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Cardiovascular responses to stress utilising anticipatory singing tasks.

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Conflict of interest statement

The authors of this paper do not foresee any potential sources of conflict of interest either from relationships, financial or otherwise that might be perceived as influencing an author's objectivity.

Ethics

This study was submitted to and approved by the University Ethics Committee

Abstract

Models of psychobiological stress reactivity have a foundation in the measurement of responses to standardised stress tasks. Tasks with anticipatory phases have been proposed as an effective method of stress induction, either as a stand-alone task or replacement constituent elements for existing stressor paradigms. Tasks utilising singing as a primary stressor have been proposed but the efficacy of these tasks have not been demonstrated while maintaining adherence to a resting/ reactivity/recovery framework desirable for HRV measurement. This study examines the viability of an anticipatory sing-a-song task as a method for inducing mental stress and examines the utility of the task with specific reference to measures of cardiovascular reactivity and recovery activity, and standard protocols to examine HRV reactivity and recovery. Participants completed a dual task with a maths task and an anticipation of singing component. Responses were examined according to a resting/reactivity/recovery paradigm and the findings indicate that the sing-a-song stimulus is effective in generating a stress response. Significant differences in heart rate and self-reported stress between baseline and stressor conditions were detected, with greater magnitude differences between baseline and anticipatory phases. This study has demonstrated the viability of the anticipation of singing as a standardised stressor using cardiovascular measures and has described variants of this task that may be used for repeated measures study designs.

Keywords

Heart Rate Variability, Stress Testing, Measurement, Anticipatory Stress, Social Evaluative Stress

Introduction

Models of psychobiological stress reactivity have a foundation in the measurement of responses to standardized stress tasks. To delineate these responses, a dedicated literature has examined the efficacy and methodological applications of stress tasks, with a focus on acute reactivity via chronic psychosocial stress (Chida & Hamer, 2008). These stressors have included cognitive tasks such as, serial subtraction, public speaking, stress interview, emotion induction, and social evaluative stress. These tasks have been utilized to elucidate differential responses in biology, such as hypothalamic pituitary adrenal (HPA) activation, via cortisol, adrenocorticotropic hormone (ACTH), vasopressin, dehydroepiandrosterone (DHEA), and cardiovascular activation (Allen et al., 2014). Demographic and psychosocial variables have also been examined in relation to stress task efficacy including age (Antelmi et al., 2004) and gender (e.g., Koenig & Thayer, 2016; Kudielka et al., 2004) and pubertal stage (e.g., Sumter et al., 2010). Further study has sought to examine pathophysiological processes of physical illness brought about by sustained hypertensions in response to stress challenge (e.g., Thayer et al., 2010) and psychopathologies, for example, depression (Burke et al., 2005; Young et al., 2000) and anxiety (Gerra et al., 2000; Young et al., 2004).

In order to better model ecologically valid stressors, tasks that utilize a combination of psychosocial procedures such as the Trier Social Stress Task (TSST; Kirschbaum et al., 1993) have been developed. The TSST has emerged as the most widely used stress test (Kudielka et al., 2007), and has been used to reliably induce stress, enabling the relationship between stress and physiological responses to be examined (Allen et al., 2017). Although widely employed by researchers, a number of challenges of the TSST exist, including, methodological resources load (Allen et al., 2017), potential confounds such as movement (standing) and speaking (Brouwer & Hogervorst, 2014), and habituation challenges, when participants are required to complete repeated measures, in particular in relation to HPA activation (Arvidson et al., 2017; Schommer et al., 2003). Reducing habituation effects presents a need to identify and validate for replacement task elements in existing stressor paradigms, such as variation of math task or novel types of social evaluative threat, although it is noted that while habitation has been observed for HPA activation, cardiovascular (CV) reactivity and blood pressure appear less susceptible to habituation effects (Boyle et al., 2016). Given the challenges indicated above, there is a need to identify and verify possible replacement elements or stand-alone tasks with demonstrated equivalence of efficacy.

Brouwer and Hogervorst (2014) proposed the Sing-A- Song Stress Test (SSST) as a task for inducing mental stress with the aim of addressing a number of the challenges inherent in the TSST such as movement, standing, and speaking. The SSST elaborates on a methodology proposed by Hoffman et al. (2006) designed to examine reactivity in shy/non-shy participants using anticipation of a singing task to induce a stress response. Hoffmans' original methodology informed participants that they would be required to give a speech and to sing-a-song. The SSST further developed this task using computer-based stimuli while standardizing presentation and the inclusion of time locking of stimuli to reduce confounds. The task was efficacious, but the timed periods did not allow examination of heart rate variability (HRV) measures. We propose the examination of the viability of the anticipatory singing task in order to adhere to standardized methods for measurement of cardiovascular activity, including a Resting/Reactivity/ Recovery (RRR) structure (Laborde et al., 2017; Shaffer & Ginsberg, 2017), which allows for delineation between tonic and phasic HRV between conditions. This design has been proposed by Laborde as a standardized procedural outlay to allow for examination of change in HRV across conditions.

Cardiovascular activity is a widely used measure in psychophysiological science, and changes in cardiovascular parameters are related to individual differences, such as personality (Huang et al., 2013) and risk factors for health and morbidity (Kemp & Quintana, 2013), and are relatively easily measured in comparison to other biological systems (e.g., the cortisol response). The most commonly used indicators of cardiovascular activity are blood pressure, heart rate reactivity, and heart rate variability. The reactivity hypothesis (Blascovich & Katkin, 1993) posits that heightened cardiovascular reactivity, typically reported as increase of beats per minute (BPM) and blood pressure (mm HG), observed in response to stress can promote sustained hypertensions that contribute to the development of cardiovascular disease (Allen et al., 2017; Everson et al., 1997; Thayer et al., 2012). HRV has also been widely used in psychophysiological study (Laborde et al., 2017; Quintana & Heathers, 2014). HRV describes a range of measures, including time domain and frequency components of the heartbeat that have been associated with affect (Kreibig, 2010; Porges, 2001) and social functioning (Porges, 2001; Quintana et al., 2012). Typically, time domain measures describe variability in successive R–R peak intervals or variations in inter-beat interval (IBI). Root mean square of successive differences (RMSSD) reflects the beat-to-beat variance in IBI for cardiovascular activity and has been associated with high frequency (HF) power

(Shaffer & Ginsberg, 2017). In addition, frequency domain measures are based on the assumption that each range is indicative of the “power” or signal energy for that given band; ultra-low frequency (ULF), very low frequency (VLF), low frequency (LF), and high frequency (HF). ULF has been associated with circadian rhythm and metabolic demands (Shaffer et al., 2014). VLF has been linked to physical movement (Bernardi et al., 1996) and parasympathetic activation (Taylor et al., 1998). The LF band has been associated with baroreceptor activity, and subsequently blood pressure (Shaffer & Ginsberg, 2017), and has been proposed as an index of sympathetic outflow (Reyes del Paso et al., 2013), although inclusion of LF is questionable and disagreement exists regarding its use as an index of sympathetic activation (Billman, 2011, 2013; Heathers & Goodwin, 2017). HF components of HRV have been reliably associated with vagal tone (Porges, 2007), and is therefore correlated with stress, permitting its use as measure to reflect parasympathetic activity (Laborde et al., 2017). Finally, LF/HF ratio has been proposed as an index of auto- nomic balance (ChuDuc et al., 2013; Quintana & Heathers, 2014), although as mentioned, because the inclusion of LF bands is questionable, employing the LF/HF ratio carries related concerns. While debate exists regarding the various HRV components, for the purposes of the current study time domain measures of BPM, IBI, RMSSD, and frequency components of HF are including following recommendations by Laborde (Laborde, Mosley, & Thayer, 2017) and Quintana (Quintana & Heathers, 2014) for experimental planning and data reporting using HRV measures.

In this study, we examine the inclusion of an anticipatory sing-a-song task as a task element for inducing mental stress while retaining adherence to a RRR design suitable for HRV analysis. Initial findings from the SSST, as demon- strated by Brouwer and Hogervorst (2014) indicate a reliable induction of a stress response with promising avenues using an anticipatory sing-a-song task that addresses confounds such as standing, speaking, researcher interaction, and also reduces methodological resource load. While the SSST has advantages, particularly for short-term CV measures (< 10-minute epochs), to our knowledge it has not been examined using more granulated measures of HRV that offer advantages in interpretation of autonomic function (Quintana & Heathers, 2014). A number of methodological considerations arise when considering the use of the SSST with HRV measures, such as matched timing of epochs of sufficient duration for HRV measures. This study employs full spectrum electrocardiogram (ECG) analysis and presents reactivity measures (BPM), time domain measures (IBI and RMSSD), and frequency domain measures of high frequency (HF). A number of adjustments are suggested to align the procedures of the SSST with recommendations (Laborde et al., 2017) for experimental planning, data analysis, and data reporting for HRV type studies utilizing a RRR design with anticipation of singing stressor stimuli included along with established stressor protocols.

Method

Participants

Participants (n = 45, Male = 21, Female = 24, aged: 19–65 years, M = 31.89, SD = 11.34) were recruited from the general population using convenience sampling. Calls for participation were advertised using social media and university email. Participants were contacted after initial expression of interest and given a schedule for laboratory attendance.

Design

This study uses a within-subject laboratory-based design to evaluate the viability of an anticipatory singing task utilizing cardiovascular measures. The task includes two components, a standardized math task followed by an anticipatory “sing-a-song” task in RRR segments. Patterns of HRV are used to assess the efficacy of the two task components, separately, in combination, and compared to baseline and recovery.

Materials and measures

Physical Environment and Laboratory Conditions

Measurements were taken in the laboratory, located on the ground floor of the psychology building. The room has no windows except a one-way observation mirror on one wall. Lighting and room temperature were kept consistent for all participants and was managed at 18.5°C. The room is equipped with visible camera and microphone recording equipment. Participants sat on a stationary office chair for the measurement period to minimize movement.

Physical measures & Confounds

Physical characteristics of the participants were recorded in the laboratory; Participant height(cm) and weight (kg) were measured, and BMI was calculated. Age & gender were self-reported. Participants self-reported physiological and lifestyle factors (both stable and transient) that could cause potential confounds in the heart rate data medical conditions or medications including, cardio-active medications, antidepressants or anti-hypertensives. These measures were used to preclude participants from the study.

Electrocardiogram Measurement

Electrocardiogram (ECG) measurement for this study was carried out using the Actiheart™ (Brage et al., 2006) mea- surement device. Actiheart is a validated (Kristiansen et al., 2011) non-invasive method of measuring physical activity and energy expenditure (PAEE) (Crouter et al., 2008). The Actiheart is attached over the skin, via an electrodermal patch, and is used to measure ECG heart rate and movement. The device is capable of capturing the full QRS spectrum for defined epochs and uses the resultant data in its proprietary software to calculate and resolve HRV measures using the Pan Tompkins QRS detection algorithm (Pan & Tompkins, 1985). R–R intervals for at least 16 heart beats are captured for each epoch and aver- aged. The correction of artifacts was completed by the deletion of the values outside of a

±25% range and removed and the remaining data re-averaged. In addition to assessment and correction of the R–R signal quality, the software presents an additional quality index (range: 0–1) for each epoch that can be examined by the user and excluded if necessary. For the purposes of the current study, this additional quality control was included and any epochs with a suboptimal quality rating (< 1/perfect score) was excluded from the analysis. The Actiheart device also returns measures of physical activity for the participant. The device measures movement in "counts of unit per time." This time/second measurement is then applied by the proletary software to the user defined epoch (CamNtech, 2010) returning the activity measure for the epoch. Only epochs where no physical activity was detected are included.

Resting - Baseline

Baseline measures (Fishel, Muth, & Hoover, 2007) were collected once the participant was fitted with the ECG device and sitting resting in a chair for 10 minutes. Participants are required not to move to reduce artifacts in their baseline heart rate data. Participants were required to remain seated on a chair with legs their knees at a 90-degree angle and both feet flat on the floor. Participants were not asked to keep their eyes closed but were asked not to move if possible. No instructions were given with regards to hand position. Measures of HR and movement were recorded during the baseline period.

Reactivity – Maths task

The maths task component is a computer-based maths task written and presented on Psycopy Software (Peirce, 2019). Similar to the maths element of the standard Trier Social Stress Task, participants are required to complete a serial subtraction task while under social evaluative threat provided by constant observation of a researcher, seated close to the participant. The maths task is presented to participants and they are told that they must achieve a fictitious score and the number of iterations of the test that they must achieve. This element is included to increase perceived external social evaluative threat. Scripted comments, such as "Most people have done much better by now" were added at predetermined times during the task to increase the evaluative element

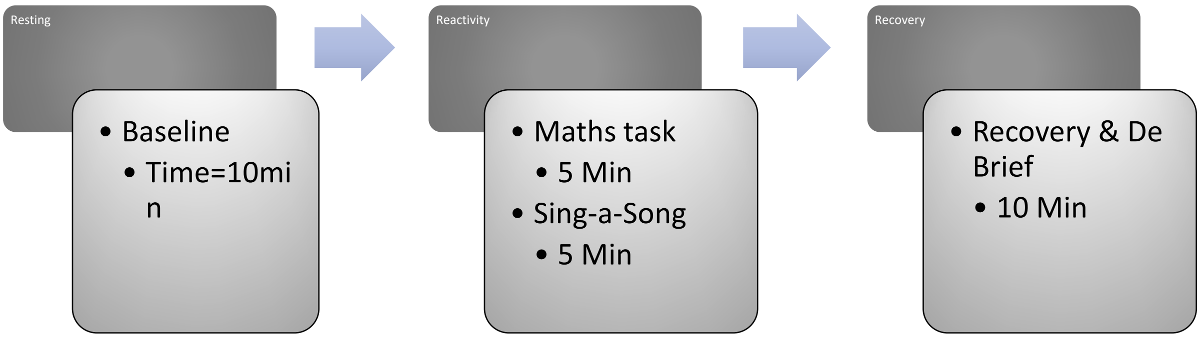
Reactivity- Singing task

The singing phase of the reactivity section is an adaptation of the stressor paradigm outlined by Brouwer & Hogervorst (2014) employing 'singing' as a primary stressor as a variant of the 'talking' task used in the original Trier Social Stress Task (Kirschbaum et al., 1993). The variant of the Sing-A-Song used in the current studies represents an adaptation of the task proposed by Brouwer with the inclusion of considerations for heart rate variability measurement. In the current study the variation introduces an anticipatory stress phase induced by a surprise instruction to sing a song. The singing phase commences once the researcher hands the participant a copy of song lyrics. It is indicated to the participant that they will be required to sing the song to the researcher following a 5-minute preparation period. The singing-phase requires the participant to sit in the same position as the baseline and maths phase, with the addition of a camera and microphone, introduced by the researcher to "record the singing session". The researcher is present, sitting at right angles, in close proximity to the participant as they prepare to sing. A verbal minute by minute countdown is included – increasing to every fifteen seconds for the final minute- followed by a ten second countdown at the end of the anticipatory period. At the end of the anticipatory phase the participant is told that they are not required to sing. The five-minute preparation period constitutes the entire singing anticipatory phase.

Recovery phase
A five-minute recovery phase was completed by all participants. The recovery phase, which mirrored the baseline measurement was included to allow a secondary comparison to the task situation (Quintana & Heathers, 2014). Recovery consisted of five minutes of rest sitting in a chair in the laboratory while continuing to wear the ECG device. Participants are again instructed not to move to reduce artifacts in the HR data.

Self-report affect
A self-report affect scale was included at all periods of measurement during the laboratory task. The affect scale requires participants to self-report five dimensions of subjective affect, on a rating of 1-5, including, measures of relaxation, stress, control and anxiety the self-report measures were included to allow for quantification of participant self-perceived affect during the task.

Procedure



Laboratory attendance
Participants were contacted and asked to attend the laboratory. Participants were required to refrain from caffeinated drinks and smoking for at least one hour prior to attendance. Participants were greeted by a researcher dressed in a white laboratory coat in a friendly but neutral manner. Participants were then escorted to the laboratory. Once the consent procedures were completed, the participants were asked to complete the self-report measures. Data was anonymised to comply with the requirements set out by the of Ethics committee and data protection legislation.

ECG procedure

Actiheart is attached directly to the skin via two conductive ECG electrodes. The first electrode was attached at the V2 fourth intercostal space immediately left of the sternum. The second electrode was placed approximately ten centimetres away on the V5 anterior axillary line at the same horizontal plane as the V4. These positions follow the recommendations outlined by the product designer (CamNtech, 2010) and by Brage et al (Brage et al., 2006) for patch location on signal integrity. Although the manufacturer recommends two possible positions (the second is also aligned with the V2 and V5 but is underneath the breast/pectoral muscle) it was decided to use the single above position in order to maintain consistency of collection method for all participants due to different physical characteristics such as breast tissue or body fat composition (Rautaharju, Park, Rautaharju, & Crow, 1998). Once patch placement was completed the participants moved on to signal testing.

Signal testing

A signal test is required to ensure the integrity of the signal and that accurate recording of the full r-r wave complex of the heartbeat is being captured. This involves a short recording period of 12 minutes for each participant. The Actiheart software provides a specific function for signal quality testing. The software analysis tests the signal and provides the user with an overall pass/fail score for the entire session. All participants successfully completed sufficient signal quality tests.

Baseline

Baseline measurements were completed by all participants (5 minutes acclimatisation / 5 minutes measurement) of rest sitting in a comfortable chair in the laboratory prior to engaging in the stressor phases

Maths task

Participants were informed they would be required to pass an “intelligence” test in order to participate any further. Participants were then told they had to maintain an 80% standard to be successful. The importance of completing the task, possible consequences of failure and significance of the maintaining successful scores was emphasised by the researcher. The maths task procedure takes 5 minutes to complete. A number of experimental elements are managed to maintain homogeneity of presentation during the task; firstly, the researcher sits in close proximity to increase social evaluative elements of the task, secondly, the researcher comments are scripted and carefully managed, thirdly, the researcher maintains a neutral manner throughout the presentation. The screen presentation ensures homogeneity of stimulus as it is central to participant focus throughout the task.

Numbers are presented to the participants with a blank box to type answers. In order to increase the task difficulty, each task is timed, and timing is represented by a clock countdown on the screen. If participants are slower than the allocated time it represents a failure of that particular task. Each failure/pass is also accompanied by a sound and image, with a low sound and green tick/correct mark for a pass, and a loud noise and visual red X for a “fail”.

Participants progress was observed by the researcher and scripted comments were mentioned at random times, regardless of participant progress. These included statements such as, “As you can see, most people have a slightly higher score by now” and “We need to speed up your progress if you are going to be able to proceed in the experiment”. Participants were also reminded by the researcher throughout the task of a fictitious average score of other participants. All participants completed the maths task.

Sing a song task

Immediately following the maths task, the participants were informed by the researcher that they would be required to complete an additional task as part of their “evaluation”. This test would require them to ‘sing the song’ into a microphone in front of the researcher. Participants were also told that a camera and microphone would be used to record them singing and this would be later used as part of their evaluation. Participants were then given a copy of the song they would be required to sing and instructed to familiarise themselves during a five-minute waiting period. During this period, the researcher was in close proximity and the countdown provided. When the countdown period ended participants were informed they would not have to sing. Participants then moved to the recovery phase.

Recovery phase

A recovery phase of five minutes followed directly after the stressor periods. During this time, the participants were required to sit still to mirror the baseline measurement. Participants were debriefed in line with ethical procedures for laboratory stress protocols and fully informed of the nature of the study.

Data Analysis

HRV data for this study was accessed using the Actiheart proprietary software, the software enables researchers to visualise and export HRV data. The software uses returns measures of beats per minute (BPM), Inter-beat interval IBI (Maximum, minimum and average measures of IBI are returned), root mean of successive square differences (RMSSD), standard deviation of NN intervals (SDNN). Also, frequency domain measures of High Frequency (HF), Very Low Frequency (VLF), Low frequency (LF) and finally Low frequency/High frequency ratio (LF/HF) are captured. For the purposes of the current study, measures of BPM, IBI, RMSSD and HF components only are reported, this is owing to disagreement and reliability regarding the LF components. Actiheart returns ECG data for defined epochs and its’ proprietary algorithm sorts and cleans data prior to presenting it to the user. Examination of movement is permitted using movement indices returned by the ECG measurement device. For the purposes of the current study an additional quality control procedure was implemented at data analysis phase and epochs with sub optimal quality ratings was excluded from the analysis.

Ethics

This study was approved by the University Ethics Committee.

Analysis

Overview

The following results describe the mean differences between test conditions for time domain and frequency components of HR. Repeated measures ANOVA's were conducted to compare of BPM, IBI, RMSSD and High Frequency HRV during baseline, maths stress task, Sing-a-Song anticipation, and recovery phases. Results for each measure are detailed including self-report momentary affect scales. Gender and age comparisons are also included.

Table 1. Analysis of Variance for Resting, Reactivity and Recovery Conditions

Measure	**Baseline**		**Maths**		**Singing**		**Recovery**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
*BPM **	66.20	9.30	78.05	11.29	79.10	14.18	71.02	10.23
Range	49 - 83		52 - 101		53 - 115		51 - 102	
*IBI **	922.82	132.72	784.63	116.81	782.67	131.96	862.7	116.22
Range	723-1218		598-1135		517-1124		586-1169	
*RMSSD**	57.24	37.06	43.97	31.00	40.43	23.94	66.08	48.29
Range	12.28 - 208.80		14.08 - 191.43		9.53 - 106.60		14.67 - 200.73	
*HF **	929.50	1000.49	858.00	1525.25	606.33	669.72	1068.2	1306.20
Range	28 - 4048		37 - 9356		18 - 2733		22 - 5912	

Figure (i) Mean BPM for Baseline, Maths, Singing and Recovery conditions.

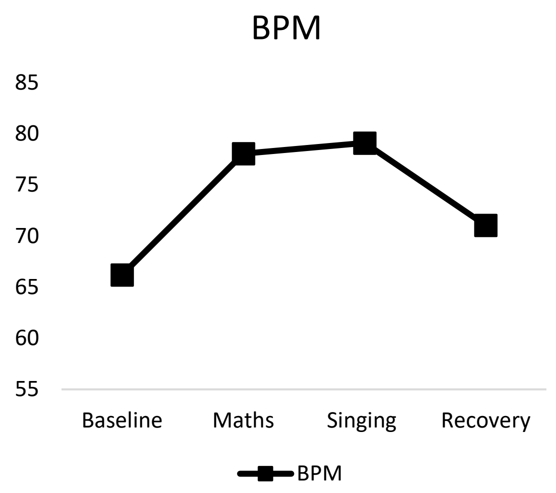


Figure (ii) Mean IBI for Baseline, Maths, Singing and Recovery conditions

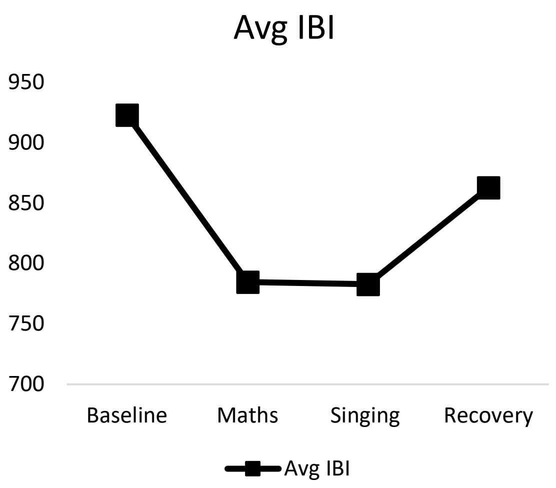


Figure (iii) Mean RMSSD for Baseline, Maths, Singing and Recovery conditions.

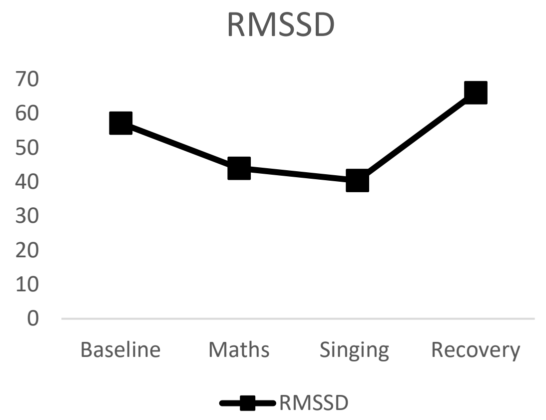
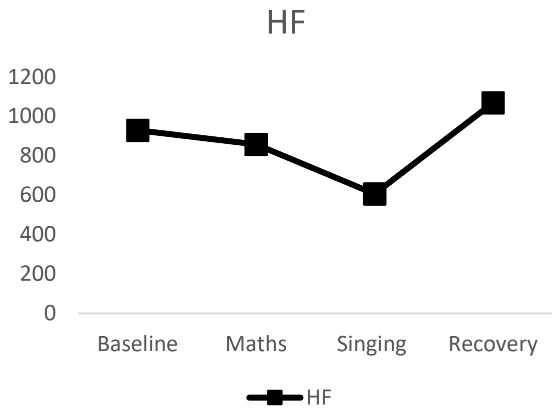


Figure (iv) Mean HF for Baseline, Maths, Singing and Recovery conditions.



Beats Per Minute

A one-way repeated measures ANOVA was conducted to compare BPM during Baseline, Maths stress task, Sing-A-Song anticipation, and recovery. There was a significant overall effect for BPM, Wilks' Lambda = .28, F (3, 37) = 32.55, p < .000, multivariate partial eta squared = .725. Post hoc tests using a Bonferroni correction showed differences from baseline BPM (M= 66.2, SD=9.30) and (i) maths condition for BPM (Mean difference = 11.85, p < .000; (ii) Sing a song condition BPM (Mean difference = 12.90, p <.000) (iii) Recovery (Mean difference = 4.825, p < .000). Differences were also detected between recovery BPM (M=71.03, SD=10.24) and (i) maths condition for BPM (Mean difference = 7.03, p < .000; (ii) Sing a song condition BPM (Mean difference = 8.08, p < .000)

No statistically significant differences were found for BPM between the two stressor conditions.

Inter-Beat Interval

A one-way repeated measures ANOVA was conducted to compare IBI during Baseline, Maths stress task, Sing-A-Song anticipation, and recovery. There was a significant effect for IBI, Wilks' Lambda = .27, F (3,37) = 33.82, p < .000, multivariate partial eta squared = .733 Post hoc tests using a Bonferroni correction showed differences from baseline (M=922.82, SD =132.71) and (i) maths condition for IBI (Mean difference = -138.2, p < .000 (ii) Sing a song condition IBI (Mean difference = -139.95, p < .000) (iii) Recovery (Mean difference = -60.09, p < .000). Differences were also detected between recovery IBI (M=862.74, SD=116.22) and (i) maths condition for IBI (Mean difference = -78.11, p < .000; (ii) Sing a song condition IBI (Mean difference = -79.86, p < .000). No statistically significant differences were found for IBI between the two stressor conditions.

RMSSD

A one-way repeated measures ANOVA was conducted to compare RMSSD during Baseline, Maths stress task, Sing-A-Song anticipation, and recovery. There was a significant effect for RMSSD, Wilks' Lambda = .552, F (3, 37) = 9.99, p < .000, multivariate partial eta squared = .448 Post hoc tests using a Bonferroni correction showed differences from baseline RMSSD (M= 57.24, SD=37.06) and (i) Sing a song condition RMSSD (Mean difference = -16.79, p = .002). Differences were also detected between recovery RMSSD (M= 66.08, SD=48.28) and Sing a song condition RMSSD (Mean difference = -25.64, p = .001). No statistically significant differences were found for RMSSD between baseline and maths task or recovery and maths task.

High Frequency

A one-way repeated measures ANOVA was conducted to compare the HF component of HR during Baseline, Maths stress task, Sing-a-Song anticipation, and recovery. There was an overall significant effect for HF, Wilks' Lambda = .724, F (3, 37) = 4.698, p = .007, multivariate partial eta squared = .276 Post hoc tests using a Bonferroni correction showed significant differences between the sing-a-song condition (M= 606.33, SD=669.71) and (i) Baseline condition for BPM (Mean difference = - 323.18, p = .017; (ii) Recovery condition

(Mean difference = 461.96, p < .036). No significant differences in HF were found between the Maths task and baseline or recovery.

Gender differences

Analysis of HR revealed mean differences for males and females. Independent-samples t-tests were conducted to compare mean scores for each component of HR for males and females across resting, maths, sing-a-song and recovery conditions. There were no significant differences in BPM, IBI, RMSSD and HF scores across all conditions. It should be noted however that at baseline, one average males had lower mean BPM (*M* = 63.76, *SD* = 9.68) than females (*M* = 68.95 *SD* = 8.07) although this did not reach statistical significance (*t* (41) = 1.859, *p* = .071, two-tailed). At baseline males also had, on average, higher IBI (*M* = 959.86, *SD* = 146.58) and females (*M* = 881.4, *SD* = 101.75) although this did not reach statistical significance (*t* (41) = 1.981, *p* = .07, two-tailed).

Self-report-affect

A one-way repeated measures ANOVA was conducted to compare self-report stress during Baseline, Maths stress task, Sing-A-Song anticipation, and recovery. There was a significant overall effect of time, Wilks' Lambda = .38, *F* (3, 39) = 21.23, *p* < .000, multivariate partial eta squared = .62. Post hoc tests using a Bonferroni correction showed differences from baseline (M=1.45, SD =80) and (i) maths condition (Mean difference = 1.52, p < .000; (ii) Sing a song condition (Mean difference = 1.71, p < .000) and (iii) Recovery (Mean difference = .50, p = .039). No statistically significant differences were found for Self-report affect between the two stressor conditions. Post hoc tests using a Bonferroni correction also showed differences between baseline (M=1.45, SD=.80) and (i) recovery (Mean difference = .50, p = .039

Discussion

In this study, we examined the inclusion of an anticipatory sing-a-song task as a task element for inducing mental stress while adhering to a RRR design suitable for HRV analysis. Findings indicate that the sing-a-song stimulus is effective in generating a stress response in laboratory in addition to the included math task. We detected statistically significant differences in HR measures and in self-report stress measures between baseline and stressor conditions and note that the peak response was the anticipatory singing task.

Analysis of cardiovascular measures indicates significant differences between baseline and stressor conditions for BPM, IBI, RMSSD, and HF. Significant within-subject differences were detected between baseline and stressor conditions for BPM, with greater magnitude differences between baseline and the sing-a-song task. A main effect was also detected for IBI, with differences between baseline and both stressor conditions. Overall differences between baseline stressor conditions were detected in RMSSD, although post hoc analysis showed significant differences between baseline and singing task only. HF also showed a main effect for the combined stressor tasks but was not significantly different between baseline and math task. The finding that RMSSD and HF only differed between baseline and singing task is interesting given the expectation that the math task, which combines both evaluative stress and a greater cognitive load, would have a higher magnitude pattern than the anticipatory singing task. Given that RMSSD is reflective of vagally mediated changes (Thayer & Lane, 2000), that reflect parasympathetic activ- ity and is correlated with HF, this could indicate that the anticipatory singing task, given its singular social evaluative task component, is reflective of a social interaction, while the cognitive elements may be contributing to increased BPM and decreased IBI. This underlines the importance of combining CV measures when examining elements of stressor tasks and also when considering what type of procedure to employ in laboratory stress task. No significant differences were detected on any measure between the stressor conditions. In alignment with the physiological data, participants reported significant differences in self reports' affective state for both math task and anticipatory singing task. No differences were detected between the stressor conditions.

Brouwer and Hogervorst (2014) cite challenges including movement or body positioning (standing) and speaking as possible confounds in the TSST and have demonstrated the potential of the SSST in addressing these. The method- ology in the current study, utilizing both math task and anticipatory components that can be completed while in a seated, steady postural position, can reduce the likelihood of measures being affected by speaking and standing. In addition to evidencing the efficacy of the anticipatory singing task, the current study has demonstrated that the task is robust when adapted to a RRR outlay. The adjustments to the procedure align the task with recommendations for experimental planning, data analysis, and data reporting for HR type studies using cardiovascular measures to indicate psychobiological reactivity and recovery (Laborde et al., 2017; Quintana & Heathers, 2014). These include standardized baseline measurements with equivalence of time periods for stressor conditions and enable sufficient duration to allow calculation and comparison of both time domain and frequency components. Despite changes, the anticipatory singing task remained efficacious in combination with the math task. This indicates its potential for use within future studies that comprise a variety of tasks with various cognitive and evaluative elements. Allen et al. (2017) described requirement for replacement elements or standalone tasks that address habituation to repeated use of the TSST.

While the proof of concept has been demonstrated, there are limitations with the current study. It provides initial evidence of the efficacy singing task in combination with the math task, but we have not directly examined the singing task alone. In addition, the tasks are not counterbalanced, yet we note that given the element of deception in the singing task, namely, that singing will be required when it is not the stressors could not be counterbalanced. If required, this could be addressed in the future study by actually asking participants to sing, which could constitute another stressor measurement period. An alternative strategy is to revise the procedure so that as part of the instructions given in the anticipatory phase, participants are told that only half of the participants were required to sing, and this will be decided at random, and at end of preparatory period. Doing this would not require any participant to perform the task, thus retain the time and resource advantages, protect the future efficacy of the task, as participants would not presume they will not actually be required to sing in future tasks, and reduce the likelihood of participants presuming other tasks will not be necessary, and so retains the researcher-participant trust as well as integrity of the stress challenge. It may also serve to enhance the stressor, as unpredictability has been reported to amplify stress (Peters et al., 2017). Future research using the anticipation only variant of the SSST should consider this task in combi- nation with other stressor task, using different cognitive load and evaluative elements and in relation to individual difference factors which may amplify or ameliorate the magnitude of response to anticipated singing.

Despite these limitations, the findings remain robust as standard Trier procedures include dual stressor tasks routinely (Allen et al., 2017; Birkett, 2011; Kudielka et al., 2007) and are sufficient for stress induction procedures. In addition, the dual stressor with combined time or 5 min allow matching of the 10-minute baseline and recovery periods. The conclusion of the current study is that the SSST carries distinct advantages that include lower resource demands, reduced participant time burden, and retention of the social evaluative and cognitive performance challenges that are crucial components of effective stressors. In addition to these advantages, the SSST variant tested in this study is efficacious evidenced by the non-invasive, sensitive measures of cardiovascular activity. This study was intended as a proof of concept and has demonstrated the efficacy of an anticipatory singing task as a laboratory stressor, and as a task replacement to the TSST with consideration for adherence to HRV measurement.

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