Heart Rate Variability during acute psychosocial stress: a randomized cross-over trial of verbal and non-verbal laboratory stressors

Agostino Brugnera[a] MA, Cristina Zarbo[a] MA, Mika P. Tarvainen[b] PhD, Paolo Marchettini[c] MD, Roberta Adorni[a] PhD, Angelo Compare[a] PhD

[a] Department of Human and Social Sciences, University of Bergamo, Bergamo, Italy

[b] Department of Applied Physics, University of Eastern Finland and Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland

[c] Department of Neurology, San Raffaele Hospital & Pain Center, Centro Diagnostico Italiano, Milano, Italy

Corresponding Author:

Agostino Brugnera, Department of Human and Social Sciences, University of Bergamo, Italy

P.le S. Agostino, 2 - 24129 Bergamo

Email: agostino.brugnera@unibg.it

Phone: +39 3402231353

Running title: HRV AND ACUTE LABORATORY STRESSORS

Declarations of interest: none

Abstract

Acute psychosocial stress is typically investigated in laboratory settings using protocols with distinctive characteristics. For example, some tasks involve the action of speaking, which seems to alter Heart Rate Variability (HRV) through acute changes in respiration patterns. However, it is still unknown which task induces the strongest subjective and autonomic stress response.

The present cross-over randomized trial sought to investigate the differences in perceived stress and in linear and non-linear analyses of HRV between three different verbal (Speech and Stroop) and non-verbal (Montreal Imaging Stress Task; MIST) stress tasks, in a sample of 60 healthy adults (51.7% females; mean age = 25.6 ± 3.83 years). Analyses were run controlling for respiration rates. Participants reported similar levels of perceived stress across the three tasks. However, MIST induced a stronger cardiovascular response than Speech and Stroop tasks, even after controlling for respiration rates. Finally, women reported higher levels of perceived stress and lower HRV both at rest and in response to acute psychosocial stressors, compared to men.

Taken together, our results suggest the presence of gender-related differences during psychophysiological experiments on stress. They also suggest that verbal activity masked the vagal withdrawal through altered respiration patterns imposed by speaking. Therefore, our findings support the use of highly-standardized math task, such as MIST, as a valid and reliable alternative to verbal protocols during laboratory studies on stress.

Keywords: Stress, HRV, non-linear analyses, Speech task, MIST, Stroop

1. INTRODUCTION

Acute stress, or the physiological reaction to internal or external events perceived as stressful (i.e., stressors), has been largely investigated in laboratory settings using physiological (e.g., pain, hunger) or psychosocial (e.g., math task, social exclusion, achievement situations) stressors (Kogler et al., 2015). Both these laboratory stressors cause a similar subjective, emotional, and physiological stress response (Kogler et al., 2015), and induce physiological reactions comparable to those experienced during real-life stressful situations (Henze et al., 2017).

The laboratory tasks allow researchers to evaluate cardiovascular functioning in standardized conditions, while monitoring or reducing the influence of confounding factors, and manipulating experimental variables in order to draw causal inferences (Chida & Steptoe, 2010). Not surprisingly, stress tasks were combined during laboratory experiments to ensure more sustained cardiovascular and subjective stress responses (Boyle et al., 2016; Skoluda et al., 2015). In addition, self-reported measures of situational stress (i.e., manipulation checks) are usually administered during experiments to ensure that participants have experienced the desired elevated levels of state anxiety or situational stress. For example, the Stress Rating Questionnaire (SRQ) is a recently-developed instrument that assesses changes in situational stress along five bipolar dimensions: Calm to Nervous, Fearless to Fearful, Relaxed to Anxious, Unconcerned to Worried, and Comfortable to Tense (Edwards et al., 2015). Each dimension is rated on a 7-point semantic differential type scale. Previous studies found that the scale has a good convergent, predictive and internal validity (Brugnera et al., 2017; Edwards, Edwards, & Lyvers, 2015).

Psychosocial stress has been evaluated using various protocols. For example, the “Montreal Imaging Stress Task” (MIST) is a computerized and highly-standardized mental arithmetic task with social evaluative threat components (Dedovic et al., 2005). Other commonly-used “verbal” protocols (i.e., requiring participants to verbalize their responses), are the Speech task (where participants are asked to prepare and present a videotaped speech to defend themselves from a false accusation of shoplifting; Saab, Matthews, Stoney, & McDonald, 1989), the Trier Social Stress Task (Kirschbaum, Pirke, & Hellhammer, 1993), and the Stroop Color-Word task (where individuals have to read loud visually incongruent stimuli, such as color names written with a different color, as quickly as possible; MacLeod, 1991). These stress tasks may lead to different patterns of cardiovascular activity: therefore, their investigation may be of particular relevance for researchers interested in the acute physiological effect of stressors.

A reliable and well-investigated biomarker of ANS activity during laboratory stress tasks is Heart Rate Variability (HRV). Indeed, the ANS modulates the heart’s rhythm together with other physiological systems (e.g., arterial baroreflex), slowing or accelerating HR through the activation of the parasympathetic (vagus) or sympathetic nerves, respectively (Shaffer, McCraty, & Zerr, 2014). The two branches of ANS can interact in complex ways, so that the autonomic activity could be fully described along two dimensions, namely coactivation-coinhibition and uncoupled-reciprocal (for a review of the "autonomic space model", see Berntson, Cacioppo, Quigley, & Fabro, 1994). Through the non-invasive assessment of the variations in the beat-to-beat interval around its mean value, HRV provides a picture of the dynamic balance between sympathetic and parasympathetic branches of ANS (Malik et al., 1996). Interestingly, these HR changes are strongly influenced by respiration rate, the timing of respiration, and tidal volume (Grossman, Wilhelm, & Spoerle, 2004). The coupling between respiration and heart period is a well-known phenomenon (for a review, see Quintana & Heathers, 2014) influenced by a centrally integrated cardio-respiratory network, baroreflex, and by the feedback activity of the mechanical stretch-receptor on the lungs (Ben-Tal, Shamailov, & Paton, 2014; Quintana & Heathers, 2014).

During the last 20 years, different measurements of Heart Rate Variability were developed. For example, time domain analyses provide basilar information on the variability of HR, using simple statistical methods on the entire RR series (e.g., standard deviation). On the contrary, frequency domain analyses quantify the autonomic dynamics through spectral methods, which extracts two components, namely i) the low frequencies (LF; 0.04 – 0.15 Hz), which reflect sympathetic/parasympathetic activity; and ii) the high frequencies (HF; 0.15–0.40 Hz), considered a well-established and reliable marker of vagal activity (Malik et al., 1996). However, it is well known that heart dynamics are chaotic, being influenced by non-linear interactions between the different physiological systems previously cited (Dimitriev, Saperova, & Dimitriev, 2016; Malik et al., 1996; Shaffer et al., 2014). This led to the development of various non-linear measures of HRV, each describing a specific aspect of the non-linear dynamics of heart rate (Dimitriev et al., 2016). One of the easiest indices to interpret is Poincaré Plot, or the graphical representation in a Cartesian plane of the correlation between successive RR intervals (Brennan, Palaniswami, & Kamen, 2001). Other methods, such as Correlation Dimension and Detrended Fluctuation Analysis, are derived from fractal geometry. The Correlation Dimension describes the “structure of the attractor approximating the fractal dimension” within the RR series, while the Detrended Fluctuation Analysis examines the fractal correlation properties of heart rate dynamics, evidencing short-range and long-range correlations in the signal (Dimitriev et al., 2016). Finally, sample entropy measures the system randomness and predictability (i.e., the repetition of patterns in the RR intervals; Dimitriev et al., 2016).

Past evidence demonstrated that laboratory psychosocial stress tasks are associated with increased heart rates, a sympathovagal balance characterized by sympathetic predominance, and a reduced “complexity” of the cardiac signal (e.g., decreased Sample Entropy and Correlation Dimension; Castaldo et al., 2015). These responses seem to be influenced by sociodemographic variables, such as gender. Indeed, women generally report higher levels of perceived stress and experience a stronger cardiovascular reactivity to stressors, compared to men (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Kudielka, Hellhammer, & Kirschbaum, 2000; Whited & Larkin, 2009). However, to the best of our knowledge, no studies investigated linear and non-linear measures of HRV across multiple psychosocial stress tasks. Previous literature, for example, focused exclusively on endocrine responses to multiple tasks, or examined the simple heart rate, which is a rather unspecific index of cardiovascular activity (see for example Al'Absi et al., 1997; Boyle et al., 2016; Hellhammer & Schubert, 2012; Henze et al., 2017; Kelsey, Ornduff, & Alpert, 2007; Kudielka, Hellhammer, & Kirschbaum, 2007; Quaedflieg, Meyer, & Smeets, 2013; Skoluda et al., 2015; Wolfram, Bellingrath, Feuerhahn, & Kudielka, 2013). Moreover, the most commonly-used tasks have distinctive characteristics: some verbal tasks focus only on the social-evaluative aspects of stress (i.e., Speech) or have a prominent executive functioning component (i.e., Stroop), while other non-verbal tasks focus on computerized mental arithmetic challenges (i.e., MIST). Previous findings (see for example Schlotz, 2013; Segerstrom & Nes, 2007; Skoluda et al., 2015) support the notion that different protocols (e.g., verbal vs non-verbal; prominent social-evaluative aspects vs prominent executive functioning components) lead to different cardiovascular responses. In addition, a potential confounding variable in experiments on stress is the respiration rate. Previous evidence suggested that tasks involving the action of speaking alter the breathing rate and “generate a confounding effect by bringing respiratory sinus arrhythmia (a predominantly vagal effect) into the non-respiratory low frequencies of HRV” (Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001; Bernardi et al., 2000). To date, no studies examined the role of respiration during a series of repeated acute stress protocols.

Thus, the present randomized, within-subjects crossover trial investigated the effect of different verbal (Speech and Stroop) or non-verbal (MIST) stress tasks on Heart Rate Variability, adjusting for respiration rates and sequence order, in a sample of healthy adults. We tested the following hypotheses: i) we expected lower breathing frequencies during the verbal tasks and different levels of perceived stress during the three stress protocols (i.e., higher during speech and lower during the other two tasks), as suggested by Skoluda and colleagues (2015); ii) we expected specific, distinct patterns of Heart Rate Variability between verbal (Speech and Stroop) and non-verbal (MIST) stress tasks (e.g., higher heart rates and sympathetic activity during Speech and Stroop), in accordance with previous literature (Bernardi et al., 2001). We examined time, frequency, and non-linear indexes with the aim of providing a comprehensive overview of the assessment and interpretation of HRV and a clear and detailed picture of the autonomic activity during the entire experiment. By identifying which stress task induces the strongest subjective, autonomic, and cardiovascular response, we can help researchers run psychophysiological experiments that are more methodologically-sound and valid in assessing the multifaceted phenomenon of stress.

2. MATERIAL AND METHODS

2.1 Participants

A total of 60 Caucasian participants (51.7% females) with a mean age of 25.60 years (SD = 3.83 years; range: 19-34) volunteered for the experiment. Participants were university students (56.7%) recruited from undergraduate courses at University of Bergamo (Italy), or self-referred adults (43.3%) which responded to media advertisements (e.g., websites) between September 2016 and January 2017. Their mean BMI was 22.53 kg\m^2 (SD = 3.69; range: 17.10 – 32.46). Fifteen of the participants (25%) were smokers, 48 (80%) were regular coffee drinkers, and 46 (76.7%) consumed alcohol at least once per month. Eight (13.3%)

regularly used oral contraceptives. Finally, 31 (43.3%) practiced regular sport (> 1 hour per week).

Participants were asked to refrain from consuming caffeinated drinks, and smoking at least 2 hours prior to testing, and from exhaustive exercise at least 24 hours before the test. They underwent the study protocol between 9AM to 12 PM, or between 15 to 18 PM. All participants were free of any neurological, psychiatric, or other medical (e.g., cardiologic) illnesses as assessed by a semi-structured interview, administered by an expert doctoral-level psychologist.

The study was conducted in accordance with APA (2017) ethical standards for the treatment of human experimental volunteers; each participant provided written consent in compliance with the Declaration of Helsinki (2013).

2.2 Procedure

During the entire experiment, participants were seated in a comfortable chair, in front of a computer (or a video camera, during the Speech task), in a silent, temperature-controlled room. Two experimenters remained in the room for the entire procedure. After the positioning of the sensor, participants were asked to relax for at least 10 minutes. The rest period (baseline) was followed by three stress tasks designed to induce moderate psychosocial stress (MIST, Stroop Color-Word and Speech tasks) and by a recovery. The order of presentation of the stress tasks was randomized for each subject. Each task was preceded by instructions, the preparation of the experimental setting, and\or a specific training phase (as in the case of MIST) which took approximately 5-7 minutes. During rest, participants were sitting with a normal posture (i.e., feet flat on the floor and knees at a 90° angle), and they were asked to relax (i.e., restrict their movements and speaking) and breathe spontaneously (Laborde, Mosley, & Thayer, 2017). During the other conditions, participants were asked to limit their movements. All conditions lasted five minutes in accordance to International Guidelines (Malik et al., 1996). Before starting the experiment and after each stress task\recovery, participants completed manipulation-check questionnaires (Stress Rating Questionnaire and Task Engagement). An electrocardiogram was collected during the entire procedure. At the end of the experiment, all participants were debriefed on the purpose of the study. The entire procedure took approximately 60 minutes.

2.2 Measures and Instruments

2.2.1 Stress Rating Questionnaire (SRQ)

The Stress Rating Questionnaire (Edwards et al., 2015) is a self-report 5-item questionnaire developed to assess change in stress awareness (Edwards, Burt, & Lipp, 2006). Current stress is rated on a 7-point Likert scale (1 to 7) on five bipolar dimensions, ranging from Calm to Nervous, Fearless to Fearful, Relaxed to Anxious, Unconcerned to Worried, and Comfortable to Tense. Total scores range from 5 to 35. Higher scores indicate higher self-reported state of stress. An internally-adapted version of the original measure was used in this study; internal consistency at rest was good (Cronbach's $\alpha = .89$; inter-item correlation = 0.53).

2.2.2 Task Engagement (TE)

Task Engagement (TE) was assessed by asking participants how much stressed they felt in that moment using a single item 10-point Likert scale (range: 0 to 10), where 0 indicates the lowest level of stress and 10 being the highest level of stress.

2.2.3 Montreal Imaging Stress Task (MIST)

The "Montreal Imaging Stress Task" is a computerized protocol used to induce psychosocial stress in participants (MIST; Dedovic et al., 2005). The protocol has two test conditions (control and experimental). In the control condition, participants had to solve a series of simple arithmetic operations (sums and subtractions) displayed on the computer screen. In the experimental condition, the same type of arithmetic operations was displayed, but the participants were under a time constraint and a social pressure induced by the experimenter. As described by Dedovic *et al.* (2005), the time limit of the operations was manipulated to be just beyond the individual's "mental capacity". Indeed, the experimental session was preceded by a 2-min training session; time recorded during the training session was used to set a default time limit for the experimental condition. During the experimental condition, after three correct responses the time limit decreased by 10%; whereas after three incorrect responses the time limit increased by 10% (see Dedovic et al., 2005 for the details of this procedure). MIST is an effective protocol and has been extensively adopted in previous studies on stress responses (Brugnera et al., 2017; Kogler et al., 2015). In the present study, only the Experimental condition was analyzed. Indeed, psychophysiological differences between MIST's Control and Experimental conditions were already evaluated in previous research (Brugnera et al., 2017). In regards to posture, participants completed the task sitting still in front of the computer screen.

2.2.4 Stroop Color-Word Task

The Stroop Color-Word task (Stroop, 1935) is a widely adopted neuropsychological task used to assess the attentional capacities, executive functioning, effortful control and processing speed of the participants (Laird et al., 2005; MacLeod, 1991; Segerstrom & Nes, 2007). Time-limited variations of this test were previously used to induce moderate psychosocial stress (see for example Mauri, Cipresso, Balgera, Villamira, & Riva, 2011). During the task, "subjects view color names presented in various ink colors and are instructed to name the presented ink color" (Laird et al., 2005). In the incongruent (Stroop interference) condition, color names are presented in non-matching ink colors (e.g., the word "green" presented in red ink; Laird et al., 2005). In the present study, we adopted a 5-minute computerized version of the Stroop task: after a brief training period (1 minute), participants were instructed to read as quickly as possible on a computer screen eight color names presented simultaneously in matching (congruent condition) and non-matching ink colors (incongruent condition). Stimuli (i.e., color names) changed after a few seconds, thus making it difficult to complete the task (i.e., reading all the color names printed on screen). In addition, the time available to read the printed colours decreased progressively during the task (from 4 to 2 seconds). The present version of the Stroop task was adopted in previous physiological studies, evidencing its effectiveness in inducing stress (Cipresso et al., 2012; Mauri et al., 2011). In regards to posture, participants completed the task sitting still in front of the computer screen.

2.2.5 Speech Task

We adopted a widely-used speech task (Cacioppo, Uchino, & Berntson, 1994; Saab et al., 1989) to induce social-evaluative stress. Participants were asked to imagine that they were shopping in a mall when they were falsely accused of shoplifting by a plainclothes security guard. Then, they were asked to prepare and deliver a speech in front of a video camera to defend themselves and their actions in front of a magistrate. Participants had 2 minutes to prepare the speech, and 5 minutes to deliver it. During the 5-min speech, they were instructed to tell (i) their side of the story, (ii) what they said to the shop manager after they were stopped by the security guard, why the security guard suspected them and why he was wrong, (iii) what disciplinary actions should be taken against the guard, (iv) how they exonerated themselves from the false accusation, and (v) a summary of their points (Cacioppo et al., 1994). As suggested by Saab et al. (1989), subjects were informed that the videotapes of their speeches would later be evaluated by a panel of three experimenters, which would give them a score based on several dimensions (i.e., poise, articulation, and appearance). Subjects were asked to continue talking until instructed otherwise. A similar speech task was used in previous studies to elicit cardiovascular stress responses (Cacioppo et al., 1994). In regards to posture, participants completed the task sitting still in front of the tripod-mounted video camera.

2.2.6 ECG Measurements

Electrocardiogram (ECG) was collected continuously throughout the protocol using “Pulse”, a wearable device with a sampling rate of 256 Hz, designed by STMicroelectronics and manufactured by MR&D (Italy). Wearable devices increase the comfort of the subject and are less invasive compared to non-wearable ones, thus supporting their use during psychophysiological studies on stress. The device was fixed to the centre of the person’s chest using an elastic band, which contained the electrodes. This positioning corresponds to lead 1 of a standard 12-lead ECG. The Pulse sensor filtered the signal with a bandpass filter (0.05 – 40 Hz). The raw ECG signal was stored within the wearable device in a European Data File format (EDF+), which was later transferred to a portable computer. The EDF+ file was passed to Kubios HRV software (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014), which was used for all HRV analyses. Kubios HRV applied an adaptive QRS detector algorithm to extract the beat-to-beat RR intervals from the ECG data. Later, the ECG of each participant was visually inspected to detect and correct artifacts (i.e., missing or extra beats) using a piecewise cubic spline interpolation method. All data were included in the analyses thanks to their overall good quality (<1% artifacts). Data were pre-processed using a smoothness priors based detrending approach, thus removing very low frequency components (< 0.04 Hz) of HRV (Tarvainen, Ranta-Aho, & Karjalainen, 2002). Finally, an ECG-derived Respiration Rate (EDR) was computed from raw-ECG throughout the procedure via a built-in algorithm of Kubios HRV software: the algorithm examined the alterations of the amplitude of the R-peak caused by chest movements during each respiratory cycle. Under stationary conditions (i.e., short-term registrations), the EDR is considered a reliable index of respiratory rates (Tarvainen, 2017): a previous study found a reasonable agreement between EDR and a reference respiratory rate derived from nasal/oral airflow (Cysarz et al., 2008)

2.2.7 Time and Frequency domain analyses of HRV

Regarding the time-domain analyses, four methods were applied to the raw RR data, namely mean HR, SDNN (standard deviation of normal to normal R-R intervals), RMSSD (the square root of the mean of the squares of the successive differences between adjacent NNs), and the Baevsky’s Stress Index. The latter was computed according to the guidelines (AMo/2*Mo*MxDMn; Baevsky, Kirillov, & Kletskin, 1984), where Mo is the mode (the most common RR interval), AMo is the amplitude of the mode, and MxDMn is the variation range of the RR interval values in that specific HRV sample.

Regarding frequency-domain analyses, the interbeat interval time series were interpolated with a rate of 4 Hz using a cubic spline interpolation to have equidistantly sampled data for spectral analysis. The power spectral density (PSD) was calculated by means of a Fast Fourier Transform (FFT) using the Welch’s periodogram method, with a 150s Hanning window and an overlap of 50%. The spectral components of interest, the Low Frequency (LF) and the High Frequency (HF), were evaluated in fixed frequency bands (LF= 0.04 – 0.15; HF = 0.15–0.40 Hz). Analyses were performed on the Total Power of HRV (i.e., the integration of the entire PSD region, 0.04 – 0.40 Hz), on absolute power values of HF (HFpow) and LF (LFpow) calculated with FFT, on HF and LF expressed in normalized units, and on ratio between LF and HF band powers (LF/HF).

2.2.8 Non-linear analyses of HRV

In addition to the time- and frequency-domain methods, four non-linear methods were applied to the raw RR data. They were computed using Kubios HRV software (calculation formulas for all indexes can be found in Tarvainen, 2017). (i) Poincaré plot is a scatter plot of the correlations between successive RR intervals. The graph can be quantitatively analyzed using two measures (i.e., SD1 and SD2) that describe the short- and the long-term variability in the signal, respectively. SD1 is strongly influenced by parasympathetic activity, while SD2 is more affected by the overall variability of the signal (Tarvainen, 2017). (ii) Sample entropy (SampEn) is a measure of the randomness (i.e., unpredictability) and irregularity of the NN interval series. Lower values suggest an increased regularity in the signal, whereas higher values suggest a more random time series (Tarvainen, 2017). SampEn was computed setting m (embedding dimensions) to a value of m = 2, and r (tolerance) to a fraction of the standard deviation of the RR data (r = 0.2 * SDNN). (iii) Detrended fluctuation analysis (DFA) measures the fractal correlation properties within the signal at different time scales, divided into short-term fluctuations (α1; modulated by the sympathetic system) and long-term fluctuations (α2; modulated by both sympathetic and vagal activity; Dimitriev et al., 2016). The short-term fluctuations slope was estimated within the range 4-16 beats, and long-term fluctuations within 16-64 beat correspondingly. (iv) Correlation dimension (D2) is a measure of the geometry of the attractor approximating the fractal dimension, and it gives information on the minimum number of dynamic variables needed to model the underlying system. Correlation Dimension decreases when the autonomous balancing shifts towards sympathetic regulation (Dimitriev et al., 2016). We computed D2 by setting m (embedding dimensions) to a value of m = 10.

2.4 Statistical Analyses

The sample size was calculated on the basis of a previous publication (Quintana, 2017). Assuming an alpha level of .05 and a power of .80, about 61 participants were required to detect medium effect sizes (*d* = 0.5) on vagally mediated HRV measures (e.g. RMSSD, HF).

We investigated hypothesis 1 about the efficacy of the procedure and changes in respiration rates using Linear Mixed Models (LMM) on the repeated measurements of SRQ, TE, and Respiration Rates, taking into account the sequence of the tasks. In order to investigate hypothesis 2 about changes in HRV indexes during the experiment, we used Linear Mixed Models on the repeated measurements of each index, taking into account the sequence of the task and controlling for respiration rates. In all LMM analyses, Time, Sequence of the task, and the covariate (Respiration Rates) were treated as fixed effects, while the covariance matrix was modeled without restrictions (i.e., unstructured): parameters were estimated using Restricted Maximum Likelihood. We tested other models (e.g., different covariance matrices or Sequence\Respiration Rates modeled as a random effect), which all had a worse fit of the data according to the Akaike's Information Criterion (AIC) test. Effect sizes (i.e., the percentage of variance explained by predictors) were assessed and reported using pseudo-R^2 (Hox, 2002). Bonferroni corrected pairwise comparisons of estimated marginal means were used as post-hoc tests. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.0. All statistical tests were two-sided; a p-value $\leq .05$ was considered significant.

3. RESULTS

3.1 Preliminary Analyses

Univariate normality was assessed by examining box-plots, histograms, and skewness and kurtosis values (Tabachnick & Fidell, 2007). SDNN, RMSSD, SD1, and SD2 were slightly positively skewed, while Total Power, HFpow, LFpow, HF/LF ratio, and Baevsky’s Stress Index were moderately positively skewed. A square-root or a log10 transformation corrected the non-normality, respectively. The transformed variables were consequently used in all analyses (untransformed means, standard deviations, skewness, and kurtosis values are reported in Supplementary Table S1). The presence of outliers was investigated using standardized scores and box plots (Tabachnick & Fidell, 2007): analyses did not reveal any outlier. Finally, we tested the effect of the study population (university students and workers), age, and gender on HRV indexes. The effects of both age and study population were not significant. However, women reported higher levels of perceived stress, had higher respiration rates, and lower HRV (i.e., higher levels of HR, SDNN, Stress Index, DFA α2, and lower levels of RMSSD, LF and Total power, SD1, SD2, and Correlation Dimension D2) during the entire procedure, compared to men. However, in both genders, the direction of effects during the experiment was similar to that noted for the entire sample (e.g., stress led to an increased sympathetic activity; see section 3.3). Means, SD, *t* and *p* values during each condition for both groups (M and F) are reported in Supplementary Table S2.

3.2 Hypothesis 1

We tested the hypothesis of a significant change in manipulation check questionnaires and in respiration rates during the procedure, using Linear Mixed Models. In all analyses, Time was the only statistically significant fixed effect ($p < 0.001$; see Table 1). According to Pseudo-R^2, the models explained between 18 and 25% of the variance.

Regarding manipulation checks, post-hocs showed that subjective stress (evaluated using SRQ and TE questionnaires) increased from Rest to the stress tasks, and then decreased during Recovery. Interestingly, no differences were found between MIST, Speech and Stroop tasks. Regarding respiration rates, post-hocs showed significant decreases in EDR between MIST and the other stress tasks. Results support the addition of EDR as a covariate in HRV analyses and suggest that stress tasks which involve the action of speaking lead to a lower respiratory frequency. F and *p* values for fixed effects, as well as estimated marginal means, *p* values and significant post-hocs between conditions are reported in Table 1 and 2, respectively.

3.3 Hypothesis 2

We used Linear Mixed Models to investigate changes in HRV indexes between stress tasks. In all analyses, Time was a statistically significant fixed effect ($p < 0.001$). Sequence of the task reached significance only for HR ($p = 0.024$) and DFA α1 ($p = 0.030$): in both cases, post-hoc comparisons showed that individuals who were randomly assigned to the sequence “MIST, Speech, Stroop” had significantly lower means compared to those who were assigned to the sequence “Speech, Stroop, MIST”. Respiration Rate was a statistically significant factor in all LMM analyses, suggesting that EDR (considered a reliable index of respiration rates; Cysarz et al., 2008) contributes significantly to all HRV indexes, with the notable exception of Correlation Dimension D2 (see Table 2). The frequency of respiration in our study was comprised between 9 (0.15 Hz) and 24 (0.40 Hz) cycles per minute (see Table 2) so that HF corresponded to vagal tone (Laborde et al., 2017). Finally, according to Pseudo-R^2, the models explained between 9 and 31% of the variance. Table 1 shows F values, p values and effect sizes (pseudo R^2) for the three fixed effects.

Regarding time-domain analyses, each mean HR was significantly different from each other. Compared to rest and recovery, all stressful conditions led to higher Heart Rates, with the highest frequency reached during the Speech task. During Recovery, all HRV indexes returned to the rest status, or were lower (as in the case of HR, for example) or higher (as in the case of SDNN, for example) compared to the initial resting condition. Interestingly, SDNN (a measure of the overall variability of HRV) was lower during MIST than during any other condition. Finally, MIST was the only task associated with significant increases in the Baevsky’s Stress Index, a Time Domain measure computed from RR intervals’ histogram (Baevsky et al., 1984); however, compared to recovery, all stress tasks led to significantly higher Stress Index values.

With regards to the frequency domain analyses, MIST was associated with a significant reduction in Total Power, and with a contemporary and similar decrease in the absolute power of both HF and LF frequencies, which led to invariant changes in the LF/HF ratio (or in LF and HF powers expressed in normalized units). Therefore, according to the autonomic space model (Berntson et al., 1994), the ANS activity during the MIST was characterized by a sympathovagal coinhibition. Noticeably, the other stress tasks were associated with significant decreases in HFnu and with significant increases in Low Frequencies (expressed both in absolute values and in normalized units) and in LF/HF ratio.

With regards to non-linear analyses, Poincaré Plot SD1 decreased from rest to the stress tasks due to the parasympathetic withdrawal even if these decreases where not significant in the case of Stroop. Interestingly, Poincaré Plot SD2 decreased from the initial resting condition only during MIST. Correlation dimension D2 was lower during MIST in comparison to the Speech and the Stroop condition: results suggest a stronger regularity and a change towards a more periodic behavior of the heart rate (Schubert et al., 2009) during the computerized arithmetic task. Finally, Sample Entropy reached its highest value during MIST, and the mean was significantly different from all the others. As such, the hypothesis advanced by Vuksanovic and colleagues (2007) of a dependency of SampEn on the magnitude of the modulation in HF band was not confirmed in our data, which showed opposite findings (i.e., decreases in HF associated with increases in complexity of the signal, as measured by SampEn). To further investigate this observation, we plotted the RR time series of all subjects during all conditions. We found more rhythmical low-frequency fluctuations (i.e., higher regularity in the signal) during Speech and Stroop compared to MIST, probably due to different respiration characteristics during the verbal and non-verbal tasks. Figure 1 shows the RR time series of three representative subjects. These findings are in accordance with the increase in LF power observed during the verbal tasks: in addition, LF power during MIST was significantly and negatively correlated with SampEn (*r* = - .26; *p* = 0.04), while the correlations during the other tasks did not reach statistical significance (*r* = – 0.08 and 0.11). Finally, the short-term fluctuation slope (α1) of Detrended Fluctuation Analysis (DFA) increased during all stress tasks compared to the resting conditions, probably due to the cardiac parasympathetic withdrawal (Dimitriev et al., 2016). However, no changes were observed for the long-term fluctuation slope (α2), with the exception of a slight significant increase during the Stroop.

Concluding, results suggest that stress tasks lead to specific cardiovascular changes based on their characteristics. It is worth noticing that analyses were performed controlling for respiration rates, thus removing the contribution of EDR during the post-hoc comparisons. Table 2 shows the estimated marginal means, standard errors, *p* values and significant post-hocs between conditions.

4. DISCUSSION

The present study investigated cardiovascular responses in a sample of healthy adults of both sexes during three commonly-used psychosocial stress tasks, adopting a randomized cross-over design. In regards to hypothesis 1, results showed that both verbal stress tasks were characterized by a significant decrease in respiration rates compared to MIST and spontaneous breathing (i.e., rest) in the Speech task. The literature on the effects of stressful tasks on respiration is rather contradictory: for example, Bernardi and colleagues (2000) found non-significant changes during both silent or aloud mental arithmetic, while other authors found reduced (Schubert et al., 2009) or increased respiration rates during speech tasks (Hernando et al., 2016; Widjaja, Orini, Vlemincx, & Van Huffel, 2013). Additionally, our findings on perceived stress suggested that acute psychosocial stress tasks involving social actions (e.g., speaking in front of a video camera, as in the case of the Speech task) are perceived to be as stressful as more cognitive tasks, such as MIST. Few studies have investigated the psychological responses to repeated laboratory stressors (see for example Skoluda et al., 2015). Due to the adoption of a reliable questionnaire on perceived stress (i.e., SRQ), the present study extends the literature on this topic suggesting a dissociation between stress appraisal and autonomic stress responses.

In regards to Heart Rate Variability analyses (hypothesis 2), stress led to higher heart rates, a sympathetic shift in sympathovagal balance, and a reduced complexity of the cardiac signal, in accordance with the previous literature (Brugnera et al., 2017; Castaldo et al., 2015). However, MIST induced the strongest cardiovascular response compared to the other stress tasks. Among the most interesting results, MIST was associated with a specific profile of cardiovascular activity, characterized by sympathovagal coinhibition (i.e., decreases in both HF and LF power). Considering that our analyses were performed controlling for respiration rates, we propose that the observed differences between MIST and the other verbal tasks (which showed only a significant increase in LF power) are attributable to other variables associated with the complex respiratory patterns of speaking, such as timing of respiration, tidal volume (i.e., respiratory depth), metabolic demand, and central respiratory drive (Grossman et al., 2004; Schubert et al., 2009). Moreover, post-hoc comparisons on HF and LF transformed in normalized units showed different results between the stress tasks (i.e., no significant changes in both indexes from rest to MIST; significant decreases in HFnu and increases in LFnu from rest to the two verbal tasks). The computational formula for LFnu and HFnu requires dividing the absolute power of that specific frequency band by the total power (Tarvainen, 2017): however, in our experiment, we found large differences in total power between MIST and the verbal tasks (see Table 2). Therefore, we suggest always including the absolute power (ms^2) of High and Low frequencies of HRV in the statistical analyses in order to draw precise and valid conclusions on the ANS activity during stress tasks. Caution should also be exercised when interpreting LF/HF ratio or LFnu and HFnu, controversial measures of sympathovagal balance (Billman, 2013): our results showed significant increases from rest only during the two verbal tasks, suggestive of a sympathovagal shift to sympathetic dominance. MIST was characterized by a contemporary and similar decrease in both low and high frequencies of HRV (see Table 2), so that the ratio did not change compared to rest, despite the sympathovagal inhibition previously described. As such, LF/HF ratio (and LF and HF in normalized units) should always be interpreted taking into account the absolute powers of LF and HF bands. With regards to non-linear analyses, we found that MIST was surprisingly characterized by higher levels of Sample Entropy (i.e., an increased randomness of the time series). This is probably due to the effect of respiration characteristics on this index (see Figure 1). Our results suggest that the unpredictability and irregularity of the NN interval series could increase despite a sympathovagal withdrawal induced by a specific task. Future studies should attempt to replicate these findings. In addition, we suggest always examining the correlations between LF (expressed in absolute power) and this non-linear index, while plotting also the spontaneous fluctuations in RR time series: this would highlight any respiratory-induced low-frequency regularities that could explain the increased randomness of the signal during specific tasks.

Finally, our study adds further information to the literature on gender-related differences during psychophysiological studies on stress. We found that women reported higher levels of perceived stress and had a lower HRV (e.g., higher heart rates) during the entire experiment compared to men, in accordance with previous literature (Kelly et al., 2008; Kudielka et al., 2004; Kudielka et al., 2000; Whited & Larkin, 2009). As reported by Kelly and colleagues (2008), the observed sex differences could be attributed to psychological (e.g., differences in coping styles, emotion regulation strategies, emotional expressiveness, and sensitivity), sociocultural (e.g., gender roles) or biological (e.g., genetic and hormonal influences) factors.

This study has some limitations. First, the lack of clear predictions (i.e., more specific, directed hypotheses) is a shortcoming which limits the interpretability of our findings, increasing the likelihood of false-positive effects. Second, administering the stressors during the same day, within a procedure that took up to 60 minutes, could have induced a habituation\sensitization effect, flattening the physiological responses of the participants. However, we randomized the order of the stress tasks to reduce this problem; in addition, Kelsey and colleagues (2004) found that evaluative observation (i.e., the presence of experimenters during the stress tasks, as in our study) disrupt the habituation effect. Third, our device registered the ECG with a sampling rate of 256 Hz, which could determine a low temporal accuracy. However, Kubios HRV software interpolates the R-wave at 2000 Hz before fiducial point detection using a cubic spline method, which considerably improves the accuracy (Tarvainen, 2017). Fourth, we controlled the analyses only for respiration rates, without measuring variables such as depth or amplitude of respiration. Future studies should replicate our findings using these respiratory indexes as covariates. Fifth, we focused exclusively on HRV, without considering other well-established neuroendocrine indexes such as cortisol. However, the long laboratory procedures required to reliably assess cortisol (for a review, see Dickerson & Kemeny, 2004) would have prolonged an already lengthy experiment. In addition, neuroendocrine changes to multiple stress tasks were already investigated in previous studies (see for example Al'Absi et al., 1997; Boyle et al., 2016), while a clear picture of changes in linear and nonlinear HRV indexes during the same tasks was still missing. Finally, we recruited a sample of young, healthy Caucasian participants. Therefore, our results might not generalize to other populations (i.e., older adults) and cultures.

Concluding, the present study provides a number of new insights on several methodological and psychophysiological areas. We found that stressors with distinctive characteristics seem to lead to a different autonomic modulation: specifically, MIST induced a stronger cardiovascular response than Speech and Stroop Color-Word tasks. Speech tasks have been generally considered more ecologically valid than other acute laboratory stressors, due to their resemblance with every-day stressful situations (see for example Henze et al., 2017; van Eck, Nicolson, Berkhof, & Sulon, 1996). Recall that psychosocial stressors induce psychophysiological responses similar to those experienced during physical (e.g., cold pressure test) or real-life stressful situations (e.g., an oral exam; Henze et al., 2017; Kogler et al., 2015). Hence, even if we recognize that the selection of a task depends on the specific objective of a study, our results seem to support the use of highly-standardized math tasks (i.e., MIST) as a valid and reliable alternative to other physical, real-life or psychosocial stressors during cardiovascular studies on stress.

Our findings also suggest taking caution when interpreting cardiovascular responses collected during verbal protocols: speaking imposes complex changes in respiratory patterns that could alter the HRV, affecting its interpretation and limiting the generalizability of results (Bernardi et al., 2001; Hernando et al., 2016). The cardiovascular activity observed during Speech and Stroop-Color Word task could be attributed to i) a reduced efficacy of these tasks, ii) the action of speaking or to iii) the results of effortful control or executive functioning, which can alter HRV (Segerstrom & Nes, 2007). However, the subjective stress appraisal was similar across the tasks. Therefore, having controlled the analyses for respiration rates, we hypothesize that verbal activity could have masked the vagal withdrawal during the verbal tasks through a complex interaction between changes in tidal volume, metabolic demands, timing of respiration, and central respiratory drive (Grossman et al., 2004; Schubert et al., 2009). Consequently, we urge authors to adjust HRV analyses for EDR (a reliable but largely underused index of respiratory frequency, that can be easily computed from ECG) and -if possible- for other respiratory indexes such as respiration amplitude. This is especially important if researchers are interested in using speech tasks during their experiments.

Acknowledgements: We are grateful to Prof. Fabio Previdi (School of Engineering, University of Bergamo) for his help in revising the manuscript.

REFERENCES

Al'Absi, M., Bongard, S., Buchanan, T., Pincomb, G. A., Licinio, J., & Lovallo, W. R. (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology, 34*(3), 266-275. doi:10.1111/j.1469-8986.1997.tb02397.x

American Psychological Association. (2017). Ethical principles of psychologists and code of conduct. (2002, Amended June 1, 2010 and January 1, 2017). Retrieved from http://www.apa.org/ethics/code/index.aspx

Baevsky, R. M., Kirillov, O. I., & Kletskin, S. Z. (1984). *Mathematical analysis of heart rate changes under stress [Matematicheskiy analiz izmeneniy serdechnogo ritma pri stresse]*. Moscow: Nauka.

Ben-Tal, A., Shamailov, S. S., & Paton, J. F. R. (2014). Central regulation of heart rate and the appearance of Respiratory Sinus Arrhythmia: new insights from mathematical modeling. *Mathematical biosciences*, *255*, 71-82. doi:10.1016/j.mbs.2014.06.015

Bernardi, L., Porta, C., Gabutti, A., Spicuzza, L., & Sleight, P. (2001). Modulatory effects of respiration. *Autonomic Neuroscience: Basic and Clinical*, *90*(1-2), 47-56. doi:10.1016/S1566-0702(01)00267-3

Bernardi, L., Wdowczyk-Szulc, J., Valenti, C., Castoldi, S., Passino, C., Spadacini, G., & Sleight, P. (2000). Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability. *Journal of the American College of Cardiology, 35*(6), 1462-1469. doi:10.1016/S0735-1097(00)00595-7

Berntson, G. G., Cacioppo, J. T., Quigley, K. S., & Fabro, V. T. (1994). Autonomic space and psychophysiological response. *Psychophysiology, 31*(1), 44-61. doi:10.1111/j.1469-8986.1994.tb01024.x

Billman, G. E. (2013). The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Frontiers in Physiology, 4 FEB*. doi:10.3389/fphys.2013.00026

Boyle, N. B., Lawton, C., Arkbåge, K., West, S. G., Thorell, L., Hofman, D., . . . Dye, L. (2016). Stress responses to repeated exposure to a combined physical and social evaluative laboratory stressor in young healthy males. *Psychoneuroendocrinology, 63,* 119-127. doi:10.1016/j.psyneuen.2015.09.025

Brennan, M., Palaniswami, M., & Kamen, P. (2001). Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? *Ieee Transactions on Biomedical Engineering, 48*. doi:10.1109/10.959330

Brugnera, A., Zarbo, C., Adorni, R., Tasca, G. A., Rabboni, M., Bondi, E., . . . Sakatani, K. (2017). Cortical and cardiovascular responses to acute stressors and their relations with psychological distress. *International Journal of Psychophysiology, 114,* 38-46. doi:10.1016/j.ijpsycho.2017.02.002

Cacioppo, J. T., Uchino, B. N., & Berntson, G. G. (1994). Individual differences in the autonomic origins of heart rate reactivity: The psychometrics of respiratory sinus arrhythmia and preejection period. *Psychophysiology, 31*(4), 412-419. doi:10.1111/j.1469-8986.1994.tb02449.x

Castaldo, R., Melillo, P., Bracale, U., Caserta, M., Triassi, M., & Pecchia, L. (2015). Acute mental stress assessment via short term HRV analysis in healthy adults: A systematic review with meta-analysis. *Biomedical Signal Processing and Control, 18,* 370-377. doi:10.1016/j.bspc.2015.02.012

Chida, Y., & Steptoe, A. (2010). Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: A meta-analysis of prospective evidence. *Hypertension, 55*(4), 1026-1032. doi:10.1161/HYPERTENSIONAHA.109.146621

Cipresso, P., Serino, S., Villani, D., Repetto, C., Sellitti, L., Albani, G., . . . Riva, G. (2012). Is your phone so smart to affect your state? An exploratory study based on psychophysiological measures. *Neurocomputing, 84*, 23-30. doi:10.1016/j.neucom.2011.12.027

Cysarz, D., Zerm, R., Bettermann, H., Frühwirth, M., Moser, M., & Kröz, M. (2008). Comparison of respiratory rates derived from heart rate variability, ECG amplitude, and nasal/oral airflow. *Annals of Biomedical Engineering, 36*(12), 2085-2094. doi:10.1007/s10439-008-9580-2

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry & Neuroscience, 30*(5), 319-325.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychological Bulletin, 130*(3), 355-391. doi:10.1037/0033-2909.130.3.355

Dimitriev, D. A., Saperova, E. V., & Dimitriev, A. D. (2016). State Anxiety and Nonlinear Dynamics of Heart Rate Variability in Students. *PloS one, 11*(1), e0146131. doi:10.1371/journal.pone.0146131

Edwards, E. J., Edwards, M. S., & Lyvers, M. (2015). Cognitive trait anxiety, situational stress, and mental effort predict shifting efficiency: Implications for attentional control theory. *Emotion, 15*(3), 350-359. doi:10.1037/emo0000051

Edwards, M. S., Burt, J. S., & Lipp, O. V. (2006). Selective processing of masked and unmasked verbal threat material in anxiety: Influence of an immediate acute stressor. *Cognition & Emotion, 20*(6), 812-835. doi:10.1080/02699930500375761

Grossman, P., Wilhelm, F. H., & Spoerle, M. (2004). Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. *American Journal of Physiology-Heart and Circulatory Physiology, 287*(2), H728-H734. doi:10.1152/ajpheart.00825.2003

Hellhammer, J., & Schubert, M. (2012). The physiological response to Trier Social Stress Test relates to subjective measures of stress during but not before or after the test. *Psychoneuroendocrinology, 37*(1), 119-124. doi:10.1016/j.psyneuen.2011.05.012

Henze, G. I., Zänkert, S., Urschler, D. F., Hiltl, T. J., Kudielka, B. M., Pruessner, J. C., & Wüst, S. (2017). Testing the ecological validity of the Trier Social Stress Test: Association with real-life exam stress. *Psychoneuroendocrinology, 75*, 52-55. doi:10.1016/j.psyneuen.2016.10.002

Hernando, A., Lázaro, J., Gil, E., Arza, A., Garzón, J. M., López-Antón, R., . . . Bailón, R. (2016). Inclusion of Respiratory Frequency Information in Heart Rate Variability Analysis for Stress Assessment. *IEEE Journal of Biomedical and Health Informatics, 20*(4), 1016-1025. doi:10.1109/JBHI.2016.2553578

Hox, J. (2002). *Multilevel analyses: Techniques and applications*. Mahwah, NJ: Erlbaum.

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal of behavior therapy and experimental psychiatry, 39*(1), 87-98. doi:10.1016/j.jbtep.2007.02.003

Kelsey, R. M., Ornduff, S. R., & Alpert, B. S. (2007). Reliability of cardiovascular reactivity to stress: Internal consistency. *Psychophysiology, 44*(2), 216-225. doi:10.1111/j.1469-8986.2007.00499.x

Kelsey, R. M., Soderlund, K., & Arthur, C. M. (2004). Cardiovascular reactivity and adaptation to recurrent psychological stress: Replication and extension. *Psychophysiology, 41*(6), 924-934. doi:10.1111/j.1469-8986.2004.00245.x

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier social stress test' - A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81.

Kogler, L., Muller, V. I., Chang, A., Eickhoff, S. B., Fox, P. T., Gur, R. C., & Derntl, B. (2015). Psychosocial versus physiological stress - Meta-analyses on deactivations and activations of the neural correlates of stress reactions. *Neuroimage, 119*, 235-251. doi:10.1016/j.neuroimage.2015.06.059

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: The impact of age and gender. *International Journal of Behavioral Medicine, 11*(2), 116-121. doi:10.1207/s15327558ijbm1102_8

Kudielka, B. M., Hellhammer, D. H., & Kirschbaum, C. (2000). Sex differences in human stress response. In G. Fink (Ed.), *Stress Consequences: Mental, Neuropsychological and Socioeconomic* (Vol. 3). San Diego: Academic Press.

Kudielka, B. M., Hellhammer, D. H., & Kirschbaum, C. (2007). Ten years of research with the Trier Social Stress Test—revisited. In E. Harmon-Jones & P. Winkielman (Eds.), *Social Neuroscience: Integrating Biological and Psychological Explanations of Social Behavior* (pp. 56-83). New York, NY: Guilford Press.

Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting. *Frontiers in Psychology, 8*, 213. doi:10.3389/fpsyg.2017.00213

Laird, A. R., McMillan, K. M., Lancaster, J. L., Kochunov, P., Turkeltaub, P. E., Pardo, J. V., & Fox, P. T. (2005). A comparison of label-based review and ALE meta-analysis in the stroop task. *Human Brain Mapping, 25*(1), 6-21. doi:10.1002/hbm.20129

MacLeod, C. M. (1991). Half a century of research on the Stroop effect: an integrative review. *Psychological Bulletin, 109*(2), 163-203.

Malik, M., Camm, A. J., Bigger Jr, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., . . . Singer, D. H. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17*(3), 354-381.

Mauri, M., Cipresso, P., Balgera, A., Villamira, M., & Riva, G. (2011). Why is Facebook so successful? Psychophysiological measures describe a core flow state while using Facebook. *Cyberpsychology, Behavior, and Social Networking, 14*(12), 723-731. doi:10.1089/cyber.2010.0377

Quaedflieg, C. W. E. M., Meyer, T., & Smeets, T. (2013). The imaging Maastricht Acute Stress Test (iMAST): A neuroimaging compatible psychophysiological stressor. *Psychophysiology, 50*(8), 758-766. doi:10.1111/psyp.12058

Quintana, D. S. (2017). Statistical considerations for reporting and planning heart rate variability case-control studies. *Psychophysiology, 54*(3), 344-349. doi:10.1111/psyp.12798

Quintana, D. S., & Heathers, J. A. J. (2014). Considerations in the assessment of heart rate variability in biobehavioral research. *Frontiers in Psychology, 5*, 805. doi:10.3389/fpsyg.2014.00805

Saab, P. G., Matthews, K. A., Stoney, C. M., & McDonald, R. H. (1989). Premenopausal and postmenopausal women differ in their cardiovascular and neuroendocrine responses to behavioral stressors. *Psychophysiology, 26*(3), 270-280.

Schlotz, W. (2013). Stress Reactivity. In M. D. Gellman & J. R. Turner (Eds.), *Encyclopedia of Behavioral Medicine* (pp. 1891-1894). New York, NY: Springer.

Schubert, C., Lambertz, M., Nelesen, R. A., Bardwell, W., Choi, J. B., & Dimsdale, J. E. (2009). Effects of stress on heart rate complexity-A comparison between short-term and chronic stress. *Biological Psychology, 80*(3), 325-332. doi:10.1016/j.biopsycho.2008.11.005

Segerstrom, S. C., & Nes, L. S. (2007). Heart rate variability reflects self-regulatory strength, effort, and fatigue. *Psychological science, 18*(3), 275-281.

Shaffer, F., McCraty, R., & Zerr, C. L. (2014). A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in Psychology, 5*. doi:10.3389/fpsyg.2014.01040

Skoluda, N., Strahler, J., Schlotz, W., Niederberger, L., Marques, S., Fischer, S., . . . Nater, U. M. (2015). Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. *Psychoneuroendocrinology, 51*, 227-236. doi:10.1016/j.psyneuen.2014.10.002

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18*(6), 643-662. doi:10.1037/h0054651

Tabachnick, B. G., & Fidell, L. S. (2007). *Using multivariate statistics* (5th ed ed.). Boston, MA: Pearson Education, Inc.

Tarvainen, M. P. (2017). Kubios HRV User’s guide. Kubios Ltd, Kuopio, Finland. Retrieved from http://www.kubios.com/support

Tarvainen, M. P., Niskanen, J. P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV--heart rate variability analysis software. *Comput Methods Programs Biomed, 113*(1), 210-220. doi:10.1016/j.cmpb.2013.07.024

Tarvainen, M. P., Ranta-Aho, P. O., & Karjalainen, P. A. (2002). An advanced detrending method with application to HRV analysis. *IEEE Trans Biomed Eng, 49*(2), 172-175. doi:10.1109/10.979357

van Eck, M. M. M., Nicolson, N. A., Berkhof, H., & Sulon, J. (1996). Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. *Biological Psychology, 43*(1), 69-84. doi:https://doi.org/10.1016/0301-0511(95)05159-7

Vuksanović, V., & Gal, V. (2007). Heart rate variability in mental stress aloud. *Medical engineering & physics, 29*(3), 344-349.

Whited, M. C., & Larkin, K. T. (2009). Sex Differences in Cardiovascular Reactivity. *Journal of Psychophysiology, 23*(2), 77-84. doi:10.1027/0269-8803.23.2.77

Widjaja, D., Orini, M., Vlemincx, E., & Van Huffel, S. (2013). Cardiorespiratory dynamic response to mental stress: A multivariate time-frequency analysis. *Computational and Mathematical Methods in Medicine, 2013*. doi:10.1155/2013/451857

Wolfram, M., Bellingrath, S., Feuerhahn, N., & Kudielka, B. M. (2013). Cortisol responses to naturalistic and laboratory stress in student teachers: Comparison with a non-stress control day. *Stress and Health, 29*(2), 143-149. doi:10.1002/smi.2439

World Medical Association. (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *Journal of the American Medical Association, 310*(20), 2191-2194. doi:10.1001/jama.2013.281053

Table 1. F and *p* values of Fixed effects (Time, Sequence of the tasks, Respiration Rates), and effect sizes (Pseudo R^2) for all variables.

	Time			**Sequence**			**Respiration Rate**			**Pseudo R^2**
	DF (4)	**F**	***p***	**DF (5)**	**F**	***p***	**DF (1)**	**F**	***p***	
Psychological variables										
SRQ	58.84	33.31	< 0.001	53.97	0.710	0.622	/	/	/	0.18
TE	58.73	25.88	< 0.001	53.25	1.160	0.340	/	/	/	0.25
Physiological variables										
EDR	59.00	8.470	< 0.001	54.00	0.630	0.680	/	/	/	0.12
HR	60.29	43.42	< 0.001	54.03	2.840	0.024	173.97	16.65	< 0.001	0.30
SDNN	59.85	19.44	< 0.001	53.82	1.310	0.273	214.13	27.06	< 0.001	0.23
RMSSD	60.86	9.760	< 0.001	54.14	1.330	0.266	210.06	14.38	< 0.001	0.16
Stress Index	59.98	14.72	< 0.001	53.68	1.571	0.184	221.01	20.99	< 0.001	0.23
Total Power	59.63	20.00	< 0.001	53.39	1.124	0.359	211.17	19.05	< 0.001	0.22
HFpow	60.43	6.710	< 0.001	54.25	1.360	0.253	229.13	7.220	0.008	0.10
LFpow	59.50	24.03	< 0.001	52.94	0.990	0.434	215.46	21.31	< 0.001	0.27
HFnu	60.68	13.52	< 0.001	53.73	0.660	0.656	247.79	4.690	0.031	0.21
LFnu	60.67	13.57	< 0.001	53.72	0.660	0.652	247.84	4.710	0.031	0.21
LF/HF	60.82	13.21	< 0.001	53.74	0.760	0.581	254.34	5.620	0.019	0.21
SD1	60.86	9.770	< 0.001	54.14	1.330	0.266	210.06	14.39	< 0.001	0.16

SD2	59.57	21.79	< 0.001	53.70	1.220	0.315	216.69	28.10	< 0.001	0.25
Sample Entropy	59.54	29.65	< 0.001	53.63	1.670	0.158	218.87	10.08	0.002	0.31
D2	59.67	5.480	0.001	54.36	1.170	0.335	246.86	2.420	0.121	0.11
DFA α1	60.73	30.09	< 0.001	54.10	2.700	0.030	249.96	9.430	0.002	0.29
DFA α2	60.56	2.950	0.027	53.20	0.600	0.704	247.23	8.920	0.003	0.09

Notes: *N* = 60. DF = Degrees of Freedom; SRQ = Stress Rating Questionnaire; TE = Task Engagement; EDR = ECG-Derived Respiration Rate (in Hertz); HR = Heart Rate; SDNN = Standard Deviation of NN intervals (square-root transformed); RMSSD = Root Mean Square of Successive Differences (square-root transformed); Stress Index = Baevsky's Stress Index (log10 transformed); Total Power = total power of HRV Spectrum (log10 transformed); HFpow = absolute power of High Frequencies (log10 transformed); LFpow = absolute power of Low Frequencies (log10 transformed); HFnu = High Frequency expressed in normalized units; LFnu = Low Frequency expressed in normalized units; LF/HF = LF HF ratio (log10 transformed); SD1 = Poincaré plot SD1 (square-root transformed); SD2 = Poincaré Plot SD2 (square-root transformed); D2 = Correlation Dimension D2; DFA α1 = short-term fluctuation slope (alpha 1) of Detrended Fluctuation Analysis; DFA α2 = long-term fluctuation slope (alpha 2) of Detrended Fluctuation Analysis.

Table 2. Estimated marginal means, Standard Errors and Bonferroni's corrected post-hocs for all variables, during each condition.

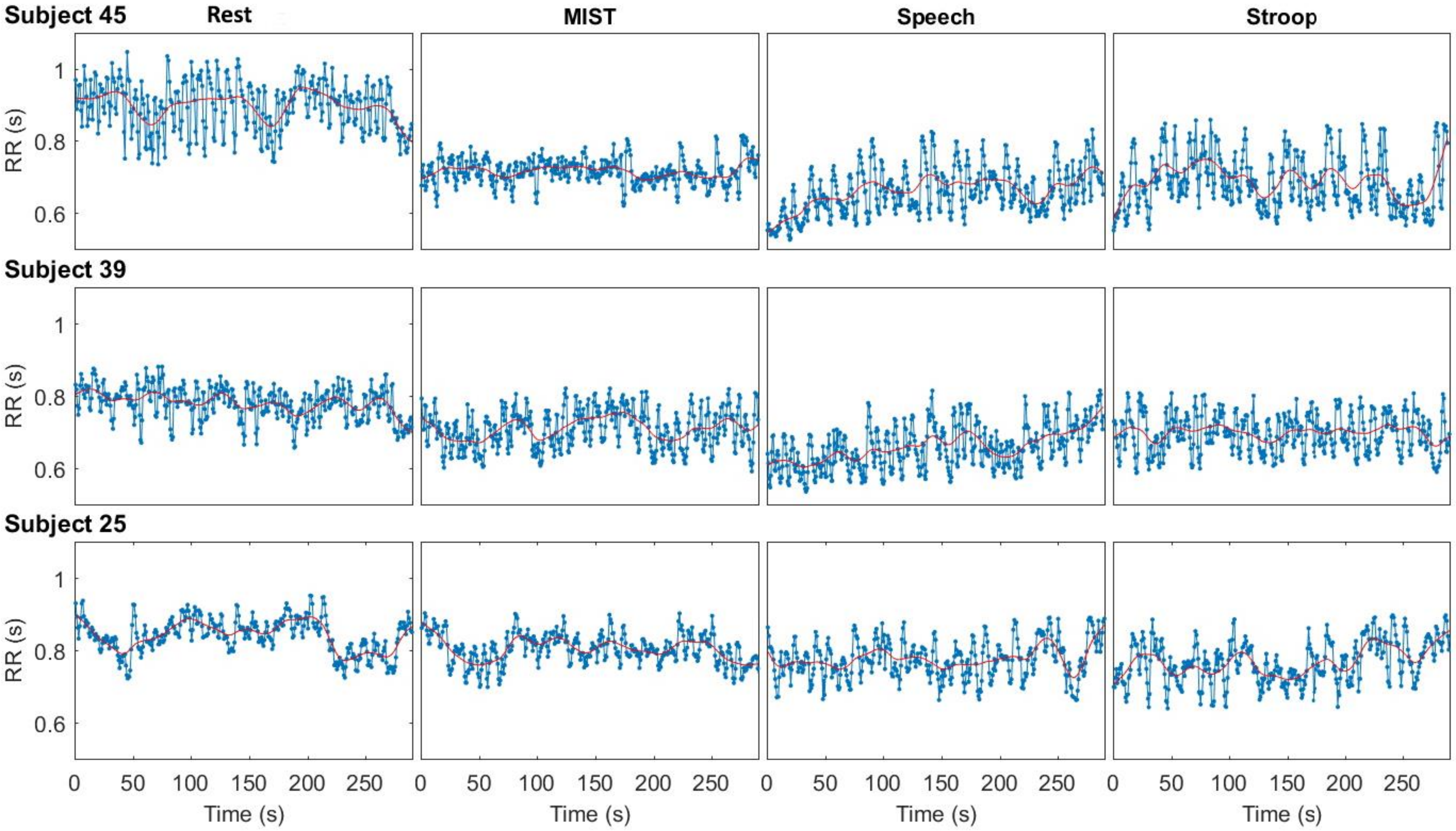
	Rest		MIST		Speech		Stroop		Recovery	
Psychological variables										
SRQ	14.74 (0.72)	b, c, d, e	19.26 (0.95)	a, e	18.76 (0.91)	a, e	18.46 (0.96)	a, e	12.25 (0.71)	a, b, c, d
TE	3.15 (0.26)	b, c, d	5.13 (0.31)	a, e	4.46 (0.28)	a, e	4.83 (0.33)	a, e	2.46 (0.25)	b, c, d
Physiological variables										
EDR	0.195 (0.006)	c	0.195 (0.006)	c, d	0.171 (0.004)	a, b	0.178 (0.005)	b	0.186 (0.006)	
HR	77.46 (1.40)	b, c, d, e	82.14 (1.57)	a, c, d, e	91.03 (1.72)	a, b, d, e	86.52 (1.63)	a, b, c, e	75.12 (1.21)	a, b, c, d
SDNN	6.59 (0.16)	b, e	6.06 (0.15)	a, c, d, e	6.67 (0.18)	b	6.86 (0.16)	b	7.01 (0.18)	a, b
RMSSD	6.12 (0.19)	b, c	5.62 (0.17)	a, d, e	5.60 (0.19)	a, d, e	5.90 (0.18)	b, c, e	6.39 (0.20)	b, c, d
Stress Index	2.09 (0.04)	b, e	2.26 (0.04)	a, d, e	2.19 (0.04)	e	2.12 (0.04)	b, e	2.00 (0.04)	a, b, c, d
Total Power	3.22 (0.05)	b	3.03 (0.05)	a, c, d, e	3.23 (0.05)	b	3.31 (0.04)	b	3.31 (0.05)	b
HFpow	2.77 (0.06)	b	2.58 (0.05)	a, d, e	2.64 (0.06)		2.71 (0.05)	b	2.79 (0.06)	b
LFpow	2.93 (0.05)	b, d, e	2.78 (0.05)	a, c, d, e	3.05 (0.05)	b	3.12 (0.04)	a, b	3.06 (0.05)	a, b
HFnu	40.84 (2.50)	c, d	38.25 (1.85)	c, d	28.35 (1.56)	a, b, e	28.22 (1.58)	a, b, e	36.05 (2.18)	c, d
LFnu	59.08 (2.51)	c, d	61.65 (1.85)	c, d	71.57 (1.57)	a, b, e	71.72 (1.58)	a, b, e	63.86 (2.19)	c, d
LF/HF	0.19 (0.05)	c, d	0.23 (0.04)	c, d	0.43 (0.03)	a, b	0.44 (0.03)	a, b, e	0.29 (0.05)	d
SD1	5.15 (0.16)	b, c	4.73 (0.14)	a, d, e	4.71 (0.16)	a, d, e	4.96 (0.15)	b, c, e	5.37 (0.17)	b, c, d
SD2	7.40 (0.18)	b, d, e	6.81 (0.16)	a, c, d, e	7.66 (0.20)	b	7.83 (0.18)	a, b	7.91 (0.20)	a, b
Sample Entropy	1.50 (0.04)	b, c, d	1.66 (0.03)	a, c, d, e	1.30 (0.03)	a, b, e	1.38 (0.04)	a, b	1.49 (0.04)	b, c
D2	2.71 (0.20)	a	2.16 (0.21)	a, c, d, e	2.78 (0.19)	b	2.98 (0.16)	b	2.86 (0.19)	b

DFA α1	1.13 (0.03) $^{b, c, d}$	1.21 (0.03) $^{a, c, d}$	1.41 (0.02) $^{a, b, d, e}$	1.34 (0.03) $^{a, b, c, e}$	1.20 (0.03) $^{c, d}$
DFA α2	0.33 (0.02) ^{d}	0.35 (0.02)	0.35 (0.02)	0.38 (0.02) ^{a}	0.34 (0.02)

Notes: *N* = 60. MIST = Montreal Imaging Stress Task; SRQ = Stress Rating Questionnaire; TE = Task Engagement; EDR = ECG-Derived Respiration Rate (in Hertz); HR = Heart Rate; SDNN = Standard Deviation of NN intervals (square-root transformed); RMSSD = Root Mean Square of Successive Differences (square-root transformed); Stress Index = Baevsky's Stress Index (log10 transformed); Total Power = total power of HRV Spectrum (log10 transformed); HFpow = absolute power of High Frequencies (log10 transformed); LFpow = absolute power of Low Frequencies (log10 transformed); HFnu = High Frequency expressed in normalized units; LFnu = Low Frequency expressed in normalized units; LF/HF = LF HF ratio (log10 transformed); SD1 = Poincaré plot SD1 (square-root transformed); SD2 = Poincaré Plot SD2 (square-root transformed); SampEn = Sample Entropy; D2 = Correlation Dimension D2; DFA α1 = short-term fluctuation slope (alpha 1) of Detrended Fluctuation Analysis; DFA α2 = long-term fluctuation slope (alpha 2) of Detrended Fluctuation Analysis. All post-hocs were Bonferroni's corrected.

Significant ($p < 0.05$) reference condition in comparisons: ^{a} = Rest; ^{b} = MIST; ^{c} = Speech; ^{d} = Stroop; ^{e} = Recovery.

Figure 1. Spontaneous fluctuations in RR time series in three subjects (45, 39, 25). The Sample Entropy could have increased during MIST due to the presence of less rhythmical low-frequency fluctuations in the RR time series, in comparison to Speech and Stroop.



Notes: The SampEn values were as following: Subject 25: Rest 1.56, MIST 1.67, Speech 1.36, Stroop 1.42; Subject 39: Rest 1.45, MIST 1.61, Speech 1.37, Stroop 1.34; Subject 45: Rest 1.59, MIST 1.64, Speech 1.22, Stroop 1.34.

Supplementary Materials (for online publication only)

Supplementary Table S1. Means, Standard Deviations (SD), Skewness and Kurtosis values for the untransformed HRV variables (*N* = 60).

	Mean	**SD**	**Skewness**	**Kurtosis**
SDNN Baseline	44.63	18.67	1.11	2.17
SDNN MIST E	37.39	14.11	0.68	-0.14
SDNN Speech	47.66	18.50	1.24	2.36
SDNN Stroop	49.32	17.50	1.03	1.54
SDNN Recovery	51.21	20.31	0.61	-0.29
RMSSD Baseline	38.75	19.10	1.26	2.52
RMSSD MIST E	32.30	14.97	0.98	0.42
RMSSD Speech	33.67	16.80	1.63	4.53
RMSSD Stroop	36.71	17.84	1.56	3.06
RMSSD Recovery	42.59	20.15	1.06	1.54
pNN50 Baseline	17.15	15.59	1.23	1.74
pNN50 MIST E	12.03	12.42	1.16	0.41
pNN50 Speech	12.05	10.83	1.60	3.42
pNN50 Stroop	14.21	12.16	1.57	2.59
pNN50 Recovery	19.48	16.03	0.86	0.54
SI Baseline	161.62	117.54	1.49	2.48
SI MIST E	235.72	168.89	1.38	1.62
SI Speech	190.11	161.85	2.19	5.43
SI Stroop	159.19	112.97	1.76	3.53
SI Recovery	128.21	105.94	2.31	6.68
HF Power Baseline	844.00	884.46	2.88	11.04
HF Power MIST E	511.22	468.85	1.69	2.61
HF Power Speech	693.17	759.93	3.12	13.42
HF Power Stroop	763.09	832.33	2.65	8.42
HF Power Recovery	959.20	1112.73	2.61	7.66

LF Power Baseline	1372.93	1622.93	3.35	14.17
LF Power MIST E	858.92	736.84	1.34	1.14
LF Power Speech	1781.06	1804.93	2.97	10.66
LF Power Stroop	1910.52	1699.14	2.90	12.07
LF Power Recovery	1836.29	1978.17	2.29	5.11
Total Power Baseline	2306.83	2051.40	2.25	7.27
Total Power MIST E	1436.63	1162.46	1.31	0.89
Total Power Speech	2579.67	2425.63	2.42	6.40
Total Power Stroop	2816.42	2400.55	2.38	7.35
Total Power Recovery	2938.66	2518.58	1.41	1.23
LF/HF Ratio Baseline	2.28	2.00	1.31	1.02
LF/HF Ratio MIST E	2.12	1.60	2.16	6.51
LF/HF Ratio Speech	3.30	1.76	0.79	0.01
LF/HF Ratio Stroop	3.35	1.93	1.18	1.76
LF/HF Ratio Recovery	2.89	2.73	1.72	2.28
SD1 Baseline	27.44	13.53	1.26	2.52
SD1 MIST E	22.87	10.60	0.98	0.42
SD1 Speech	23.84	11.90	1.63	4.54
SD1 Stroop	25.99	12.64	1.56	3.06
SD1 Recovery	30.16	14.27	1.06	1.54
SD2 Baseline	56.47	23.58	1.07	1.90
SD2 MIST E	47.37	17.69	0.71	-0.04
SD2 Speech	62.87	23.73	1.22	2.37
SD2 Stroop	64.48	22.06	0.94	1.38
SD2 Recovery	65.39	26.02	0.63	-0.19

Notes: SDNN = Standard Deviation of NN intervals; RMSSD = Root Mean Square of Successive Differences; Stress Index = Baevsky’s Stress Index; Total Power = total power of HRV Spectrum; HF Power = absolute power of High Frequencies; LF Power = absolute power of Low Frequencies; SD1 = Poincaré plot SD1; SD2 = Poincaré Plot SD2.

Supplementary Table S2. Means, Standard Deviations (SD), *t* and *p* values for all psychophysiological indexes, between Women and Men.

Variable	**Women** (***N*** **= 31)**	**Men** (***N*** **= 29)**	***t*** **value** **(*df*** **= 58)**	***p*** **value**	**Effect size** ***(Cohen's d)***
SRQ Baseline	16.06 (5.43)	12.83 (4.47)	2.509	0.015	0.65
SRQ MIST E	21.00 (6.18)	16.90 (7.45)	2.329	0.023	0.60
SRQ Speech	20.03 (6.74)	16.90 (6.42)	1.842	0.071	0.48
SRQ Stroop	20.32 (6.39)	15.97 (7.30)	2.465	0.017	0.64
SRQ Recovery	14.00 (5.27)	10.14 (3.81)	3.220	0.002	0.84
EDR Baseline	0.21 (0.05)	0.17 (0.03)	3.791	< 0.001	0.98
EDR MIST E	0.20 (0.04)	0.19 (0.04)	1.139	0.259	0.29
EDR Speech	0.18 (0.03)	0.16 (0.02)	2.367	0.021	0.61
EDR Stroop	0.19 (0.04)	0.17 (0.03)	2.041	0.046	0.53
EDR Recovery	0.19 (0.05)	0.18 (0.04)	1.177	0.244	0.30
HR Baseline	81.86 (11.59)	74.94 (8.88)	2.585	0.012	0.67
HR MIST E	87.02 (11.65)	79.17 (10.81)	2.701	0.009	0.70
HR Speech	95.02 (14.35)	87.08 (11.08)	2.387	0.020	0.62
HR Stroop	90.46 (14.30)	83.20 (11.01)	2.194	0.032	0.57
HR Recovery	77.69 (10.15)	73.90 (6.82)	1.687	0.097	0.44
sqrtSDNN Baseline	6.15 (1.39)	6.97 (1.20)	-2.447	0.017	-0.63
sqrtSDNN MIST E	5.84 (1.19)	6.20 (1.06)	-1.238	0.221	-0.32
sqrtSDNN Speech	6.34 (1.20)	7.26 (1.22)	-2.946	0.005	-0.76
sqrtSDNN Stroop	6.45 (1.13)	7.42 (1.09)	-3.399	0.001	-0.88
sqrtSDNN Recovery	6.67 (1.41)	7.39 (1.33)	-2.032	0.047	-0.52
sqrtRMSSD Baseline	5.83 (1.55)	6.28 (1.37)	-1.172	0.246	-0.30
sqrtRMSSD MIST E	5.37 (1.32)	5.73 (1.20)	-1.110	0.272	-0.29

sqrtRMSSD Speech	5.25 (1.40)	6.06 (1.23)	-2.369	0.021	-0.61
sqrtRMSSD Stroop	5.50 (1.37)	6.34 (1.25)	-2.497	0.015	-0.65
sqrtRMSSD Recovery	6.21 (1.53)	6.51 (1.46)	-0.765	0.448	-0.20
logSI Baseline	2.20 (0.33)	2.00 (0.27)	2.588	0.012	0.67
logSI MIST E	2.32 (0.32)	2.22 (0.29)	1.303	0.198	0.34
logSI Speech	2.26 (0.31)	2.05 (0.29)	2.647	0.010	0.68
logSI Stroop	2.21 (0.30)	2.00 (0.26)	2.846	0.006	0.74
logSI Recovery	2.09 (0.33)	1.89 (0.24)	2.646	0.010	0.68
logTotPower Baseline	3.10 (0.40)	3.34 (0.30)	-2.587	0.012	-0.67
logTotPower MIST E	2.95 (0.36)	3.10 (0.33)	-1.713	0.092	-0.44
logTotPower Speech	3.14 (0.39)	3.40 (0.32)	-2.825	0.006	-0.73
logTotPower Stroop	3.20 (0.32)	3.47 (0.28)	-3.514	0.001	-0.91
logTotPower Recovery	3.23 (0.40)	3.41 (0.34)	-1.848	0.070	-0.48
logLF.pow Baseline	2.77 (0.46)	3.10 (0.34)	-3.146	0.003	-0.81
logLF.pow MIST E	2.68 (0.39)	2.89 (0.35)	-2.199	0.032	-0.57
logLF.pow Speech	2.95 (0.37)	3.25 (0.32)	-3.285	0.002	-0.85
logLF.pow Stroop	3.01 (0.31)	3.31 (0.28)	-3.932	< 0.001	-1.02
logLF.pow Recovery	2.94 (0.41)	3.22 (0.36)	-2.755	0.008	-0.71
sqrtSD1 Baseline	4.91 (1.30)	5.29 (1.16)	-1.172	0.246	-0.30
sqrtSD1 MIST E	4.52 (1.11)	4.82 (1.01)	-1.111	0.271	-0.29
sqrtSD1 Speech	4.42 (1.17)	5.10 (1.03)	-2.369	0.021	-0.61
sqrtSD1 Stroop	4.62 (1.15)	5.34 (1.05)	-2.497	0.015	-0.65
sqrtSD1 Recovery	5.23 (1.29)	5.48 (1.23)	-0.765	0.447	-0.20
sqrtSD2 Baseline	6.86 (1.54)	7.90 (1.33)	-2.780	0.007	-0.72
sqrtSD2 MIST E	6.57 (1.32)	6.97 (1.19)	-1.231	0.223	-0.32
sqrtSD2 Speech	7.29 (1.31)	8.35 (1.39)	-3.035	0.004	-0.78
sqrtSD2 Stroop	7.38 (1.23)	8.50 (1.22)	-3.548	0.001	-0.92
sqrtSD2 Recovery	7.49 (1.58)	8.40 (1.50)	-2.304	0.025	-0.60

Samp.En Baseline	1.55 (0.30)	1.45 (0.29)	1.369	0.176	0.35
Samp.En MIST E	1.65 (0.23)	1.68 (0.26)	-0.460	0.647	-0.12
Samp.En Speech	1.31 (0.27)	1.25 (0.26)	0.931	0.355	0.24
Samp.En Stroop	1.38 (0.29)	1.35 (0.30)	0.378	0.707	0.10
Samp.En Recovery	1.57 (0.28)	1.40 (0.30)	2.159	0.035	0.56
D2 Baseline	2.28 (1.70)	2.97 (1.13)	-1.852	0.069	-0.48
D2 MIST E	1.78 (1.63)	2.35 (1.48)	-1.415	0.162	-0.37
D2 Speech	2.38 (1.53)	3.15 (1.12)	-2.225	0.030	-0.57
D2 Stroop	2.55 (1.47)	3.32 (0.61)	-2.615	0.011	-0.68
D2 Recovery	2.75 (1.51)	2.82 (1.12)	-0.197	0.845	-0.05
DFA.a2 Baseline	0.37 (0.13)	0.30 (0.11)	2.265	0.027	0.59
DFA.a2 MIST E	0.40 (0.14)	0.31 (0.09)	3.061	0.003	0.79
DFA.a2 Speech	0.38 (0.13)	0.29 (0.09)	3.152	0.003	0.81
DFA.a2 Stroop	0.41 (0.14)	0.34 (0.09)	2.237	0.029	0.58
DFA.a2 Recovery	0.38 (0.14)	0.31 (0.12)	1.840	0.071	0.48

Notes: SRQ = Stress Rating Questionnaire; MIST = Montreal Imaging Stress Task; EDR = ECG-Derived Respiration Rate (in Hertz); HR = Heart Rate; SDNN = Standard Deviation of NN intervals (square-root transformed); RMSSD = Root Mean Square of Successive Differences (square-root transformed); Stress Index = Baevsky's Stress Index (log10 transformed); Total Power = total power of HRV Spectrum (log10 transformed); LFpow = absolute power of Low Frequencies (log10 transformed); SD1 = Poincaré plot SD1 (square-root transformed); SD2 = Poincaré Plot SD2 (square-root transformed); DFA α2 = long-term fluctuation slope (alpha 2) of Detrended Fluctuation Analysis.