Rhythmic Emotional Stimulation and Its Influence on Heart Rate Variability: Investigating Visual and Acoustic Cues as Potential Tools for Stress Modulation

Antonio Castells-Delgado[1,2], Mahtab Mohammadpoor Faskhodi[1],
Mireya Fernández Chimeno[1], Miquel A. García-González[1]

[1] Department of Biomedical Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain
[2] Centre for Languages, Culture and Communication (Humanities), Imperial College London, United Kingdom

Date: 13 October 2024

Suggested citation

Castells-Delgado A, Mohammadpoor Faskhodi M, Fernández Chimeno M, García-González MA. *Rhythmic Emotional Stimulation and Its Influence on Heart Rate Variability: Investigating Visual and Acoustic Cues as Potential Tools for Stress Modulation*. Universitat Politècnica de Catalunya (2024). doi.org/10.25560/126862

Corresponding author

Antonio Castells-Delgado
Email: t.castells@imperial.ac.uk.
Affiliations: Universitat Politècnica de Catalunya (PhD candidate) and Imperial College London (Teaching Fellow)

Table of Contents

List of Abbreviations and Acronyms

HR	Heart Rate
BPM	Beats Per Minute
HRV	Heart Rate Variability
RB	Resonant Breathing
RSMT	Rhythmic Skeletal Muscle Tension
RES	Rhythmic Emotional Stimulation
RVES	Rhythmic Visual Emotional Stimulation
RAES	Rhythmic Acoustic Emotional Stimulation
ECG, EKG	Electrocardiogram
BP	Blood Pressure
ANS	Autonomic Nervous System
SNS	Sympathetic Nervous System
PNS	Parasympathetic Nervous System
RSA	Respiratory Sinus Arrhythmia
ABP	Arterial Blood Pressure
VT	Vascular Tone
CVS	Cardiovascular System
IAPS	International Affective Picture System
IADS	International Affective Digital Sounds
IBI	Inter-beat Interval

Abstract

This study investigates the effects of Rhythmic Visual Emotional Stimulation (RVES) and Rhythmic Acoustic Emotional Stimulation (RAES) on Heart Rate Variability (HRV), specifically analyzing SDNN, RMSSD, and IBI as key physiological indicators of autonomic function. HRV is a well-established biomarker of stress and autonomic regulation, and previous research has demonstrated that rhythmic external stimuli presented at the resonant frequency of the arterial baroreflex (0.1 Hz) can enhance HRV. RVES, originally developed by Vaschillo et al. (2008), utilizes emotionally charged visual stimuli to elicit high-amplitude HRV oscillations. This study extends prior research by exploring whether RAES, using emotionally matched acoustic stimuli, can induce comparable effects.

Thirty healthy participants (15 males, 15 females) underwent both RVES and RAES protocols, with HRV measured before, during, and after each intervention. The results revealed distinct patterns in autonomic responses to the two modalities. RVES significantly increased SDNN, indicating its effectiveness in enhancing overall HRV variability, with females exhibiting stronger and more sustained HRV responses than males. RMSSD showed an initial increase during RVES but declined post-intervention, particularly in males, suggesting a short-term parasympathetic withdrawal. RAES, in contrast, had a weaker effect on HRV, producing only a modest increase in RMSSD during the intervention with no significant changes in SDNN. Additionally, BMI was found to be a moderating factor, with a significant three-way interaction between stimulation, sex, and BMI ($p = 0.023$) for IBI in RAES, highlighting individual variability in autonomic responses.

These findings suggest that RVES is a more effective tool for autonomic modulation compared to RAES, supporting previous studies on visual rhythmic entrainment of the baroreflex. The weaker effects of RAES indicate that auditory stimuli alone may not be as effective as visual stimuli in eliciting sustained HRV changes. The significant influence of sex and BMI further emphasises the importance of individual differences in autonomic regulation. Future research should explore multimodal integration (visual-auditory rhythmic stimulation), personalised stimulus design, and repeated exposure effects to optimise HRV-based interventions for stress management and autonomic health.

Keywords

Heart Rate Variability, Resonant Emotional Stimulation, Arterial Baroreflex, Gender-Specific Responses, Autonomic Entrainment, Parasympathetic Regulation.

Ethics Clearance

The studies involving human participants were reviewed and approved by the Ethics Committee on Animal and Human Experimentation (CEEAH) of the Universitat Autònoma de Barcelona (protocol code CEEAH-5745 associated with the PID2019- 107473RB-C2 research project granted by the Spanish Ministerio de Ciencia e Innovación). The patients/ participants provided their written informed consent to participate in this study.

1. Introduction

Stress has been dubbed the *"Health Epidemic of the 21st Century"* by the World Health Organisation and recent data shows it is costing businesses up to $300 billion a year in the US alone (Fink, 2017). Evidence also suggests higher levels of stress in urban areas compared to rural areas, with research showing strong links between long-term environmental city noise and stress, anxiety and depression (Beutel *et al.*, 2016). Research has also identified chronic stress as one of the major contributors to ever-increasing physical chronic disorders such as hypertension, arthritis, type II diabetes, obesity and heart disease (Hayano *et al.*, 1990; Thayer *et al.*, 2009; Seo and Lee, 2010; Cohen *et al.*, 2012).

At the same time, the search for reliable and affordable stress biomarkers has been challenging due to the historical lack of consensus on the definition and diagnosis of stress, and the costs associated with tracking the hormone cortisol and salivary enzyme alpha-amylase (Thayer *et al.*, 2009; Kim *et al.*, 2018). This led to new research into biomarkers that signal stress, particularly focusing on the link between stress and Autonomic Nervous System (ANS) function. The stress response is activated when the Parasympathetic Nervous System (PNS) branch of the ANS can no longer fulfil survival needs, leading to the activation of the Sympathetic Nervous System (SNS) branch of the ANS to cope with temporary or permanent physiological and psychological demands (Gary G. Berntson *et al.*, 1994).

This interplay between the PNS and SNS is especially apparent in its effect on the heart. This net balance between the PNS and the SNS at rest is called cardiac vagal tone (Beauchaine, 2001; Farmer *et al.*, 2016; Brock *et al.*, 2017; Laborde, Mosley and Thayer, 2017a). Increased vagal tone is broadly associated with a low HR and increased HRV Heart Rate Variability (HRV), conditions linked with optimal health levels and satisfactory self-regulation of cognitive, emotional, and social function (Laborde, Mosley and Thayer, 2017a). Decreased cardiac vagal tone, on the other hand, is associated with elevated HR and low HRV, conditions linked to several pathologies and severe physiological stress (Porges, 1995; McCraty, Atkinson and Tomasino, 2003; Seo and Lee, 2010; Kim *et al.*, 2018).

Cardiac vagal tone is proposed as an index of stress and stress vulnerability in mammals (Porges, 1992; Kim *et al.*, 2018). HRV is thus emerging as a non-invasive, reliable, and cost-effective biomarker for stress and vulnerability to stress (Porges, 1995; Beauchaine, 2001; Brock *et al.*, 2017; Laborde, Mosley and Thayer, 2017a). Studies show a strong correlation between HRV and an individual's resilience and ability to adapt to changing and challenging social and environmental contexts (Beauchaine, 2001; Berntson *et al.*, 2008).

As a result of the ubiquitous link between HRV and stress, there has been a growing interest within both the research community and the wellbeing and fitness industries to harness this potential and reverse engineer this biological phenomenon by aiming to improve our resilience towards stressors by directly training and maximising HRV (Mussgay *et al.*, 2006; Lehrer *et al.*, 2013; Lehrer and Gevirtz, 2014; Russo, Santarelli and O'Rourke, 2017; Noble and Hochman, 2019; Shaffer and Meehan, 2020a). Resonant stimulation of the arterial baroreflex is the preferred method of achieving this objective (Vaschillo, Vaschillo, Buckman, *et al.*, 2011a).

The arterial baroreflex is a negative feedback loop mechanism that acts through both the PNS and the SNS branches of the ANS to balance and buffer rapid Arterial Blood Pressure (ABP) changes. Consistent with all negative feedback loop mechanisms, the arterial baroreflex has a resonant frequency of around 0.1Hz (HR change to ABP variations) and approximately 0.04Hz (vascular tone

(VT) changes to ABP variations) (Vaschillo, Vaschillo, Buckman, *et al.*, 2011a). Thus, stimulating the arterial baroreflex at its resonant frequencies will create high-amplitude rhythmic oscillations of HR (0.1Hz) and VT (0.04Hz).

By stimulating the baroreflex at a frequency of 0.1 Hz, high-amplitude rhythmic variations in HRV are induced, optimizing HRV levels. Resonant stimulation of the arterial baroreflex, aimed at maximizing heart rate variability (HRV), can be compared to strength and endurance training, where the benefits extend beyond the training session itself. Regular practice can improve baseline HRV over time, meaning that the heart becomes more adaptable and efficient even outside of practice sessions (Dworkin *et al.*, 1994; Steffen *et al.*, 2017). Baroreflex stimulation also conditions the autonomic nervous system, strengthening the vagal tone, which can lead to improved heart rate control, reduced blood pressure variability, and enhanced resilience to stress (Shaffer and Meehan, 2020b). Thus resonant stimulation of the arterial baroreflex has a cumulative effect which results in significant improvements in HRV and autonomic regulation over time (Lalanza *et al.*, 2023). Thus, this practice is expected to enhance HRV over time, thereby increasing resilience to stress (Lehrer *et al.*, 2009, 2003; Giardino, Chan and Borson, 2004; Mussgay *et al.*, 2006; Karavidas *et al.*, 2007; Siepmann *et al.*, 2008; Hallman *et al.*, 2011; Henriques *et al.*, 2011; Lin, Tai and Fan, 2014; Meule *et al.*, 2012; Gevirtz, 2013; Lehrer and Gevirtz, 2014; Russo, Santarelli and O'Rourke, 2017; Shaffer and Meehan, 2020a).

Resonant stimulation of the arterial baroreflex can be achieved through three main methods. Resonant Breathing (RB) leverages respiratory sinus arrhythmia (RSA) at the baroreflex's resonant frequency of 0.1 Hz (Lehrer *et al.*, 2013). Rhythmic Skeletal Muscle Tension (RSMT) uses rhythmic tensing and relaxing of large muscle groups at this same frequency, prompting arterial blood pressure (ABP) changes that elicit a resonant response in the baroreflex system (Vaschillo, Vaschillo, Buckman, *et al.*, 2011a; Vaschillo, Vaschillo, Pandina, *et al.*, 2011a). Lastly, Rhythmic Emotional Stimulation (RES) creates ABP variations by presenting rhythmic external emotional stimuli, with visual cues (RVES) or acoustic cues (RAES) serving as the triggers (Ritz *et al.*, 2005; Grühn and Scheibe, 2008a; Vaschillo *et al.*, 2008a; Wiens, Sand and Olofsson, 2011).

The concept of using RES with images as emotional stimuli at the resonant frequency of the arterial baroreflex (RVES) was pioneered by Evgeny Vaschillo (2008c) as a novel method to investigate emotional precursors that may lead to alcohol or substance abuse, particularly in efforts to alleviate negative emotions during developmental stages in both animals and humans. Vaschillo employed images from the International Affective Picture System (IAPS), presenting negative, positive, and neutral images in 5-second intervals (5 seconds on, 5 seconds off) over a 5-minute period. This technique successfully induced high-amplitude heart rate variability (HRV) oscillations that generated rhythmic arterial blood pressure (ABP) changes, yielding results comparable to those achieved with resonant stimulation of the arterial baroreflex through RB and RSMT (Lehrer *et al.*, 2003, 2009; France, France and Patterson, 2006; Vaschillo, Vaschillo, Buckman, *et al.*, 2011a; Lehrer and Gevirtz, 2014; Russo, Santarelli and O'Rourke, 2017). Notably, Vaschillo's experiments demonstrated a stronger resonance effect in response to negative-valence images compared to neutral or positive ones, with HRV variations occurring independently of respiratory sinus arrhythmia (RSA) since respiration parameters remained constant. This supports the theory that baroreflex resonance mechanisms, rather than RSA, drive high-amplitude HRV oscillations (Vaschillo *et al.*, 2008a; Bates *et al.*, 2022).

Research on emotion using acoustic stimuli has traditionally been less common than visual-based studies, likely due to the static nature of visual stimuli, which made them easier to control and

manipulate, unlike acoustic stimuli that vary over time (Bradley and Lang, 2000; Gerdes, Wieser and Alpers, 2014). Technological advancements in data storage and digital manipulation, however, have since made acoustic stimuli equally viable, leading researchers to increasingly use sound for studying attention and emotion (Fabiani *et al.*, 1996; Czigler *et al.*, 2007; Armony *et al.*, 2015). Early studies with acoustic stimuli faced challenges, as the sounds were often selected based on limited emotional ratings from small participant pools, resulting in inconsistencies that complicated comparisons and replications across studies (Bradley and Lang, 2000).

To address this, the NIMH Centre for the Study of Emotion and Attention developed the International Affective Digitized Sound System (IADS), a standardized non-verbal sound library for research. Released in 1999, IADS-1 included 110 six-second sounds rated for valence, arousal, and dominance using the Self-Assessment Manikin (SAM) method, and was later expanded in 2007 with IADS-2 (Fernández-Abascal *et al.*, 2008). Designed to minimise cultural bias, the library includes universal, easily identifiable sounds (e.g., thunderstorms, crying babies) but acknowledges the inherent complexity and symbolism in emotional interpretation (Bradley and Lang, 2000; Yang *et al.*, 2018). Notably, IADS sounds avoid habituation effects seen in other stimuli like white noise, making them suitable for sustained emotional engagement (Martin-Soelch *et al.*, 2006).

Building on the success of RVES, which uses visual stimuli at the resonant frequency of the arterial baroreflex to evoke high-amplitude HRV oscillations, the question arises whether Rhythmic Acoustic Emotional Stimulation (RAES)—using sounds of comparable emotional valence—could similarly enhance HRV performance. RAES offers practical advantages over RVES, being less invasive and not requiring participants to remain stationary in front of a screen, thus enabling a broader range of applications and settings for emotional intervention.

2. Methodology

2.1 The Experiments

The study involved three experimental conditions—RVES, RAES, and RB—to evaluate and compare their effectiveness in modulating the cardiovascular system (CVS) through baroreflex stimulation. RVES and RAES were structured following Vaschillo's RVES protocols, with RVES using visually presented emotional stimuli and RAES employing acoustic emotional stimuli (Vaschillo *et al.*, 2008a; Vaschillo, Vaschillo, Pandina, *et al.*, 2011b, 2011a). To serve as a control, RB, a proven method for resonant baroreflex stimulation at 0.1 Hz, was administered last, minimizing its known impact on CVS responses that might otherwise carry over into RES tasks (Lehrer et al., 2003; Vaschillo et al., 2008a). CVS baseline readings were taken at the study's outset and repeated between tasks to assess recovery to baseline levels. The experimental timeline proceeded sequentially through each condition, ensuring controlled comparisons across interventions.

2.1.1 Vanilla Baseline (Code V1)

Baseline cardiovascular (CVS) readings were taken for each participant at the start of the experiments using a 5-minute vanilla baseline protocol, as developed by Jennings et al. (1992). The same protocol

was also conducted between tasks to assess recovery of baseline CVS levels. During the baseline test, participants completed a low-demand mental task: they watched squares change colour every 10 seconds on a screen and counted the blue squares, ensuring consistency in cognitive engagement across participants (Jennings *et al.*, 1992; Vaschillo *et al.*, 2008a; Vaschillo, Vaschillo, Pandina, *et al.*, 2011b, 2011a).

2.1.2 RVES Experiment (Code IM)

Participants underwent visual stimulation using the same IAPS images employed in Vaschillo's original studies. Each participant was randomly assigned one of two image sets, each containing three emotional valence blocks (negative, positive, and neutral). To account for content appropriateness, image sets varied by gender. Each block consisted of 15 images, shown for 5 seconds on and 5 seconds off, to match the resonant frequency of the arterial baroreflex at 0.1 Hz. A 1-minute rest was included between blocks. Figure 1 illustrates the experimental setup, and Table 1 lists the specific IAPS codes used (Vaschillo *et al.*, 2008a).

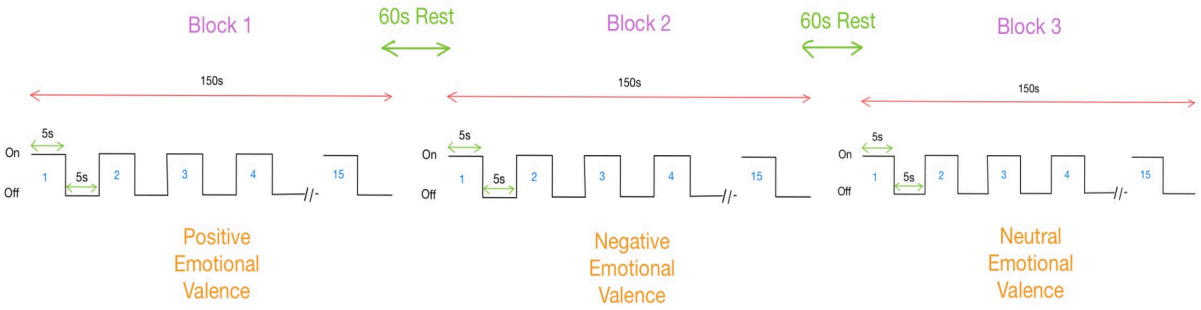


Figure 1. The 3 blocks of RVES with a 1 min period of rest in between. Both block presentation and images within the block are randomised.

Set 1			Set 2		
Neutral	**Negative**	**Positive**	**Neutral**	**Negative**	**Positive**
2215	1070	1710	2200	1300	2050
2221	2683	2630[1] (4210)	2480	3000	2216
2440	2730	4500[1] (4220)	2570	3130	4510[1] (4180)
5120	3010	4607[2]	5130	3140	4660[2]
5500	3100	4608[2]	5520	3530	4680[2]
7000	3500	4626	7010	6210	5621
7040	6260	5470	7025	6230	5629
7050	6300	5910	7031	6312	7200
7090	6313	7270	7060	6350	7330
7100	6821	8030	7080	6415	7502

Set 1			Set 2		
Neutral	**Negative**	**Positive**	**Neutral**	**Negative**	**Positive**
2215	1070	1710	2200	1300	2050
2221	2683	2630[1] (4210)	2480	3000	2216
2440	2730	4500[1] (4220)	2570	3130	4510[1] (4180)
5120	3010	4607[2]	5130	3140	4660[2]
5500	3100	4608[2]	5520	3530	4680[2]
7224	9050	8170	7150	6570	8080
7705	9410	8185	7234	9250	8180
7110	9635.1	8190	7700	9420	8370
9210	9800	8200	9070	9570	8470
9360	9810	8490	9090	9910	8501

[1]Erotic pictures that were different for each gender. Parenthesis indicates pictures for males.
[2]Pictures of erotic couples.

Table 1. Both sets of images from the IAPS for the RVES experiments. Each set contains 3 blocks of 15 images each of neutral, positive and negative emotional valence (Vaschillo *et al.*, 2008a).

2.1.3 Vanilla Baseline (Code V2)

A vanilla baseline was conducted between the RVES and RAES experiments to evaluate any shifts in CVS baseline levels.

2.1.4 RAES Experiment (Code SO)

RAES experiments followed the RVES protocol, substituting visual with acoustic stimuli from the IADS database. Participants listened to one of two randomized sets of sounds, each with three blocks representing positive, negative, and neutral emotional valences. Each block consisted of 15 sounds, played in 5-second intervals (5 seconds on, 5 seconds off) at the resonant frequency of 0.1 Hz, with a 1-minute rest between blocks. The chosen sounds in each set were selected to closely match their IAPS image counterparts in terms of arousal and valence values (Vaschillo et al., 2008a). Table 2 lists the IADS codes used.

Set 1			Set 2		
Neutral	**Negative**	**Positive**	**Neutral**	**Negative**	**Positive**
700	310	226	246	134	811
708	711	206	130	290	270
262	244	171	722	279	377
723	285	365	702	286	366
113	277	716	724	292	820
322	422	110	701	713	353

Set 1			Set 2		
Neutral	**Negative**	**Positive**	**Neutral**	**Negative**	**Positive**
700	310	226	246	134	811
708	711	206	130	290	270
262	244	171	722	279	377
723	285	365	702	286	366
113	277	716	724	292	820
728	420	351	102	600	367
602	288	815	720	703	809
172	284	810	382	424	816
812	712	352	170	115	360
698	241	311	410	296	355
729	278	817	320	261	215
358	276	220	710	275	151
627	260	717	114	501	230
252	255	813	243	709	150

Table 2. IADS sounds for Sets 1 and 2 both for males and females. Sound presentation was done in random order.

2.1.5 Vanilla Baseline (V3)

Following the RAES experiment, a vanilla baseline was taken before proceeding with the RB task to monitor any residual baseline changes.

2.1.6 RB Experiment

The final RB experiment consisted of three blocks of deep breathing, each lasting 150 seconds, with a 1-minute rest between blocks. Participants were guided to inhale for 5 seconds and exhale for 5 seconds, with visual cues provided to maintain a steady breathing pattern aligned with the 0.1 Hz baroreflex frequency.

2.2 The Participants

Thirty healthy individuals (15 males, 15 females) without a history of cardiac issues were selected for the study. Participants were instructed to abstain from caffeine, alcohol, and vigorous exercise for at least 12 hours before each experimental session. Written consent was obtained from all participants (Salahuddin *et al.*, 2007), who also provided demographic information such as age, weight, height, and occupation. To minimize variations due to circadian rhythms, sessions were conducted between 10 a.m. and 2 p.m. (Vaschillo, Vaschillo, Pandina, *et al.*, 2011b). Each participant was assigned a unique 4-digit identifier, randomly generated between 1000 and 1999. Participant demographics are presented in Table 3.

Descriptives

Descriptives

	Occupation	Sex	Age	BMI	Weight(Kg)	Height(m)
N	30	30	30	30	30	30
Missing	0	0	0	0	0	0
Mean			33.8	24.5	71.6	1.71
Median			33.5	24.5	70.0	1.72
Standard deviation			8.08	3.29	11.3	0.0835
Minimum			23	19.1	50	1.56
Maximum			60	36.1	96	1.86

Frequencies

Frequencies of Occupation

Occupation	Counts	% of Total	Cumulative %
Researcher	2	6.7%	6.7%
Student	5	16.7%	23.3%
Professional	1	3.3%	26.7%
PhD Student	19	63.3%	90.0%
Academic	3	10.0%	100.0%

Frequencies of Sex

Sex	Counts	% of Total	Cumulative %
F	15	50.0%	50.0%
M	15	50.0%	100.0%

Table 3. Descriptives of the participants.

2.3 The experiment setup

The experiments took place in a sound-isolated, dimly lit room to minimize external stimuli that could influence outcomes (Calvert, Spence and Stein, 2004; Deroy, Chen and Spence, 2014). Visual stimuli were presented on a 29-inch LG monitor (model 29UM68), with low lighting to enhance contrast. Auditory stimuli were delivered via Sennheiser HD-202 closed-back headphones. Electrocardiogram (ECG) and respiration data were recorded for each participant using a Biopac MP36 data acquisition system, which included SS29L electrodes and an SS5LB respiratory transducer, all managed through Acknowledge software. The open-source software PsychoPy controlled the presentation of both visual and auditory stimuli.

2.4 Data analysis

ECG and respiratory data from Acknowledge files were imported into Kubios HRV Scientific for analysis, with experiment start markers automatically carried over during the import of the .acq files.

Since both RVES and RAES blocks were presented in random order, PsychoPy logs were reviewed for each participant to confirm accurate data labelling.

To ensure data integrity and account for potential cardiac irregularities, we processed all inter-beat interval (IBI) data using Kubios HRV Premium with the automatic beat correction algorithm set to a 0.3 threshold. This threshold corresponds to a commonly used criterion in HRV analysis, whereby any RR interval deviating by more than 30% from the local average is automatically flagged and corrected through interpolation. This method allows for effective removal of ectopic beats (such as premature ventricular contractions – PVCs) and signal artefacts, without compromising the underlying physiological variability of the heart rate signal. While PVCs were not manually annotated, the applied correction settings reliably detect and adjust for such anomalies. This approach ensures that the resulting HRV metrics reflect true autonomic modulation, minimising distortion from non-physiological sources.

2.4.1 Choice of Time-Domain Metrics Over Frequency-Domain Analysis

While frequency-domain metrics, particularly low-frequency power (LF-PSD, 0.04–0.15 Hz), are frequently used to assess autonomic activity, their interpretation in emotional interventions presents significant limitations. LF-PSD is influenced by both parasympathetic (vagal) and sympathetic activity, meaning that an increase in LF power does not unequivocally indicate baroreflex resonance (Berntson *et al.*, 2008; Billman, 2013). Emotional stimulation, by design, induces sympathetic activation, which can also elevate LF power (Beauchaine, 2001). Consequently, relying on LF-PSD as an index of baroreflex resonance in this context would introduce ambiguity, as increases in this measure could be attributed to sympathetic arousal rather than resonance effects (Vaschillo *et al.*, 2008a).

Given these confounds, time-domain metrics (RMSSD and SDNN) were selected as the primary HRV indices, as they provide a more direct measure of beat-to-beat variability without conflating sympathetic and parasympathetic contributions (Laborde, Mosley and Thayer, 2017b). RMSSD is particularly suited for short-term recordings, as it reflects high-frequency parasympathetic modulation (Shaffer and Ginsberg, 2017a), while SDNN captures overall HRV fluctuations, making it a useful index of autonomic control (G. G. Berntson *et al.*, 1994). This approach aligns with existing recommendations for studies investigating short-term emotional or cognitive effects on autonomic function (Esco and Flatt, 2014; Baek *et al.*, 2015). Thus, the findings in this study are interpreted in a framework that minimizes misattributions related to sympathetic activation, ensuring that observed HRV changes can be more confidently linked to the intended emotional stimulation rather than non-specific autonomic shifts (Lehrer and Gevirtz, 2014).

In addition to RMSSD and SDNN, mean heart rate (HR) was included as a variable of interest to provide a complementary perspective on autonomic regulation. While HRV indices reflect beat-to-beat variability in cardiac control, mean HR is a direct indicator of autonomic balance, as it is modulated by both parasympathetic and sympathetic inputs (Shaffer and Ginsberg, 2017b). Studies in emotional and stress-related autonomic responses often include mean HR as a control variable to contextualize HRV findings, as it ensures that HRV changes are not confounded by gross shifts in cardiovascular activity (Berntson *et al.*, 2008).

To ensure mean HR is expressed in a format comparable to HRV measures, which are typically reported in milliseconds, we calculated the Inter-Beat Interval (IBI) using the transformation: IBI = 60000 / Mean HR. This conversion allows for direct comparison with other time-domain HRV metrics.

This conversion expresses the mean time between consecutive heartbeats (inter-beat interval, IBI) in milliseconds (ms), allowing for a consistent temporal unit across all HRV-related analyses. This is particularly relevant in autonomic research, where higher mean HR is typically associated with reduced parasympathetic tone and increased sympathetic drive, while a lower mean HR indicates stronger vagal influence (Gary G. Berntson *et al.*, 1994; Beauchaine, 2001). Including mean HR in the analysis helps disentangle HRV changes driven by specific autonomic adjustments versus those influenced by shifts in overall cardiac demand (Laborde, Mosley and Thayer, 2017a).

2.4.2 Statistical Analysis

Statistical analysis was conducted using Jamovi, an open-source platform. As no direct transfer method was available, Kubios HRV Scientific data were first exported into Excel, with key variables manually input for each participant. SDNN and RMSSD values during interventions were compared to pre- and post-intervention baselines, and baseline measurements before and after interventions were also analyzed.

While earlier studies suggested stronger HRV responses to negatively valenced stimuli Vaschillo et al. (2008b; 2011b), we adopted a more conservative and comprehensive approach by analyzing the peak reactivity of the autonomic nervous system. We defined the 'MAX' metric as the maximum absolute deviation from baseline among the three stimulation conditions (positive, neutral, and negative). This approach was chosen to account for the high degree of inter-individual variability in emotional processing and the well-documented limitations of standardized image databases, such as the IAPS (Ribeiro, Pompéia and Bueno, 2005; Grühn and Scheibe, 2008b; Domínguez, Tapia and Troncoso, 2011; Lohani, Gupta and Srinivasan, 2013). Given that certain 'neutral' stimuli (e.g., environmental pollution) or 'positive' stimuli (e.g., erotica) may carry different emotional valences today compared to when these libraries were first validated, selecting the maximum physiological response ensures that we capture the true engagement of the baroreflex arc, regardless of the pre-assigned category of the stimulus. By focusing on the magnitude of the autonomic shift rather than a specific valence, we avoid the bias of outdated categorical labels and provide a more accurate representation of the individual's maximum capacity for rhythmic entrainment. The variables analyzed are detailed in Tables 4 and 5.

RVES Experiments	
Name	**Variable**
RMSSD IM Stim-V1 MAX	Maximum RMSSD deviation from baseline (V1) in response to image-based RES, indicating the largest deviation across all stimuli types relative to pre-intervention levels.
RMSSD IM Stim-V2 MAX	Maximum RMSSD deviation from baseline (V2) in response to image-based RES, indicating the largest deviation across all stimuli types relative to post-intervention levels.
RMSSD IM	The difference in RMSSD between post-intervention (V2) and pre-intervention (V1) baselines,

V2-V1	indicating overall intervention effect.
SDNN IM Stim-V1 MAX	Maximum SDNN deviation from baseline (V1) in response to image-based RES, indicating the largest change across all stimuli types relative to pre-intervention levels.
SDNN IM Stim-V2 MAX	Maximum SDNN deviation from baseline (V2) in response to image-based RES, indicating the largest change across all stimuli types relative to post-intervention levels.
SDNN IM V2-V1	The difference in SDNN between post-intervention (V2) and pre-intervention (V1) baselines, reflecting the overall impact of the intervention on SDNN.
IBI IM Stim-V1 MAX	Maximum IBI deviation from baseline (V1) in response to image-based RES, indicating the largest change across all stimuli types relative to pre-intervention levels.
IBI IM Stim-V2 MAX	Maximum IBI deviation from baseline (V2) in response to image-based RES, indicating the largest change across all stimuli types relative to post-intervention levels.
IBI IM V2-V1	The difference in IBI between post-intervention (V2) and pre-intervention (V1) baselines, reflecting the overall impact of the intervention on IBI.

Table 4. Variables used during RVES experiments and analysis.

RAES Experiments	
Name	**Variable**
RMSSD SO Stim-V2 MAX	Maximum RMSSD deviation from baseline (V2) in response to acoustic-based RES, indicating the largest deviation across all stimuli types relative to pre-intervention levels.
RMSSD SO Stim-V3 MAX	Maximum RMSSD deviation from baseline (V3) in response to acoustic-based RES, indicating the largest deviation across all stimuli types relative to post-intervention levels.
RMSSD SO V3-V2	The difference in RMSSD between post-intervention (V3) and pre-intervention (V2) baselines, indicating overall intervention effect.
SDNN SO Stim-V2 MAX	Maximum SDNN deviation from baseline (V2) in response to acoustic-based RES, indicating the largest change across all stimuli types relative to pre-intervention levels.
SDNN SO Stim-V3 MAX	Maximum SDNN deviation from baseline (V3) in response to acoustic-based RES, indicating the largest change across all stimuli types relative to post-intervention levels.
SDNN SO V3-V2	The difference in SDNN between post-intervention (V3) and pre-intervention (V2) baselines, reflecting the overall impact of the intervention on SDNN.
IBI SO Stim-V1 MAX	Maximum IBI deviation from baseline (V1) in response to acoustic-based RES, indicating the largest change across all stimuli types relative to pre-intervention levels.
IBI SO Stim-V2 MAX	Maximum IBI deviation from baseline (V2) in response to acoustic-based RES, indicating the largest change across all stimuli types relative to post-intervention levels.
IBI SO V2-V1	The difference in IBI between post-intervention (V2) and pre-intervention (V1) baselines, reflecting the overall impact of the intervention on IBI.

Table 5. Variables used during RAES experiments and analysis.

2.4.3 Normality of variables

Normality was assessed for each variable using the Shapiro–Wilk test, complemented by visual inspection of histograms and Q–Q plots.

Although log transformation is a commonly recommended method to normalize HRV data, in this dataset it did not reduce the number of outliers. It was not possible to apply log transformation to the difference variables as these include both positive and negative values, representing bidirectional changes in HRV. Applying log transformation would require altering the data structure (e.g., adding a constant or reflecting negative values), which would undermine the directional interpretability of the physiological response. Preserving raw values was essential to assess whether the interventions increased or decreased HRV, in line with the core aims of the study.

Instead, outliers were identified and excluded using the standardized method applied by Jamovi box plots, which is based on the 1.5×IQR rule. This means that any data point falling outside the range of Q1 - 1.5 * IQR and Q3 + 1.5 * IQR is considered an outlier and will be marked with a dot in the box plot. Q1 and Q3 represent the 25th and 75th percentiles of the data. IQR (interquartile range) is calculated as Q3 – Q1. Thus a value is flagged as an outlier if it falls below Q1 – 1.5 × IQR or above Q3 + 1.5 × IQR

This method is widely accepted in statistical reporting (Beyer, 1981) and offers an objective, non-parametric way to minimize the influence of extreme values without distorting the main distribution. In some cases, Jamovi also flags extreme outliers beyond ±3 × IQR, though these were rare in the present dataset. Only individual data points were excluded—not entire participants—and the number of exclusions per variable was limited. This suggests the presence of either genuine physiological variability—such as atypical autonomic responses—or possible data inconsistencies, such as signal artefacts or deviations in adherence to the breathing protocol. While each exclusion was applied at the individual data point level and based on a consistent statistical rule (1.5×IQR), this should be acknowledged as a limitation. These exclusions may modestly reduce statistical power and introduce interpretive constraints. Future studies should consider tracking such participants more closely to explore whether their responses reflect trait-level differences or methodological artefacts.

Importantly, these outlier exclusions were applied solely to the descriptive analyses to support visualisation and summary of central tendencies. The Repeated Measures ANOVA was conducted using the full sample (N = 30) with no outlier exclusion, ensuring that all participants contributed to the core statistical inferences. Therefore, the identified limitations pertain primarily to descriptive interpretations, and do not affect the robustness of the main inferential results.

3. Results

3.1 Respiration Control and Potential Confounds

To ensure that observed HRV changes were driven by emotional stimulation rather than respiratory entrainment, participants were instructed to breathe naturally throughout the experiment, without any imposed breathing rhythm. Given that paced breathing at 0.1 Hz (six breaths per minute) is known to induce resonance effects in HRV through baroreflex stimulation, it was critical to confirm that participants did not unintentionally breathe at this frequency. Respiration rates were recorded and analyzed across all conditions as shown in Table 6.

Descriptives

	Resp Freq V1	Resp Freq IM+	Resp Freq IM=	Resp Freq IM-	Resp Freq V2	Resp Freq SO+	Resp Freq SO=	Resp Freq SO-	Resp Freq V3
N	30	30	30	30	30	30	30	30	30
Mean	0.314	0.298	0.302	0.299	0.310	0.303	0.303	0.301	0.306
Median	0.330	0.315	0.320	0.320	0.315	0.315	0.315	0.315	0.310
Standard deviation	0.0770	0.0780	0.0741	0.0798	0.0608	0.0650	0.0669	0.0718	0.0624
Variance	0.00594	0.00608	0.00550	0.00636	0.00370	0.00423	0.00448	0.00516	0.00389
IQR	0.0950	0.118	0.120	0.0950	0.0650	0.0825	0.105	0.107	0.0850
Range	0.310	0.320	0.310	0.320	0.260	0.250	0.280	0.280	0.270
Skewness	-0.169	0.128	0.134	-0.141	-0.108	-0.297	-0.361	-0.266	0.115
Std. error skewness	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427

Table 6. Breathing Rate Analysis during RVES and RAES interventions.

The results indicate that mean respiration frequencies ranged between 0.298 Hz and 0.314 Hz, corresponding to an average breathing rate of 17.88 to 18.84 breaths per minute—well above the resonance frequency threshold. Additionally, the standard deviation and range values suggest natural variability in breathing patterns, further confirming the absence of a structured breathing effect. By ensuring that participants did not engage in slow-paced breathing, we created the necessary conditions to evaluate whether baroreflex activation occurred as a result of the intended intervention—emotional stimulation—rather than as an artefact of respiratory influences.

3.2 Preliminary RVES Descriptives Analysis

A summary of the descriptive statistics for the RVES variables of analysis, with outliers removed, is presented in Tables 7, 8 and 9. Box plots can be seen in Figures 2, 3 and 4.

Descriptives

	RMSSD IM Stim-V1 MAX	RMSSD IM Stim-V2 MAX	RMSSD IM V2-V1
Mean	1.50	2.73	-0.722
Median	2.61	3.08	-1.14
Standard deviation	4.67	4.40	4.09

Table 7. Summary of descriptive analysis for RMSSD during RVES experiments.

Descriptives

	SDNN IM Stim-V1 MAX	SDNN IM Stim-V2 MAX	SDNN IM V2-V1
Mean	3.76	0.793	1.91
Median	5.12	-1.78	2.38
Standard deviation	6.57	7.55	1.94

Table 8. Summary of descriptive analysis for SDNN during RVES experiments.

Descriptives

	IBI IM Stim-V1 MAX	IBI IM Stim-V2 MAX	IBI IM V2-V1
Mean	28.5	10.2	14.0
Median	30.0	19.1	19.1
Standard deviation	23.4	28.9	21.0

Table 9. Summary of descriptive analysis for IBI during RVES experiments.

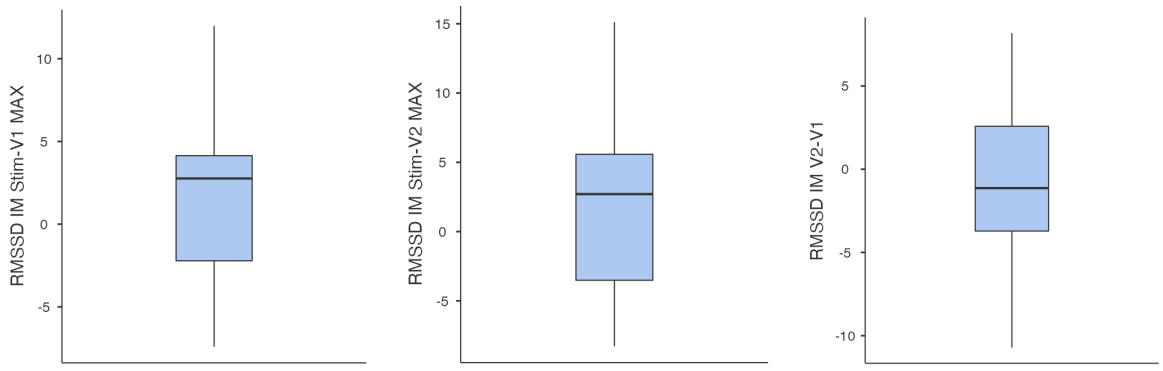


Figure 2. Box plots for RVES RMSSD with outliers removed.

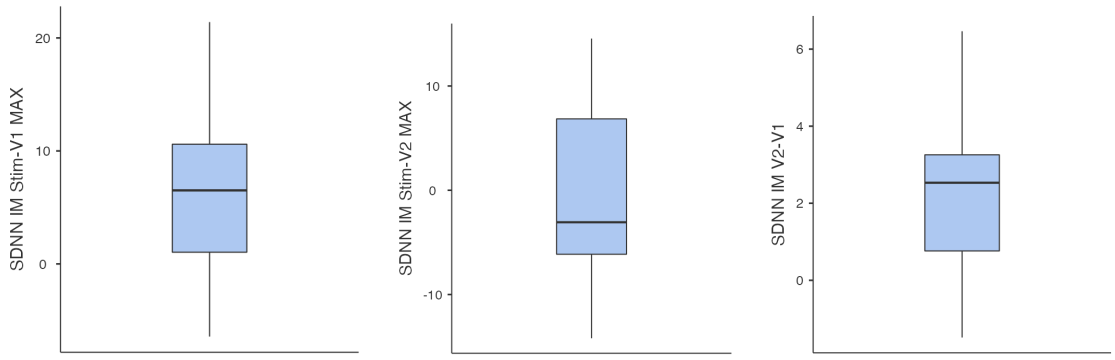


Figure 3. Box plots for RVES SDNN with outliers removed.

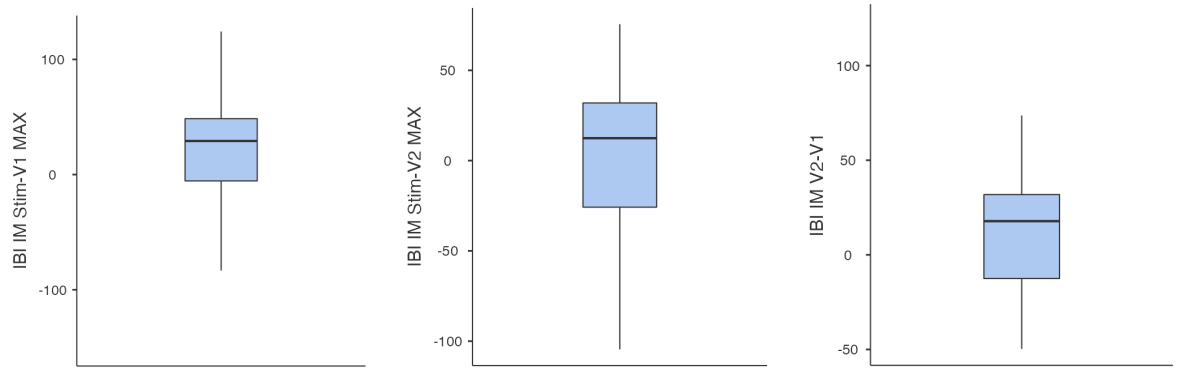


Figure 4. Box plots for RVES IBI with outliers removed.

Analysis of RVES Effects on HRV (RMSSD, SDNN, IBI – Combined Sample)

Across the full sample, RMSSD increased (+1.50ms) during the RVES intervention, indicating a moderate enhancement in parasympathetic activity. However, this was followed by a post-intervention decline (−2.73ms) resulting in a net reduction below baseline. This pattern suggests a short-term vagal activation during the intervention that was not sustained post-exposure, and may indicate a rebound effect once the emotional stimulation ceased.

For SDNN, which reflects overall heart rate variability, there was a clear increase (+3.76ms) during the intervention, followed by a slight decrease (−0.793ms) afterward. The post-intervention levels remained above baseline, suggesting a sustained improvement in global autonomic regulation as a result of RVES.

IBI increased substantially (+28.5ms) during the intervention—consistent with a slower heart rate and parasympathetic dominance—and then decreased (−10.2ms) post-intervention. Despite the drop, IBI remained elevated compared to pre-intervention levels, pointing to a partial retention of autonomic slowing.

In summary, RVES induced measurable improvements in HRV and heart rate during stimulation, particularly in SDNN and IBI, with partial retention after the intervention. However, RMSSD showed

a less stable response, with the post-intervention value falling below baseline, potentially indicating a transient vagal effect. These findings highlight RVES as a potentially effective stimulus for short-term autonomic enhancement, especially in overall HRV and cardiac deceleration, though its parasympathetic effects may vary in duration and intensity depending on the metric analyzed.

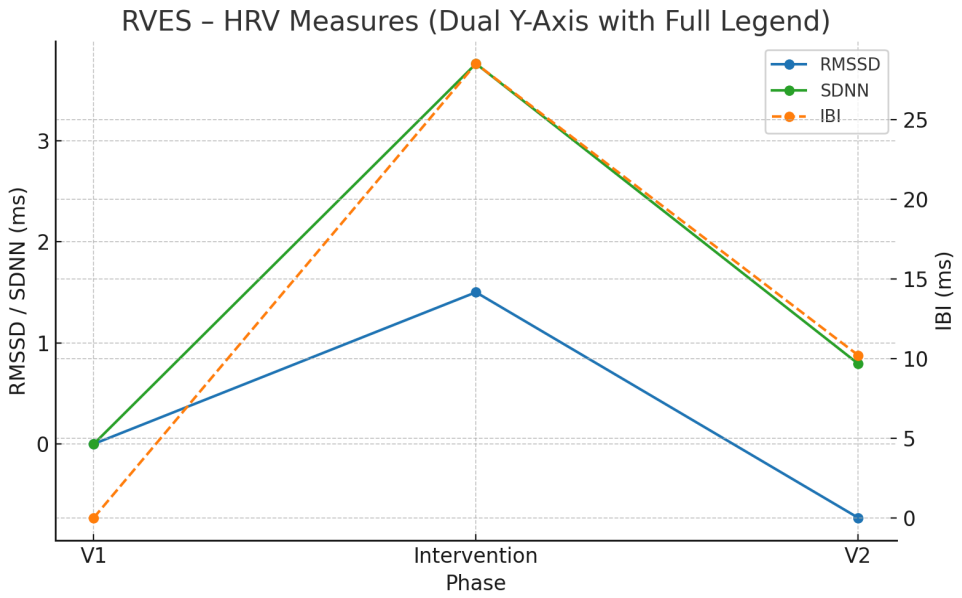


Figure 5. Variations of mean values (normalised) of SDNN, RMSSD and IBI duringthe RVES intervention.

3.2.1 RVES Descriptives Analysis by Sex

To explore gender-based patterns, descriptive statistics were further analyzed by dividing the data by sex, with outliers removed to maintain normality. Summarized descriptive statistics are presented in Tables 10, 11 and 12, with box plots shown in Figures 6, 7 and 8.

Descriptives

	Sex	RMSSD IM Stim-V1 MAX	RMSSD IM Stim-V2 MAX	RMSSD IM V2-V1
Mean	F	4.04	2.22	1.09
	M	-1.05	3.24	-2.53
Median	F	4.08	-0.897	1.66
	M	0.557	3.24	-2.76
Standard deviation	F	3.77	6.02	4.47
	M	4.18	2.08	2.84

Table 10. Summary of descriptive analysis for RMSSD during RVES experiments split by sex.

Descriptives

	Sex	SDNN IM Stim-V1 MAX	SDNN IM Stim-V2 MAX	SDNN IM V2-V1
Mean	F	4.85	1.81	2.39
	M	2.93	0.0301	1.55
Median	F	3.95	1.27	2.94
	M	5.51	-3.18	1.95
Standard deviation	F	6.45	7.22	1.29
	M	6.82	8.01	2.31

Table 11. Summary of descriptive analysis for SDNN during RVES experiments split by sex.

Descriptives

	Sex	IBI IM Stim-V1 MAX	IBI IM Stim-V2 MAX	IBI IM V2-V1
Mean	F	38.2	8.64	21.1
	M	21.2	11.3	8.62
Median	F	33.7	19.1	19.9
	M	29.0	20.4	5.90
Standard deviation	F	15.1	30.0	9.09
	M	26.3	29.3	25.9

Table 12. Summary of descriptive analysis for IBI during RVES experiments split by sex.

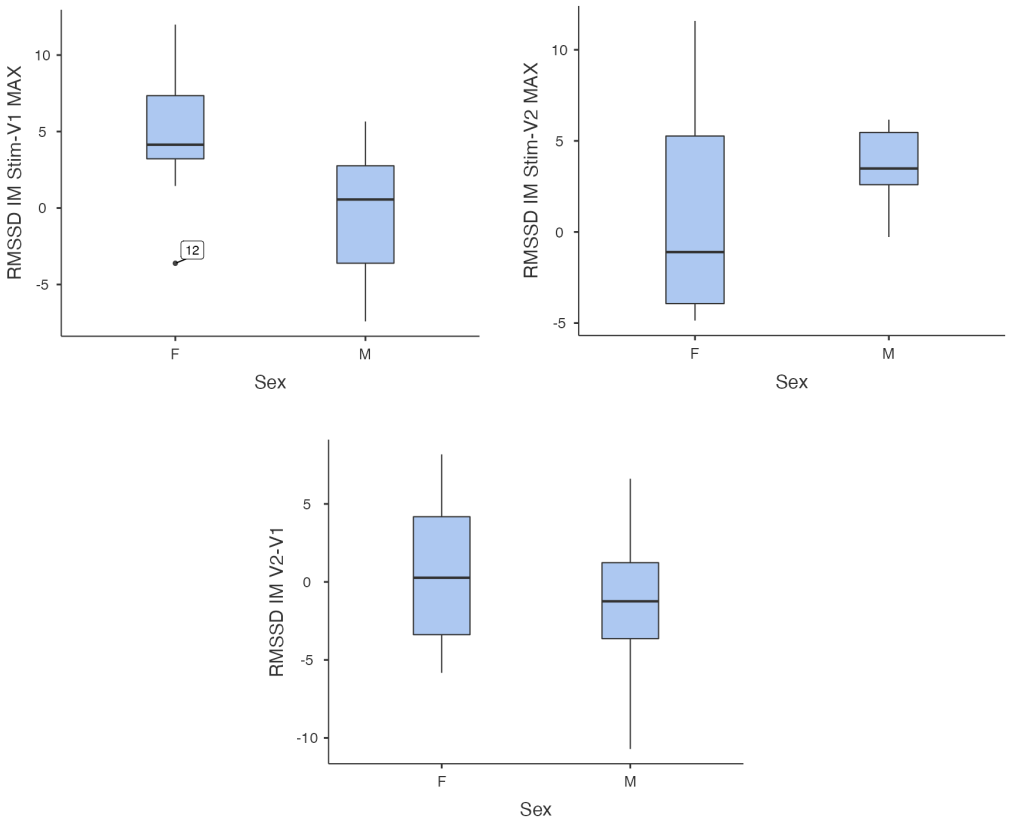


Figure 6. Box plots for RVES RMSSD with outliers removed split by sex.

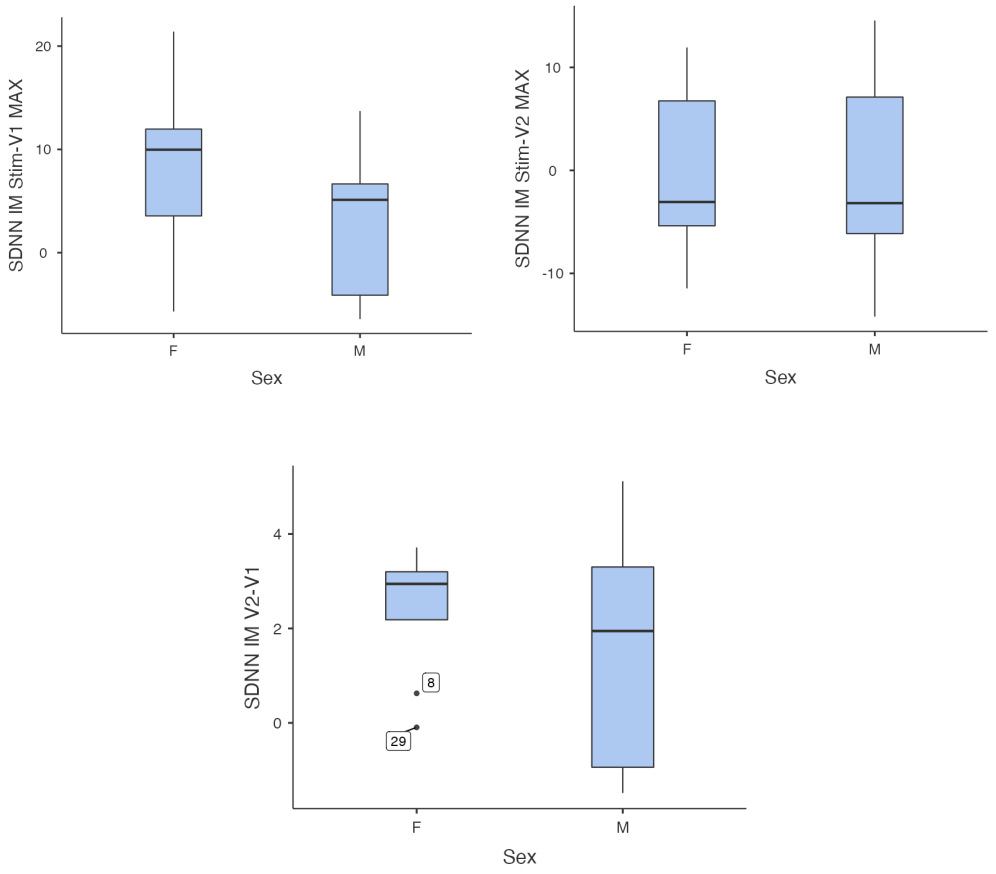
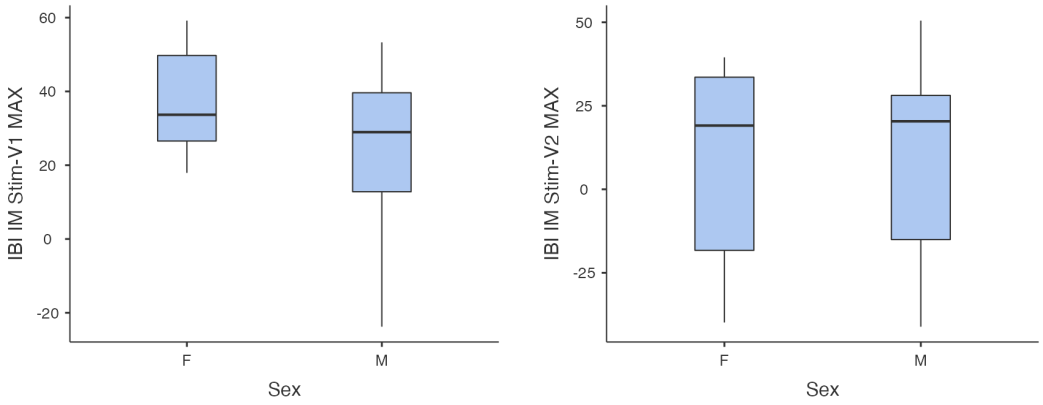


Figure 7. Box plots for RVES SDNN with outliers removed and split by sex.



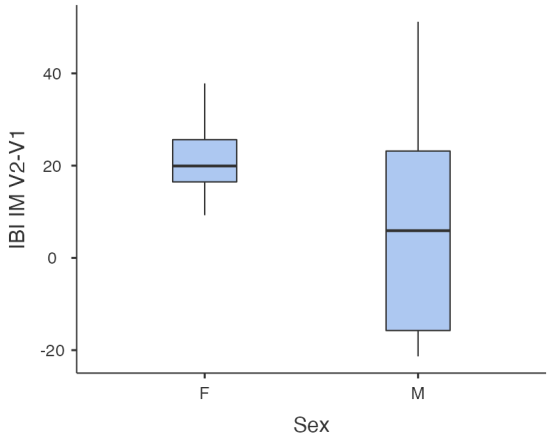


Figure 8. Box plots for RVES IBI with outliers removed and split by sex.

Sex-Based Analysis of RVES Effects on HRV (RMSSD, SDNN, IBI)

In the analysis of RMSSD, females exhibited a marked increase (+4.04ms) during the RVES intervention, followed by a modest decrease (−2.22ms) post-intervention, resulting in a net sustained gain. In contrast, males showed a decline (−1.05ms) during the intervention and a further drop (−3.24ms) afterward. This divergent pattern suggests that females experienced a robust parasympathetic activation during RVES, with partial retention post-intervention, whereas males not only failed to benefit but demonstrated a continued reduction in vagal tone.

For SDNN, both sexes exhibited increases during the intervention, though the effect was more pronounced in females (+4.85ms) compared to males (+2.93ms). Post-intervention, females experienced a slight decline (−1.81ms), while SDNN in males remained relatively stable (−0.03ms). This suggests that the intervention temporarily enhanced overall HRV in both sexes, with a stronger initial response in females and a more persistent retention in males.

In terms of IBI, females showed a substantial increase (+38.2ms) during the intervention, indicative of heart rate slowing and parasympathetic activation, followed by a post-intervention decrease (−8.64ms) still above baseline. Males also experienced a positive shift during the intervention (+21.2ms) but exhibited a more substantial rebound drop (−11.3ms) afterward. This pattern suggests that although both sexes experienced a resonant slowing of heart rate during RVES, females retained more of the benefit post-intervention, while males displayed a quicker return toward baseline or potentially sympathetic rebound.

Overall, RVES elicited stronger autonomic responses in females across all three metrics (RMSSD, SDNN, IBI), both during and after the intervention. These results align with previous literature suggesting heightened vagal responsiveness to emotional stimulation in females, and support the notion that RVES may be more effective for female populations when targeting parasympathetic activation and HRV-based stress modulation.

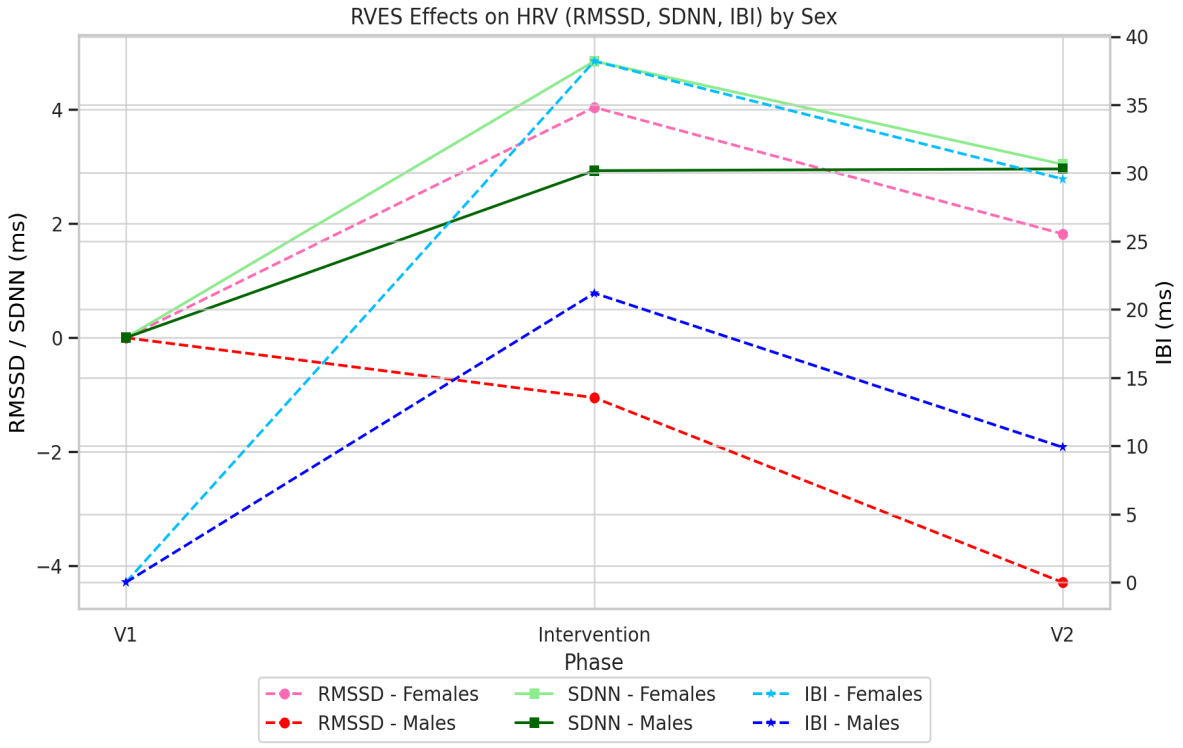


Figure 9. Variations of mean values (normalised) SDNN, RMSSD and IBI duringthe RVES intervention split by sex.

3.3 Preliminary RAES Descriptives Analysis

A summary of the descriptive statistics for the RAES variables of analysis, with outliers removed, is presented in Tables 13, 14 and 15. Box plots can be seen in Figures 10, 11 and 12.

Descriptives

	RMSSD SO Stim-V2 MAX	RMSSD SO Stim-V3 MAX	RMSSD SO V3-V2
Mean	0.942	2.04	-0.826
Median	1.68	1.58	-1.21
Standard deviation	3.90	5.45	2.97

Table 13. Summary of descriptive analysis for RMSSD during RAES experiments.

Descriptives

	SDNN SO Stim-V2 MAX	SDNN SO Stim-V3 MAX	SDNN SO V3-V2
Mean	-3.13	-0.991	-1.09
Median	-4.51	-3.64	-0.537
Standard deviation	5.89	8.50	4.23

Table 14. Summary of descriptive analysis for SDNN during RAES experiments.

Descriptives

	IBI SO Stim-V2 MAX	IBI SO Stim-V3 MAX	IBI SO V3-V2
Mean	28.1	20.7	1.84
Median	30.3	22.8	6.79
Standard deviation	9.80	33.7	27.4

Table 15. Summary of descriptive analysis for IBI during RAES experiments.

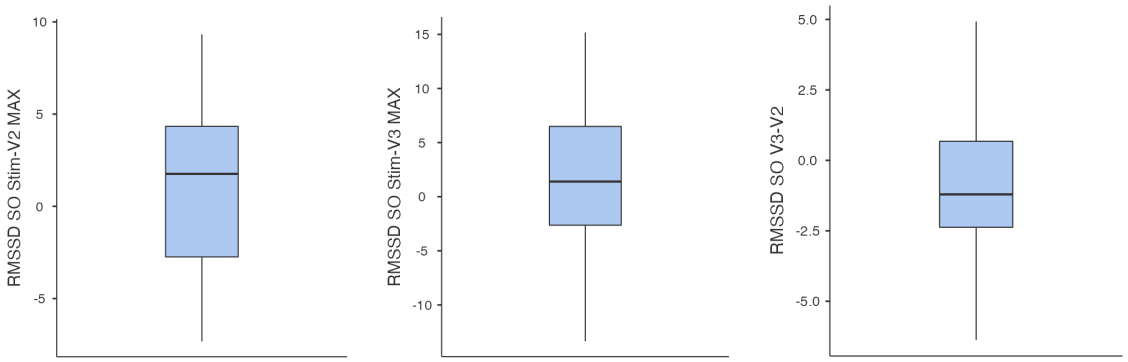


Figure 10. Box plots for RAES RMSSD with outliers removed.

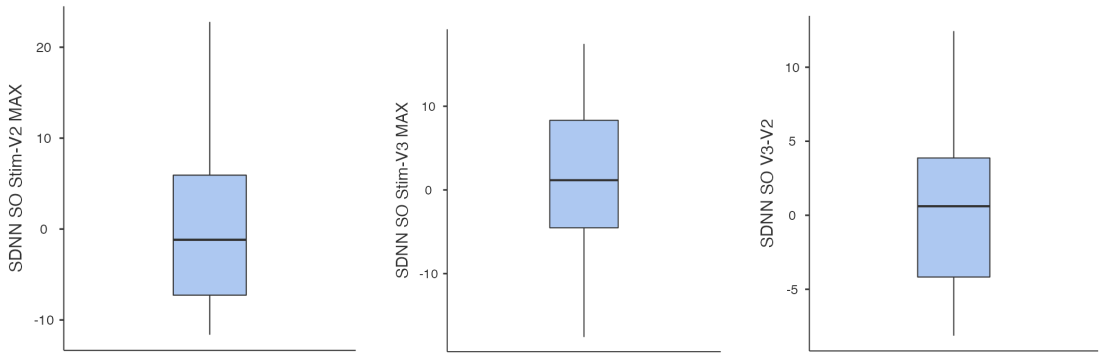


Figure 11. Box plots for RAES SDNN with outliers removed.

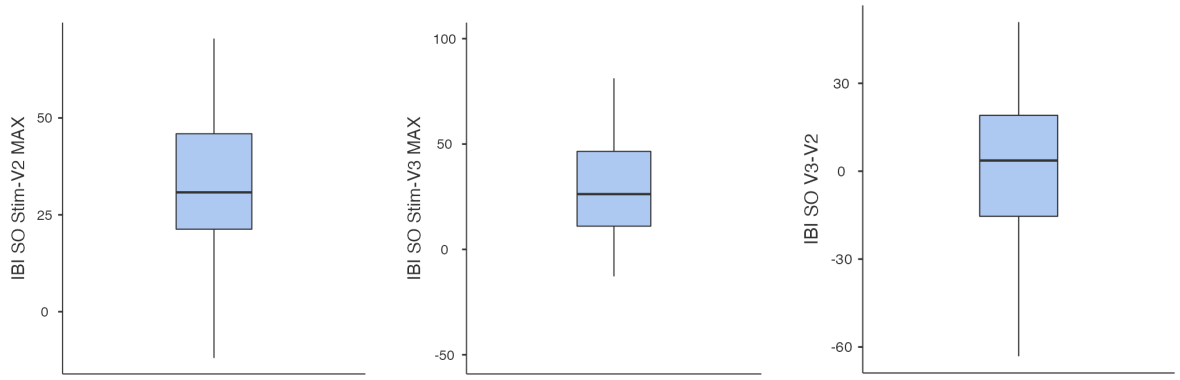


Figure 12. Box plots for RAES IBI with outliers removed.

Analysis of RAES Effects on HRV (RMSSD, SDNN, IBI – Combined Sample)

In the RAES condition, RMSSD increased modestly (+0.942ms) during the intervention, suggesting a slight enhancement in parasympathetic (vagal) activity. However, this was followed by a notable post-intervention decrease (−2.04 ms) resulting in RMSSD levels falling below the pre-intervention baseline. This pattern indicates that RAES produced only a mild vagal activation, which was not sustained and may have been followed by a reactive drop in vagal tone once the stimulation ended, a pattern we already saw in RVES.

SDNN, reflecting overall HRV and capturing both sympathetic and parasympathetic inputs, showed a decline (−3.13ms) during the intervention, followed by a slight post-intervention recovery (+0.991ms). Despite this rebound, SDNN values remained lower than baseline, suggesting that RAES had a limited or even dampening effect on global autonomic variability, especially when compared to the more consistent and sustained increases observed during RVES.

In contrast, IBI increased considerably (+28.1ms) during the intervention, consistent with reduced heart rate and parasympathetic activation. However, a post-intervention drop (−20.7ms) brought IBI values closer to baseline, though still slightly elevated overall. This trajectory suggests that RAES induced a temporary slowing of heart rate, but the effect was less sustained than with RVES.

In summary, RAES elicited weaker autonomic responses than RVES, with only small improvements in RMSSD and IBI during stimulation, and an overall decline in SDNN. These findings point to limited efficacy of acoustic rhythmic stimulation (RAES) in modulating HRV, at least when compared to visual stimulation. The patterns observed suggest that RAES may be less effective in promoting sustained parasympathetic engagement or global autonomic flexibility.

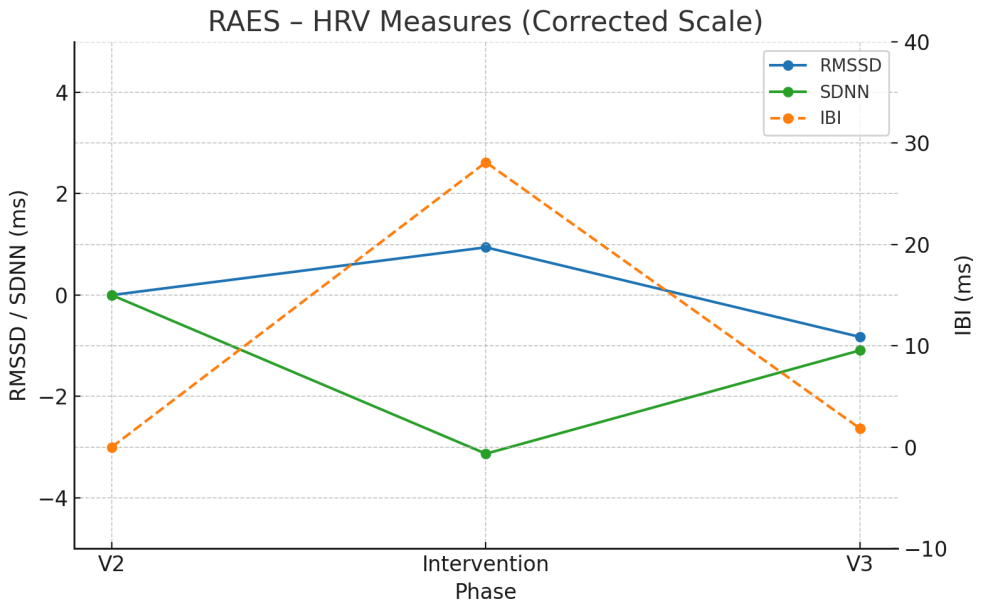


Figure 13. Variations of mean values (normalised) of SDNN, RMSSD and IBI duringthe RAES intervention.

3.3.1 RAES Descriptives Analysis by Sex

To explore gender-based patterns, descriptive statistics were further analyzed by dividing the data by sex, with outliers removed to maintain normality. Summarized descriptive statistics are presented in Tables 16, 17 and 18, with box plots shown in Figures 14, 15 and 16.

Descriptives

	Sex	RMSSD SO Stim-V2 MAX	RMSSD SO Stim-V3 MAX	RMSSD SO V3-V2
Mean	F	2.69	3.85	-0.983
	M	0.0480	-1.57	0.775
Median	F	3.40	3.73	-1.36
	M	1.37	-2.54	0.205
Standard deviation	F	2.26	2.74	1.47
	M	4.13	4.90	2.62

Table 16. Summary of descriptive analysis for RMSSD during RAES experiments split by sex.

Descriptives

	Sex	SDNN SO Stim-V2 MAX	SDNN SO Stim-V3 MAX	SDNN SO V3-V2
Mean	F	-0.192	1.41	-1.60
	M	-5.81	-3.18	-0.620
Median	F	0.277	-0.907	0.0355
	M	-7.12	-4.38	-1.03
Standard deviation	F	6.34	8.28	4.18
	M	4.08	8.47	4.43

Table 17. Summary of descriptive analysis for SDNN during RAES experiments split by sex.

Descriptives

	Sex	IBI SO Stim-V2 MAX	IBI SO Stim-V3 MAX	IBI SO V3-V2
Mean	F	29.5	31.9	-6.30
	M	35.5	22.4	10.8
Median	F	30.6	29.3	-5.35
	M	30.8	23.2	7.71
Standard deviation	F	19.1	39.0	23.6
	M	22.6	28.5	28.0

Table 18. Summary of descriptive analysis for IBI during RAES experiments split by sex.

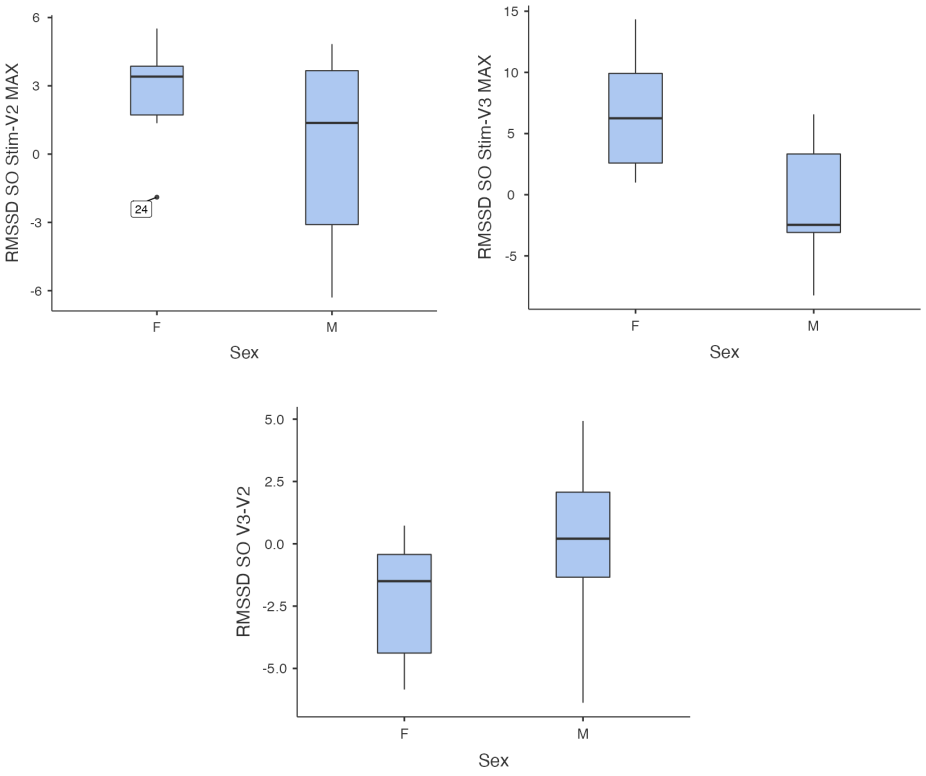


Figure 14. Box plots for RAES RMSSD with outliers removed split by sex.

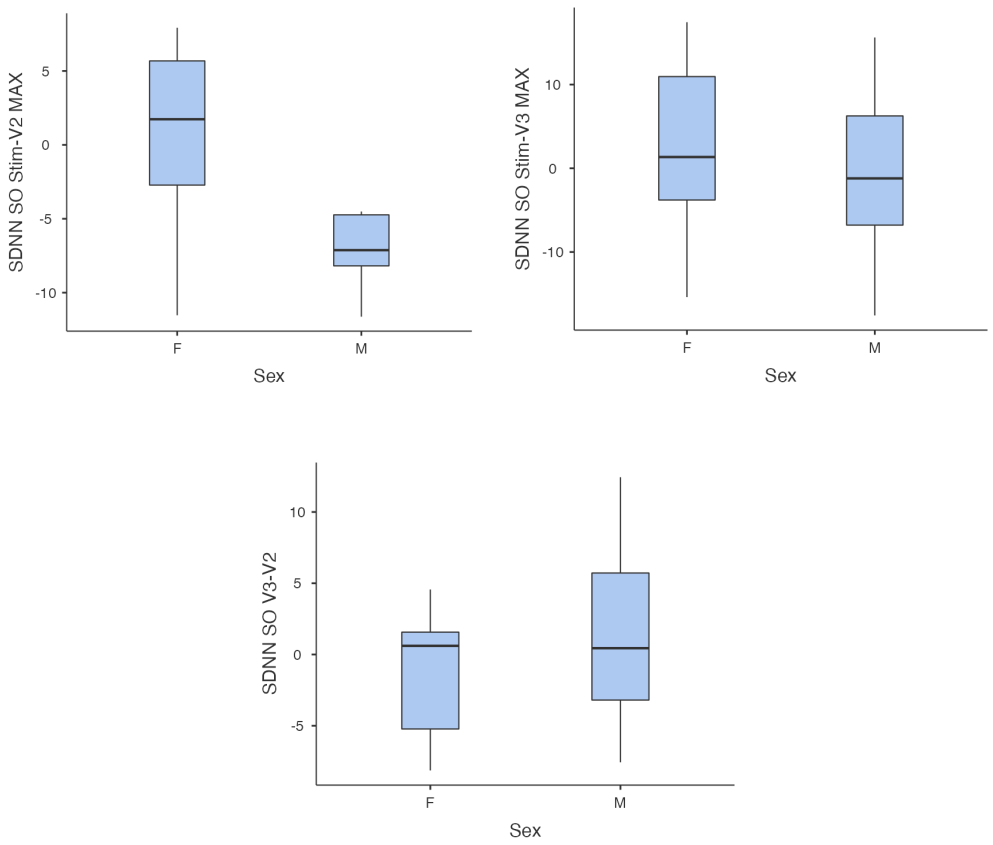


Figure 15. Box plots for RAES SDNN with outliers removed and split by sex.

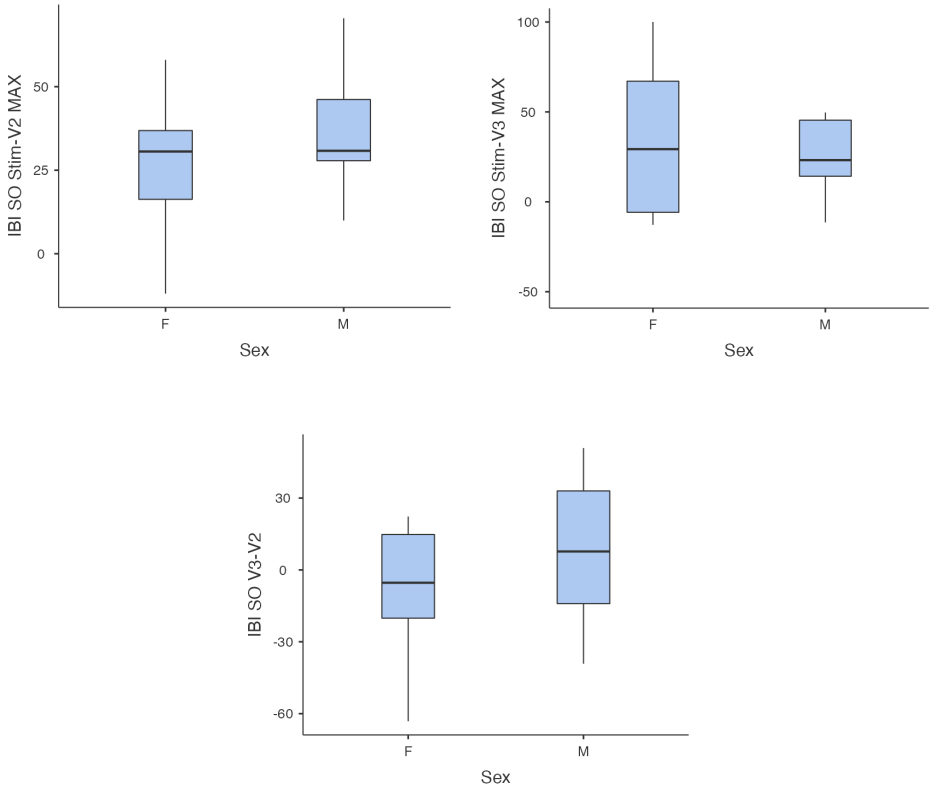


Figure 16. Box plots for RAES IBI with outliers removed and split by sex.

Sex-Based Analysis of RAES Effects on HRV (RMSSD, SDNN, IBI)

In the analysis of RMSSD, females exhibited a clear increase (+2.69 ms) during the RAES intervention, followed by a sharper post-intervention decline (−3.85 ms), ultimately resulting in RMSSD values below baseline. In contrast, males showed a marginal increase during the intervention (+0.048 ms), followed by a continued post-intervention rise (+1.57 ms), suggesting a more gradual but sustained parasympathetic activation. Compared to RVES, where females demonstrated both stronger and more stable RMSSD improvements, the RAES intervention induced a more transient effect in females and a delayed effect in males, highlighting a fundamental difference in how emotional acoustic stimuli are processed across sexes.

For SDNN, the results reveal a more complex picture. Females exhibited a small decline during the intervention (−0.192 ms), followed by a larger drop post-intervention (−1.41 ms), resulting in a net reduction in overall HRV. Males, on the other hand, showed a marked decline during the intervention (−5.81 ms), followed by a partial rebound post-intervention (+3.18 ms), though still below baseline. These findings contrast with RVES, where both sexes demonstrated SDNN improvements during the intervention. The RAES pattern suggests that acoustic stimulation may have had a suppressive or fatiguing effect on overall autonomic variability, particularly in females, while males showed greater adaptability after the initial drop.

In terms of IBI, females experienced a strong increase during the intervention (+29.5 ms), indicative of parasympathetic activation, followed by a pronounced post-intervention decrease (−31.9 ms), resulting in a net heart rate acceleration beyond baseline. Males also showed an initial IBI increase (+35.5 ms), but their post-intervention drop (−22.4 ms) was less severe, leaving them with a heart rate still slower than at baseline. Compared to RVES, where IBI benefits were more sustained in both sexes, the RAES intervention triggered larger autonomic rebounds, particularly in females.

Overall, RAES produced less stable and less favorable autonomic outcomes compared to RVES, especially for females. While females responded more strongly during the RAES intervention, these gains were not maintained and were followed by greater post-intervention declines. Males, by contrast, demonstrated weaker initial responses but more stable trajectories. These findings suggest that acoustic rhythmic stimulation may be less effective than visual stimuli in eliciting sustained autonomic improvements, particularly in female populations. They also reinforce the importance of considering sex-based differences when evaluating the design and impact of HRV-based interventions.

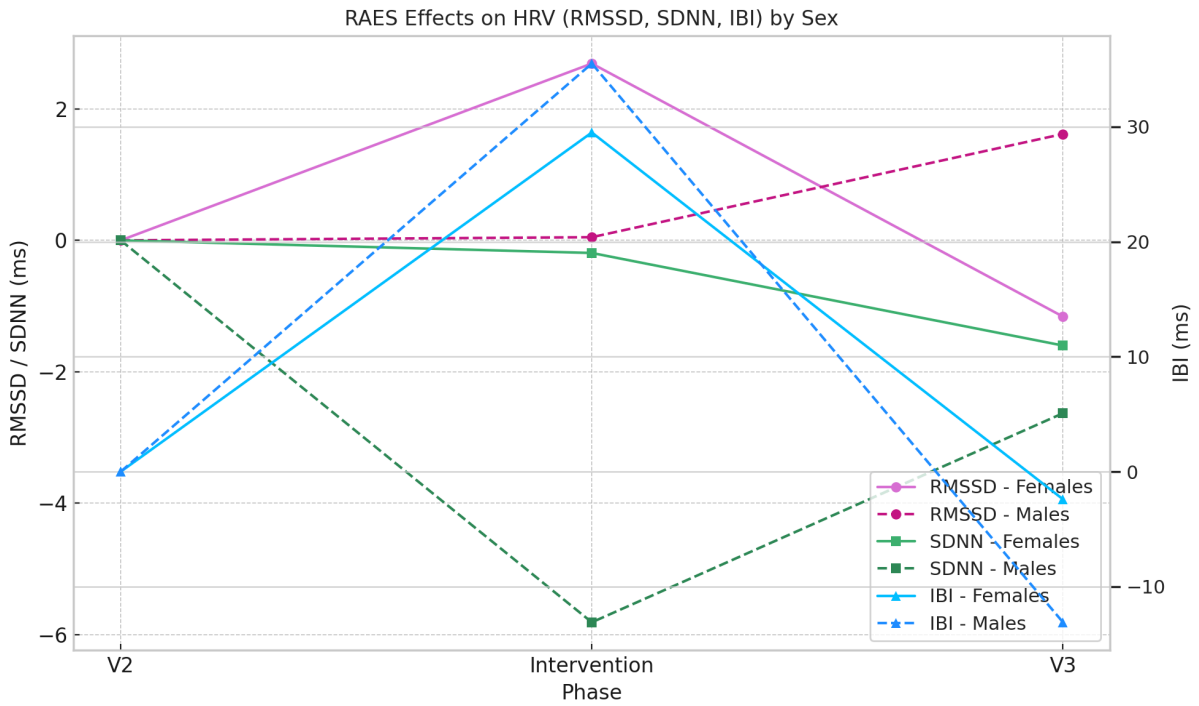


Figure 17. Variations of mean values (normalised) for SDNN, RMSSD and IBI duringthe RVES intervention split by sex.

3.4 Independent Samples T-Test

Independent samples t-tests were employed to directly compare mean HRV responses between male and female participants during the RVES and RAES interventions. This approach allowed us to assess gender-based differences in RMSSD and SDNN by determining if observed trends were statistically significant. Conducting t-tests for each condition provided a straightforward method to evaluate HRV changes across genders, complementing the broader patterns identified in the descriptive analysis

3.4.1 RVES Independent Samples T-Test

Independent samples t-tests were conducted to assess sex-based differences in HRV responses during and after the RVES intervention. A trend towards significance was found for RMSSD during the intervention ($p = 0.054$), suggesting that females may experience stronger parasympathetic activation than males during visual emotional stimulation. No significant sex differences were observed in RMSSD post-intervention or in the overall RMSSD change from baseline ($p > 0.2$). For SDNN, although descriptive data suggested that females exhibited greater sustained increases (V2–V1), the t-test revealed a statistically significant difference in favour of females ($p = 0.044$), indicating stronger retention of global HRV improvements post-intervention. For IBI, the difference between sexes during the intervention approached significance ($p = 0.100$), with descriptive data also showing a larger increase in females. No significant differences were found post-intervention or in overall IBI change. These findings partially support the descriptive analyses, which consistently indicated that females responded more robustly to RVES across all three metrics. While not all t-test results reached statistical significance, the trends align with the visual and descriptive patterns, reinforcing the

interpretation that RVES may be more effective in enhancing vagal and autonomic activity in female participants.

Tables 19, 20 and 21 summarize the t-test results for RVES, while Figures 18, 19 and 20 display the descriptive plots for visual reference of these trends across groups.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
RMSSD IM Stim-V1 MAX	Student's t	2.0408	21.0	0.054	4.948	2.42
RMSSD IM Stim-V2 MAX	Student's t	0.0906	21.0	0.929	0.239	2.64
RMSSD IM V2-V1	Student's t	1.2497	21.0	0.225	2.484	1.99

Note. $H_a\ \mu_F \neq \mu_M$

Table 19. Independent samples T-Test for RVES RMSSD.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
SDNN IM Stim-V1 MAX	Student's t	1.215	22.0	0.237	3.182	2.62
SDNN IM Stim-V2 MAX	Student's t	-0.196	22.0	0.847	-0.627	3.20
SDNN IM V2-V1	Student's t	2.133	22.0	0.044	2.316	1.09

Note. $H_a\ \mu_F \neq \mu_M$

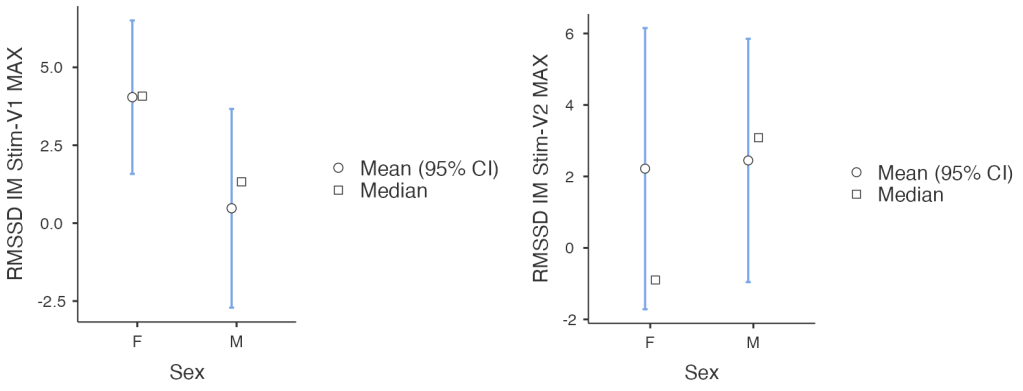
Table 20. Independent samples T-Test for RVES SDNN.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
IBI IM Stim-V1 MAX	Student's t	1.728	19.0	0.100	16.99	9.84
IBI IM Stim-V2 MAX	Student's t	-0.207	19.0	0.838	-2.70	13.05
IBI IM V2-V1	Student's t	1.376 [a]	19.0	0.185	12.48	9.07

Note. $H_a\ \mu_F \neq \mu_M$

Table 21. Independent samples T-Test for RVES IBI.



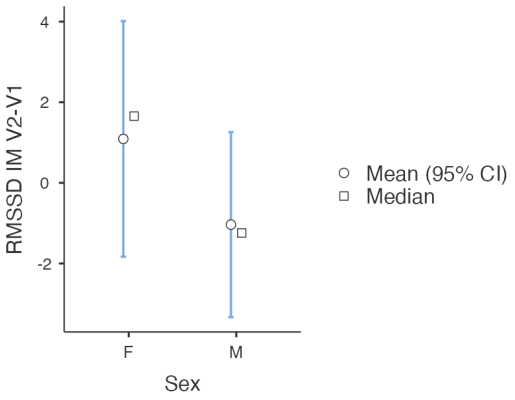


Figure 18. Descriptive plots for image RES RMSSD independent samples T-Test.

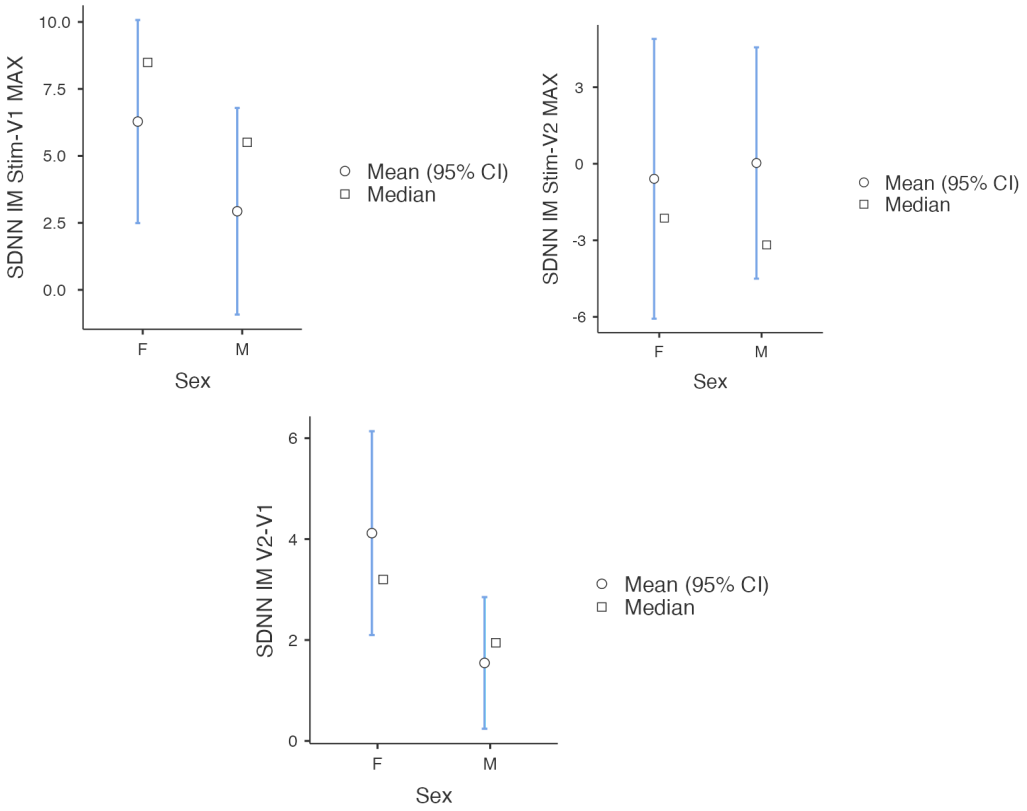


Figure 19. Descriptive plots for image RES SDNN independent samples T-Test.

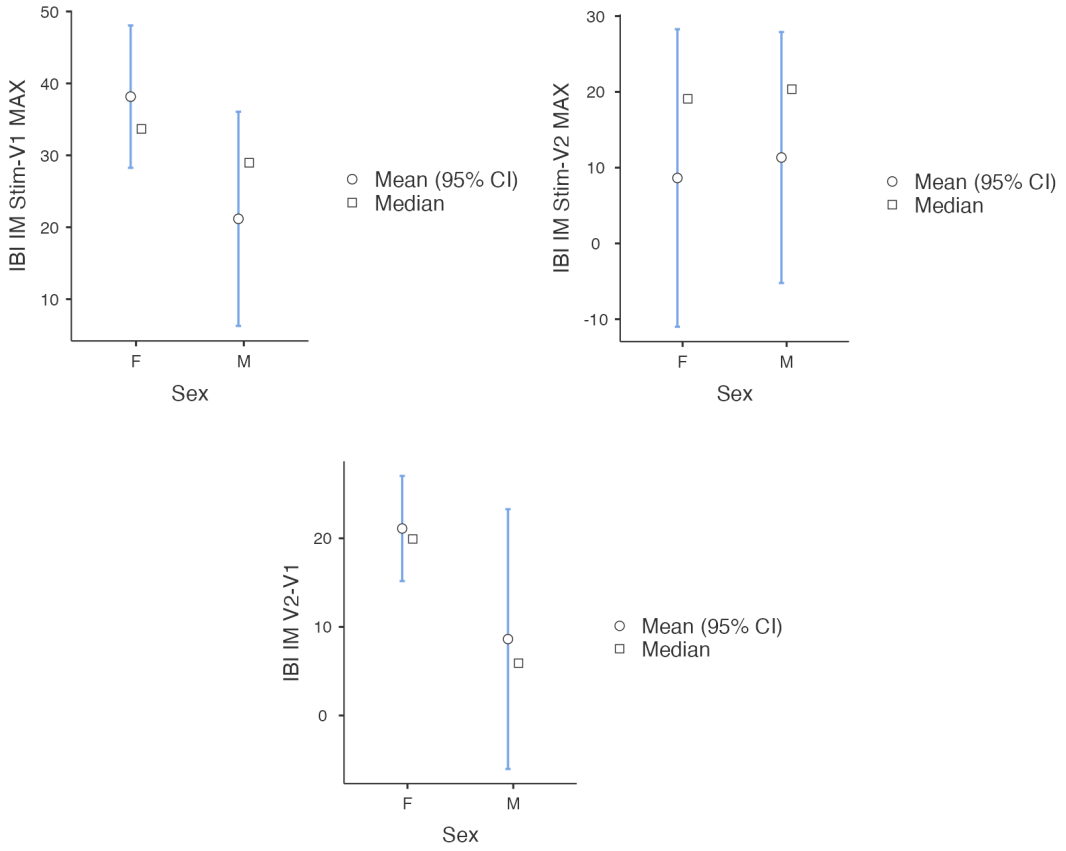


Figure 20. Descriptive plots for image RES IBI independent samples T-Test.

3.4.2 RAES Independent Samples T-Test

Independent samples t-tests were conducted to assess sex-based differences in HRV responses to the RAES intervention. For RMSSD, no significant sex difference was observed during the intervention ($p = 0.169$), but the post-intervention (V3) comparison revealed a statistically significant difference ($p = 0.011$), with females showing substantially higher RMSSD than males. This finding aligns with the descriptive data, where females showed a strong parasympathetic increase during intervention (+2.69 ms) but a sharp decline afterwards (−3.85 ms), whereas males showed a delayed increase across both phases. Despite this, the overall RMSSD change (V3–V2) did not differ significantly between sexes ($p = 0.145$).

For SDNN, the intervention phase showed a significant difference between sexes ($p = 0.029$), with males showing a greater reduction (−5.81 ms) than females (−0.192 ms). Although the post-intervention SDNN comparison approached significance ($p = 0.069$), the overall change (V3–V2) remained statistically non-significant ($p = 0.449$). This reinforces the descriptive observation that females displayed a consistent decline in SDNN throughout, while males showed partial recovery post-intervention.

Regarding IBI, no significant sex differences were observed at any phase ($p > 0.29$), though descriptively females exhibited a sharper autonomic rebound post-intervention (−31.9 ms) compared to males (−22.4 ms). Males, however, had a larger initial increase in IBI during the intervention (+35.5 ms vs. +29.5 ms).

In summary, while RMSSD and SDNN showed some statistically significant sex-based differences during and after RAES, these effects were less consistent than those found in the RVES condition. The results suggest that RAES may elicit more variable and less robust parasympathetic effects, particularly in males. By contrast, females continue to show stronger post-intervention RMSSD responses, echoing the patterns seen in RVES. However, the limited number of significant findings in RAES and the high variability observed—particularly in IBI—highlight the need for further research with larger samples to clarify these sex-based patterns.

Tables 22, 23 and 24 present the t-test results for RAES, with descriptive plots in Figures 21, 22 and 23.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
RMSSD SO Stim-V2 MAX	Student's t	1.42	23.0	0.169	3.02	2.13
RMSSD SO Stim-V3 MAX	Student's t	2.76	23.0	0.011	7.30	2.65
RMSSD SO V3-V2	Student's t	-1.51	23.0	0.145	-2.60	1.72

Note. $H_a\ \mu_F \neq \mu_M$

Table 22. Independent samples T-Test for RAES RMSSD.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
SDNN SO Stim-V2 MAX	Student's t	2.330	23.0	0.029	6.99	3.00
SDNN SO Stim-V3 MAX	Student's t	1.910	23.0	0.069	6.60	3.46
SDNN SO V3-V2	Student's t	-0.771	23.0	0.449	-1.45	1.88

Note. $H_a\ \mu_F \neq \mu_M$

Table 23. Independent samples T-Test for RAES SDNN.

Independent Samples T-Test

		Statistic	df	p	Mean difference	SE difference
IBI SO Stim-V2 MAX	Student's t	-1.073	16.0	0.299	-4.94	4.60
IBI SO Stim-V3 MAX	Student's t	0.161	16.0	0.874	2.64	16.37
IBI SO V3-V2	Student's t	-0.593	16.0	0.561	-7.81	13.16

Note. $H_a\ \mu_F \neq \mu_M$

Table 24. Independent samples T-Test for RAES IBI.

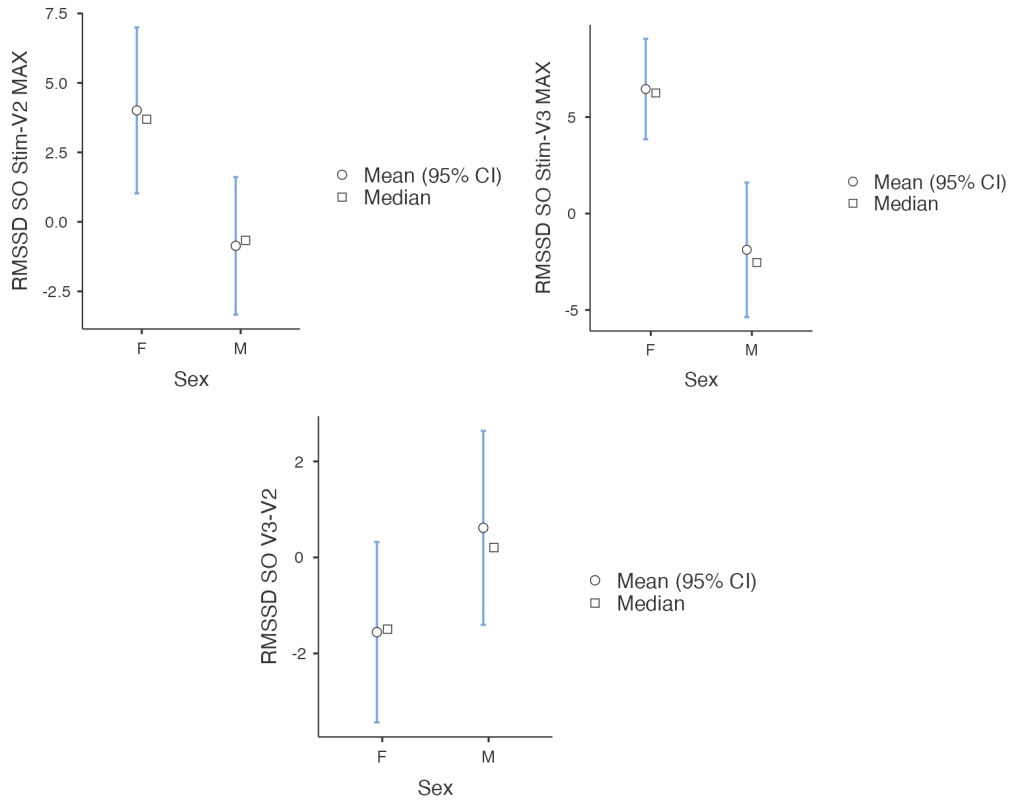


Figure 21. Descriptive plots for acoustic RES RMSSD independent samples T-Test.

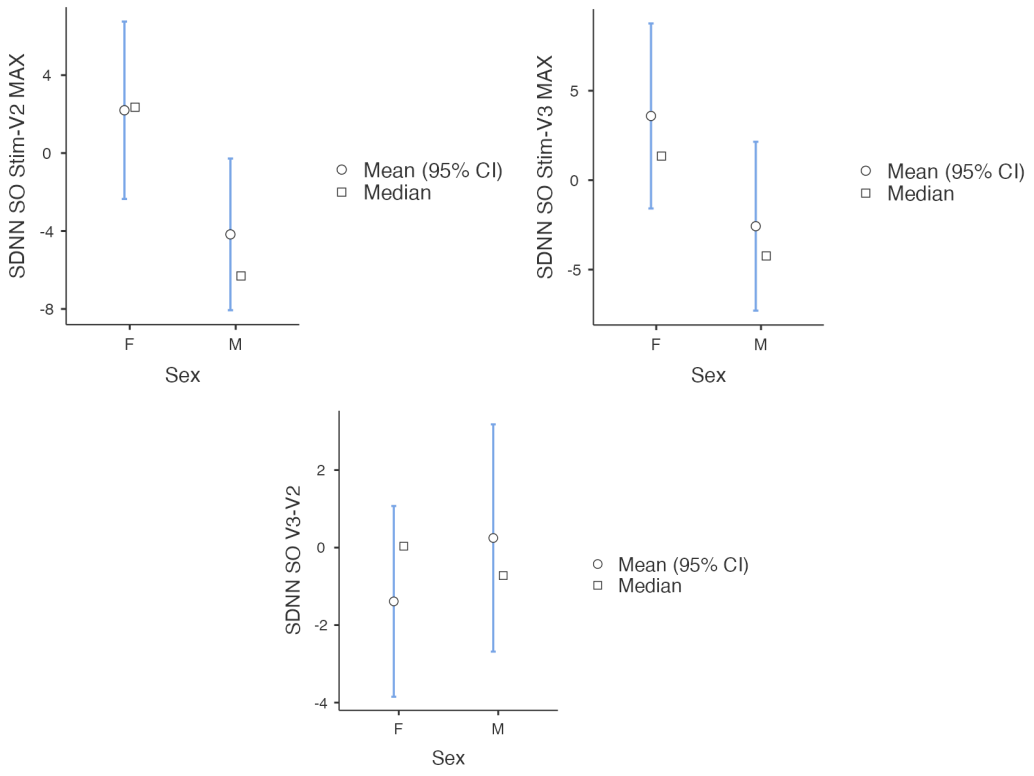


Figure 22. Descriptive plots for acoustic RES SDNN independent samples T-Test.

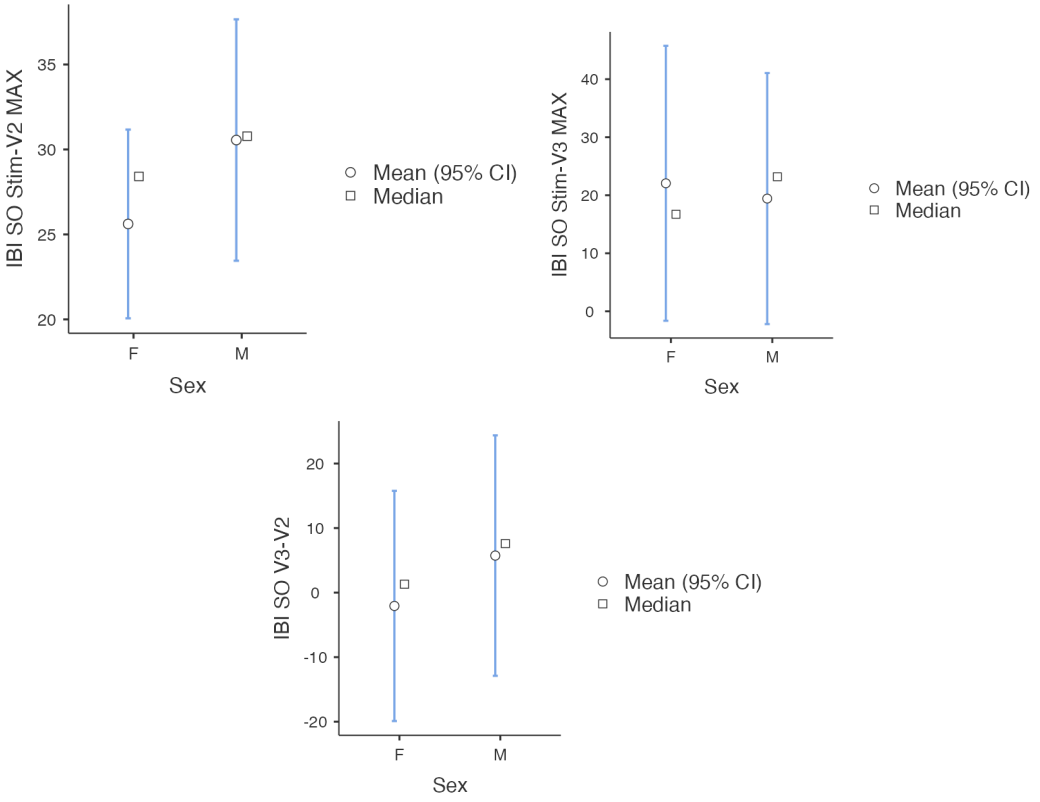


Figure 23. Descriptive plots for acoustic RES IBI independent samples T-Test.

3.5 Repeated Measures ANOVA

A repeated measures ANOVA was performed to assess the effects of RVES on HRV metrics, focusing on RMSSD and SDNN across positive, neutral, and negative stimuli, as well as their interaction with sex. This analysis aimed to explore the variability of HRV responses and the potential influence of gender, BMI and age.

3.5.1 RVES Repeated Measures ANOVA

Homogeneity of variance tests confirmed that this assumption was met across variables. A repeated measures ANOVA was subsequently performed to assess RMSSD, SDNN and IBI changes during the RVES intervention. The findings are presented in Table 25, 26 and 27.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
RMSSD IM Stim	27.7	4	6.92	0.167	0.954	0.008
RMSSD IM Stim ✻ Sex	165.1	4	41.28	0.999	0.413	0.043
RMSSD IM Stim ✻ Age	58.3	4	14.59	0.353	0.841	0.016
RMSSD IM Stim ✻ BMI	30.0	4	7.49	0.181	0.948	0.008
RMSSD IM Stim ✻ BMI Category	474.0	8	59.26	1.434	0.194	0.115
RMSSD IM Stim ✻ Sex ✻ BMI Category	1235.5	8	154.44	3.737	<.001	0.254
Residual	3636.4	88	41.32			

Note. Type 3 Sums of Squares

Table 25. Repeated measures ANOVA for RMSSD RVES.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
SDNN IM Stim	123	4	30.7	0.646	0.631	0.029
SDNN IM Stim ✻ Sex	218	4	54.5	1.149	0.339	0.050
SDNN IM Stim ✻ Age	114	4	28.6	0.603	0.662	0.027
SDNN IM Stim ✻ BMI	101	4	25.3	0.534	0.711	0.024
SDNN IM Stim ✻ BMI Category	637	8	79.6	1.677	0.115	0.132
SDNN IM Stim ✻ Sex ✻ BMI Category	365	8	45.6	0.960	0.473	0.080
Residual	4178	88	47.5			

Note. Type 3 Sums of Squares

Table 26. Repeated measures ANOVA for SDNN RVES.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
IBI IM Stim	500	4	125	0.937	0.450	0.067
IBI IM Stim ✻ Sex	401	4	100	0.752	0.561	0.055
IBI IM Stim ✻ BMI Category	3333	8	417	3.127	0.006	0.325
IBI IM Stim ✻ Age	567	4	142	1.064	0.384	0.076
IBI IM Stim ✻ BMI	535	4	134	1.004	0.414	0.072
IBI IM Stim ✻ Sex ✻ BMI Category	2491	8	311	2.336	0.032	0.264
Residual	6929	52	133			

Note. Type 3 Sums of Squares

Table 27. Repeated measures ANOVA for IBI RVES.

The repeated measures ANOVA revealed no significant main effect of stimulation (Stim) on RMSSD ($F = 0.167$, $p = 0.954$, $\eta^2p = 0.008$), nor any significant two-way interactions with sex ($p = 0.413$), age ($p = 0.841$), or BMI ($p = 0.948$). The interaction with BMI category was also non-significant ($p =$

0.194, $\eta^2p = 0.115$). However, a significant three-way interaction was observed between stimulation, sex, and BMI category ($F = 3.737$, $p < .001$, $\eta^2p = 0.254$), suggesting that BMI may moderate sex-based differences in RMSSD response to RVES. Despite the absence of overall RMSSD effects, this interaction highlights complex modulations influenced by individual characteristics.

In the case of SDNN, neither the main effect of stimulation ($F = 0.646$, $p = 0.631$, $\eta^2p = 0.029$) nor its interactions with sex ($p = 0.339$), age ($p = 0.662$), BMI ($p = 0.711$), or the triple interaction ($p = 0.473$) reached statistical significance. The closest to significance was the interaction between stimulation and BMI category ($p = 0.115$, $\eta^2p = 0.132$), indicating a possible—but unconfirmed—moderating role.

For IBI, the main effect of stimulation was again non-significant ($F = 0.937$, $p = 0.450$, $\eta^2p = 0.067$). However, two interactions reached statistical significance: stimulation ✻ BMI category ($F = 3.127$, $p = 0.006$, $\eta^2p = 0.325$), and the three-way interaction stimulation ✻ sex ✻ BMI category ($F = 2.336$, $p = 0.032$, $\eta^2p = 0.264$). These moderate-to-large effect sizes suggest that IBI responses to RVES are not uniform, and are substantially influenced by BMI and its interaction with sex—highlighting a nontrivial degree of interindividual variability in autonomic response.

In summary, while RVES did not yield strong overall effects across the full sample, it revealed meaningful three-way interactions in both RMSSD and IBI, driven by sex and BMI category. These results underscore the importance of considering individual physiological traits when designing and interpreting resonance-based HRV interventions.

3.5.2 RAES Repeated Measures ANOVA

Homogeneity of variance tests confirmed that this assumption was met across variables. A repeated measures ANOVA was subsequently performed to assess RMSSD and SDNN changes during the RAES intervention. The findings are presented in Tables 28, 29 and 30.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
RMSSD SO Stim	9.59	4	2.40	0.0789	0.989	0.004
RMSSD SO Stim ✻ Sex	296.22	4	74.06	2.4364	0.053	0.100
RMSSD SO Stim ✻ Age	311.05	4	77.76	2.5584	0.044	0.104
RMSSD SO Stim ✻ BMI	32.43	4	8.11	0.2667	0.899	0.012
RMSSD SO Stim ✻ BMI Category	104.87	8	13.11	0.4313	0.899	0.038
RMSSD SO Stim ✻ Sex ✻ BMI Category	300.64	8	37.58	1.2364	0.288	0.101
Residual	2674.83	88	30.40			

Note. Type 3 Sums of Squares

Table 28. Repeated measures ANOVA for RMSSD RAES.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
SDNN SO Stim	22.0	4	5.49	0.0908	0.985	0.004
SDNN SO Stim ✻ Sex	17.4	4	4.36	0.0721	0.990	0.003
SDNN SO Stim ✻ Age	331.0	4	82.74	1.3686	0.251	0.059
SDNN SO Stim ✻ BMI	37.0	4	9.24	0.1528	0.961	0.007
SDNN SO Stim ✻ BMI Category	249.8	8	31.22	0.5164	0.841	0.045
SDNN SO Stim ✻ Sex ✻ BMI Category	400.4	8	50.05	0.8277	0.580	0.070
Residual	5320.6	88	60.46			

Note. Type 3 Sums of Squares

Table 29. Repeated measures ANOVA for SDNN RAES.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p	η^2_p
IBI SO Stim	196.6	4	49.2	0.2667	0.898	0.026
IBI SO Stim ✻ Sex	117.1	4	29.3	0.1589	0.958	0.016
IBI SO Stim ✻ BMI Category	1397.8	8	174.7	0.9482	0.489	0.159
IBI SO Stim ✻ Age	402.8	4	100.7	0.5465	0.703	0.052
IBI SO Stim ✻ BMI	55.9	4	14.0	0.0759	0.989	0.008
IBI SO Stim ✻ Sex ✻ BMI Category	3797.5	8	474.7	2.5759	0.023	0.340
Residual	7371.1	40	184.3			

Note. Type 3 Sums of Squares

Table 30. Repeated measures ANOVA for IBI RAES.

The repeated measures ANOVA for the RAES condition did not show a significant main effect of stimulation on RMSSD ($F = 0.079$, $p = 0.989$, $\eta^2p = 0.004$), nor significant interactions with BMI category ($p = 0.899$), BMI ($p = 0.899$), or the three-way interaction ($p = 0.288$). However, significant interaction effects emerged for age ($F = 2.56$, $p = 0.044$, $\eta^2p = 0.104$), and a marginal interaction between stimulation and sex ($F = 2.44$, $p = 0.053$, $\eta^2p = 0.100$), indicating moderate effect sizes. These results suggest that age and sex may moderate RMSSD responses to RAES, though the effect of stimulation itself was negligible.

In contrast to RMSSD, SDNN outcomes showed even weaker effects. Neither the main effect of stimulation ($F = 0.091$, $p = 0.985$, $\eta^2p = 0.004$) nor any two-way or three-way interactions reached statistical significance. The largest effect size emerged in the stimulation ✻ BMI category interaction ($\eta^2p = 0.045$), but it remained non-significant ($p = 0.841$), further underscoring the lack of consistent modulation in overall HRV (SDNN) under RAES conditions.

For IBI, the main effect of stimulation and all two-way interactions (sex, age, BMI, BMI category) were non-significant (all $p > 0.15$), with very small effect sizes (η^2p ranging from 0.008 to 0.159). However, a significant three-way interaction between stimulation, sex, and BMI category was found ($F = 2.576$, $p = 0.023$, $\eta^2p = 0.340$), indicating a large effect size. This suggests that while RAES did

not influence IBI consistently across participants, there is substantial variation depending on the combined influence of sex and BMI category.

In summary, RAES did not produce a main effect on any of the three HRV markers (RMSSD, SDNN, IBI). Nonetheless, the data reveal meaningful moderating roles for age, sex, and BMI category, particularly in RMSSD and IBI. The findings highlight the importance of considering individual physiological differences, and suggest that RAES may exert selective effects that are not uniformly observable across the sample but may emerge in subgroups defined by sex, age, and body composition. These results contrast with the RVES findings, where stronger group-level responses were detected, reinforcing the idea that acoustic stimulation may require more individualised calibration or that it elicits weaker autonomic entrainment compared to visual rhythmic stimulation.

3.5.3 Power Analysis

A power analysis was conducted to assess the statistical strength of the ANOVA results for RVES and RAES HRV measures (RMSSD, SDNN, and IBI). The observed power for the current study, with a sample size of N=30, was low across all measures, particularly for RAES. For RVES, the observed power was 0.19 for RMSSD, 0.15 for SDNN, and 0.24 for IBI, suggesting that the study lacked sufficient power to detect significant effects. RAES demonstrated even lower observed power, with values below 10% for RMSSD and SDNN, indicating a high likelihood of Type II error (false negatives).

To achieve the conventional standard of 80% power at an alpha level of 0.05, we would require larger samples. These findings suggest that the study was underpowered, and that the non-significant ANOVA results may have been influenced by insufficient statistical power rather than a true absence of effect. Given these results, future research should prioritize increasing sample sizes, particularly for RAES. Additionally, alternative experimental designs, such as within-subject designs, could be considered to enhance statistical power and improve the detection of HRV changes.

4. Discussion and Conclusions

This study investigated the effects of Rhythmic Visual Emotional Stimulation (RVES) and Rhythmic Acoustic Emotional Stimulation (RAES) on Heart Rate Variability (HRV), focusing on time-domain measures (RMSSD and SDNN) and inter-beat interval (IBI) to assess autonomic nervous system modulation. Building upon Vaschillo's foundational RVES protocol, the study evaluated whether acoustic stimuli delivered at 0.1 Hz could induce similar baroreflex resonance effects, while also exploring sex and BMI as potential moderating variables.

4.1 Summary of Findings

RVES produced clear and consistent increases in HRV, especially in SDNN and IBI, during the intervention phase. These increases were largely sustained post-intervention, particularly in females, supporting the potential of RVES as a resonant, non-invasive method for modulating autonomic

balance. RMSSD also increased during stimulation but decreased afterward, especially in males, suggesting that RVES elicits stronger parasympathetic activation in females with more stable post-intervention effects. These patterns are consistent with prior evidence showing that rhythmic visual stimuli at the baroreflex resonance frequency can enhance both vagal tone and global HRV (Vaschillo et al., 2008a; Lehrer & Gevirtz, 2014).

By contrast, RAES showed a more modest and less consistent effect. RMSSD increased only slightly during stimulation and decreased significantly post-intervention, particularly in females. SDNN remained relatively unchanged in both sexes, and while IBI increased during stimulation—suggesting heart rate slowing—it dropped sharply afterward, indicating poor retention of autonomic benefit. These outcomes suggest that acoustic stimulation alone may be less effective than visual stimulation in engaging the baroreflex and sustaining parasympathetic activation. The weaker response could relate to the nature of the sounds used or the less direct engagement of visual-attentional circuits implicated in HRV modulation.

4.2 Sex and BMI as Moderators

Sex emerged as a significant moderator in both interventions. In RVES, females showed stronger improvements across all HRV indices, with greater and more sustained gains in RMSSD, SDNN, and IBI compared to males. In RAES, the results were more complex: although females again showed higher RMSSD during stimulation, they experienced a sharper post-intervention decline, suggesting a greater initial responsiveness followed by faster autonomic rebound. Males exhibited more modest changes but a more gradual recovery, particularly in IBI. These patterns are consistent with known sex differences in vagal flexibility, emotional processing, and baroreceptor sensitivity (Laborde et al., 2017a; Shaffer & Meehan, 2020a).

The role of BMI was particularly pronounced in the RAES condition, where significant three-way interactions (stimulation ✻ sex ✻ BMI category) emerged for both RMSSD and IBI. These findings suggest that individuals with different BMI profiles may vary in how they physiologically respond to auditory rhythmic stimuli, possibly due to differences in metabolic load, cardiovascular fitness, or sympathetic/parasympathetic balance (Hallman et al., 2011). Notably, these interactions were not observed in RVES, reinforcing the idea that RAES responses are more susceptible to interindividual variability, whereas RVES produces more consistent autonomic effects.

4.3 Statistical Insights

Repeated Measures ANOVA showed a clear and statistically significant three-way interaction in RVES for RMSSD ($p < .001$), again supporting the role of sex and BMI in shaping autonomic responses. For RAES, the main effects were non-significant, but interaction effects with age and sex approached significance, and the sex ✻ BMI interaction for IBI was significant ($p = .023$, $\eta^2p = 0.340$), indicating a large effect size. These outcomes reinforce that group-level effects in RAES are weak, but individual-level moderators matter.

Independent t-tests further confirmed significant sex differences in RAES, particularly in RMSSD post-intervention ($p = 0.011$), while no such differences were statistically confirmed in RVES despite

large descriptive contrasts. These results imply that RAES effects vary more sharply by sex, while RVES delivers more uniform benefits across individuals, especially in females.

4.4 Limitations

Despite the insights provided by this study, several limitations warrant consideration. Primarily, the sample size of 30 participants may have limited the statistical power required to detect smaller effects, particularly within complex interaction models or when stratifying data by sex and BMI; indeed, power analyses indicated particularly low sensitivity for RAES-related tests.

Regarding data integrity, while outliers were excluded from descriptive summaries using a standard 1.5×IQR rule to ensure representativeness, all inferential analyses (e.g., ANOVA and t-tests) utilized the full dataset to maintain statistical robustness. Furthermore, the stimulus design was matched for valence but not specifically optimized for acoustic baroreflex engagement; thus, it remains uncertain whether alternative auditory characteristics, such as binaural beats or frequency-specific modulations, could elicit stronger autonomic responses. It must also be noted that the observed effects are acute, and longitudinal research is necessary to evaluate the cumulative impact of repeated rhythmic stimulation for therapeutic use.

Finally, the absence of a time-matched control condition, such as neutral rhythmic auditory stimulation or passive exposure, means that factors such as habituation or time-on-task effects cannot be entirely excluded. Future investigations should address these constraints by employing counterbalanced within-subject designs with control stimuli matched for both rhythm and duration.

4.5 Contribution to the Field and Research Significance

This study makes a novel contribution to the growing body of literature exploring HRV-based interventions for emotional and autonomic regulation by directly comparing rhythmic visual and auditory emotional stimulation at the baroreflex resonance frequency (0.1 Hz). While prior research has established the efficacy of RVES in producing resonant baroreflex stimulation (Vaschillo et al., 2008a), the potential of RAES—auditory rhythmic emotional stimulation—has remained largely unexplored. This research fills a critical gap by directly testing whether RAES can elicit comparable HRV effects using parallel protocols and emotionally matched stimuli.

Our findings reaffirm the role of RVES as a potent non-invasive intervention for increasing HRV, particularly SDNN, which is considered a robust marker of overall autonomic adaptability. This supports and extends prior work linking rhythmic visual cues to enhanced baroreflex resonance and autonomic flexibility (Lehrer and Gevirtz, 2014; Lehrer, Vaschillo, and Vidali, 2020). Importantly, our study shows that RMSSD and IBI also respond to RVES, though with different time-course dynamics across sexes—offering a more nuanced understanding of how parasympathetic and heart rate effects manifest during and after stimulation.

In contrast, RAES produced weaker and more inconsistent effects, both at the group level and across HRV metrics. This highlights a potential modality-specific limitation: while emotionally salient acoustic stimuli are known to provoke strong affective responses (Bradley and Lang, 2017), they may

not produce the precise mechanical oscillations in arterial blood pressure needed to fully engage the baroreflex loop. Previous studies exploring binaural beats, resonant soundscapes, or music therapy have typically not controlled for breathing rhythm or resonance frequency, making direct comparisons difficult. Our study adds clarity to this literature by controlling for these variables and showing that sound alone may not reliably produce resonant HRV effects—unless perhaps paired with breathwork or multisensory cues.

A further contribution lies in our exploration of individual differences. The significant Sex ✻ BMI ✻ Stimulation interactions, particularly in the RAES condition, underscore the non-universality of physiological responses to rhythmic stimulation. While sex differences in HRV and baroreflex sensitivity are well established (Shaffer and Meehan, 2020a; Laborde et al., 2017a), few studies have linked these to differential responsiveness to rhythmic interventions. The interaction with BMI is also noteworthy, aligning with findings that metabolic health and adiposity influence autonomic tone and reactivity (Hallman et al., 2011). These findings position your study within a more personalized and precision-oriented model of HRV research, emphasizing the need to tailor interventions based not only on emotional content or stimulus modality but also on physiological and demographic profiles.

In sum, this study bridges several critical gaps in the existing literature by systematically comparing visual and auditory emotional stimulation within a baroreflex resonance protocol. Our findings clarify the practical limits of RAES as a stand-alone modality for HRV enhancement while simultaneously introducing sex and BMI interactions into the broader HRV entrainment discourse. By identifying these individual differences, this research suggests pivotal new directions for personalized, multimodal, and repeated-exposure designs. Ultimately, these contributions support a growing movement toward evidence-based, individualized stress interventions, leveraging emotion-based entrainment and resonant stimulation as emerging therapeutic tools for autonomic health.

Together, these contributions support a growing movement toward evidence-based, individualized stress interventions, leveraging HRV biofeedback, emotion-based entrainment, and resonant stimulation as emerging therapeutic tools.

4.6 Conclusions and Implications

This study provides robust evidence for the differential impact of rhythmic sensory stimulation on autonomic regulation. Our findings confirm that Rhythmic Visual Emotional Stimulation (RVES) is a potent driver of cardiovascular oscillations, significantly enhancing global heart rate variability (SDNN), parasympathetic tone (RMSSD), and inter-beat intervals (IBI). This reinforces the role of the visual pathway as a primary gateway for baroreflex entrainment at 0.1 Hz, likely due to a more direct sensory-autonomic coupling between the visual cortex and brainstem regulatory centers.

The modest and inconsistent results observed for Rhythmic Acoustic Emotional Stimulation (RAES) suggest that auditory cues alone may lack the necessary physiological "gain" to induce full systemic resonance in the baroreflex arc. However, the discovery of a significant three-way interaction between stimulation modality, sex, and BMI for IBI ($p = 0.023$) is a pivotal finding. It suggests that autonomic receptivity to sound is highly individualized, with lower-BMI females exhibiting the most pronounced response. This highlights a crucial implication for clinical practice: one-size-fits-all interventions for stress management are likely to be suboptimal.

These findings carry significant clinical and practical implications for the field of autonomic health. Primarily, the results underscore the necessity for personalized autonomic therapy, as HRV-based interventions must account for individual physiological profiles to maximize efficacy. Clinicians should prioritize visual paradigms, such as RVES, for rapid autonomic modulation; conversely, the implementation of acoustic methods may require higher degrees of personalization, specifically considering sex and body composition, to overcome the lower responsiveness observed in certain subgroups. Furthermore, given its non-invasive nature and accessibility, RVES emerges as a scalable, non-pharmacological tool for enhancing autonomic resilience in populations characterized by vagal withdrawal, including those suffering from chronic stress or anxiety. Ultimately, the relative weakness of single-modality acoustic stimulation suggests that future rhythmic protocols should transition toward multimodal integration. By combining visual and auditory cues, researchers may harness synergistic effects that could potentially overcome individual resistance to entrainment and optimize the homeostatic engagement of the baroreflex arc.

Future research should investigate the longitudinal stability of these autonomic shifts and explore the neuroimaging correlates of sensory-autonomic coupling to map the exact pathways of entrainment. Additionally, the role of BMI as a moderator warrants further study to determine if metabolic or adipose-related factors influence the efficiency of autonomic feedback loops during rhythmic stimulation.

5. Bibliography

Armony, J.L. *et al.* (2015) 'The specificity of neural responses to music and their relation to voice processing: an fMRI-adaptation study', *Neuroscience Letters*, 593, pp. 35–39. doi:10.1016/j.neulet.2015.03.011.

Baek, H.J. *et al.* (2015) 'Reliability of ultra-short-term analysis as a surrogate of standard 5-min analysis of heart rate variability', *Telemedicine Journal and E-Health: The Official Journal of the American Telemedicine Association*, 21(5), pp. 404–414. doi:10.1089/tmj.2014.0104.

Bates, M.E. *et al.* (2022) 'The Process of Heart Rate Variability, Resonance at 0.1 hz, and the Three Baroreflex Loops: A Tribute to Evgeny Vaschillo', *Applied Psychophysiology and Biofeedback* [Preprint]. doi:10.1007/s10484-022-09544-4.

Beauchaine, T. (2001) 'Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology', *Development and Psychopathology*, 13(2), pp. 183–214. doi:10.1017/s0954579401002012.

Berntson, Gary G. *et al.* (1994) 'Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades', *Psychophysiology*, 31(6), pp. 599–608. doi:10.1111/j.1469-8986.1994.tb02352.x.

Berntson, G. G. *et al.* (1994) 'Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades', *Psychophysiology*, 31(6), pp. 599–608. doi:10.1111/j.1469-8986.1994.tb02352.x.

Berntson, G.G. *et al.* (2008) 'Cardiac autonomic balance versus cardiac regulatory capacity', *Psychophysiology*, 45(4), pp. 643–652. doi:10.1111/j.1469-8986.2008.00652.x.

Beutel, M.E. *et al.* (2016) 'Noise Annoyance Is Associated with Depression and Anxiety in the General Population- The Contribution of Aircraft Noise', *PLoS ONE*, 11(5). doi:10.1371/journal.pone.0155357.

Beyer, H. (1981) 'Tukey, John W.: Exploratory Data Analysis. Addison-Wesley Publishing Company Reading, Mass. — Menlo Park, Cal., London, Amsterdam, Don Mills, Ontario, Sydney 1977, XVI, 688 S.', *Biometrical Journal*, 23(4), pp. 413–414. doi:10.1002/bimj.4710230408.

Billman, G.E. (2013) 'The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance', *Frontiers in Physiology*, 4, p. 26. doi:10.3389/fphys.2013.00026.

Bradley, M.M. and Lang, P.J. (2000) 'Affective reactions to acoustic stimuli', p. 12.

Brock, C. *et al.* (2017) 'Cardiac vagal tone, a non-invasive measure of parasympathetic tone, is a clinically relevant tool in Type 1 diabetes mellitus', *Diabetic Medicine: A Journal of the*

British Diabetic Association, 34(10), pp. 1428–1434. doi:10.1111/dme.13421.

Calvert, G., Spence, C. and Stein, B.E. (2004) *The Handbook of Multisensory Processes*. 1st edition. Cambridge, Mass: MIT Press.

Cohen, S. *et al.* (2012) 'Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk', *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), pp. 5995–5999. doi:10.1073/pnas.1118355109.

Czigler, I. *et al.* (2007) 'Event-related potential study to aversive auditory stimuli', *Neuroscience Letters*, 420(3), pp. 251–256. doi:10.1016/j.neulet.2007.05.007.

Deroy, O., Chen, Y.-C. and Spence, C. (2014) 'Multisensory constraints on awareness', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 369, p. 20130207. doi:10.1098/rstb.2013.0207.

Domínguez, M.D., Tapia, A.M.F. and Troncoso, R.M. (2011) 'Adding support to cross-cultural emotional assessment: validation of the international affective picture system in a chilean sample', *Universitas Psychologica*, 10(2), pp. 521–533. doi:10.11144/Javeriana.upsy10-2.asce.

Dworkin, B.R. *et al.* (1994) 'Central effects of baroreceptor activation in humans: attenuation of skeletal reflexes and pain perception.', *Proceedings of the National Academy of Sciences of the United States of America*, 91(14), pp. 6329–6333. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC44195/ (Accessed: 23 May 2022).

Esco, M.R. and Flatt, A.A. (2014) 'Ultra-Short-Term Heart Rate Variability Indexes at Rest and Post-Exercise in Athletes: Evaluating the Agreement with Accepted Recommendations', *Journal of Sports Science & Medicine*, 13(3), pp. 535–541. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126289/ (Accessed: 8 November 2021).

Fabiani, M. *et al.* (1996) 'Naming norms for brief environmental sounds: effects of age and dementia', *Psychophysiology*, 33(4), pp. 462–475. doi:10.1111/j.1469-8986.1996.tb01072.x.

Farmer, D.G.S. *et al.* (2016) 'Brainstem sources of cardiac vagal tone and respiratory sinus arrhythmia', *The Journal of Physiology*, 594(24), pp. 7249–7265. doi:10.1113/JP273164.

Fernández-Abascal, E.G. *et al.* (2008) '[The International Affective Digitized Sounds (IADS): Spanish norms]', *Psicothema*, 20(1), pp. 104–113.

Fink, G. (2017) 'Stress: Concepts, Definition and History', in. doi:10.1016/B978-0-12-809324-5.02208-2.

France, C.R., France, J.L. and Patterson, S.M. (2006) 'Blood pressure and cerebral oxygenation

responses to skeletal muscle tension: a comparison of two physical maneuvers to prevent vasovagal reactions', *Clinical Physiology and Functional Imaging*, 26(1), pp. 21–25. doi:10.1111/j.1475-097X.2005.00642.x.

Gerdes, A.B.M., Wieser, M.J. and Alpers, G.W. (2014) 'Emotional pictures and sounds: a review of multimodal interactions of emotion cues in multiple domains', *Frontiers in Psychology*, 5. Available at: https://www.frontiersin.org/articles/10.3389/fpsyg.2014.01351 (Accessed: 26 October 2022).

Gevirtz, R. (2013) 'The Promise of Heart Rate Variability Biofeedback: Evidence-Based Applications', *Biofeedback*, 41, pp. 110–120. doi:10.5298/1081-5937-41.3.01.

Giardino, N.D., Chan, L. and Borson, S. (2004) 'Combined heart rate variability and pulse oximetry biofeedback for chronic obstructive pulmonary disease: preliminary findings', *Applied Psychophysiology and Biofeedback*, 29(2), pp. 121–133. doi:10.1023/b:apbi.0000026638.64386.89.

Grühn, D. and Scheibe, S. (2008a) 'Age-related differences in valence and arousal ratings of pictures from the International Affective Picture System (IAPS): Do ratings become more extreme with age?', *Behavior Research Methods*, 40(2), pp. 512–521. doi:10.3758/BRM.40.2.512.

Grühn, D. and Scheibe, S. (2008b) 'Age-related differences in valence and arousal ratings of pictures from the International Affective Picture System (IAPS): Do ratings become more extreme with age?', *Behavior Research Methods*, 40(2), pp. 512–521. doi:10.3758/BRM.40.2.512.

Hallman, D.M. *et al.* (2011) 'Effects of heart rate variability biofeedback in subjects with stress-related chronic neck pain: a pilot study', *Applied Psychophysiology and Biofeedback*, 36(2), pp. 71–80. doi:10.1007/s10484-011-9147-0.

Hayano, J. *et al.* (1990) 'Decreased magnitude of heart rate spectral components in coronary artery disease. Its relation to angiographic severity', *Circulation*, 81(4), pp. 1217–1224. doi:10.1161/01.cir.81.4.1217.

Henriques, G. *et al.* (2011) 'Exploring the effectiveness of a computer-based heart rate variability biofeedback program in reducing anxiety in college students', *Applied Psychophysiology and Biofeedback*, 36(2), pp. 101–112. doi:10.1007/s10484-011-9151-4.

Jennings, J.R. *et al.* (1992) 'Alternate cardiovascular baseline assessment techniques: vanilla or resting baseline', *Psychophysiology*, 29(6), pp. 742–750. doi:10.1111/j.1469-8986.1992.tb02052.x.

Karavidas, M.K. *et al.* (2007) 'Preliminary results of an open label study of heart rate variability biofeedback for the treatment of major depression', *Applied Psychophysiology and Biofeedback*, 32(1), pp. 19–30. doi:10.1007/s10484-006-9029-z.

Kim, H.-G. *et al.* (2018) 'Stress and Heart Rate Variability: A Meta-Analysis and Review of the

Literature', *Psychiatry Investigation*, 15(3), pp. 235–245. doi:10.30773/pi.2017.08.17.

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

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

Lalanza, J.F. *et al.* (2023) 'Methods for Heart Rate Variability Biofeedback (HRVB): A Systematic Review and Guidelines', *Applied Psychophysiology and Biofeedback*, 48(3), pp. 275–297. doi:10.1007/s10484-023-09582-6.

Lehrer, P. *et al.* (2009) 'Effects of rhythmical muscle tension at 0.1Hz on cardiovascular resonance and the baroreflex', *Biological Psychology*, 81(1), pp. 24–30. doi:10.1016/j.biopsycho.2009.01.003.

Lehrer, P. *et al.* (2013) 'Protocol for Heart Rate Variability Biofeedback Training', *Biofeedback*, 41, pp. 98–109. doi:10.5298/1081-5937-41.3.08.

Lehrer, P.M. *et al.* (2003) 'Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow', *Psychosomatic Medicine*, 65(5), pp. 796–805. doi:10.1097/01.psy.0000089200.81962.19.

Lehrer, P.M. and Gevirtz, R. (2014) 'Heart rate variability biofeedback: how and why does it work?', *Frontiers in Psychology*, 5, p. 756. doi:10.3389/fpsyg.2014.00756.

Lin, I.M., Tai, L.Y. and Fan, S.Y. (2014) 'Breathing at a rate of 5.5breaths per minute with equal inhalation-to-exhalation ratio increases heart rate variability', *International Journal of Psychophysiology*, 91(3), pp. 206–211. doi:10.1016/j.ijpsycho.2013.12.006.

Lohani, M., Gupta, R. and Srinivasan, N. (2013) 'Cross-Cultural Evaluation of the International Affective Picture System on an Indian Sample', *Psychological Studies*, 58(3), pp. 233–241. doi:10.1007/s12646-013-0196-8.

Martin-Soelch, C. *et al.* (2006) 'Anxiety trait modulates psychophysiological reactions, but not habituation processes related to affective auditory stimuli', *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 61(2), pp. 87–97. doi:10.1016/j.ijpsycho.2005.07.009.

McCraty, R., Atkinson, M. and Tomasino, D. (2003) 'Impact of a workplace stress reduction program on blood pressure and emotional health in hypertensive employees', *Journal of Alternative and Complementary Medicine (New York, N.Y.)*, 9(3), pp. 355–369. doi:10.1089/107555303765551589.

Meule, A. *et al.* (2012) 'Heart rate variability biofeedback reduces food cravings in high food cravers', *Applied Psychophysiology and Biofeedback*, 37(4), pp. 241–251. doi:10.1007/s10484-012-9197-y.

Mussgay, L. *et al.* (2006) 'Effects of heart rate variability biofeedback in reducing blood pressure for the treatment of essential hypertension', *Journal of Psychophysiology*, 20, pp. 118–118.

Noble, D.J. and Hochman, S. (2019) 'Hypothesis: Pulmonary Afferent Activity Patterns During Slow, Deep Breathing Contribute to the Neural Induction of Physiological Relaxation', *Frontiers in Physiology*, 10, p. 1176. doi:10.3389/fphys.2019.01176.

Porges, S. (1992) 'Vagal Tone: A Physiologic Marker of Stress Vulnerability', *Pediatrics*, 90, pp. 498–504. doi:10.1542/peds.90.3.498.

Porges, S.W. (1995) 'Cardiac vagal tone: a physiological index of stress', *Neuroscience and Biobehavioral Reviews*, 19(2), pp. 225–233. doi:10.1016/0149-7634(94)00066-a.

Ribeiro, R.L., Pompéia, S. and Bueno, O.F.A. (2005) 'Comparison of Brazilian and American norms for the International Affective Picture System (IAPS)', *Brazilian Journal of Psychiatry*, 27, pp. 208–215. doi:10.1590/S1516-44462005000300009.

Ritz, T. *et al.* (2005) 'Airways, respiration, and respiratory sinus arrhythmia during picture viewing', *Psychophysiology*, 42(5), pp. 568–578. doi:10.1111/j.1469-8986.2005.00312.x.

Russo, M.A., Santarelli, D.M. and O'Rourke, D. (2017) 'The physiological effects of slow breathing in the healthy human', *Breathe*, 13(4), pp. 298–309. doi:10.1183/20734735.009817.

Salahuddin, L. *et al.* (2007) 'Ultra Short Term Analysis of Heart Rate Variability for Monitoring Mental Stress in Mobile Settings', in *2007 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. *2007 29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, pp. 4656–4659. doi:10.1109/IEMBS.2007.4353378.

Seo, S.-H. and Lee, J.-T. (2010) *Stress and EEG*, *Convergence and Hybrid Information Technologies*. IntechOpen. doi:10.5772/9651.

Shaffer, F. and Ginsberg, J.P. (2017a) 'An Overview of Heart Rate Variability Metrics and Norms', *Frontiers in Public Health*, 5. doi:10.3389/fpubh.2017.00258.

Shaffer, F. and Ginsberg, J.P. (2017b) 'An Overview of Heart Rate Variability Metrics and Norms', *Frontiers in Public Health*, 5, p. 258. doi:10.3389/fpubh.2017.00258.

Shaffer, F. and Meehan, Z.M. (2020a) 'A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback', *Frontiers in Neuroscience*, 14, p. 1055.

doi:10.3389/fnins.2020.570400.

Shaffer, F. and Meehan, Z.M. (2020b) ‘A Practical Guide to Resonance Frequency Assessment for Heart Rate Variability Biofeedback’, *Frontiers in Neuroscience*, 14. doi:10.3389/fnins.2020.570400.

Siepmann, M. *et al.* (2008) ‘A pilot study on the effects of heart rate variability biofeedback in patients with depression and in healthy subjects’, *Applied Psychophysiology and Biofeedback*, 33(4), pp. 195–201. doi:10.1007/s10484-008-9064-z.

Steffen, P.R. *et al.* (2017) ‘The Impact of Resonance Frequency Breathing on Measures of Heart Rate Variability, Blood Pressure, and Mood’, *Frontiers in Public Health*, 5. doi:10.3389/fpubh.2017.00222.

Thayer, J.F. *et al.* (2009) ‘Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health’, *Annals of Behavioral Medicine: A Publication of the Society of Behavioral Medicine*, 37(2), pp. 141–153. doi:10.1007/s12160-009-9101-z.

Vaschillo, E.G. *et al.* (2008a) ‘Heart rate variability response to alcohol, placebo, and emotional picture cue challenges: effects of 0.1-Hz stimulation’, *Psychophysiology*, 45(5), pp. 847–858. doi:10.1111/j.1469-8986.2008.00673.x.

Vaschillo, E.G. *et al.* (2008b) ‘Heart rate variability response to alcohol, placebo, and emotional picture cue challenges: effects of 0.1-Hz stimulation’, *Psychophysiology*, 45(5), pp. 847–858. doi:10.1111/j.1469-8986.2008.00673.x.

Vaschillo, E.G. *et al.* (2008c) *RES 0.1Hz*.

Vaschillo, E.G., Vaschillo, B., Pandina, R.J., *et al.* (2011a) ‘Resonances in the Cardiovascular System Caused by Rhythmical Muscle Tension’, *Psychophysiology*, 48(7), pp. 927–936. doi:10.1111/j.1469-8986.2010.01156.x.

Vaschillo, E.G., Vaschillo, B., Pandina, R.J., *et al.* (2011b) ‘Resonances in the cardiovascular system caused by rhythmical muscle tension’, *Psychophysiology*, 48(7), pp. 927–936. doi:10.1111/j.1469-8986.2010.01156.x.

Vaschillo, E.G., Vaschillo, B., Buckman, J.F., *et al.* (2011a) ‘The Investigation and Clinical Significance of Resonance in the Heart Rate and Vascular Tone Baroreflexes’, in Fred, A., Filipe, J., and Gamboa, H. (eds) *Biomedical Engineering Systems and Technologies*. Berlin, Heidelberg: Springer Berlin Heidelberg (Communications in Computer and Information Science), pp. 224–237. doi:10.1007/978-3-642-18472-7_18.

Vaschillo, E.G., Vaschillo, B., Buckman, J.F., *et al.* (2011b) ‘The Investigation and Clinical Significance of Resonance in the Heart Rate and Vascular Tone Baroreflexes’, in Fred, A.,

Filipe, J., and Gamboa, H. (eds) *Biomedical Engineering Systems and Technologies*. Berlin, Heidelberg: Springer Berlin Heidelberg (Communications in Computer and Information Science), pp. 224–237. doi:10.1007/978-3-642-18472-7_18.

Wiens, S., Sand, A. and Olofsson, J.K. (2011) 'Nonemotional features suppress early and enhance late emotional electrocortical responses to negative pictures', *Biological Psychology*, 86(1), pp. 83–89. doi:10.1016/j.biopsycho.2010.11.001.

Yang, W. *et al.* (2018) 'Affective auditory stimulus database: An expanded version of the International Affective Digitized Sounds (IADS-E)', *Behavior Research Methods*, 50(4), pp. 1415–1429. doi:10.3758/s13428-018-1027-6.