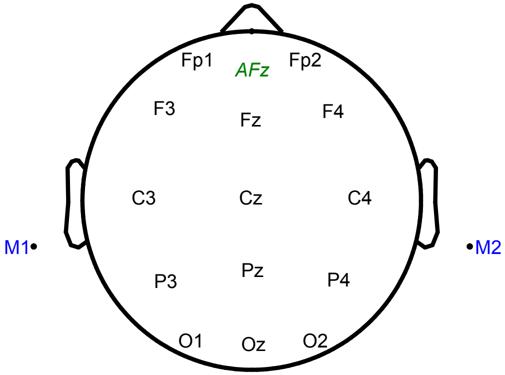

Figure 2.1. EEG electrode placement scheme in the main study of interoception.

2.2.3. Experimental procedure

Upon arrival, the participants filled in a form of informed consent, a questionnaire on their demographic data, health and mental state due to any criteria that would prevent their inclusion in the study and the MAIA^{LT} questionnaire. Participants were seated in a chair ~ 0.8 m in front of the LCD screen in an electrically shielded room where they completed all subsequent tasks.

Participants were asked to sit still and fixate on a bright gray cross against a black background on the computer screen for 10 min (resting state condition). For the evaluation of interoceptive accuracy, the participants performed a modified version of the Schandry (1981) heartbeat counting task (HCT) (see full dissertation for more details). Psychophysiological data were recorded during the resting state and during HCT.

2.2.4. The processing of psychophysiological data

For offline data processing, all psychophysiological signals were FIR band-pass filtered between 0.3 and 30 Hz using EEGLAB for

MATLAB (Delorme and Makeig, 2004) and custom MATLAB scripts. Later, R peaks in the EKG recording were identified using a modified Pan-Tompkins (1985) algorithm, and event markers for each R peak were added to EKG and EEG. EEG and EOG were further subjected to manual cleaning. Independent component analysis (ICA) was used to identify and remove horizontal and vertical eye movements. Noisy EEG channels were interpolated.

EEG signals were cut into epochs of 800 ms: from −200 ms to 600 ms relative to the R peaks. All EEG epochs were baseline-corrected by subtracting the mean of the baseline period (window from −200 ms to −50 ms relative to R peaks). Epochs were rejected from the analysis if the voltage had exceeded ± 50 µV in any of the channels. Only epochs created to R peaks with the distance to the next R peak of 750 ms or longer were included into further analyses. These EEG epochs were averaged into heartbeat evoked potentials (HEP).

2.2.5. Statistical analysis

2.2.5.1. Analysis of heartbeat-evoked potentials (HEP)

Mean HEP amplitudes of 12 channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2) in a 400–600 ms time window relative to the EKG R peak were compared across hemispheres – left (F3, C3, P3, O3), midline (Fz, Cz, Pz, Oz), right (F4, C4, P4, O4) – and four areas – frontal (F3, Fz, F4), center (C3, Cz, C4), parietal (P3, Pz, P4), occipital (O3, Oz, O4) – with repeated measures ANOVA analysis and the participants’ genders being included as a between-subject variable. The sphericity assumption was checked with the Mauchly sphericity test, and a Greenhouse-Geisser adjustment was applied when necessary.

2.2.5.2. Analysis of heart rate variability (HRV)

For the heart rate variability (HRV) analysis, 5 min of the representative EKG from the end of the recordings were selected. Kubios HRV 2.2 software (Tarvainen et al., 2014) was used to obtain the mean heart rate and HRV parameters: three time-domain HRV parameters – the standard deviation of R-R intervals (SDNN), the square root of mean-squared successive heart period differences (RMSSD) and the proportion derived by dividing the number of the interval differences of successive NN intervals greater than 50 ms by the total number of RR intervals (pNN50); four frequency-domain HRV parameters after spectrum calculation using the fast, Fourier transform-based Welch's periodogram method – total power (< 0.4 Hz), power in the low frequency range ($0.04 \text{ Hz} \leq \text{LF} < 0.15 \text{ Hz}$), power in the high frequency range ($0.15 \text{ Hz} \leq \text{HF} < 0.4 \text{ Hz}$) and the LF/HF ratio; two nonlinear HRV parameters – approximate entropy and sample entropy (Berntson et al., 2007; Task Force, 1996). Time-domain and frequency-domain HRV parameters were log-transformed (base *e*).

2.2.5.3. The associations of HEPs with other variables

HEP amplitudes across 12 channels (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, O2) and 204 time samples (in the time window from 400 to 600 ms after the R peak) are significantly associated with other variables (e.g., the MAIA scores). A cluster-based permutation tests (Maris and Oostenveld, 2007) on the two-tailed t-scores were performed in a "Mass Univariate ERP Toolbox" (Groppe et al., 2011) to identify these time windows and scalp areas. A cluster-based permutation test allows to correct for multiple comparisons over the multiple time samples and channel locations.

In the case of a correlational analysis between HEP amplitudes and other variables, the t-values of Pearson correlation were used (the initial code of "Mass Univariate ERP Toolbox" that used the

Student t-test was changed); a similar approach was used in other HEP studies (e.g., Babo-Rebelo et al., 2016b).

As the cluster-based permutation analysis was run on the low number of electrodes, no electrode clusters were formed (i.e., all 12 electrodes were treated as distinct); however, all t-scores corresponding to the uncorrected p-values of 0.05 or less were grouped into clusters on the basis of temporal adjacency. To establish the likelihood that a cluster was obtained by chance, the HEP difference's waveforms were shuffled 20 000 times with respect to the relevant variable (e.g., an HRV parameter or a score of the MAIA scale). The remaining parameters for the "Mass Univariate ERP Toolbox" remained at default values. The sum of the t-scores in each cluster is the "mass" of that cluster. The most extreme cluster mass in each of the sets of tests was recorded and used to estimate the distribution of the null hypothesis. The permutation cluster mass percentile ranking of each cluster from the observed data was used to derive its p-value (i.e., the *permutation's conditional p-value*).

If there was a HEP cluster where the amplitudes had differed between heartbeat perceivers groups, then mean amplitudes in the cluster were calculated. If there was a cluster of correlations, a Pearson r or Spearman ρ correlation coefficient would then be calculated between the mean HEP amplitude in the cluster and the relevant variable (e.g., an HRV parameter or a MAIA scale score).

2.2.5.4. HEP differences between heart rate acceleration and deceleration

The grouping of EEG epochs was based on the duration of EKG R–R intervals (RRI) and their distance from the epoch under consideration. First, four scenarios based on the distance of RRI from the considered epoch were elaborated (see Figure 2.2):

- in the "Not shifted" ("Shifted by 0") scenario, the assignment of a particular epoch was performed after comparing two RRIs overlapping this particular epoch (i.e., RRI_{-1} and RRI_0);

- in the “Shifted by –1” scenario, the assignment of a particular epoch was performed after comparing the RRI before the epoch (i.e., RRI$_{-2}$) and the RRI overlapping the first 200 ms of the epoch (i.e., RRI$_{-1}$);
- in the “Shifted by +1” scenario, the assignment of a particular epoch was performed after comparing the RRI overlapping this epoch at the highest degree (i.e., RRI$_0$) and the subsequent RRI (i.e., RRI$_{+1}$);
- in the “Shifted by +2” scenario, the assignment of a particular epoch was performed after comparing the RRI appearing after this epoch (i.e., RRI$_{+1}$) and the subsequent RRI (i.e., RRI$_{+2}$).

Second, all EEG epochs within the scenario were classified into two subgroups depending on the duration of the adjacent RRIs: 1) epochs related to the RRI longer than the previous RRI (*RRI*+

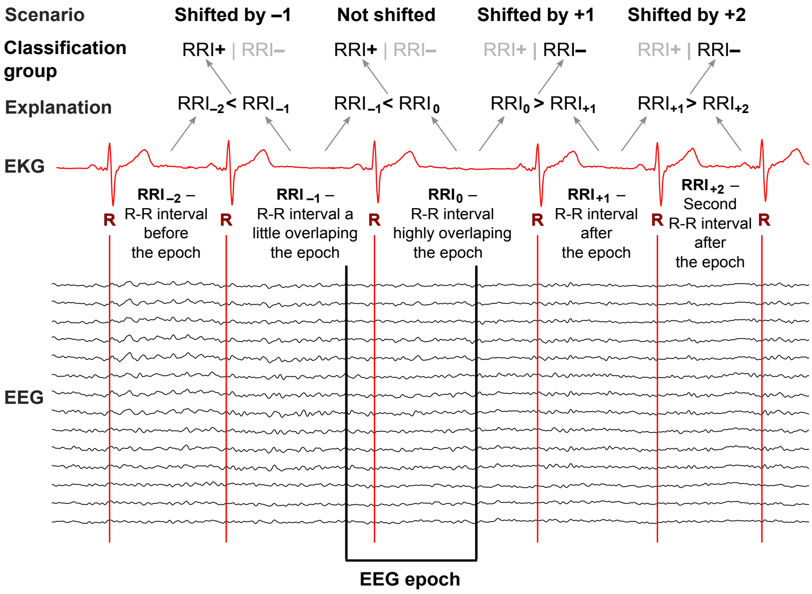

Figure 2.2. An example of the decision-making process during the classification of a particular EEG epoch into a RRI+ or RRI– group in “Shifted by –1”, “Not shifted” (“Shifted by 0”), “Shifted by +1” and “Shifted by +2” scenarios. Gray text marks non-assigned groups.

subgroup, i.e., heart rate deceleration); 2) epochs related to the RRI shorter than the previous RRI (*RRI– subgroup*, i.e., heart rate acceleration). However, epochs were not assigned to any subgroup if the duration of the adjacent RRIs was equal. Such a classification resulted in eight groups of epochs for each subject. The epochs were averaged into HEPs, resulting in eight HEP groups correspondingly.

Third, for each scenario separately, difference's waveforms (for every subject and EEG channel) were computed by subtracting the HEP for a RRI– subgroup from the HEP for a RRI+ subgroup. These difference's waveforms were further used for statistical evaluation.

To identify the time windows and scalp areas where the brain activity, as reflected in the HEP amplitudes, had significantly differed between the RRI+ and RRI– tendencies, a cluster-based permutation statistical analysis on the two-tailed Student t-scores across 12 channels and 512 time samples (in the time window from 100 to 600 ms after the R peak) was performed in a "Mass Univariate ERP Toolbox" similarly as described in the above chapter.

2.2.5.5. Other aspects of statistical analysis

Outliers were defined as data less than $Q_1 - 3 \times IQR$ or more than $Q_3 + 3 \times IQR$[2]. The significance level was set to $\alpha = 0,05$. The statistical analysis was performed using *R, IBM SPSS Statistics 22* statistics packages and *MATLAB*.

2.3. Supplementary study with males

A supplementary study of interoception with males was conducted, as males and females differed according to various aspects of interoception; however, more males' data were rejected from study of interoception due to various reasons.[3] Also, it was noticed that the strongest associations between HEP and the aspects

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[2] Q_1 – first quartile, Q_3 – third quartile, IQR – interquartile range.

[3] See full dissertation for more detailed explanations.

of body awareness (as evaluated by the MAIA^{LT}) can be linked to emotion regulation.

2.3.1. Participants

The study sample consisted of 20 male volunteers aged between 20 and 31 years. The volunteers had no history of heart activity-related disorders and were not taking any medication that have might affected their mental or physiological processes.

2.3.2. Psychophysiological measurements

For the psychophysiological evaluation, EEG, EOG and EKG signals were registered with the same *ASA-Lab* system and the same parameters as in the main study of interoception, except that the EEG signal was registered at 64 scalp sites (see Figure 2.3). The reference was the average of mastoids (M1 and M2). In addition, belts were placed on the abdomen and respiration was registered; however, respiration data will not be further mentioned in the summary of this dissertation.[4]

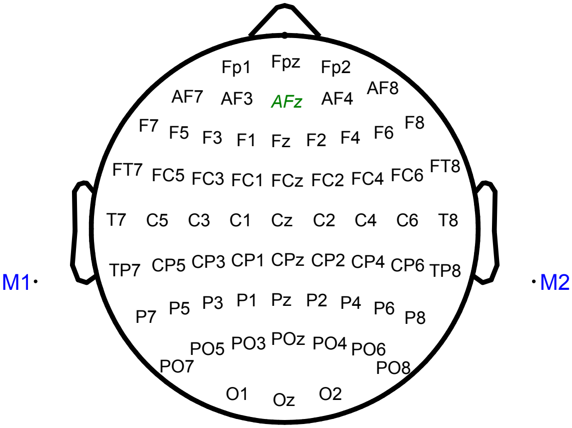

Figure 2.3. EEG electrode scheme in the supplementary study.

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[4] See full dissertation for respiration registration and results.

2.3.3. Emotion Regulation Questionnaire

The *Emotion Regulation Questionnaire (ERQ)* consists of 10 items grouped into two scales and is designed to assess individual differences in the habitual use of two emotion regulation strategies: *cognitive reappraisal* and *expressive suppression* (Gross and John, 2003). Cognitive reappraisal is defined as a form of cognitive change that involves construing a potentially emotion-eliciting situation in a way that changes its emotional impact. Expressive suppression is defined as a form of response modulation that involves inhibiting ongoing emotion-expressive behavior.

Maslenikova and Bulotaitė (2013) translated the ERQ into Lithuanian and assessed its psychometric characteristics. The Lithuanian version of the ERQ showed the appropriate indicators of reliability (internal consistency) for scientific purposes (Cronbach's $\alpha \geq 0{,}6$), with a Cronbach's $\alpha = 0.804$ for *Cognitive reappraisal* and Cronbach's $\alpha = 0.662$ for *Expressive suppression* scale.

2.3.4. Experimental procedure

Upon arrival, the participants filled in a form of informed consent and a questionnaire on their demographic data, health and mental state due to any criteria that would prevent their inclusion in the study. Then, the participants were seated in a chair in an electrically shielded room and asked to sit still and fixate on a bright gray cross against a black background on the computer screen for 5 min (resting state condition). Subsequently, emotional images and sounds were presented, and participants were asked to evaluate them (this task was the interest of another researcher and the data gathered during this part of the procedure were not included in this dissertation). During the break of this task, participants filled in the Lithuanian versions of the MAIA and ERQ questionnaires. Finally, the participants were again asked to sit still and fixate on a gray cross against a black background on the computer screen for 5 min. EEG, EOG, EKG and respiration were being registered during the whole session.

2.3.5. The processing of psychophysiological data

The processing of psychophysiological data was similar to how processing was conducted in the earlier study on interoception (see Chapter 2.2.4). Data gathered from 12 electrodes near the border of the EEG cap were excluded from the EEG analysis; thus, data from 50 electrodes remained in the EEG analysis.

2.3.6. Statistical analysis

Statistical analysis was similar to the analysis in the earlier study of interoception (see Chapter 2.2.5).

Statistical analysis was done on the data merged from both of the mentioned studies. EEG data had a different number of channels both in the main study of interoception and in the supplementary study; thus, data from 12 EEG channels (same as in the study of interoception) were selected for the HEP analysis of merged data.

The studies of this dissertation were part of a larger study that was approved by the Vilnius Regional Biomedical Research Ethics Committee (license 6B-12-240 issued in 2012-07-11).

3. RESULTS

3.1. HEP

Heartbeat-evoked potentials (HEP) reflecting the electrical activity of the brain are the core of this dissertation. After merging psychophysiological data from the main study of interoception and the supplementary study, the data from both studies (43 participants) was used in the grand analysis. The grand averaged topography plot of HEP in 400–600 ms window is presented in Figure 3.1.

The evaluation of the area (frontal vs. central vs. parietal vs. occipital) and the hemisphere (left vs. midline vs. right) using a 4 × 3 ANOVA analysis indicated that mean HEP amplitudes in the 400–600 ms window differed across the anterior-posterior direction ($F_{2.1, 89.3} = 7.500$, $p < 0.001$, $\eta^2 = 0.152$, see Figure 3.2). The *post hoc* comparison revealed that amplitudes at the central ($M = 0.15$, $SE = 0.09$, $p = 0.002$) and parietal ($M = 0.20$, $SE = 0.09$, $p = 0.005$) areas were more positive as compared to the frontal area ($M = -0.16$, $SE = 0.10$). No significant effects of the hemisphere ($F_{2, 84} = 0.119$, $p = 0.888$, $\eta^2 = 0.003$) or interaction between the area and the hemispheric factors ($F_{3.96, 166.4} = 0.441$, $p = 0.851$, $\eta^2 = 0.010$) were observed. The gender factor did not influence the results ($F_{1, 40} = 0.17$, $p = 0.896$).

HEP topography (see Figure 3.2) at diastole is dissimilar to the topography of cardiac field projections in the scalp (Dirlich et al., 1997). Therefore, within the analyzed time window, the source of the observed HEP should lie in neural activity.

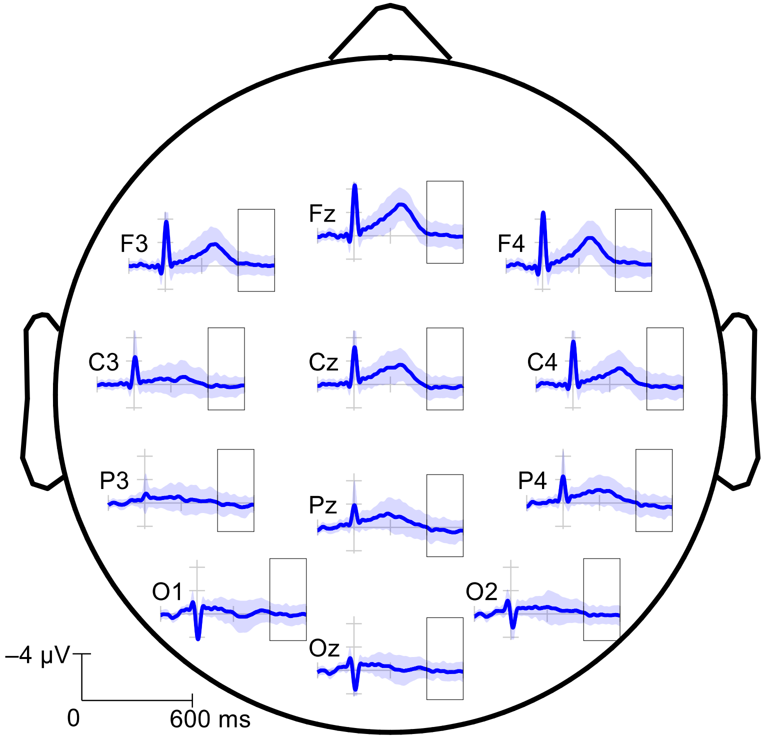

Figure 3.1. A topographical representation of grand-averaged HEP waveforms (N = 43). The shaded area denotes standard deviation. The frame indicates a 400–600 ms time window of analysis.

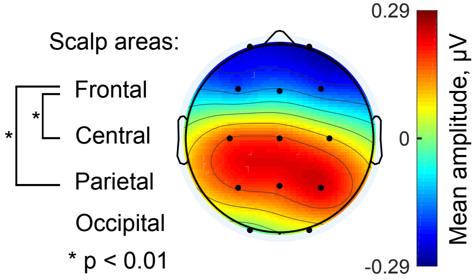

Figure 3.2. A topographical representation of the mean HEP amplitudes in a 400–600 ms time window relative to the EKG R peak (N = 43). The significant differences between amplitudes across the frontal (F3, Fz, F4), central (C3, Cz, C4) and parietal (P3, Pz, P4) areas are marked with *.

3.2. Subjectively evaluated body awareness

3.2.1. The psychometric characteristics of Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* questionnaire

The tendencies and abilities of body awareness were subjectively evaluated using the Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* (MAIA^{LT}).

Reliability as the internal consistency of the MAIA^{LT} was acceptable (i.e., Cronbach's $\alpha \geq 0.7$) for five out of eight scales – *Attention regulation, Emotional awareness, Self-regulation, Body listening* and *Trusting*. Reliability for the *Not-worrying* scale (Cronbach's $\alpha = 0.632$) was questionable ($0.7 > \alpha \geq 0.6$) yet still sufficient for scientific purposes. Reliability for the *Not-distracting* and *Noticing* scales was poor ($\alpha < 0.6$) and unacceptable ($\alpha < 0.5$) respectively; consequently, these two scales were not included into further analysis.

For the evaluation of the validity of the MAIA^{LT} structure, a confirmatory factor analysis was performed after rejecting the *Not-distracting* and *Trusting* scales. The confirmatory factor analysis showed that a six-factor solution (i.e., with the remaining six scales) provided an adequate fit to the data ($\chi^2 = 760.91$, df = 260, $p < 0.001$; RMSEA = 0.072 and SRMR = 0.072), so these two fit indices were acceptable (≤ 0.08). Moreover, IFI = 0.852 and CFI = 0.850, so these two fit indices were close to acceptance. Therefore, a six-factor solution supports the original structure of the MAIA with six scales.

Overall, the psychometric characteristics of the MAIA^{LT} with six out of eight scales – *Not-worrying, Attention regulation, Emotional awareness, Self-regulation, Body listening* and *Trusting* – are sufficient for scientific purposes.

3.2.2. HEP associations with the qualitative aspects of body awareness

According to a cluster-based permutation test, statistically significant correlations were observed between HEP amplitudes and scores on the *Not-worrying* and *Emotional awareness* scales of the MAIA^{LT} (Figure 3.3). In the males' subsample (N = 26), the statistically significant clusters of correlations were observed between HEP amplitudes at frontal scalp sites and scores on the *Attention regulation* and *Self-regulation* scales of the MAIA^{LT} (see Figure 3.3). In the females' subsample (N = 16), no statistically significant clusters of correlations were observed between the HEP amplitudes and scores on the MAIA^{LT} scales; however, in this subsample, the permutation test indicated a tendency for a cluster of correlations to exist between HEP amplitudes at centroparietal areas and scores on the MAIA's *Not-worrying* scale (see Table 3.1). Generally, the electrical activity of the brain (as evaluated by HEP amplitudes) correlated with four aspects of body awareness that had been subjectively evaluated using the MAIA questionnaire. In most cases, these correlations between HEP and the qualitative aspects of interoception differed between males and females.

Table 3.1. Scalp sites and time windows where the correlation's clusters were identified between HEP amplitudes and MAIA scores according to a cluster-based permutation test in the **females'** subsample (N = 16). Clusters with permutations' conditional p-values ≤ 0.1 are listed.

MAIA scale	Scalp site	Time interval, ms	Permutation's conditional p-value	Correlation's coefficient	Correlation's p-value
Not-Worrying	Cz	452–531	0.100	r = 0.691	0.0030
	P4	498–599	0.097	r = 0.581	0.018

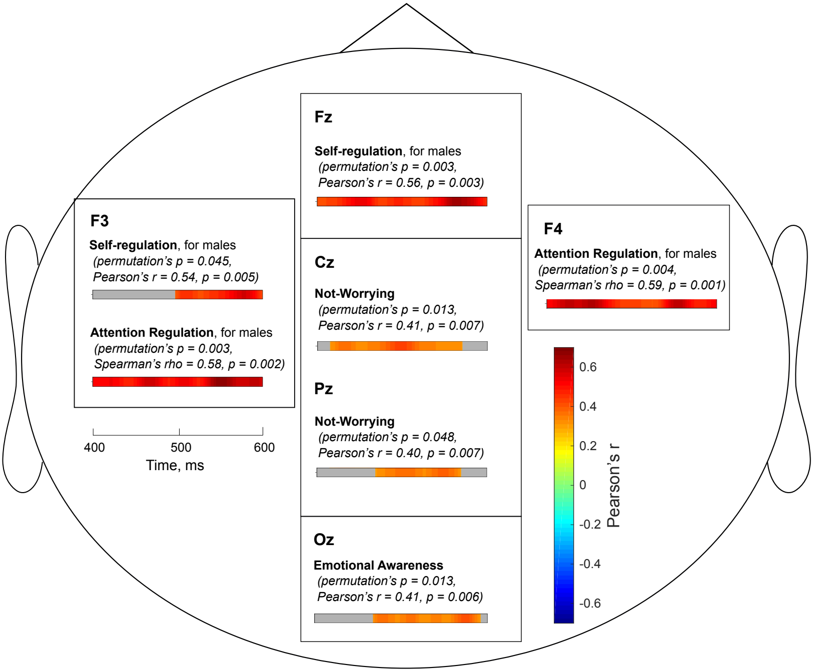

Figure 3.3. Overview scalp sites (frames) and time windows (colorful bars) where a cluster-based permutation test identified statistically significant clusters of correlation between HEP amplitudes and MAIA scores in all samples and genders' subsamples. The brackets contain the permutation's conditional p-values and correlation's values between mean HEP amplitudes in cluster and MAIA scores. Colors within the colorful bars depict Pearson's correlations at clusters' time points.

3.3. HRV

According to the cluster-based permutation test, statistically significant associations were observed between HEP amplitudes at the frontal areas and three heart rate variability (HRV) parameters: pNN50, HF and LF/HF ratio (see Figure 3.4). In the males' subsample (N = 25), no statistically significant associations were observed between the HEP amplitudes and HRV parameters. In the

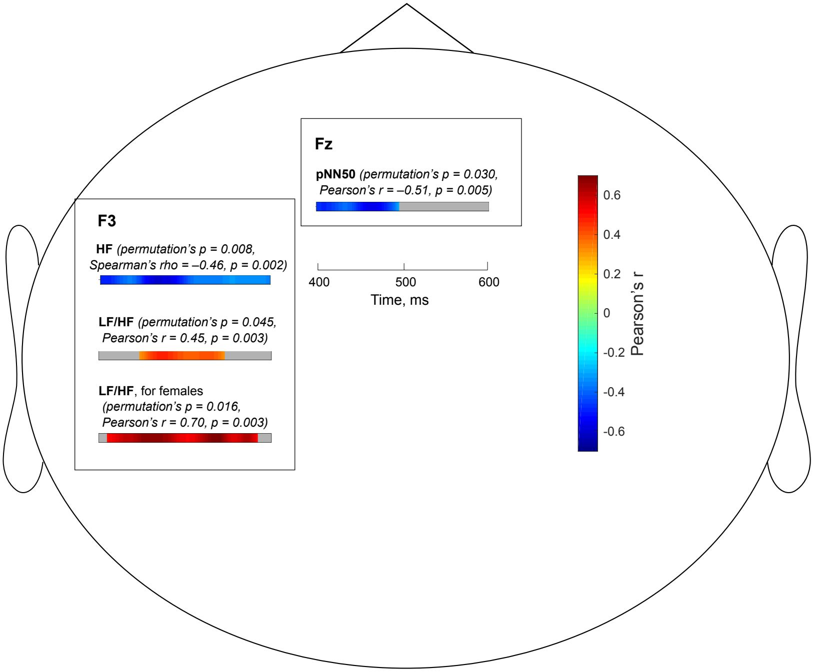

Figure 3.4. An overview of the scalp sites (frames) and time windows (colorful bars) where a cluster-based permutation test identified statistically significant clusters of correlation between HEP amplitudes and HRV parameters in all samples and genders' subsamples. The brackets contain the permutation's conditional p-values and correlation's values between mean HEP amplitudes in cluster and HRV parameter. Colors within the colorful bars depict Pearson's correlations at clusters' time points.

females' subsample (N = 16), statistically significant associations were observed between HEP amplitudes at the frontal areas and the LF/HF ratio. Generally, the HEP amplitudes at the frontal areas were associated with those HRV parameters that indicate rapid heart rate regulation via the autonomic nervous system (ANS).

3.4. HEP differences between heart rate acceleration and deceleration

The sequence of EKG R–R intervals (RRI) was used to identify heart rate acceleration and deceleration, assign EEG epochs into RRI– and RRI+ groups correspondingly and create the HEP difference's waveforms of these two groups. After merging the psychophysiological data derived from both the main study of interoception and the supplementary study, the HEP difference's waveforms of 46 participants (28 males, 18 females) were analyzed using a cluster-based permutation test.

In the "Not shifted" ("Shifted by 0") scenario, the HEP amplitudes in the middle of diastole (around 380–600 ms after the R peak) were more positive at centroparietal scalp sites in the RRI+ subgroup as compared to amplitudes in the RRI– subgroup according to a cluster-based permutation test (see Table 3.2 and Figure 3.5).

In the "Shifted by –1" and "Shifted by +1" scenarios, the HEP amplitudes between the RRI+ and RRI– subgroups: all cluster-based permutations' conditional p-values ≥ 0.328 for "Shifted by –1" scenario and ≥ 0.767 for "Shifted by +1" scenario.

Table 3.2. Scalp sites and time windows showing significant differences of HEP amplitudes between RRI+ and RRI– subgroups according to a cluster-based permutation test in the "Not shifted" scenario.

Scalp site	Time interval, ms	Permutation's conditional p-value	Mean amplitude difference between RRI+ and RRI– subgroups, μV
Pz	378–599	0.0081	0.731
P3	377–599	0.0084	0.605
Cz	386–599	0.011	0.608
C4	463–599	0.044	0.485
O1	477–599	0.046	0.540

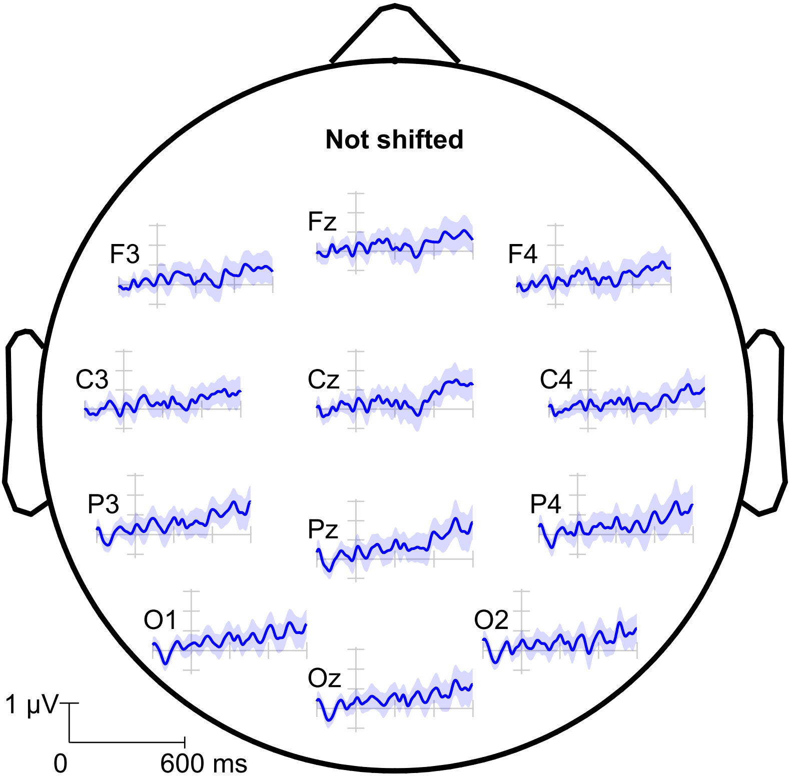

Figure 3.5. A topographical representation of the grand-averaged difference's waveforms that were obtained by subtracting the HEP for the RRI– subgroup (a shorter subsequent RRI) from the HEP for RRI+ (a longer subsequent RRI) subgroup in "Not shifted" scenario. The shaded area denotes a 95% confidence interval. The frame indicates a 100–600 ms time window used in analysis.

In the "Shifted by +2" scenario, the HEP amplitudes of the RRI+ subgroup were more negative in the time window from 330 ms to 458 ms after the R peak over the right frontal area (F4) (permutations' conditional p = 0.041) than the HEP amplitudes of the RRI– subgroup (the median of mean amplitude difference was –0.33 µV).

Generally, the highest differences across the HEP amplitudes between the RRI+ and RRI– groups were observed right before the lengthening of heart cycle (i.e., in the “Not shifted” scenario), and no differences were found when the EEG epochs were shifted by one heart cycle in either direction.

3.5. Emotion regulation strategies

HEP amplitudes were not associated with the *Emotion Regulation Questionnaire (ERQ)* scores according to a cluster-based permutation test ($N = 13$) in the analyzed 50 scalp sites and 400–600 ms time window (a cluster could have been made by spatial (distance ≤ 5 cm) and/or temporal adjacency): all permutations’ conditional p-values ≥ 0.27 for the *Cognitive Reappraisal* scale and ≥ 0.27 for the *Expressive Suppression* scale.

4. DISCUSSION

The aim of this dissertation was to investigate the relations that the electrical activity of the brain has with interoception (viewing it as a multidimensional construct) and heart rate regulation. As compared to other methods, heartbeat-evoked potentials (HEP) possess a good temporal resolution. It must be noted that until now, researchers have been analyzing HEP almost only in the context of interoception (viewing it as unidimensional) and have usually been investigating HEP amplitudes during systole, while this dissertation provides knowledge about HEP amplitudes in terms of the brain-heart interactions at diastole.

Mean HEP amplitudes in the 400–600 ms time window after the EKG R peak were higher (i.e., more positive mathematically, though these amplitudes had both positive and negative absolute values) at the central and parietal areas as compared to the frontal area. This observation is in accordance with studies by Dirlich et al. (1998) and Schulz et al. (2015). Müller et al. (2015) analyzed HEPs at diastole, and their participants had somewhat higher (however non-significant) amplitudes at the vertex. On the contrary, Pollatos et al. (2016) reported higher amplitudes at diastole over the frontocentral sites – however, their reference was Cz, while the studies of this dissertation and the studies of Schulz et al. (2015) chose mastoids for reference, Dirlich et al. (1998) chose the average of the two earlobe-electrodes (close to mastoids), and Müller et al. (2015) chose the average of all EEG electrodes for reference.

4.1. Subjectively evaluated body awareness

4.1.1. Psychometric characteristics of the MAIA^{LT}

The qualitative aspects of body awareness were subjectively evaluated using the participants’ responses about their tendencies and abilities, and this was done using a Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* (MAIA^{LT}) questionnaire.

Reliability as internal consistency for six out of eight scales – *Not-worrying, Attention regulation, Emotional awareness, Self-regulation, Body listening* and *Trusting* – was sufficient for scientific purposes (Cronbach's $\alpha \geq 0.6$) in the MAIA^{LT} and in the MAIA versions of other languages (Bornemann et al., 2015; Brytek-Matera and Kozieł, 2015; Calì et al., 2015; Mehling et al., 2013; Mehling et al., 2012; Valenzuela-Moguillansky and Reyes-Reyes, 2015), although only the *Not-worrying* scale had unacceptable reliability in Italian (Calì et al., 2015) and Spanish (Valenzuela-Moguillansky and Reyes-Reyes, 2015) versions. The *Not-distracting* scale was consistently reported to have had the lowest reliability as internal consistency across almost all versions of the MAIA (Bornemann et al., 2015; Brytek-Matera and Kozieł, 2015; Calì et al., 2015; Mehling et al., 2013; Mehling et al., 2012). Thus, in our study, the poor reliability of the *Noticing* scale and the unacceptable reliability of the *Not-distracting* scale is present presumably not because of the conducted translation into the Lithuanian language or any potential peculiarities of our sample.

The validity of the MAIA^{LT} structure is acceptable with six out of eight scales, i.e., after the rejection of the *Not-distracting* and *Trusting* scales. Other known MAIA studies have evaluated the validity of the questionnaire (Bornemann et al., 2015; Calì et al., 2015; Mehling et al., 2013; Mehling et al., 2012; Valenzuela-Moguillansky and Reyes-Reyes, 2015), and they did not try to reject these two scales.

Overall, the psychometric characteristics of the MAIA^{LT} are similar to the MAIA versions of other languages, and the MAIA^{LT} with six out of eight scales is sufficient for scientific purposes.

4.1.2. HEP associations with subjectively evaluated body awareness

The studies of this dissertation revealed relations between HEP amplitudes and scores on four MAIA^{LT} scales – *Not-worrying, Emotional awareness, Attention regulation* and *Self-regulation*.

In this dissertation, the association between HEP and the MAIA's ***Emotional awareness*** was one of the strongest (r = 0.41) in the total sample (N = 43): higher HEP amplitudes at the occipital area were associated with a better awareness of the connection between body sensations and emotional states.

The remaining associations between HEP amplitudes and the qualitative aspects of interoceptive awareness (i.e., scores on the *Not-worrying, Attention regulation* and *Self-regulation* scales) were different for males and females. Scores on two of the mentioned scales – *Not-worrying* and *Attention regulation* – were previously shown to be among the few scales that formed a factor having neural correlates as assessed by fMRI (Stern et al., 2017).

From all of the analyzed HEP associations with scores on the MAIA, the strongest association was with the ***Not-worrying*** scale at the vertex (r = 0.69) and at the right parietal scalp site (r = 0.58) in the females' subsample. However, the existence of these two clusters was only a tendency according to a cluster-based permutation test – presumably because of the low number of females (N = 16). Otherwise, similar statistically significant clusters were found at the central and parietal scalp sites when the data of both genders were analyzed together, even though the correlations did come out weaker (N = 43, r = 0.4). Higher HEP amplitudes were associated with a lesser tendency to worry or experience emotional distress with the sensations of pain or discomfort. The trait measured by the *Not-worrying* scale reflect the basic ideas of mindfulness training, i.e., cultivating an awareness and a non-judgmental acceptance of body sensations (Farb et al., 2013; Haase et al., 2016; Keng et al., 2011;

Manuello et al., 2016). Mindfulness practices were, in turn, proved to enhance the regulation of emotions as well as reduce stress and anxiety in healthy and clinical populations (Chiesa and Serretti, 2009; Grossman et al., 2004; Hölzel et al., 2013). In line with that, Müller et al. (2015) have showed that higher HEP amplitudes at diastole in the parietal and occipital areas were associated with better emotion regulation (lower scores on *Emotional dysregulation* as measured by the *Difficulties in Emotion Regulation Scale, DERS*). The majority of the *DERS* subscales had strongest associations with the *Not-worrying* scale out of all MAIA scales (Mehling et al., 2012).

Qualities measured by the MAIA's ***Not-worrying***, ***Attention regulation*** and ***Self-regulation*** scales have associations with emotion regulation. However, there were no significant associations between HEP and emotion regulation strategies – cognitive reappraisal and expressive suppression – as measured by the *Emotion Regulation Questionnaire*.

Males and females have different neural mechanisms of emotional processes (including emotion perception and emotion regulation) (Kong et al., 2014; Mak et al., 2009; McRae et al., 2008; Whittle et al., 2011). Gender differences in emotional processes may help in explaining the dissertation's findings that HEPs have different associations with the qualitative aspects of body awareness for genders.

Higher HEP amplitudes at frontal scalp sites were associated with a better ability to control/sustain attention to body sensations (higher scores on the *Attention regulation* scale) and an ability to use this strategy to regulate distress (higher scores on *the Self-regulation* scale) in the males' (not the females') subsample. However, clusters associated with these two MAIA scales did only partially overlap.

In the majority of cases, clusters of the observed HEP associations with specific MAIA^{LT} scales did not overlap in time and scalp sites. Thus, some of the qualitative aspects of interoceptive awareness have neural correlates that presumably are different depending on the particular dimension of interoception. This supports the recent notion that interoception is a multidimensional construct (e.g., Garfinkel et al., 2015; Mehling et al., 2012), and supports the need to evaluate interoception simultaneously using subjective and objective methods (e.g., Grossi et al., 2014).

4.2. Heart regulation

4.2.1. HEP associations with HRV

Relations between HEP amplitudes at diastole and HRV were found in the frontal areas. Other researchers (Gray et al., 2007; Schandry and Montoya, 1996) have noticed that HEP amplitudes at diastole in the frontal areas have associations with mechanical heart parameters. They suggested that the mechanical strength and velocity of myocardial action enhance afferent transmission and further afferent information processing in the brain. However, HRV in general allows to evaluate the tonic activity of ANS efferents. In the studies of this dissertation, lower HEP amplitudes were associated with a higher HRV high frequency (HF) spectral power and pNN50, both indicating higher parasympathetic activity. The same interpretation can be attributed to an observation that higher HEP correlated with a higher ratio between an HRV low and high spectral power (LF/HF). These associations are in line with the mentioned findings on mechanical heart parameters found by Gray et al. (2007), Schandry and Montoya (1996). Thus, according to the findings in this dissertation regarding the relations between HEP and HRV, the observations made by the mentioned researchers could also be re-interpreted as associations with efferent regulation.

Fast changes of the heart rate (HRV HF) are often linked to respiratory sinus arrhythmia (Draghici and Taylor, 2016; Task Force, 1996); however, while speaking with oversimplification, respiration does not enhance parasympathetic activity, while it only suppresses parasympathetic activity at inspiration phase (Dergacheva et al., 2010; Palma and Benarroch, 2014). A higher HF can be attributed to higher parasympathetic tonic activity provided by brain areas higher than the brainstem. For example, the human salience network is crucial for maintaining the ANS basal parasympathetic outflow (Guo et al., 2016).

4.2.2. HEP differences between heart rate acceleration and deceleration

A new approach for analyzing the relation between brain activities and heart rate regulation was used in this dissertation: the heartbeat-based EEG epochs were created, grouped and evaluated in respect to the relative duration (prolongation vs. shortening) of the R–R intervals (RRI) at various distances from the R peak and were used to create the HEP difference's waveform. The contrasting of the prolongation vs. shortening of the RRI allowed to remove the shared artifacts and the afferent sensory information, also enabling the detection of the electrical activity dynamics at brain areas higher than the brainstem that were contributing to the regulation of the heart rate even during the resting state.

After applying this approach, the main finding was as follows: the HEP amplitudes at diastole (380–600 ms after R peak) were higher at the centroparietal areas right before the postponing of the heartbeat (i.e., the prolongation of the RRI corresponding to the HEP epoch under consideration), and no differences were found if the analyses had been shifted by one heart cycle in either direction. Sympathetic activity is slow (Berger et al., 1989; Spear et al., 1979) and is minimal at the resting state, high tonic parasympathetic (vagal) influence dominates (Palma and Benarroch, 2014; Smetana and Malik, 2013); thus, differences between the adjacent RRI can almost entirely be attributed to parasympathetic activity, where even a fast increase in the heart rate during resting conditions is linked to the removal of parasympathetic activity and not to the increase of sympathetic activity. Also, this is in line with the knowledge that the parasympathetic effect on SAN is near immediate (Berger et al., 1989; Spear et al., 1979) and has a notable effect for less than 800 ms (Spear et al., 1979). The results have confirmed our hypothesis that the electrical activity of the brain, as reflected in the HEPs, differs between periods of relatively more vs. less strong parasympathetic activity, i.e., during the prolongation vs. shortening of the RRIs.

4.3. Overview

The findings in this dissertation reveal the associations of HEP amplitudes at diastole with subjectively evaluated tendencies and abilities of body awareness and an ANS efferent regulation of heart rate.

4.4. Limitations

In the studies included in this dissertation, the qualitative aspects of body awareness were assessed only using questionnaires when the participants reflected on their general lives; however, future studies could include behavioral tasks producing particular body sensations. The control of the heart rate is performed through multiple hierarchical levels that could be controlled better. The current experimental setting did not allow for performing source localization in order to identify brain structures involved in HEPs. Studies included in this dissertation had a low number of females; thus, future studies could investigate gender differences after including more females and by expanding the sample with younger and older participants, persons with altered interoception or heart rate regulation and persons that regularly do practices in altering their interoception or heart rate regulation.

CONCLUSIONS

1. The Lithuanian version of the *Multidimensional Assessment of Interoceptive Awareness* with six of the eight scales – *Not-worrying, Attention regulation, Emotional awareness, Self-regulation, Body listening* and *Trusting* – has sufficient psychometric characteristics for use in scientific research.
2. A subjectively evaluated body awareness has associations with heartbeat-evoked potentials at diastole:
    a) higher amplitudes at vertex and parietal scalp sites are associated with a lower tendency to worry in the presence of pain or discomfort sensations;
    b) higher amplitudes at occipital scalp sites are associated with a better awareness of the connection between body sensations and emotional states;
    c) in the males' sample, higher amplitudes at frontal scalp sites are associated with the ability to sustain/control attention to body sensations, and the ability to regulate distress by attention to body sensations.
3. Heartbeat evoked potentials' amplitudes were higher in the centroparietal scalp sites at diastole before heart rate deceleration.
4. Heartbeat-evoked potentials at frontal scalp sites at diastole are associated with heart rate variability: lower amplitudes are associated with heart rate variability parameters indicating a higher parasympathetic activity.

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ACKNOWLEDGMENTS

I am sincerely thankful to everyone for their help to preparing this dissertation:

- to *Aida Grabauskaitė* for her aid in accomplishing my studies and for the support while investigating interoception;
- to my supervisor, *dr. Rytis Stanikūnas*, for the time, support and the shared insights as well as for the broad view into dissertation-related topics;
- to my past supervisor, *dr. Inga Griškova-Bulanova*, for her contribution to my papers;
- to *dr. Ramunė Grikšienė* and *dr. Kastytis Dapšys* for reviewing the initial version of my dissertation and for their hints on bettering the work;
- to *Benedikta Lataitytė* for help in registering data in a supplementary study;
- to other members of the *Department of Neurobiology and Biophysics* at Vilnius University for various things;
- to *dr. Algimantas Švegžda* for his comments on data analysis and writing of the dissertation;
- to *dr. Rūta Sargautytė* for her insights;
- to *dr. Wolf E. Mehling* and his coworkers for the MAIA questionnaire and for consultations while translating it into Lithuanian.

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PUBLICATIONS

Publications included in the dissertation

Articles published in reviewed scientific journals from the ISI Web of Science list:

- **Baranauskas M, Grabauskaitė A, Griškova-Bulanova I**. Brain responses and self-reported indices of interoception: Heartbeat evoked potentials are inversely associated with worrying about body sensations. *Physiology & Behavior.* **2017**;180:1–7.
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- **Baranauskas M, Grabauskaitė A, Griškova-Bulanova I**. Psychometric Characteristics of Lithuanian Version of Multidimensional Assessment of Interoceptive Awareness (MAIA^{LT}). *Neurologijos seminarai*. **2016**;20(70):202–6.

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Publications not included in the dissertation

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potencialų. *International "Virtual Instruments in Biomedicine" Conference,* Klaipėda, **2014**.

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- **Noreika D, <u>Baranauskas M</u>, Stonkus R, Griškova-Bulanova I, Grikšienė R**. Subjektyvių ir objektyvių nuovargio parametrų išsiskyrimas ir lytiniai skirtumai mintinio sukimo užduotyje. *International "Virtual Instruments in Biomedicine" Conference*, Klaipėda, **2013**.

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