

**The effects of musical tempo and non-invasive  
neuromodulation on autonomic control of the heart**

Beatrice Emily Bretherton

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The candidate confirms that the work submitted is his/her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 2 of this thesis includes work that has been published in a jointly authored publication. Details of the publication include:

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## Abstract

Music is viewed as conferring health benefits, with tempo being the most influential parameter for altering human physiology and psychology. However, this work has used stimuli that manipulate multiple musical parameters at a time. Therefore, this thesis investigated the effects of musical tempo manipulations on cardiovascular autonomic function and subjective responses.

Tempo manipulations comprised of stepped (sudden) increases and decreases in the speed of a simple beat pattern and heart rate variability estimated autonomic balance. Shifts towards parasympathetic predominance occurred for the stepped decrease in tempo stimulus but not for the stepped increase in tempo. When using more musically sophisticated stimuli, greatest vagal tone occurred for the slowest tempo (60bpm) of the stepped decrease in tempo stimulus. Autonomic function did not differ between an experimental (melody and rhythm) and control group (rhythm only). However, the latter experienced greater subjective arousal than the former.

Growing interest in wearable technologies led to the testing of a wearable device that combined relaxation music with transcutaneous vagal nerve stimulation (tVNS). tVNS is a non-invasive neuromodulatory technique that administers small electrical impulses to the outer ear to stimulate the auricular vagus nerve. Both stimuli individually promote shifts towards parasympathetic predominance. It was anticipated that music combined with tVNS would elicit the greatest shifts towards parasympathetic predominance. However, the sham was equally as effective as music only, tVNS only, and their combination at altering autonomic activity. Autonomic responses to all stimuli employed in the thesis were predicted by baseline LF%. These findings suggest that music and wearables may be susceptible to placebo effects.



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## List of Abbreviations

- ABVN – auricular branch of the vagus nerve
- ACTH – adrenocorticotrophic hormone
- ADHD - attention-deficit/hyperactivity disorder
- ANOVA – analysis of variance
- ANS – autonomic nervous system
- AT – altered tempo
- AV – atrioventricular node
- BIM - breathe with interactive music, music-guided breathing device
- BP – blood pressure
- bpm – beats per minute
- BRS – baroreflex sensitivity
- cpm – cycles per minute
- CVRR – coefficient of variance of adjacent interbeat intervals, time-domain heart rate variability parameter
- $\Delta$ RR – range of interbeat intervals (maximum interbeat interval minus minimum interbeat interval)
- DBP – diastolic blood pressure
- ECG – electrocardiogram
- EDA – electrodermal activity
- EEG – electroencephalography
- EMG – electromyography
- ENS – enteric nervous system
- FFT – fast fourier transform
- FPA – finger pulse amplitude
- FPTT – pulse transmission time to finger
- GA – gestational age

GSR – galvanic skin response

HRBM - heart rate-based music

HF – high frequency component of frequency-domain heart rate variability

HF% - percentage of high frequency power relative to low and very low frequency out of total power

HPA – hypothalamic-pituitary-adrenal

HR – heart rate

HRV – heart rate variability

IQR RR – interquartile range (difference between the first and third quartiles) of interbeat intervals

JT interval – difference between the QT interval (see below) and the QRS interval (see below)

KMO - Kaiser-Meyer-Olkin

LF – low frequency component of frequency-domain heart rate variability

LF/HF – ratio of low frequency to higher frequency heart rate variability

LF% - percentage of low frequency power relative to high and very low frequency power out of total power

MAP – mean arterial pressure

Max RR – maximum interbeat interval

Max-Q3 – difference between maximum and third quartile interbeat intervals

MCS – minimally conscious state

Min RR – minimum interbeat interval

MSNA – muscle sympathetic nerve activity

NN50 – number of interbeat intervals that are larger than 50ms, time-domain heart rate variability parameter

nSD1 – normalised short-term variability in interbeat intervals, non-linear heart rate variability parameter

nSD2 – normalised short- and long-term variability in interbeat intervals, non-linear heart rate variability parameter

NTS – nucleus tractus solitarius

nuLF – normalised low frequency component of frequency-domain heart rate variability

nuHF – normalised high frequency component of frequency-domain heart rate variability

nuLF/HF – normalised ratio of low frequency to higher frequency heart rate variability

PNS – parasympathetic nervous system

pRR50 - percentage of the number of interbeat intervals that are larger than 50ms, time-domain heart rate variability parameter

PTSD – post-traumatic stress disorder

Q amplitude/wave – initial negative deflection of a heart beat

QoL – quality of life

QRS interval – duration of the QRS complex, starting at the Q wave (initial negative deflection of a heart beat) and finishing at the S wave (final negative deflection of a heart beat)

QT interval – time taken between the Q wave (initial negative deflection of a heart beat) and the first positive deflection following the QRS complex

QTc interval – the QT interval corrected for heart rate

Q1 RR – first quartile (25<sup>th</sup> percentile) of the interbeat intervals

Q1-min RR – difference between first quartile and minimum interbeat intervals

Q3 RR – third quartile (75<sup>th</sup> percentile) of the interbeat intervals

R wave/amplitude – initial positive deflection of a heart beat

RR – interbeat interval

RMSSD – square root of the mean of the squared differences between adjacent interbeat intervals, time-domain heart rate variability parameter

RSA – respiratory sinus arrhythmia

RVLM – rostral ventrolateral medulla

S – total variability in adjacent interbeat intervals, non-linear heart rate variability parameter

S amplitude/wave – final negative deflection of a heart beat

SA – sinoatrial node

SBP – systolic blood pressure

SCL – skin conductance level

SCR – skin conductance response

SD – standard deviation

SD1 – short-term variability in interbeat intervals, non-linear heart rate variability parameter

SD1/SD2 – ratio of short-term variability to short- and long-term variability in interbeat intervals, non-linear heart rate variability parameter

SD2 – combination of short- and long-term variability in interbeat intervals, non-linear heart rate variability parameter

SD2/SD1 – ratio short- and long-term variability to short-term variability in interbeat intervals, non-linear heart rate variability parameter

SDANN – standard deviation of the average interbeat intervals in all five-minute segments of a 24-hour ECG recording, time-domain heart rate variability parameter

SDRR – standard deviation of normal interbeat (RR) intervals

SDSD – standard deviation of the difference between adjacent interbeat intervals, time-domain heart rate variability parameter

SEM – standard error of the mean

SNS – sympathetic nervous system

SSNA – skin sympathetic nerve activity

STAI - state-trait anxiety inventory

ST height – different in amplitude between the S and T waves

T wave/amplitude – first positive deflection following the QRS complex

TEM – finger temperature

TENS – transcutaneous electrical nerve stimulation

tVNS – transcutaneous vagal nerve stimulation

VAS – visual analogue scale

VLf – very low frequency component of heart rate variability

VNS – vagal nerve stimulation

VS – vegetative state



# **Chapter 1. General introduction: The autonomic nervous system and music**

The human body is highly sensitive to changes in the external environment. Indeed, changes in human physiology can be elicited by a range of different stimuli. Music is one of these stimuli. As well as eliciting changes in human physiology, it is adept at modulating motor activity (Fraisse, 1982), neurochemistry (see Chanda & Levitin, 2013 for a review), cognition (e.g. Schellenberg, Nakata, Hunter & Tamoto, 2007) and affect (Juslin & Sloboda, 2013). The perceived influential power of music is demonstrated by its pervasiveness in a variety of settings, including educational establishments, hospital wards, operating theatres, commercial properties, marketing campaigns, films and television shows and as an aesthetic product in its own right. Music has also been used to negative effect, for instance, in concentration camps (Eckhard, 2001) and detention centres (Chornik, 2013), to repel and/or control teens (Hirsch, 2007) and to compete in the external soundscape (Cloonan & Johnson, 2002). In addition, performing music is associated with physical and mental health issues (Williamon & Thompson, 2006), and music-induced hearing loss (Backus, Clark & Williamon, 2007; Backus & Williamon, 2009).

From this, it is clear that music plays an important role in our lives and culture: people deliberately use music in order to alter emotions, mood, cognitions, behaviour and bodily processes. In addition, music can be used in ethical as well as morally questionable ways. However, music is generally used with good intentions and believed to provide beneficial effects. This anecdotal evidence is beginning to be supported by research investigating the impact of music on the autonomic nervous system.

## **1.1 The autonomic nervous system**

### **1.1.1 The autonomic nervous system**

The autonomic nervous system (ANS) is a complex neural network that is responsible for maintaining homeostasis. The ANS innervates smooth muscle organs and tissues, projecting to viscera including the human heart, lungs, sweat glands, blood vessels, abdominal and pelvic organs, external genitalia and gastrointestinal and urinary tracts (Charkoudian & Wallin, 2014; Porges, 2011). Autonomic reflexes continuously and unceasingly adjust the

body's physiology, and in turn human behaviour, to meet the demands of the changing external world. These adjustments are phasic responses that occur in very short time scales, and can range from adjustments in respiration and blood chemistry, to changes in immune responses and reproductive status. Therefore, the ANS is not just limited to controlling 'emergency' responses which may require large alterations in metabolic and cardiovascular demands. The ANS also plays a crucial role in immune system responses and reproductive behaviours (and their accompanying physiological processes) (Squire et al., 2012). The ANS carries out these adjustments automatically and without conscious input by communicating with the central nervous system, particularly the brainstem and spinal cord (Porges, 2011; Squire et al., 2012). This is evolutionary adaptive as it allows the individual to focus on other functions which require conscious awareness. Impairments in autonomic function can result in the loss of the ability of the ANS to rapidly alter visceral function and homeostasis. This inability to meet the body's physiological demands can have detrimental consequences on health. Indeed, impaired ANS function has been found in numerous conditions, including depression, autism, emotional dysregulation, diabetes, heart failure and Crohn's Disease (Krabs, Enk, Teich & Koelsch, 2015; Porges, 2011).

The ANS has three divisions the: Sympathetic Nervous System (SNS), Parasympathetic Nervous System (PNS) and Enteric Nervous System (ENS).

### **1.1.2 The sympathetic and parasympathetic nervous systems**

The SNS and PNS are considered to act in an antagonistic manner (Porges, 2011; Xhyheri, Manfrini, Mazzolini, Pizzi & Bugiardini, 2012). For instance, the SNS promotes catabolic activity: it mobilises energy resources by increasing metabolic output to prepare an individual for intense muscular and motor action (Porges, 2011). These behaviours are known as 'fight or flight' responses and aim to protect individuals when exposed to external challenges. Activation of the SNS is associated with: increases in heart rate, the force at which the heart contracts and blood pressure; mydriasis (pupil dilation) and skin vasoconstriction; and the inhibition of digestive and

restorative functions which occur following the withdrawal of blood from intestinal organs in order to provide greater availability of oxygenated blood to be transported to the heart, lungs, brain and skeletal muscles (Porges, 2011). In contrast, the PNS increases anabolic activity: it promotes gastrointestinal processes so that the human body can digest food and effectively absorb nutrients. It also facilitates the efficient use and flow of energy between energy stores in the body (Porges, 2011; Squire et al., 2012). These functions also known as 'rest and digest' activities and aim to promote digestive, developmental and restorative functions. Activation of the PNS is associated with decreases: in heart rate, the force at which the heart contracts and blood pressure; miosis (pupil constriction); skin vasodilation and the facilitation of digestive and restorative functions (Porges, 2011, see Figure 1.1 for a summary).

Although the typical responses of the SNS and PNS in emergency situations illustrate the type of processes each control, these examples do not provide an accurate reflection of how the two divisions work in reality when an individual is not faced with threatening stimuli. Rather than one division being 'on' whilst the other is 'off', the SNS and PNS are tonically active. This means that both divisions are constantly active with one being more dominant than the other at a particular point in time. For example, in the absence of external challenges, vagal tone (PNS activity) is dominant. However, when a change in the external environment occurs, vagal tone is withdrawn and SNS activity increases. A change in the external environment does not necessarily need to be big in order to result in shifts in balance between the two sub-divisions. Indeed, subtle, as well as profound changes have been found to attenuate the balance between the sympathetic and parasympathetic nervous systems (Porges, 2011). For instance, a slight increase in ambient temperature can result in increased sympathetic activity and reduced parasympathetic activity. This tonic relationship between the SNS and PNS allows the ANS to finely adjust physiological responses in accordance with cardiovascular, metabolic, digestive, immune system and reproductive demands in order to achieve ideal visceral function and homeostasis.

The SNS and PNS can be distinguished based on their function. But these two divisions can also be differentiated based on their neural characteristics. Indeed, preganglionic neurons of the SNS are located in the thoracic and lumbar spinal cord (sections T1-L3) and its postganglionic neurons innervate the head, thorax, trunk and limbs in addition to viscera. In contrast, preganglionic neurons of the PNS are located in a longitudinal column in the brainstem and sacral spinal cord (sections S2-S4). The preganglionic neurons of the brainstem project to the cerebral aqueduct system which in turn project through: cranial nerves III, VII and IX to target organs in the head; and cranial nerve X (the vagus nerve) to target organs throughout the digestive tract. The preganglionic neurons of the sacral spinal cord exit the ventral roots of the spinal cord to target organs, including the kidneys, bladder, colon and reproductive organs. Therefore, unlike the sympathetic preganglionic neurons, those of the PNS only project to viscera.

Additionally, in comparison to the postganglionic neurons of the SNS which are located distant to the target organs and have long axons, those of the PNS are located near or in the target organs and tend to have shorter axons. Furthermore, although the SNS and PNS use the same preganglionic transmitter (acetylcholine), most SNS postganglionic neurons are catecholaminergic and release norepinephrine, whereas PNS postganglionic neurons are cholinergic and primarily release acetylcholine. These neural differences between the two divisions allow the ANS to have dynamic and finely tuned control over the target organs and ultimately human behaviour.

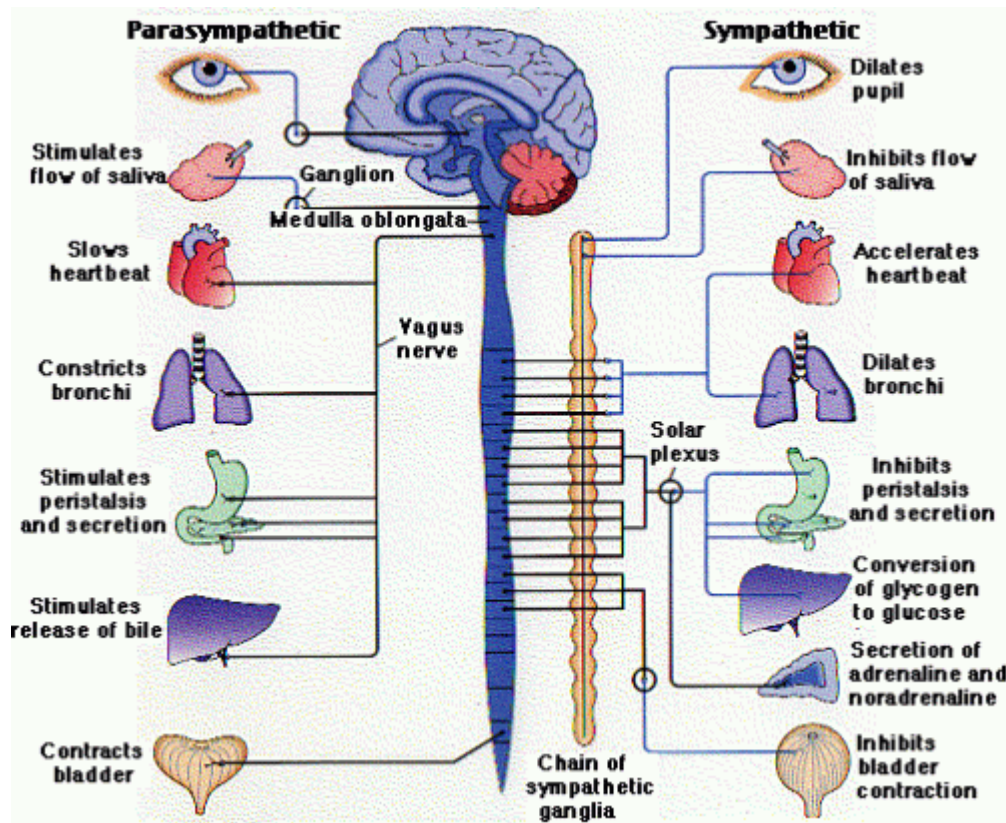


Figure 1.1 Diagram of the SNS and PNS. Image comes from: <https://blog.coturnix.org/2010/08/24/bio101-physiology-regulation-and-control/>

### 1.1.3 Cardiovascular autonomic function

The brain regulates the cardiovascular system via sympathetic and parasympathetic divisions of the ANS (Mitchell, 1953). Activity in the cortex sends information to the hypothalamus which in turn regulates activity of the medulla oblongata, located in the brainstem. The Nucleus Tractus Solitarius (NTS) is one group of nuclei found in the medulla. The NTS is a particularly important network that contributes to autonomic regulation because it has outputs to both sympathetic and parasympathetic (vagal) neurons (Mitchell, 1953). NTS activity regulates sympathetic neurons located in the Rostral Ventrolateral Medulla (RVLM) and parasympathetic neurons in the Dorsal Vagal Nucleus and Nucleus Ambiguus (Mitchell, 1953). Efferent fibres of sympathetic and parasympathetic nerves travel to the heart where they modulate its activity. Parasympathetic fibres innervate the right and left vagus nerves which travel down both sides of the neck (Mitchell, 1953). The right vagus nerve innervates the Sinoatrial (SA) node and the left innervates the atrioventricular (AV) node. The SA node generates the electrical pulses

and the AV node slows the electrical signal sent by the SA node before it passes on to the ventricles. Sympathetic fibres also innervate the SA node and both efferents supply the atria and ventricles (Mitchell, 1953). Due to the dual innervation, it allows the ANS to finely tune cardiovascular responses to changing external stimuli.

The cardiovascular system also includes blood vessels which are only innervated by the SNS. Research by Marshall (1982) demonstrated that sympathetic nerve fibres surrounded arterial vessels, but this was not the case for capillaries or venous vessels. This was determined by investigating the distribution of adrenergic nerves which were implicated in the SNS and innervated smooth muscles, like blood vessels (Marshall, 1982). In addition, Marshall (1982) also revealed that stimulation of sympathetic nerve fibres provoked constriction responses in arterial vessels but not in capillaries or venous vessels. Similar results were reported by McGregor (1965) who showed that stimulation of sympathetic nerve fibres increased perfusion pressure in rats. In addition, this vasoconstrictor response was eliminated following the administration of bretylium or guanethidine (which blocks the release of norepinephrine) which bind to  $\alpha_2$ -adrenergic receptors (negative feedback receptors that prevent the further release of neurotransmitters e.g. norepinephrine). In contrast, administering noradrenalin resulted in significantly greater perfusion pressure compared to values obtained in the absence of drug administration. A similar increase in perfusion pressure during sympathetic nerve stimulation was observed for angiotensin, which is another vasoconstrictor drug. In contrast, parasympathetic blocking drugs, atropine and physostigmine, had no significant effect on sympathetic nerve stimulation, demonstrating that the PNS does not innervate blood vessels. Sympathetic nerve fibres are also responsible for vasodilation. Unlike vasoconstriction which is driven by  $\alpha$ -adrenergic receptors, vasodilation is driven by  $\beta_2$ -adrenergic receptors. These receptors have a strong affinity for binding to epinephrine and facilitate the dilation of blood vessels (Lefkowitz, 1976).

#### **1.1.4 Measuring cardiovascular autonomic function**

Since sympathetic and parasympathetic nerves innervate the heart, and sympathetic nerves innervate blood vessels, an indication of the relative activity in both divisions can be obtained. This can be achieved by undertaking non-invasive and invasive measures.

##### **1.1.4.1 Non-invasive measures**

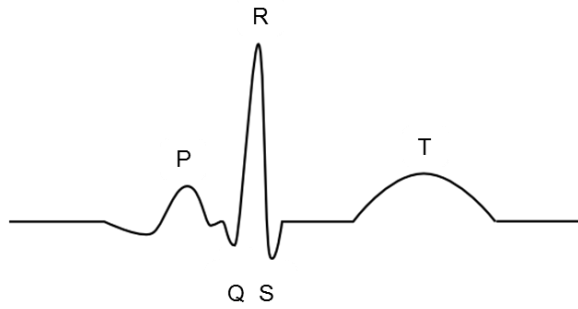
Non-invasive measures of cardiovascular autonomic function indirectly quantify (i.e. provide estimates of) the relative contribution of sympathetic and parasympathetic activity.

###### **1.1.4.1.1 Heart rate**

Heart rate (HR) is quantified as the number of beats per minute (bpm) (Patel et al., 2013) and ranges from 60 to 80bpm in healthy participants (Krabs et al., 2015). As the heart is innervated by sympathetic and vagus nerves, increases in sympathetic input (and/or a withdrawal in vagal tone) leads to increases in HR. In contrast, increases in vagal tone (and/or a withdrawal in sympathetic activity) leads to decreases in HR. Therefore, HR can be used as an indicator of fluctuations in sympathetic and parasympathetic activity.

HR can be measured by an Electrocardiogram (ECG). ECG involves placing microelectrodes on the upper torso and recording the electrical activity of the human heart. The resultant trace facilitates the identification of the PQRST complex (see Figure 1.2). The P wave, which resembles a small initial peak, represents depolarisation of the SA node. The Q wave results from a depolarisation of the interventricular septum, which separates the left and right ventricles, and resembles a small negative deflection. The R wave is a large positive peak in electrical activity and characterises the depolarisation of the ventricles. The last depolarisation of the ventricles is defined as the S wave and can be identified as a small negative deflection following the R wave. The T wave forms the final part of the cardiac cycle and represents repolarisation of the ventricles (Ashley & Niebauer, 2004).





*Figure 1.2: A heartbeat consists of five waves: P, Q, R, S and T waves. It is known as the PQRST complex.*

#### **1.1.4.1.2 Heart rate variability**

HR varies naturally from beat to beat and such variation is associated with good health. This variation is known as heart rate variability (HRV) and is typically derived from the time interval between consecutive R waves (termed RR interval). High levels of variance in RR interval (greater HRV) has been taken to reflect autonomic flexibility and an adaptive capacity for efficient regulation of physiological and psychological responding (Hauschildt, Peters, Mortiz & Jelinek, 2011; Porges, 2011). In contrast, low levels of variance in RR interval (lower HRV) is associated with poorer health because it indicates autonomic rigidity and a limited capacity for regulating physiological and psychological responses relative to the demands of external challenges (Porges, 2011). The variance in RR interval results from shifts in balance between the SNS and PNS (Patel et al., 2013). Evidence for this sympathetic and parasympathetic-mediated control of HRV comes from pharmacological blockade experiments. For instance, Akselrod et al.'s (1981) pioneering study on dogs, showed that blockade of parasympathetic activity with glycopyrrolate eliminated HRV above 0.06Hz. However, sympathetic blockade with propranolol inconsistently reduced HRV around 0.04Hz. In addition, combined parasympathetic and sympathetic blockade completely abolished fluctuations in HR, demonstrating that both sympathetic and parasympathetic activity contribute to variations in RR interval. Akselrod et al. (1985) also showed that parasympathetic blockade with glycopyrrolate increased HR by 139% and reduced fluctuations in HR. Moreover, sympathetic blockade with propranolol decreased HR by 8% and increased HR fluctuations by 50%.

Similar results were also reported by Rimoldi et al. (1990) who applied spectral analysis to recorded RR intervals. They identified two peaks: one ranging from 0.04-0.16 Hz, termed the low frequency (LF) component; and a second ranging from 0.16-0.4Hz, termed the high frequency (HF) component. Administration of atropine raised HR by 31%, and significantly reduced the LF and HF components and their ratio (LF/HF). In addition, following ganglionic blockade with trimethapan (which blocks sympathetic and parasympathetic activity) no spectral components could be identified and variation in RR interval was completely eliminated. Also, stellectomy (the excision of the left and right stellate ganglions which are a collection of sympathetic nerves) abolished the LF component and boosted the HF component.

Using a different paradigm, Pomeranz et al. (1985) manipulated sympathetic outflow by requiring participants to undertake postural changes: sitting in a supine position and standing. Rimoldi et al.'s (1990) spectral analysis of the RR intervals identified two peaks: one between 0.04 and 0.12 Hz, the LF component; and a second between 0.224 and 0.28Hz, the HF component. Prior to drug infusion, standing was associated with increases in the peak within the LF component and decreases in the peak within the HF component. Following atropine administration (which blocks parasympathetic activity) the peak in the HF component was significantly reduced during sitting and standing. Combining atropine with propranolol resulted in no significant change in LF component. In addition, administration of propranolol by itself had no impact on the area of the HF component. In contrast, for the peak in the LF component, propranolol alone reduced the peak of the LF component during standing only whilst atropine alone reduced the peak during both sitting and standing. Combining the two drugs led to further suppression of the LF component band peak.

Other physical activities are associated with increases in HR and hence shifts towards sympathetic predominance, these include postural changes in the form of a tilt, and physical exercise. Indeed, Furlan et al. (1993) reported increases in a LF component (centred around 0.1Hz) during a tilt task (changing from a supine position to a 90° upright position). In addition, one hour following exercise was associated with heightened HR and an enlarged

HF component. Mental tasks have also been implicated as altering sympathetic and parasympathetic activity. For instance, Bernardi et al. (2000) revealed that silent reading decreased mean RR interval and increased power in a LF component (ranging from 0.03 to 0.14Hz). Furthermore, mental arithmetic significantly reduced mean RR interval and a HF component ( $> 0.15\text{Hz}$ ). Therefore, these findings not only suggest that parasympathetic and sympathetic contribute to variations in HR, but they can also be indirectly measured by using spectral analysis of a sequence of RR intervals. This is known as frequency-domain HRV.

Frequency-domain analysis is typically used for short-term recordings (Camm et al., 1996). It involves producing a tachogram, which plots the difference in time between each RR interval, and applying spectral density analysis. This splits the tachogram into different frequency components which are considered to represent specific physiological functions. In a five-minute recording, the spectral density analysis identifies four distinct frequency components. Table 1.1 provides a summary of the four components and the autonomic functions they are considered to represent. It is also possible to derive a fifth component, but only from 24-hour recordings (Camm et al., 1996). The function of this frequency component is still under debate (Xhyheri et al., 2012).

Quantification of the variance in RR interval can also be achieved by performing time-domain analysis. Time-domain HRV is typically conducted with long-term recordings taken over 24 hours (Camm et al., 1996). The most widely used HRV parameters for time-domain analysis are detailed in Table 1.1. These parameters take the RR interval as their starting point and apply different formulas of dispersion. For instance, standard deviation and root mean square. In response to growing interest in HRV, Camm et al. (1996) provide guidelines for identifying abnormal HRV parameters and are as follows:  $\text{SDNN} < 70\text{ms}$ ;  $\text{SDANN} < 50\text{ms}$ ;  $\text{RMSSD} < 15\text{ms}$ ; and  $\text{pNN50} < 0.75\%$ . Since HRV is commonly used in clinical settings as an indicator of health status, these guidelines enable medical professionals to help diagnose physiological diseases; to determine the risk of a patient experiencing serious complications and to predict prognosis (Camm et al., 1996). In addition, time-domain HRV is considered to be a reliable method

for identifying individuals at risk of developing respiratory, cardiovascular, neurological and developmental diseases (Porges, 2011). Indeed, low parameters have been found to be associated with diabetes, hypertension, coronary artery disease and sudden death (Camm et al., 1996).

*Table 1.1: Frequency- and time-domain HRV parameters.*

	Parameter	Units	Definition <sup>1, 2, 3, 4, 5</sup>
Freq- domain HRV	Total power	$\mu\text{s}^2$	Frequency band: 0.00 – 0.4Hz. Represents overall variability.
	VLF power	$\mu\text{s}^2$	Frequency band: 0.00 - 0.04Hz. Hypothesised to reflect thermoregulatory functions, but little conclusive evidence (Xhyheri et al., 2012).
	LF power	$\mu\text{s}^2$	Frequency band: 0.04 to 0.15Hz. Previously considered to represent sympathetic activity. Based on research by Malliani, Pagani, Lombardi and Cerutti, (1991), Kamath and Fallen (1993), Rimoldi et al. (1990) and Montano et al. (1994) who found that it had a similar oscillation frequency to that of Mayer waves (oscillations in arterial blood pressure) and correlated with the LF component of systolic blood pressure variability. Present understanding states it is a combination of sympathetic and parasympathetic activity.
	HF power	$\mu\text{s}^2$	Frequency band: 0.15 to 0.4Hz. Represents parasympathetic (vagal) activity.
	LF/HF	-	Ratio of LF to HF power. Initially considered to represent sympathovagal balance, but disputed (Billman, 2013).
Time- domain HRV	SDRR	ms	Standard deviation (SD) of all normal RR intervals. Represents overall variability
	SDANN	ms	SD of the average RR intervals in all five-minute segments of a 24-hour ECG recording. Represents long-term variability.
	CVRR	-	The coefficient of variance of adjacent RR intervals
	SDSD	ms	SD of the difference between adjacent RR intervals
	RMSSD	ms	The square root of the mean of the squared differences between adjacent RR intervals. Represents short-term variability.
	NN50	Count	The number of pairs of adjacent RR intervals that differ by more than 50ms.
	pRR50	%	The percentage of the number of RR intervals that are larger than 50ms

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<sup>1</sup> Bilchick and Berger (2006)

<sup>2</sup> Camm et al. (1996)

<sup>3</sup> Stein, Bosner, Kleiger and Conger (1994)

<sup>4</sup> Xhyheri et al. (2012)

<sup>5</sup> However, Reyes Del Paso, Langewitz, Mulder, Roon and Duschek (2013) argue all frequency-domain HRV components are under vagal control.

The frequency- and time-domain parameters are strongly correlated with each other. Table 1.2 provides a summary of the correlations between the two types.

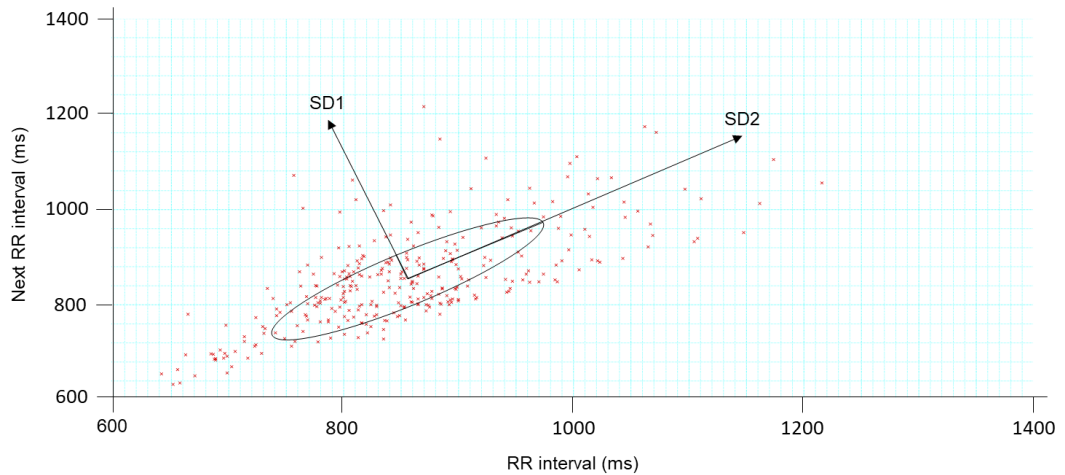
*Table 1.2: Correlations between the frequency- and time-domain HRV parameters.*

<b>Frequency-domain parameter</b>	<b>Time-domain parameter</b>
Total power	SDRR
ULF power	SDANN
HF power	SDSD
HF power	RMSSD
HF power	NN50
HF power	pRR50

A third measure of HRV concerns non-linear analysis. Non-linear HRV measures are relatively new and a less common measure for investigating the changes in sympathetic and parasympathetic activity. Non-linear analysis entails generating a scatterplot which plots current cardiac cycle length (current RR interval) against the subsequent RR interval. This is called a Poincaré plot with each point representing two successive heart beats (Guzik et al., 2007). It generates a visual representation of the temporal correlations between the RR intervals (Georgieva-Tsaneva, Gospodinova & Gospodinova, 2014). Analysis of the plot involves fitting an ellipse with its centre point of the markings (Tulppo, Mäkikallio, Seppänen, Laukkanen & Huikuri, 1998). In healthy volunteers, the Poincaré plot typically resembles a comet shape, whereby a long RR interval is followed by another long RR interval (Woo et al., 1992).

From the Poincaré plot it is possible to derive quantitative measures. These include SD1 and SD2. SD2 is the standard deviation of the projection of the Poincaré plot on the line of identity (the length of the ellipse, see Figure 1.3). Similar to LF power, the physiological meaning of SD2 is less well-defined than SD1 (Mourot et al., 2004). It correlates with SDRR (overall variability), LF power and HF power so is thought to represent both long- and short-term

variations in HR (Guzik et al., 2007). SD1 is the standard deviation of projection of the Poincaré plot on the line perpendicular to the line of identity (width of the ellipse, see Figure 1.3). It is interpreted to be a short-term measure of HRV and correlates with HF power (Kamen, Krum & Tonkin, 1996; Guzik et al., 2007).



*Figure 1.3: Example Poincaré plot. Each data point plots the current RR interval against the following RR interval. SD1 is the width of the ellipse and reflects vagal activity. SD2 is the length of the ellipse and reflects overall variability.*

From SD1 and SD2, additional measures can be derived, including those presented in Table 1.3.

Table 1.3: Non-linear HRV parameters.

Measure	Calculation	Interpretation
SD1	$\sqrt{\frac{1}{2}[SDRR(\Delta RR)]^2}$ <p>Where:</p> $\Delta RR = RR_n - RR_{n+1}$	Short-term variability, analogous to HF power.
SD2	$\sqrt{2[SDRR(RR)]^2 - \frac{1}{2}[SD(\Delta RR)]^2}$	Combination of short- and long-term variability.
nSD1	$\left(\frac{SD1}{RR\ interval}\right) \times 1000$	Absolute SD1 value normalised relative to average interval.
nSD2	$\left(\frac{SD2}{RR\ interval}\right) \times 1000$	Absolute SD2 value normalised relative to RR interval.
S	$\pi \times SD1 \times SD2$	Total area of the ellipse. Represents total variability in the RR signal (Georgieva-Tsaneva et al., 2014).
SD2/SD1	$SD2/SD1$	Balance between long- and short-term variations in HR. Analogous to LF/HF (Guzik et al., 2007).
SD1/SD2	$SD1/SD2$	Balance between short- and long-term variations in HR.

#### 1.1.4.1.3 Blood pressure

Blood pressure (BP) is conceptualised as the amount of cardiac force exerted on blood vessels in the body when blood is being pumped around the body (Charkoudian & Wallin, 2014; Patel et al., 2013). It is typically expressed as two types: systolic (SBP) which refers to the pressure in arteries when the heart contracts; and diastolic (DBP) which refers to the pressure in arteries between heart beats (Charkoudian & Wallin, 2014; Patel et al., 2013). Mean arterial pressure can also be calculated from systolic and



diastolic blood pressures. This measure provides an indication of the average pressure in the arteries during a single cardiac cycle. A healthy resting blood pressure in adults is approximately 120/80mmHg (Babbs, 2012). As mentioned above, sympathetic fibres innervate blood vessels (mainly arterial vessels) and control vasoconstriction and vasodilation. Vasoconstriction is associated with increases in blood pressure due to the narrowing of blood vessels. In contrast, vasodilation is associated with decreases in blood pressure due to the widening of blood vessels. Therefore, the measurement of SBP and DBP can provide an indication of fluctuations in sympathetic activity.

Although blood vessels receive sympathetic innervation, parasympathetic input also plays an important role. For instance, parasympathetic innervation of the salivary glands provokes vasodilation and increases in salivary secretion. Evidence for this comes from Izumi and Karita (1994) who demonstrated that stimulation of the vagus nerve in cats prompted increases in saliva and blood flow in the submandibular gland, which is one of the major salivary glands located in the floor of the mouth. Interestingly, these effects were abolished when the chorda tympani nerve was sectioned. This nerve contains parasympathetic fibres that extend from the facial nerve to the lower lip (Izumi, 1995). In a follow-up study, Takahashi, Izumi and Karita (1995) showed that stimulation of the chorda tympani, lingual (branch of the trigeminal nerve involved in facial sensations and motor functions), glossopharyngeal (also contains parasympathetic fibres that project to the lower lip, (Izumi, 1995)) and vagus nerves in cats also elicited increases in salivation and vasodilator responses in the parotid gland (major salivary gland in the mouth). Therefore, this suggests that parasympathetic vasodilator fibres exist in the lower lip of cats.

In a more recent study, it also transpires that these fibres may also exist in rats. Indeed, Mizuta, Karita and Izumi (2000) showed that stimulation of the lingual nerve and chorda-lingual nerve in rats was associated with increases in blood flow in the submandibular gland. The cutting of the chorda tympani resulted in no change in blood flow in the submandibular gland following stimulation of the lingual nerve. Therefore, although these results demonstrate that parasympathetic nerve fibres are not directly involved in

blood vessel regulation, parasympathetic reflex processes do impact glandular blood flow in both cats and rats. As sympathetic blockade with phentolamine and propranolol failed to have any significant impact on salivary secretion and blood flow in the submandibular gland (Izumi & Karita, 1994), it appears that sympathetic activity exerts little impact on glandular blood flow and salivary secretion (Proctor & Carpenter, 2007). However, since this research was conducted in cats, it still remains unclear whether these findings also apply to humans.

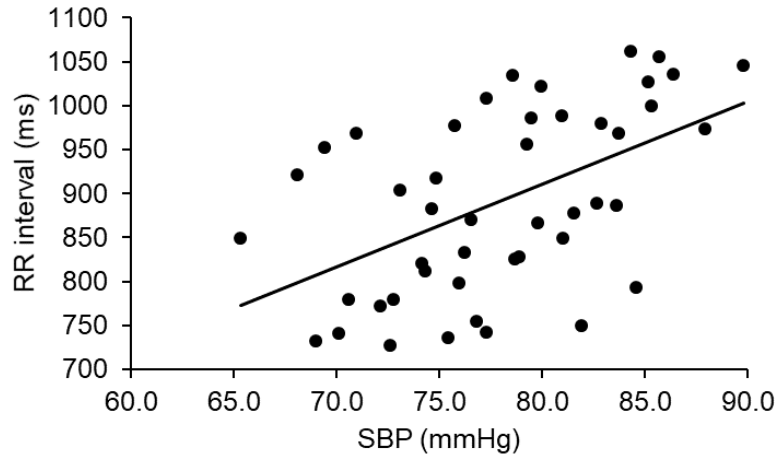
#### **1.1.4.1.4 Baroreflex sensitivity**

Blood pressure is regulated by a range of neural and vascular mechanisms. These include baroreceptors which detect changes in blood pressure and relay information to the NTS. When baroreceptors detect increases in blood pressure, this leads to increased activity of baroreceptor NTS neurons and the subsequent inhibition of RVLM sympathetic neurons. This then causes decreases in sympathetic, cardiac stimulation, vasoconstriction and decreases in blood pressure. Therefore, NTS baroreceptor neurons track increases and decreases in blood pressure which occur in each cardiac cycle in order to beneficially regulate blood pressure in the arteries. This negative feedback system is known as the arterial baroreflex and functions to control blood pressure (Charkoudian & Wallin, 2014). The responsiveness of the arterial baroreflex to fluctuations in blood pressure is known as baroreflex sensitivity (BRS) (Patel et al., 2013). Higher baroreflex sensitivity represents greater vascular and neural responsiveness to changes in blood pressure, whereas lower baroreflex sensitivity means less vascular and neural responsiveness to changes in blood pressure (Charkoudian & Wallin, 2014). Two non-invasive techniques for quantifying spontaneous BRS include the spectral method and the sequence method.

Spectral BRS is derived from applying spectral density analysis to continuous blood pressure and heart rate recordings. It involves detecting SBP peaks in the waveform, as well as R peaks in an ECG trace, and applying FFT to the RR interval/SBP trace. BRS can then be computed by calculating the square root of the ratio of RR interval over SBP power, otherwise known as the  $\alpha$  coefficient (Robbe et al., 1987). The technique

focuses on two frequency regions: 0.01Hz, which is known as LF  $\alpha$  BRS; and 0.2-0.3Hz, which is known as HF  $\alpha$  BRS (Parati, di Rienzo & Mancia, 2000). Normal LF and HF  $\alpha$  BRS values tend to be  $\geq 20.00$ . Spectral spontaneous BRS relies on the ECG and blood pressure traces displaying high coherence, where oscillations in both variables are linearly related (as RR interval increases so does SBP). Therefore, in individuals who experience ectopic heart beats, or who have attenuated BP variability, it is difficult to derive spectral BRS.

The sequence method is characterised by detecting a sequence of three or more beats during which there is either: a progressive increase in SBP accompanied with a lengthening of RR intervals; or a progressive decrease in SBP accompanied with the shortening in RR interval (Parati et al., 1988; see Figure 1.4). This asymmetry in the baroreceptor response is well-known and illustrates the dynamic and monitoring nature of arterial baroreceptors and the ANS (La Rovere, Pinna & Raczak, 2008). Identifying valid sequences involves strict criteria. For example, sequences must be at least three beats in length and have consecutive SBP and RR interval changes  $\geq 1$ mmHg (Parati et al., 1988) and 2ms (Laude, Baudrie & Elghozi, 2009), respectively. There is great debate in the literature concerning these criteria. Some researchers argue for stricter thresholds (e.g. four beats or more and a change in SBP and RR interval of 1mmHg and 5ms respectively, Parati et al., 2000) whereas others argue for criteria that minimise the risk of missing true positives (three beats or more and a change in SBP and RR interval of 1mmHg and 2ms respectively, Laude et al., 2009). Nonetheless, the BRS values are obtained by calculating the slope of the regression line between SBP and RR interval changes (Parati et al., 1988, see Figure 1.4). This includes the average of: 'up' sequences only (progressively increasing SBP and lengthening RR interval, termed up BRS); 'down' sequences only (progressively decreasing SBP and shortening RR interval, termed down BRS) and the average of all (up and down) sequences (termed mean BRS). Therefore, unlike the spectral technique, the sequence technique facilitates an assessment of baroreceptor stimulation and inhibition to be made (Parati et al., 2000).



*Figure 1.4: Example up BRS regression line: as SBP increases, RR intervals become longer. Slope of the line: 40.07 ms/mmHg.*

#### **1.1.4.1.5 Respiration**

Unlike heart rate and blood pressure which are products of neural interactions between the SNS and PNS, respiration is a motor function (Squire et al., 2012). Neural networks in the medulla oblongata generate respiratory motor output. The rhythm is relayed to premotor and interneurons which transform the rhythm into a mechanically efficient respiratory pattern. These respiratory motor-neurons include spinal motor-neurons which control diaphragm and rib cage muscle activity, and in turn the rate of pumping of the lungs; and cranial motor-neurons which adjust the tongue, glottis, trachea and bronchi during each respiratory cycle to alter resistance in the airways (Squire et al., 2012). The regulation of oxygen and carbon dioxide in arterial blood is one of the main goals of homeostasis and therefore requires constant measurement. This constant measurement is achieved by chemoreceptors located in arteries and mechanoreceptors found in the airways. Appropriate ventilation also requires the respiratory pattern to be adjusted in response to human behaviours, such as posture, physical activity levels and cardiovascular output.

Respiration is coordinated with the cardiovascular system. For instance, the respiratory and cardiac pumps must work in tandem to ensure that the required levels of oxygen and carbon dioxide are delivered throughout the body. This may involve increasing the oscillating rhythm of respiratory motor output when low levels of oxygen are detected by chemoreceptors. In turn,

this leads to increases in HR and BP, and elevations in oxygen (Squire et al., 2012). This is because diaphragm movement (during inhalation and exhalation) alters intrapleural pressure (the pressure in the pleura<sup>6</sup>) in order to create a pressure gradient for the air outside to move into the lungs (during inhalation), and for the air in the lungs to move out (during exhalation). This in turn alters the aortic pressure. As pressure detectors are located in the aorta and are sensitive to transmural (and therefore intrapleural pressure) they activate the baroreflex which adjusts HR to compensate for these respiratory-induced changes in intrapleural pressure (Sayers, 1973).

Therefore, respiratory oscillations can be identified in RR interval sequences, frequency-domain HRV and BP. In RR interval sequences, inhalation is accompanied with a shortening of RR intervals (accelerating HR), and exhalation is accompanied with the lengthening of RR intervals (decelerating HR). This coupling between respiration and HR follows an inverted U-shaped curve and is known as respiratory sinus arrhythmia (RSA). Frequency-domain HRV also has a large respiratory influence. The HF component of HRV centres around respiratory frequency (Hirsch & Bishop, 1981) and can therefore be modulated by changes in respiration rate, tidal volume and static lung volume (Hirsch & Bishop, 1981). Indeed, respiration rates lower than 10 breaths per minute can result in convergence between the frequency components in the HRV spectrum (Bilchick & Berger, 2006; Camm et al., 1996). Therefore, due to the influence respiration has on modulating HR, HRV and BP, it is crucial to capture respiratory variables when measuring cardiovascular autonomic function. Furthermore, findings from studies which neither measure nor control for respiration rate must be carefully considered.

Respiration can be measured using a pneumograph. A pneumograph is a tube placed around the upper torso that continuously measures chest movement as individuals inhale and exhale. From the recording of

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<sup>6</sup> Pleura is a large, thin sheet of membrane located around the outside of the lungs. It also lines the inside of the chest cavity. There is a small space between the two layers of the pleura which filled with fluid. Therefore, intrapleural pressure refers to the pressure in the fluid between the layers of pleura.

respiration, it is possible to derive respiration rate which is conceptualised as the number of breaths per minute and occasionally as cycles per minute (which is synonymous with the former). As well as respiration rate, numerous other respiratory parameters can be derived. These include: tidal volume, which refers to the total amount of air that enters the lungs during inhalation or exhalation; inspiratory reserve volume, which is the maximal volume of air than can be inhaled after normal tidal inhalation; expiratory reserve, which refers to the maximal volume of air that can be exhaled after normal tidal exhalation; residual volume, which is the volume of gas remaining in the lungs following maximal exhalation; and vital capacity, which refers to the volume change that occurs between maximal inspiration and expiration (AARC clinical practice guideline, 2001).

#### **1.1.4.2 Invasive measures**

Unlike indirect measures of cardiovascular autonomic function, invasive measures directly measure ANS activity. Although there is no method to measure parasympathetic activity, a validated technique exists to measure sympathetic nerve activity. This is known as microneurography and involves inserting microelectrode needles into a sympathetic nerve.

##### **1.1.4.2.1 Microneurography**

The microneurography technique was first pioneered in the late 1960s by Hagbarth and Vallbo (1968) who inserted tungsten microelectrodes into the peroneal nerve and recorded efferent post-ganglionic sympathetic nerve impulses from multiple sympathetic nerve fibres. This technique was subsequently refined by Macefield, Wallin and Vallbo (1994) who demonstrated that the impulse behaviour of single units can be obtained. These single units were classified as sympathetic vasoconstrictor efferents due to their occurrence in diastole and their firing of one per cardiac cycle. Due to these advances in single-unit recording it is now possible to analyse the firing characteristics of these sympathetic neurons in terms of the number of impulses generated per cardiac cycle and per minute (Grassi et al., 1992; Lambert, Hering, Schlaich and Lambert, 2012). In addition, when combined with non-invasive measures of cardiovascular autonomic function, the data obtained from the single-unit recording technique can provide a

more comprehensive account of how sympathetic activity is influenced by external stimuli and is implicated in various health conditions.

## **1.2 The effects of music on cardiovascular autonomic function**

Music is a multidimensional auditory stimulus and defined as organised sound whose purpose is to elicit an aesthetic response (Kellaris & Kent, 1993). It is multidimensional in nature because it is the product of three objective dimensions: time, pitch and texture (Bruner, 1990). These three major dimensions are fundamental to all other musical parameters, including tempo, meter and rhythm (parameters of the time dimension); melody, harmony and tonality (parameters of the pitch dimension); and timbre, articulation and dynamics (parameters of the texture dimension).

However, parallels between music and noise can be drawn. For instance, noise can have tempo, meter, rhythm, timbre, articulation and dynamics. Nevertheless, research looking at the impact of music on autonomic activity suggests otherwise. That is, music provokes responses that are unique to itself and distinct from noise. Support for this claim comes from research by Sakamoto, Hayashi, Sugiura and Tsujikawa (2002) who investigated the effect of steady noise, fluctuating noise and music on blood pressure. Thirty-five healthy females were exposed to seven auditory stimuli: a steady noise that had a wide octave band; classical music; popular music; an arrangement made for medical and health use, and fluctuating noises that were synchronised with each musical piece. A five-minute baseline period was obtained prior to participants listening to the first 130 seconds of each stimulus. Significant effects on participants occurred. This included increases from baseline in SBP and DBP for the classical and popular music stimuli but not for the fluctuating noise stimuli. These findings demonstrate that music provokes physiological responses that are distinct from those elicited by noises.

Further support for the argument that music is distinguishable from sound comes from Gomez and Danuser (2004) who compared physiological responses to environmental sounds and music. The study involved recruiting

a sample of 31 healthy participants (16 males) and presenting them with 16 environmental sounds and 16 musical fragments each lasting 30 seconds in duration. The environmental sound stimuli included excerpts of bird song, sea waves crashing, water drops, a pneumatic hammer, waterfall, a flying airplane along with other sounds. The musical stimuli were all instrumental and included many well-known pieces such as Holst's *The Planets*, Dvorak's *Symphony No.9*, Elgar's *Enigmatic Variations* and Ravel's *Piano Concerto in G major*. Participants were asked to rate how aroused and positive they felt after each stimulus whilst respiration rate, skin conductance level<sup>7</sup> (SCL) and HR were continuously recorded. The data were analysed by exploring the relationships between the self-report and physiological responses for the environmental sounds and musical stimuli.

For the sounds, inspiratory time, expiratory time and SCL were unrelated to subjective arousal and valence. In contrast, inspiratory time and expiratory time were negatively associated with subjective arousal and valence for the musical stimuli. Also, SCL was positively correlated with subjective arousal. This suggests that rate of inhalation and expiration and SCL were impacted by the musical stimuli but not the sound stimuli. In addition, HR for the sound stimuli was positively associated with subjective arousal whereas no association between the two variables occurred for the musical stimuli. Therefore, the results not only suggest that listeners differentially respond to music and environmental sounds, but they also imply that music has unique characteristics that are responsible for these changes.

### **1.2.1 Autonomic effects of music are determined by its musical structure**

One of the first papers published examining the impact of music on cardiovascular and respiratory function was by Ellis and Brighthouse (1952). This study aimed to explore the impact of music on HR and respiration rate. Thirty-six students (18 males) were exposed to four minutes of three famous

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<sup>7</sup> Also known as electrodermal activity (EDA) or Galvanic Skin Response (GSR). Measures the electrical changes that occur at the skin's surface. Based on the premise the skin's conductance increases with shifts towards sympathetic predominance. Due to an increase in sweat secreted by pores in the skin SCL refers to the raw level of electrical conductance of the skin. Considered to represent a tonic (slowly changing) response.



musical pieces: Hall's Blue Interval; Debussy's Prelude to the Afternoon Faun; and Liszt's Hungarian Rhapsody No. 2. To ascertain whether the music modulated HR and respiration, participants underwent a baseline condition prior to hearing the pieces, and a recovery condition, after hearing the pieces, both lasting four minutes in duration. HR and respiration were recorded throughout and derived for each minute of the conditions.

The results revealed that respiration rate was significantly higher during the musical stimuli compared to the baseline period, except for the Debussy piece during the first minute, which had a similar respiration rate to that of baseline. HR was similar for all time points when compared to baseline for all stimuli except Liszt's Hungarian Rhapsody at the second minute of the piece. At this point in the Hungarian Rhapsody, HR was significantly higher compared to that during the baseline period. As a result, these findings demonstrate that the stimuli employed here significantly impacted respiration rate and to a lesser extent HR. In addition, they also provide an indication of how these measures vary over the duration of a musical stimulus. That is, autonomic activity during a musical stimulus varies depending on the interactions between the musical parameters.

Ellis and Brighouse (1952) clearly showed that the structure of music significantly impacts autonomic function. Further evidence in support of this finding also comes from Bernardi et al. (2009) who found that changes in autonomic activity patterns are closely related to specific musical characteristics. In this study, continuous measures of heart rate, blood pressure, skin vasomotion, mid-cerebral artery flow and respiration were obtained whilst 24 participants (15 males) listened to five contrasting pieces of music. The authors then examined whether the cardiovascular and respiratory measures were significantly different from baseline measures at any point in the musical pieces. Also, unlike Ellis and Brighouse (1952) who provided no possible reasons for the observed changes in respiration rate and HR during the musical stimuli, Bernardi et al. (2009) aimed to determine which musical parameters alterations were responsible for the observed changes in cardiovascular autonomic function.

The authors found that alterations in BP and HR were closely related to changes in musical dynamics. In particular, crescendos were accompanied with a decrease in skin vasomotion and an increase in SBP and DBP. HR also appeared to be responsive to changes in the music envelope: changes in dynamics coincided with greater variation in HR. Interestingly, the impact of sound intensity on autonomic activity not only manifests in adults, but also in fetuses. For instance, Kisilevsky, Hains, Jacquet, Granier-Deferre and Lecanuet (2004) found that fetuses at 28-32 weeks gestational age (GA) showed no change in HR over the duration of a five-minute recording of Brahms' Lullaby played at four different intensities (95, 100, 105 or 110 dBa). In contrast, fetuses between 33 and 36 weeks gestational age and term fetuses showed accelerations in HR, suggesting that the fetuses from 33 weeks GA attend to changes in the auditory environment.

Findings by Mikutta et al. (2013) demonstrate that the fluctuations in the sound intensity of a musical stimuli interact with other musical parameters. The study involved exposing 20 students to two recorded performances of Chopin's Tristesse which were played in two contrasting musical interpretations. In the first interpretation, the piece was played in a strict metric shape that had few rhythmic alterations and sparse phrasing of the melody. In contrast, the second interpretation had greater variations in the metric shape which resulted in greater rhythmic alterations, intense melodic phrasing and suspenseful changes in the music that accentuated rhythmically and melodically important parts in the piece. Therefore, the first interpretation was more restricting and less expressive than the second. Prior to listening to the two different versions, participants rested during a 10-minute baseline condition. They were then presented with the two interpretations which were interspaced with a two-minute resting period to allow physiological measures to stabilise. Unlike Bernardi et al. (2009) who obtained a range of continuous measures of cardiovascular autonomic function, Mikutta et al. (2013) concentrated on two only: HR and respiration. To ascertain how changes in sympathetic and parasympathetic activity contributed to the fluctuations in HR, LF, HF and LF/HF were also analysed. HR positively correlated with sound intensity for both interpretations; however, the strength of the correlation was higher for the second

interpretation than the first. This difference in strength was considered a result of differences in the direction of the correlation at specific moments in the two pieces. For instance, a positive correlation between sound intensity and HR persisted throughout the piece during the second interpretation. This was the same for the first interpretation but the relationship became negative during the middle section of the piece. Combined with the findings of Bernardi et al.'s (2009) study, these results demonstrate that HR is sensitive to the expressive qualities of a musical stimulus.

Similar to Ellis and Brighthouse (1952), Mikutta et al. (2013) also found that HR and respiration rate were significantly higher during both interpretations than during baseline. This implies that regardless of the nature of the music, listening to musical stimuli provoke increases in sympathetic predominance. However, as HR and respiration rate were significantly higher during the first interpretation than in the second, this could be due to the greater levels of emotional expressivity that occurred in the first interpretation. However, LF, HF and LF/HF did not significantly differ between the two interpretations. As a result, no information pertaining to the relative change in sympathetic and parasympathetic activity that was responsible for the change in HR was generated. Nevertheless, Mikutta et al. (2013), as well as Nakahara et al.'s (2009) findings support the claim that musical expressiveness impacts autonomic activity.

Adopting a different paradigm, Nakahara et al. (2009) asked 13 classical pianists to perform 35 bars of Bach's Well-Tempered Clavier under two different performance requirements. This constituted: an expressive performance, which involved playing the excerpt with emotion; and a non-expressive performance, which required the pianists to perform the excerpt devoid of emotion. Nakahara et al. (2009) then asked the pianists to listen to the two different performances. HR was continuously recorded and total power, LF power, HF power and LF/HF derived.

Interestingly, the expressive performance was associated with significantly higher HR than the non-expressive performance and perception and the expressive perception. In addition, LF/HF was highest and HF power lowest during the expressive performance. As a result, this illustrates two points.

Firstly, consistent with the results of Bernardi et al. (2009) and Mikutta et al. (2013), HR was highly sensitive to the changes in musical expressivity. Secondly, the results postulate that the increase in HR was due to a withdrawal in vagal tone, as opposed to an increase in sympathetic activity. Therefore, this reinforces the claim that performing music, as well as listening to music, can have anxiolytic effects. Further support for the beneficial effects of performing music comes from a study by Vickhoff et al. (2013).

This comprised of case studies in addition to a group study. The group study required 11 healthy 18-year olds to undertake three singing tasks as a choir. Each singing task was five minutes in duration and consisted of humming and singing a hymn and mantra. A five-minute baseline preceded the first singing task, which involved reading an emotionally neutral text, and the same five-minute baseline finished the study. The three tasks had contrasting levels of temporal structure. Unlike humming which had no temporal structure, the mantra had a two-bar structure and the hymn had a four-bar structure that was indicated by melody, pauses and breathing instructions. HR was recorded throughout and RMSSD and LF/HF derived.

Analysis of the data revealed that unlike the hymn and mantra, humming was not associated with an increase in RMSSD when compared to baseline. However, it did induce regular HR fluctuations despite these not being consistent between participants. In contrast, the mantra elicited the highest RMSSD values and was associated with the greatest amount of HR fluctuations and coherence in HRV at the 0.1Hz frequency. These results also emerged when exploring the effects of the three tasks on five individual participants. However, they also discovered that respiration and HR accelerated and decelerated in unison, providing evidence of an entrainment effect between singers. In addition, RSA (the coherence between HRV and respiration) was strongest for the hymn and mantra compared to any other condition. This suggests that singing not only boosts HRV, but it also enhances vagal tone. Therefore, it appears that parasympathetic activity can be enhanced by the structural characteristics of music, as well as its expressive qualities.

In addition to these qualities, Pérez-Lloret et al. (2014) demonstrated that musical styles can also impact cardiovascular autonomic function. For instance, Pérez-Lloret et al. (2014) exposed 28 participants to three relaxing musical genres and a period of a silence on one testing occasion. The three styles of relaxing music which were used included: classical, which consisted of The Blue Danube by Johann Strauss; new age, which was represented by Enya's Only Time; and Romantic which constituted The Day You Will Love Me by Carlos Gardel and Alfredo La Pera. The silence and stimuli were 3.5 minutes in duration. HR was recorded throughout and the following HRV variables derived: mean RR interval, total power, VLF power, LF power, HF power and their percentage values, scaling component  $\alpha_1$  (non-linear analysis which quantifies short-term variation in RR) and sample entropy (also a non-linear measure which quantifies the regularity and complexity of an RR interval signal).

Although total power, VLF power, LF power and HR did not significantly differ between the silence and three musical stimuli, HF power was significantly lower during the new age stimulus compared to the silence. In contrast, HF power during the classical and romantic stimuli was similar to HF power during silence. LF/HF was significantly higher during the new age stimulus compared to silence, but was significantly lower during the classical and romantic stimuli compared to silence. Finally, sample entropy for the new age stimulus was significantly lower compared to silence. Therefore, it appears that the new age stimulus was associated with a shift towards sympathetic predominance, whilst the classical and romantic stimuli had little impact on autonomic activity. Interestingly, similar patterns emerged in a more recent study by Kume et al. (2017) who investigated the impact of relaxation music on autonomic activity. After presenting participants with environmental music (ambient music combined with natural sounds, e.g. birdsong, bubbling creeks and a weak breeze) and silence, no significant differences in LF power, HF power or LF/HF emerged (between music and silence). However, HR significantly decreased during the music compared to the silence. Therefore, although it may appear that relaxation music has little effect on autonomic activity, when compared to more arousing music (e.g. New Age music), relaxation music is associated with decreases in

sympathetic activity (da Silva et al., 2014) and/or increases in vagal tone (Iwanaga, Kobayashi & Kawasaki, 2005; van der Zwaag, Westerink & van den Broek, 2011).

Despite these findings, there is also evidence to suggest that mode impacts cardiac autonomic activity. For instance, Proverbio et al. (2015) demonstrated that atonal and tonal music induce different physiological responses. This involved presenting a sample of 50 participants (25 males) with either three tonal or three atonal musical excerpts. Each musical excerpt was one-minute in duration and presented three times at different stages during the testing session and HR and BP were continuously recorded throughout. Analysis of the data revealed that HR was significantly lower during the atonal excerpts compared to the tonal excerpts. But SBP and DBP were significantly higher during the atonal excerpts than during the tonal excerpts.

At first sight, the findings seem to demonstrate that physiological differences between tonal and atonal music exist. But this view is called into question given the somewhat contradictory HR and BP findings. Indeed, seeing that HR was significantly lower during the atonal excerpt, it would have been expected that SBP and DBP would also be significantly lower during this excerpt. However, this was not observed. Moreover, due to a lack of HRV parameters, it is unclear how the two excerpts modulated sympathetic and parasympathetic activity. A possible reason accounting for the contrasting HR and BP responses may be the repeated exposure of each stimulus. This is something to bear in mind given that repeated exposure to a musical stimulus has been found to influence autonomic function (Iwanaga et al., 2005).

Nevertheless, happy music (which is characterised by a major mode) has been found to be associated with significantly greater BP and skin conductance responses (SCR<sup>8</sup>) (Khalifa, Roy, Rainville, Bella & Peretz, 2008) than sad music (which is characterised by a minor mode). In addition, Gomez and Danuser (2007) identified significant quadratic relationships

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<sup>8</sup> Refers to abrupt changes in the conductance of the skin. Visual characteristics of these changes include a sudden peak followed by a slow decay in the signal. Considered to reflect a phasic (rapidly changing) response.

between mode and: inspiratory time, expiratory time, minute ventilation and HR. Therefore, inspiratory and expiratory times were shortest for very clear examples of major and minor modes. Also, minute ventilation and HR were highest for excerpts in a major mode. Similarly, Etzel, Johnsen, Dickerson, Tranel and Adolphs (2006) showed that HR and respiration decelerated during sad excerpts, whilst respiration quickened during happy excerpts. Taken together these results demonstrate that mode significantly effects autonomic activity. More specifically, major modes (or happy sounding pieces) are associated with increases in HR and respiration rate and minor modes (or sad-sounding pieces) are associated with decreases in HR and respiration rate.

Other musical characteristics have also been found to impact temporal breathing pattern. For instance, Sakaguchi and Aiba (2016) investigated how breath timing is associated with the events and internal characteristics of a musical performance. This involved recoding the breathing of 15 professional and amateur pianists whilst they played 10 different excerpts. These ranged in complexity and included: four-octave C major scale, a piano exercise by Hanon, Mozart's Sonata Pathétique and Debussy's Clair de Lune. The order of the excerpts was fixed and commenced with a 200-second resting period. Respiration increased when the music was played faster. In addition, expiration occurred 1 second after the onset of the first note; the pianists were more likely to inhale during rest notes, particularly for Mozart's Piano Sonata No. 16, K.545, 2nd movement and expiration was more likely to occur during slurs than inspiration. Therefore, these results suggest that low-level musical features, such as slurs and pauses, also induce significant changes in respiratory patterns.

Table 1.4 provides a summary of the physiological correlates of changes in a number of musical parameters.

Table 1.4: Studies investigating the impact of music on cardiovascular autonomic function.

Paper	Outcomes																
	HR	SBP	DBP	HF power	LF/HF	RMSSD	RSA	Sample entropy	RRTi	SD2	SCR	SCL	Respiration rate	Minute ventilation	Inspiratory time	Expiratory time	Musical parameter
Ellis and Brighthouse (1952)	↑												↑				Unknown
Bernardi et al. (2009)	↑	↑	↑														Crescendos
Kisilevsky et al. (2004)	↑																Intensity
Mikutta et al. (2013)	↑																Intensity & expressiveness
Nakahara et al. (2009)	↑			↓	↑												Expressiveness
Vickhoff et al. (2013)						↑	↑										Slow singing - tempo?
Pérez-Lloret et al. (2014)				New Age: ↓	New Age: ↑			New Age: ↓									Genre
Kume et al. (2017)	↓																Genre
da Silva et al. (2014)									Heavy metal: ↓	Heavy metal: ↓							Genre
Iwanaga et al. (2005)	Excitative: ↑	Excitative: ↑	Excitative: ↑										Excitative: ↑				Genre
van der Zwaag et al. (2011)					↓												Tempo
Proverbio et al. (2015)	Atonal: ↓	Atonal: ↑	Atonal: ↑														Percussiveness (timbre)
Khalfa et al. (2008)		Major: ↑	Major: ↑														Mode
Gomez and Danuser (2008)	Major: ↑													Major: ↑	Major & minor: ↓	Major & minor: ↓	Mode
Etzel et al. (2006)	Minor: ↓												Minor: ↓				Mode
Sakaguchi et al. (2016)													↑		Rests	Slurs	Tempo



### 1.2.2 Music-guided breathing

The sensitivity of respiration to changing musical parameters has been drawn upon by a number of labs who have investigated the impact of music-guided breathing on cardiovascular autonomic activity. As hypertension and diabetes are associated with elevated sympathetic activity, HR and BP, and slow-breathing has been shown to reduce BP, devices that use music to induce slow, deep breathing have been tested.

One of these devices is called RESPeRATE. It consists of a belt that is positioned on the upper torso which expands with inspiration and contracts with expiration. This respiration belt is connected to a box which generates musical patterns which in turn are presented to users via ear phones. The device interactively guides the user towards adopting slow breathing by continuously monitoring breathing and generating music based on inspiration and expiration time to which the user is required to synchronise. The device is pre-programmed to slow respiration rate to  $< 10$  breaths/min in sessions that are 15 minutes in duration.

The effectiveness of this device in reducing BP in hypertensives has been tested in a number of studies. Most studies demonstrate that utilisation of the device, typically over an eight-week period, results in decreases in SBP, DBP and MAP (Elliott et al., 2004; Meles et al., 2004; Schein et al., 2009; Viskoper et al., 2003). However, one study detected no significant change in SBP, DBP or HR between those who used the device and those who did not (Logtenberg et al., 2007). In addition, there are greater inconsistencies in the impacts of this device on HR. For instance, Meles et al. (2004), Schien et al. (2009) and Viskoper et al. (2003) reported no significant change in HR. In contrast, Grossman et al. (2001) found that use of the Breathe with Interactive Music (BIM) device was associated with decreases in HR significantly.

Another device similar to RESPeRATE has also received interest, particularly when treating hypertensive samples. The BIM device also consists of a respiration belt positioned on the upper abdomen which is connected to a control unit that sends music-like sound sequences to the user via ear-phones. The auditory sequences are composed in real-time,

therefore enabling the generation of a sound pattern tailored to the user's breathing pattern. The music-like patterns are slightly offset to encourage the user to have a prolonged expiration. Sessions normally last for 10 minutes.

Testing of the BIM device shows positive results. For instance, Schein et al. (2001) found that SBP, DBP and MAP were significantly lower post-intervention compared to baseline in an intervention group (using the BIM for 10 minutes once/day for eight weeks) and an active control group (listening to synthesised music that had a non-identifiable rhythm on a Walkman for the same duration). In addition, there were significantly greater reductions in DBP and MAP in the intervention compared to the control group and post-intervention respiration rate was low: 8.4 breaths/min. Similar results have also been reported by Grossman et al. (2001). Indeed, this group of researchers found that reductions in office SBP and MAP were significantly greater in the intervention group compared to the control (synthesised music with a non-identifiable rhythm played on a Walkman). For home measures, there was a significantly greater reduction in DBP, MAP and HR in the intervention group compared to the control. In addition, average respiration rate for the intervention group was 7.1 breaths/min. An interesting relationship between pre- and post-intervention BP emerged: those with higher pre-intervention BP in the intervention showed larger reductions whilst those in the control group showed larger increases. Together these findings imply that music-guided breathing does reduce BP and that it may be possible to identify individuals who show more pronounced responses.

It is plausible that the observed effects for the RESPeRATE and BIM were enhanced by the fact that these are professional-looking devices (see Chapters 4 and 5 for a discussion). However, the beneficial effects observed were also replicated in a study which avoided using devices. Modesti et al. (2010) randomly allocated participants with essential hypertension to one of four groups: intervention group which performed the Buteyko and pranayama breathing technique whilst listening to music (which had a slow rhythm and tempo); slow music control group (same slow music in the absence of slow breathing); reading control group (reading a book or magazine in the absence of slow breathing). All participants were required to

undertake these sessions for 30 minutes/day, three hours after lunch for a period of six months. Follow-up visits occurred at one week and one, three and six months. An additional follow-up for the intervention group only was scheduled for six months after the end of the treatment. The Buteyko and pranayama breathing technique was taught by a practitioner and involved synchronising respiration to a slow musical rhythm (between four and six breaths/min) and maintaining the same rate while undertaking abdominal breathing where expiration was twice as long as that of inspiration. Training sessions with the therapist occurred one week and one, three and six months after commencement of the treatment. Twenty-four hour BP was measured along with day-time, night-time and office BP and HR, antihypertensive treatment and Quality of Life (QoL).

Analysis of the results showed that 24-hour and night-time SBP significantly decreased in the intervention group and the reductions observed in both groups were significantly larger in the intervention group compared to the control groups. DBP, HR, antihypertensive treatment and QoL did not significantly change. However, general positive affect was found to be significantly associated with low treatment efficacy. Meaning that those who had a less positive outlook responded better to the intervention than those who had a more positive like outlook. Similar results were found in a later study by Modesti, Ferrari, Bazzini and Boddi (2015) who adopted a similar study procedure but incorporated additional measures of autonomic activity (RR interval, nuLF, nuHF, LF/HF, renal resistive index<sup>9</sup>, and spectral and sequence BRS) and scheduled the follow-up visits at one, four and eight week intervals.

In this more recent study, Modesti et al. (2015) showed that 24-hour SBP, DBP and renal resistive index were significantly lower upon completion compared to baseline for the intervention group only. These reductions were apparent from the first week onwards. Spectral and sequence BRS were also significantly enhanced at the two-month follow-up visit compared to

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<sup>9</sup> Renal resistive index is derived by Doppler ultrasound and is a hemodynamic index. In Modesti et al.'s (2010) study the ultrasound was applied to the left and right kidneys (as high blood pressure can damage the kidneys). It is derived by the following formula:

$$\frac{\text{peak systolic velocity} - \text{end diastolic velocity}}{\text{peak systolic velocity}}$$

baseline. Also, mean RR was significantly longer after the first week and progressively increased over the duration of the study. nuHF was significantly higher after the first week and further increased at one month. As a significant decrease in nuLF was also observed after two months, LF/HF emerged as being significantly lower at the one-month follow-up visit compared to baseline. Overall, these results demonstrate that tailoring breathing to slow music, even in the absence of a medical device, can have profound benefits for individuals with hypertension.

Table 1.5 below provides a summary of the studies discussed above and their outcomes.

*Table 1.5: Studies investigating the impact of music-guided breathing on human physiology. ↑ = significantly higher compared to control; ↓ = significantly lower compared to control; - = no significant difference compared to control.*

Paper	Device	SBP	DBP	MAP	HR	RR interval	nuLF	nuHF	LF/HF	BRS	Respiration rate	Renal resistive index
Elliott et al. (2004)	RESPeRATE	↓										
Grossman et al. (2001)	BIM	↓	↓	↓	↓							
Logtenberg et al. (2007)	RESPeRATE	-	-	-	-						-	
Meles et al. (2004)	RESPeRATE	↓	↓	-	-						↓	
Modesti et al. (2010)	Buteyko and pranayama breathing and slow music	↓	-									
Modesti et al. (2015)	Buteyko and pranayama breathing and slow music	↓	↓			↑	↓	↑	↓	↑		↓
Schein et al. (2001)	BIM	-	↓	↓	-							
Schein et al. (2009)	RESPeRATE	↓	↓		-							
Viskoper et al. (2003)	RESPeRATE	↓	↓		-							

### 1.2.3 Music aids anxiety and stress to promote relaxation

The psychological underpinning of anxiety rests in the emotional experience of fear. Moreover, anxiety is physiologically characterised by attenuated HRV, a withdrawal of vagal tone and increases in HR, BP and sympathetic activation (Guzetta, 1989). As music has been found to boost HRV and vagal tone in healthy volunteers, one would anticipate music to confer beneficial effects for individuals experiencing anxiety. Indeed, research investigating the beneficial effects of music for individuals experiencing heightened anxiety suggests that this is the case.

For instance, Wu, Huang, Lee, Wang and Shih (2017) compared the physiological effects of exposing participants to soothing music to a usual care control in a group of patients undergoing craniotomy. As this procedure is accomplished whilst patients remain awake, it can provoke a great deal of anxiety leading to physiological abnormalities during the procedure. Consequently, there is high demand for using alternative therapies, such as music, to help to relieve patient anxiety. The study involved randomly allocating participants to either a music or usual care group. Those in the music group chose their most preferred track out of an experimenter-selected set of six while in the waiting room. They then listened to the music while in the operating theatre. In contrast, the usual care group received no music whilst in the operating theatre. Lower state anxiety in the music group emerged along with lower HR, SBP, DBP and respiration rate. Together these findings demonstrate that in this group of patients, soothing music was effective at relieving both psychological and the physiological correlates of anxiety.

The anxiety-relieving effects of music have also been observed in patients waiting for cardiac catheterisation (Hamel, 2011). In this study patients waiting to be taken for cardiac catheterisation were randomly allocated to one of two groups: a music intervention group, which received 20 minutes of Halpern's Trance-Zendance; or a control group, which sat in silence while waiting for their procedure. HR, BP and state anxiety were obtained immediately before and after the condition. Analysis of the data revealed that state anxiety (as measured by the state sub-scale of the State-Trait Anxiety Inventory (STAI)) decreased to a significantly greater extent in the music intervention group compared to the control group. In addition, HR and SBP significantly decreased between the two time periods in the music intervention group, yet increased in the control. Taken together these results illustrate that even in a different group of patients, music helps relieve the subjective and physiological characteristics of anxiety in this patient group.

Similar results have also been replicated in additional patient groups including those undergoing: cross-section with stripping of the great saphenous vein (Jiménez- Jiménez, García-Escalona, Martín-López, de Vera-Vera & de Haro, 2013), anaesthesia (Bae, Lim, Hur & Lee, 2014),

mechanical ventilation (Lee, Chung, Chan & Chan, 2005), chemotherapy (Lin, Hsieh, Hsu, Fetzer & Hsu, 2011) and colonoscopy (Smolen, Topp & Singer, 2002); and in patients with: myocardial infarction (White, 1992), cerebrovascular disease and dementia (Okada et al., 2009), coronary heart disease (Gupta & Gupta, 2015) and Leukaemia (Nguyen, Nilsson, Hellstrom & Bengtson, 2010). However, mixed results have been reported in patients undergoing: angiography or percutaneous coronary intervention (Bally, Campbell, Chesnick & Tranmer, 2003; Buffum et al., 2006; Nilsson, 2009), percutaneous transluminal coronary angioplasty (Forooghy, Tabrizi, Hajizadeh & Pishgoo, 2015), cardiac computed tomography (Ng et al., 2016) and coronary artery bypass and/or valve replacement surgery (Sendelbach, Halm, Doran, Miller & Gaillard, 2006). Growing interest in the effect of music in dental anxiety has also been observed. For instance, playing music during dental procedures has been associated with significantly lower salivary cortisol, SBP, DBP, HR and body temperature compared to undergoing the procedure in silence (Mejía-Rubalcava, Alanís-Tavira, Mendieta-Zerón & Sánchez-Pérez, 2015). Consequently, music appears to have profound anxiolytic effects in some patient samples who are prone to experiencing high levels of anxiety.

Like anxiety, stress can also have negative impacts on physiological well-being, particularly if it is experienced over long periods of time. The hypothalamic-pituitary-adrenal (HPA) axis is one of the most prominent stress systems in the human body (Linnemann, Strahler & Nater, 2016). The HPA axis is a feedback mechanism between the hypothalamus, pituitary gland and adrenal cortex which regulates stress responses and more crucially the release of cortisol. Cortisol is secreted by the adrenal cortex, which is stimulated by the release of adrenocorticotrophic hormone (ACTH) by the pituitary gland. Stimulation of pituitary gland requires input from the hypothalamus which releases corticotrophin releasing hormone. As the hypothalamus contains cells which can contribute to sympathetic nerve activity via connections to the spinal cord and medulla oblongata (Mitchell, 1953), HPA axis output also influences activity in the ANS. For instance, increases in cortisol during stress result in increases in glucose and the mobilisation of energy resources to prepare for fight or flight. In turn, this

leads to the suppression of bodily processes that are energy demanding, such as immune-system responses (Krout, 2007; Linnemann et al., 2016). An additional marker of stress is salivary alpha-amylase. Unlike cortisol which is a HPA axis stress marker, salivary amylase is an ANS stress marker (Nater & Rohleder, 2009). This is based on results from animal studies which found that sympathetic and parasympathetic stimulation increased the concentration of salivary alpha-amylase. In addition, in human studies salivary alpha-amylase was negatively correlated with RMSSD and positively correlated with LF/HF, RSA and SCL (see Nater & Rohleder, 2009 for a review). Therefore, if music can reduce stress, and particularly salivary cortisol and alpha-amylase, this should lead to both positive psychological and physiological states.

In studies which experimentally-induce stress, music appears to significantly aid in stress recovery. For instance, Labbé, Schmidt, Babin and Pharr (2007) administered a challenging test to 56 participants. The test contained 80 simple mathematical calculations and 16 challenging operations that were to be completed in 45 seconds. In addition, there were eight number memory items (required participants to memorise single digits in 9-10 strings); 12 verbal analogies and the spelling of 14 difficult words. They were then exposed to one of four conditions: self-selected music, classical music, heavy metal music or silence. HR, respiration and SCR were continuously recorded along with subjective state anxiety, anger and relaxation.

HR during the self-selected music significantly decreased post-stress, whilst no change occurred for participants who listened to the classical and heavy metal music or those exposed to silence. However, participants listening to classical and heavy metal music exhibited significantly lower respiration rates than those in silence or who listened to self-selected music. In addition, SCR significantly lowered in all conditions (self-selected, classical, heavy metal and silence). For the subjective measures, state anxiety decreased for self-selected and classical music; relaxation increased for all conditions except heavy metal music; and anger decreased for silence and self-selected music. Taking the physiological and subjective results together, it is evident that heavy metal music confers little benefit to listeners following a stressor. In contrast, self-selected music, classical music and silence were

effective at boosting recovery from stress. This finding corroborated those from an earlier study (Burns et al., 2002).

Indeed, following the completion of the mental rotations task and exposure to silence or heavy metal, classical or self-selected music, HR was significantly lower in the classical music group compared to those who sat in silence or listened to self-selected music. In addition, state anxiety was significantly lower for those who were exposed to silence, classical music and self-selected music, but not heavy metal music. Also, relaxation significantly decreased following exposure to all conditions except heavy metal music. Consequently, these findings reinforce the notion that classical and self-selected music confer physiological and psychological benefits following a stressor. However, as these studies investigated the acute effects of music on stress and stress is often a chronic condition (developing and persisting over long durations), Helsing, Västfjäll, Bjälkebring, Juslin and Hartig (2016) conducted a more ecologically valid investigation by looking at the chronic effects of music on stress.

This involved running two groups: a control group, who were instructed to relax for 30 minutes/day for three weeks; and an experimental group, who listened to their own preferred music every day for two weeks. For one week, the experimental group chose relaxing music and in the other week they listened to energising music. They also had a baseline week, which constituted relaxing for 30 minutes every day. Participants completed a questionnaire that measured self-reported stress, emotions and health every day. In addition, salivary cortisol was measured two consecutive days every week. The results showed that when participants in the experimental group listened to music, they experienced more intense positive emotions and less stress compared to baseline. But, there was no difference between the relaxing and energising music, suggesting that type of music did not matter. However, there was a tendency for cortisol to be lower during the second week compared to the baseline week, implying that the relaxing music may have reduced physiological stress to a greater extent.

The failure to detect a statistically significant decrease in cortisol as a result of the intervention is consistent with what was reported by Linnemann et al.



(2016). Indeed, these researchers found that listening to music for 30 minutes five times a day for thirty days did not facilitate decreases in salivary cortisol or salivary alpha-amylase. However, they did show that salivary cortisol and alpha-amylase significantly decreased when participants listened to music in the presence of other people. As a result, this suggests that the social aspects of listening to music can further enhance the stress-relieving effects of music listening.

In contrast to the previous research which induced stress via a cognitive task, Sokhadze (2007) elicited stress by employing aversive stimuli, which had previously been validated to provoke disgust responses. After exposure to the stimuli, 29 participants were then presented with pleasant music, sad music and white noise in three consecutive sessions. The stimuli were successful in modulating autonomic activity, due to significant decreases in HR, LF/HF, peak blood flow, blood flow velocity and increases in HF power and SCR. But, while listening to pleasant music HF power and SCR decreased whilst peak blood flow and blood flow velocity increased. In contrast, listening to the sad music led to increases in HR and peak blood flow, and exposure to the white noise significantly reduced peak blood flow. Interestingly, some of these effects persisted post-auditory stimulation. For instance, peak blood flow and blood flow velocity remained significantly higher and SCR significantly lower compared to post-stress induction for the pleasant music. In addition, the post-sad music was associated with significantly higher blood flow velocity and significantly lower SCR compared to the stress-induction in addition to the increase in HR and peak blood flow. Finally, for the white noise stimulus, peak blood flow remained significantly higher post-stimulus compared to stress-induction together with the decrease in SCR. As both pleasant and sad music appear to facilitate recovery from stress, perhaps other musical parameters are more effective at promoting stress recovery.

In response, Nakajima, Tanaka, Mima and Izumi (2016) explored the effect of modulating different frequencies of musical stimuli on stress. Different to the previous stress-related experiments, Nakajima et al. (2016) exposed participants to a 90-second stressful noise (scratching sound). They then presented them with three, 224-second versions of the third movement of

Mozart's Horn Concerto No. 2 in E-flat major. One was the original version, one amplified the high-frequency components of the excerpt and the third amplified the low-frequency components. The stressful noise followed 90-seconds of white noise and the order of the musical stimuli were counterbalanced between participants. HR was continuously recorded throughout and nuLF, nuHF and LF/HF were derived for all stimuli. Nakajima et al. (2016) derived a 'stress recovery ratio' which involved subtracting values for the stress noise from the white noise and dividing it by the value obtained by subtracting stress noise from the music stimulus. The high-frequency modulated stimulus had a significantly greater stress recovery ratio than the low-frequency version for nuHF. However, neither nuLF nor LF/HF significantly differed between the stimuli. As no change in sympathetic predominance occurred, these results suggest that the high-frequency component of music plays a greater role in relieving auditory-induced stress than the low-frequency components of sound.

Performing music, especially in front of an audience, is a stressful undertaking. As a result, research looking at the effects of music performance on autonomic activity has been performed. This work has reported significant changes in the predominance of sympathetic and parasympathetic activity. For instance, in a study comparing the autonomic differences between a low-stress (rehearsal) and high-stress (competition) situation, Yoshie, Kudo and Ohtsuki (2009) showed that HR and sweat rate were significantly higher during the competition compared to the rehearsal. In addition, Fancourt, Aufegger and Williamon (2015) revealed that low-stress singing (in the absence of an audience) was associated with significantly lower levels of cortisol than high-stress singing (in front of an audience). More recently, Chanwimalueang et al. (2017) also demonstrated that high-stress performance (auditioning in front of an audience) was associated with significant decreases in the structural complexity of HRV compared to a low-stress condition (performing in the absence of an audience).

As a result, these findings imply that performing in high-stress conditions lead to changes in autonomic function, characterised by shifts towards sympathetic predominance. This postulation was supported by Nakahara et

al. (2009) who showed that music performance was associated with significantly higher LF/HF compared to listening to the same performance. But despite significant decreases in SDRR between low- and high-stress conditions, LF power, HF power and LF/HF also decreased demonstrating that there are some inconsistencies in the effect of music performance-induced stress on autonomic function. Indeed, this may be due to numerous factors, including stimuli used, sample characteristics and type of stress-inducing task.

#### **1.2.4 Music and disorders of consciousness**

As well as being a form of relaxation, music is also being used as a prognostic indicator in patients with disorders of consciousness. Disorders of consciousness are characterised by extensive psychological loss and deprivations in communication, sensory interpretation and behavioural responding, as a result of altered states of consciousness (Owen, 2008). Disorders of consciousness include: comas, vegetative states, minimally conscious states and sedation. A coma is a state in which individuals demonstrate no awareness of the self or their environment. They are also unresponsive to internal and external stimuli and normally lie supine with their eyes constantly closed (Puggina & da Silva, 2014). In contrast, vegetative states (VS) refer to comatose patients who spontaneously open their eyes but who continue to remain unaware of themselves as well as their environment (Puggina & Silva, 2015). Increased levels of awareness compared to those exhibited by patients in a coma and VS are shown by those who are in a minimally conscious state (MCS). This sub-category is characterised by a self-awareness and awareness of their surroundings which still remains limited and problems in communicating with other individuals (Puggina & Silva, 2015). The final sub-category, sedation, tends to be drug-induced and can be a result of psychoticism and/or a pathophysiological condition. It is typically presented as greater awareness of the self and environment compared to the other three sub-categories, with the patient also showing enhanced communication abilities (Puggina & Silva, 2015). Clinically differentiating between these sub-categories of disorders of consciousness is no easy task. This is mainly due to the lack of objective

measures in quantifying the observed behaviours of patients (Puggina & Silva, 2015). Due to this reliance on behavioural measures, diagnostic errors are often made. In addition, it reinforces the need to develop other, less subjective measures that can more reliably detect the subtle differences between the types of disorders of consciousness, as well as the recovery process.

Since patient groups, as well as healthy individuals, show autonomic sensitivity to musical stimuli, there is growing interest in the effectiveness of using music as a diagnostic tool. Indeed, research with this group of patients is beginning to show that music can provide reliable information that can be used when diagnosing individuals with these conditions. Some of this initial evidence came from pilot work by Aldridge, Gustorff and Hannich (1990). In this study five patients with severe coma were exposed to live improvised music while HR and respiration were recorded, along with electroencephalography (EEG) and body movements. Although the data were not quantitatively analysed, it was observed that respiration rates became slower and deeper; HR decreased over the duration of the session; body movements augmented (e.g. grabbing hand movements, turning the head and opening the eyes); and EEG changed from theta activity to alpha or beta activity. As no statistical analysis was performed and no control was employed, the diagnostic power of music remained unclear. Fortunately, more recent research has employed more robust methodologies.

For instance, in a case study Jones, Hux, Morton and Anderson and Knepper (1994) presented a 16-year old comatose patient with six different conditions: rock n roll music (personal favourite); classical music (least preferred musical style); natural sounds; a personal message from family and friends; and silence in the form of baseline and recovery conditions. HR and respiration rate were recorded throughout in addition to body movements and facial expressions. Unlike Aldridge et al. (1990) who examined the acute effects of music on comatose patients, Jones et al. (1994) looked at the chronic effects. This was achieved by presenting the patient with the stimuli over a two-week period. Data analysis revealed that HR and respiration rate were significantly lower during the rock n roll stimulus compared to baseline, and HR and respiration rate were

significantly higher during the personal message and natural sounds compared to baseline. Interestingly, HR and respiration rate were not significantly different during classical music compared to baseline. However, body movements increased during the personal message only, and no significant differences emerged in the number of facial expressions detected. As a consequence, this study highlights the influential nature patient preferred music has on eliciting responses in individuals with comas.

Beneficial effects of music have also been reported in VS patients. For example, Riganello, Candelieri, Quintieri, Conforti and Dolce (2010) conducted a cross-over study which involved exposing participants to pieces by: Boccherini, Tchaikovsky, Mussorgsky and Grieg as well as a baseline (silence) condition. Two of the stimuli were presented on one day, with a baseline period coming before music presentation. To ensure autonomic changes had fully washed out, a 20-minute rest period was inserted between the two trials. In contrast, for the control group of healthy participants, all music stimuli were presented in one day. HR was continuously recorded throughout and nuLF and LF/HF derived. The healthy controls also provided subjective ratings concerning the emotions the musical stimuli represented.

nuLF power was significantly different between the two groups for the Grieg (happy-sounding) and Mussorgsky (sad-sounding) pieces: healthy controls had significantly higher nuLF power than patients. A significant difference in nuLF power in patients between Boccherini (lowest musical complexity) and Mussorgsky (highest musical complexity) also emerged: nuLF power was significantly lower for the Mussorgsky piece compared to the Boccherini piece. Additionally, sample entropy was significantly different between the two groups for the Mussorgsky piece only: healthy controls had significantly higher values than patients. In addition, there was a significant difference in sample entropy in patients only between the Boccherini and Mussorgsky pieces: sample entropy was significantly lower for the Mussorgsky piece than for the Boccherini. Although differences between the two groups emerged, the results illustrate that VS patients differentially respond to different musical pieces. As a result, this suggests that even with limited awareness of themselves and their environment, VS patients are able to respond to music with different affective qualities. As changes in many

musical characteristics have been shown to be associated with fluctuations in autonomic activity, perhaps the differential responses of VS patients are also a result of the detection of changing musical characteristics.

Findings from O'Kelly et al. (2013) not also support those of Riganello et al. (2010), but they show that MCS patients also respond to different musical stimuli. This study involved presenting VS and MCS patient groups and healthy controls with a five-minute (silence) baseline period followed by four different stimuli: live performance of a preferred song; improvised vocal melody incorporating the patient's name; recordings of disliked music and recordings of white noise. A two-minute washout (silence) period was interspaced between the individual stimuli. HR, respiration and EEG were recorded in both groups, and body movements, vocalisations and blink rate were recorded for patients only. In healthy controls, EEG power (globally and within alpha, beta, theta and delta bandwidths) and respiration rates were significantly higher for the live performance of a preferred song compared to baseline and the other stimuli. There were no statistically significant differences between any of the conditions for HR and HRV parameters. As there was heterogeneity in patient responses, the two patient groups were analysed separately and case studies were performed on three patients.

In VS patients, frontal and frontal midline theta activity were significantly higher for the live performance of a preferred song compared to baseline. No differences between baseline and the stimuli emerged in HR, HRV, respiration, body movements, head movements and vocalisations. However, blink rate was significantly higher in the live performance of a preferred song compared to baseline. In MCS patients, frontal alpha activity was significantly higher for the live performance of a preferred song compared to baseline. This was also the case for frontal and frontal midline theta activity. Similar to the group of VS patients, there were no significant differences between the conditions in HR, HRV, respiration, body movements, vocalisations or blink rate.

Exploration of the data at the individual level revealed different responses to the stimuli. For instance, in one VS patient, alpha and beta activity, LF power

and blink rate were significantly higher, and RMSSD and HF power significantly lower during the live performance of a preferred song. Whereas, in another VS patient, EEG power was significantly higher for the white noise compared to baseline and the other stimuli in all bandwidths except for: frontal and frontal midline theta activity which was significantly higher for the personal message; and beta activity which was significantly higher for the live performance of a preferred song. This patient also exhibited significantly lower HR for all stimuli compared to baseline as well as heightened RMSSD for the live performance of a preferred song and attenuated RMSSD for the disliked music. The number of head movements was significantly lower for the personal message and disliked music compared to baseline. Taken together, O'Kelly et al.'s (2013) results suggest that at the group level responsiveness in two groups of patients is boosted for the live performance of a preferred song. But, as there is heterogeneity in responses between patients, it is also crucial to consider individual-level changes in autonomic (and brain) activity in order to generate the best possible account of prognosis and recovery progress. Indeed, this between-subject variation may account for the mixed results that have been reported in patients diagnosed with coma (Puggina & Silva, 2015), VS (Wilson, Cranny & Andrews, 1992; Puggina & Silva, 2015) and sedation (Puggina & Silva, 2015).

The following Table 1.6 provides a summary of the studies discussed above investigating the impact of music in diagnosing and treating patients with disorders of consciousness.

Table 1.6: Studies investigating the role of music in disorders of consciousness. For all papers except Riganello et al. (2010): ↑ = significantly higher compared to control; ↓ = significantly lower compared to control; - = no significant difference compared to control. For Riganello et al. (2010): ↓ = significantly lower in Mussorgsky compared to Grieg.

Paper	Sample	SBP	DBP	HR	RMSSD	LF power	HF power	nuLF	Sample entropy	Respiration rate	Oxygen saturation	Skin tempertaure	EEG	Body movements	Head movements	Facial expressions	Eye movements/blink rate	Vocalisations
Aldridge et al. (1990)	Coma			↓						↓			Theta to beta and alpha	Fine body movements				
Jones et al. (1994)	Coma			↓						↓				-		-		
O'Kelly et al. (2013)	VS group			-		-	-			-			↑	-	-		-	-
	MCS group			-		-	-			-			↑	-	-		-	-
	VS case 1			-	↓	↑	↓						↑				↑	
	VS case 2			↓	↑	-	-						↑					
Riganello et al. (2010)	VS and healthy controls							↓	↓									
Puggina and Silva (2014)	Coma and VS	-	-	-							-	-				-		↑
	Sedation	-	-	-							-	-				-		-
Wilson et al. (1994)	VS patient 1													↑				↑
	VS patient 2													↑				↑
	VS patient 3													-				-
	VS patient 4													-				-



### 1.3 Thesis rationale

The reviewed literature clearly shows that music has multiple benefits in both healthy and patient samples. This includes attenuating anxiety in patients waiting to undergo medical procedures and patient concern about an underlying health condition. In addition, music is effective at reducing the psychological and physiological correlates of stress, in a range of samples, therefore boosting relaxation. Music can also be used as a tool to guide breathing in order to reduce BP in hypertensives. This effect is driven by the coupling between respiration and autonomic activity, and the sensitivity of breathing to changing musical characteristics. The use of music, particularly pieces that are preferred by patients, can also be used as a diagnostic tool to aid with discriminating between different sub-categories of disorders of consciousness and as a measure of prognosis and recovery. Therefore, music seems to have many positive attributes which are worth examining.

Some of these positive attributes may be due to the interaction between specific musical parameters. Indeed, research investigating the impact of distinct musical parameters in healthy samples have shown that the ANS is highly sensitive to changes in musical stimuli. Many musical parameters have been explored and include: dynamics/intensity, expressivity/musical interpretation, musical styles/genres, mode, slurs, pauses, note onset, pitch and tempo.

Out of all of the musical parameters explored, tempo could be considered to be one of the most fundamental characteristics of music. Indeed, it determines the temporal structure of a piece and, as shown with the music-guided breathing work, can facilitate entrainment. Further support for this claim comes from work in the music and exercise literature, particularly that by Karageorghis and colleagues, and in the music and movement literature (Fraisie, 1982). In addition, it could be argued that without temporal structure, music is no longer music. Therefore, due to tempo's fundamental nature to music, the current project explores the effects of different tempi on cardiovascular autonomic function.

The current project comprises three research studies (see Chapters 2-4). The first is a pilot study (Chapter 2) looking at the effects of stepped increases and decreases in tempo on non-invasive measures of cardiovascular autonomic function. It tests a specific methodology that employs tightly controlled auditory stimuli that manipulate tempo only.

Based on the results of the pilot, the second (a follow-up study, Chapter 3) builds on its methodology to include an invasive measure of autonomic activity as well as subjective measures of musical emotion. In this study, the musical complexity of the auditory stimuli is developed with the hope of increasing ecological validity. This includes using a nursery rhyme and a control group to investigate the possible contribution of melody in influencing autonomic responses to tempo manipulations.

The results of the follow-up study inform the design of the final study (Chapter 4). This involves testing a previously validated piece of relaxation music in combination with a form of non-invasive neuromodulation. This study comes in response to the call for determining whether relaxation music has any real effect on human physiology, or if the proposed effects are purely conjecture. In addition, the study explores the effectiveness of a 'music therapy' wearable device in promoting shifts towards greater parasympathetic predominance. This is important work, given the sudden increase in the number of wearable tracking technologies (e.g. Fitbit, Pip, Muse headband and Viatom Checkme) and music/health related wearables (e.g. MusicGlove<sup>10</sup>, MedRhythms<sup>11</sup>, Vibeat<sup>12</sup> and Touché<sup>13</sup>) available to purchase.

The final chapter (Chapter 5) provides a general discussion of four main outcomes.

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<sup>10</sup> <https://www.wired.com/2015/03/music-glove/>: accessed 07.09.2017

<sup>11</sup> <https://pitchfork.com/features/article/9976-the-doctor-is-in-your-pocket-how-apps-are-harnessing-musics-healing-powers/>: accessed 07.09.2017

<sup>12</sup> <https://www.dezeen.com/2016/08/07/liron-gino-design-vibeat-listening-devices-wearable-hearing-impaired-tactile-music/>: accessed 07.09.2017

<sup>13</sup> <https://www.dezeen.com/2017/06/30/royal-college-art-graduate-marie-tricaud-touche-system-musicians-compose-perform-vibrations/>: access 07.09.2017

**Chapter 2. The impact of tempo  
manipulations on cardiovascular autonomic  
function**

## 2.1 Introduction

Tempo, the speed of music, is considered to be one of the most basic musical parameters (Duerr, 1981). It is defined as the rate of periodic events (beats) in a given time interval, so is quantified as the number of beats per minute (bpm) (McAuley, 2010). Research exploring the importance of different musical parameters has highlighted that tempo is one of the most influential musical parameters in everyday life. For instance, tempo can promote synchronicity between motor actions and the temporal characteristics of a piece of music. This can translate into determining the type and duration of a dancer's movements; how quickly a conductor moves his arms and how quickly a listener taps his or her feet. This bodily movement entrainment also influences larger-scale behaviour, such as driving (Brodsky, 2001), shopper behaviour (Eroglu, Machleit & Chebat, 2005), drinking speed (McElrea & Standing, 1992) and restaurant diner behaviour (Caldwell & Hibbert, 1999). This may well be influenced by the power of preferred tempo on human actions.

Reducing medication consumption and side-effect occurrence is a big concern in present-day society. This has led to examining the effectiveness of alternative therapies (treatments not traditionally used in western medical practice). Examples of alternative therapies include, massage, Reiki, acupuncture, herbal remedies, yoga and music. Due to the prevalence of music across cultures and the anecdotal evidence that individuals use music as a form of self-help/medication, the impact of music on health and well-being has received a great deal of interest. Moreover, given this drive for medication reduction and the fundamental role tempo plays in music, there has also been increasing interest in exploring the impact of tempo on cardiovascular autonomic activity. The following section provides a summary of research investigating the impact of tempo on autonomic control of the heart in healthy volunteers.

### 2.1.1 The impact of musical tempo on autonomic control of the heart

Music is the culmination of many acoustic characteristics, including, tempo, rhythmic, dynamics and articulation. Of these musical parameters, tempo is

thought to be one of the most important determinants of music-related effects on cardiovascular autonomic function. Some of the earliest supporting evidence comes from work by Iwanaga (1995a, 1995b) who was interested in exploring the relationship between preferred musical tempo and HR. The first experiment involved recording HR during a resting period and whilst participants searched for their preferred tempo in an ascending and descending series of 440Hz pure tones. The tempo of the tones ranged from 10 to 300cpm (cycles per minute, synonymous with beats per minute). Iwanaga (1995a) identified that participants showed a preference for tempi that were one, one and a half and two times as fast as their resting HR. Preference was measured by participants adjusting a control dial when asked to search for their most favoured tempo. This is taken to suggest that resting HR is related to, and significantly influences, preferred musical tempo.

As the stimuli employed in this first study shared little resemblance to 'real music', Iwanaga (1995b) replaced the pure tones with a simple musical piece: the theme of Disney's 'It's a small world'. A similar experimental procedure was implemented: participants searched for their preferred tempo of the theme in an ascending and descending series. As the theme to the tune was short, the theme was repeatedly played without a pause. In contrast to Iwanaga's first study, the results showed that participants preferred a tempo that was the same as their own HR. However, when examining the ascending and descending series separately, preferred tempo differed between the two series. That is participants preferred a faster tempo in the descending stimulus (1.00, 1.17, 1.33, 1.50 and 1.67 times resting HR) compared to the ascending stimulus (same tempo as resting HR). This suggests that the initial tempo of the two series influenced participant responses.

Interestingly, the influential power of increasingly slower tempo transpired in a more recent experiment by van Dyck et al. (2017). This study built on the methodology of Iwanaga's (1995a, 1995b) studies in three ways. Firstly, confounding variables, such as: stimulus familiarity, musical preference, time of day of conducting the experiment and participant consumption of coffee, alcohol and nicotine were controlled for. This is because these factors have

all been found to influence cardiovascular autonomic function (see method section below). Secondly, the influence of gender and formal music training were also tested. This is important given that at baseline young females have significantly higher HRV and lower sympathetic predominance than young males (Antelmi et al., 2004; Kuo et al., 1999; Stein, Kleiger & Rottman, 1997). Also, musicians have been found to show greater sensitivity to changes in musical stimuli than non-musicians (Bernardi, Porta & Sleight, 2006). Finally, van Dyck et al. (2017) used more ecologically valid stimuli: non-vocal, ambient music that had a simple rhythmical structure.

The procedure required participants to undergo a nine-minute resting period to allow HR to stabilise; followed by a 60 second silent condition, during which the final 45 seconds were used to ascertain participant HR. Next participants underwent a 'heart rate-based music (HRBM) condition. This lasted for 60 seconds, during which participants heard a musical stimulus that had a tempo that was the same or a similar speed as participants HR. Finally, there was a 60-second altered tempo (AT) condition, in which the tempo of the stimulus was the same as the HRBM condition or increased or decreased by 15%, 30%, 45%. The trial was repeated seven times, interspersed with a 15 second period of silence, to ensure all tempo manipulations (+/- 0%, 15%, 30%, 45%) were addressed.

HR was significantly higher during HRBM compared to the 45-second period of silence, suggesting that the stimuli had an arousal effect. In addition, HR was significantly higher during the HRBM condition compared to the AT condition when the tempi of the stimuli were decreased by 30% and 45%. However, HR did not significantly differ between the two conditions when the tempo remained unchanged, decreased by 15% or increased by 15%, 30% or 45%. Although the results failed to support van Dyck et al.'s (2017) hypothesis (HR changes would be proportional to changes in musical tempo), they are partly consistent with Iwanaga's (1995a, 1995b) argument that changes in HR and tempo are related. Furthermore, the experiment reinforces the notion that the effects of music on autonomic function are partly regulated by tempo. Nevertheless, as gender and formal music training did not significantly modulate the differences observed, this raises questions regarding possible inconsistencies in the literature. Indeed, these

discrepancies may be a result of methodological differences (e.g. samples, stimuli employed and musical background classification differences and measures of autonomic function). Considering that respiration determines HR and other measures like BP, HRV and BRS, a major weakness with van Dyck et al.'s (2017) experiment concerns the lack of measuring respiration rate. Consequently, it remains plausible that the observed effects were driven by changes in participant breathing. Fortunately, in an experiment investigating the impact of tempo manipulations on HR responses, Watanabe, Oishi and Kashino (2017) held respiration rate constant and considered other measures of cardiovascular autonomic function.

Watanabe et al. (2017) adopted a novel paradigm with the hope of examining the impact of baseline HR on HR responses induced by changes in tempo. This comprised three experiments. The first consisted of two conditions: a five-minute baseline period, during which respiration rate was held constant at 15cpm; followed by a five-minute stimulation period in which respiration was maintained at 20cpm whilst listening to an 80bpm drum sound sequence. Results revealed a negative correlation between baseline HR and change in HR between baseline and stimulation. This suggests that HR during the stimulus shifted towards the 80bpm tempo.

The second experiment developed these initial results by presenting participants with two conditions: three-minute baseline, where respiration rate was maintained at 15cpm; followed by a five-minute constant tempo stimulus which increased baseline HR by a factor of 0, 5, 10 or 20 whilst holding respiration rate at 20cpm. HR significantly increased when tempo was 10, 15 and 20 times faster than baseline HR. Although no change in HF power<sup>14</sup> resulted, LF/HF<sup>15</sup> was significantly higher for the former two tempo manipulations when compared to baseline LF/HF. In the third experiment, the stimulus gradually increased in tempo by 0%, 1%, 2%, 3% or 4% more per minute than baseline HR. Apart from this difference, the procedure was the same as that used in the first experiment. The results showed that in the

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<sup>14</sup> Reflects parasympathetic (vagal) activity.

<sup>15</sup> Ratio of LF power (combined sympathetic and parasympathetic activity) to HF power. Provides an index of balance between the SNS and PNS. Increases in LF/HF reflect shifts towards sympathetic predominance and decreases in LF/HF reflect shifts towards parasympathetic predominance.

first minute of the stimulus, HR did not significantly change for any tempo increase percentages. However, from the second minute onwards HR was significantly higher for an increase in tempo of 2% compared to baseline HR. However, HF power did not significantly differ between baseline and the five tempi increase percentages. Despite this, the 2% tempo increase had significantly higher LF/HF compared to baseline. Consequently, this suggests that HR most effectively followed a gradually increasing tempo when the increase was 2% per minute. Additionally, even during controlled respiration rate, baseline HR was an important factor in determining how listeners responded to stimuli with different tempi. Therefore, the results of Iwanaga (1995a, 1995b), van Dyck et al (2017) and Watanabe et al. (2017) provide clear evidence in support of a HR and tempo relationship.

Since Watanabe et al.'s (2017) study assessed HF power and LF/HF, the findings also provide detail pertaining to the mechanism responsible for the observed increases in HR. As no change in HF manifested whilst LF/HF increased, it could be argued that the increase in HR was due to an increase in sympathetic activity and no change in vagal tone. However, the reliability of this claim is contentious due its reliance on two measures (one of which is contested: LF/HF). Therefore, consultation of other measures of cardiac autonomic function would have been beneficial. One study which adopted a more comprehensive set of autonomic function measures was conducted by Bernardi et al. (2006).

In this study, respiration rate<sup>16</sup>, minute ventilation<sup>17</sup>, end tidal carbon dioxide<sup>18</sup>, mid-cerebral artery flow<sup>19</sup>, RR interval<sup>20</sup>, LF/HF, SBP<sup>21</sup>, DBP<sup>22</sup> and

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<sup>16</sup> Number of complete breathing cycles (one inhalation followed by one exhalation) in a minute.

<sup>17</sup> Amount of gas inhaled and exhaled from a participant's lungs in a minute.

<sup>18</sup> Amount of carbon dioxide exhaled (mmHg). Measured via plethysmography using a nasal cannula and side stream capnography. This involves detecting concentrations of carbon dioxide by using infrared light. A photodetector compares the amount of light absorbed by carbon dioxide molecules with the amount absorbed by other molecules to determine the concentrations in carbon dioxide exhaled.

<sup>19</sup> Non-invasive evaluation of peak velocity of SBP in the middle cerebral artery (a major artery that supplies blood to the brain). This was measured by applying transcranial Doppler ultrasound at a depth of 35-55mm on the temporal lobe of the non-dominant side of the brain. The ultrasound transmitted and received sound waves to determine how blood flow changed through the middle cerebral artery.

<sup>20</sup> Time interval between consecutive R-peaks.

<sup>21</sup> Pressure in the arteries when the heart contracts.

<sup>22</sup> Pressure in the arteries when the heart is at rest, immediately before the heart contracts.



$\alpha$  BRS<sup>23</sup> were measured. Twenty-four healthy participants were exposed to six different musical stimuli, all varying in musical tempo (see Table 2.1 for a summary). Prior to the stimuli, a five-minute baseline recording was obtained. Additionally, a two-minute period of silence was randomly inserted in either the first or second trial (participants attended on two occasions in order to explore the effect of familiarity on cardiac autonomic function).

*Table 2.1: The six music genres and their corresponding tempi employed by Bernardi et al. (2006).*

<b>Music genre</b>	<b>Tempo (bpm)</b>
Raga	55
Slow classical	70
Dodecaphonic	76
Rap	103
Techno	136
Fast classical	150

HR was significantly higher during the raga stimulus and significantly lower during the fast classical stimulus compared to baseline. Furthermore, HR during all stimuli except raga was significantly lower compared to the random silence. Examination of LF/HF revealed significantly higher values in the rap, techno and fast classical music stimuli compared to baseline and the random silence. Also, SBP was significantly lower in baseline compared to techno, and the random period of silence compared to techno and fast classical. A different pattern emerged for DBP: values were significantly higher during slow classical compared to the random period of silence. Interestingly,  $\alpha$  BRS was significantly higher during the fast classical stimulus compared to baseline and the random silence. Breathing frequency was significantly higher during the three faster tempi (rap, techno and fast classical) compared to baseline and the random period of silence. Also, percentage change in minute ventilation was significantly higher during the techno and fast classical stimuli than during baseline. Although, mid-cerebral artery flow velocity during the six stimuli did not differ from baseline, values were significantly lower during the random period of silence compared to all six stimuli.

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<sup>23</sup> Spectral BRS derived by applying power spectrum density analysis to continuous blood pressure and heart rate recordings.

Taken together these findings suggest that passive listening to music that has a tempo greater than 100bpm leads to shifts towards sympathetic predominance. These results are consistent with those of Watanabe et al. (2017) and Gomez and Danuser (2007) who adopted a more rigorous approach than Bernardi et al. (2006) to explore the extent to which structural aspects of music determine autonomic function. This involved exposing 31 participants to 16 noises and 16 instrumental musical fragments of 30 seconds in duration whilst monitoring respiration, SCL and HR. There were positive correlations between tempo and: HR, SCL and minute ventilation; and negative correlations between tempo and: inspiration time and expiration time. This means that the faster the tempo the faster the HR, and the greater the SCL and volume of gas inhaled and exhaled.

This positive relationship between physiological arousal and tempo also emerged in a more recent experiment by Chuen, Sears and McAdams (2016), who manipulated pitch, timbre, dynamics, rhythm, as well as tempo in a controlled manner. As tempo is the main musical parameter of interest, only the experiment which manipulated this parameter will be discussed. Forty participants (20 males) were exposed to four conditions. In each condition participants heard bassoon-like reference tones played at 80bpm. At the end of the sequences they then heard a target tone which had the same timbre, tone duration and loudness (65 dB SPL) and either the same tempo (80bpm) or a faster tempo (100bpm, 120bpm or 140bpm). HR, SCR (skin conductance response), respiration and facial motor activity<sup>24</sup> were recorded throughout and analysed.

In contrast to van Dyck et al. (2017), HR decreased during the sequence that played the tones at the same tempo (80bpm). Furthermore, contrary to Bernardi et al.'s (2006) findings, HR did not significantly change when the target tones of 100bpm and 140bpm were played. Nevertheless, HR did increase during the 120bpm target tone, suggesting that in this sample 120bpm was particularly arousing. Despite no impact on facial motor activity

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<sup>24</sup> Measures the electrical activity of the corrugator supercillii (small muscle close to the eye located near to the eyebrow that draws the eyebrow down to produce frowning) and zygomaticus major (muscle that extends from the cheekbone to the corner of the mouth to produce a smile). The measurement of the electrical activity of muscles is called electromyography (EMG).

transpiring, a positive relationship between the tempo manipulations and SCR magnitudes emerged. Consistent with Gomez and Danuser's (2007) findings, faster target tones were associated with greater SCR magnitudes. Interestingly, the tempo manipulations failed to significantly impact respiration rate. This is contrary to the results of Bernardi et al. (2006), yet implies that the changes observed in Chuen et al.'s (2016) study were not driven by alterations in breathing.

Clearly there are inconsistencies in the literature regarding certain measures (e.g. respiration). In addition, there are some studies which report the opposite effect of faster tempi on human physiology. For instance, Dousty, Daneshvar and Haghjoo's (2011) study on the effects of sedative<sup>25</sup> and arousal<sup>26</sup> music on electrocardiography revealed that arousal music had significantly lower R peaks compared to sedative music and silence. This implied that heart contractions were significantly smaller in arousal music than in sedative music. Therefore, this suggests that the relationship between faster tempi and physiological arousal may not be linear. Indeed, this view is supported by Chuen et al.'s (2016) HR results. Nevertheless, it is generally accepted that faster tempi are associated with shifts towards sympathetic predominance. This claim is also substantiated by da Silva et al.'s (2014) results which showed that heavy metal music, which was characterised by a faster tempo, had significantly lower SD2<sup>27</sup> compared to baseline. Moreover, inspection of the Poincaré plots illustrated that there was less dispersion, and therefore lower RR interval variability during heavy metal music compared to baseline. In addition, Iwanaga et al. (2005) demonstrated that HF power was significantly higher during sedative music than excitative music. Also, van der Zwaag et al. (2011) showed that RMSSD<sup>28</sup> increased during slow music compared to fast music and Dillman Carpentier and Potter (2007) found that SCL was significantly greater during fast music compared to slow music. Nevertheless, there are numerous

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<sup>25</sup> One-minute excerpt of piano music that had a tempo of 19bpm.

<sup>26</sup> Fifty-two-second excerpt of 'trance-like music' that had a tempo of 120bpm.

<sup>27</sup> Non-linear measure of HRV. Represents a combination of short- and long-term variability in RR intervals. Decreases in SD2 reflect reductions in overall variability and increases represent increases in overall variability.

<sup>28</sup> Time-domain HRV measure. Represents short-term variability in RR interval. Correlates with HF power (parasympathetic activity).

methodological limitations associated with these research studies which will now be discussed. Table 2.2 provides a summary of the studies performed investigating the impact of different musical tempi of cardiovascular autonomic function.

### **2.1.2 Study rationale**

Tempo significantly impacts cardiovascular autonomic function in a directional manner: faster tempi are associated with increases in HR, BP and respiration and shifts towards sympathetic predominance. However, one of the most common issues with this research concerns the stimuli that are used. As detailed in Table 2.2, most research has used complex musical stimuli, which alter numerous parameters simultaneously. This renders it difficult to ascertain whether changes in a particular parameter are associated with the observed cardiovascular changes. To overcome this issue, some studies have focussed on manipulating one musical parameter whilst keeping all others consistent (e.g. Chuen et al., 2016; Iwanaga, 1995a, 1995b; Watanabe et al., 2017). This controlled, experimental approach has a particular strength: it can facilitate a more granular account of the impact of tempo manipulations on cardiac autonomic function. This can be developed by systematically increasing the complexity of the stimuli, leading to a more robust and comprehensive account of the effect of music on human physiology.

Another limitation associated with previous work concerns the differentiation between 'fast and slow tempi' or 'sedative and arousal music', as well as the use of different tempi across research studies. Indeed, discrepancies in the literature may be a result of these tempo classification differences.

Consequently, this calls for an approach that explores individual tempi, rather than a higher-level tempo definition. Differences in measures of cardiac autonomic function are also prevalent in the literature. This can be seen in Table 2.2 which also summarises the measures that have been employed in previous work. As these measures consider different aspects of human physiology and the impact of tempo on autonomic function has not been fully delineated, it is important to employ numerous measures.

Differences in baseline cardiovascular autonomic function exist between groups (see method section below). Also, some researchers have commented that autonomic responses to musical stimuli differ between individuals. For instance, differences have been identified between: males and females (Vlachopoulos et al., 2015; Nater, Abbruzzese, Krebs & Ehlert, 2006); older and younger listeners (Hilz et al., 2014); individuals with differing musical backgrounds (Mikutta et al., 2013; Virtala, Huotilainen, Partanen, & Tervaniemi, 2014; Haas, Distenfeld & Axen, 1986) and individuals with different personalities (Rawlings & Leow, 2008). However, out of the very few studies which looked at the impact of tempo on autonomic function, only two (Bernardi et al., 2006; van Dyck et al., 2017, see Table 2.2) considered individual differences. Moreover, results from these two studies are contradictory: Bernardi et al. (2006) showed that musicians were more sensitive to changes in tempo than non-musicians (only for respiration), whereas van Dyck et al. (2017) detected no significant differences. This is probably due to methodological differences: an issue which must be addressed in future work. Furthermore, baseline HR appears to interact with HR responses to tempo manipulations (Watanabe et al., 2017). Therefore, exploring the impact of demographic information and baseline readings on autonomic responses to music would be of benefit. This is because to enhance comprehension of why people respond to music in different ways and to identify which musical stimuli will most benefit certain people, it is crucial to investigate different response patterns to musical stimuli.

Inconsistencies exist in the literature regarding the effect of repetition on autonomic activity during exposure to tempo manipulations. For instance, Bernardi et al. (2006) failed to detect any differences in responses between the two visits. In contrast, Iwanaga et al. (2005) demonstrated that HR decreased with increasing repetition.

Therefore, to overcome some of the limitations associated with previous work the current study will:

1. Develop and use simple and tightly controlled auditory stimuli that manipulate tempo only. Although the auditory stimuli may have limited

ecological validity, it is hoped that this will facilitate the establishment of a clearer relationship between tempo manipulations and cardiovascular autonomic function.

2. Employ multiple measures of cardiovascular autonomic function, including: time, frequency and non-linear HRV parameters, RR interval measures, ECG morphology, BP, BRS and respiration rate. It is anticipated that this will facilitate a more comprehensive and granular account of the changes that result from tempo manipulations.
3. Explore whether baseline readings influence the relationship between tempo manipulations and changes in cardiovascular autonomic function.



### **2.1.3 Hypothesis**

Tempo has been shown to be one of the most influential musical parameters. Fast tempi (e.g. > 100bpm) are associated with increases in HR and BP and a shift towards sympathetic predominance. In contrast, slower tempi are accompanied with decreases in these measures. Therefore, the study hypothesised that stepped increases in tempo will increase sympathetic predominance, and stepped decreases in tempo will enhance parasympathetic predominance.

### **2.1.4 Aims and objectives**

By employing simple auditory stimuli, the study aimed to examine the effects of stepped increases and decreases in tempo on cardiac autonomic function. The primary outcome measure was LF/HF.

The objectives were to:

1. Determine the extent to which measures of autonomic function changed from baseline during two auditory stimuli (stepped increase in tempo, stepped decrease in tempo) in two testing occasions.  
Research question: Is there an effect of stimulus?
2. Investigate the extent to which change in cardiac autonomic function from baseline to stimulus differed between the two testing occasions (visit 1, visit 2). Research question: Is the first visit associated with greater changes than the second visit?
3. Examine the extent to which baseline measures predict response patterns to the two auditory stimuli. Research question: Is it possible to predict response type based on baseline readings?
4. Assess whether data analysis software impacts the reported results. This final objective emerged as a result of the introduction of a second physiological analysis program (LabChart) at the time of running the final study (see Chapter 4). Although there were similarities between the initial program (Spike2) and LabChart, the



latter utilised a different algorithm to perform frequency-domain HRV analysis and was more automated than Spike2. This led to examining the extent to which values from LabChart differed from those obtained from Spike2. Research question: Does computer software (Spike2 or LabChart) impact the results?

## **2.2 Method**

### **2.2.1 Participants**

Twenty-eight participants were initially recruited for the study (12 males, mean age: 25.68 years, SD: 3.87 years, minimum: 18 years, maximum: 37 years). The inclusion criteria for participation in the study were healthy males and females aged between 18 and 60 years. The exclusion criteria were a history of heart disease, diabetes, hypertension and severe auditory impairments. Individuals with these conditions were excluded from taking part because these conditions are associated with impaired autonomic function and the aim of the study was to investigate the impact of sudden changes in the speed of music in healthy volunteers only. Appendix A.1. provides a summary of the initial characteristics of the initial sample of 28 participants.

To avoid complications arising from cardiovascular circadian patterns, the study was conducted between 14:00 and 16:00 on each testing occasion. Participants were asked to avoid caffeine, nicotine, alcohol and strenuous exercise for 12 hours before the study. They were also asked to have a light breakfast and lunch, and to empty their bladder before the study commenced. The study was approved by the University of Leeds Ethics Committee (PVAR 14-050) and written informed consent was obtained from all participants prior to testing.

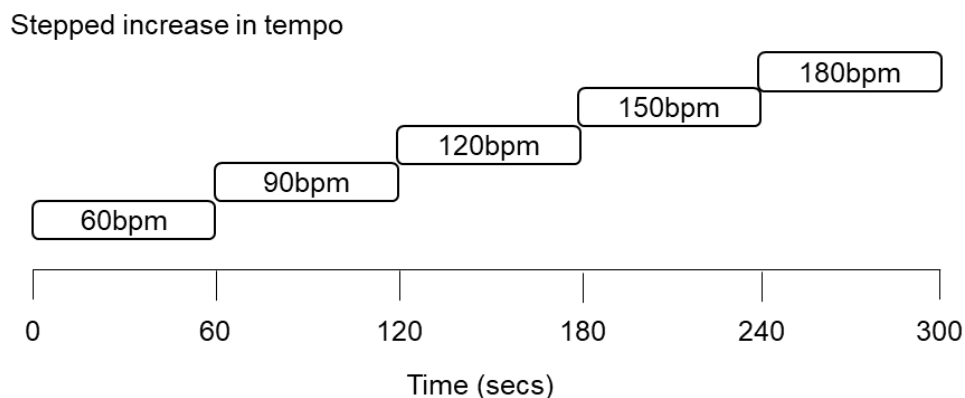
### **2.2.1 Materials**

#### **2.2.1.1 Auditory stimuli**

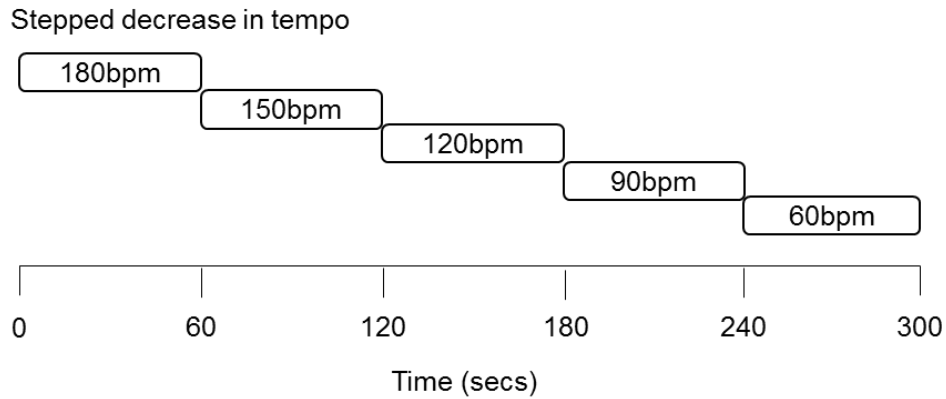
The auditory stimuli were created using Python with the Pygame library, recorded as a .wav file using Audacity and uploaded onto an MP3 player. A simple Python code was developed to generate the auditory stimuli using MIDI instruments, rather than a music composition program, such as Sibelius. This was because the code afforded greater efficiency for controlling musical parameters, including tempo, rhythm, volume and pitch.

Stepped changes in tempo were employed, as opposed to gradual tempo changes. This was because stepped tempo changes have been shown to be

more easily detected by listeners than gradual changes (Wang, 1983; Geringer & Madsen, 2003; Grondin & Laforest, 2004). This led to the development of two stepped tempo change stimuli: a stepped increase in tempo stimulus and a stepped decrease in tempo stimulus. Both stimuli consisted of five individual tempi: 60bpm (Adagio), 90bpm (Andante), 120bpm (Allegro), 150bpm (Vivace) and 180bpm (Presto). Each tempo played for 60 seconds so that the stepped increase and decrease in tempo conditions were five minutes in duration. This was based on frequency-domain HRV analysis guidelines from Camm et al. (1996) which recommended employing five-minute recordings. The tempi were organised in ascending order for the stepped increase in tempo stimulus (see Figure 2.1) and organised in descending order for the stepped decrease in tempo stimulus (see Figure 2.2). The beats in both stimuli were uniform (they had the same pitch, rhythm and volume). This was so that no specific metre or rhythm was expressed, and the changes in tempo were obvious and unconfounded by other parameters.



*Figure 2.1: The five tempi of the stepped increase in tempo stimulus. Comprised of 60bpm, 90bpm, 120bpm, 150bpm and 180bpm. Each tempo was 60-seconds in duration.*



*Figure 2.2: The five tempi of the stepped decrease in tempo stimulus. Comprised of 180bpm, 150bpm, 120bpm, 90bpm and 60bpm. Each tempo was 60-seconds in duration.*

### 2.2.1.2 Questionnaires

Participants were asked to complete two different questionnaires at the beginning of the first visit. Information obtained from these questionnaires aided with the analysis of the outcome measures and included the following:

1. Health questionnaire (see Appendix A.2.). Devised for the purposes of the study, this required participants to provide information pertaining to their age, medical history and lifestyle, e.g. smoking and physical activity habits. These questions were included because these variables have been found to impact cardiovascular autonomic activity. For instance, older age is associated with decreases in HRV (Antelmi et al., 2004; Melo et al., 2005; Stein et al., 1997; Umetani, Singer, McCraty & Atkinson, 1998) and BRS (Laitinen et al., 1998) and a shift towards sympathetic predominance (Kuo et al., 1999; Stein et al., 1997). These age effects interact with gender, whereby autonomic function is attenuated in females upon reaching the menopause, which results in no differences between older males and older females in autonomic activity (Kuo et al., 1999; Stein et al., 1997). Also, smokers have lower vagal tone than non-smokers, as well as higher HR and BP (Hayano et al., 1990; Niedermaier et al., 1993, Narkiewicz et al., 1998; Barutcu et al., 2005; Hering et al., 2006). Additionally, physically active individuals have higher resting HRV (de Meersman, 1993; Melo et al., 2005; Stein,

Ehsani, Domitrovich, Kleiger & Rottman, 1999) and lower resting HR than their sedentary counterparts (Jurca, Church, Morss, Jordan & Earnest, 2004).

2. Musical background questionnaire (see Appendix A.3.). Also devised for the purposes of the study. This required participants to provide information about their music listening habits (e.g. amount of time spent listening to music and preferred music genres), and formal music training. It was particularly important to correctly differentiate between musically trained (musicians) and not musically trained (non-musicians) participants. This is because musicians have been found to have lower respiration rates at baseline compared to non-musicians (Bernardi et al., 2006). In addition, brain imaging research demonstrates that there are structural and functional differences between musicians and non-musicians (Schmithorst & Wilke, 2002; Gaser & Schlaug, 2003; Bermudez & Zatorre, 2005; Patston, Kirk, Rolfe, Corballis & Tippett, 2007).

## **2.2.2 Apparatus**

### **2.2.2.1 MP3 player**

A SanDisk Sansa Fuse+ MP3 player was used to present the musical stimulus to participants.

### **2.2.2.2 Headphones**

Sony MDR-P180 wired over-the-head stereo headphones were used to play the auditory stimuli to participants.

### **2.2.2.3 Physiology equipment**

#### **2.2.2.3.1 Heart rate**

A three-lead ECG was used to monitor and record HR. This involved attaching electrode pads (Ambu, UK) to the right collarbone and to either side of the bottom of the ribcage. Also, an Omron upper-arm digital BP

monitor was used to obtain instantaneous measures of HR (and BP) at the end of each recording.

#### **2.2.2.3.2 Blood pressure**

A Finometer device (Finometer Medical Systems B.V., Arnhem, Netherlands) was used to continuously monitor and record blood pressure. This involved attaching one small inflatable cuff onto the middle phalanx of the middle finger on the left hand. The cuff detected changes in blood volume as a result of heart contractions by using the photoplethysmography method. Photoplethysmography involves emitting infrared light and detecting the amount of infrared light that is reflected. Fluctuations in the amount of infrared light reflected varies as a result of changes in blood volume and can therefore provide an estimate of blood pressure. The finger cuff used the incoming photoplethysmography data to adjust the amount of pressure in the cuff to ensure that the diameter of the arteries was kept constant. This is known as the volume clamp method. The non-invasive blood pressure device corrected for hydrostatic pressure changes that resulted from changes in hand position relative to the heart. This was achieved by using the height correction unit, which consisted of two sensors. One sensor was placed on top of the finger cuff and the other on the upper torso at a similar vertical height as the heart. The finger cuff and height correction unit were connected to the main unit which produced a blood pressure waveform in Spike2 (CED UK). To maximise accuracy of the blood pressure waveform a calibration setting was used. This setting calculated the blood pressure waveform by determining the point at which pressure in the arteries matched that in the finger cuff. The calibration setting was left on until blood pressure values were 10mmHg within the average of measures of SBP and DBP taken from the Omron BP monitor. If a discrepancy >10mmHg persisted between the Finometer device and Omron monitor, a variety of adjustments were made. These involved:

1. Reapplying the finger cuff
2. Warming the hands
3. Applying the finger cuff to a different finger (e.g. ring or index fingers)

### **2.2.2.3.3 Respiration**

Respiration rate per minute was recorded using a piezo-electric transducer (Pneumotrace, UFI, USA). This was placed around the upper thorax to monitor and record chest movement during breathing.

### **2.2.3 Procedure**

Figure 2.3 provides a summary of the study procedure. Participants attended the University of Leeds on two occasions spaced at least one week apart. At the beginning of the first visit, informed consent was obtained and the health and musical background questionnaires administered. The height and weight of participants were obtained at the beginning of every visit.

Participants were then asked to lie in a semi-supine position on a couch with a memory foam mattress with pillows supporting the head and lower back.

Physiological equipment which continuously recorded HR, BP and respiration were then attached to participants on each testing occasion.

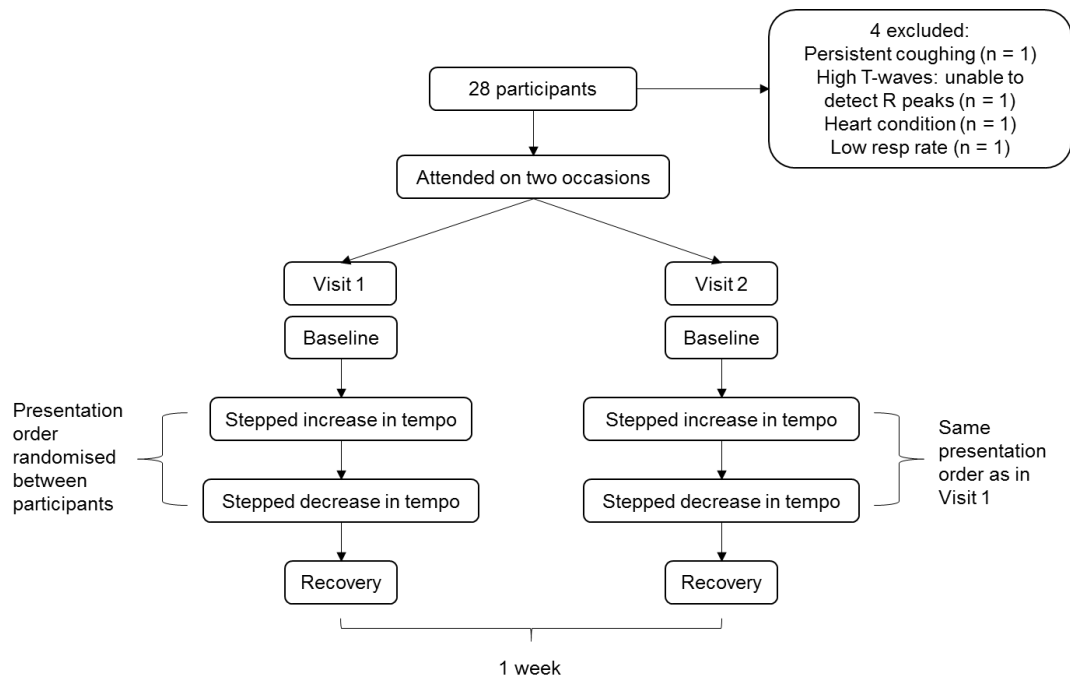
Participants underwent an adaptation period of approximately three minutes which allowed cardiovascular measures to stabilise before commencing the baseline condition. Data collection commenced following stabilisation of HR and BP.

In each visit, participants underwent four five-minute conditions: baseline (rested in silence with the headphones in place); stepped increase in tempo (rested while listening to the tempo increase stimulus); stepped decrease in tempo (rested while listening to the tempo decrease stimulus) and recovery (same as baseline). Participants were asked to close their eyes during all four conditions. The order of the baseline and recovery conditions was fixed: baseline was always first and recovery was always last. In contrast, the presentation order of the stimuli (stepped increase or stepped decrease in tempo) was randomised between participants and remained the same in both visits. The stepped increase and decrease in tempo stimuli ran back-to-back (there was no break between the two stimuli). HR, BP and respiration were continuously recorded during the four conditions. At the end of each condition, brachial HR and BP were measured three times using the Omron blood pressure monitor. Following this, an adaptation period of three minutes

was implemented to allow HR and BP to stabilise before commencement of the subsequent section.

Participants were asked to remain still, close their eyes and to refrain from talking and falling asleep during the recordings. The study room was kept at a constant temperature of  $21 \pm 2$  degrees Celsius.

Upon completion of the data collection, physiological recording equipment was detached from participants.



*Figure 2.3: Procedure employed in the pilot study. Twenty-eight participants attended on two occasions during which they experienced baseline, stepped increase in tempo, stepped decrease in tempo and recovery conditions.*

#### 2.2.4 Data acquisition

The audio signal was digitised (Power 1401, CED, UK), passed to a Dell laptop and visualised in a data channel in Spike2 software (CED, UK). ECG, BP and respiration signals were split into two channels and fed into two data amplification systems (Coulbourn Lab Sinc V, Coulbourn Ltd, USA and Neurolog, CED, UK) and sampled at 12 kHz. These data were also digitised (Power 1401, CED, UK) and passed to a Dell laptop to be visualised in real-time and continuously recorded in individual data channels in Spike2 software. Each data channel was independently calibrated before digitisation of the incoming signals.



### 2.2.5 Data analysis

Two computer software programs were used to analyse the recorded data: Spike2 (CED, UK) and LabChart (ADInstruments). As the data were recorded in Spike2, they were initially analysed off-line using this software. An equipment upgrade at the time of running the final study (see Chapter 4) resulted in also using LabChart. Although there were similarities between the two programs (e.g. both recorded HR, BP and respiration), there were differences (e.g. frequency-domain HRV was derived using different algorithms; and only LabChart derived time-domain HRV, non-linear HRV and ECG parameters). Therefore, it was important to determine whether computer program (Spike2 or LabChart) and hence algorithm impacted the results (final study objective). This resulted in analysing the data in LabChart as well as Spike2.

To perform the data analysis in LabChart, the Spike2 file was exported as a text file and imported into LabChart. The same time points were used in both analyses. As recordings of five-minutes were recommended by the Task Force (1996), the entirety of each of the four conditions was analysed. The onset and offset of the auditory stimuli were taken from the auditory stimulus channel and from lab notes in the case of the baseline and recovery conditions.

The third study objective was to assess the extent to which cardiac autonomic function differed between the two visits. This resulted in calculating absolute change between:

1. Baseline and stepped increase in tempo
2. Baseline and stepped decrease in tempo

These calculations were based on the testing of a range of approaches in the final study (see Chapter 4). Absolute change values were calculated for every measure in Excel for both the Spike2 and LabChart data.

To address the final objective, percentage change values were derived for all measures. The following formula was used:

$$\left( \frac{(\text{new value} - \text{old value})}{\text{old value}} \right) \times 100$$

The subsequent two sections detail the data analysis that was performed when using Spike2 and LabChart.

### **2.2.5.1 Spike2**

#### **2.2.5.1.1 HRV**

The ECG trace was visually inspected to ensure there were no abnormalities, such as ectopic beats. Next, R peaks, from the recordings were identified by setting a threshold (around 3.00mV) and the resulting RR intervals were used to produce a tachogram in a memory channel. The tachogram was inspected to ensure all R peaks were detected. If not all R peaks were detected, two approaches were used:

1. The threshold was lowered
2. The R peaks were manually entered

If ectopic beats were identified, they were removed from the tachogram and an R peak was manually interpolated. The memory channel was then used to produce a virtual channel on which power spectral density analysis was performed. The virtual channel was resampled at 5Hz and the DC removal process applied to offset the channel to zero. The Fast Fourier Transform was then applied (512 point; 50% overlap) with a Hanning window to calculate the power spectrum of HRV. The power spectrum was divided into the VLF (0.00 – 0.04Hz), LF (0.04 - 0.15Hz) and HF (0.15 - 0.40Hz) components. These data were then exported to Excel (2013) and nuLF, nuHF and LF/HF calculated using the formulae in Table 2.3. Baseline LF/HF was normalised to 1 (nuLF/HF) to eliminate between-participant differences in baseline LF/HF. The formula employed is also provided in Table 2.3.

Table 2.3: Spike2 calculations for nuLF, nuHF, LF/HF and nuLF/HF.

Parameter	Formula
nuLF	$\frac{LF \text{ power}}{(Total \text{ power} - VLF \text{ power})}$
nuHF	$\frac{HF \text{ power}}{(Total \text{ power} - VLF \text{ power})}$
LF/HF	$\frac{nuLF}{nuHF}$
nuLF/HF	$\frac{LF/HF \text{ for condition of interest}}{Baseline LF/HF}$

### 2.2.5.1.2 Heart rate

Average HR was calculated by using the tachogram that was produced when deriving HRV. To determine average HR in each condition, the y axis of the memory channel was compressed and a horizontal cursor placed through the middle of all data points.

### 2.2.5.1.3 Blood pressure

Average SBP and DBP were ascertained by placing a horizontal cursor through the systolic peaks and diastolic troughs in the recordings obtained via the finometer. MAP was calculated using the following formula:

$$\frac{(SBP + (2 \times DBP))}{3}$$

### 2.2.5.1.4 Spontaneous spectral BRS

The ECG and BP traces were visually inspected to ensure there were no abnormalities. Next, R peaks and systolic peaks were identified by using the active cursors mode. This entailed using two cursors for both measures. One cursor identified the current peak whilst the other identified the previous peak. The active cursors method generated two memory channels: one displayed the RR interval and the other displayed the time interval between each systolic peak. The systolic peak memory channel values were then divided by the RR-interval memory channel values in a virtual channel. The virtual channel was resampled at 5Hz and DC removal process applied to

offset the channel to zero. The FFT was then applied (512 point; 50% overlap) with a Hanning window to calculate the power spectrum of BRS (same as that for HRV). These data were then exported to Excel (2013) and  $\alpha$  (LF) BRS computed with the following formula:

$$\sqrt{\frac{LF\ HRV\ power}{LF\ SBP\ power}}$$

### 2.2.5.1.5 Respiration

The respiration trace was visually inspected to ensure that all respiration rates were > 10 breaths/minute. If respiration rates were < 10 breaths/minute, participants were excluded. This is because low respiration rates (< 10 breaths/minute) can result in convergence in the frequency components in the HRV spectrum (Bilchick & Berger, 2006; Camm et al., 1996). Respiration rate was derived by identifying each peak in the trace.

### 2.2.5.2 LabChart

#### 2.2.5.2.1 HRV

The ECG trace was visually inspected to ensure there were no abnormalities, such as ectopic beats. If ectopic beats were detected, they and the subsequent beat were removed from the analysis. No interpolation was performed in these cases. The HRV module in LabChart was used to derive time, frequency and non-linear indices. The following time-domain HRV measures were obtained: average HR, SDRR, CVRR, SDSD, RMSSD and pRR50. All measures were then exported to Excel (2016).

The Lomb-Periodogram algorithm was used to derive the frequency-domain HRV indices. This algorithm is typically used for detecting and characterising periodic signals in unevenly-sampled time-series data (Delane, Bohórquez, Gupta & Schiavenato, 2016; VanderPlas, 2017). As HRV data is not uniform, the Lomb-Periodogram algorithm has been argued to be a more appropriate method than the FFT for performing power spectral density analysis on an ECG signal to derive frequency-domain HRV (Delane, Bohórquez, Gupta & Schiavenato, 2016). This argument is particularly strong given that the FFT requires evenly sampled data (Delane et al., 2016; VanderPlas, 2017).

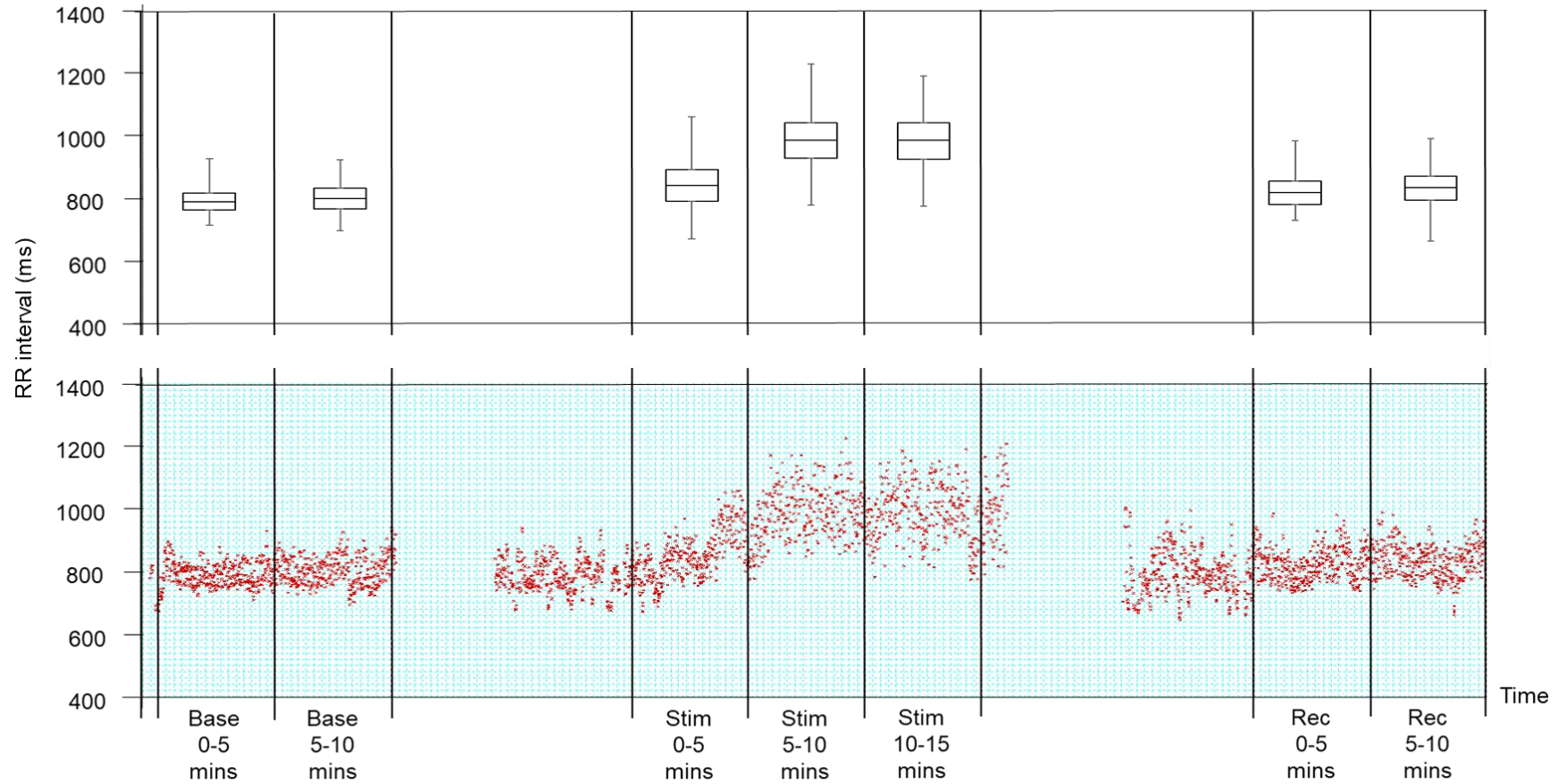
Moreover, in a study comparing the two algorithms, the error rate for HF power was lower when using the Lomb-Periodogram algorithm compared to the FFT (Delane et al., 2016). In turn, this suggests that the Lomb algorithm is more appropriate for deriving frequency-domain HRV.

The power spectrum was divided into the VLF (0.00 – 0.04Hz), LF (0.04 - 0.15Hz) and HF (0.15 - 0.40Hz) components. This analysis derived the following frequency-domain HRV measures: total power, VLF power, LF power and HF power in absolute values; percentage of VLF, LF and HF power (VLF%, LF% and HF% respectively); normalised LF and HF power (nuLF and nuHF respectively); and LF/HF. These measures were also exported Excel (2016) and baseline LF/HF was normalised to 1 (nuLF/HF).

Non-linear HRV indices were also derived and exported to Excel. These included, SD1 and SD2. Other non-linear HRV measures were calculated in Excel including: nSD1, nSD2, SD1/SD2, SD2/SD1 and S.

#### **2.2.5.2.2 RR interval**

The ECG module was used to analyse RR interval (time between each R peak). Visual representations of the RR interval over time were produced by generating RR/time graphs for the entire study for each participant. This facilitated an assessment of the extent to which variability in RR changed during the different stimuli. For example, in the final study (see Chapter 4) clear changes in the dispersion in RR interval occurred. This prompted an exploration into the ways in which this dispersion could be analysed graphically and quantitatively. This resulted in deriving box plots which shared similar visual patterns with the RR/time plots (see Figure 2.4).



*Figure 2.4: An example RR interval over time plot with corresponding box-plots for one participant showing how RR interval dispersion changed during the study. Box plots were generated for each of the five-minute time periods indicated. They too provided visual illustrations of how RR interval dispersion fluctuated.*

The advantage of using box plots was two-fold:

1. They showed visually that the dispersion in RR changed during the stimuli.
2. They used quantitative measures (e.g. median, minimum, maximum, first quartile and third quartile) which could be statistically analysed (see Figure 2.5).

Figure 2.5 summarises the RR interval measures that were derived for each time period of interest:

RR measure (ms)	Calculation
Mean RR	The mean of all RR intervals in the section of interest. Provided in an output table in LabChart.
Median RR	The median RR interval. Calculated by obtaining all RR intervals in LabChart, exporting to Excel (2016) and applying the 'median' function.
Mode RR	The most common RR interval. Calculated by obtaining all RR intervals, exporting to Excel and applying the 'mode' function.
Min RR	The minimum RR interval. Provided in an output table in LabChart.
Max RR	The maximum RR interval. Provided in an output table in LabChart.
$\Delta$ RR	Min RR subtracted from max RR (range). Derived in Excel.
Q1 RR	First quartile of all RR intervals in the section of interest. Calculated by obtaining all RR intervals in LabChart, exporting to Excel and applying the 'quartile' function.
Q3 RR	Third quartile of all RR intervals in the section of interest. Calculated by obtaining all RR intervals in LabChart, exporting to Excel and applying the 'quartile' function.
IQR RR	The interquartile range of all RR intervals in the section of interest. Calculated by subtracting Q1 RR from Q3 RR. Derived to explore whether the middle 50% of the set of RR intervals changed during the stimulation periods.
Q1-min RR	The difference between Q1 and min RR calculated in Excel. Derived to determine whether the lower 25% of the set of RR intervals changed.
Max-Q3 RR	The difference between max and Q3 RR calculated in Excel. Derived to determine whether the upper 25% of the set of RR intervals changed.

Figure 2.5: Summary of RR interval measures derived with an illustration of the quantitative measures derived from the box plots.

### 2.2.5.2.3 ECG

Figure 2.6 details the ECG measures that were derived using the ECG module.

ECG measure	Unit	Calculation
Q amplitude	mV	The initial negative deflection (depolarisation) of the QRS complex. Represents septal depolarisation.
R amplitude	mV	The initial positive deflection (repolarisation) of the QRS complex. Represents early ventricular depolarisation.
S amplitude	mV	The final negative deflection of the QRS complex. Represents late ventricular depolarisation.
T amplitude	mV	The first positive deflection after the QRS. Represents repolarisation of the ventricles.
ST height	mV	The amplitude of the ST segment (time between the end of the S wave and the start of the T wave).
QRS interval	s	Time taken for the QRS complex: from the onset of the Q wave to the completion of the S wave. Represents ventricular depolarisation.
QT interval	s	Time taken from the onset of the Q wave to the completion of the T wave.
QTc interval	s	The QT interval corrected for heart rate. Two formulas were used depending on heart rate: 1. The Bazett formula was used for heart rates $\geq 60$ bpm 2. The Fridericia formula was employed for heart rates $< 60$ bpm
JT interval	s	QTc interval - QRS interval

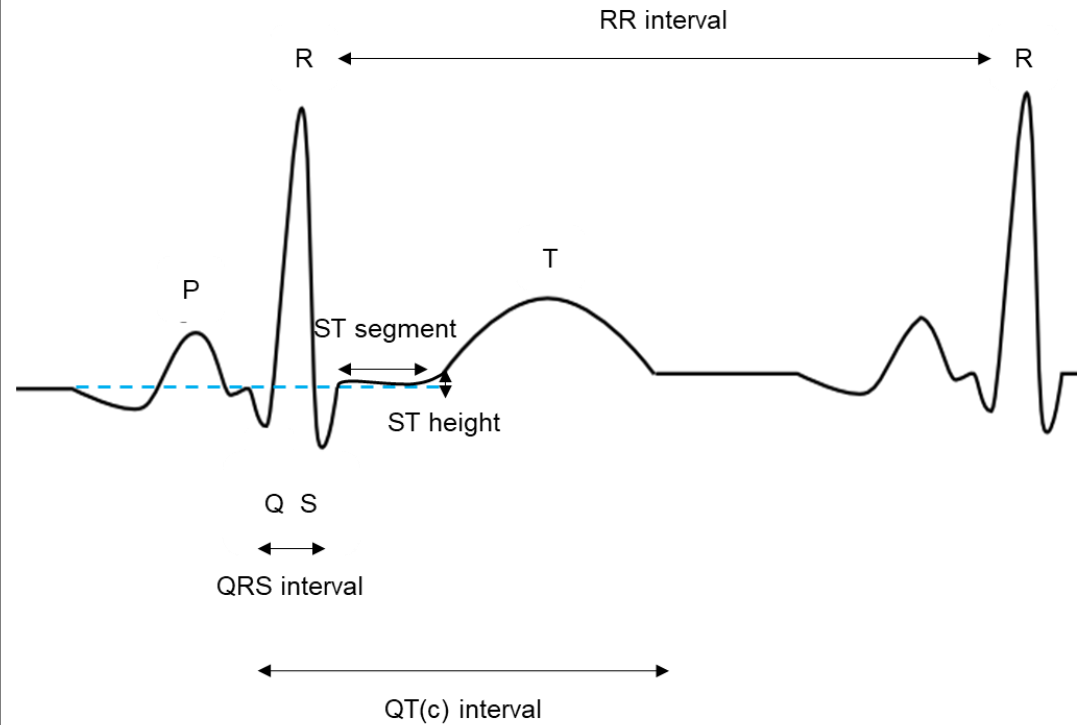


Figure 2.6: ECG measures derived included the amplitude of Q, R, S, T and ST waves and the duration of the QRS, QT(c) and JT intervals. The corresponding diagram illustrates the location of these variables in the PQRST complex.



There is some debate in the literature concerning the most reliable formula to use when correcting the QT interval for HR. Work by Puddu et al. (1988) showed that the Fridericia formula was more accurate for HRs < 60bpm compared to Bazett. Similar results were reported by Karjalainen, Viitasalo, Mänttari and Manninen (1994) and Goldenberg, Moss and Zareba (2006) who found that unlike Fridericia, the Bazett formula under-adjusts at low HRs and the Framingham formula over-adjusts at low HRs. As a result, it was decided that Bazett was most suitable for HRs between 60bpm and 100bpm, and Fridericia was to be used when HRs were < 60bpm. Table 2.4 summarises the Bazett and Fridericia formulas.

*Table 2.4: Bazett and Fridericia formulas used to calculate QTc.*

QTc formula	Calculation
Bazett	$\frac{QT}{\sqrt{RR}}$
Fridericia	$\frac{QT}{\sqrt[3]{RR}}$

#### **2.2.5.2.4 Blood pressure**

The blood pressure LabChart module was used to derive SBP and DBP. MAP was calculated using the same formula as above.

#### **2.2.5.2.5 Spontaneous sequence BRS**

Sections of interest in the ECG and BP traces (the five-minute recordings) were exported from LabChart as a text file. They were then imported back into LabChart and visually inspected to ensure there were no abnormalities. If abnormalities existed, the RR and corresponding beat-to-beat SBP value (along with the subsequent value) were removed. Interpolation was not performed. A macro was applied to derive up, down and mean BRS. The macro defined spontaneous sequences as three or more consecutive cycles of either: increases in SBP coupled with increases in RR interval (up sequences); or decreases in SBP coupled with decreases in RR (down sequences) (Laude et al., 2009). The blood pressure change had to be at least 1 mmHg/heartbeat (Parati et al., 1988) and the change in RR had to be at least 2ms (Laude et al., 2009). After the macro had identified the

sequences of interest, the SBP, RR interval and time data were exported to Excel (2016). Linear regression was then performed between the SBP and RR interval data points in each sequence and only sequences with a correlation coefficient  $> 0.85$  were accepted for further analysis (Parati et al., 1988). For these selected sequences, the slope of the function of  $\Delta$  RR and  $\Delta$  SBP was calculated and averaged separately for up and down sequences. The slopes of all selected sequences (both up and down) were also averaged to derive mean BRS.

#### **2.2.5.2.6 Respiration**

Respiration was analysed in the same way as Spike2.

#### **2.2.6 Statistical analysis**

The Shapiro-Wilk test was used to test for normality. If data were not normally distributed non-parametric equivalents were implemented.

To address the first and second objectives (is there an effect of stimulus and is one visit associated with greater changes than the other visit?) repeated measure ANOVAs (or Friedman tests) were performed. For the first objective, these tests were conducted on the raw values where the within-participants variable was condition (baseline, stepped increase in tempo, stepped decrease in tempo, recovery). For the second objective the repeated measure ANOVAs (or Friedman tests) were performed on the absolute change values where the within-participants variable was visit (visit 1, visit 2).

To fulfil the third objective (is it possible to predict response type based on baseline readings?) linear regressions were performed between baseline and percentage change values. To examine differences between responder types, independent sample t-tests (or Mann-Whitney U) and one-way ANOVAs (or Kruskal-Wallis tests) were performed.

For statistically significant ANOVAs, Bonferroni pairwise comparisons were performed. The Greenhouse-Geisser correction was used when data did not meet sphericity.

For statistically significant Friedman tests, pairwise comparisons were performed using the Wilcoxon Signed-Ranks test. As the Wilcoxon did not automatically adjust the alpha level for multiple comparisons, the p-value was adjusted by dividing it by the number of pairwise comparisons made. So, in the case of three pairwise comparisons for a statistically significant Friedman test, the alpha level was set to 0.017. In the instance of six pairwise comparisons the alpha level was set to 0.008.

Data in this and the forthcoming chapters are presented as the group mean  $\pm$  one standard error of the mean (SEM) unless stated otherwise. Two-tailed statistical tests were employed in all cases. All statistical analyses were performed in SPSS (version 23).

### 2.2.7 Study failures

In total, 28 volunteers took part in the study. Data from four participants were excluded, the reason and their characteristics are presented in Table 2.5.

Appendix A.4. provides a summary of the final sample of 24 participants (12 males).

*Table 2.5: Pilot study failures.*

	Gender	Age (yrs)	Physically active?	Formally music trained?	Reason
1	M	18	Y	N	Persistent coughing
2	F	29	N	Y	High T-waves: unable to detect R-peaks
3	M	23	N	N	Heart condition
4	F	32	Y	Y	Respiration rate < 10 breaths per minute

## **2.3 Results**

The study aimed to examine the effects of stepped increases and decreases in tempo on cardiac autonomic function. It was anticipated that the simple auditory stimuli would be effective at modulating autonomic activity.

Specifically, the stepped increase in tempo stimulus was hypothesised to be associated with shifts towards sympathetic predominance (indexed by increases in LF/HF) compared to baseline. It was also predicted that the stepped decrease in tempo stimulus would be associated with shifts towards parasympathetic predominance (indexed by reductions in LF/HF) relative to baseline. The results that follow report both within- and between-visit analyses for the full sample.

### **2.3.1 Spike2 vs. LabChart**

The fourth study objective sought to determine the extent to which computer program (Spike2 or LabChart) influenced the results. This involved a two-stage process:

1. Comparing the raw values of parameters that were derived in both programs
2. Comparing the results from statistical analyses

#### **2.3.1.1 Raw values**

Ten parameters that were derived in both programs were examined. These included: HR, SBP, DBP, MAP, total (HRV) power, VLF power, LF power, HF power and LF/HF. As shown in Table 2.6, the raw HR and SBP values were broadly consistent, despite LabChart automatically detecting the R and systolic peaks and Spike2 requiring calculation by hand. However, Table 2.6 also illustrates that there were greater differences between the two programs for the DBP and MAP values. Perhaps this was due to greater difficulty in detecting the diastolic trough by eye in the Spike2 compressed trace.

Table 2.6: Raw values for HR and BP obtained in Spike2 and LabChart.

		Visit 1		Visit 2	
		Spike2	LabChart	Spike2	LabChart
<b>HR (bpm)</b>	Baseline	71.86 (1.86)	71.41 (1.98)	72.42 (1.81)	72.09 (1.91)
	Stepped increase in tempo	69.47 (1.77)	68.70 (1.77)	71.05 (1.73)	70.33 (1.94)
	Stepped decrease in tempo	68.58 (1.83)	68.20 (1.89)	69.84 (1.87)	69.62 (1.93)
	Recovery	69.14 (1.75)	68.86 (1.92)	71.87 (1.99)	71.03 (1.96)
<b>SBP (mmHg)</b>	Baseline	111.67 (2.96)	110.39 (3.21)	108.40 (3.21)	109.71 (3.54)
	Stepped increase in tempo	114.55 (3.01)	114.45 (3.33)	115.05 (2.74)	116.28 (3.97)
	Stepped decrease in tempo	114.37 (2.80)	115.21 (3.13)	114.92 (2.81)	116.25 (4.14)
	Recovery	118.40 (3.46)	123.05 (4.22)	118.41 (3.19)	123.33 (4.20)
<b>DBP (mmHg)</b>	Baseline	55.43 (2.08)	46.82 (1.95)	56.12 (2.64)	46.12 (2.34)
	Stepped increase in tempo	56.11 (1.53)	45.46 (1.69)	59.46 (2.00)	46.74 (2.13)
	Stepped decrease in tempo	54.99 (1.57)	44.78 (1.70)	58.09 (1.76)	46.56 (2.34)
	Recovery	59.71 (2.21)	49.98 (2.54)	62.40 (2.16)	51.49 (2.60)
<b>MAP (mmHg)</b>	Baseline	74.18 (2.17)	68.01 (2.26)	73.54 (2.67)	67.32 (2.59)
	Stepped increase in tempo	75.59 (1.75)	68.46 (2.04)	77.99 (2.05)	69.92 (2.58)
	Stepped decrease in tempo	74.78 (1.73)	68.25 (1.95)	77.04 (1.92)	69.79 (2.81)
	Recovery	75.82 (4.17)	69.32 (4.59)	81.07 (2.34)	75.43 (2.97)

Examination of Table 2.7 demonstrates that there were large differences between the two programs for total, VLF, LF and HF (HRV) power. This could be due to the use of different algorithms: Spike2 used the FFT whereas LabChart used the Lomb-Periodogram algorithm. These differences manifested in the LF/HF raw values. Indeed, despite the direction of change in LF/HF between the four conditions remaining consistent in Visit 1, the pattern observed in the Spike2 values did not occur in LabChart.

*Table 2.7: Raw values for frequency-domain HRV parameters obtained in Spike2 and LabChart.*

		Visit 1		Visit 2	
		Spike2 (ms <sup>2</sup> )	LabChart (μs <sup>2</sup> )	Spike2 (ms <sup>2</sup> )	LabChart (μs <sup>2</sup> )
<b>Total power</b>	Baseline	484.65 (102.06)	3225.58 (527.45)	290.36 (40.52)	2464.86 (375.51)
	Stepped increase in tempo	333.67 (67.99)	2964.30 (450.64)	269.75 (33.89)	2694.41 (319.02)
	Stepped decrease in tempo	387.96 (80.89)	3472.37 (461.12)	311.76 (45.15)	2957.20 (406.17)
	Recovery	321.28 (44.43)	2994.84 (398.14)	341.61 (63.32)	2848.25 (496.81)
<b>VLF power</b>	Baseline	52.03 (10.64)	772.38 (98.67)	40.63 (7.28)	799.91 (174.44)
	Stepped increase in tempo	39.25 (5.71)	927.07 (175.70)	39.53 (7.64)	1096.90 (212.66)
	Stepped decrease in tempo	37.10 (7.93)	842.15 (139.92)	49.26 (9.32)	1058.39 (179.50)
	Recovery	50.90 (9.71)	975.07 (192.26)	64.97 (12.64)	1047.66 (206.84)
<b>LF power</b>	Baseline	224.95 (65.88)	1131.76 (300.58)	114.94 (15.89)	704.20 (128.24)
	Stepped increase in tempo	155.41 (45.67)	947.69 (256.55)	121.74 (15.06)	680.18 (114.12)
	Stepped decrease in tempo	176.90 (54.01)	1055.87 (243.74)	139.96 (24.90)	887.46 (184.44)
	Recovery	151.18 (28.47)	902.47 (186.20)	161.36 (42.55)	871.02 (265.78)
<b>HF power</b>	Baseline	207.66 (57.86)	1334.51 (289.61)	134.79 (29.34)	970.49 (186.69)
	Stepped increase in tempo	139.02 (34.08)	1104.66 (146.23)	108.48 (17.27)	926.98 (145.76)
	Stepped decrease in tempo	173.96 (40.86)	1591.88 (290.94)	122.54 (19.07)	1022.97 (169.60)
	Recovery	119.20 (18.67)	1128.39 (194.87)	115.28 (21.73)	936.97 (198.17)
<b>LF/HF</b>	Baseline	1.54 (0.24)	1.35 (0.28)	1.47 (0.22)	1.25 (0.19)
	Stepped increase in tempo	1.76 (0.54)	1.43 (0.53)	1.66 (0.26)	1.08 (0.22)
	Stepped decrease in tempo	1.43 (0.42)	1.14 (0.29)	1.44 (0.23)	1.22 (0.22)
	Recovery	1.60 (0.24)	1.21 (0.24)	1.84 (0.35)	1.63 (0.40)

### 2.3.1.2 Statistical analysis results

The Spike2 and LabChart data underwent similar statistical tests as detailed above. It was imperative to ascertain whether the Spike2 data were associated with different statistical results compared to LabChart, as there could have been issues with reproducibility and reliability.

Table 2.8 provides a summary of the statistical test outcomes for the within-visit analyses (Research question: Is there an effect of stimulus?). The table shows that when exploring the impact of the auditory stimuli on autonomic function within each visit the results are broadly consistent (as indicated by the yellow highlighter). Differences between Spike2 and LabChart emerged in Visit 1 for total and HF power: which were significantly higher in baseline than in the stepped increase in tempo stimulus for the Spike2 data; and for SBP: which was significantly higher in recovery compared to the two auditory stimuli for the LabChart data. Differences also transpired in Visit 2 in the LabChart data for SBP: which was significantly higher in baseline than during the stepped increase in tempo stimulus; and DBP: which was significantly higher in recovery than baseline.

Table 2.8: Summary of outcomes from the within-visit analyses for Spike2 and LabChart data.

	Visit 1		Visit 2	
	Spike2	LabChart	Spike2	LabChart
Total power	Baseline > Tempo increase			
HF power	Baseline > Recovery Tempo decrease > Tempo increase	Tempo decrease > Tempo increase		
HR	Baseline > Tempo increase > Tempo decrease > Recovery	Baseline > Tempo increase > Tempo decrease > Recovery	Tempo decrease < Recovery	Tempo decrease < Recovery
SBP	Recovery > Baseline	Recovery > Baseline > Tempo increase > Tempo decrease	Baseline < Tempo decrease < Recovery	Baseline < Tempo increase < Tempo decrease < Recovery
DBP				Baseline < Recovery
MAP			Baseline < Recovery	Baseline < Recovery

As the study also sought to determine whether visit had an impact on physiological responses to the stimuli (study objective 3), statistical tests were performed on the absolute change values for the Spike2 and LabChart data. Table 2.9 provides a summary of the statistical test outcomes for the between-visit analyses.

The table shows that different statistical outcomes emerged between the two computer programs. Indeed, differences between the two visits occurred in the Spike2 data only, despite the same statistical procedure being implemented and an identical sample being used. For the stepped increase in tempo stimulus, the decrease in total and HF power (between baseline and the tempo increase stimulus) was significantly greater in visit 1 compared to visit 2. Also, the decrease in LF power that occurred in visit 1 was significantly different to the increase that emerged in visit 2. For the stepped decrease in tempo stimulus, the decrease in total and LF power that emerged in visit 1 was significantly different to the increase that transpired in visit 2.

*Table 2.9: Summary of outcomes from the between-visit analyses for Spike2 and LabChart data.*

	Tempo increase		Tempo decrease	
	Spike2	LabChart	Spike2	LabChart
<b>Total power</b>	Visit 1 (↓↓) · Visit 2 (↓)		Visit 1 (↓) · Visit 2 (↑)	
<b>LF power</b>	Visit 1 (↓) · Visit 2 (↑)		Visit 1 (↓) · Visit 2 (↑)	
<b>HF power</b>	Visit 1 (↓↓) · Visit 2 (↓)			

Taken together these results demonstrate that the computer program moderately impacted the results. This was particularly the case when examining the discrepant between-visit results (see Table 2.9).

Nevertheless, as these differences emerged for only three variables, the importance of these differences is questionable. This is particularly noteworthy given there were no discrepancies for the primary outcome measure (LF/HF), these are indirect measures of autonomic function and there remains contention surrounding frequency-domain HRV analysis (Billman, 2013). Therefore, computer program use may not have had as



large an impact as initially thought. Indeed, using LabChart over Spike2 may be associated with greater benefits:

1. LabChart has greater functionality, allowing the derivation of more variables than Spike2 (e.g. time-domain and non-linear HRV, spontaneous sequence BRS and ECG parameters). In turn, this facilitates greater exploration of the effects of tempo manipulations on cardiovascular autonomic function.
2. LabChart avoids deriving parameters by hand, therefore reducing the risk of human error and improving reliability. For instance, the LabChart HRV module automatically computes HRV, whereas Spike2 requires the user to complete each step in the process. Also, when deriving HR and BP, LabChart automatically detects the R and systolic peaks and diastolic troughs (which can be checked and altered by the user if incorrect), whereas Spike2 requires the user to condense the trace and place a cursor through the peaks and troughs.
3. LabChart is more commonly used by labs investigating cardiovascular autonomic function than Spike2. This renders comparing results and conclusions more valid and reduces the risk of different computer programs being responsible for inconsistencies in the literature.

Considering these Spike2-LabChart comparisons the decision was made to use LabChart as the preferred computer program. Therefore, the following reported results come from the LabChart analysed data.

### **2.3.2 The effects of the auditory stimuli on cardiac autonomic control**

First, differences in baseline measures between the two visits were ascertained. Statistically significant differences in baseline HF, SD1, nSD1, up BRS and mean BRS were identified. See Table 2.10 for a summary. For all variables, baseline readings were significantly higher in visit 1 than in visit 2. Thus, the between-visit analyses needed to control for these differences in baseline measures. This was achieved by using the absolute change values.

Table 2.10: Statistically significant differences in baseline measures between visit 1 and visit 2.

Measure	Visit 1 mean (SEM)	Visit 2 mean (SEM)	t-test (or equivalent) p-value
HF power	1334.51 (295.84)	970.49 (190.71)	0.043
SD1	33.00 (3.35)	28.66 (2.96)	0.050
nSD1	37.64 (3.25)	32.93 (3.00)	0.041
Up BRS	17.01 (1.51)	13.72 (1.14)	0.027
Mean BRS	17.04 (1.53)	13.87 (1.11)	0.022

### 2.3.2.1 Time-domain HRV analysis

#### 2.3.2.1.1 Visit 1

In the first visit, a statistically significant effect of the auditory stimuli occurred on HR ( $p < 0.001$ ). Further analysis revealed that HR was significantly higher in baseline compared to the stepped increase and decrease in tempo stimuli (both  $p < 0.001$ ) and recovery ( $p = 0.004$ , see Figure 2.7).

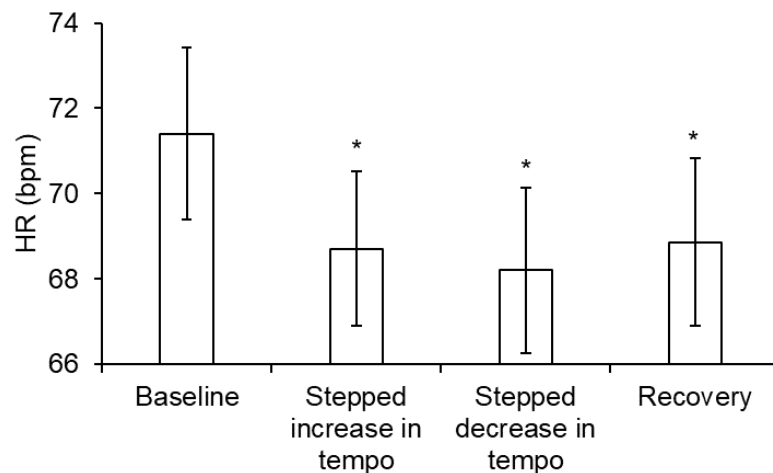
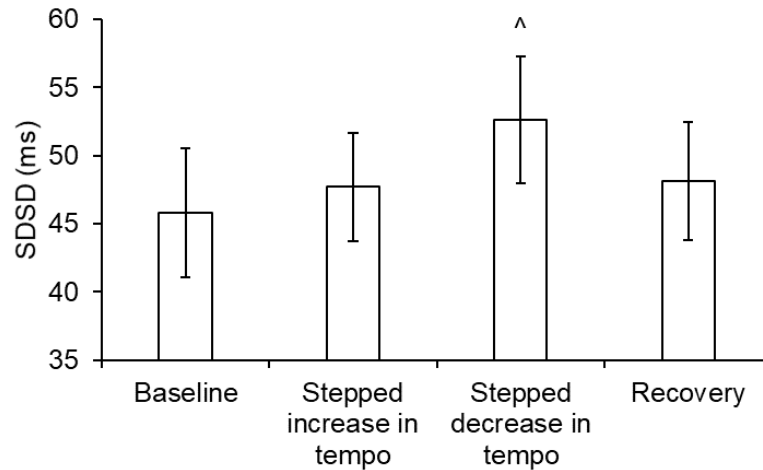


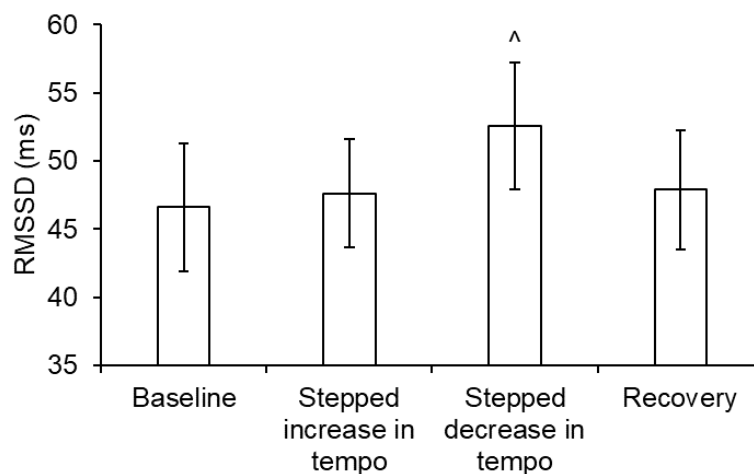
Figure 2.7: HR was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

SDSD was also found to be significantly impacted by the two stimuli ( $p = 0.011$ ). During the stepped decrease in tempo stimulus, SDSD was significantly higher than that during the stepped increase in tempo stimulus ( $p = 0.001$ , see Figure 2.8). No other significant differences emerged ( $p > 0.008$ ).



*Figure 2.8: SDSD was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. ^ = significantly different to the stepped increase in tempo stimulus.*

A similar pattern emerged for RMSSD (Friedman test:  $p = 0.011$ ). As shown in Figure 2.9, RMSSD during the stepped decrease in tempo stimulus was significantly lower than that during the stepped increase in tempo stimulus ( $p = 0.001$ ).



*Figure 2.9: RMSSD was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. ^ = significantly different to the stepped increase in tempo stimulus.*

Finally, there was a significant effect of the auditory stimuli on pRR50 ( $p = 0.034$ ). Figure 2.10 illustrates that pRR50 was significantly higher during the stepped decrease in tempo stimulus compared to baseline ( $p = 0.007$ ). No other significant differences were observed ( $p > 0.008$ ).

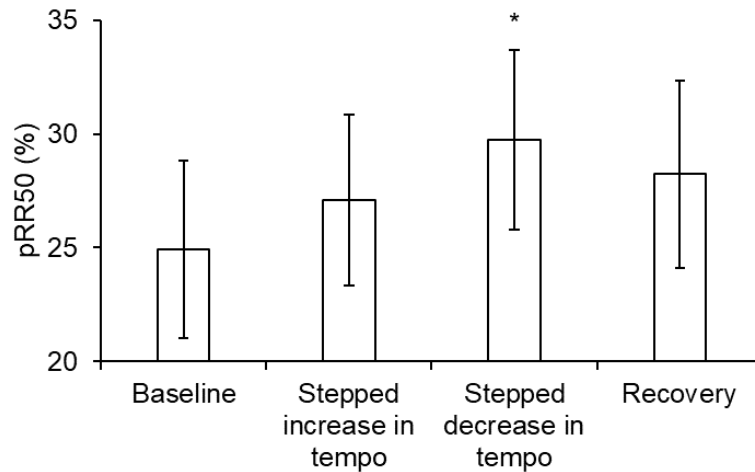


Figure 2.10: pRR50 was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

### 2.3.2.1.2 Visit 2

The only statistically significant difference that emerged was for HR ( $p = 0.030$ ). Bonferroni pairwise comparisons revealed that HR during the stepped decrease in tempo stimulus was significantly lower than that for recovery ( $p = 0.036$ , see Figure 2.11).

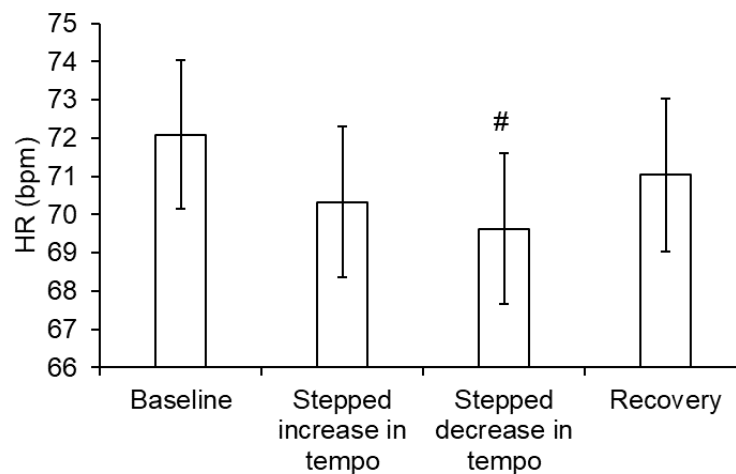


Figure 2.11: HR was significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. # = significantly different to recovery.

### 2.3.2.1.3 Between-visit comparison

No statistically significant differences emerged between the two visits for either stimulus ( $p > 0.05$ ).

### 2.3.2.2 Frequency-domain HRV analysis

#### 2.3.2.2.1 Visit 1

The only significant effect of the auditory stimuli on frequency-domain HRV occurred for HF power ( $p = 0.030$ ). As illustrated in Figure 2.12, HF power was significantly higher during the stepped decrease in tempo stimulus compared to the stepped increase in tempo stimulus ( $p = 0.003$ ).

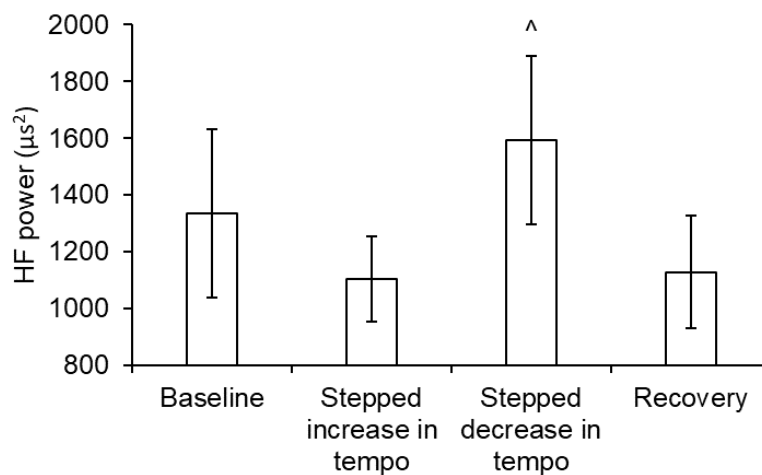


Figure 2.12: HF power was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. ^ = significantly different to stepped increase in tempo.

#### 2.3.2.2.2 Visit 2

No statistically significant differences emerged for any of the frequency-domain HRV parameters ( $p > 0.05$ ).

### 2.3.2.2.3 Between-visit comparison

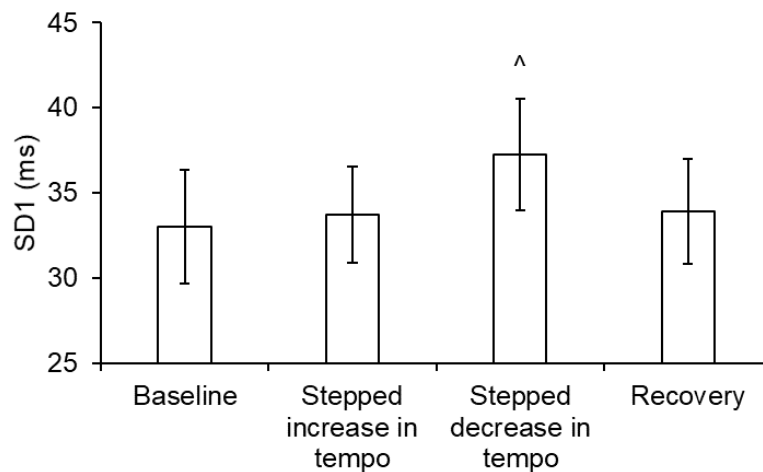
No statistically significant differences emerged between the two visits for either stimulus ( $p > 0.05$ ).

### 2.3.2.3 Non-linear HRV analysis

#### 2.3.2.3.1 Visit 1

Similar to the patterns observed in SDSD, RMSSD and HF power, effects on SD1 and S also transpired ( $p = 0.011$  and  $p = 0.029$  respectively). SD1 and S during the stepped decrease in tempo stimulus were significantly higher than that during the stepped increase in tempo stimulus (both  $p = 0.001$ , see Figure 2.13a and Figure 2.13b). It should be noted that the similarity in results between SDSD, RMSSD and SD1 is due to them being identical metrics of HRV (Ciccone et al., 2017).

a.



b.

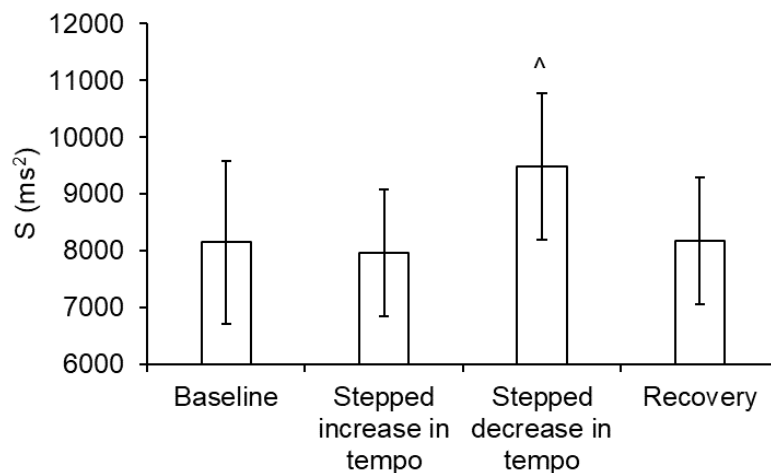


Figure 2.13: SD1 (a) and S (b) were significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. ^ = significantly different to stepped increase in tempo.

### **2.3.2.3.2 Visit 2**

No statistically significant differences emerged for any of the non-linear HRV parameters in this visit ( $p > 0.05$ ).

### **2.3.2.3.3 Between-visit comparison**

No statistically significant differences emerged between the two visits for either stimulus ( $p > 0.05$ ).

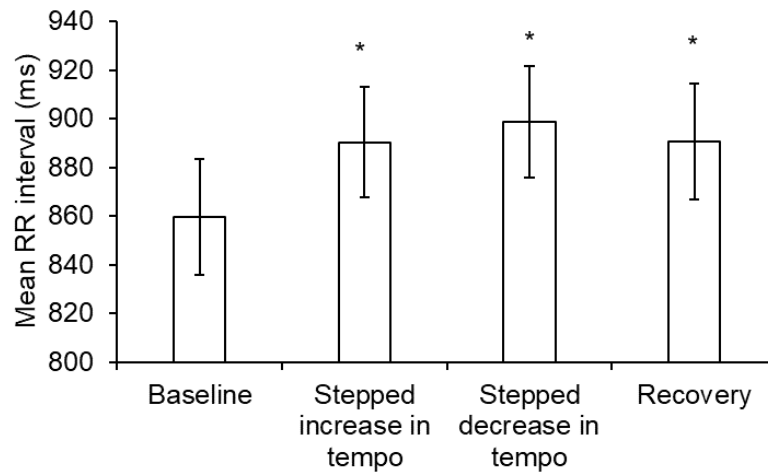
### **2.3.2.4 RR analysis**

#### **2.3.2.4.1 Visit 1**

Mean RR interval was significantly impacted by the stimuli ( $p < 0.001$ ). Bonferroni pairwise comparisons revealed that baseline mean RR interval was significantly greater during the stepped increase in tempo stimulus ( $p < 0.001$ ), stepped decrease in tempo stimulus ( $p = 0.001$ ) and recovery condition ( $p = 0.021$ , see Figure 2.14a).

Similar findings emerged when examining median RR interval (ANOVA:  $p < 0.001$ ). For instance, median RR during baseline was significantly lower than during the stepped increase in tempo stimulus ( $p < 0.001$ ), stepped decrease in tempo stimulus ( $p = 0.001$ ) and recovery condition ( $p = 0.010$ , see Figure 2.14b).

a.



b.

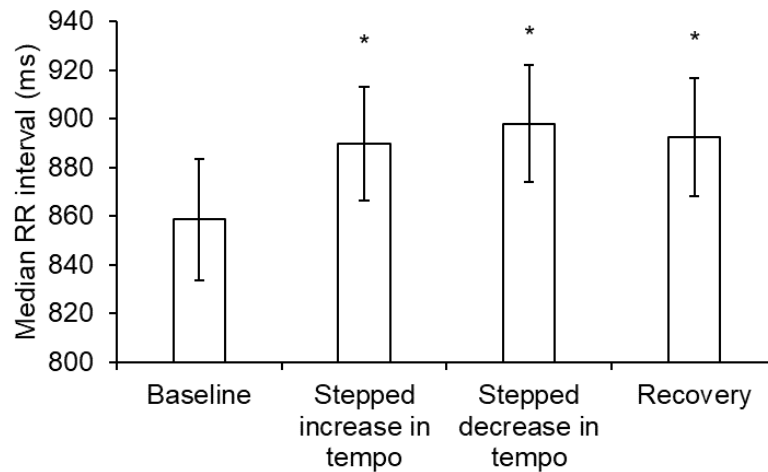
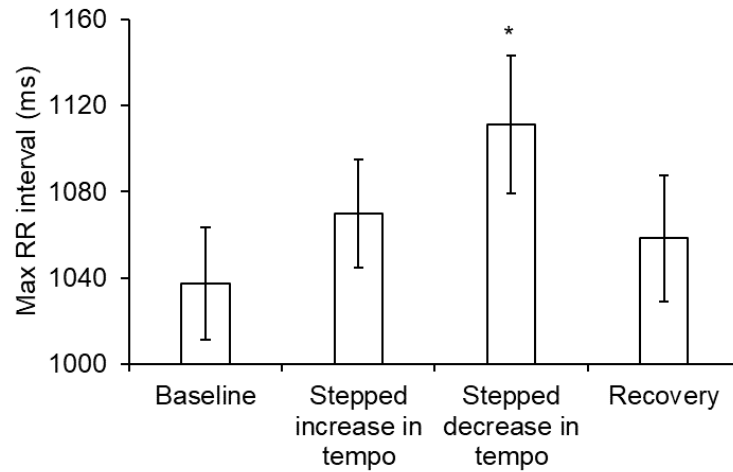


Figure 2.14: Mean (a) and median (b) RR intervals were significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

Although min and  $\Delta$  RR did not change between the four conditions ( $p > 0.05$ ), max RR did ( $p = 0.001$ ). Indeed, Bonferroni identified a significant difference between baseline in the stepped decrease in tempo stimulus ( $p = 0.002$ ). Examination of Figure 2.15 shows that max RR was significantly higher during the stepped decrease in tempo stimulus than in baseline.

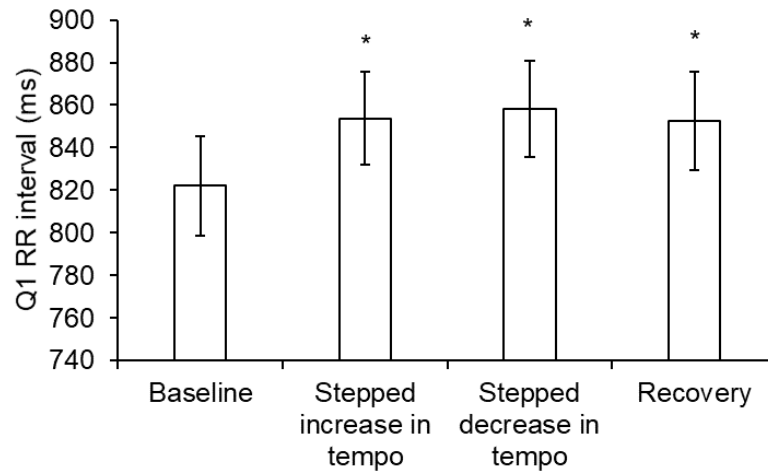




*Figure 2.15: Max RR interval was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.*

Q1 and Q3 RR intervals were significantly impacted by the stimuli (both  $p < 0.001$ ). As demonstrated in Figure 2.16a and b, Q1 and Q3 RR intervals were significantly lower during baseline than during the stepped increase in tempo stimulus ( $p < 0.001$  and  $p = 0.001$  respectively), the stepped decrease in tempo stimulus ( $p = 0.002$  and  $p = 0.001$ ) and the recovery condition ( $p = 0.024$  and  $p = 0.012$ ). Despite these differences, IQR RR was not found to significantly differ ( $p > 0.05$ ).

a.



b.

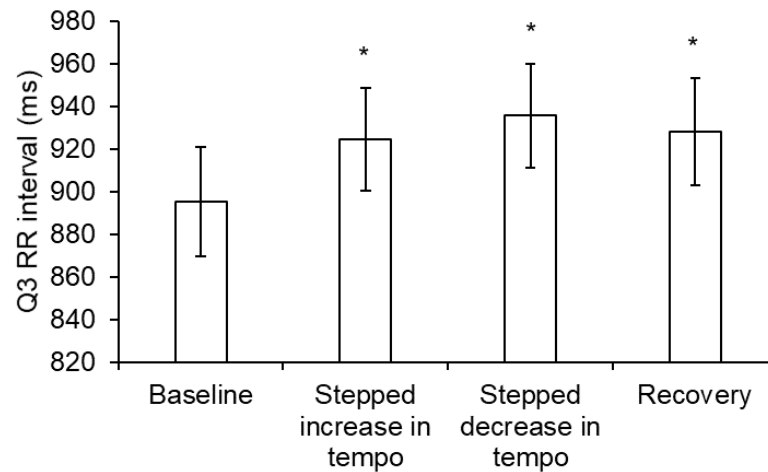


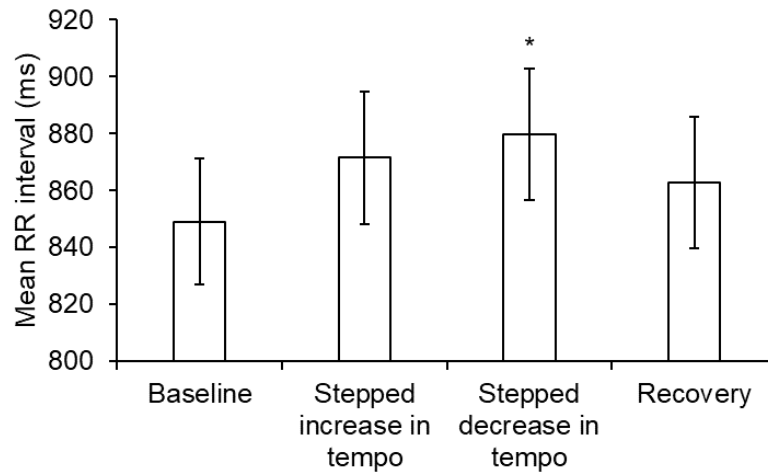
Figure 2.16: Q1 (a) and Q3 (b) RR intervals were significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 2.3.2.4.2 Visit 2

Like visit 1, a significant effect of the stimuli occurred on mean RR interval ( $p = 0.003$ ). However, the only significant difference that emerged with Bonferroni was between baseline and the stepped decrease in tempo stimulus ( $p = 0.027$ ). As shown in Figure 2.17a, mean RR was significantly higher during the stepped decrease in tempo stimulus compared to baseline ( $p = 0.027$ ).

A similar pattern emerged for median RR interval ( $p = 0.002$ ). Median RR during baseline was significantly lower than that during the stepped decrease in tempo stimulus ( $p = 0.028$ , see Figure 2.17b).

a.



b.

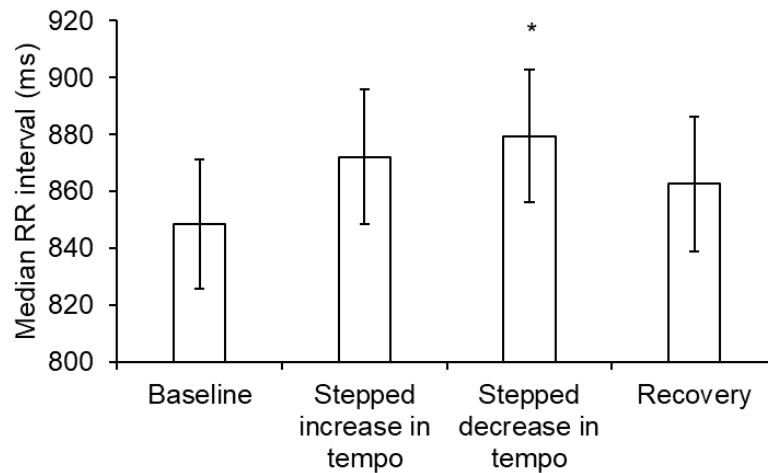
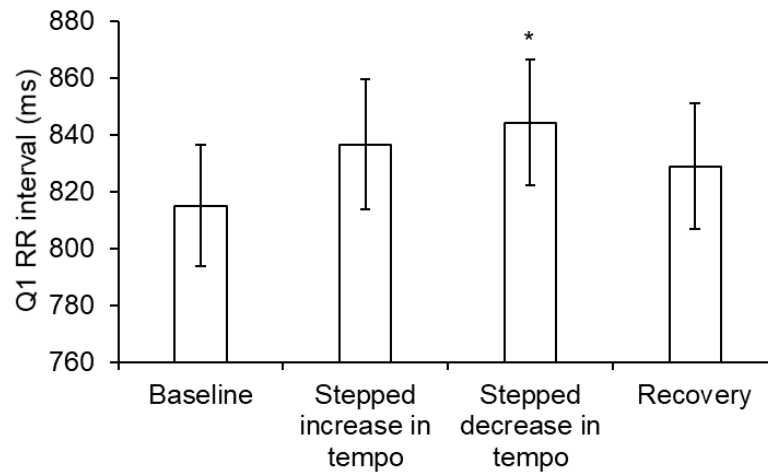


Figure 2.17: Mean (a) and median (b) RR intervals were significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

In contrast to visit 1 which found no significant change in Q1 RR intervals, a significant effect emerged in visit 2 ( $p = 0.010$ ). As shown in Figure 2.18a, Q1 RR was significantly greater during the stepped decrease in tempo stimulus compared to baseline ( $p = 0.035$ ). Although the same differences were found for Q3 RR (ANOVA:  $p = 0.003$ ; Bonferroni:  $p = 0.025$ , see Figure 2.18b), no significant change in IQR RR was detected ( $p > 0.05$ ).

a.



b.

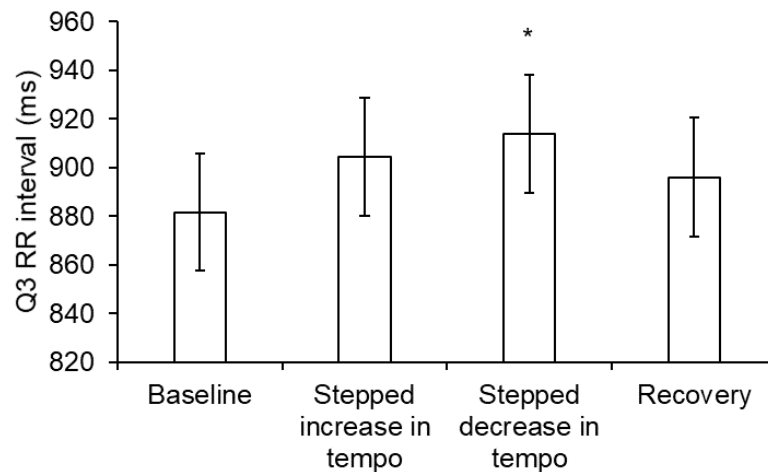


Figure 2.18: Q1 (a) and Q3 (b) RR intervals were significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

### 2.3.2.4.3 Between-visit comparison

No statistically significant differences emerged between the two visits for either stimulus ( $p > 0.05$ ).

### 2.3.2.5 ECG analysis

#### 2.3.2.5.1 Visit 1

A statistically significant effect of the auditory stimuli on QT interval emerged ( $p = 0.021$ ). As shown in Figure 2.19, QT interval was significantly greater during the stepped increase in tempo stimulus compared to baseline ( $p =$

0.004). However, this difference failed to emerge after correcting for HR (QTc:  $p > 0.05$ ).

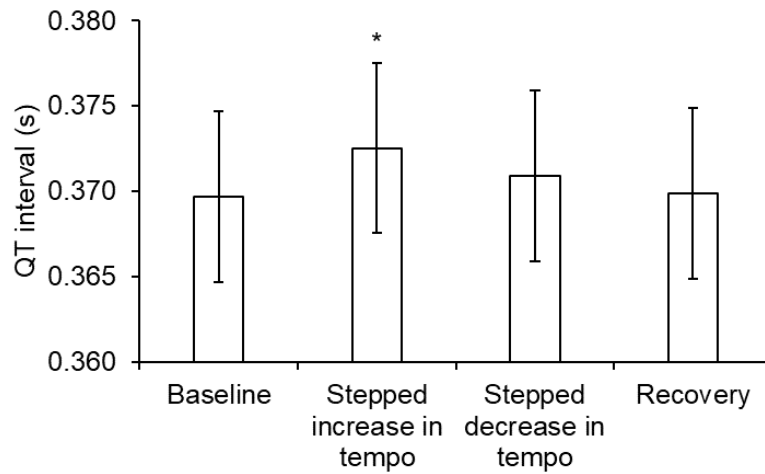


Figure 2.19: QT interval was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

Nevertheless, statistically significant differences transpired for Q amplitude ( $p < 0.001$ ). Bonferroni pairwise comparisons demonstrated that Q amplitude during the stepped increase in tempo stimulus was significantly smaller than that during baseline ( $p = 0.034$ ). In addition, recovery Q amplitude was significantly smaller than that during baseline ( $p = 0.002$ ), the stepped increase in tempo stimulus ( $p = 0.030$ ) and the stepped decrease in tempo stimulus ( $p = 0.004$ , see Figure 2.20).

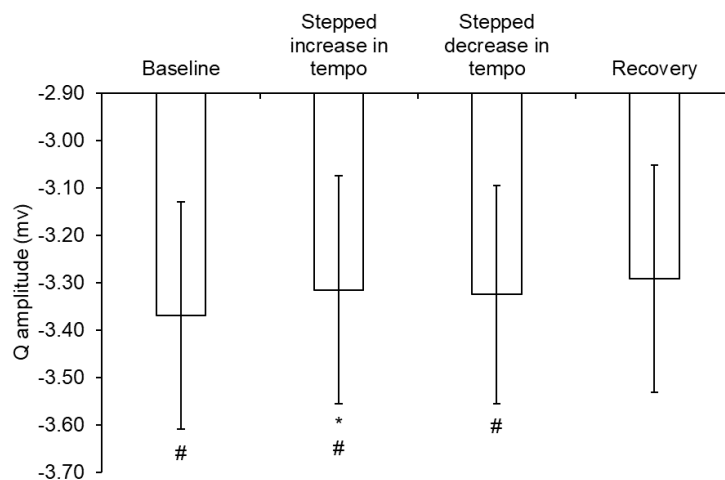
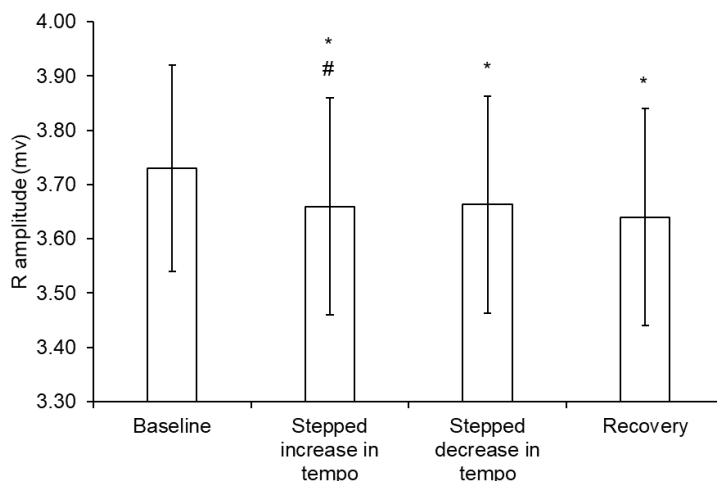


Figure 2.20: Q amplitude was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Interestingly, R amplitude was also significantly impacted by the stimuli ( $p < 0.001$ ). As illustrated in Figure 2.21, R amplitude was significantly lower during the stepped increase in tempo stimulus ( $p < 0.001$ ), stepped decrease in tempo stimulus ( $p = 0.001$ ) and recovery condition ( $p < 0.001$ ) compared to baseline. Furthermore, the difference between recovery and the stepped increase in tempo stimulus just reached significance ( $p = 0.008$ ): R amplitude was significantly smaller in the former compared to the latter.



*Figure 2.21: R amplitude was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.*

Finally, a significant effect on T amplitude also occurred ( $p < 0.001$ ). T amplitude was significantly smaller in the two stimuli (stepped increase in tempo:  $p = 0.002$ ; stepped decrease in tempo:  $p = 0.003$ ) and recovery ( $p = 0.019$ ) compared to baseline (see Figure 2.22).

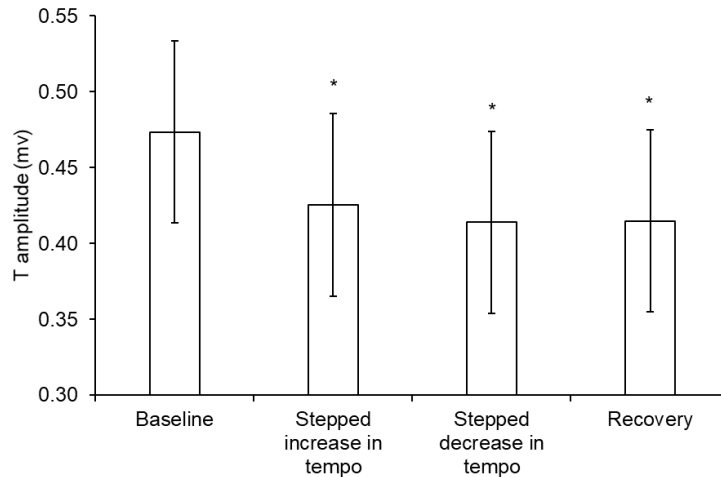


Figure 2.22: T amplitude was significantly impacted by the auditory stimuli in visit 1. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

### 2.3.2.5.2 Visit 2

In visit 2, a significant effect on Q amplitude also occurred ( $p = 0.001$ ).

Bonferroni pairwise comparisons demonstrated that Q amplitude was significantly smaller in the stepped decrease in tempo stimulus ( $p = 0.033$ ) and recovery condition ( $p = 0.014$ ) compared to baseline (see Figure 2.23).

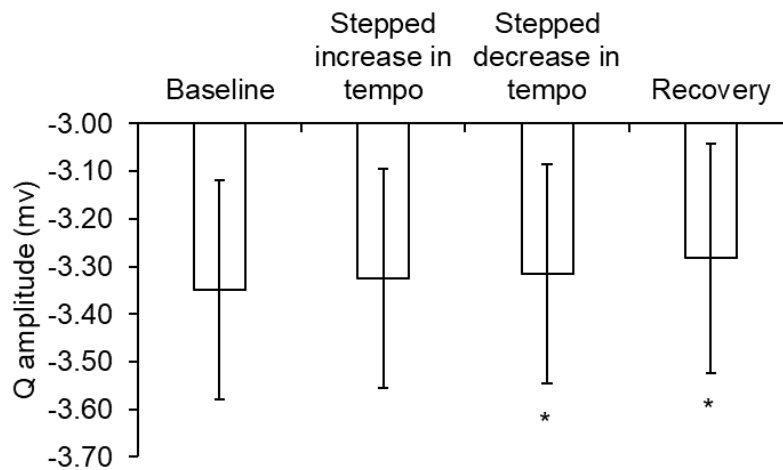


Figure 2.23: Q amplitude was significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

S amplitude also significantly differed between the conditions ( $p = 0.029$ ). As shown in Figure 2.24, S amplitude was significantly lower in recovery than baseline ( $p = 0.030$ ).

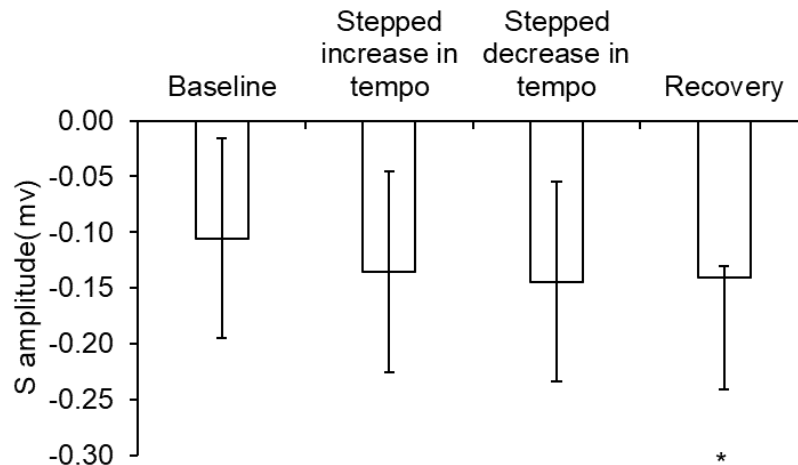


Figure 2.24: S amplitude was significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.

Finally, the changes in T amplitude observed in visit 1 also manifested in visit 2 ( $p < 0.001$ ). Indeed, Bonferroni pairwise comparisons demonstrated that T amplitude was significantly smaller in the stepped increase in tempo stimulus ( $p = 0.006$ ), stepped decrease in tempo stimulus ( $p = 0.001$ ) and recovery condition ( $p = 0.013$ ) compared to baseline (see Figure 2.25). No other significant differences emerged ( $p > 0.05$ ).

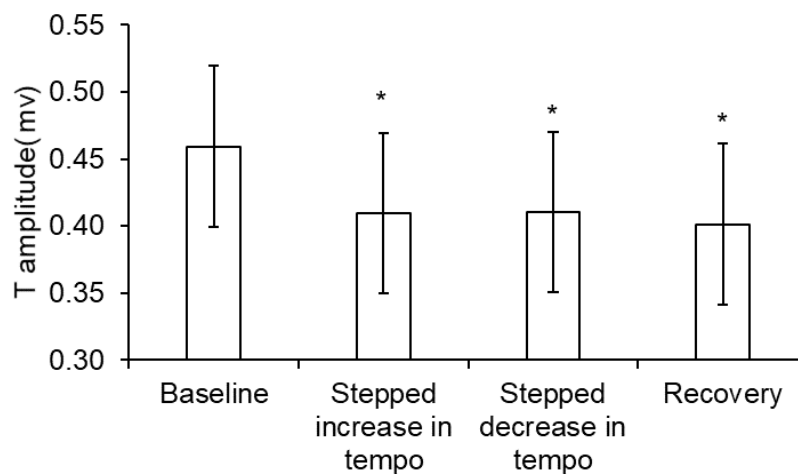
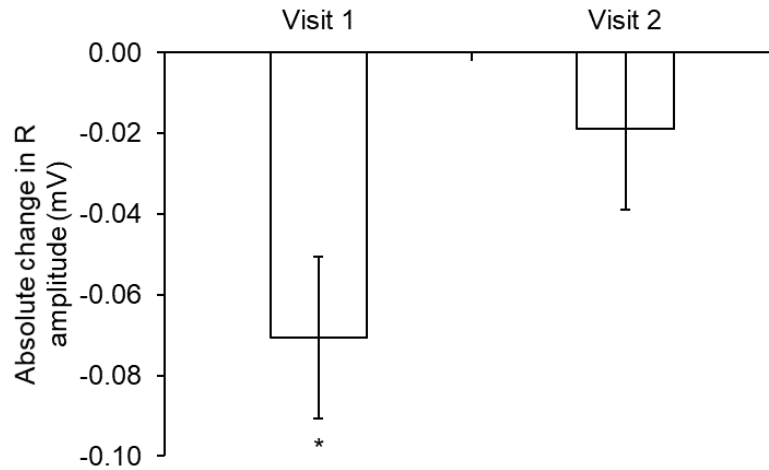


Figure 2.25: T amplitude was significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to baseline.



### 2.3.2.5.3 Between-visit comparison

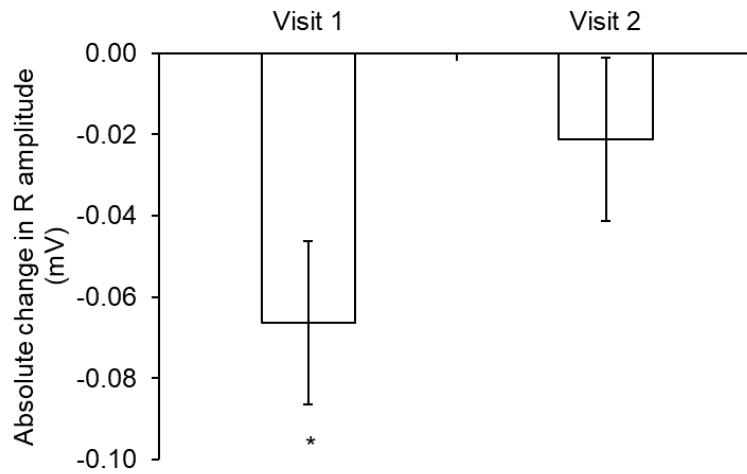
A statistically significant difference in R amplitude emerged between the two visits for the stepped increase in tempo stimulus ( $p = 0.013$ ). As shown in Figure 2.26, change in R amplitude between baseline and the stimulus was significantly greater during visit 1 than visit 2.



*Figure 2.26: The decrease in R amplitude between baseline and the stepped increase in tempo stimulus was significantly greater in visit 1 than in visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to stepped increase in tempo.*

A similar pattern in R amplitude between visits 1 and 2 also occurred for the stepped decrease in tempo stimulus ( $p = 0.030$ ). Like the stepped increase in tempo stimulus, change in R amplitude was significantly greater in visit 1 than visit 2 for the stepped decrease in tempo stimulus (see Figure 2.27a). However, as shown in Figure 2.27b, a significantly greater decrease in S amplitude was detected in visit 2 when compared to visit 1 ( $p = 0.043$ ) for the stepped decrease in tempo stimulus.

a.



b.

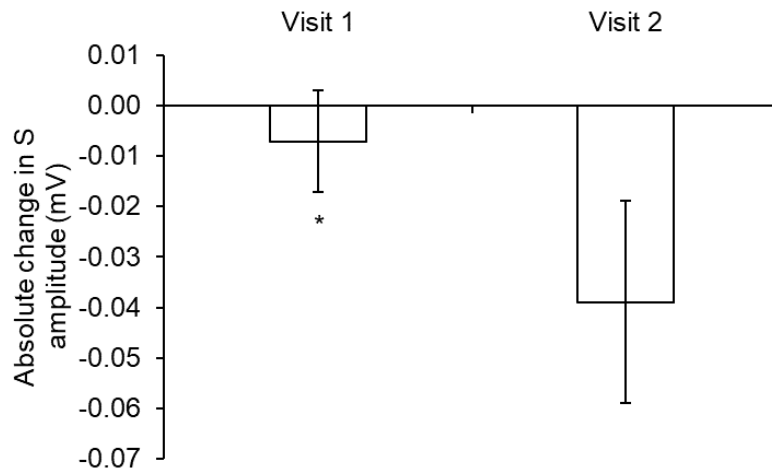


Figure 2.27: The decrease in R amplitude (a) between baseline and the stepped decrease in tempo stimulus was significantly greater in visit 1 than in visit 2. S amplitude (b) was less pronounced in visit 1 compared to visit 2. Data presented as the mean  $\pm$  1 SEM. \* = significantly different to visit 2.

### 2.3.2.6 BP, BRS and respiration

#### 2.3.2.6.1 Visit 1

The only significant effect that occurred was for SBP ( $p < 0.001$ ). Bonferroni pairwise comparisons revealed that SBP was significantly higher during recovery than baseline ( $p = 0.005$ ) and during the stepped increase ( $p = 0.038$ ) and decrease ( $p = 0.044$ ) in tempo stimuli (see Figure 2.28).

No statistically significant differences emerged for any of the BRS measures or for respiration ( $p > 0.05$ ).

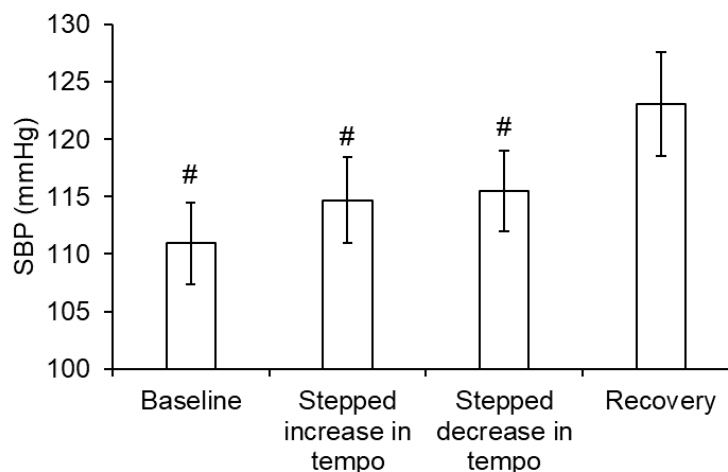


Figure 2.28: SBP in visit 1 significantly differed between the four conditions. Data presented as the mean  $\pm$  1 SEM. # = significantly different to recovery.

### 2.3.2.6.2 Visit 2

Similar to visit 1, a significant effect of the auditory stimuli on SBP occurred in visit 2 ( $p < 0.001$ ). Recovery SBP was significantly higher than that during baseline ( $p = 0.049$ ) and the stepped increase ( $p = 0.030$ ) and decrease ( $p < 0.001$ ) in tempo stimuli (see Figure 2.29).

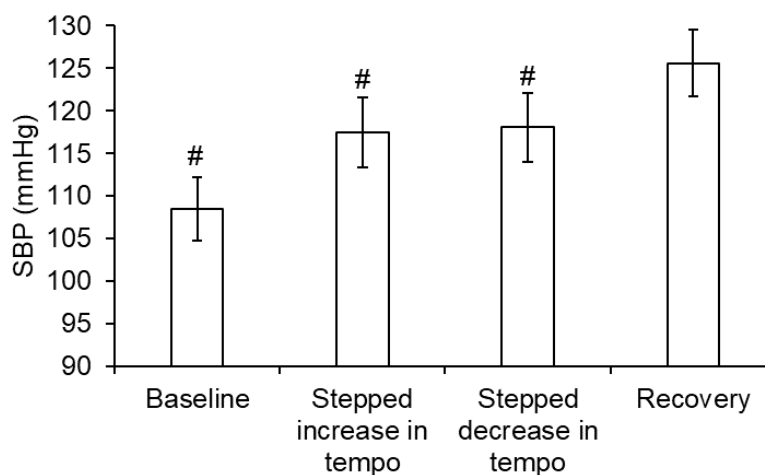
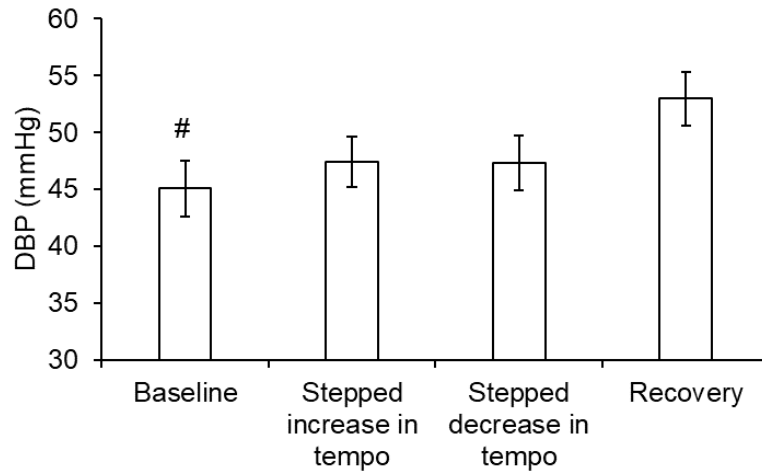


Figure 2.29: SBP was significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. # = significantly different to recovery.

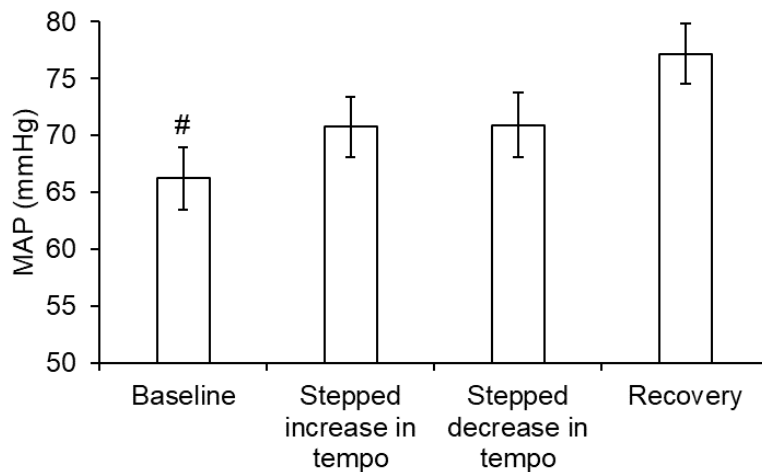
However, unlike visit 1, DBP was also found to significantly change over the course of the four conditions ( $p = 0.007$ ). Further examination revealed that recovery DBP was significantly greater than baseline DBP ( $p = 0.002$ , see Figure 2.30a). These differences manifested in MAP (ANOVA:  $p < 0.001$ ),

whereby recovery MAP was significantly higher than baseline MAP ( $p = 0.001$ , see Figure 2.30b).

**a.**



**b.**



*Figure 2.30: DBP (a) and MAP (b) were significantly impacted by the auditory stimuli in visit 2. Data presented as the mean  $\pm$  1 SEM. # = significantly different to recovery.*

No statistically significant differences emerged for any of the BRS measures or for respiration ( $p > 0.05$ ).

### **2.3.2.6.3 Between-visit comparison**

No statistically significant differences emerged between the two visits for either stimulus ( $p > 0.05$ ).

### 2.3.2.7 Summary of the cardiovascular autonomic results

To aid with summarising the above cardiovascular autonomic results, the following tables were generated. This entailed grouping together all the measures that were considered to reflect parasympathetic activity (see Table 2.11), and all remaining measures (see Table 2.12).

#### 2.3.2.7.1 Parasympathetic measures summary

Table 2.11 summarises the changes in parasympathetic measures that occurred for the stepped increase in tempo and stepped decrease in tempo stimuli. A marked difference between the two visits clearly exists: no changes in vagal tone occurred for either stimulus in visit 2. In contrast, all changes in vagal tone occurred for either stimulus in visit 1. In contrast, all changes that transpired in the first visit applied to the stepped decrease in tempo stimulus only. This means that the stepped decrease in tempo stimulus during the first visit was associated with the greatest shift towards parasympathetic predominance.

*Table 2.11: Summary of changes in measures of parasympathetic activity.*

Parameter	Visit 1		Visit 2	
	Stepped increase in tempo	Stepped decrease in tempo	Stepped increase in tempo	Stepped decrease in tempo
SDSD		↑		
RMSSD		↑		
pRR50		↑		
HF power		↑		
SD1		↑		

No statistically significant differences in any measure of parasympathetic activity occurred between the two visits for either stimulus. This is mildly surprising given that all measures of parasympathetic activity increased during the stepped decrease in tempo stimulus in visit 1, whilst none occurred in visit 2.

#### 2.3.2.7.2 Remaining cardiovascular autonomic measures summaries

Table 2.12 summarises the significant differences that occurred for the stepped increase in tempo and stepped decrease in tempo stimuli. Inspection of the table shows that no meaningful differences emerged between the two stimuli in visit 1. That is, the stepped increase and decrease in tempo stimuli showed similar increases in overall variability.

In contrast, the stepped decrease in tempo stimulus in visit 2 was associated with more variables that changed compared to the stepped increase in tempo stimulus. This means that greater variability in cardiac autonomic activity transpired for the stepped decrease in tempo stimulus.

*Table 2.12: Summary of changes in remaining measures of cardiovascular autonomic function.*

Parameter	Visit 1		Visit 2	
	Stepped increase in tempo	Stepped decrease in tempo	Stepped increase in tempo	Stepped decrease in tempo
HR	↓	↓		↓
S		↑		
Mean RR	↑	↑		↑
Median RR	↑	↑		↑
Max RR		↑		
Q1 RR	↑	↑		↑
Q3 RR	↑	↑		↑
QT interval	↑			
Q amplitude	↑			
R amplitude	↓	↓		
T amplitude	↓	↓	↓	↓
SBP	↓	↓	↓	↓

Only two measures significantly differed between visit 1 and visit 2 for the stepped increase and decrease in tempo stimuli, these were R and S amplitude (see Table 2.13). The decrease in R amplitude was significantly greater in visit 1 for both stimuli, whereas the decrease in S amplitude was significantly greater in visit 2 compared to visit 1 for the stepped decrease in tempo stimulus only. This suggests that heart contractions were weaker during visit 1 than visit 2, pointing to the claim that participants were less physiologically aroused during their first visit compared to their second visit.

*Table 2.13: Summary of changes in remaining measures of cardiovascular autonomic function that occurred between the two visits.*

Parameter	Stepped increase in tempo stimulus	Stepped decrease in tempo stimulus
R amplitude	Greater decrease in Visit 1	Greater decrease in Visit 1
S amplitude		Greater decrease in Visit 2

### 2.3.3 Predicting physiological response to the auditory stimuli

The literature frequently reports the occurrence of participants responding to auditory stimuli in different ways (Ellis & Brighthouse, 1952; Siddle & Heron, 1976; Haas et al., 1986; Khalifa, Peretz, Blondin & Manon, 2002).

Examination of the data at the level of individual participants revealed that not all participants responded in the same direction. That is, some showed an increase in LF/HF between baseline and the stepped increase in tempo stimulus whilst others showed a decrease. If one of the aims of using music in a therapeutic manner is to reduce sympathetic dominance, it would be of interest to explore the extent to which it is possible to predict the direction of change in measures of sympathetic and parasympathetic activity. Indeed, despite using a different stimulus, Clancy et al. (2014) showed that baseline LF/HF significantly predicted percentage change to transcutaneous vagal nerve stimulation (tVNS). Therefore, a series of regressions was performed investigating the plausibility of predicting physiological responses to the two auditory stimuli. Table 2.14 summarises the measures of interest and their regression outcomes.

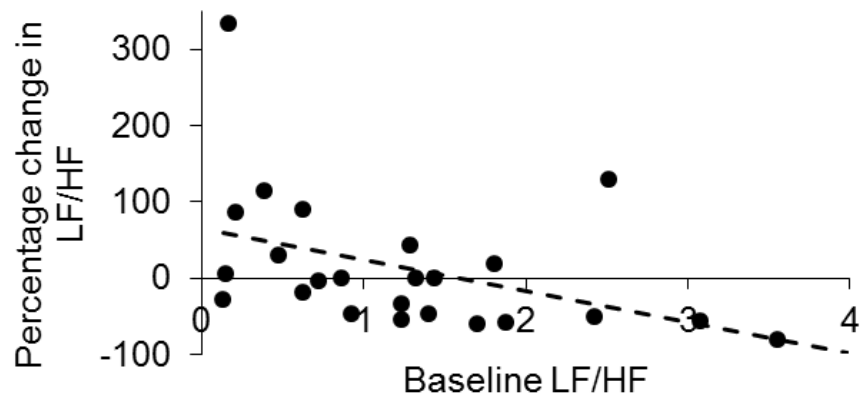
### 2.3.3.1 Regression analyses

Table 2.14: Summary of statistically significant linear regressions.

	Visit 1						Visit 2					
	Baseline → Tempo increase		Baseline → Tempo decrease		Tempo increase → Tempo decrease		Baseline → Tempo increase		Baseline → Tempo decrease		Tempo increase → Tempo decrease	
	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value
LF power												
HF power	0.181	0.038			0.180	0.039	0.228	0.018	0.164	0.050		
LF% power							0.283	0.007	0.432	< 0.001		
HF% power	0.284	0.007							0.175	0.042		
nuLF							0.325	0.004	0.312	0.005		
nuHF			0.165	0.049			0.299	0.006	0.224	0.019		
LF/HF							0.180	0.039				
SDRR							0.253	0.012	0.190	0.033		
SDSD							0.236	0.016	0.251	0.013		
RMSSD							0.236	0.016	0.251	0.013		
SD1							0.236	0.016	0.251	0.013		
SD2							0.219	0.021	0.199	0.029		
nSD1							0.194	0.031	0.283	0.007		
nSD2							0.174	0.043	0.191	0.033		
SD1/SD2	0.295	0.006							0.246	0.014		
SD2/SD1	0.249	0.013							0.267	0.010		
S							0.281	0.008	0.171	0.044		
Mean RR	0.209	0.025										
Median RR	0.239	0.015										
Δ RR									0.173	0.043		
IQR RR							0.320	0.004	0.176	0.041		

Partially supporting Clancy et al.'s (2014) findings, baseline LF/HF significantly predicted percentage change in LF/HF between baseline and the stepped increase in tempo stimulus in visit 2 ( $R^2 = 0.180$ ,  $p = 0.039$ ). Indeed, Figure 2.31 illustrates that the higher the baseline LF/HF, the greater the decrease in LF/HF. Meaning that participants who had a low baseline LF/HF experienced a larger increase in LF/HF when presented with the

stepped increase in tempo stimulus in visit 2 than those with a higher baseline LF/HF.



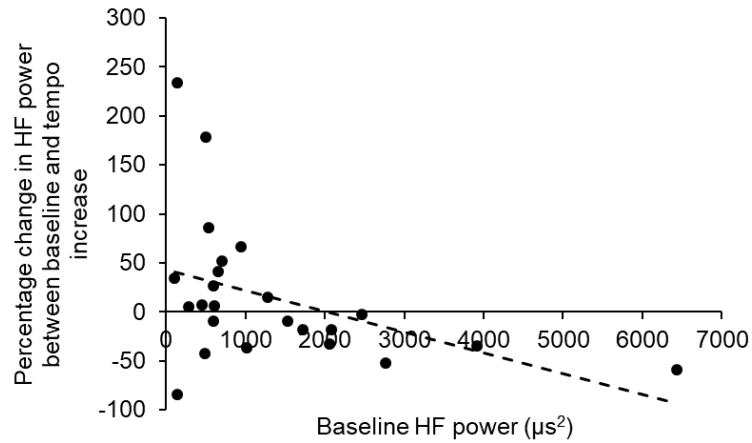
*Figure 2.31: Baseline LF/HF significantly predicted response to the stepped increase in tempo stimulus. Reductions in LF% between baseline and the stepped increase in tempo stimulus increased with higher baseline LF/HF ( $p = 0.039$ ).*

However, baseline LF/HF did not significantly predict any of the other regression comparisons (e.g. visit 2 baseline  $\rightarrow$  tempo decrease).

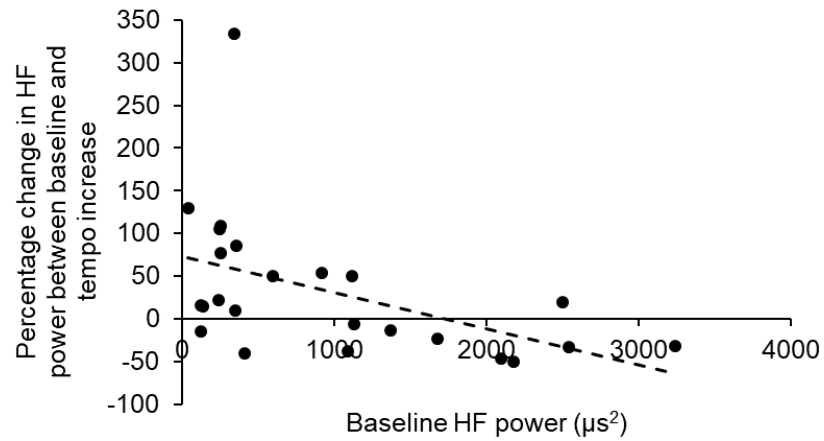
Interestingly, the variable that predicted the most regressions was HF power. Indeed, Figure 2.32a-c show that high baseline HF power was associated with greater reductions in HF power during both stimuli. This means that participants who had low HF power during baseline encountered an increase during the stimuli, whereas those who had high HF power in baseline experienced a reduction.



a.



b.



c.

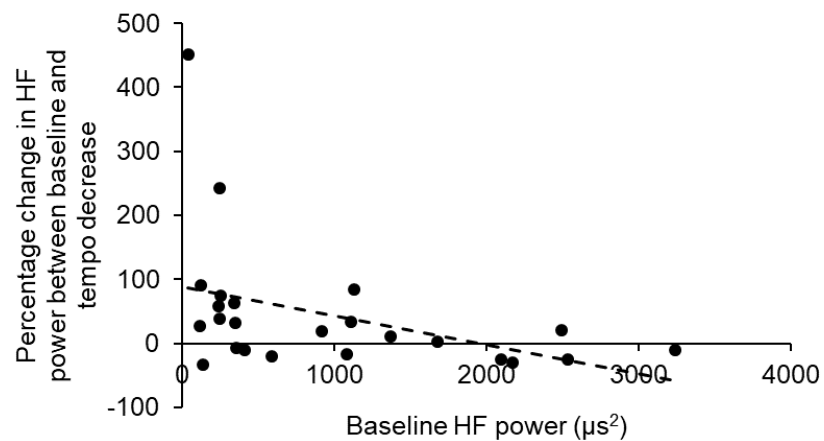
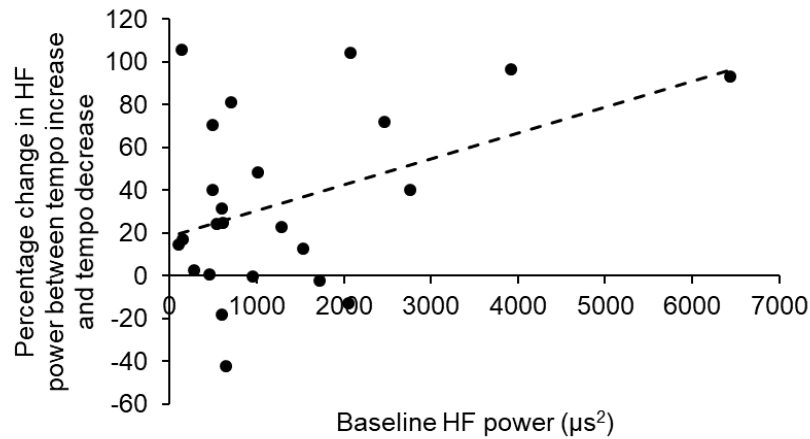
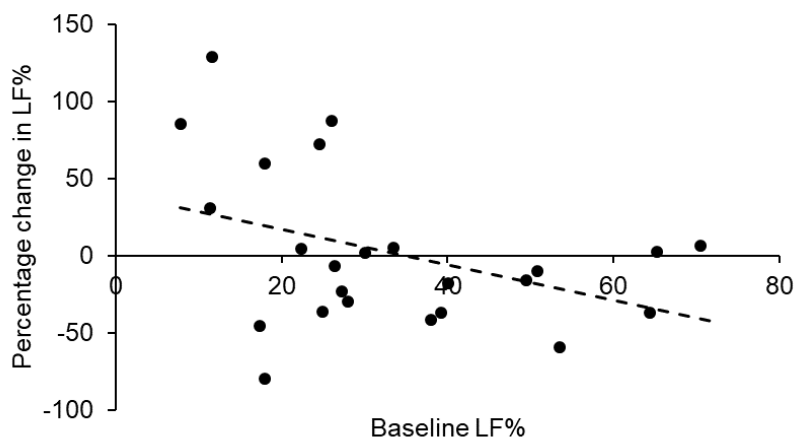


Figure 2.32: In visit 1 (a) and visit 2 (b) reductions in HF power between baseline and stepped increase in tempo increased with higher baseline HF power ( $p = 0.038$  and  $0.018$  respectively). This was also the case in visit 2 for the stepped decrease in tempo stimulus (c,  $p = 0.050$ ).

In contrast, a different pattern emerged when plotting baseline HF power against percentage change in HF power between the stepped increase and decrease in tempo stimuli in visit 1 (see Figure 2.33). Indeed, when exploring response patterns between the two auditory stimuli the higher a participant's baseline HF power, the greater the increase in HF power between the two auditory stimuli.



a.



b.

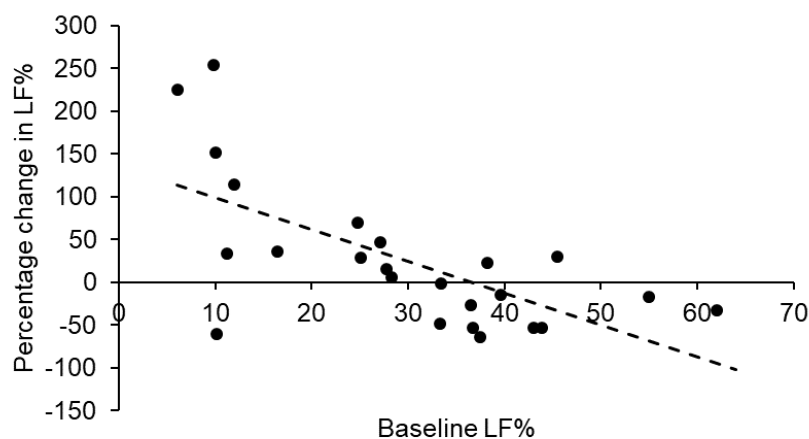


Figure 2.34: Baseline LF% significantly predicted response to the stepped increase and decrease in tempo stimuli in visit 2. In the stepped increase in tempo (a) and stepped decrease in tempo (b) stimuli, reductions in LF% between baseline and the stimuli increased with higher baseline LF% ( $p = 0.007$  and  $p < 0.001$  respectively).

### 2.3.3.2 Defining a response to the auditory stimuli

Due to these significant relationships, participants were subsequently categorised based on percentage change in LF% between baseline and the stepped decrease in tempo stimulus for each visit (this permitted changes in responder definition between the visits). Two approaches were tested:

1. Two-level response definition:
  - a. Non-responder:  $< 20\%$  change in LF%
  - b. Responder:  $\geq 20\%$  change in LF%
2. Three-level response definition:

- a. Non-responder: < 20% change in LF%
- b. Type 1 responder:  $\geq$  20% decrease in LF%
- c. Type 2 responder:  $\geq$  20% increase in LF%

This involved examining the extent to which baseline LF% for each visit differed between the responder types.

#### **2.3.3.2.1 Baseline LF% and the stepped increase in tempo stimulus**

For the regression between baseline LF% and the stepped increase in tempo stimulus, the first approach failed to detect statistically significant differences between non-responders ( $n = 15$ ) and responders in visit 1 ( $p > 0.05$ ). This was also the case for visit 2 (non-responders:  $n = 9$ ;  $p > 0.05$ ).

Testing of the second approach led to no statistically significant differences between non-responders ( $n = 15$ ), type 1 responders ( $n = 6$ ) and type 2 responders in visit 1 ( $p > 0.05$ ). However, in visit 2 the ANOVA reached significance ( $p = 0.042$ ). Bonferroni pairwise comparisons revealed that type 1 responders had significantly higher baseline LF% (mean: 34.64; SEM: 4.48) than type 2 responders (mean: 13.22; SEM: 4.04;  $p = 0.041$ ).

As the three-level response definition reached statistical significance, the same analyses were performed on LF% during the stepped increase in tempo stimulus in both visits. No statistically significant differences between the three response types emerged in either visit ( $p > 0.05$ ).

Consistency in response type between the visits was then investigated. Nine participants (37.50%) showed consistent responses (same response type) in both visits. Appendix A.5. summarises the characteristics of those who showed consistent and inconsistent responses. Analysis of these characteristics revealed no statistically significant differences between the two groups ( $p > 0.05$ ).

As a  $\geq 20\%$  change in LF% (regardless of direction) could be interpreted as a response, consistency was re-evaluated. Only six participants (25.00%) of the sample always responded (either type 1 or type 2 responder) and the remaining 18 participants were inconsistent responders (non-responders, type 1 or type 2 responders in any given visit). Appendix A.6. summarises

the characteristics of these two groups. Analysis of the characteristics of the two groups revealed no statistically significant differences ( $p > 0.05$ ).

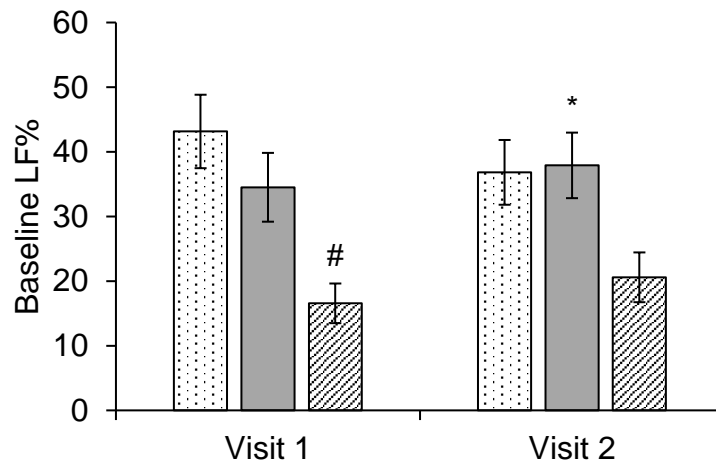
### **2.3.3.2.2 Baseline LF% and the stepped decrease in tempo stimulus**

For the regression between baseline LF% and the stepped decrease in tempo stimulus, the first approach revealed a significant difference in baseline LF% between non-responders and responders in visit 1 ( $p = 0.031$ ). Non-responders ( $n = 9$ ) had significantly higher LF% at baseline (mean: 43.15; SEM: 5.86) than responders (mean: 27.33; SEM: 4.07). No statistically significant difference emerged in visit 2 ( $p > 0.05$ ).

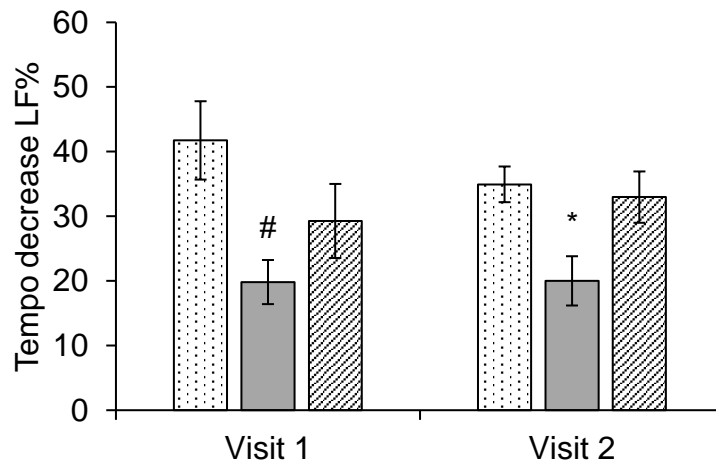
Statistically significant differences in LF% emerged for the three-level definition: visit 1 ( $p = 0.010$ ) and visit 2 ( $p = 0.017$ ). Exploration of these effects revealed that in visit 1, non-responders ( $n = 9$ ) had significantly higher baseline LF% than type 2 responders ( $n = 6$ ) ( $p = 0.008$ , see Figure 2.35a). But in visit 2, type 1 responders ( $n = 8$ ) had significantly higher baseline LF% than type 2 responders ( $n = 11$ ) ( $p = 0.028$ ). Although the same statistically significant differences did not emerge in both visits, Figure 2.35a depicts a similar pattern: type 2 responders had the lowest baseline LF%.

As the three-level response definition reached statistical significance, the same analyses were performed on LF% during the stepped decrease in tempo stimulus. For all visits, significant differences between the three responder types were identified (visit 1:  $p = 0.016$ ; visit 2:  $p = 0.017$ ). As shown in Figure 2.35b, LF% during the stepped decrease in tempo stimulus in visit 1 was significantly lower in type 1 responders than non-responders ( $p = 0.013$ ). However, in visit 2 this difference did not transpire ( $p > 0.05$ ). Instead, type 1 responders had significantly lower LF% than type 2 responders ( $p = 0.028$ ). Nevertheless, Figure 2.35b shows that a similar pattern emerged in both visits.

a.



b.



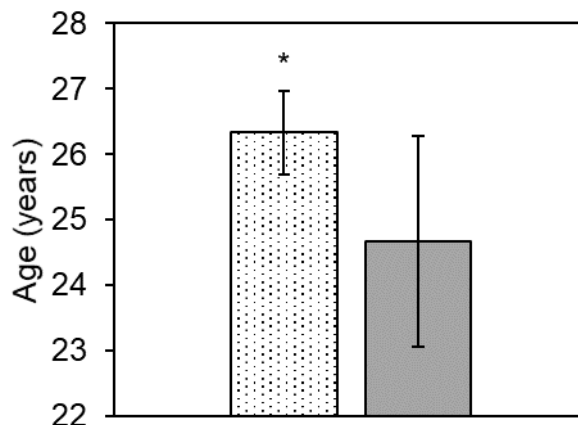
□ Non-responder    ■ Type 1 responder    ▨ Type 2 responder

Figure 2.35: Baseline LF% (a) was significantly lower in type 2 responders than non-responders in visit 1 and significantly higher in type 1 responders than type 2 responders. Tempo decrease LF% (b) was significantly lower in type 1 responders than non-responders in visit 1 and type 2 responders in visit 2. Data presented as mean  $\pm$  1 SEM. \* = significantly different to type 2 responders; # = significantly different to non-responders.

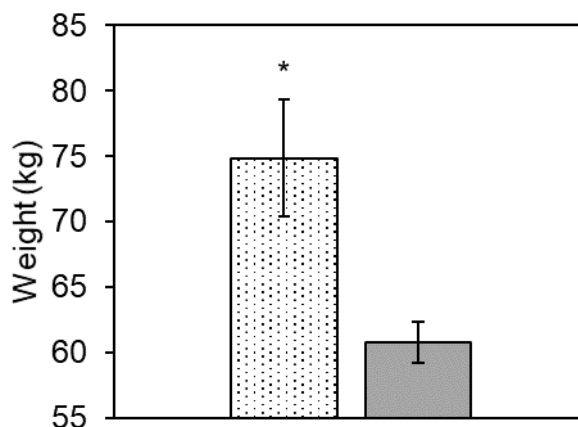
Consistency in response type between the visits was then explored. Nine participants (37.50%) showed consistent responses (same response type) in all four visits. Appendix A.7. summarises the characteristics of these two groups. Analysis of these group characteristics revealed that participants who showed consistent responses in both visits were significantly younger

and had a lower weight than those who showed inconsistent responses ( $p = 0.033$  and  $p = 0.039$  respectively, see Figure 2.36a and b).

a.



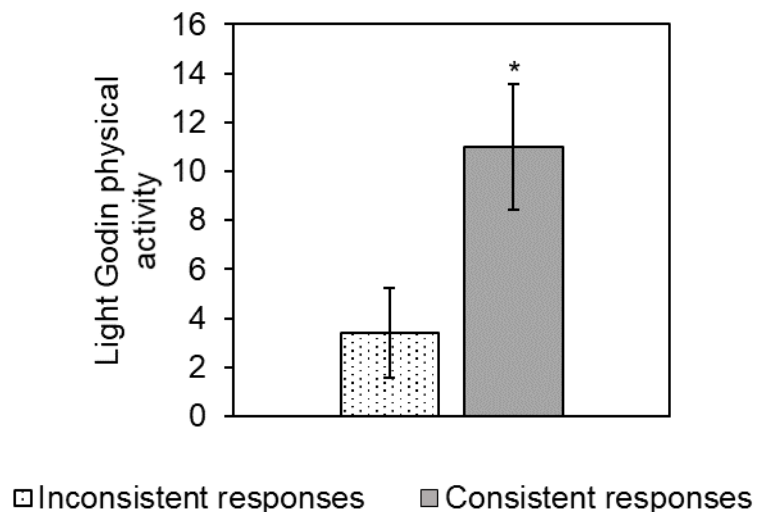
b.



□ Inconsistent responses    ■ Consistent responses

Figure 2.36: Age (a) and weight (b) significantly differed between inconsistent and consistent responses. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference.

Additionally, participants who showed consistent responses undertook significantly more light Godin physical activity than those who showed inconsistent responses ( $p = 0.007$ , see Figure 2.37).



*Figure 2.37: Light Godin physical activity significantly differed between inconsistent and consistent responses. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference.*

As a  $\geq 20\%$  change in LF% (regardless of direction) could be interpreted as a response, consistency was re-evaluated. Thirteen participants (54.17%) of the sample always responded (either type 1 or type 2 responder) and the remaining 11 participants were inconsistent responders (non-responders, type 1 or type 2 responders in any given visit). Appendix A.8. summarises the characteristics of these two groups. Analysis of the two groups' characteristics revealed no statistically significant differences ( $p > 0.05$ ).



## 2.4 Discussion

### 2.4.1 Main findings

This study examined the effects of stepped increases and decreases in tempo on cardiac autonomic function. This entailed exploring the impact of two different computer programs (Spike2 and LabChart) on the results. Although similar statistical outcomes resulted when examining within each visit, discrepancies emerged when exploring the between-visit differences. Indeed, it could be argued that detecting statistical significance was easier with Spike2 than with LabChart, particularly when using absolute change values. This outcome may have been exacerbated by the fact that the raw values derived in LabChart were slightly different to those obtained in Spike2. This was particularly the case for the frequency-domain HRV raw values, which was not completely surprising given that the two programs used different algorithms. This finding has important impact when comparing results between studies. Indeed, discrepancies in results reported in the literature may in part be due to the use of different computer programs. Therefore, the type of computer program used to analyse physiological measures, particularly the frequency-domain HRV parameters, should be carefully considered in future work.

Results from the LabChart analysis illustrated that significant changes in physiological measures occurred within each visit. In the first visit, the stepped decrease in tempo stimulus was associated with a shift towards parasympathetic predominance, as indexed by heightened HF power, RMSSD, pRR50, SD1 and nSD1, and elongated RR intervals. In contrast, many of these variables failed to significantly differ between the four conditions in visit 2. This led to a surprising finding that responses to the two auditory stimuli were similar in both visits. However, this finding is consistent with that of Bernardi et al. (2006) who identified no repetition effect. Indeed, the only significant differences that emerged between the two visits concerned the amplitude of the R and S waves. The R peak was not as high in visit 1 as it was in visit 2 for both auditory stimuli. Additionally, the S wave was not as low in visit 1 compared to visit 2 for the stepped decrease in

tempo stimulus. Taken together these findings suggest that the force at which participant's hearts contracted in visit 1 was less than that in visit 2. This is unexpected given that participants were more familiar with the experimenter, study room, procedure and stimuli on the second visit. However, as the change in R and S amplitude were small in magnitude (e.g. R amplitude stepped increase in tempo visit 1: -0.071; visit 2: -0.019; R amplitude stepped decrease in tempo visit 1: -0.066; visit 2: -0.021; S amplitude stepped decrease in tempo visit 1: -0.007; visit 2: -0.040), the meaningfulness of these results is questionable. Moreover, as these differences were not substantiated by similar patterns in other variables, e.g. HF power, SD1, RR interval, the reliability of these findings is tenuous.

Baseline HR has previously been shown to interact with HR responses to tempo manipulations (Watanabe et al., 2017). However, the results from the study show for the first time that responses to simple auditory stimuli that manipulate tempo can be predicted by other baseline measures. This is a novel finding and important given that the use of music as an alternative therapy is increasingly prevalent. Baseline LF% was a significant predictor of how participants responded to the stepped increase and decrease in tempo stimuli in visit 2. Although other variables predicted responses to the stimuli (like LF/HF), baseline LF% was one of the strongest predictors. Moreover, classification of participants based on percentage change in LF% between baseline and the two stimuli demonstrated that significant differences emerged in baseline LF% between the three responder types. This is a key outcome because it suggests that individuals who may respond better can be identified at the outset of an intervention employing music.

This identification could be aided by knowing which individuals are more likely to respond consistently well when exposed to stimuli on multiple occasions. Indeed, results showed that participants who were the same responder type (non-responder, type 1 responder, type 2 responder) in both visits tended to be younger, weigh less and undertake more light physical activity than those who showed inconsistent responses. Perhaps this is counterintuitive as the findings suggests that individuals who engage with health-positive behaviours may respond most positively. Indeed, this finding may have a psychological basis, underpinned by personal outlook

concerning health-related behaviours. As the main finding was that physiological responses were significantly impacted by the auditory stimuli, this finding will be discussed in more detail below.

#### **2.4.2 The (simple) stepped increase and decrease in tempo stimuli altered cardiac autonomic function**

Previous research shows that tempo has significant impacts on autonomic control of the heart in healthy volunteers. However, most work investigating the impact of music on autonomic function has used stimuli that manipulate multiple musical parameters at a time (e.g. Bernardi et al., 2006; Iwanaga et al., 2005; van Dyck et al., 2017; van der Zwaag et al., 2011). This renders it difficult to ascertain the extent to which tempo really does impact physiological state. Furthermore, studies investigating the impact of music (and more specifically tempo) have used limited physiological measures. Consequently, this called for an experiment that carefully manipulated tempo and adopted a comprehensive set of measures of cardiac autonomic function.

The results clearly show that the impoverished stimuli successfully altered cardiovascular autonomic function. More importantly, responses to the stepped decrease in tempo stimulus were consistent with the experimental hypothesis: a progressively slower beat was associated with greater parasympathetic predominance compared to baseline. This result is consistent with the literature. Indeed, van der Zwaag et al. (2011) found that RMSSD was significantly higher during slow music compared to fast music. Also, Iwanaga et al. (2005) revealed that HF power was significantly higher during sedative music compared to excitative music. Furthermore, Iwanaga (1995b) and van Dyck et al. (2017) showed that stimuli decreasing in tempo were more effective at boosting autonomic wellbeing than those that increased in tempo. This makes sense because the aim of most music interventions is to enhance relaxation.

Although the stepped decrease in tempo stimulus promoted parasympathetic predominance, the reason underpinning this effect is unclear. For instance, the effect may have been cumulative i.e. the five increasingly slower tempi may have linearly facilitated shifts towards

parasympathetic predominance. Alternatively, the effect may have been driven by a specific tempo (such as 60bpm, which is closest to the preferred range of tempi (Iwanaga, 1995a, 1995b)). As frequency-domain HRV requires a minimum of two minutes (Camm et al., 1996), it was not plausible to run the analyses on the individual tempi of the stepped increase and decrease in tempo stimuli. Further examination of the changes that occur for individual tempi in a stimulus comprised of sudden (stepped) changes in tempo would be worthwhile.

The results also reinforce the importance of adopting numerous measures. The reason for this is two-fold: 1) numerous measures need to be adopted to obtain a comprehensive account of an observable effect; 2) stimuli impact different aspects of human physiology, so a range of measures, each of which considers different autonomic functions, needs to be incorporated. In addition, it is important to explore other, less common measures (e.g. Q1 RR, Q3 RR and IQR RR) to examine the data in different ways. This point is particularly relevant given the debate in the literature concerning which physiological characteristics different measures represent. For instance, although HF power is generally accepted to represent vagal tone, there are disputes with regards to LF power (unclear if it is purely sympathetic or a combination of sympathetic and vagal activity) and therefore LF/HF. This issue is exacerbated by the fact that different algorithms can be applied to a tachogram to derive frequency-domain HRV parameters, and these algorithms produce different results. Consequently, it is prudent to explore other variables that are more mathematically secure.

### **2.4.3 Conclusion**

This study examined the effect of simple auditory stimuli that manipulated tempo on cardiac autonomic control. A key finding was that the carefully controlled stimuli successfully modulated listener physiology. This provides evidence that the paradigm adopted here worked. Furthermore, parasympathetic predominance was enhanced during the stepped decrease in tempo stimulus. This is consistent with previous work which shows that slower tempi are associated with a shift towards parasympathetic predominance (Iwanaga et al., 2005; van der Zwaag et al., 2011). A novel

finding from the study emerged: it is possible to predict responses to the stepped increase and decrease in tempo stimuli based on resting LF%. Furthermore, young participants who weighed less and undertook high levels of light physical activity appeared to show consistent responses between the two stimuli and two visits. This suggests that individuals who are more likely to respond positively to tempo manipulations can be identified from the outset.

Study findings also had methodological implications. For instance, the results benefitted from adopting a range of physiological measures, including non-linear HRV, ECG analysis, BRS and less conventional RR interval measures (e.g. Q1 RR, Q3 RR, and IQR RR). However, insight from subjective interpretations of the stimuli would have enhanced the study outcomes. Nevertheless, it appears that unsophisticated auditory stimuli, that manipulate tempo in controlled conditions, provoke responses that are consistent with those seen when using 'real' musical stimuli. This finding has implications when designing experimental paradigms investigating the impact of tempo (and music more generally) on cardiovascular autonomic function.

**Chapter 3. The effects of stepped changes in tempo on autonomic function and subjective emotion**

### **3.1 Introduction**

The pilot study demonstrated that the simple and tightly controlled stimuli were successful in modulating cardiovascular autonomic function in a way consistent with the literature. The stepped decrease in tempo stimulus was associated with a shift towards parasympathetic predominance, as indexed by enhanced HF power, RMSSD, pRR50, SD1 and nSD1, and elongated RR intervals. Although the two tempo manipulation stimuli made less impact in the second visit, statistical analysis showed that change in physiological responses did not significantly differ between the two visits. Finally, baseline LF% predicted responses to the two stimuli, where the higher the baseline LF%, the greater the decrease in LF% between baseline and auditory stimulus. Therefore, these results provided support for adopting a paradigm that manipulated tempo whilst holding all other musical parameters. However, the study highlighted four areas that could be refined to improve outcomes.

#### **3.1.1 Incorporating a direct measure of sympathetic activity: microneurography**

All measures employed in the pilot indirectly measured autonomic activity. That is, only approximate measures of sympathetic and parasympathetic activity were obtained. Direct measures of sympathetic activity are possible and can be achieved by performing microneurography. Microneurography involves directly recording muscle sympathetic nerve activity (MSNA) from the peroneal nerve in the lower leg (Hagbarth & Vallbo, 1968). Unlike HR, BP and HRV, microneurography generates hard, biological evidence of sympathetic activity. Although this is an invasive procedure, combining it with the indirect measures would make it possible to establish whether sympathetic activity (as well as vagal tone) changes with tempo. As a result, the patterns that emerged in the non-invasive measures would be substantiated. No other research investigating the influence of music on physiology has employed this method (see Table 3.1). Therefore, the current study employs microneurography in order to generate direct biological

evidence of sympathetic activity alterations that result from tempo manipulations.

### **3.1.2 Developing the auditory stimuli**

An additional area that could be improved concerns the stimuli. As mentioned in the introduction of Chapter 2, most research looking at the impact of tempo on cardiovascular autonomic function has used complex musical stimuli, which alter numerous parameters simultaneously. As a result, this makes it difficult to ascertain whether increases in tempo are actually associated with enhanced sympathetic activity. To overcome this issue, the pilot study used simple and tightly controlled stimuli (in the form of a drum beat), that manipulated tempo whilst holding all other parameters constant. Although equally impoverished stimuli have been used before, such as those used by Iwanaga (1995a), Chuen et al. (2016) and Watanabe et al. (2017), the pilot study shared little resemblance to real music (e.g. there was no rhythm or melody). This limits the ecological validity of the findings and renders it difficult to see how the results could be applied to real music. Therefore, the current study uses more musically sophisticated stimuli which manipulate tempo whilst keeping all other parameters constant.

In the pilot, it was not feasible to ascertain the extent to which cardiovascular autonomic activity changed with the five individual tempi (60bpm, 90bpm, 120bpm, 150bpm and 180bpm). Therefore, based on recommendations from Camm et al. (1996), who stated that a minimum of 120 seconds is required to perform frequency-domain HRV analysis, the duration of the individual tempi was doubled. This was important to do because it was not clear from the pilot whether the shift towards parasympathetic predominance in the stepped decrease in tempo stimulus was driven by a particular tempo or a cumulative effect. Moreover, the relationship between the five tempi and changes in vagal tone could not be explored. This is unfortunate given that research by Karageorghis et al. (2011) identified a quartic relationship between preferred musical tempo and exercise intensity. Slow tempo music was least preferred for all exercise intensities, whilst preference for fast tempo increased with exercise intensity to a greater extent than that for



medium and very fast tempi. Therefore, the current study increases the duration of the individual tempi to at least 120 seconds in order to permit the reliable derivation of frequency-domain HRV.

In the pilot, the stepped increase and decrease in tempo stimuli were played continuously without a break. As a result, carry-over effects may have occurred. This means that the changes in autonomic activity that transpired for the first stimulus, may not have had sufficient time to wash out before commencement of the second stimulus. As the order of the stimuli was randomised between participants, it is unlikely that an order effect happened. Nevertheless, it remains that the physiological responses to the second stimulus may have been influenced by responses to the first. Due to the continuity between the two stimuli, it was also not possible to determine whether brachial BP and HR differed between the four conditions. This is unfortunate given that McConnell, Froeliger, Garland, Ives and Sforzo (2014) found that post-exercise binaural beats boosted HF power and attenuated LF power and LF/HF to a greater extent in the first two minutes of post-exercise relaxation than placebo binaural beats. In addition, Wallert and Madison (2014) showed that music with a decreasing tempo lowered post-exercise LF/HF to a significantly larger extent than music with an increasing tempo. Also, Chafin, Roy, Gerin and Christenfeld (2004) demonstrated that classical music facilitated SBP recovery following a stressful task. Although these results demonstrate that music can improve recovery from physical and mental stress, they also show that music has effects post-stimulation. Therefore, in contrast to the pilot, the current study removes the continuity between the stepped increase and decrease in tempo stimuli in order to minimise carry-over effects and to explore post-stimulation brachial changes.

It cannot be ignored that the pilot study did not use a control condition or control group. Although baseline autonomic function was ascertained (which vaguely functioned as a control), no comparison was made between a stimulus that had an unchanging tempo and one that had a variable tempo. Consequently, it was not possible to determine whether a stimulus that had a changing tempo elicited significantly different physiological responses

compared to one that had a consistent speed. This point has not been addressed in the literature as most studies have tended to adopt one of two approaches:

1. Comparing a stimulus with an unvaried fast tempo with a stimulus that has an unchanging slow tempo (either with or without a no music control) (e.g. Bernardi et al., 2006; Gomez & Danuser, 2007; da Silva et al., 2014; van der Zwaag et al., 2011).
2. Comparing a stimulus which gradually increases in tempo whilst the other gradually decreases (either with or without a no music control) (e.g. Watanabe et al., 2017; Iwanaga, 1995a, 1995b).

Therefore, the current study employs a control stimulus, in the form of a stimulus with an unvaried (stable) tempo.

Music consists of many musical parameters all of which interact with one another over the course of a piece to influence affect (Dalla Bella, Peretz, Rousseau & Gosselin, 2001; Gagnon & Peretz, 2003; Hunter, Schellenberg & Schimmack, 2010). To boost the ecological validity of physiological responses to tempo manipulations, other musical parameters should be incorporated. These could include rhythmic and/or melodic phrases that are played at different tempi. However, the inclusion of each additional parameter requires testing to determine how physiological responses change with tempo and rhythmic and/or melodic additions. This requires a control group of some description. A good example of a control group used in research in this area was implemented by Khalfa et al. (2008). This involved allocating participants to one of three groups: experimental group, where participants heard a tune that had a melody and rhythm; rhythm control group, where participants heard the same tune but with the rhythm removed; melody control group, where participants heard the same tune but with the melody removed. This permitted an assessment of the interactive effects of tempo, melody and rhythm to be made. Therefore, similar to Khalfa et al. (2002), the current study implements a melody control group. It is hoped that this will aid with assessing the contribution of melody to physiological responses to tempo manipulations.

### 3.1.3 Developing the baseline and recovery conditions

Recommendations by Camm et al. (1996) promote the analysis of five minute recordings for HRV derivation. This led to implementing baseline and recovery conditions that were five minutes in duration in the initial study. Although a three-minute stabilisation period was implemented in the pilot prior to starting the conditions, orienting responses could have resulted in artificial baseline and recovery measures (Watanabe et al., 2017). This means that the readings obtained in the baseline and recovery conditions may not have been an accurate reflection of how participants were at rest. There is concern in the literature pertaining to what constitutes a true baseline or 'resting state' (Quintana & Heathers, 2014). This issue is exacerbated by the fact that participants are out of their normal environment; are interacting with an 'experimenter' (which has its own challenges); and are normally required to undertake tasks peculiar to everyday life. Therefore, obtaining a reliable baseline reading can be challenging.

Several recommendations have been made to enhance the reliability and validity of baseline conditions. Firstly, to control for differences between participants in baseline readings, a within-participants design is preferred over a between-participants design. Also, implementing the Vanilla baseline has also been recommended. This requires participants to perform a task requiring minimal cognitive effort e.g. a simple counting task (Quintana & Heathers, 2014). Finally, since breathing drives cardiovascular autonomic function a consistent respiration rate has been advocated, (Watanabe et al., 2017). However, these recommendations are not always practical given the objectives and design of research studies. For instance, although paced breathing eliminates the confound of fluctuating respiration rate, it could undermine the ecological validity of some research outcomes. In addition, implementing the Vanilla baseline may provoke unanticipated stress responses, particularly if a counting task is implemented. Also, although the gold-standard is a within-participants, issues with participant recruitment and fall-out may be encountered.

Therefore, despite these recommendations, breathing was not paced, participants rested with their eyes closed and a between-participants design was adopted. In addition, the current study employed baseline and recovery conditions that were 10 minutes in duration. This was done to ensure that a five-minute section taken from the 10-minute recording would not include an orienting response. However, as it was not clear from the literature which five-minute section should be taken, the current study aimed to explore which five-minute section was most valid.

### **3.1.4 Measures of subjective emotion**

The final area for improvement concerns the psychological impacts of changes in the speed of music. Indeed, it cannot be ignored that the pilot primarily focussed on the physiological changes that resulted from tempo manipulations. Therefore, this provided little information pertaining to how participants felt during the auditory stimuli. This is an important aspect given that numerous studies have demonstrated that music impacts a range of affective responses. In healthy samples at rest these include: subjective feelings of arousal (Holbrook & Anand, 1990; Kellaris & Kent, 1993; Iwanaga & Moroki, 1999; Burns et al., 2002; Gomez & Danuser, 2004, 2007; Iwanaga et al., 2005; van der Zwaag et al., 2011; Mikutta et al., 2013; Proverbio et al., 2015; Jiang, Rickson & Jiang, 2016), valence (Gomez & Danuser, 2004, Khalfa et al., 2002; Etzel et al., 2006; Khalfa et al., 2008; van der Zwaag et al., 2011; Proverbio et al., 2015; Jiang et al., 2016), tension (Iwanaga & Moroki, 1999; Iwanaga et al., 2005; van der Zwaag et al., 2011; Jiang et al., 2016), surprise (Kellaris & Kent, 1993), anxiety (Burns et al., 2002), and pain (Mitchell, MacDonald & Brodie, 2006).

The affective space that is considered to account for subjective responses to music is two-dimensional in nature. One dimension is defined by valence (e.g. positivity/negativity and pleasantness/unpleasantness) and the other dimension is defined by arousal (e.g. stimulated/relaxed) (Russell & Barrett, 1999). These dimensions can be measured using Likert Scales and Visual Analogue Scales (VAS) and are considered to exist in two categories of musical emotion: experienced and perceived music emotion. The former

refers to emotions that are felt by listeners whilst and/or after listening to a musical stimulus. For example, a listener may feel energised or relaxed, happy or sad after listening to a piece of music. Perceived musical emotion refers to the idea that music may represent emotions, for instance, a piece of music may sound happy, cheerful and energised (Gomez & Danuser, 2007). The emotions a listener experiences whilst or after being exposed to a piece of music are considered to be determined by the musical structure the stimulus (Gomez & Danuser, 2007). For instance, music played in a major mode with a fast tempo is normally associated with experienced positive valence and high levels of arousal. In contrast, music played in a minor mode with a slow tempo is generally associated with experienced negative valence and low levels of arousal. Similarly, the emotions a listener perceives the music to personify are determined by how the music is structured. For example, major mode fast tempo music is perceived as happy whereas minor mode slow tempo music is interpreted as being sad (Khalifa et al., 2002).

Since PNS preganglionic neurons located in the longitudinal column of the brainstem project to some of the cranial nerves (which have been implicated as a requisite for experiencing emotion), it comes as little surprise to know that the ANS is considered to participate in affective responses to musical stimuli. Indeed, there is a long-standing debate in the literature concerning patterns in emotion that are unique to specific autonomic responses. This view was first expounded by William James in the 1890s who argued that emotions were a result of specific patterns in autonomic activity. That is, emotional experiences were a predictable result of alterations in human physiology and determined by physiological responses. Most of the early research supporting this claim tested the congruence between emotional experiences and facial motor activity (facial expressions) and came from a specific group of researchers (Ekman and colleagues: Ekman, Levenson, & Friesen, 1983; Levenson, Ekman, & Friesen, 1990; Levenson, Friesen, Ekman & Carstensen, 1991; Levenson, Heider, Ekman & Friesen, 1992). Indeed, they claimed that: 1) there is a basic set of emotions, each of which has its own unique set of features (including autonomic activity patterns); 2)

these emotions (and their unique features) are universal (they are shared cross-culturally) (Ekman, 1992).

However, this position was undermined by Schachter and Singer's (1962) work which showed that the same state of physiological arousal can be defined by different emotional labels. More recently, a meta-analysis by Cacioppo, Berntson, Larsen, Poehlmann and Ito (2000) showed that some emotional experiences had unique physiological patterns. For instance, anger and fear had higher HR than that of happiness. However, other emotional experiences had non-specific patterns of autonomic activity. They also maintained that some measures of autonomic function were more reliable in differentiating between emotions. For example, HR was more reliable than respiration. Interestingly, Cacioppo et al. (2000) do acknowledge the limited range of measures of cardiac autonomic function that have been used, along with the range of emotion-eliciting stimuli employed (video clips and pictures are most common).

As music has long been associated with prompting different emotional responses (Cooke, 1959), applying the physiology-specific emotion debate to this stimulus is of interest. This is because listening to music is anecdotally claimed to be mainly motivated by the emotional changes it can provoke. One of the first studies investigating the psychophysiological impacts of music was conducted by Krumhansl (1997). This involved measuring a range of physiological parameters, including, RR interval, RSA<sup>29</sup>, SBP, DBP, MAP and SCL<sup>30</sup>, whilst listening to six musical excerpts. Two represented fear, two represented sadness and the final two represented happiness. Participants were also asked to provide continuous ratings of one of four emotions: sadness, fear, happiness or tension.

The results showed that the sad ratings correlated most strongly with DBP (0.41), SBP (0.37), MAP (0.37) and SCL (-0.36). In contrast, the happy

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<sup>29</sup> RSA refers to the coupling between RR interval and respiration rate. Characterised by the shortening of RR intervals during inspiration and the elongation of RR intervals during expiration.

<sup>30</sup> Skin Conductance Level: a non-invasive measure of autonomic function, taken to reflect alterations in sympathetic activity. Represents tonic (slow) changes in autonomic activity.

ratings correlated most strongly with finger pulse amplitude (FPA) (0.24) and finger temperature (TEM) (0.21). Fear was strongly correlated with FPA (-0.31), MAP (-0.25), DBP (-0.24) and SBP (-0.23). Finally, the tension ratings were most strongly related to FPA (-0.35), TEM (-0.24) and pulse transmission time to finger (FPTT) (0.23). When exploring the changes in physiology for the individual excerpts, some emotion-specific changes occurred. For instance, the largest changes in RR interval, SBP, DBP and MAP emerged for the sad excerpts. TEM varied to a greater extent for sad and fear excerpts. Also, changes in FPTT and FPA were largest for the fear excerpts and fluctuations in respiration rate were most pronounced for the fear and happy excerpts.

Although some physiological changes were specific to some of the emotions, particularly sad excerpts, Ekman and colleagues' view that all emotions are determined by specific patterns of autonomic activity was only partially supported. This is surprising, particularly for the fear ratings, given that the removal of the adrenal glands has been associated with the loss of fear. Evidence for this comes from work by Takahashi and Rubin (1993) who showed that infant rats (10 days old) who had undergone adrenalectomy (the removal of both adrenal glands) showed extinguished fear responses (freezing<sup>31</sup>) when confronted with a male rat. These findings have been replicated, for instance, by Moriceau, Roth, Okotoghaide and Sullivan (2004) who demonstrated that freezing behaviours were eliminated in infant rats who had undergone adrenalectomy and were presented with novel male odour. This was not the case in controls (who had intact adrenal glands). Indeed, controls showed immobility/freezing responses that lasted around 38 seconds. Nonetheless, Krumhansl's (1997) finding that not all musical emotions were associated with unique physiological patterns may have been due to a reliance on correlations. However, Krumhansl (1997) did set the precedence for not only investigating the impact of different music on affective responses, but for also determining whether musical emotions are a result of unique physiological response patterns.

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<sup>31</sup> Freezing: immobile posture, where the head is elevated and stationary (Takahashi, 1995).

More recent work by Khalfa et al. (2002) tested the claim that experienced musical emotions are associated with different autonomic responses. Thirty-seven healthy participants (12 males) were exposed to 28 musical clips that were seven seconds in duration. All clips were taken from movie soundtracks and rated as unfamiliar by all participants. SCR<sup>32</sup> was recorded throughout and participants rated their experienced arousal and valence after each clip. Khalfa et al. (2002) found that SCR was significantly higher for the stimuli which induced feelings of fear and happiness compared to those which induced feelings of sadness and peacefulness. Interestingly, no significant differences in SCR were found between fear and happiness, and sadness and peacefulness.

Initially, these findings suggest that experienced happiness and fear as induced by music, can be distinguished from experienced sadness and pleasantness based on SCR. However, the stimuli employed here were very short (seven seconds in duration) and not previously validated. In addition, as demonstrated by Krumhansl (1997), not all measures of autonomic function differed between affective responses (e.g. there was no significant difference in SCRs between happiness and fear). Therefore, relying on one measure of autonomic activity (SCR) may have resulted in either a false positive, or an incomplete account. Fortunately, Etzel et al. (2006) examined time-domain HRV to determine whether cardiovascular and respiratory patterns could distinguish between different moods.

The study involved exposing 18 healthy participants (8 males) to classical music taken from movie soundtracks which had been previously reported to induce one of three target emotions: happiness, sadness and fear. Stimuli ranged from 74-189 seconds in duration. Respiration rate and HR were recorded throughout and participants rated experienced happiness, sadness and fear on nine-point Likert Scales. Time-domain measures of HRV were derived from the HR data. These included: SDRR and SDSD. RSA was also derived from the HR recordings. Results illustrated that each of the clips

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<sup>32</sup> Skin Conductance Response: another non-invasive measure of autonomic function, taken to reflect alterations in sympathetic activity. Unlike SCL, SCR represents phasic (rapid changes) in sympathetic activity.



successfully induced the targeted mood. However, none of the HRV measures significantly differed between the induced happiness, sadness and fear moods. Despite this, inspection of the HR data showed that values were significantly lower during the sadness mood induction compared to baseline. In addition, HR was significantly higher during the fear induction than during baseline. Differences between the mood inductions also emerged for expiration and breath length: both variables were significantly longer during the sadness induction than during the happiness and fear inductions. But, no significant differences transpired between the happiness and fear inductions.

Together, these results suggest that sadness and fear can be differentiated based on respiratory measures and HR. Indeed, these findings are consistent with those of Gomez and Danuser (2004) who showed that respiratory parameter responses can distinguish between different musical induced emotions. However, it should be noted that all of these studies failed to use a control (group or condition). Although it is difficult to determine an appropriate control for studies using music, a control in these cases could have constituted a musical excerpt that has previously been validated as neutral, or the induction of a neutral emotion. One study which employed an interesting control was conducted by Khalfa et al. (2008).

The study aimed to assess whether affective and autonomic function differences existed between happy and sad excerpts. Fifty participants (29 males) were presented with six original musical excerpts from the classical repertoire which had been used previously (Peretz, Gagnon & Bouchard, 1998). Three of the excerpts were validated as 'happy' and the remaining three as 'sad'. Two versions of all six musical excerpts were created from the originals. This included a rhythm version, which involved removing pitch variations from the original excerpt; and a tempo version, which involved removing pitch variations. Each excerpt was 15 seconds in duration and repeated four times to create a one-minute stimulus. Piano timbre was used in each stimulus and dynamics were held constant throughout. Following a five-minute baseline, participants were presented with each stimulus

followed by a resting period of 20 seconds during which they were required to state the perceived musical emotion of each piece (happy or sad) and to rate their experienced arousal and valence on a ten-point Likert Scale. HR, BP, skin conductance, and zygomatic and corrugator muscle activity was recorded throughout.

The study revealed that the target perceived musical emotions (happy or sad) were significantly better identified in the original excerpt than in the rhythm and tempo versions. In addition, participants experienced significantly higher levels of positive valence and arousal for the happy stimuli compared to the sad stimuli. The sad rhythm version was associated with significantly lower levels of experienced positive valence and arousal than the happy rhythm version. Also, the original versions of the happy and sad stimuli were rated as inducing more positive valence than the rhythm and tempo versions. Although none of the physiological measures significantly differed between the happy and sad stimuli, a statistically significant interaction between perceived emotion (happy or sad) and type (original version, rhythm version or tempo version) was found for SCR, DBP and respiration rate. Fast excerpts evoked significantly more SCRs than sad, happy rhythm and happy tempo excerpts. Also, DBP was significantly higher during happy excerpts compared to sad excerpts, and during the original happy excerpt compared to the happy rhythm and tempo versions. No significant differences in DBP existed between the three slow excerpts. Additionally, in contrast to the findings of Gomez and Danuser (2004) and Etzel et al. (2006), Khalifa et al. (2008) found no significant difference in respiration rate between happy and sad excerpts. However, the authors reported that respiration rate was significantly higher during the happy tempo versions than during the sad tempo versions, and in the sad and sad rhythm version compared to the sad tempo version.

This study illustrates two important points. Firstly, the results demonstrate that the inclusion of a control in music research studies is crucial. This is because meaningful differences exist between a control stimulus and an 'experimental' stimulus, thus providing further detail for establishing a

taxonomy of music-induced alterations in affect and autonomic activity. Secondly, the study reinforces the importance of adopting a multi-method approach. This is because the literature remains unclear about which autonomic activity variables show unique response patterns to certain musical emotions. So all in all, this study substantiates the rationale for including a control stimulus, a control group, and measures of self-reported emotion along with physiological measures. Even though these studies do not directly manipulate tempo, research investigating the impact of tempo manipulations on experienced and perceived music emotions has been performed.

#### **3.1.4.1 The effects of tempo on affect**

One of the first studies investigating the effects of tempo on affective responses to music was conducted by Holbrook and Anand (1990). This involved presenting 44 participants with an unfamiliar jazz piece ('I found love') played at 14 different tempi, ranging from 57bpm to 348bpm. Individual tempi were determined by increasing the previous tempo by 15%. Participants were randomly allocated to one of two groups: low arousal (eyes closed, resting); or a high arousal (working on four anagram puzzles). These two different levels of situational arousal facilitated an assessment of the relationship between tempo and perceived activity (arousal) and valence (affect). At the end of each of the 14 tempi, participants completed the following eight bipolar adjective scales to measure perceived activity: agitated/calm, slow/fast, energetic/ listless, hard/soft, passive/active, restful/exciting, lazy/busy and tense/relaxed; and six bipolar adjective scales to measure perceived affect: displeasing/pleasing, good/bad, enjoyable/unenjoyable, unpleasant/pleasant, like/dislike, ugly/beautiful.

Consistent with expectations, perceived activity linearly increased with tempo. That is, participants perceived increasingly faster tempi as having greater levels of activity. In addition, when analysing the low and high arousal groups together and separately, quadratic curves between the tempi and perceived affect emerged. For the groups combined, affect peaked at 114bpm. For the low arousal group perceived affect was highest at 96bpm,

whilst it was 118bpm for the high arousal group. These findings led Holbrook and Anand (1990) to conclude that unlike the simple positive relationship between tempo and perceived activity, the relationship between tempo and affect is more complex. This is because it depends on external factors, including baseline arousal, whether that is physiological and/or psychological.

As Holbrook and Anand (1990) employed such a wide range of tempi, the results may not be applicable to everyday listening situations. Furthermore, it is unclear from the results whether the tempo manipulations led to a distortion in the musical qualities of the track. Therefore, to overcome these limitations, Kellaris and Kent (1993) composed and produced classical and pop-style pieces which were then orthogonally manipulated to vary musical tempo, texture and tonality. Each of the three parameters were manipulated in turn, resulting in the development of nine versions of each composition. This included playing the pieces at three tempi (slow: 60bpm; moderate: 120bpm; and fast 180bpm) in three tonalities (major, minor and non-diatonic atonal). Similar self-report items to those used by Holbrook and Anand (1990) were obtained at the end of each stimulus.

Consistent with the two-dimensional model of affective space, exploratory factor analysis revealed two factors, one represented arousal and the other represented valence. An additional third factor was identified which related to emotional surprise, or participant unfamiliarity with the stimuli. Analysis of these three factors with respect to the three musical parameter manipulations revealed that valence for the classical stimulus only was significantly impacted by the three tempi: valence increased with tempo for the classical music but remained constant for the pop music. A similar pattern emerged for arousal, however arousal for the classical stimulus remained constant for all three tempi yet increased for the pop music. Surprisingly, the third factor (surprise) was not significantly impacted by the tempo manipulations at any level.

This comes as little surprise given that Gomez and Danuser (2007) discovered that tempo, along with accentuation and rhythmic articulation

best differentiated between high and low arousal stimuli. In addition, it seems that the attribution of happiness to fast music and sadness to slow music is a robust phenomenon stemming from early development. For instance, Dalla Bella et al. (2001) found that happy and sad judgements of 16 happy and 16 sad excerpts were significantly impacted by tempo and mode manipulations in adults (aged 19-27 years) and children aged 6-8 years. Although 5-year old responses did not distinguish between happy and sad music based on mode manipulations, they did differentiate between the two emotions for the tempo manipulations. Interestingly, these patterns were not observed in younger children aged 3-4 years, suggesting that musical tempo is one of the first musical parameters children aged 5 years and above use to determine the emotional quality of musical stimuli. The same developmental trajectory was also found by Mote (2011). Therefore, these findings lend further support to the claim that musical tempo is one of the most important musical parameters in determining affective responses to music.

Exploring the affective responses of individuals who have sustained brain damage can also generate information about the influence of tempo on human emotions. For example, Gosselin, Peretz, Johnsen and Adolphs (2007) conducted a study looking at the impact of amygdala damage on emotion recognition in music. This involved exploring the subjective responses of an individual (SM) who had complete bilateral damage to the amygdala and comparing her responses to controls. As the amygdala is involved in the perception of fear, it came as little surprise to find that SM's responses to the scary music were impaired. However, this was not the case when hearing the peaceful, happy and sad music. More interestingly, similar to controls, SM's arousal ratings were sensitive to changes in tempo: a positive correlation emerged between the two variables. In addition, even though the controls showed little change in valence ratings when presented with tempo variations, SM showed heightened sensitivity in her valence ratings. For example, music with faster tempi were judged as significantly more pleasant than those of the control group. Moreover, in Gosselin et al.'s (2007) second experiment in which they orthogonally manipulated mode and

tempo, SM's responses to tempo (mode and their interaction) did not significantly differ to those of the control group. As a result, this implies that even following damage to an area of the brain that is crucial for emotional processing, tempo acts as an important characteristic in determining the affective qualities of musical stimuli.

This conclusion was also contended in a research study by Peretz et al. (1998) which explored the emotional responses to music in an individual (IR) who sustained lesions in both temporal lobes that extended bilaterally to frontal areas of the brain. IR and controls rated the happiness/sadness of an original excerpt and a modified version whose tempo was set to the median of the original tempo. Although IR had difficulties recognising familiar melodies, her responses did not significantly differ from those of controls, suggesting that brain damage of this nature leaves intact the ability to appraise the affective qualities of a piece from the analysis of its tempo. However, when presented with musical stimuli in a non-emotional context (error detection paradigm), IR exhibited a deficit in detecting time shifts (delayed or early onset of a note) compared to controls. This implies that task factors (emotional-context or non-emotional context) influenced the affective analysis of musical stimuli. Furthermore, when required to detect changes in tempo and mode, IR's performance peaked when changes in both parameters were combined. This contrasted with controls who performed better when tempo deviations were alone or combined with changes in mode. As a result, this demonstrates that different cognitive strategies were implemented by IR compared to controls: controls relied more on tempo, than mode, whereas IR used information from both parameters to generate emotional judgements.

A final interesting finding from Peretz et al.'s (1998) study was that both IR and controls performed better on decreasing tempi compared to accelerating tempi. Although these data come from an error-detection task, they are consistent with the results of Iwanaga (1995a) and van Dyck et al. (2017) who showed that decelerating tempi were preferred and provoked significantly greater changes in autonomic activity compared to tempi that

increased in speed. Indeed, this may be due to better detection of decelerating tempi (Peretz et al., 1998) and may explain why listeners prefer to listen to music that has a slow tempo when aiming to boost relaxation.

However, there are some inconsistencies in the literature regarding the affective impact of tempo variations. For instance, Kamenetsky, Hill and Trehub (1997) reported no significant impact of tempo on subjective likeability and emotional expressiveness. In contrast, Webster and Weir (2005) showed that faster tempi were associated with happier appraisals whilst slower tempi provoked sadder responses. In addition, Husain, Thompson and Schellenberg (2002) revealed that listening to a fast version of Mozart's sonata K.448 increased arousal, whereas listening to the slow version decreased arousal. Gagnon and Peretz (2003) also showed that tempo was more salient than mode when generating happy/sad judgements of musical pieces. Finally, van der Zwaag et al. (2011) demonstrated that fast music elicited significantly greater levels of arousal and tension than slow music.

Despite the quantity of studies investigating the impact of tempo on affective responses to music, three main areas of improvement have become apparent:

1. Few studies have combined self-report measures with those measuring cardiac autonomic activity, despite both individually maintaining that accelerating tempi are associated with psychological and physiological arousal (see Table 3.1).
2. Similar to studies examining the autonomic effects of tempo manipulations, those adopting a psychological approach have also used stimuli that either:
  - a. Manipulate numerous musical parameters simultaneously
  - b. Or use a restricted range of tempi (e.g. 'fast and slow')

Therefore, a more granular account of the impact of tempo manipulations on affective responses cannot be obtained.

3. Little work in this area has considered the consistency in responses between subjective and physiological readings. This is important given that self-report measures could be more vulnerable to participant biases and experimenter effects. Nonetheless, consistency (ascertained via correlations) between physiological and affective responses to music has been reported in the literature. For example, van der Zwaag et al. (2011) showed that subjective arousal and tension positively correlated with SCR. Also, RMSSD was negatively related with arousal and tension. In addition, Gomez and Danuser (2004) showed that inspiration, expiration and total breath duration decreased with increases in valence and arousal, whereas mean inspiratory flow<sup>33</sup>, minute ventilation and SCR increased with valence and arousal. Finally, Khalfa et al. (2002) found that SCR positively correlated with fear and happiness.

Consequently, the current study builds on the methodology and results of the pilot and previous work in order to generate a more comprehensive account of the effects of tempo manipulations on autonomic and affective responses.

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<sup>33</sup> Inspiratory flow: amount of gas inhaled by participants.



*Table 3.1: Studies investigating the impact of tempo on autonomic function and subjective emotion.*

Paper	Time-domain HRV	Frequency-domain HRV	Non-linear HRV	RR	HR	ECG	BP	BRS	Respiration	MSNA	Experienced emotion	Perceived emotion	Real music
Bernardi et al. (2006)		✓		✓			✓	✓	✓				✓
Chuen et al. (2016)					✓				✓				
Dillman Carpentier and Potter (2007)													✓
Dalla Bella et al. (2001)												✓	✓
Dousty et al. (2011)						✓							?
Gagnon and Peretz (2003)												✓	✓
Gomez and Danuser (2007)					✓				✓		✓		✓
Gosselin et al. (2007)												✓	✓
Holbrook and Anand (1990)											✓	✓	
Husain et al. (2002)											✓		✓
Iwanaga (1995a)					✓								
Iwanaga (1995b)					✓								✓
Iwanaga et al. (2005)		✓			✓								✓
Kamenetsky et al. (1997)												✓	✓
Kellaris and Kent (1994)												✓	✓
Khalfa et al. (2008)					✓		✓		✓		✓	✓	✓
Mote (2011)												✓	✓
Peretz et al. (1998)												✓	✓
da Silva et al. (2014)				✓									✓
Watanabe et al. (2017)					✓				✓				
Webster and Weir (2005)												✓	✓
van der Zwaag et al. (2011)	✓										✓		✓
van Dyck et al. (2017)					✓								✓

### 3.1.5 Hypothesis

Changes in musical tempo are associated with changes in human physiology, as demonstrated in the literature and in the previous study. Tempo also influences subjective emotion, including the emotions experienced by listeners and the emotions they perceive music to embody. Therefore, it was hypothesised that stepped increases in tempo will increase sympathetic predominance and subjective stimulation, whilst stepped decreases in tempo will enhance parasympathetic predominance and subjective relaxation.

### 3.1.6 Aims and objectives

By employing more musically sophisticated stimuli, the study aimed to investigate the effects of sudden increases and decreases in the speed of a

simple tune on experienced musical emotion, perceived musical emotion and cardiovascular autonomic function. The primary outcome measures were LF/HF ratio and MSNA.

The objectives of the study were to:

1. Ascertain which five-minute section, out of a 10-minute baseline and 10-minute recovery recording (0-10 minutes, 0-5 minutes, 2.5-7.5 minutes, 5-10 minutes), was the most accurate measure of participants at rest. Research question: Which five-minute section is the most accurate baseline and recovery recording?
2. Determine the extent to which measures of autonomic function and subjective emotion changed from baseline during three different auditory stimuli (stepped increase in tempo, stepped decrease in tempo, stable tempo). Research question: Is there an effect of auditory stimuli?
3. Assess the extent to which cardiovascular autonomic function differed between five tempi of the stepped increase in tempo and stepped decrease in tempo stimuli (60bpm, 90bpm, 120bpm, 150bpm, 180bpm). Research question: Is there an effect of tempo?
4. Examine the extent to which autonomic and subjective responses differ between the control and experimental groups. Research question: Is there an effect of group?
5. Investigate the replicability of the two-factor model of affective space. Research question: Is the two-factor affective space model applicable?
6. Explore the consistency between autonomic and subjective responses. Research question: Do self-report responses correlate with physiological responses?

7. Examine the extent to which baseline measures predict response patterns to the auditory stimuli. Research question: Is it possible to predict response type based on baseline readings?

## **3.2 Method**

### **3.2.1 Participants**

Fifty-eight participants were initially recruited for the study. Age ranged from 22 to 72 years and included 31 females. See Appendix B.1. for a full summary of the characteristics of the full sample and for males and females separately.

Participants were randomly assigned to the control group (rhythm only stimuli) or the experimental group (rhythm and melody stimuli). Twenty-nine participants were randomly allocated to the control group (13 females) and the same number were randomly allocated to the experimental group (18 females).

The inclusion criteria for participation in the study were healthy males and females aged between 18 and 80 years. The exclusion criteria were similar to the pilot (see Chapter 2). However, obstructive sleep apnoea was also added as this condition has been found to be associated with heightened MSNA (Narkiewicz et al., 1998).

Like the previous study, similar controls were enforced to reduce the impact of confounding variables. These included: conducting the study between 14:00 and 18:00 on each testing occasion; asking participants to avoid caffeine, nicotine and strenuous exercise for 12 hours before the study, to have a light breakfast and lunch, and to empty their bladder before the study commenced. The study was approved by the University of Leeds Ethics Committee (BIOSCI 15-014) and written informed consent was obtained from all participants prior to testing.

### **3.2.1 Materials**

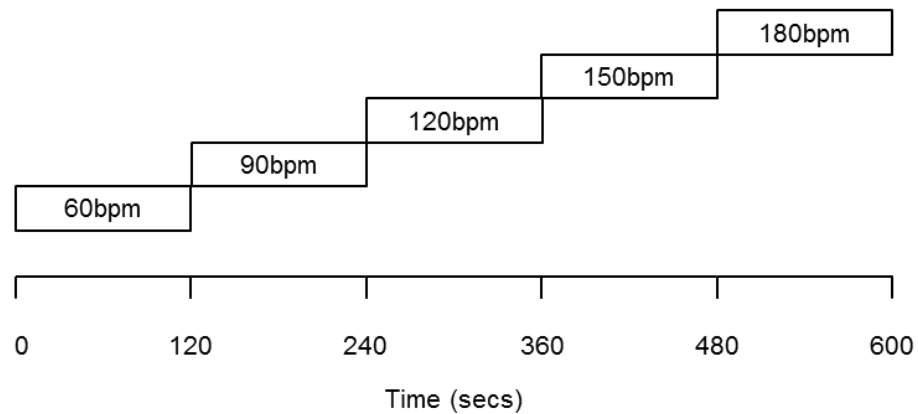
#### **3.2.1.1 Auditory stimuli**

All auditory stimuli were created in Sibelius Version 7 exported as a MIDI file, recorded in Audacity at 44100Hz at 76dB and subsequently exported as a .wav file and stored on an mp3 player.

Four nursery rhymes were initially selected as possible tunes to use in the study. These included: Baa Baa Blacksheep, Frère Jacques, Twinkle Twinkle Little Star and Mary had a Little Lamb. These tunes were initially selected because they had simple melodic and rhythmic patterns and would naturally be sung as a round (repeatedly). This latter quality was particularly important because there would be high levels of auditory repetition during the study. To determine which nursery rhyme to use in the study, three versions of each tune were created. This included stepped increase in tempo, stepped decrease in tempo and stable tempo stimuli.

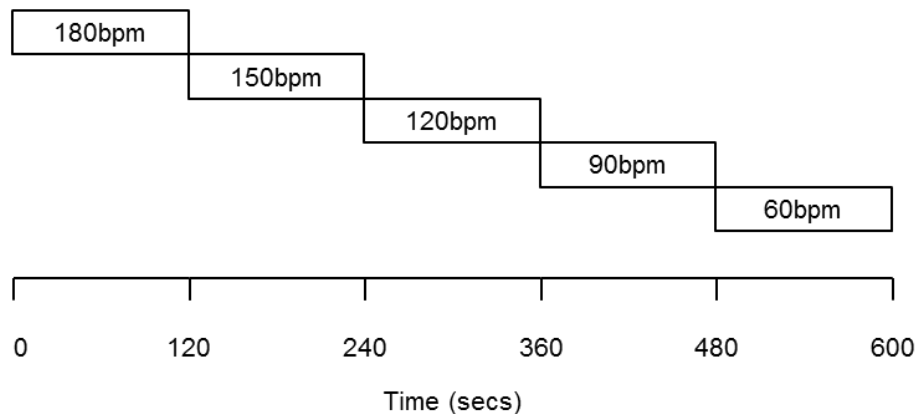
The stepped increase and stepped decrease in tempo stimuli closely resembled those used in the previous study (see Chapter 2). That is, they consisted of five separate tempi: 60bpm, 90bpm, 120bpm, 150bpm and 180bpm. However, each tempo played repeatedly for a longer duration: at least 120 seconds. A minimum duration of 120 seconds for the five individual tempi was applied to ensure valid derivation of HRV and BRS parameters for each individual tempo. This resulted in the stepped increase and decrease in tempo stimuli being at least 10 minutes in duration. The five individual tempi were organised in ascending order for the stepped increase in tempo stimulus and organised in descending order for the stepped decrease in tempo stimulus. Figure 3.1 diagrammatically illustrates the structure of the stepped increase in tempo stimulus and Figure 3.2 portrays the structure of the stepped decrease in tempo stimulus.

## Stepped increase in tempo



*Figure 3.1: The five tempi of the stepped increase in tempo stimulus. Comprised of 60bpm, 90bpm, 120bpm, 150bpm and 180bpm. Each tempo was a minimum of 120-seconds in duration.*

## Stepped decrease in tempo



*Figure 3.2: The five tempi of the stepped decrease in tempo stimulus. Comprised of 180bpm, 150bpm, 120bpm, 90bpm and 60bpm. Each tempo was a minimum of 120-seconds in duration.*

For the stable tempo stimulus, the tune was repeatedly played at a consistent speed (120bpm) for ten minutes. 120bpm was chosen as it was the mean tempo of the five individual tempi that comprised the stepped increase and stepped decrease in tempo stimuli.

The stimuli were then preliminarily tested on five participants. Frère Jacques and Twinkle Twinkle Little Star were immediately excluded as they were insufficiently interesting and resulted in participants falling asleep. This left

Baa Baa Blacksheep and Mary Had a Little Lamb which were both thought to be slightly more stimulating. Ultimately, Baa Baa Blacksheep was chosen as the final tune because it was sufficiently interesting to maintain participant interest throughout, whilst having simple melodic and rhythmic patterns. The tune was played in c major for the rhythm + melody group. However, for the control group, all pitch changes were removed by playing the tune on one note (G) whilst keeping the rhythmic characteristics intact. This note was chosen because it was the most common note in the nursery rhyme and close to the average frequency of all notes that occurred in the version given to participants in the rhythm + melody group. By including this rhythmic version of the tune, an evaluation of the extent which melody contributes to physiological and subjective responses to changes in tempo was made possible. An additional group which would have received the melody of the nursery but with the rhythm removed was also considered. However, after creating and testing the stimuli the group was not run. This was because the stimuli sounded too peculiar. This counteracted one of the aims of the study which was to increase the ecological validity of the findings. Instrumentation (piano) was the same for both groups and all other musical parameters, such as dynamics, articulation and phrasing were held constant. This was done to ensure close examination of the tempo manipulations.

The auditory stimuli were played using an mp3 player and presented to participants via wired over-the-head headphones.

### **3.2.1.2 Questionnaires**

Participants were asked to complete four different questionnaires at specific time points during the visit. These included: health, Godin Leisure Time, music background and VAS questionnaires. All four questionnaires were completed at the beginning of the study (after obtaining informed consent), and specific VAS questionnaires were administered at the end of each condition. The information obtained from these questionnaires aided with the analysis of the outcome measures.

1. Health questionnaire (see Appendix B.2.). This questionnaire was similar to that used in Chapter 2, however, extra items were added.

This included whether female participants were post-menopausal and if yes, whether they were receiving hormone replacement therapy. These questions were included because evidence suggests the menopause may influence autonomic function in females. For instance, postmenopausal women have attenuated HRV (Neves et al., 2007) and higher blood pressure (Neves et al., 2007) and MSNA (Matsukawa, Sugiyama, Watanabe, Kobayashi & Mano, 1998) than premenopausal women. Furthermore, some studies have found that hormone replacement therapies can alter autonomic function (Yildirim et al., 2002; Christ, Seyffart, Tillmann & Wehling, 2002; Farag et al., 2002; Carnethon et al., 2003; Neves et al., 2007). A third questionnaire item concerning the presence of obstructive sleep apnoea was also included.

2. Godin Leisure Time Questionnaire (see Appendix B.3.). This required participants to state the frequency of light, moderate and strenuous exercise they undertook per week. This questionnaire has been found to be a reliable and valid measure of the amount of physical activity individuals perform per week (Godin & Shephard, 1985).
3. Musical background questionnaire (see Appendix B.4.). This was also similar to the one employed in Chapter 2. However, additional items were included:
  - a. Ranking participants' top five genres of music
  - b. Stating whether participants undertook any 'music-making activities'
  - c. Preferred music for the study room

These changes were based on participant confusion in the pilot (with regards to the music genre item) and the possibility that individuals who partake in music-making activities have lower baseline sympathetic predominance than those who do not. In addition, as the suitability of music to a listener's environment is considered important (Koelsch & Jäncke, 2015), the preferred music of participants whilst



situated in the study room was obtained. Indeed, this provided useful information when needing to choose a musical stimulus in the final study (see Chapter 4).

4. VAS questionnaire. Although the project primarily focusses on autonomic function, the subjective experiences of participants was also of interest as correlations between physiological and self-report measures exist in the literature (Gomez & Danuser, 2002; Khalfa et al., 2002; van der Zwaag et al., 2011). Consequently, this led to the development and use of VAS questionnaires. The questionnaires were designed based on the circumplex model of emotion (Hevner, 1937; Russell, Ward & Pratt, 1981; Roberts & Wedell, 1994) and the experienced/perceived musical emotion distinction (Gomez & Danuser, 2007). Three different VAS questionnaires were employed: one was administered before the study (pre-baseline VAS questionnaire: see Appendix B.5.); one was administered after the baseline and recovery conditions (baseline/recovery VAS questionnaire: see Appendix B.6.) and another was administered after the auditory stimuli (stimuli VAS questionnaire: see Appendix B.7.). For all VAS questionnaires, participants were required to place a vertical line on a 100mm horizontal line which was anchored at each end by one member of an adjective pair. Experienced and perceived musical emotions were examined in the baseline/recovery and stimuli VAS questionnaires, whilst the initial VAS questionnaire measured how participants felt immediately before being connected to the equipment.

Separate scales for six experienced musical emotions (stimulation, relaxation, stress, calm, happiness and sadness) were used. This was because there is evidence suggesting that these emotions are not bipolar (being less sad does not necessarily equate to being happier) (Averill, 1980; Russell, 1989; Barrett & Russell, 1998; Russell & Carroll, 1999). In addition, it was hoped that participants could provide more accurate reports of their emotional experiences.

Due to more ambiguity concerning whether the independence of opposite emotions applies to objects (such as music) (Russell & Carroll, 1999), bipolar adjective pairs were used for the four perceived musical emotions of interest (positivity, stimulation, pleasantness and irritation).

It should be acknowledged that VASs are a less common measure for assessing subjective valence and arousal in music listeners compared to Likert Scales. But they do facilitate greater flexibility in the range of statistical tests that can be performed and can be administered quickly and simply. Moreover, they are argued to be a more sensitive measure of subjective sensations than Likert Scales (Gift, 1989).

### **3.2.2 Apparatus**

#### **3.2.2.1 MP3 player**

A SanDisk Sansa Fuse+ MP3 player was used to present the musical stimulus to participants.

#### **3.2.2.2 Headphones**

Sony MDR-P180 wired over-the-head stereo headphones were used to play the auditory stimuli to participants.

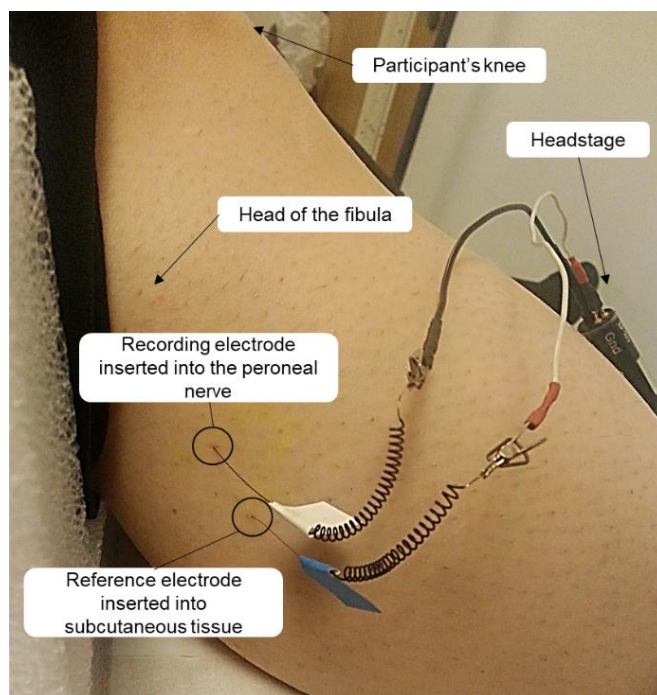
#### **3.2.2.3 Physiology equipment**

Heart rate, blood pressure and respiration were recorded using the same equipment as that was used in the previous study (see chapter 2).

#### **3.2.2.4 Microneurography**

MSNA was recorded as previously described by Macefield et al. (1994) and Greenwood, Stoker and Mary (1999). The peroneal nerve was identified by palpation and confirmed with the use of a Powerlab stimulator unit (ADInstruments, Dunedin, New Zealand). Use of the stimulator unit involved attaching electrode pads (Ambu UK) to the inner ankle and metatarsals and generating small electrical impulses (5-10mA) in LabChart (ADInstruments,

Dunedin, New Zealand) whilst moving the stimulator unit, located near the fibula, towards the foot and towards the back of the leg. Upon confirmation of the peroneal nerve, two tungsten microelectrodes (FHC Inc., USA) were inserted percutaneously. The microelectrodes were 35mm in length with a diameter of 200 $\mu$ m tapering to a tip. The recording microelectrode was epoxy insulated with an impedance of  $0.3 \pm 0.6\text{M}\Omega$ . An electrode with high impedance was used as this limits the area over which neural activity is picked up and is necessary for single unit MSNA recordings (Macefield, Elam & Wallin, 2002). The recording microelectrode was inserted into the peroneal nerve, identified by palpation and by the stimulator unit. The second microelectrode, a reference microelectrode, was inserted into subcutaneous tissue 1-2cm away from the recording electrode (see Figure 3.3). Both microelectrodes were connected to a headstage (Neurolog NL100AK, UK) which was connected to an AC pre-amplifier (x50k amplification; Neurolog NL104A, UK). The signal was passed through a Humbug (Quest Scientific, Canada) to filter out mains noise at 50Hz and a bandpass filter (0.7-2.0 kHz; Neurolog NL125/6, UK). The signal was sampled at 16 kHz and digitised (Power 1401, CED, UK).



*Figure 3.3: Microelectrode placement for microneurography. The recording electrode was inserted into the peroneal nerve just below the head of the fibula. The reference electrode was inserted into tissue just below the recording electrode.*

### 3.2.3 Procedure

#### 3.2.3.1 General procedure

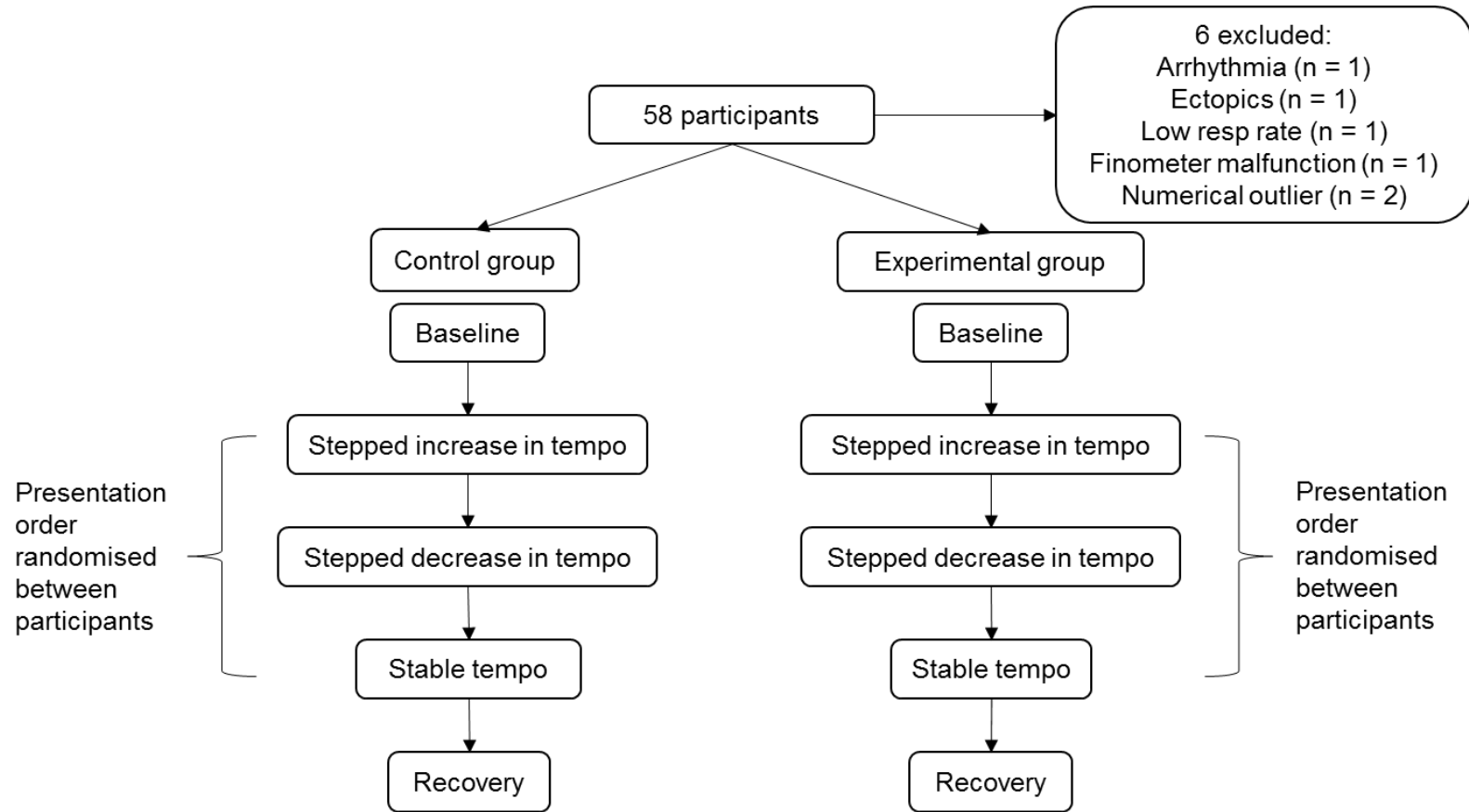
Figure 3.4 outlines the study procedure. Participants attended the University of Leeds on one occasion only. At the beginning of the study the health questionnaire, Godin Leisure Time Questionnaire, musical background questionnaire and initial VAS questionnaire were administered to participants, and their height and weight obtained. Participants were then asked to lie in a semi-supine position on a couch with a memory foam mattress with pillows supporting the head and lower back. Physiological equipment which continuously recorded respiration, HR, BP and MSNA were then attached to participants. Participants then underwent an adaptation period of approximately three minutes which allowed cardiovascular measures to stabilise. Data collection commenced following stabilisation of HR and BP.

Participants underwent five 10-minute conditions: baseline (rested in silence with the headphones in place); stable tempo (rested while listening to the

stable tempo stimulus); stepped increase in tempo (rested while listening to the tempo increase stimulus); stepped decrease in tempo (rested while listening to the tempo decrease stimulus) and recovery (same as baseline). Before the first auditory stimulus, participants listened to a c major, one octave scale starting on middle c to ensure the audio was played at a comfortable volume. The order of the baseline and recovery conditions was fixed: baseline was always first and recovery was always last. In contrast, the presentation order of the stimuli (stable tempo, stepped increase or stepped decrease in tempo) was randomised between participants. Unlike the pilot study, the stepped increase and decrease in tempo stimuli did not run back-to-back (there was a break between the two stimuli). HR, BP and respiration were continuously recorded during the five conditions. At the end of each condition, participants completed a VAS questionnaire and brachial HR and BP were measured three times using the Omron blood pressure monitor. Following this, an adaptation period of three minutes was implemented to allow HR and BP to stabilise before commencement of the subsequent condition.

Participants were asked to remain still and to refrain from talking and falling asleep during the recordings. The study room was kept at a constant temperature of  $21 \pm 2$  degrees Celsius.

Upon completion of the data collection, physiological recording equipment was detached from participants and all participants received £20 reimbursement (as funded by SEMPRES's Arnold Bentley New Initiatives Fund).



*Figure 3.4: Procedure employed in the follow-up study. Fifty-eight participants were randomly allocated to a control or experimental group. The control group received the rhythm of Baa Baa Blacksheep whilst the experimental group received the melody and rhythm. Both groups underwent baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery conditions.*

### **3.2.3.2 Microneurography procedure**

Participants who agreed to undergo microneurography completed two cardiovascular tests: the cold pressor test and the isometric handgrip test.

#### **3.2.3.2.1 Cold pressor test**

A baseline recording was obtained for one minute then the participant was asked to submerge the entire left hand and wrist into ice water (approximately 4°C). The hand was submerged for one minute unless the discomfort level was too high. This occurred for one participant only who did not complete the full minute. Following one minute of immersion, the hand was removed and placed in a towel. The cold pressor test aided with discriminating between MSNA and skin sympathetic nerve activity (SSNA). This is because SSNA is considered to remain constant throughout whilst MSNA increases consistently following a 30 second delay (Victor, Leimbach, Seals, Wallin & Mark, 1987). The cold pressor test also evoked an increase in blood pressure which has been shown to be correlated with increases in MSNA (Victor, Leimbach, Seals, Wallin & Mark, 1987; Calhoun, Mutinga, Collins, Wyss & Oparil, 1993). As a result, the response to the cold pressor test was derived from the last 30 seconds of the immersion period and compared to the baseline period.

#### **3.2.3.2.2 Isometric handgrip test**

Maximal voluntary contraction (MVC) was determined during the experimental set up by asking the participant to squeeze a handgrip as hard as possible. The handgrip was connected to a dynamometer (MIE Medical Research Ltd, UK) which provided a numerical display. A baseline recording was obtained for one minute then the participant was asked to squeeze the handgrip at 50% MVC for two minutes, unless discomfort level was too high. This occurred for one participant only who did not complete the full two minutes. During the handgrip, participants were asked to keep all other muscles as relaxed as possible and to avoid holding their breath during the handgrip. After two minutes, participants were asked to release the

handgrip. Like the cold pressor test, the isometric handgrip test can also aid with differentiating between MSNA and SSNA. This is because SSNA increases suddenly at the beginning of the test then remains constant, whereas MSNA increases throughout following a 30 second delay (Seals, 1988; Saito, Mano, Iwase, 1990). The isometric handgrip test also elicits a continuous increase in blood pressure which coincides with the gradual increase in MSNA (Seals, 1988; Saito et al., 1990). Due to the delay in MSNA, data from the second half of the test was compared to the baseline period.

#### **3.2.4 Data acquisition**

The data were acquired as per the pilot study (see Chapter 2). The microneurography signal was also digitised, passed to the laptop and visualised in a data channel in Spike2 software.

#### **3.2.5 Data analysis**

HR, HRV, SBP, DBP, MAP, BRS and respiration rate were analysed offline in LabChart. As the data were originally recorded in Spike2, the data were exported as text files, imported into LabChart and analysed as per Chapter 2. MSNA was analysed offline in Spike2. Values for each measure were derived for each condition and for the five individual tempi that comprised the stepped increase and stepped decrease in tempo stimuli.

##### **3.2.5.1 MSNA**

The blood pressure traces during the cold pressor and isometric hand grip tests were visually inspected to determine whether blood pressure increased in the second half of each test. If blood pressure failed to increase during the second half of both tests, no further MSNA analysis was performed.

Following confirmation of the required changes in blood pressure, the MSNA trace was visually inspected to identify potential action potentials. Action potentials were confirmed as originating from sympathetic vasoconstrictor fibre if:



1. Occurrence of the action potential increased over the duration of the cold pressor and isometric handgrip tests. Whereby unit frequency increased with the increases in blood pressure in the second half of the tests.
2. The action potential occurred in diastole only. If the action potential occurred in systole the unit was discounted.
3. No change in action potential activity was observed during cutaneous stimulation (stroking the dorsum of the foot).
4. Superimposition of all putative MSNA single units demonstrated that the shape and amplitude of the action potentials were consistent. Superimposing the presumed MSNA single units was performed by exporting a metafile image of each unit from Spike2 and importing it into Corel Draw (Version 6).

In total 10 MSNA units were confirmed. The cold pressor and isometric handgrip tests as well as the confirmed unit for all 10 units are presented in Appendix B.8. MSNA frequency (per min) was calculated by counting all single units that occurred in each recording. MSNA incidence (number of units per 100 heart beats) was also calculated to limit the effect of any changes in heart rate. This involved dividing the MSNA frequency by mean heart rate and multiplying by 100. Data were also normalised to 1.

### **3.2.6 Statistical analysis**

Like Chapter 2, normality was tested for via the Shapiro-Wilk test. If data were not normally distributed non-parametric equivalents were implemented. To address the first objective repeated measure ANOVAs (or Friedman tests) were performed on four time periods in the baseline and recovery recordings (0-10 minutes, 0-5 minutes, 2.5-7.5 minutes, 5-10 minutes). For statistically significant Friedman tests, pairwise comparisons were performed using the Wilcoxon Signed-Ranks test. As the Wilcoxon did not automatically adjust the alpha level for multiple comparisons, the p-value was adjusted by dividing it by the number of pairwise comparisons made. So, in the instance of six pairwise comparisons the alpha level was set to 0.008.

To examine the second and third objectives mixed mode ANOVAs were performed. For the second objective, the within-participants variable was condition (baseline, stepped increase in tempo, stepped decrease in tempo, stable tempo, recovery) and the between-participants variable was group (control, experimental). For the third objective, the within-participants variable was tempo (60bpm, 90bpm, 120bpm, 150bpm, 180bpm) and the between-participants variable was group (control, experimental). Bonferroni pairwise comparisons were performed on statistically significant main effects and interactions. The Greenhouse-Geisser correction was used when data did not meet sphericity.

To address the fourth objective Pearson correlations (or Spearman correlations for non-normally distributed variables) were conducted. As the self-report measures were obtained upon completion of each condition, the physiological measures were inputted as independent variables and the VAS as the dependent variables.

To fulfil the final objective (is it possible to predict response type based on baseline readings?) linear regressions were performed between baseline and percentage change values. To examine differences between responder types, independent sample t-tests (or Mann-Whitney U) and one-way ANOVAs (or Kruskal-Wallis tests) were performed. For significant Kruskal-Wallis tests, pairwise comparisons were performed with Mann-Whitney U tests and the alpha level adjusted to 0.017.

### **3.2.7 Study failures**

In total, 58 volunteers took part in the study. Data from six were excluded, the reason and their characteristics are presented in Table 3.2. Appendix B.9 provides a summary of the final sample of 52 participants (24 males). Appendix B.10 provides a summary of the characteristics of the two groups (control and experimental).

*Table 3.2: Follow-up study failures.*

	<b>Gender</b>	<b>Group</b>	<b>Age (yrs)</b>	<b>Post-menopausal?</b>	<b>Physically active?</b>	<b>Formally music trained?</b>	<b>Reason</b>
1	M	Experimental	39	-	Y	N	Extreme outlier
2	M	Control	36	-	N	N	Extreme outlier
3	F	Control	57	Y	N	Y	Arrhythmia
4	F	Experimental	57	Y	N	Y	≥ Two ectopic heartbeats in any given five-minute section
5	F	Control	32	-	Y	Y	Finometer malfunction
6	M	Control	25	-	Y	Y	Respiration rate < 10 breaths per minute

### **3.3 Results**

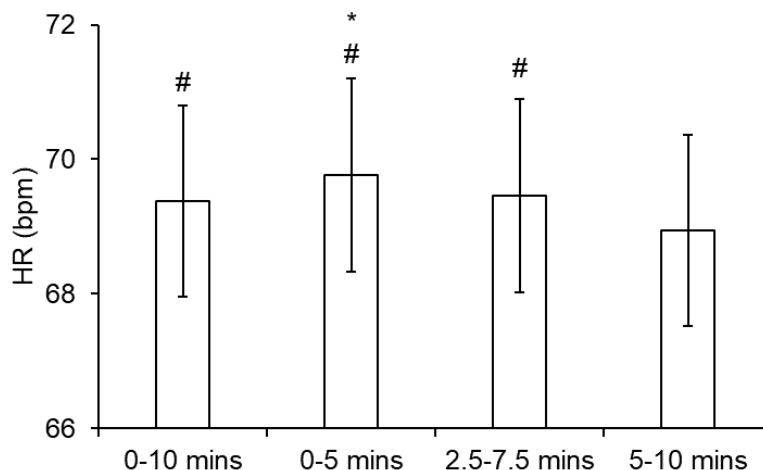
#### **3.3.1 Baseline and recovery condition time periods**

Guidelines by Camm et al. (1996) recommend analysing frequency-domain HRV in five minute chunks and time-domain HRV over 24 hours unless the design of the study dictates otherwise. The purpose of this recommendation is to standardise procedures across studies measuring HRV. However, since long-term recordings were not feasible given the study design, conditions of at least 10 minutes were employed. As LF/HF (a frequency-domain HRV parameter) was one of the primary outcome measures, all variables were measured over four time periods during the baseline and recovery recordings. This was accomplished to ascertain which of the four time periods presented the most accurate portrayal of participants at rest. The following four different time periods were explored via statistical analysis:

1. 0-10 minutes (full 10-minute condition)
2. 0-5 minutes (first five minutes)
3. 2.5-7.5 minutes (middle five minutes)
4. 5-10 minutes (last five minutes)

##### **3.3.1.1 Time-domain HRV analysis**

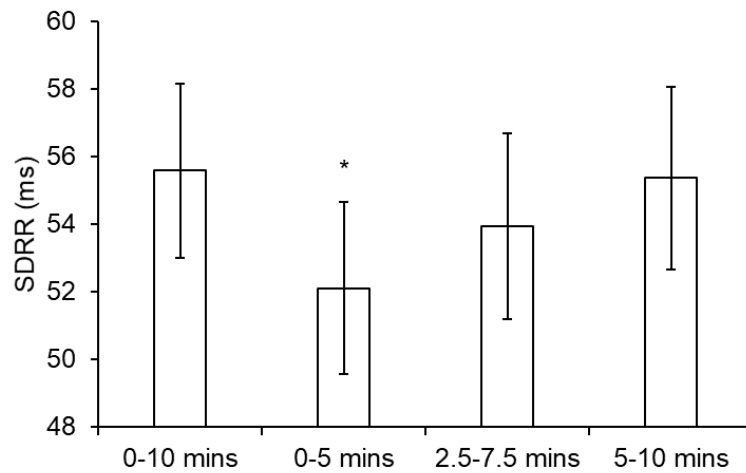
For the baseline condition, a significant difference in HR emerged between the four time periods ( $p = 0.004$ ). Bonferroni pairwise comparisons revealed that HR during 5-10 mins was significantly lower than that during 0-10 mins ( $p = 0.020$ ), 0-5 mins ( $p = 0.026$ ) and 2.5-7.5 mins ( $p = 0.014$ ). Furthermore, HR during 0-10 mins was significantly lower than that during the first five minutes ( $p = 0.035$ , see Figure 3.5).



*Figure 3.5: HR significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.*

SDRR also significantly differed between the four time periods ( $p = 0.022$ ). As illustrated in Figure 3.6a, SDRR was significantly higher during 0-10 mins than 0-5 mins ( $p < 0.001$ ). Finally, a significant effect on pRR50 was identified ( $p = 0.013$ ). Pairwise comparisons revealed that pRR50 was significantly lower during 0-5 mins than 0-10 mins ( $p = 0.006$ ), 2.5-7.5 mins ( $p = 0.007$ ) and 5-10 mins ( $p = 0.005$ ). Additionally, pRR50 was significantly higher during the last five minutes than during the full 10-minute recording ( $p = 0.004$ , see Figure 3.6b).

a.



b.

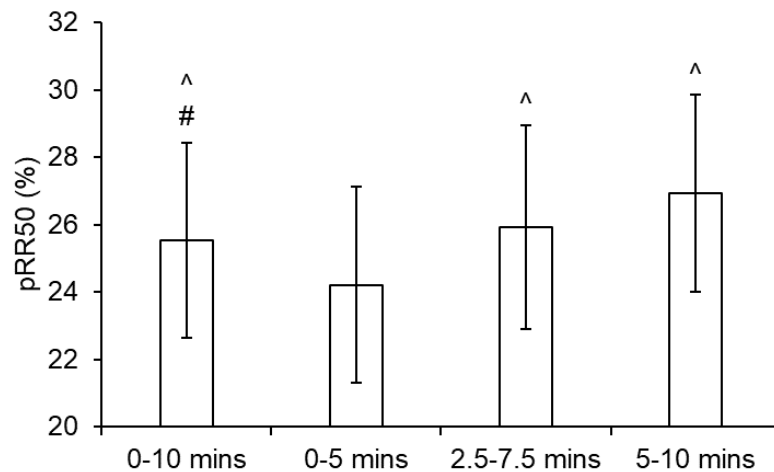


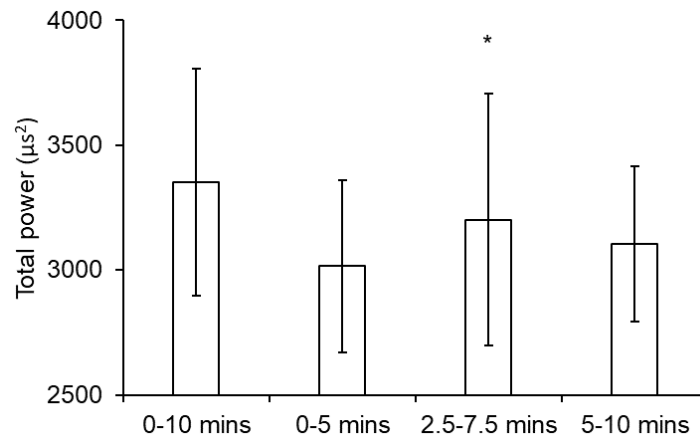
Figure 3.6: SDRR (a) and pRR50 (b) significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins; ^ = significantly different to 0-5 mins.

None of the time-domain HRV measures significantly differed between the four recovery time periods ( $p > 0.05$ ).

### 3.3.1.2 Frequency-domain HRV analysis

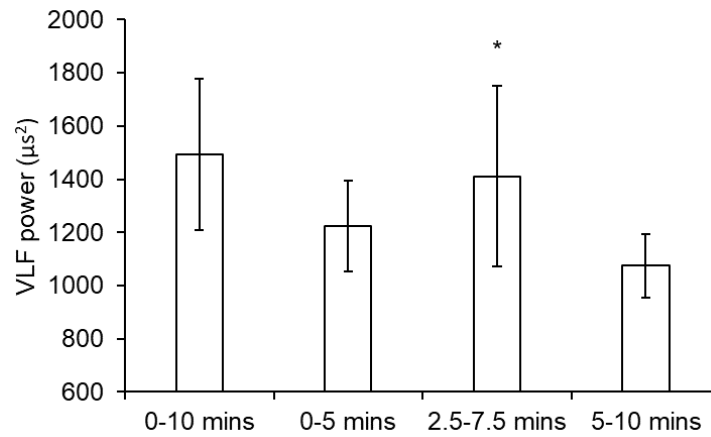
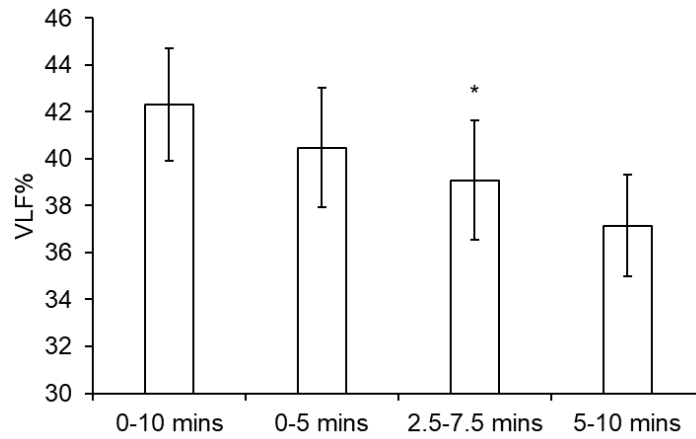
For the baseline condition, a Friedman test revealed a statistically significant difference in total power between the four time periods ( $p = 0.007$ ). As

illustrated in Figure 3.7, total power was significantly lower during 2.5-7.5 mins compared to the full 10 minutes ( $p = 0.004$ ).



*Figure 3.7: Total power significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.*

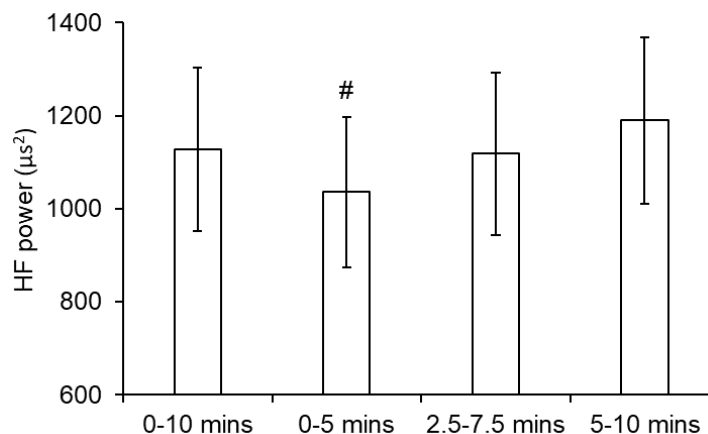
Similarly, VLF power and VLF% significantly differed between the four time periods (both  $p = 0.013$ ), with VLF power and VLF% being significantly lower during 2.5-7.5 mins than 0-10 mins ( $p = 0.002$  and  $p < 0.001$  respectively, see Figure 3.8).

**a.****b.**

*Figure 3.8: VLF power (a) and VLF% (b) significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.*

Although no statistically significant effect of time period emerged for LF power, a Friedman test detected a significant difference for HF power ( $p = 0.007$ ). As shown in Figure 3.9, HF power was significantly higher during the last five minutes than during the first five minutes ( $p = 0.006$ ).



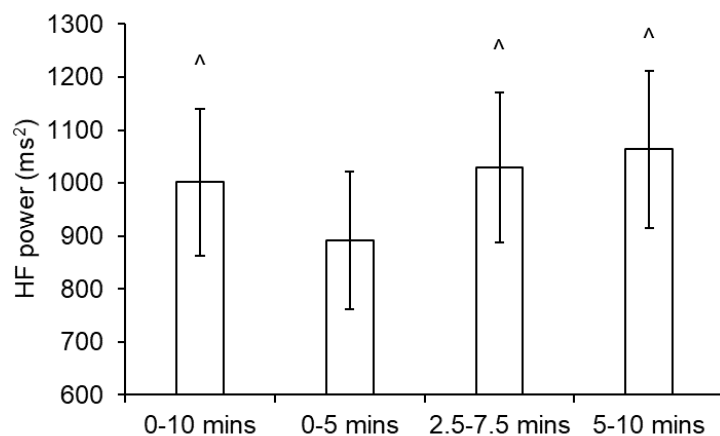


*Figure 3.9: HF power significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. # = significantly different to 5-10 mins.*

No other statistically significant differences emerged in the frequency-domain HRV measures ( $p > 0.05$ ).

For the recovery condition, HF power significantly differed between the four time periods ( $p = 0.001$ ). As depicted in Figure 3.10a, HF power was significantly lower during the first five minutes, compared to the full 10 minutes ( $p < 0.001$ ), middle five minutes ( $p = 0.001$ ) and last five minutes ( $p = 0.001$ ). As a result, an effect of recovery time periods emerged for HF% ( $p = 0.037$ ). However, the only significant difference that emerged was between 0-10 mins and 2.5-7.5 mins ( $p < 0.001$ ). Inspection of Figure 3.10b shows that HF% was higher in the latter compared to the former. No other frequency-domain HRV parameters significantly differed between the four recovery time periods ( $p > 0.05$ ).

a.



b.

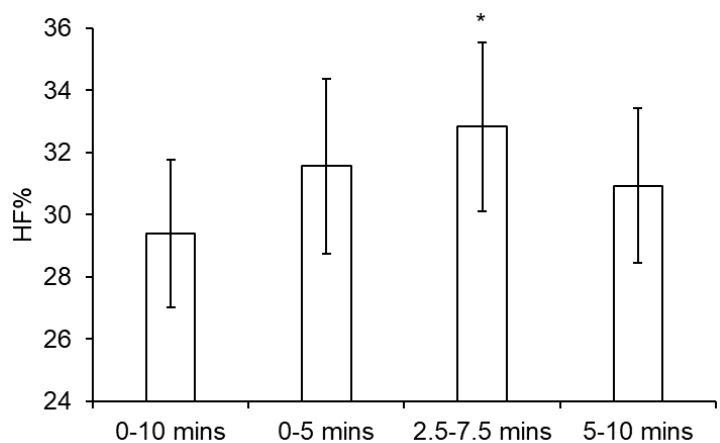


Figure 3.10: HF power (a) and HF% (b) significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; ^ = significantly different to 0-5 mins.

### 3.3.1.3 Non-linear HRV analysis

For the baseline condition, some of the non-linear measures varied between the four time periods. For instance, as shown in Figure 3.11, SD2 (Friedman test:  $p = 0.044$ ) was significantly lower during the first five minutes compared to the full ten minutes ( $p < 0.001$ ).

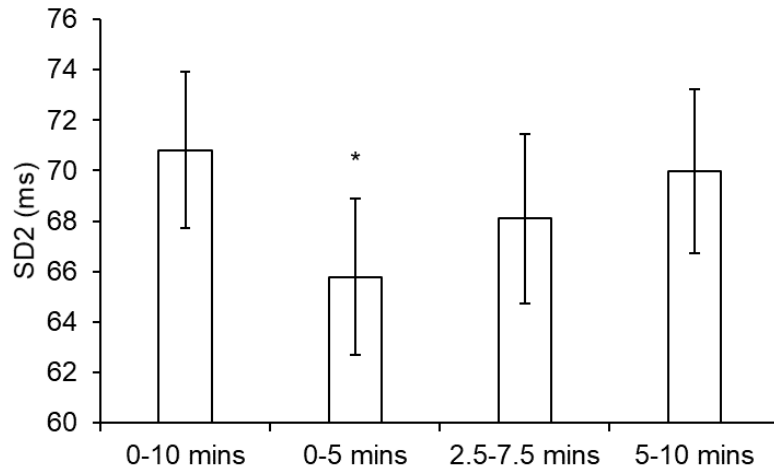


Figure 3.11: SD2 significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.

Also, although no significant difference in SD1 was detected, there was a significant effect on nSD1 ( $p = 0.022$ ). Further exploration showed that nSD1 was significantly higher during 5-10 mins than 0-10 mins ( $p = 0.002$ ) and 0-5 mins ( $p = 0.002$ , see Figure 3.12). Furthermore, nSD1 was significantly lower in 0-5 mins compared to 0-10 mins ( $p = 0.002$ ).

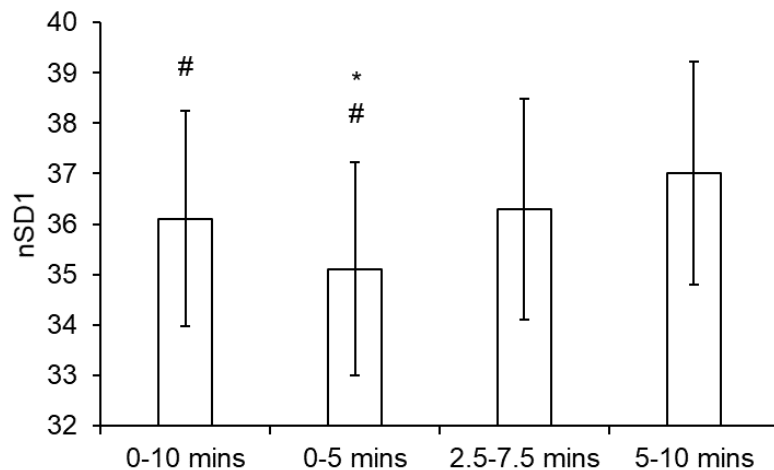
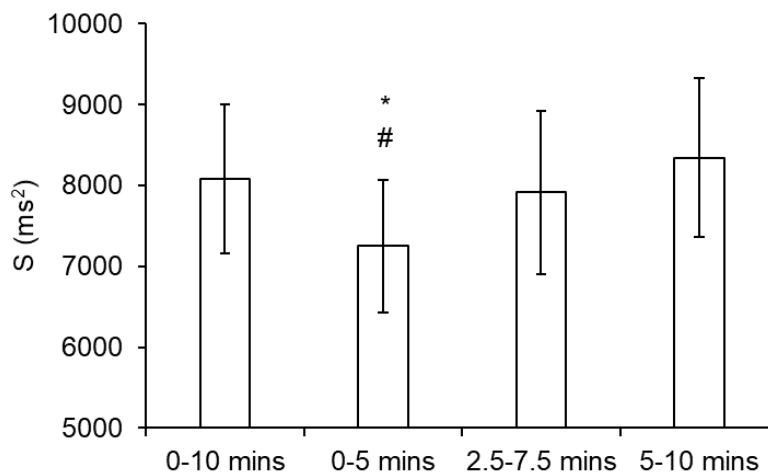


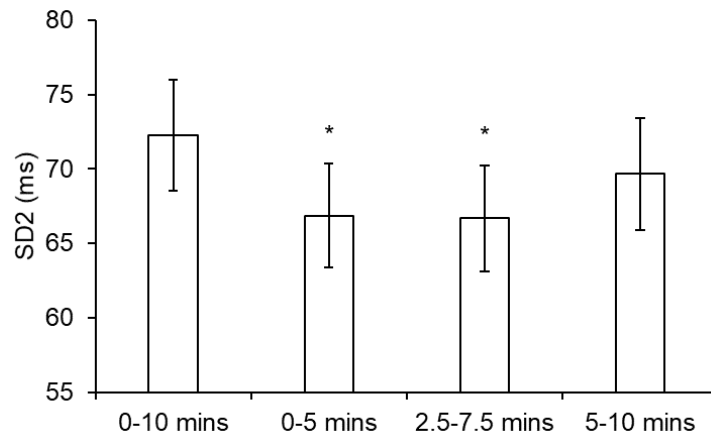
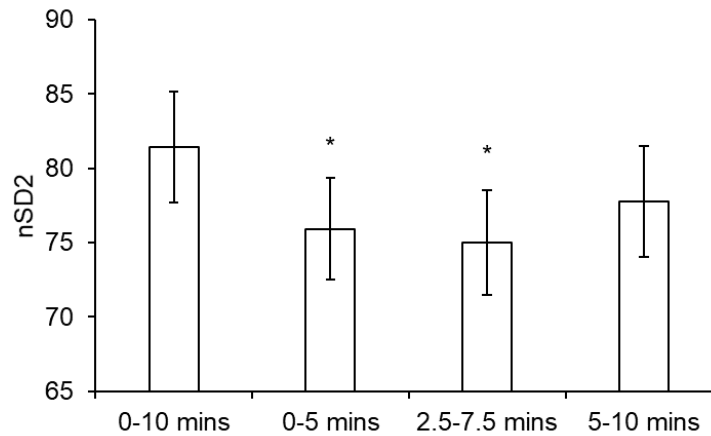
Figure 3.12: nSD1 significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.

The final variable that differed between the time periods was S ( $p = 0.005$ ). Figure 3.13 illustrates that S was significantly lower during the first five minutes than during the full recording ( $p < 0.001$ ) and final five minutes ( $p = 0.008$ ).



*Figure 3.13: S was significantly impacted by the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.*

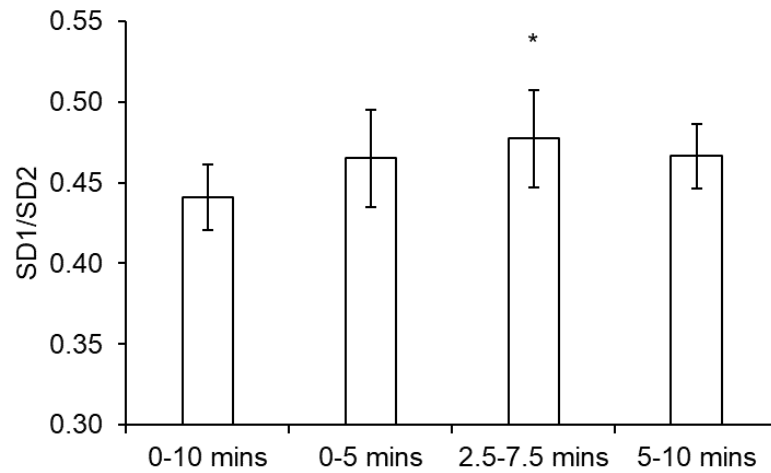
For the recovery condition, there was a significant impact of time period on SD2 ( $p = 0.033$ ). As shown in Figure 3.14a, SD2 was significantly higher during the full 10 minutes compared to the first ( $p = 0.002$ ) and middle ( $p < 0.001$ ) five minutes. Even after controlling for HR, the effect of time period on nSD2 reached statistical significance ( $p = 0.013$ ). Moreover, the same pairwise comparisons were statistically significant: nSD2 was significantly higher during 0-10 minutes compared to 0-5 mins ( $p = 0.007$ ) and 2.5-7.5 mins ( $p = 0.002$ , see Figure 3.14b).

**a.****b.**

*Figure 3.14: SD2 (a) and nSD2 (b) significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.*

Although SD1 (and nSD1) did not vary between the time periods, SD1/SD2 and SD2/SD1 did (both  $p = 0.010$ ). Indeed, examination of Figure 3.15a and b demonstrate that SD1/SD2 was significantly higher and SD2/SD1 significantly lower during the middle five minutes compared to full duration (both  $p < 0.001$ ).

a.



b.

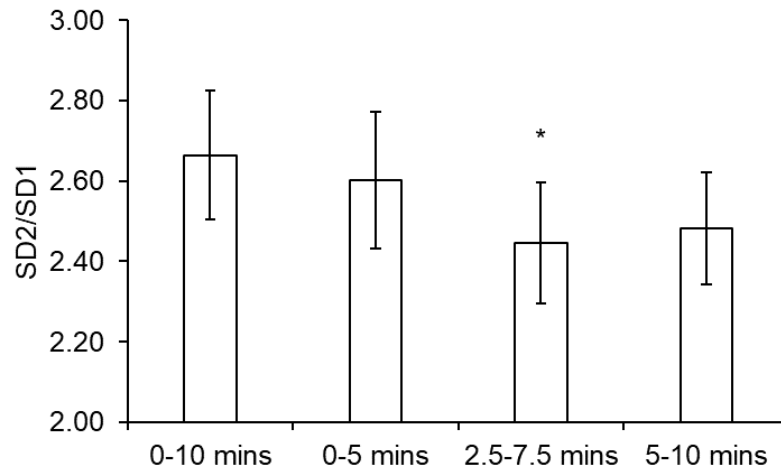
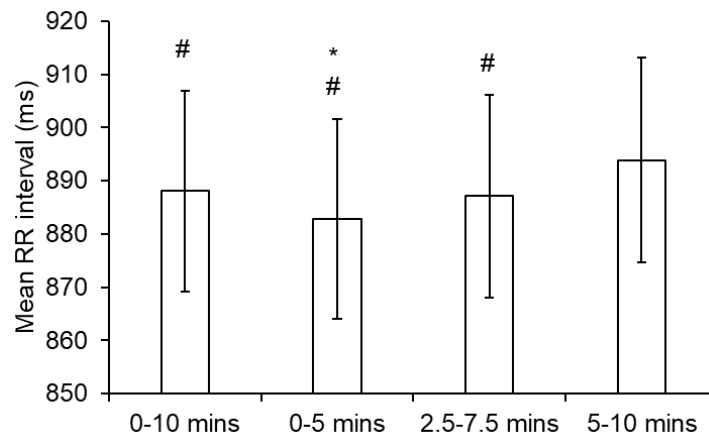
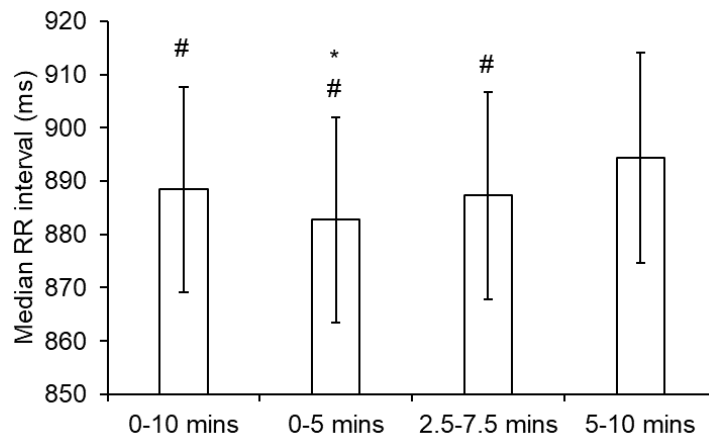


Figure 3.15:  $SD1/SD2$  (a) and  $SD2/SD1$  (b) significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.

#### 3.3.1.4 RR analysis

For the baseline condition, mean RR and median RR significantly differed between the four time periods ( $p = 0.003$  and  $p = 0.004$  respectively). In both cases, RR interval was significantly longer in the last five minutes compared to the full 10 minutes ( $p = 0.014$  and  $p = 0.039$ ), first five minutes ( $p = 0.019$  and  $p = 0.023$ ) and middle five minutes ( $p = 0.004$  and  $p = 0.006$ ). In addition, mean and median RR were significantly longer during 0-10 mins than 0-5 mins ( $p = 0.026$  and  $p = 0.016$ , see Figure 3.16a and b).

**a.****b.**

*Figure 3.16: Mean (a) and median (b) RR intervals significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.*

Min, max and  $\Delta$  RR significantly differed between the four time periods (all  $p < 0.001$ ). As shown in Figure 3.17a-c, all three variables were significantly higher in 0-10 mins than 0-5 mins, 2.5-7.5 mins and 5-10 mins (all  $p < 0.001$ ).

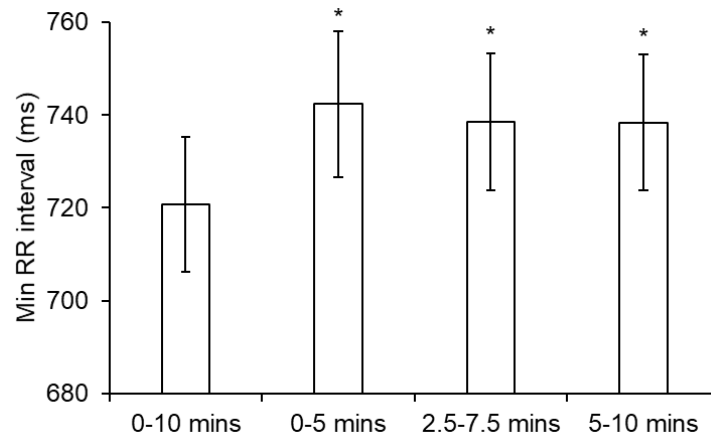
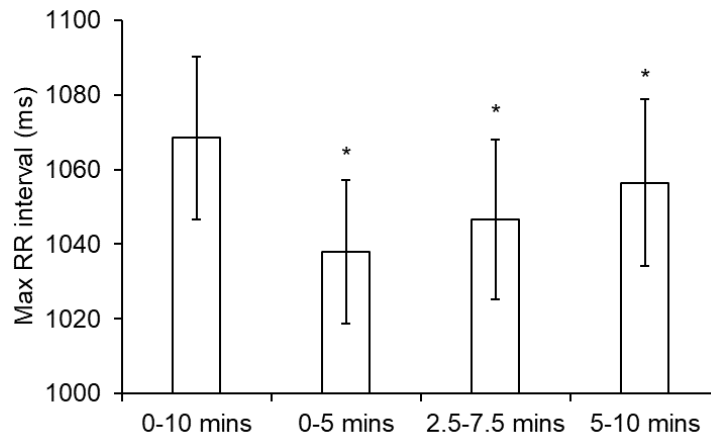
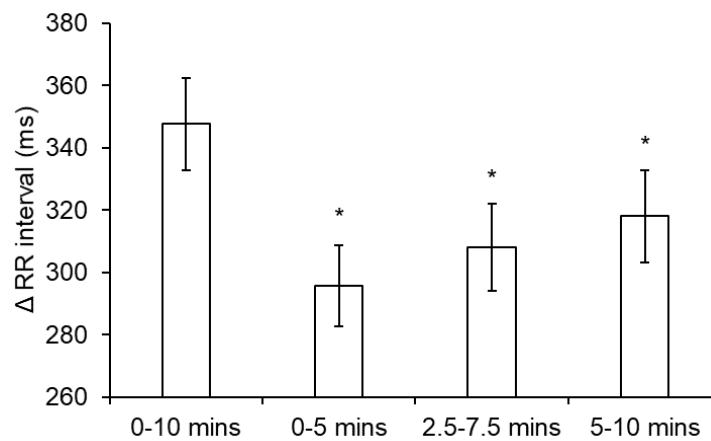
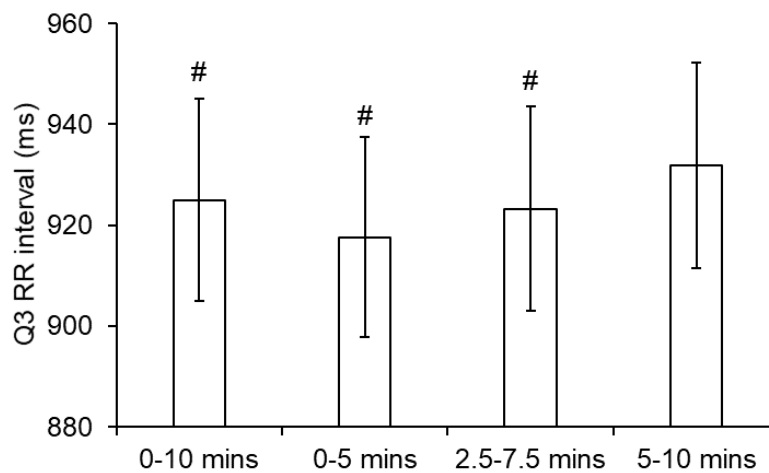
**a.****b.****c.**

Figure 3.17: Min (a), max (b) and  $\Delta$  (c) RR intervals significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.

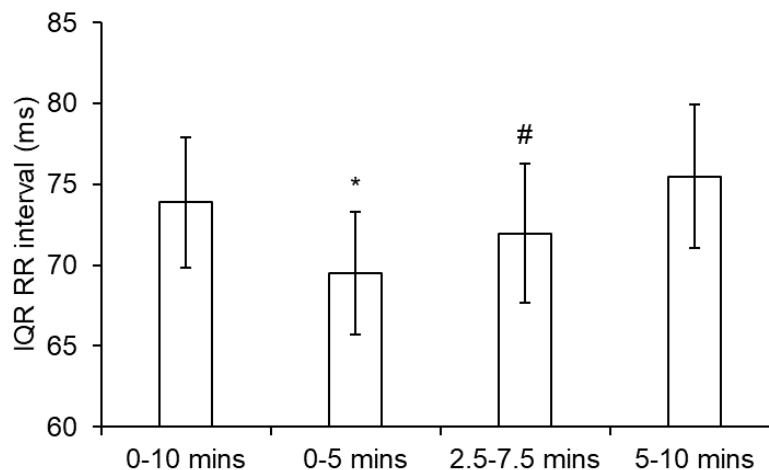


Another variable that significantly varied was Q3 RR ( $p < 0.001$ ). Bonferroni pairwise comparisons revealed that Q3 RR was significantly higher in the last five minutes than during the full 10 minutes ( $p = 0.001$ ), first five minutes ( $p = 0.001$ ) and middle five minutes ( $p < 0.001$ , see Figure 3.18a). Despite the effect on Q1 RR failing to reach statistical significant ( $p > 0.05$ ), IQR RR did ( $p < 0.001$ ). As shown in Figure 3.18b **Error! Reference source not found.**, IQR RR was significantly higher in 0-10 mins than 0-5 mins ( $p = 0.003$ ) and 5-10 mins compared to 2.5-7.5 mins ( $p = 0.006$ ).

**a.**



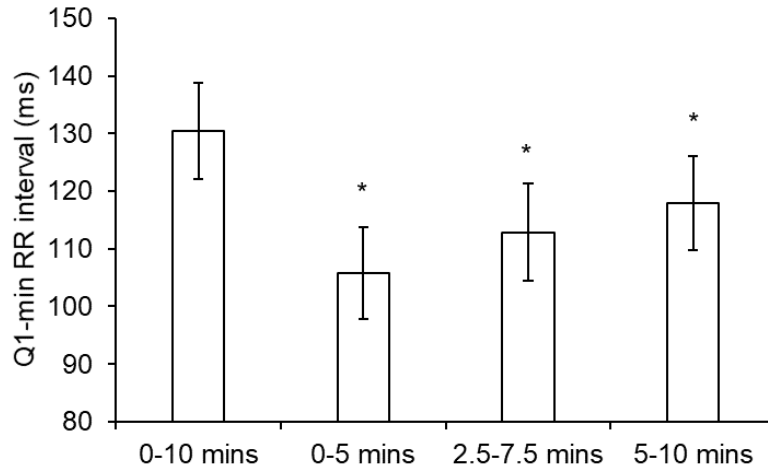
**b.**



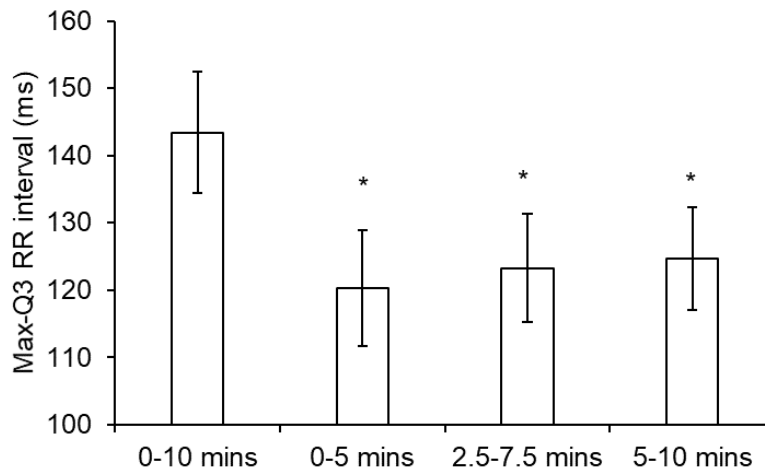
*Figure 3.18: Q3 (a) and IQR (b) RR intervals significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.*

The final two RR interval measures that were significantly impacted by the baseline time periods were: Q1-min RR and max-Q3 RR (both  $p < 0.001$ ). In both cases, RR interval was significantly greater during 0-10 mins than 0-5 mins, 2.5-7.5 mins and 5-10 mins (all  $p < 0.001$ , see Figure 3.19a and b).

**a.**



**b.**



*Figure 3.19: Q1-min (a) and max-Q3 (b) RR intervals significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.*

For the recovery condition, significant effects of time period on min, max and  $\Delta$  RR emerged (all  $p < 0.001$ ). Further analysis of these effects revealed that min RR was significantly higher during all three five-minute periods

compared to the 10-minute time period (all  $p < 0.001$ , see Figure 3.20a). In contrast, max and  $\Delta$  RR were significantly lower during the three five minute periods compared to the full recovery condition (all  $p < 0.001$ , see Figure 3.20b and Figure 3.20c).

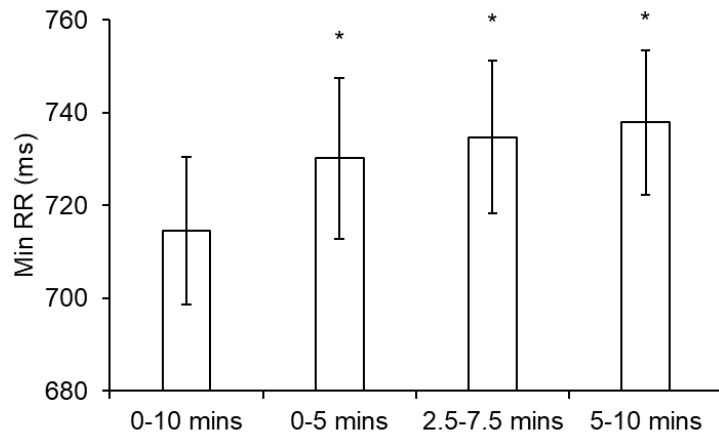
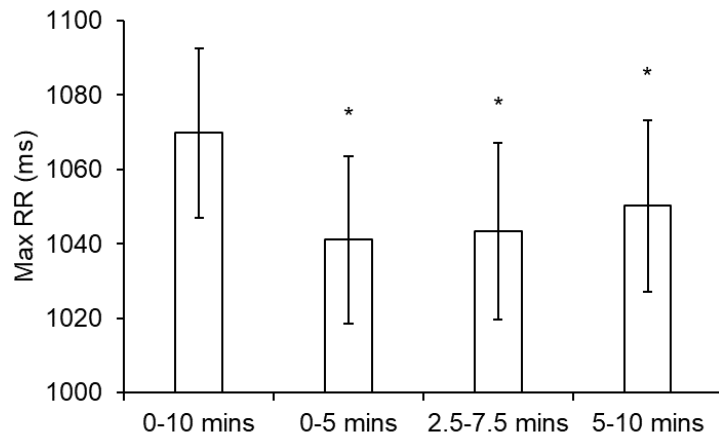
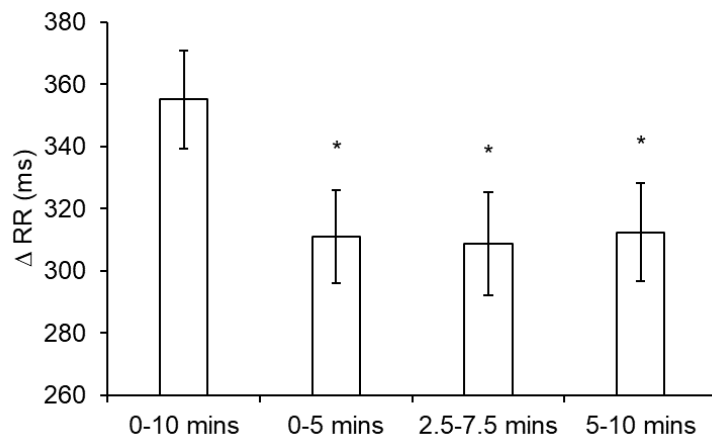
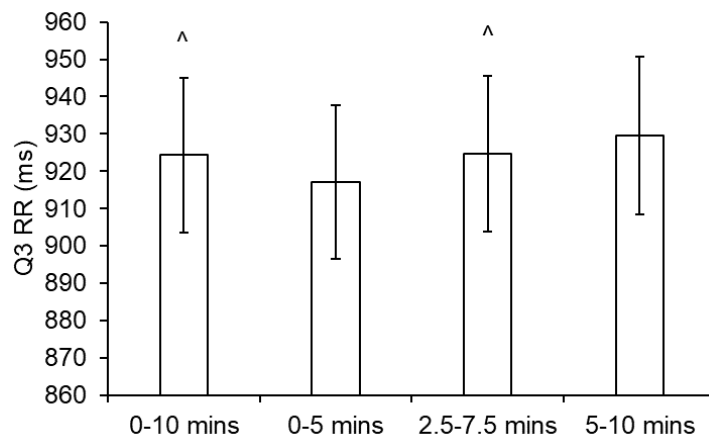
**a.****b.****c.**

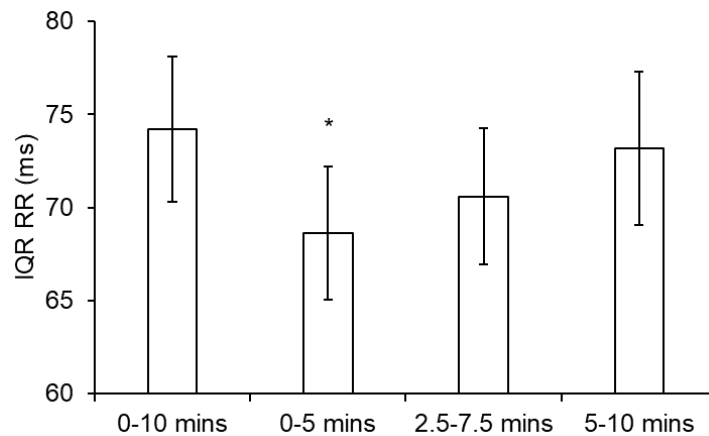
Figure 3.20: Min (a), max (b) and  $\Delta$  (c) RR intervals significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.

Q3 RR was also significantly impacted by the four time periods ( $p = 0.012$ ). As shown in Figure 3.21a, Q3 RR was significantly lower during 0-5 mins compared to 0-10 mins ( $p = 0.036$ ) and 2.5-7.5 mins ( $p = 0.034$ ). Although a significant effect of time period transpired for IQR RR ( $p = 0.031$ ), pairwise comparisons revealed contrasting differences. Indeed, Figure 3.21b illustrates that IQR RR was significantly lower during the first five minutes compared to the full 10 minutes ( $p < 0.001$ ).

**a.**



**b.**

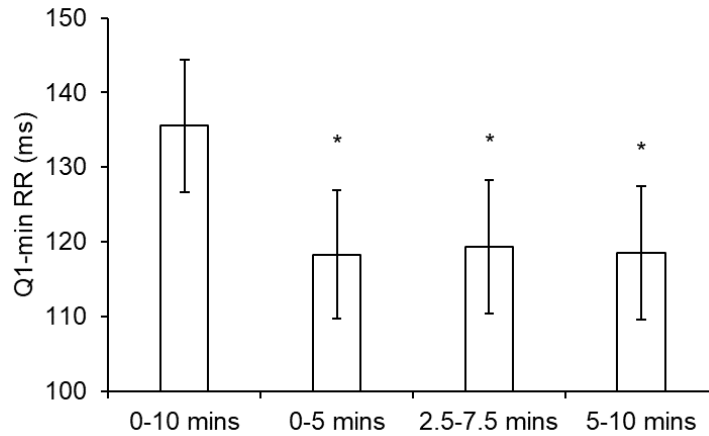


*Figure 3.21: Q3 (a) and IQR (b) RR intervals significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; ^ = significantly different to 0-5 mins.*

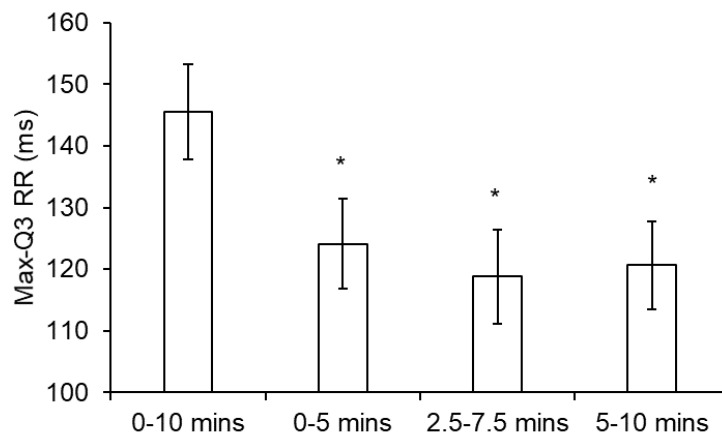
Interestingly, significant effects of time period on Q1-min RR and Max-Q3 RR emerged (both  $p < 0.001$ ). Inspection of Figure 3.22a and b show that

the same pairwise comparisons reached significance. That is, Q1-min RR and max-Q3 RR were significantly higher during 0-10 mins compared to all three five-minute time periods (all  $p < 0.001$ ).

**a.**



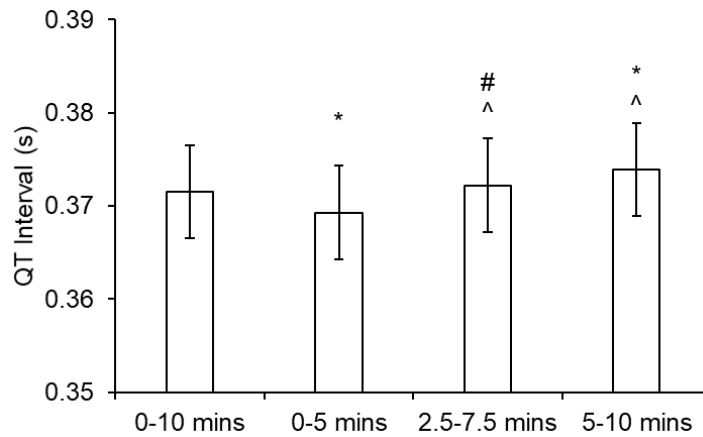
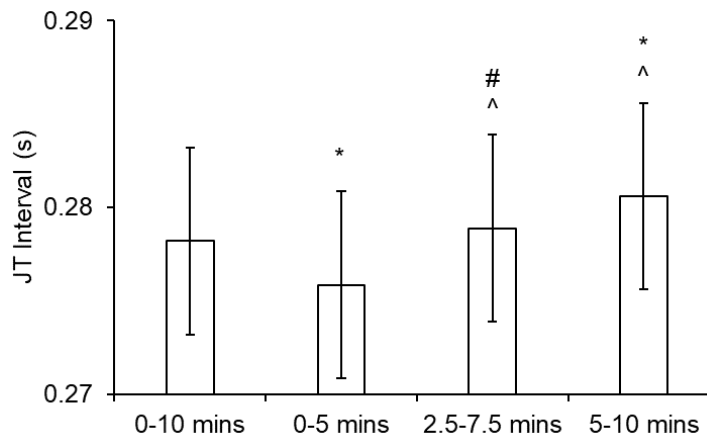
**b.**



*Figure 3.22: Q1-min (a) and max-Q3 (b) RR intervals significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins.*

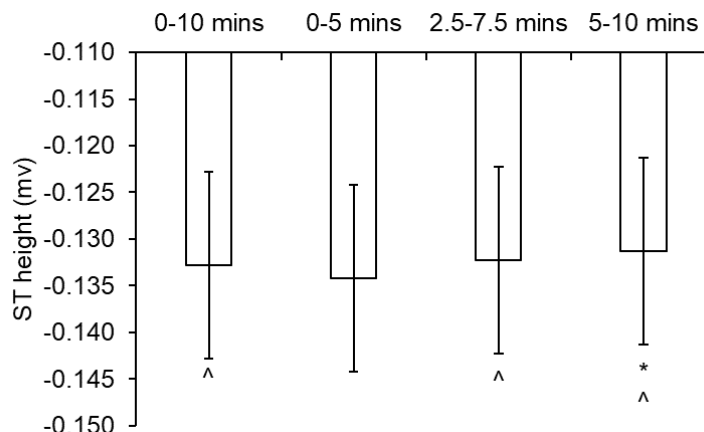
### 3.3.1.5 ECG analysis

For the baseline condition, statistically significant effects on the QT and JT intervals were identified (both  $p < 0.001$ ). For both variables, the intervals were significantly shorter for the first five minutes than for full duration and middle and last five minutes (all  $p < 0.001$ ). In addition, QT and JT intervals were significantly longer during 5-10 mins than during 0-10 mins and 2.5-7.5 mins (all  $p < 0.001$ , see Figure 3.23a and Figure 3.23b).

**a.****b.**

*Figure 3.23: QT (a) and JT (b) intervals significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins; ^ = significantly different to 0-5 mins.*

Finally, ST height significantly differed between the four time periods ( $p < 0.001$ ). As shown in Figure 3.24, ST height in 5-10 mins was significantly higher than that during 0-10 mins and 0-5 mins (both  $p = 0.003$ ). Also, ST height during 0-5 mins was significantly lower than that during 0-10 mins ( $p = 0.002$ ) and 2.5-7.5 mins ( $p < 0.001$ ).



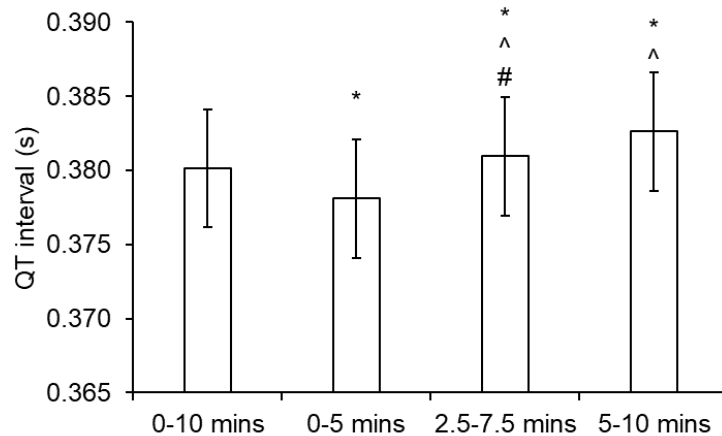
*Figure 3.24: ST height significantly differed between the four baseline time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; ^ = significantly different to 0-5 mins.*

For the recovery condition, QT interval significantly differed between the time periods ( $p < 0.001$ ). As depicted in Figure 3.25a, QT interval was significantly lower during 0-10 mins compared to 2.5-7.5 mins ( $p = 0.023$ ) and 5-10 mins ( $p = 0.001$ ). Also, QT interval was significantly higher during 0-10 minutes compared to 0-5 minutes ( $p < 0.001$ ). Significant differences also emerged between the first five minutes and middle and last five minutes: QT interval was lower during the first five minutes compared to the middle and last five minutes (both  $p < 0.001$ ). Finally, QT interval during 2.5-7.5 mins was significantly lower compared to that during 5-10 mins ( $p = 0.050$ ).

The effect of time period on JT interval also reached significance ( $p < 0.001$ ). Moreover, the same differences that were identified for the QT interval transpired for the JT interval. JT interval was significantly lower during 0-10 mins compared to 2.5-7.5 mins ( $p = 0.025$ ) and 5-10 mins ( $p = 0.001$ ). The measure was also significantly higher during 0-10 mins compared to 0-5 mins ( $p < 0.001$ ). In addition, JT interval was significantly lower during the first five minutes compared to the middle and last five minutes (both  $p < 0.001$ ). Finally, JT interval during 2.5-7.5 mins was significantly lower compared to that during 5-10 mins ( $p = 0.043$ , see Figure 3.25b).



a.



b.

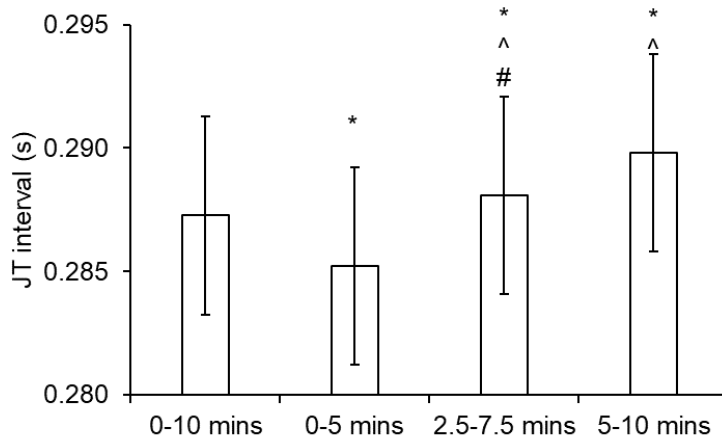


Figure 3.25: QT (a) and JT (b) intervals were significantly impacted by the four time periods of the recovery condition. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; ^ = significantly different to 0-5 mins; # = significantly different to 5-10 mins.

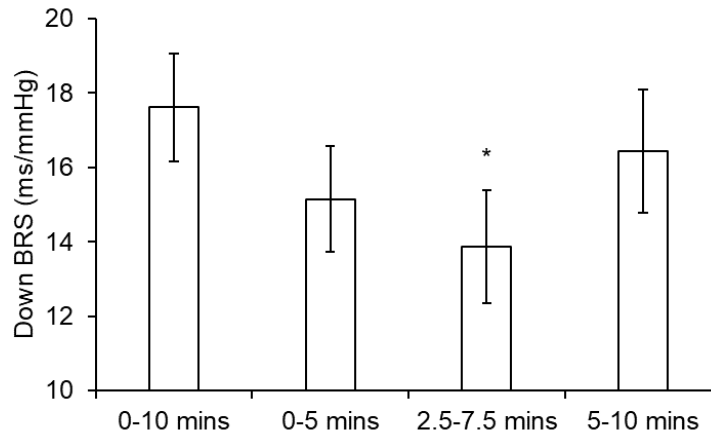
### 3.3.1.6 BP, BRS and respiration

For the baseline condition, no statistically significant differences emerged ( $p > 0.05$ ).

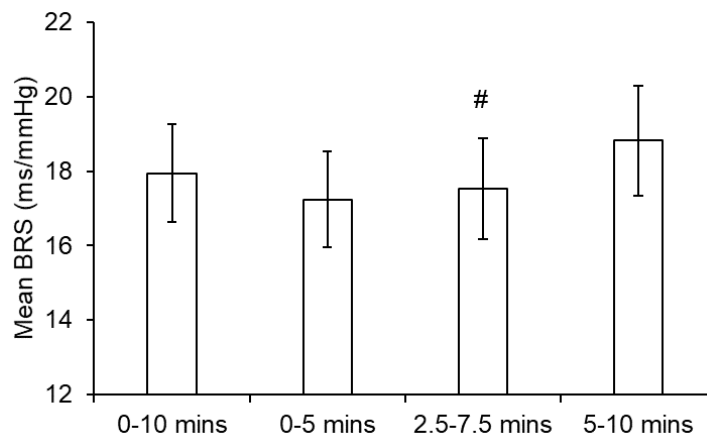
However, for the recovery condition, down BRS and mean BRS significantly differed between the four time periods ( $p = 0.004$  and  $p = 0.050$  respectively). Further analysis revealed that down BRS was significantly higher during the full condition compared to the middle five minutes ( $p = 0.005$ , see Figure 3.26a). In contrast, mean BRS was significantly lower during the middle five minutes compared to the last five minutes ( $p = 0.008$ ,

see Figure 3.26b). None of the other variables (up BRS or respiration) significantly varied between the four time periods ( $p > 0.05$ ).

**a.**



**b.**



*Figure 3.26: Down BRS (a) and mean BRS (b) significantly differed between the four recovery time periods. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 0-10 mins; # = significantly different to 5-10 mins.*

The results clearly show that the first five minutes of the baseline and recovery conditions have the lowest levels of parasympathetic predominance and attenuated time-domain HRV and RR intervals compared to the other time periods. Interestingly, the final five minutes of the baseline condition was associated with the greatest HF power and pRR50, and the longest RR intervals, QT intervals and JT intervals, suggesting that maximal relaxation was achieved at this point. These differences did not emerge to

the same extent for the recovery period, perhaps suggesting that there was no meaningful difference between using the final five minutes compared to full duration and the middle five minutes. Nevertheless, it was decided that the 5-10 minute periods of both recordings would be used as the baseline and recovery conditions. Considering the concerns expressed in the literature for obtaining accurate resting readings, the reason for this was three-fold:

1. Camm et al. (1996) recommends using recordings of five minutes in duration, therefore eliminating the full 10-minute duration.
2. Orienting responses will have most likely influenced the 0-10 and 0-5 minute time periods, but not the 2.5-7.5 and 5-10 minute time periods.
3. Parasympathetic predominance was highest during the last five minutes of the baseline condition, therefore the time period adopted for the baseline condition must be consistent with that used for the recovery condition.

### **3.3.2 The effects of tempo manipulations on cardiovascular autonomic control**

First, differences in starting and baseline measures between the two groups were ascertained. The only statistically significant difference emerged in baseline experienced relaxation as measured by VAS ( $p = 0.033$ ).

Examination of this difference revealed that the control group reported significantly higher levels of experienced relaxation (mean: 8.50; SEM: 0.28) than the experimental group (mean: 7.74; SEM: 0.27).

To determine the extent to which measures of autonomic function changed from baseline during the three auditory stimuli, mixed mode ANOVAs were performed. The within-participants variable was condition: baseline (5-10 min period), stepped increase in tempo (full duration), stepped decrease in tempo (full duration), stable tempo (full duration) and recovery (5-10 min period). The between-participants variable was group: experimental (melody and rhythm of Baa Baa Blacksheep) and control (rhythm of Baa Baa Blacksheep).

Mixed mode ANOVAs were also performed to assess the extent to which measures of autonomic function differed between five tempi of the stepped increase in tempo and stepped decrease in tempo stimulus. The within-participants variable was tempo: 60bpm, 90bpm, 120bpm, 150bpm and 180bpm. The between-participants variable was group: experimental (melody and rhythm of Baa Baa Blacksheep) and control (rhythm of Baa Baa Blacksheep).

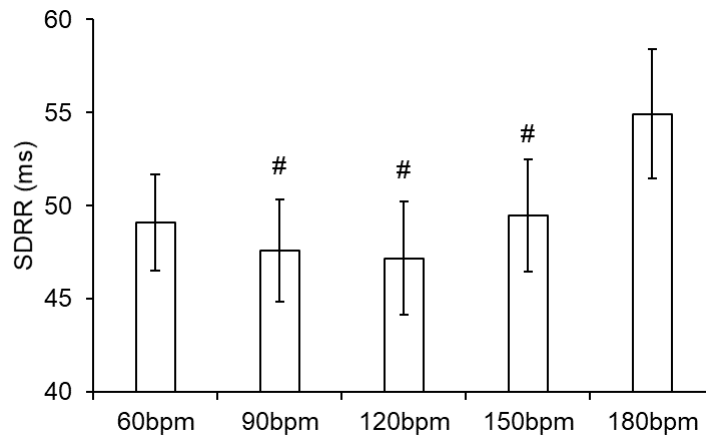
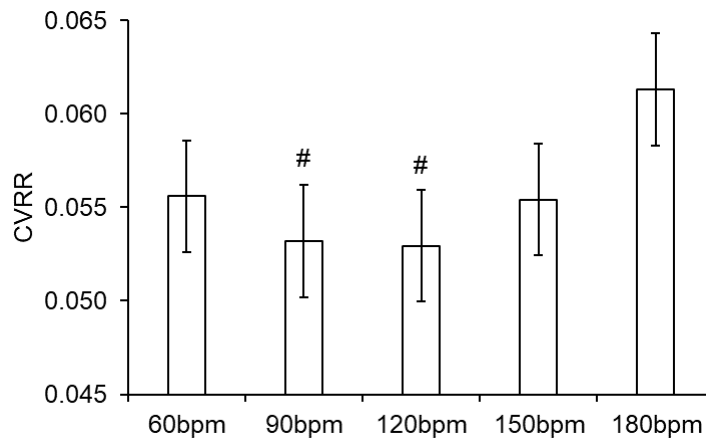
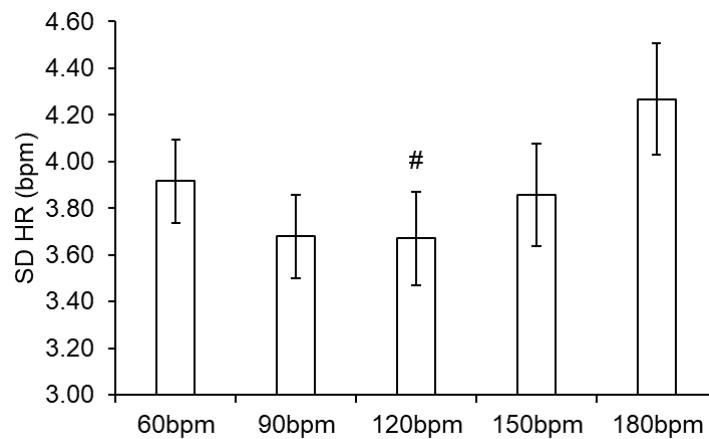
#### **3.3.2.1 Time-domain HRV analysis**

##### **3.3.2.1.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)**

No statistically significant main effects of condition or group, or their interaction emerged for any of the time-domain HRV variables ( $p > 0.05$ ).

### **3.3.2.1.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)**

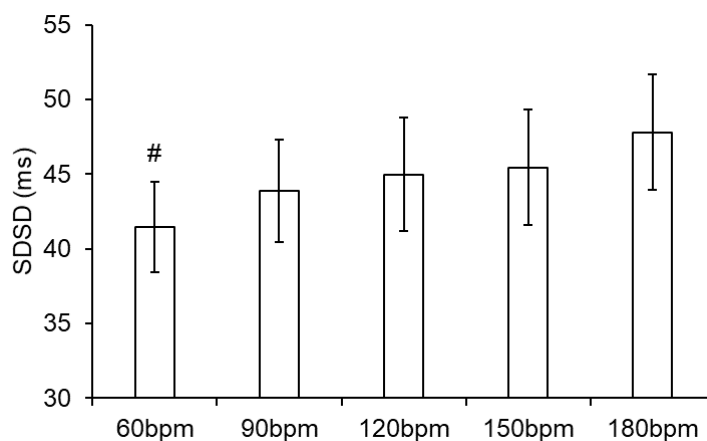
In contrast to the five-condition analysis, significant main effects of tempo were observed for SDRR ( $p = 0.001$ ), CVRR ( $p = 0.002$ ), SD HR ( $p = 0.006$ ) SDSD ( $p = 0.003$ ) and RMSSD ( $p = 0.003$ ). As illustrated in Figure 3.27a, SDRR was significantly higher during the 180bpm tempo than during the 90bpm ( $p = 0.025$ ), 120bpm ( $p = 0.003$ ) and 150bpm ( $p = 0.049$ ) tempi. For CVRR, values were significantly higher during the final tempo (180bpm) compared to the second (90bpm;  $p = 0.039$ ) and third (120bpm;  $p = 0.008$ ) tempi (see Figure 3.27b). The only significant difference that emerged during the Bonferroni pairwise comparisons for SD HR was between the 120bpm and 180bpm tempi ( $p = 0.021$ ). As shown in Figure 3.27c, SD HR was significantly higher during the latter compared to the former.

**a.****b.****c.**

*Figure 3.27: SDRR (a), CVRR (b) and SD HR (c) significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 180bpm.*

For SDSD and RMSSD the same differences emerged: both variables were significantly higher during the final tempo compared to the first (both  $p = 0.003$ , see Figure 3.28a and b).

a.



b.

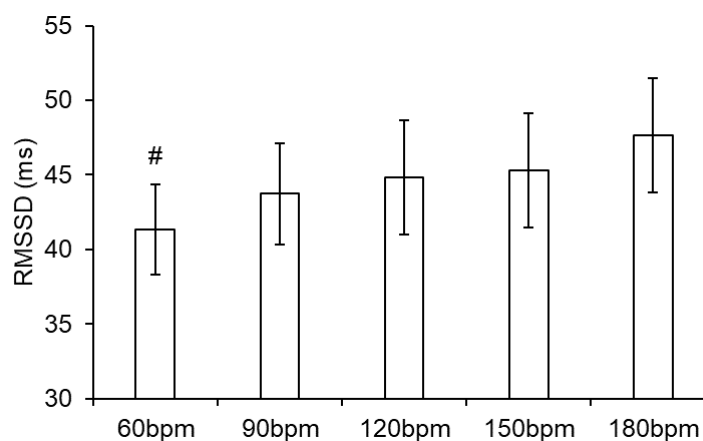
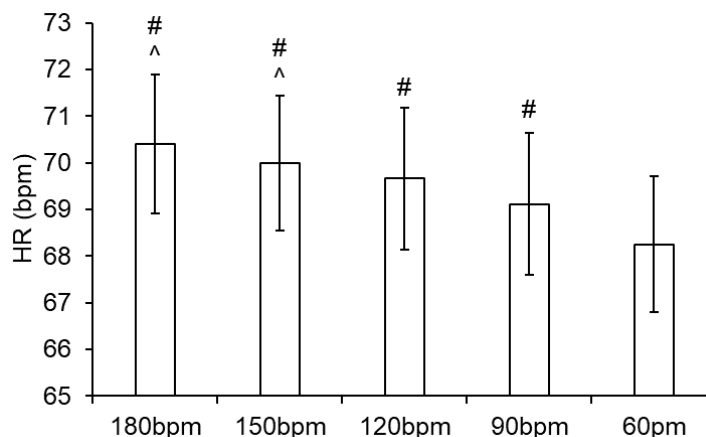


Figure 3.28: SDSD (a) and RMSSD (b) significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 180bpm.

### 3.3.2.1.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

A significant effect of tempo on HR transpired ( $p < 0.001$ ). Bonferroni pairwise comparisons demonstrated that HR was significantly lower during 60bpm compared to 180bpm ( $p = 0.001$ ), 150bpm ( $p < 0.001$ ), 120bpm ( $p = 0.003$ ) and 90bpm ( $p = 0.039$ ). In addition, HR during 90bpm was

significantly lower than that during 180bpm ( $p = 0.022$ ) and 150bpm ( $p = 0.048$ , see Figure 3.29).



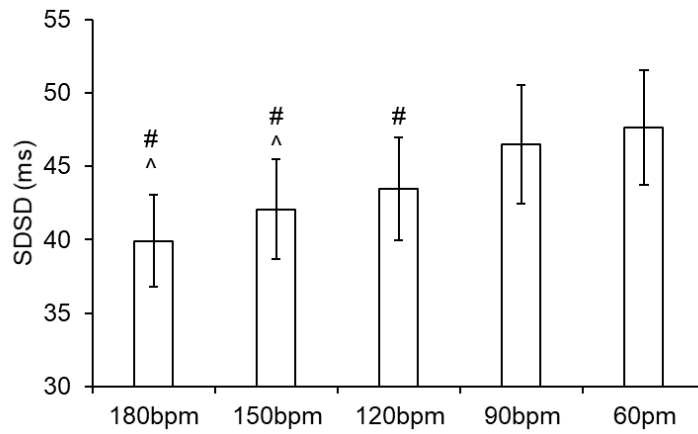
*Figure 3.29: HR significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; ^ = significantly different to 90bpm.*

Mixed mode ANOVAs performed on SDSD and RMSSD also showed that tempo had a significant impact (main effect of tempo: both  $p < 0.001$ ). As illustrated in Figure 3.30a and b, SDSD and RMSSD were significantly higher during 60bpm than 180bpm ( $p = 0.003$ ), 150bpm ( $p = 0.004$ ) and 120bpm ( $p = 0.019$ ). Furthermore, both variables were significantly higher during 90bpm than 180bpm ( $p = 0.006$ ) and 150bpm ( $p = 0.007$ ).

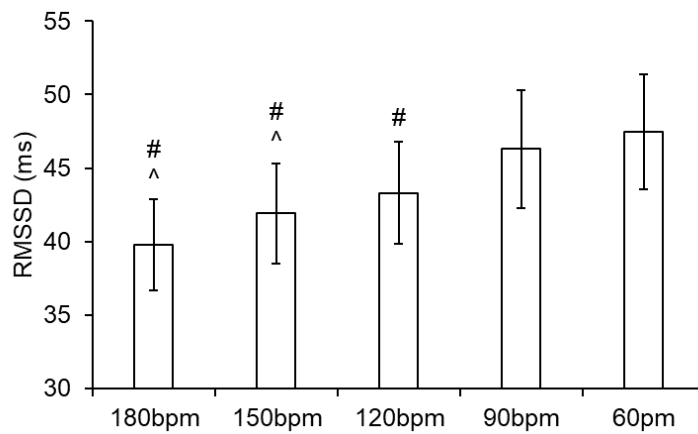
The final variable that was significantly impacted by the five tempi was pRR50 (main effect of tempo:  $p < 0.001$ ). Further examination revealed that pRR50 was significantly lower during the first tempo (180bpm) compared to the fourth (90bpm,  $p = 0.010$ ) and final (60bpm,  $p = 0.007$ ) tempi (see Figure 3.30c).



a.



b.



c.

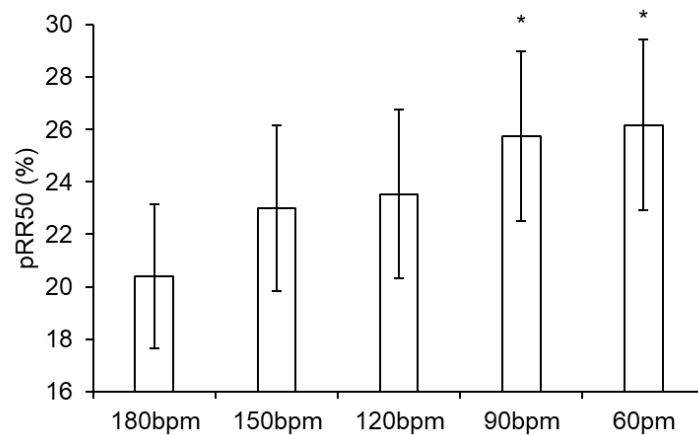


Figure 3.30: SDSD (a), RMSSD (b) and pRR50 (c) significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; \* = significantly different to 180bpm; ^ = significantly different to 90bpm.

### 3.3.2.2 Frequency-domain HRV analysis

#### 3.3.2.2.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)

The only variable that was significantly impacted by the conditions was VLF% (main effect of condition:  $p = 0.029$ ). Bonferroni pairwise comparisons revealed that VLF% was significantly lower in baseline than in the stable tempo stimulus ( $p = 0.028$ , see Figure 3.31). No other statistically significant differences emerged ( $p > 0.05$ ).

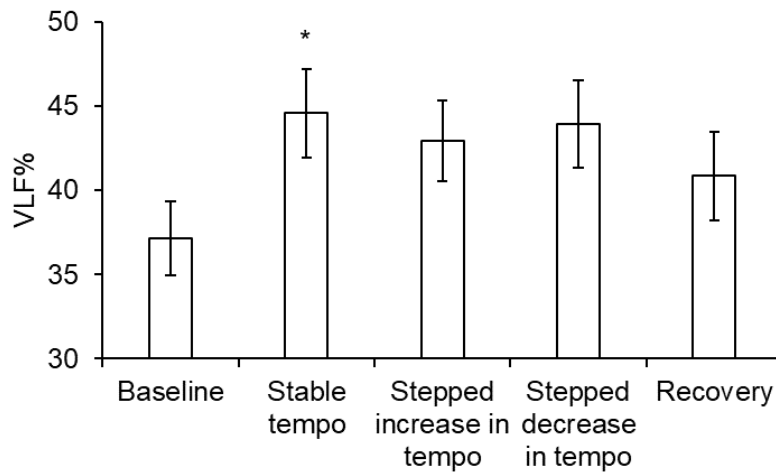


Figure 3.31: VLF% was significantly higher during the stable tempo stimulus compared to baseline. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

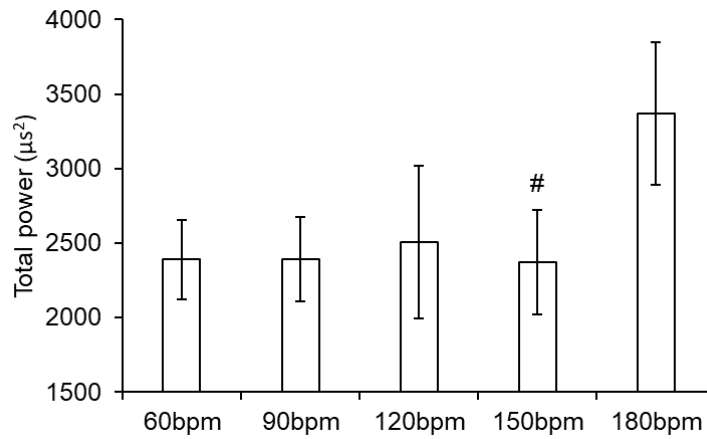
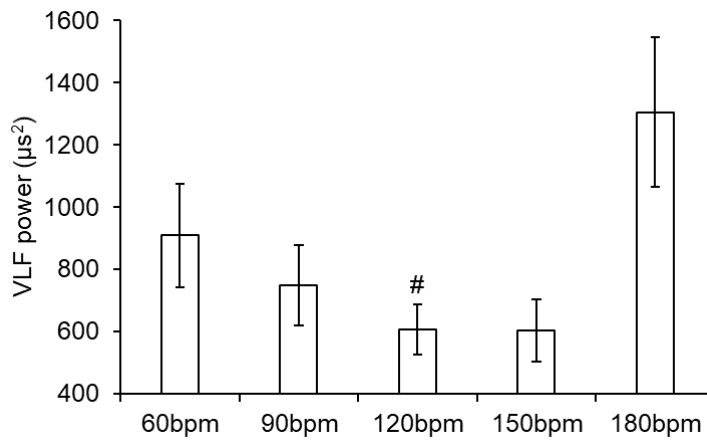
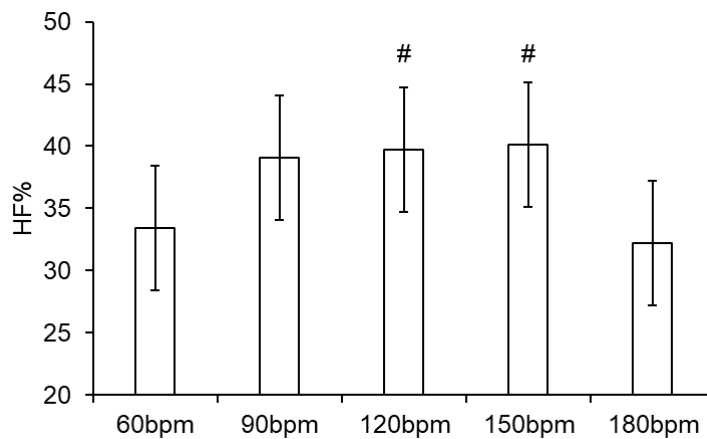
#### 3.3.2.2.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

A statistically significant main effect of tempo on total power emerged ( $p = 0.026$ ). As shown in Figure 3.32a, total power was significantly greater in the 180bpm tempo compared to the 150bpm tempo ( $p = 0.043$ ).

VLF power was also significantly influenced by the tempi (main effect of tempo:  $p = 0.010$ ). Indeed, VLF power was significantly higher during the 180bpm tempo than during the 120bpm tempo ( $p = 0.036$ , see Figure 3.32b).

The final main effect of tempo occurred for HF% ( $p = 0.010$ ): HF% was significantly lower during the final tempo (180bpm) than in the third (120bpm;  $p = 0.013$ ) and fourth (150bpm;  $p = 0.030$ ) tempi (see Figure 3.32c).

No other significant main effects or interactions emerged ( $p > 0.05$ ).

**a.****b.****c.**

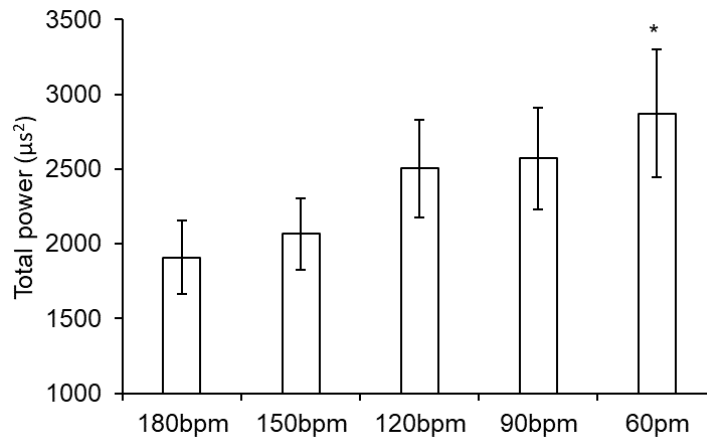
*Figure 3.32: Total power (a), VLF power (b) and HF% (c) were significantly impacted by the five tempi of the stepped increase in tempo stimulus. Data presented as mean  $\pm$  1 SEM. # = significantly different to 180bpm.*

### 3.3.2.2.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

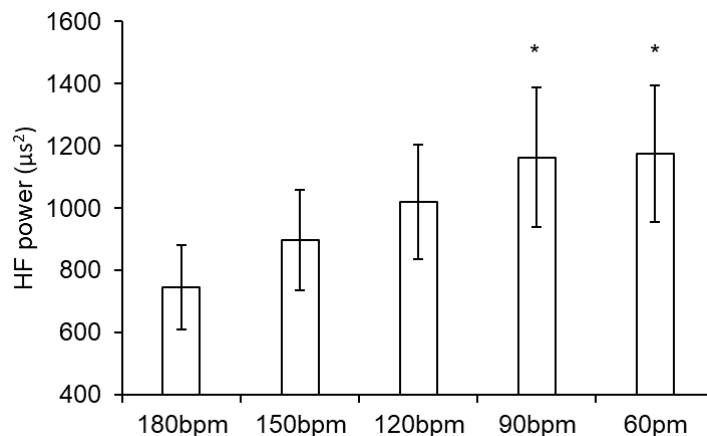
A statistically significant main effect of tempo on total power emerged ( $p = 0.015$ ). Further exploration of this effect revealed that total power was significantly higher during 60bpm than 180bpm ( $p = 0.038$ , see Figure 3.33a). The main effect of tempo on HF power also reached statistical significance ( $p = 0.001$ ). Figure 3.33b demonstrated that HF power was significantly higher in 60bpm and 90bpm compared to 180bpm ( $p = 0.018$  and  $p = 0.030$  respectively).

No other main effects of interactions were statistically significant ( $p > 0.05$ ).

**a.**



**b.**



*Figure 3.33: Total power (a) and HF power (b) were significantly impacted by the five tempi of the stepped decrease in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 180bpm.*

### 3.3.2.3 Non-linear HRV analysis

#### 3.3.2.3.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)

nSD1 was the only variable found to be significantly influenced by the five conditions (main effect of condition:  $p = 0.042$ ). As illustrated in Figure 3.34, nSD1 was significantly higher in baseline than during the stable tempo stimulus ( $p = 0.017$ ).

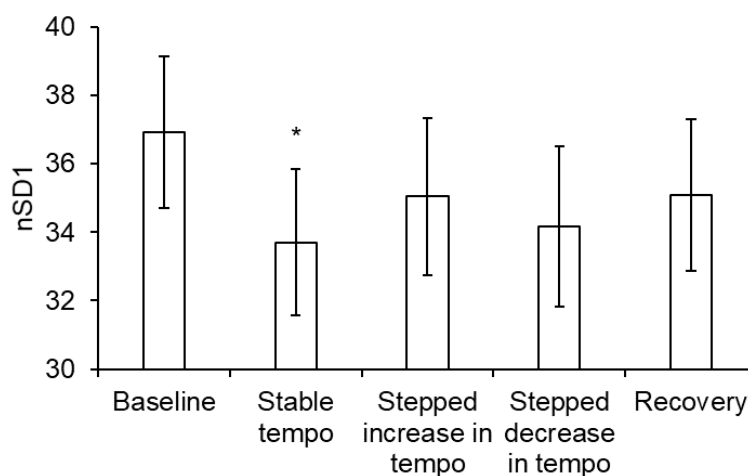
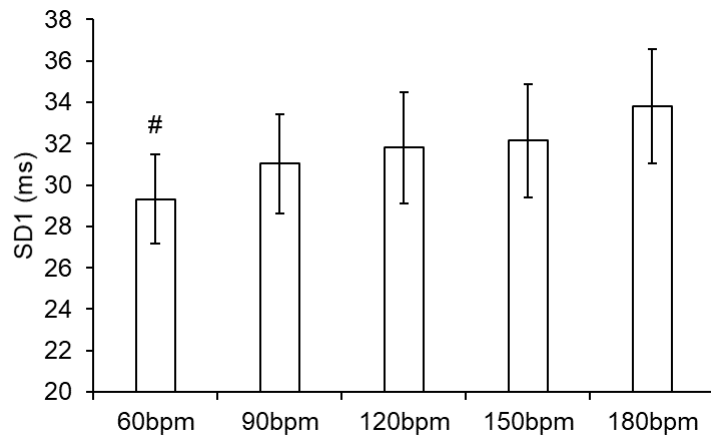
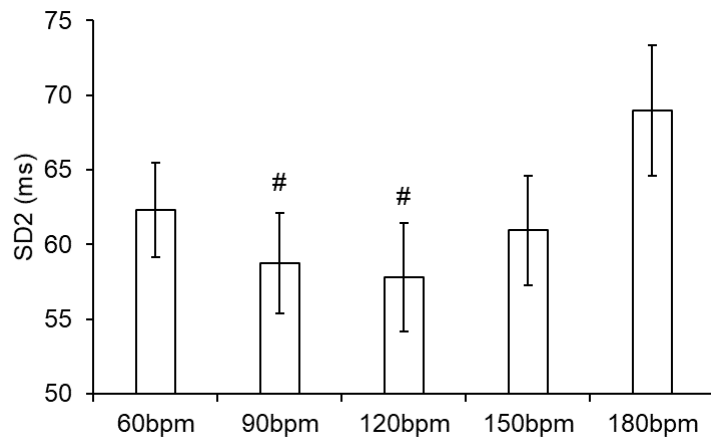


Figure 3.34: nSD1 was significantly lower during the stable tempo stimulus compared to baseline. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 3.3.2.3.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

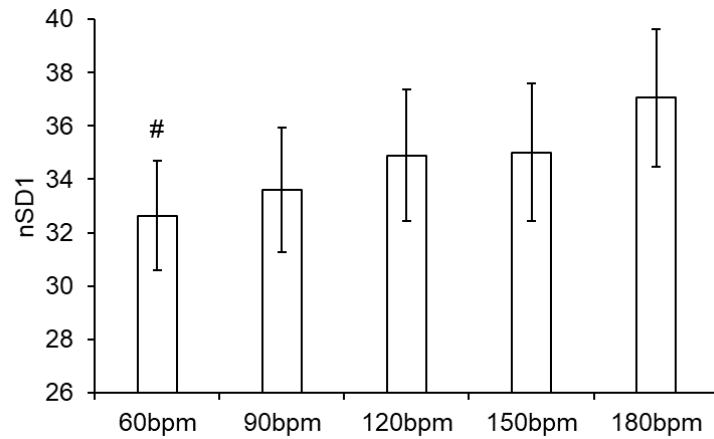
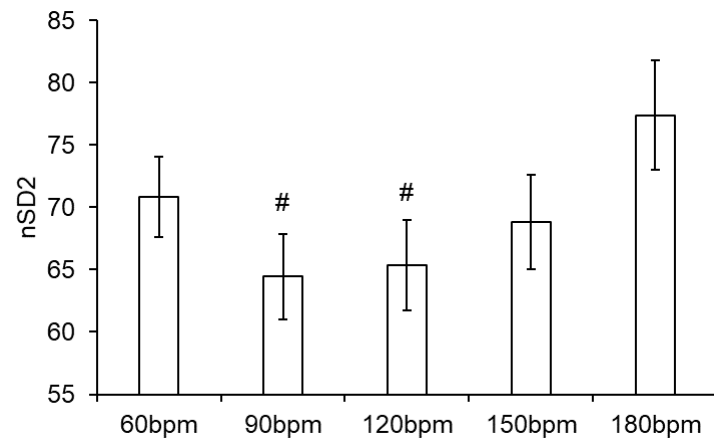
Mixed mode ANOVAs detected statistically significant main effects of tempo on SD1 ( $p = 0.003$ ), SD2 ( $p = 0.001$ ), nSD1 ( $p = 0.005$ ), nSD2 ( $p = 0.001$ ), SD1/SD2 ( $p = 0.004$ ) and SD2/SD1 ( $p = 0.034$ ).

As shown in Figure 3.35a, SD1 during 180bpm was significantly greater than that during 60bpm ( $p = 0.003$ ). For SD2, values were significantly higher during 180bpm than during 90bpm ( $p = 0.041$ ) and 120bpm ( $p = 0.004$ , see Figure 3.35b).

**a.****b.**

*Figure 3.35: SD1 (a) and SD2 (b) significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 180bpm.*

The only significant difference in nSD1 was detected between 60bpm and 180bpm: nSD1 in 60bpm was significantly lower than that in 180bpm ( $p = 0.002$ , see Figure 3.36a). Finally, significant differences in nSD2 emerged between the 180bpm tempo and: 90bpm ( $p = 0.029$ ) and 120bpm ( $p = 0.010$ , see Figure 3.36b). nSD2 during 180bpm was significantly higher than that during 90bpm and 120bpm.

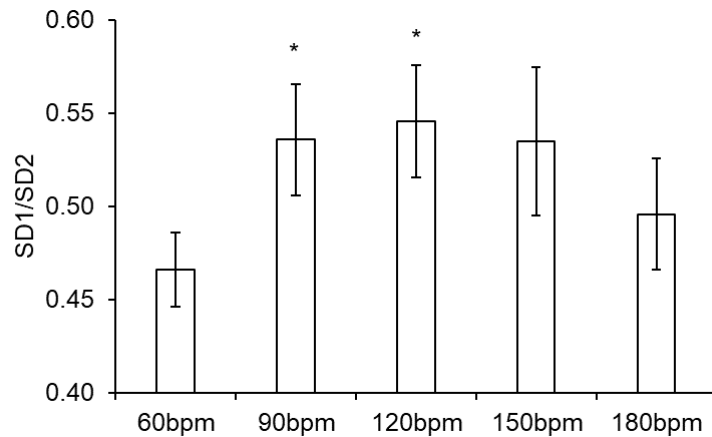
**a.****b.**

*Figure 3.36: nSD1 (a) and nSD2 (b) significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 180bpm.*

SD1/SD2 was significantly lower during 60bpm compared to 90bpm ( $p = 0.048$ ) and 120bpm ( $p = 0.010$ , see Figure 3.37a). Finally, for SD2/SD1 the only significant difference emerged between 60bpm and 120bpm ( $p = 0.016$ ). As shown in Figure 3.37b, the former was significantly higher than the latter.



a.



b.

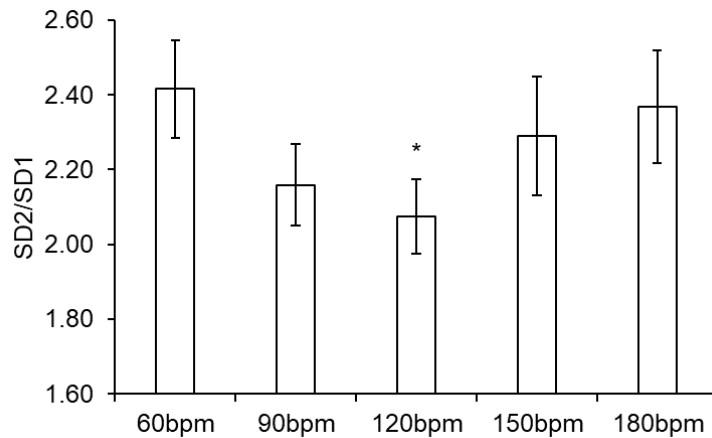
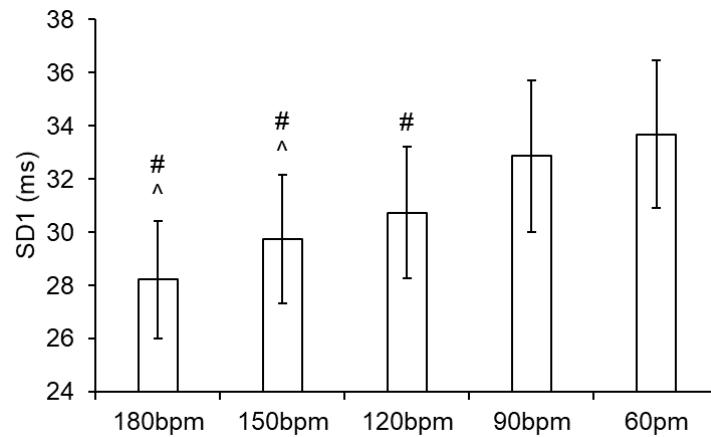


Figure 3.37:  $SD1/SD2$  (a) and  $SD2/SD1$  (b) significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 60bpm.

### 3.3.2.3.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

Mixed mode ANOVAs identified a statistically significant main effect of tempo on SD1 and nSD1 (both  $p < 0.001$ ). As highlighted in Figure 3.38, the same differences emerged following Bonferroni pairwise comparisons. Indeed, SD1 and nSD1 were significantly higher during 60bpm compared to 180bpm ( $p = 0.003$  and  $p = 0.005$  respectively), 150bpm ( $p = 0.004$  and  $p = 0.015$ ) and 120bpm ( $p = 0.019$  and  $p = 0.041$ ). In addition, SD1 and nSD1 were significantly higher in 90bpm than in 180bpm ( $p = 0.006$  and  $p = 0.008$  respectively) and 150bpm ( $p = 0.007$  and  $p = 0.017$ ).

a.



b.

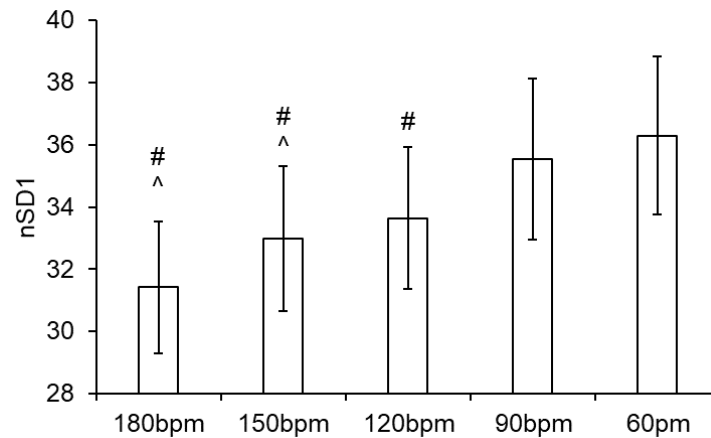


Figure 3.38: SD1(a) and nSD1 (b) were significantly impacted by the five tempi of the stepped decrease in tempo stimulus. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; ^ = significantly different to 90bpm.

### 3.3.2.4 RR analysis

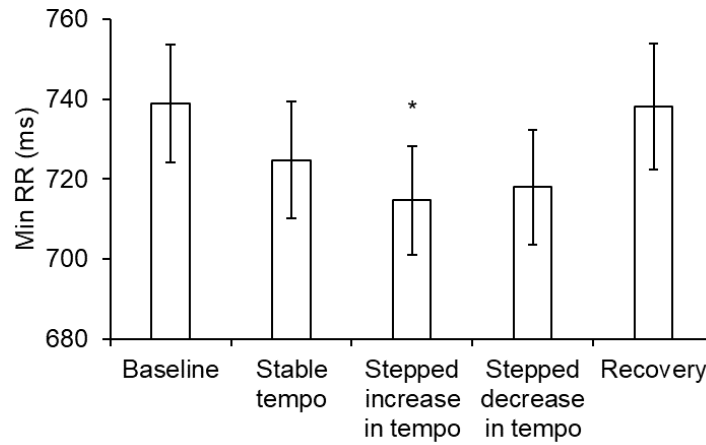
#### 3.3.2.4.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)

A statistically significant main effect of condition emerged for min RR ( $p = 0.005$ ). As illustrated in Figure 3.39a, min RR was significantly higher during baseline than during the stepped increase in tempo stimulus ( $p = 0.016$ ).

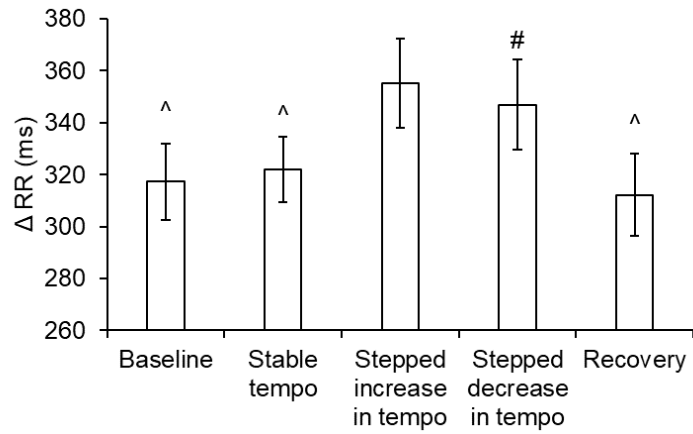
Although none of the main effects or interactions reached significance for max RR, a statistically significant main effect of condition on  $\Delta$  RR was detected ( $p < 0.001$ ). Further exploration demonstrated that  $\Delta$  RR was significantly greater during the stepped increase in tempo stimulus compared to baseline ( $p = 0.034$ ), the stable tempo stimulus ( $p = 0.038$ ) and recovery ( $p = 0.005$ ). Furthermore, as shown in Figure 3.39b,  $\Delta$  RR was significantly greater during the stepped decrease in tempo stimulus compared to recovery ( $p = 0.030$ ).

Finally, the main effect of condition on max-Q3 RR reached statistical significance ( $p < 0.001$ ). Figure 3.39c shows that max-Q3 RR was significantly greater in the stepped increase in tempo stimulus compared to baseline ( $p = 0.028$ ) and recovery ( $p = 0.001$ ). Also, the variable was significantly greater during the stepped decrease in tempo stimulus compared to recovery ( $p = 0.027$ ).

a.



b.



c.

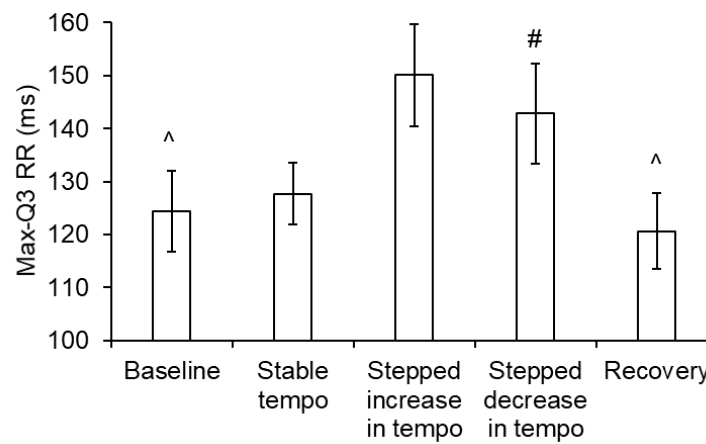


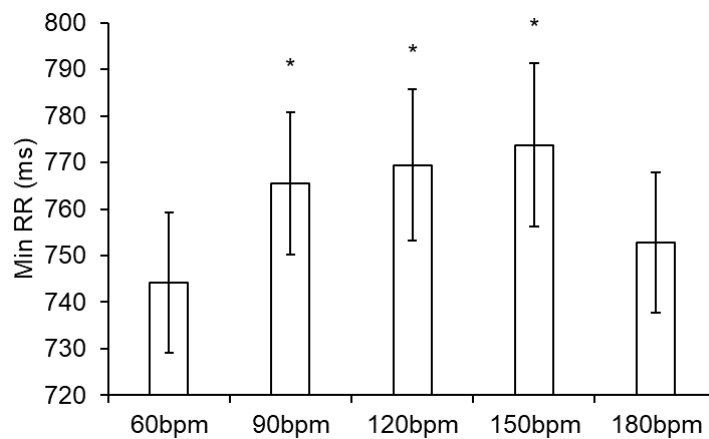
Figure 3.39: Min (a),  $\Delta$  (b) and max-Q3 (c) RR intervals were significantly impacted by the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery; <sup>^</sup> = significantly different to stepped increase in tempo.

### 3.3.2.4.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

A statistically significant main effect of tempo on min RR was identified ( $p = 0.001$ ). Further exploration of this effect demonstrated that min RR was significantly lower during 60bpm than in 90bpm ( $p = 0.019$ ), 120bpm ( $p = 0.024$ ) and 150bpm ( $p = 0.002$ , see Figure 3.40a).

$\Delta$  RR was also significantly impacted by the tempi ( $p = 0.009$ ). Indeed, Figure 3.40b illustrates that  $\Delta$  RR was significantly greater during 180bpm than 120bpm ( $p = 0.048$ ) and 150bpm ( $p = 0.039$ ).

**a.**



**b.**

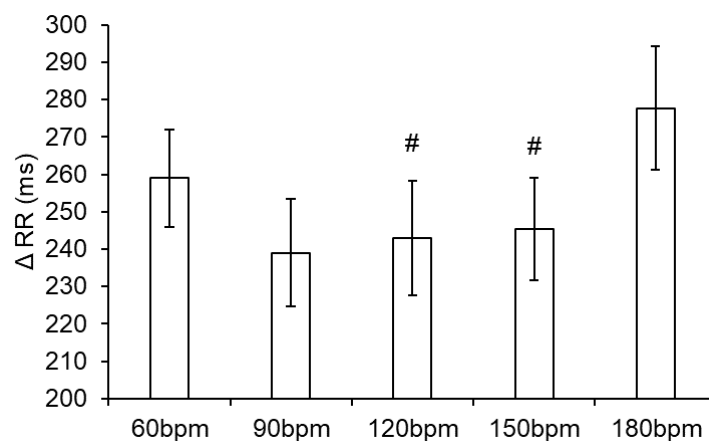
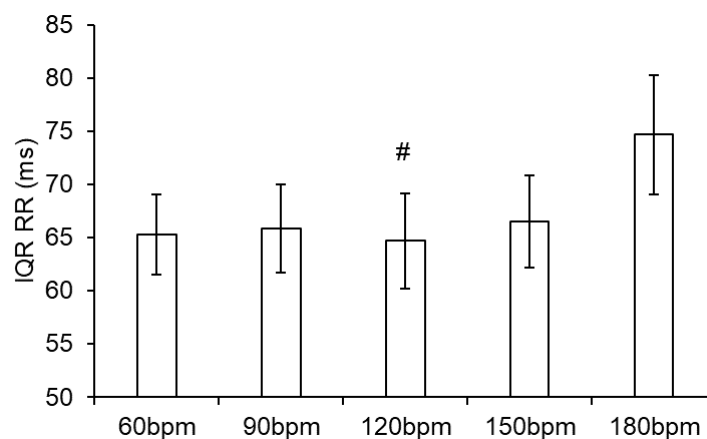


Figure 3.40: Min (a) and  $\Delta$  (b) RR intervals significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 60bpm; # = significantly different to 180bpm.

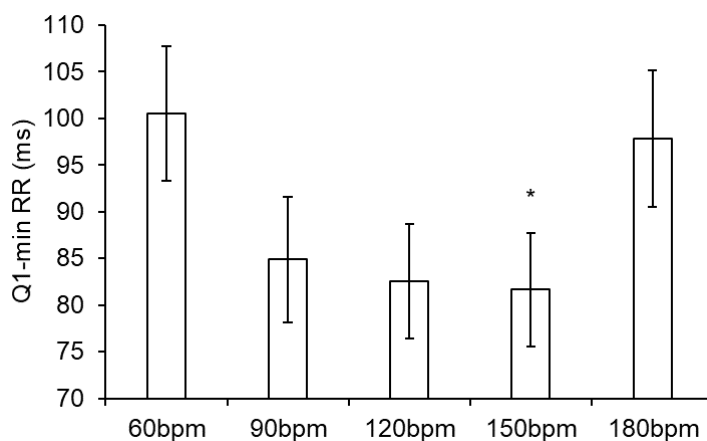
Although Q1 RR and Q3 RR were not affected by the tempi in this stimulus, a significant main effect of tempo was identified for IQR RR ( $p = 0.017$ ). Bonferroni pairwise comparisons detected one significant difference between 120bpm and 180bpm ( $p = 0.006$ ). As demonstrated in Figure 3.41a, IQR RR was significantly lower in 120bpm than 180bpm.

The final variable that differed between the five tempi was Q1-min RR ( $p = 0.004$ ). Figure 3.41b highlights that this main effect of tempo was driven by the difference between 60bpm and 150bpm: the former was significantly greater than the latter ( $p = 0.032$ ).

**a.**



**b.**



*Figure 3.41: IQR (a) and Q1-min (b) RR intervals significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 60bpm; # = significantly different to 180bpm.*

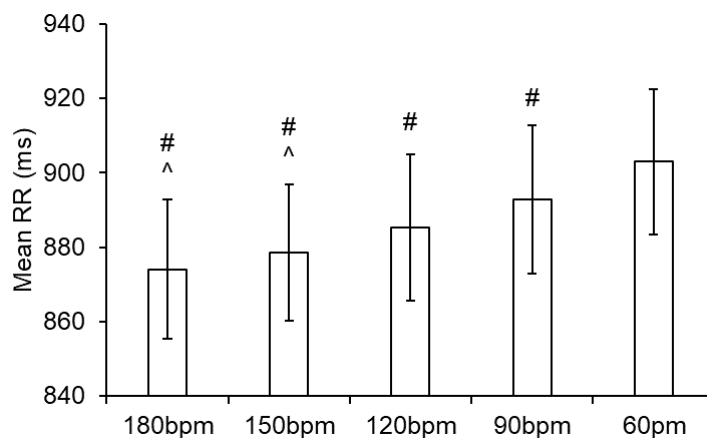
### **3.3.2.4.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)**

A mixed mode ANOVA revealed a statistically significant main effect of tempo on mean RR ( $p < 0.001$ ). Further exploration of this effect revealed that mean RR was significantly greater during 60bpm than 180bpm ( $p < 0.001$ ), 150bpm ( $p < 0.001$ ), 120bpm ( $p = 0.001$ ) and 90bpm ( $p = 0.034$ , see Figure 3.42a). Furthermore, mean RR was significantly greater in 90bpm than 180bpm ( $p = 0.011$ ) and 150bpm ( $p = 0.010$ ).

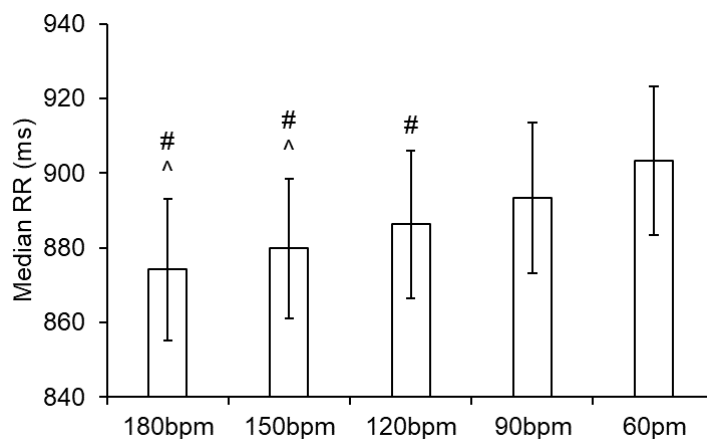
The same main effect was observed for median RR ( $p < 0.001$ ). Moreover, as shown in Figure 3.42b, Bonferroni detected similar statistically significant differences (60bpm vs: 180bpm ( $p = 0.001$ ), 150bpm ( $p < 0.001$ ) and 120bpm ( $p = 0.005$ ); 90bpm vs: 180bpm ( $p = 0.008$ ), 150bpm ( $p = 0.029$ )).

Mode RR was also found to significantly differ between the five tempi (main effect of tempo:  $p = 0.005$ ). Further examination of this effect revealed that mode RR was significantly greater during 60bpm than 180bpm ( $p = 0.021$ ) and 150bpm ( $p = 0.018$ , see Figure 3.42c).

a.



b.



c.

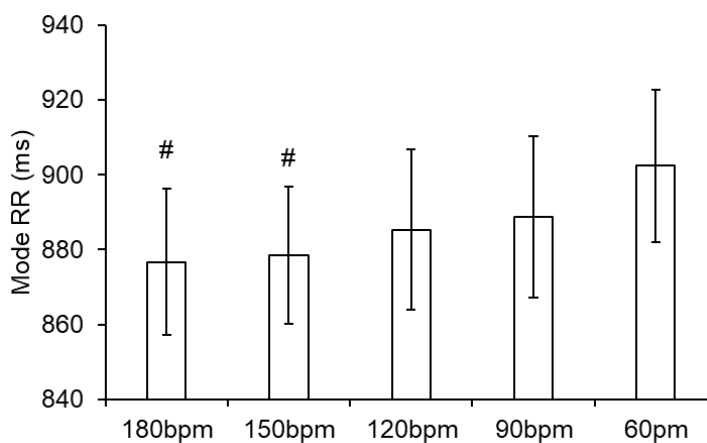


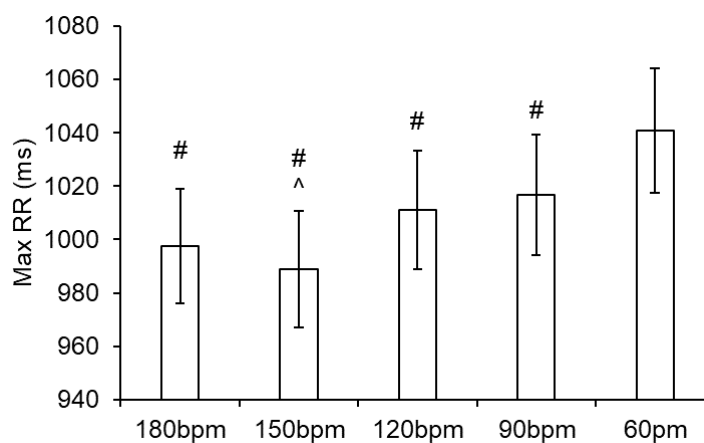
Figure 3.42: Mean (a), median (b) and mode (c) RR intervals significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; ^ = significantly different to 90bpm.



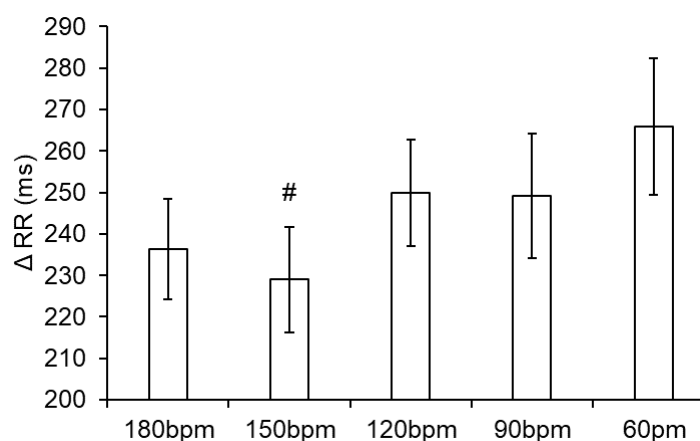
The main effect of tempo on max RR also reached significance ( $p < 0.001$ ). As highlighted in Figure 3.43a, max RR was significantly greater during the final tempo (60bpm,  $p = 0.004$ ) compared to all other tempi (180bpm:  $p = 0.004$ ; 150bpm:  $p < 0.001$ ; 120bpm:  $p = 0.014$ ; 90bpm:  $p = 0.025$ ). Also, max RR during 90bpm was significantly greater than that during 150bpm ( $p = 0.006$ ).

Although min RR did not significantly vary,  $\Delta$  RR did (main effect of tempo:  $p = 0.024$ ). As depicted in Figure 3.43b,  $\Delta$  RR was significantly larger during 60bpm than 150bpm ( $p = 0.039$ ).

**a.**



**b.**

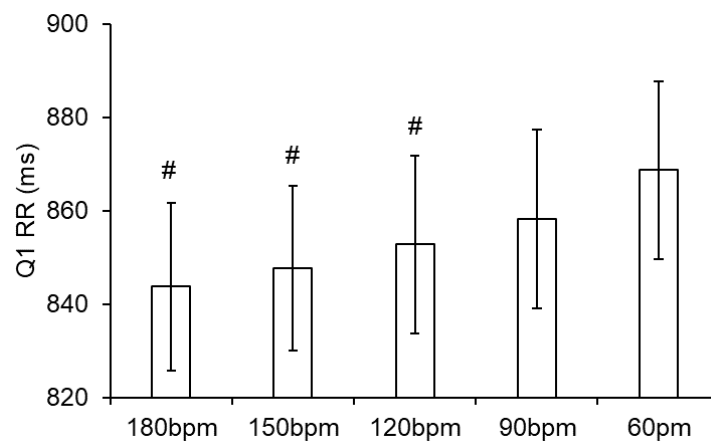


*Figure 3.43: Max (a) and  $\Delta$  (b) RR intervals significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; ^ = significantly different to 90bpm.*

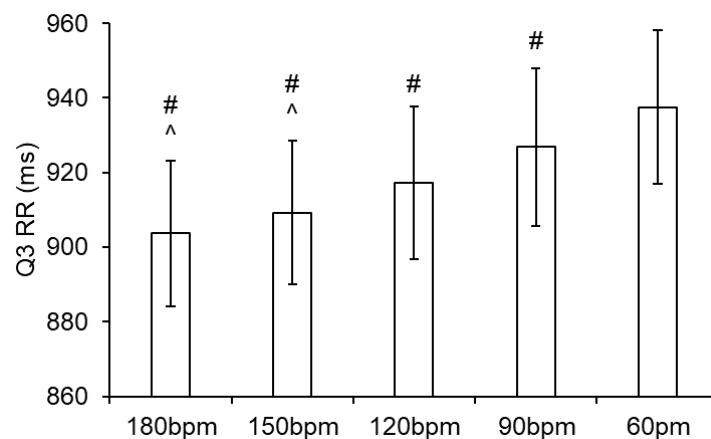
Main effects of tempo on Q1 RR and Q3 RR also transpired (both  $p < 0.001$ ). Exploration of the effect on Q1 RR revealed that this variable was significantly greater during 60bpm than 180bpm ( $p = 0.002$ ), 150bpm ( $p < 0.001$ ) and 120bpm ( $p = 0.026$ , see Figure 3.44a).

Similarly, as shown in Figure 3.44b, Q3 RR was significantly greater in 60bpm compared to all other tempi ( $p < 0.001$  for first three tempi and  $p = 0.036$  for 90bpm). Additionally, Q3 RR was significantly greater during 90bpm than 180bpm ( $p = 0.006$ ) and 150bpm ( $p = 0.005$ ).

**a.**



**b.**



*Figure 3.44: Q1 (a) and Q3 (b) RR intervals significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; ^ = significantly different to 90bpm.*

The final RR interval variable that was significantly impacted by the tempi was max-Q3 RR ( $p = 0.010$ ). Figure 3.45 shows that the only difference that emerged was between 60bpm and 150bpm ( $p = 0.015$ ): the former was significantly greater than the latter.

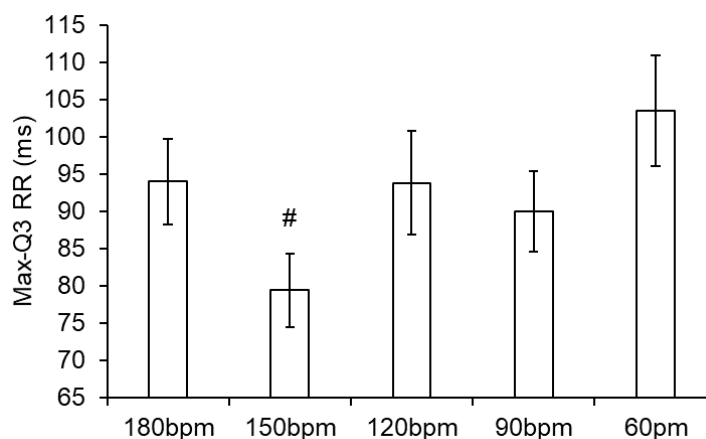
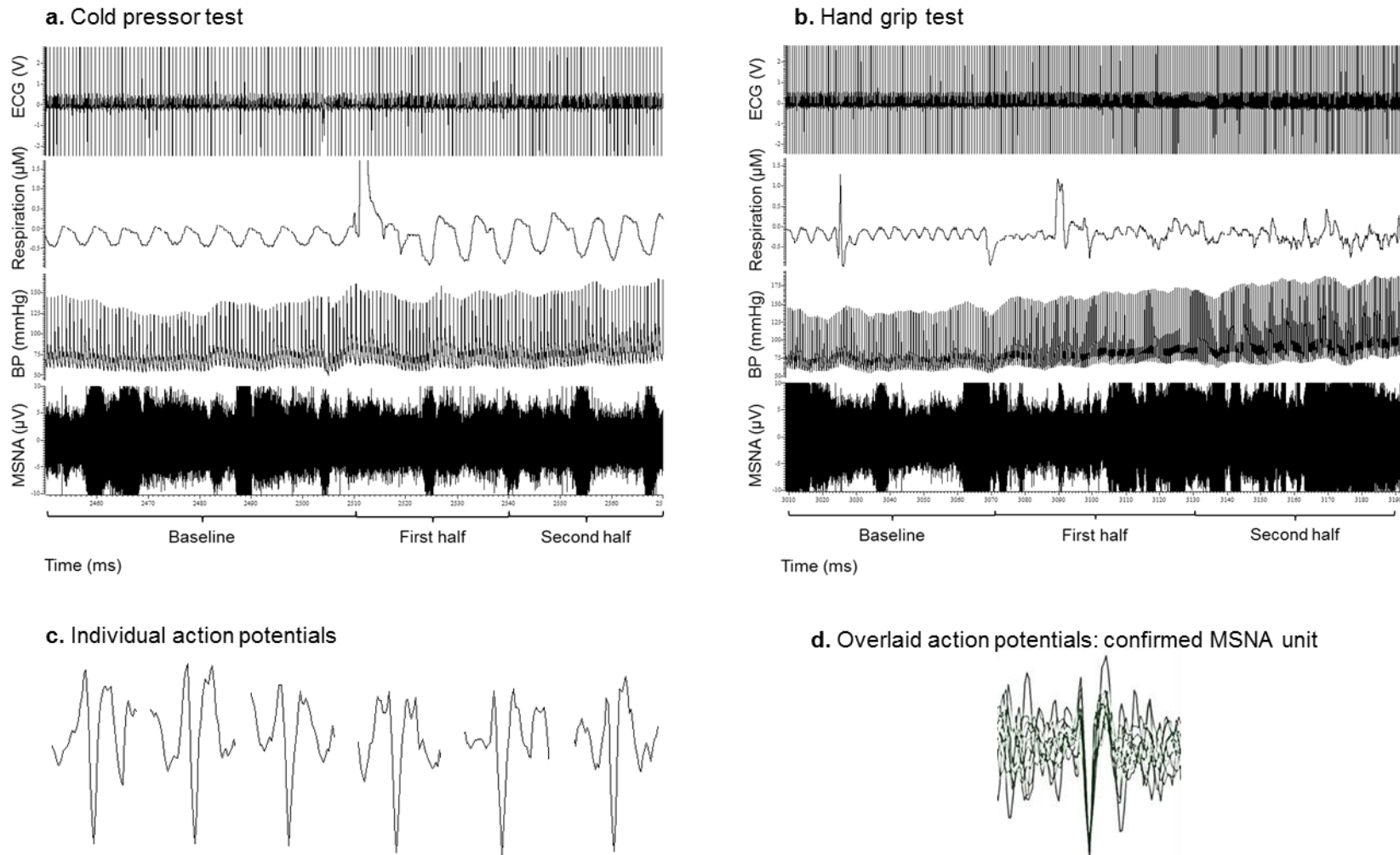


Figure 3.45: Max-Q3 RR significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm.

### 3.3.2.5 MSNA

Figure 3.46a and Figure 3.46b present example traces from cold pressor and hand grip tests. Consistent with microneurography analysis guidelines, BP increased during both tests (although this was more prominent in the hand grip test) and more importantly, during the second half of the tests. This led to the identification of single action potentials (see Figure 3.46c). These signals occurred during diastole and had similar amplitude and shape characteristics. Overlaying the action potential led to the confirmation of the MSNA unit (see Figure 3.46d).



*Figure 3.46: Example microneurography analysis. Traces from cold pressor (a) and hand grip tests (b). (c) shows putative individual action potentials that may have originated from one MSNA unit. Overlaying the individual action potentials (d) lead to confirmation of one MSNA unit.*

### **3.3.2.5.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)**

No statistically significant main effects of interactions emerged for MSNA frequency or MSNA incidence ( $n = 10$ ,  $p > 0.05$ ).

### **3.3.2.5.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)**

No statistically significant main effects of interactions emerged for MSNA frequency or MSNA incidence ( $n = 10$ ,  $p > 0.05$ ).

Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

No statistically significant main effects or interactions emerged for MSNA frequency or MSNA incidence ( $n = 10$ ,  $p > 0.05$ ).

### **3.3.2.5.3 Both groups combined**

Running a mixed mode ANOVA on the sample of 10 confirmed MSNA units may have resulted in false negatives. Therefore, the two groups were combined and analysed by running a repeated measures ANOVA with condition (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo, recovery) and tempo (60bpm, 90bpm, 120bpm, 150bpm, 180bpm) as the within-participants variable. No statistically significant main effects or interactions emerged ( $p > 0.05$ ).

### **3.3.2.6 ECG analysis**

#### **3.3.2.6.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)**

A statistically significant main effect of condition on QT interval emerged ( $p = 0.014$ ). Bonferroni pairwise comparisons revealed that QT interval was significantly longer in recovery compared to the stable tempo ( $p = 0.004$ ),

stepped increase in tempo ( $p = 0.017$ ) and stepped decrease in tempo ( $p = 0.010$ ) stimuli (see Figure 3.47a).

This main effect remained significant after controlling for HR (main effect of condition on QTc:  $p = 0.013$ ). Similar to the QT interval, QTc was significantly greater in recovery than the three stimuli (stable tempo:  $p = 0.005$ ; stepped increase in tempo:  $p = 0.022$ ; stepped decrease in tempo:  $p = 0.004$ , see Figure 3.47b).

The same main effect of condition and pairwise comparisons reached statistical significance for JT interval (main effect of condition:  $p = 0.007$ ; pairwise comparison with recovery and: stable tempo:  $p = 0.002$ ; stepped increase in tempo:  $p = 0.013$ ; stepped decrease in tempo:  $p = 0.006$ , see Figure 3.47c).

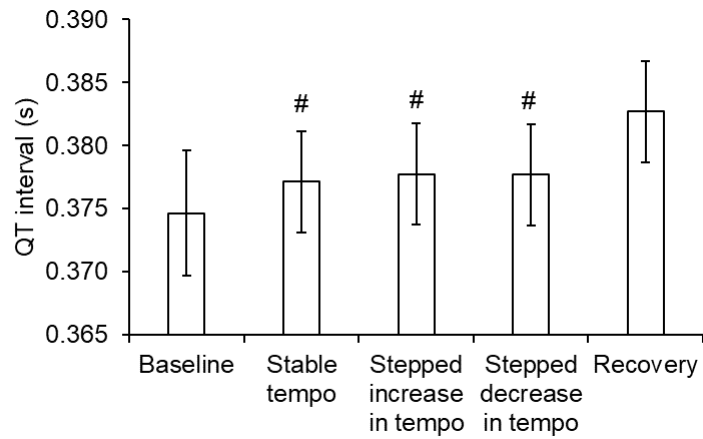
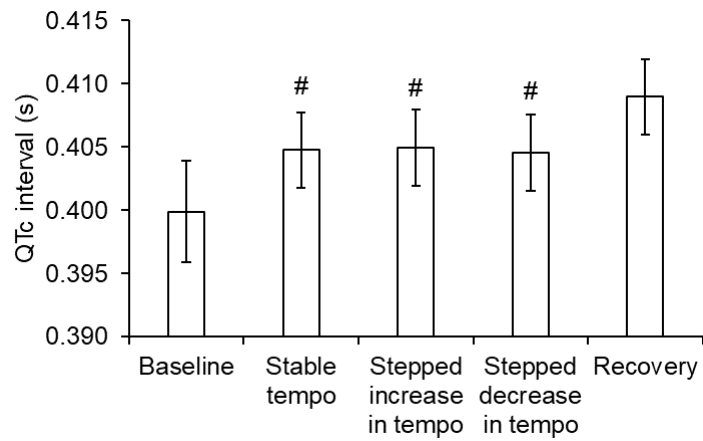
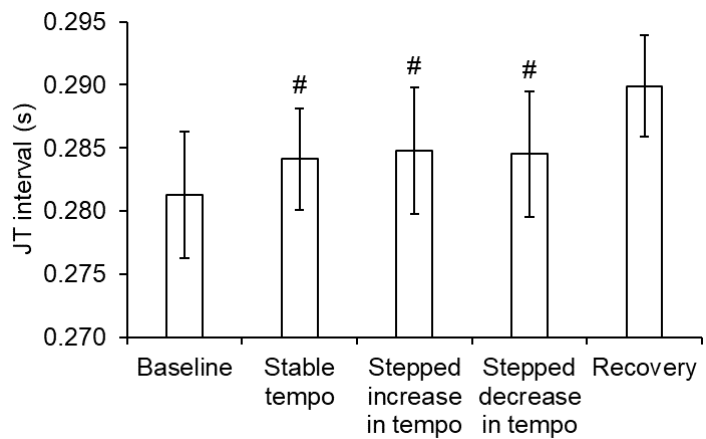
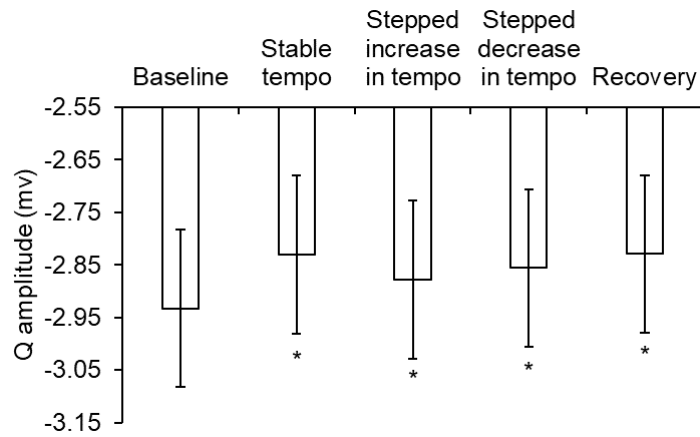
**a.****b.****c.**

Figure 3.47: QT (a), QTc (b) and JT (c) intervals significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery.

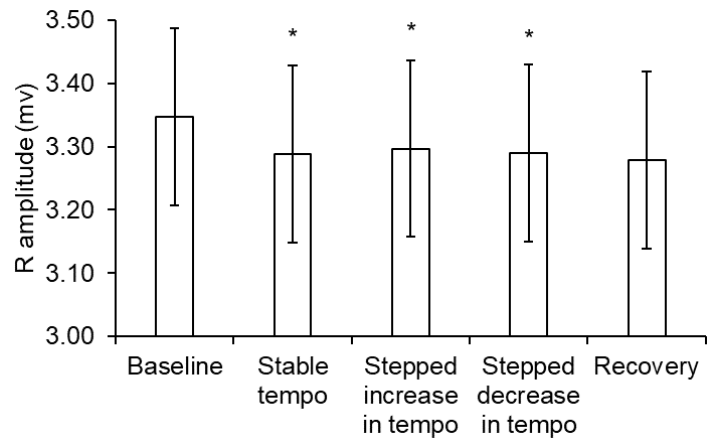
The mixed mode ANOVA revealed that Q amplitude, R amplitude and ST height were also significantly impacted by the conditions ( $p = 0.002$ ,  $p = 0.006$  and  $p = 0.001$  respectively). Q amplitude was significantly lower during baseline than compared to stable tempo ( $p = 0.025$ ), stepped increase in tempo ( $p = 0.002$ ), stepped decrease in tempo ( $p = 0.004$ ) and recovery ( $p = 0.002$ , see Figure 3.48a). In contrast, R amplitude was significantly higher during baseline than during the stable tempo ( $p = 0.023$ ), stepped increase in tempo ( $p = 0.001$ ) and stepped decrease in tempo ( $p = 0.001$ ) stimuli (see Figure 3.48b). Finally, as shown in Figure 3.48c, ST height was significantly higher during recovery than baseline ( $p = 0.009$ ) and the stable tempo stimulus ( $p = 0.028$ ).



a.



b.



c.

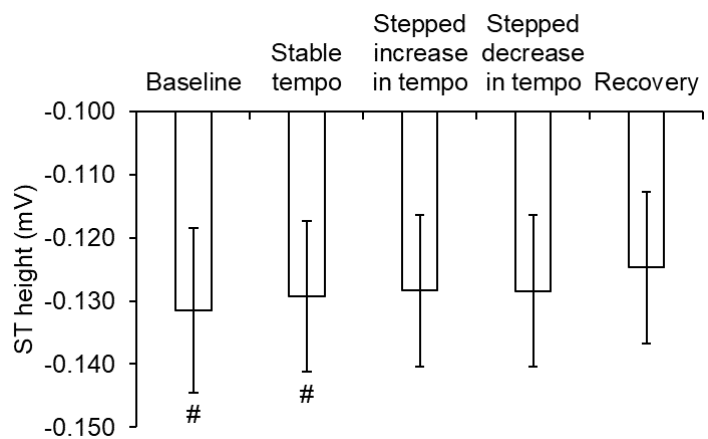


Figure 3.48: Q amplitude (a), R amplitude (b) and ST height (c) significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

### **3.3.2.6.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)**

A statistically significant main effect of tempo emerged for the QT interval ( $p < 0.001$ ). As demonstrated in Figure 3.49a, QT interval was significantly lower during the 60bpm tempo compared all other tempi (all  $p < 0.001$ ).

The main effect of tempo remained statistically significant after correcting QT for HR (QTc,  $p = 0.001$ ). Bonferroni pairwise comparisons revealed that QTc interval was significantly lower during 60bpm than during 120bpm ( $p = 0.002$ ), 150bpm ( $p = 0.001$ ) and 180bpm ( $p = 0.019$ , see Figure 3.49b).

A mixed mode ANOVA also revealed a statistically significant main effect of tempo on JT interval ( $p < 0.001$ ). Figure 3.49c shows that the JT interval during 60bpm was significantly smaller than that during all other tempi (all  $p < 0.001$ ).

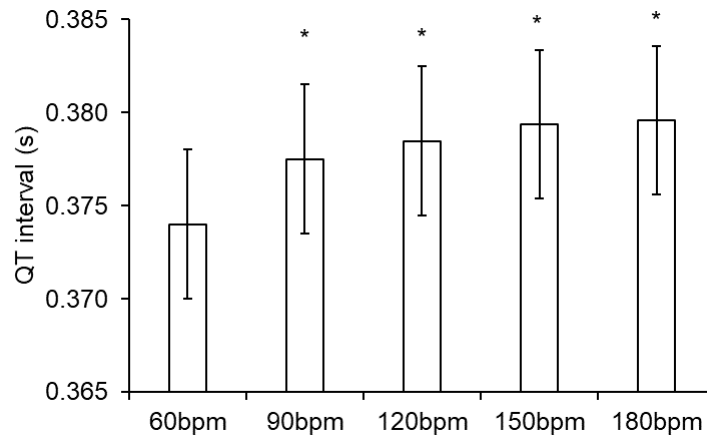
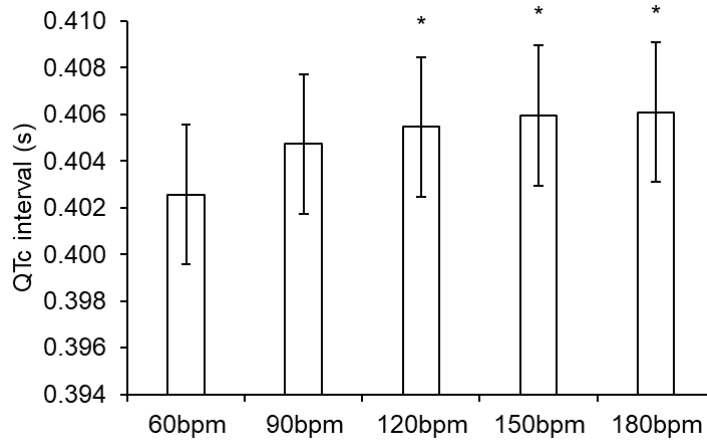
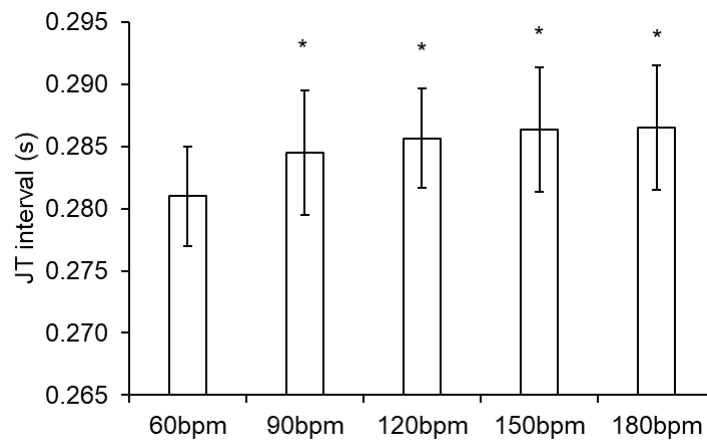
**a.****b.****c.**

Figure 3.49: QT (a), QTc (b) and JT (c) intervals significantly differed between the five increasingly faster tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 60bpm.

The final variable that was significantly impacted by the five tempi was ST height ( $p < 0.001$ ). Further examination of this effect demonstrated that ST height was significantly greater during 60bpm than during 120bpm ( $p = 0.023$ ), 150bpm ( $p < 0.001$ ) and 180bpm ( $p = 0.013$ , see Figure 3.50).

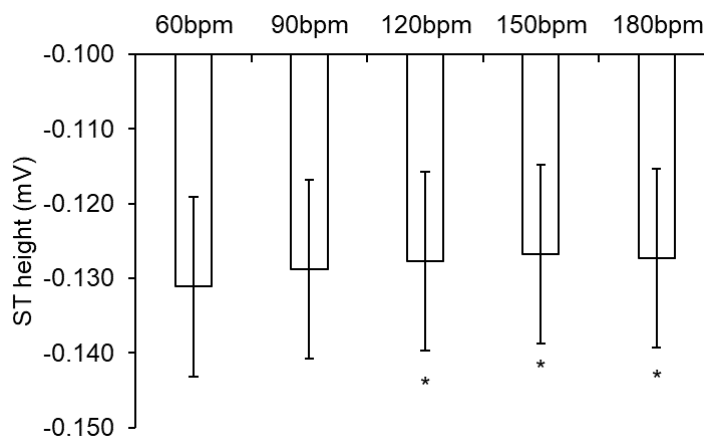
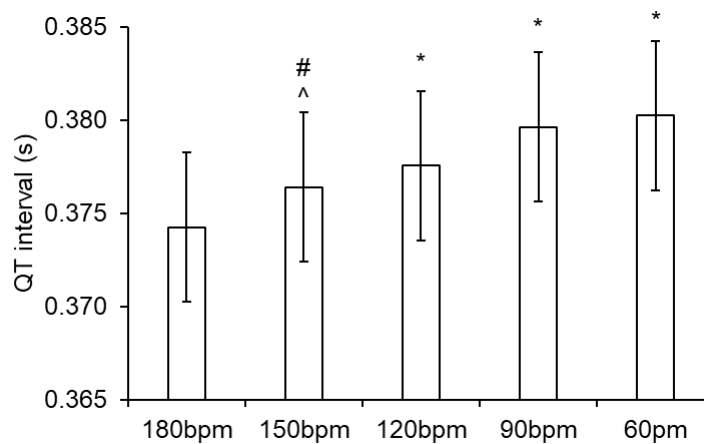
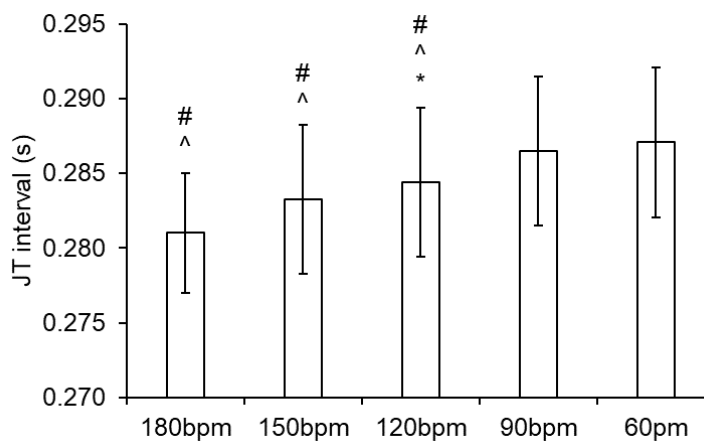


Figure 3.50: ST height significantly differed between the five increasing tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 60bpm.

### 3.3.2.6.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

A mixed mode ANOVA detected a statistically significant main effect of tempo on QT interval ( $p < 0.001$ ). Further exploration revealed that QT interval was significantly shorter during 180bpm compared to 120bpm ( $p = 0.030$ ), 90bpm ( $p < 0.001$ ) and 60bpm ( $p < 0.001$ , see Figure 3.51a). Furthermore, QT interval in 150bpm was significantly shorter than that during 90bpm ( $p < 0.001$ ) and 60bpm ( $p = 0.001$ ).

Similar to the stepped increase in tempo stimulus, a statistically significant main effect of tempo emerged for JT interval ( $p < 0.001$ ). As depicted in Figure 3.51b, JT interval was significantly greater during 60bpm than 180bpm ( $p < 0.001$ ), 150bpm ( $p < 0.001$ ) and 120bpm ( $p = 0.033$ ). Moreover, 90bpm had significantly longer JT intervals than 180bpm ( $p < 0.001$ ), 150bpm ( $p < 0.001$ ) and 120bpm ( $p = 0.043$ ). Also, JT interval was significantly longer in 120bpm than 180bpm ( $p = 0.013$ ).

**a.****b.**

*Figure 3.51: QT (a) and JT (b) intervals significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm; \* = significantly different to 180bpm; ^ = significantly different to 90bpm.*

A main effect of tempo on T amplitude also transpired ( $p = 0.002$ ). As shown in Figure 3.52, T amplitude was significantly higher in 180bpm than 90bpm ( $p = 0.042$ ) and 60bpm ( $p = 0.031$ ).

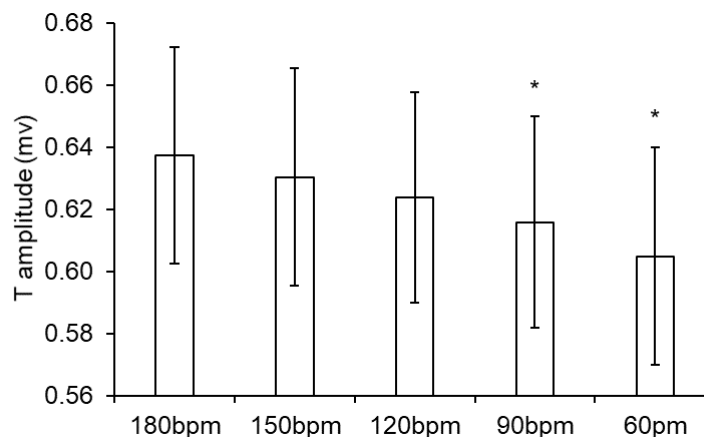


Figure 3.52: T amplitude significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. \* = significantly different to 180bpm.

### 3.3.2.7 BP, BRS and respiration

#### 3.3.2.7.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)

A mixed mode ANOVA identified a statistically significant main effect of condition ( $p < 0.001$ ) and interaction ( $p = 0.022$ ) on SBP. Further exploration of the interaction revealed no statistically significant differences between the control and experimental groups at the level of the individual conditions ( $p > 0.05$ ). However, at the level of the two groups, statistically significant differences between some of the conditions emerged for the control group only. Indeed, as shown in Figure 3.53, SBP was significantly higher during recovery than baseline ( $p < 0.001$ ), the stable tempo stimulus ( $p = 0.045$ ), the stepped increase in tempo stimulus ( $p = 0.005$ ) and the stepped decrease in tempo stimulus ( $p = 0.001$ ). In addition, SBP was significantly greater during the stepped decrease in tempo stimulus than baseline ( $p = 0.004$ ).

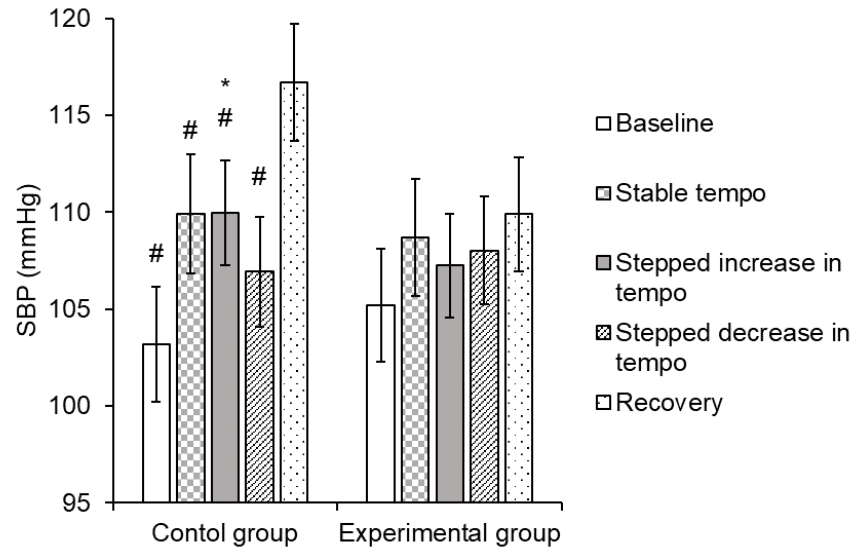


Figure 3.53: SBP significantly differed between the five conditions for the control group only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

For DBP a statistically significant main effect of condition emerged ( $p < 0.001$ ). As illustrated in Figure 3.54, DBP was significantly higher during recovery than during baseline ( $p < 0.001$ ), the stable tempo stimulus ( $p = 0.033$ ), the stepped increase in tempo stimulus ( $p = 0.010$ ) and the stepped decrease in tempo stimulus ( $p < 0.001$ ). Furthermore, baseline DBP was significantly lower than that during the stepped increase in tempo stimulus ( $p = 0.036$ ).

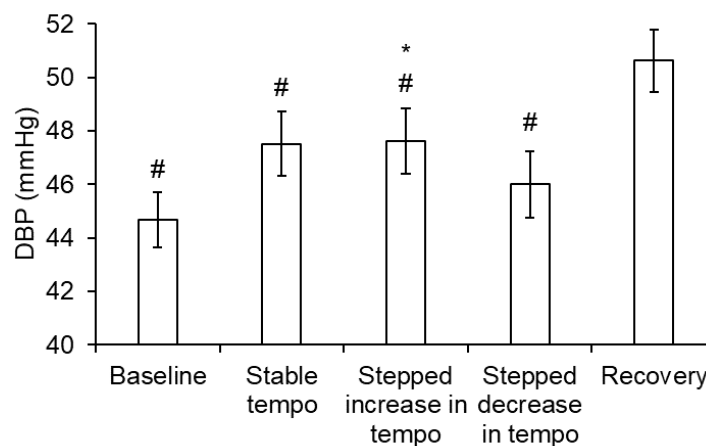
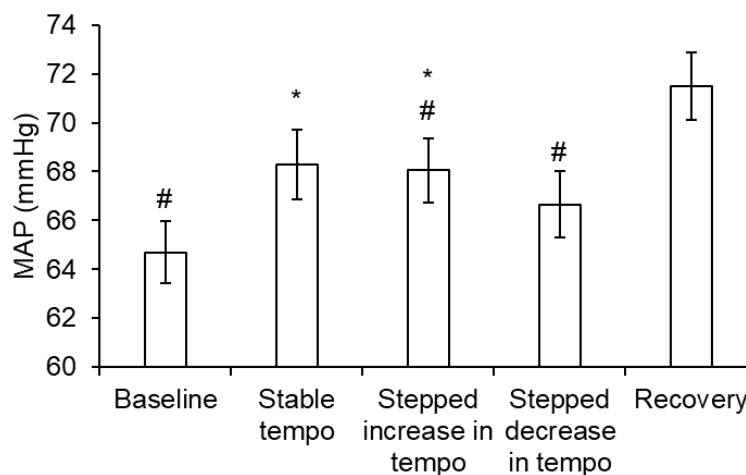


Figure 3.54: DBP significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Unsurprisingly, the main effect of condition on MAP also reached statistical significance ( $p < 0.001$ ). Figure 3.55 demonstrates that similar to SBP and DBP, MAP was significantly higher during recovery than baseline ( $p < 0.001$ ) and the stepped increase in tempo ( $p = 0.004$ ) and stepped decrease in tempo ( $p = 0.001$ ) stimuli. Furthermore, MAP was significantly lower during baseline than during the stable tempo ( $p = 0.044$ ) and stepped increase in tempo ( $p = 0.012$ ) stimuli.



*Figure 3.55: MAP significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.*

No main effects or interactions reached statistical significance for any of the BRS measures ( $p > 0.05$ ). However, there was a significant main effect of condition on respiration rate ( $p < 0.001$ ). As shown in Figure 3.56, respiration rate was significantly lower during baseline than during all three auditory stimuli (all  $p < 0.001$ ). In addition, stepped increase in tempo respiration rate was significantly higher than that during the stable tempo stimulus ( $p = 0.012$ ). Finally, recovery respiration rate was significantly lower during recovery than during the stepped increase and decrease in tempo stimuli (both  $p = 0.001$ ).



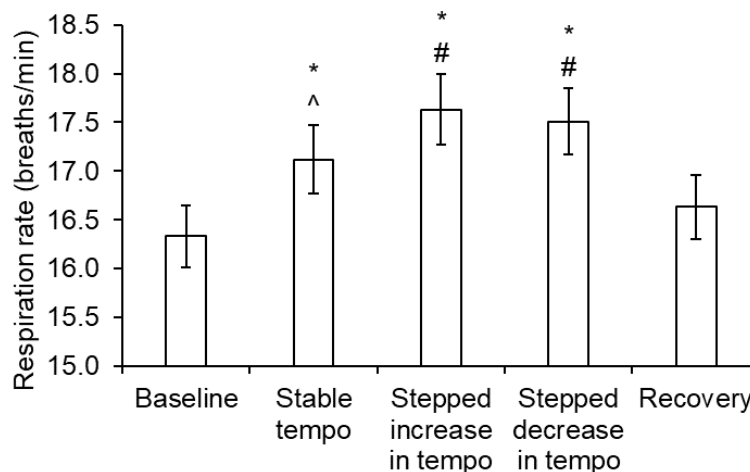


Figure 3.56: Respiration rate significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery; ^ = significantly different to stepped increase in tempo.

### 3.3.2.7.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

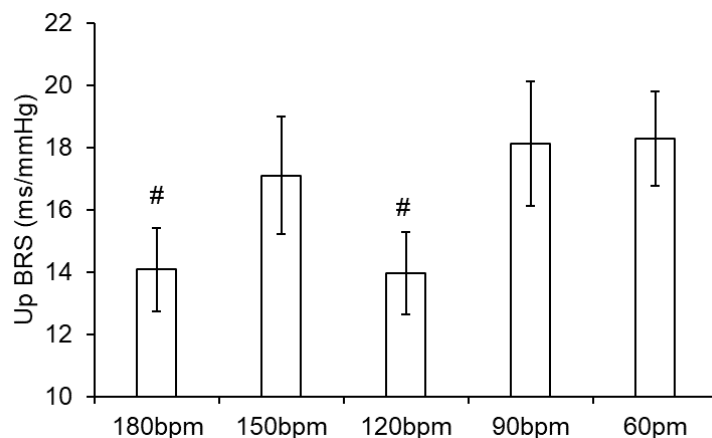
No statistically significant main effects or interactions emerged for any of the variables ( $p > 0.05$ )

### 3.3.2.7.3 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

Significant main effects of tempo on up and mean BRS emerged ( $p = 0.004$  and  $p = 0.009$  respectively). As depicted in Figure 3.57, Bonferroni detected the same statistically significant differences: up and mean BRS were significantly larger during 60bpm than 180bpm ( $p = 0.009$  and  $p = 0.022$ ) and 120bpm ( $p = 0.009$  and  $p < 0.001$ ).

No other significant differences emerged ( $p > 0.05$ ).

a.



b.

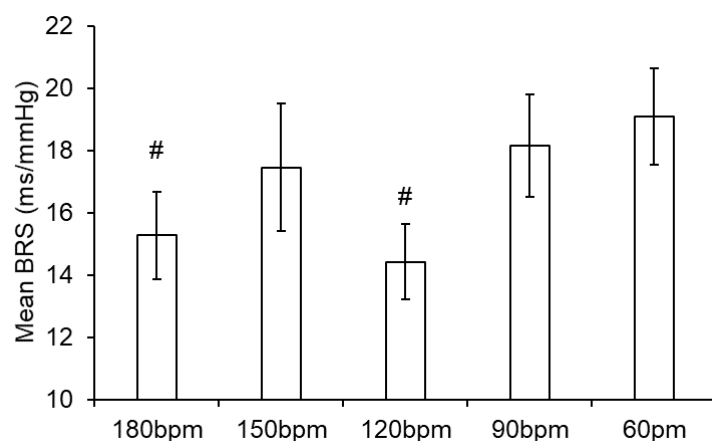


Figure 3.57: Up BRS (a) and mean BRS (b) significantly differed between the five decreasing tempi. Data presented as mean  $\pm$  1 SEM. # = significantly different to 60bpm.

### 3.3.2.8 Brachial HR and BP

No statistically significant main effects or interactions emerged for any variable ( $p > 0.05$ ).

### 3.3.2.9 The effect of blood pressure measurement

The impact of different methods of blood pressure measurement was investigated by running a two-way repeated measures ANOVA. The within-participants variables were: condition (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo, recovery); and BP method (finometer, brachial).

### 3.3.2.9.1 SBP

Statistically significant interactions were detected for SBP ( $p < 0.001$ ). Figure 3.58 shows the interaction between condition and BP method for SBP. At the level of the condition, brachial SBP was significantly higher than finometer SBP for baseline ( $p < 0.001$ ), stable tempo ( $p = 0.001$ ), stepped increase in tempo ( $p < 0.001$ ), stepped decrease in tempo ( $p < 0.001$ ) and recovery ( $p = 0.008$ ). Moreover, at the level of the BP method, no statistically significant differences emerged between the five conditions for brachial SBP ( $p > 0.05$ ). However, finometer SBP values were significantly higher in recovery than during baseline ( $p < 0.001$ ), stepped increase in tempo ( $p = 0.006$ ) and stepped decrease in tempo ( $p = 0.010$ ). Additionally, finometer SBP was significantly lower during baseline compared to the stable tempo ( $p = 0.029$ ) and stepped increase in tempo ( $p = 0.013$ ) stimuli.

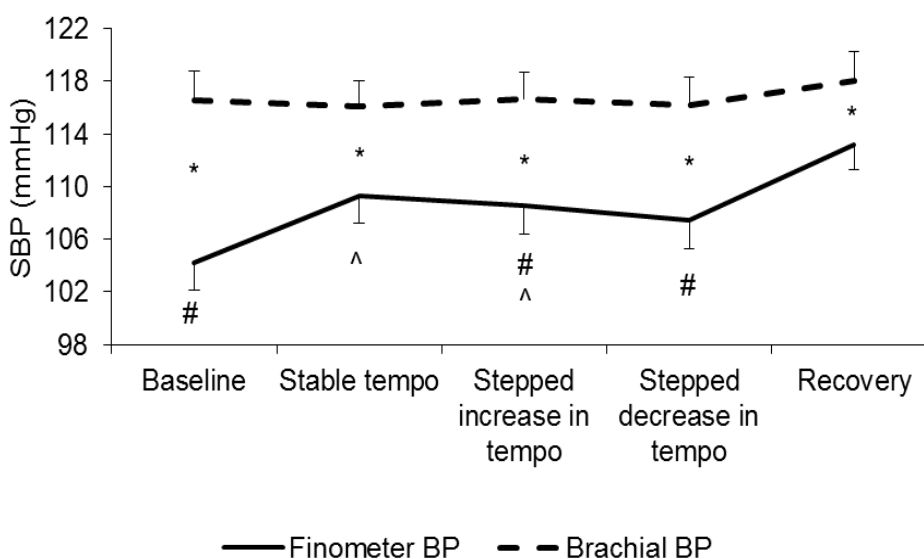
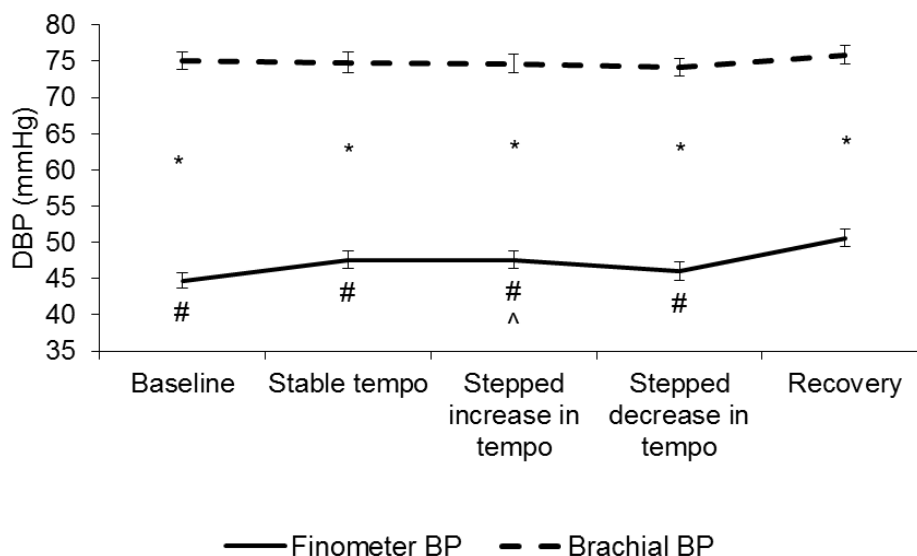


Figure 3.58: SBP was significantly impacted by condition and BP method of measurement. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two BP methods; # = significantly different to recovery; ^ = significantly different to baseline.

### 3.3.2.9.2 DBP

The repeated measures ANOVA revealed a statistically significant interaction for DBP ( $p = 0.001$ ). As illustrated in Figure 3.59, brachial DBP was significantly higher than finometer DBP for baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery (all  $p <$

0.001). Moreover, examination of this effect at the level of BP method revealed that finometer DBP was significantly higher during recovery than during baseline ( $p < 0.001$ ), stable tempo ( $p = 0.041$ ), stepped increase in tempo ( $p = 0.009$ ) and stepped decrease in tempo ( $p < 0.001$ ). In addition, finometer DBP was significantly lower in baseline than during the stepped increase in tempo stimulus ( $p = 0.042$ ). No statistically significant differences in brachial DBP emerged between the five conditions ( $p > 0.05$ ).



*Figure 3.59: DBP was significantly impacted by condition and BP method of measurement. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two BP methods; # = significantly different to recovery; ^ = significantly different to baseline.*

### 3.3.2.9.3 MAP

Unsurprisingly, the interaction for MAP also reached statistical significant ( $p < 0.001$ ). As depicted in Figure 3.60, when analysed at the level of the condition, brachial MAP was significantly greater than finometer MAP for all five conditions (all  $p < 0.001$ ). Also, when analysed at the level of BP method, finometer MAP was significantly higher during recovery compared to baseline ( $p < 0.001$ ), stepped increase in tempo ( $p = 0.004$ ) and stepped decrease in tempo ( $p = 0.001$ ). Furthermore, finometer MAP was significantly lower during baseline than during the stable tempo ( $p = 0.042$ ) and stepped increase in tempo ( $p = 0.016$ ) stimuli. No differences between any of the five conditions emerged for brachial MAP ( $p > 0.05$ ).

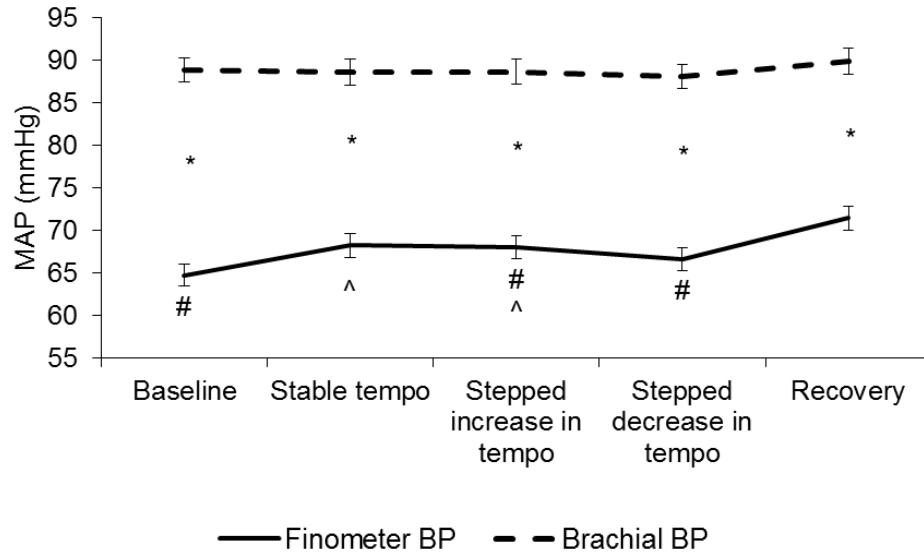


Figure 3.60: DBP was significantly impacted by condition and BP method of measurement. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two BP methods; # = significantly different to recovery; ^ = significantly different to baseline.

### 3.3.2.10 Summary of the cardiovascular autonomic results

To aid with summarising the above cardiovascular autonomic results, the following tables were generated. This entailed grouping together all measures that were considered to reflect parasympathetic activity and all remaining measures.

#### 3.3.2.10.1 Parasympathetic measures summaries

Table 3.3 summarises the changes in parasympathetic measures that occurred for the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli. It clearly shows that the stepped increase and decrease in tempo stimuli did not elicit changes in vagal tone (when compared to baseline, recovery or the stable tempo stimulus). However, the stable tempo (control) stimulus was associated with reductions in nSD1.

*Table 3.3: Summary of changes in measures of parasympathetic activity for the three stimuli.*

	Stable tempo stimulus	Stepped increase in tempo stimulus	Stepped decrease in tempo stimulus
SDSD			
RMSSD			
pRR50			
HF			
nuHF			
HF%			
SD1			
nSD1	↓		

Table 3.4 summarises the alterations in the eight measures of parasympathetic activity that occurred for the five tempi of the stepped increased in tempo stimulus. Indeed, it appears that 180bpm (the final tempo) was associated with the most increases in parasympathetic activity. This suggests that 180bpm was the most physiologically relaxing when compared to the other four tempi.

*Table 3.4: Summary of changes in measures of parasympathetic activity for the increasing tempi.*

	60bpm	90bpm	120bpm	150bpm	180bpm
SDSD					↑
RMSSD					↑
pRR50					
HF					
nuHF					
HF%			↑	↑	
SD1					↑
nSD1					↑

Table 3.5 summarises the changes in the parasympathetic measures that occurred for the stepped decrease in tempo stimulus. Here, 90bpm and 60bpm were associated with the same increases in vagal tone whilst the remaining tempi showed no significant alterations. Therefore, contrary to the stepped increase in tempo results, 90bpm and 60bpm were associated with the greatest shifts towards parasympathetic predominance.

*Table 3.5: Summary of changes in measures of parasympathetic activity for the decreasing tempi.*

	180bpm	150bpm	120bpm	90bpm	60bpm
SDSD				↑	↑
RMSSD				↑	↑
pRR50				↑	↑
HF				↑	↑
nuHF					
HF%					
SD1				↑	↑
nSD1				↑	↑

### 3.3.2.10.2 Remaining cardiovascular autonomic measures summaries

Table 3.6 summarises the significant differences that occurred for the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli. Inspection of the table shows that no meaningful differences emerged between the three stimuli. That is, all three were associated with similar changes in cardiovascular autonomic activity.

*Table 3.6: Summary of changes in remaining measures of autonomic activity for the three stimuli. Where: a = in the control group only.*

	Stable tempo stimulus	Stepped increase in tempo stimulus	Stepped decrease in tempo stimulus
VLF%	↑		
Min RR		↓	
ΔRR		↑	↑
Max-Q3 RR		↑	↑
QT interval	↓	↓	↓
QTc interval	↓	↓	↓
JT interval	↓	↓	↓
Q amplitude	↑	↑	↑
R amplitude	↓	↓	↓
ST height	↑		
SBP	↓ <sub>a</sub>	↓ <sub>a</sub>	↓ <sub>a</sub>
DBP	↓	↓	↓
MAP		↓	↓
Respiration rate			

This was also the case for the five tempi of the stepped increase in tempo stimulus (see Table 3.7 below). However, it could be argued that 90bpm, followed by 120bpm, was associated with smaller shifts towards sympathetic predominance compared to the other tempi. In addition, it should be noted

that the increase in total power that occurred for 180bpm may have been due to increases in VLF power.

*Table 3.7: Summary of changes in remaining measures of autonomic activity for the increasing tempi.*

	60bpm	90bpm	120bpm	150bpm	180bpm
CVRR		↓	↓		
SDRR		↓	↓	↓	
SD HR			↓		
Total power					↑
VLF power					↑
SD2		↓	↓		
nSD2		↓	↓		
SD1/SD2		↑	↑		
SD2/SD1			↓		
Min RR		↑	↑	↑	
ΔRR					↑
IQR RR					↑
Q1-min RR				↓	
QT interval	↓				
QTc interval	↓	↓			
JT interval	↓				
ST height	↑	↑			

Table 3.8 summarises the differences in the remaining measures of cardiovascular autonomic function that occurred for the stepped decrease in tempo stimulus. Consistent with Table 3.5, 60bpm was associated with decreases in HR and increases in HRV and BRS. Therefore, these patterns provide further support for the claim that the 60bpm tempo of the stepped decrease in tempo stimulus elicited the greatest shifts towards parasympathetic predominance.



*Table 3.8: Summary of changes in remaining measures of autonomic activity for the decreasing tempi.*

	180bpm	150bpm	120bpm	90bpm	60bpm
HR					↓
Total power					↑
Mean RR				↑	↑
Median RR				↑	↑
Mode RR					↑
Max RR				↑	↑
$\Delta$ RR					↑
Q1 RR					↑
Q3 RR				↑	↑
Max-Q3 RR					↑
QT interval				↑	↑
JT interval				↑	↑
T amplitude				↓	↓
UP BRS					↑
MEAN BRS					↑

### 3.3.2.10.3 Take-home message

From the above summary tables, autonomic activity was similar in all three auditory stimuli. This means that sympathetic and parasympathetic predominance did not fluctuate as a result of the stimuli when the full duration of each stimulus was analysed. However, analysis at the level of the tempi revealed two findings. Firstly, 180bpm in the stepped increase in tempo stimulus was associated with the greatest shifts towards parasympathetic predominance. Secondly, 60bpm in the stepped decrease in tempo stimulus elicited the greatest shifts towards parasympathetic predominance and increases in overall variability. Figure 3.61 diagrammatically illustrates these outcomes.



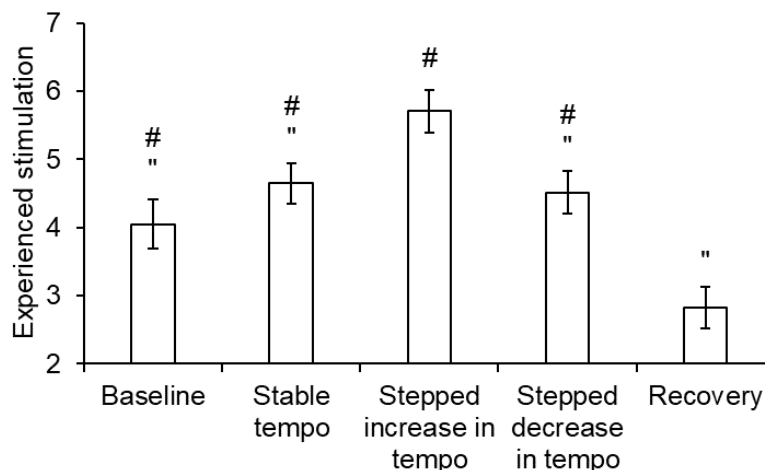


Figure 3.62: Experienced stimulation significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery; ^ = significantly different to baseline; " = significantly different to the stepped increase in tempo stimulus.

### 3.3.3.1.2 Relaxation

For experienced relaxation, the main effect of condition reached significance ( $p < 0.001$ ) as did the interaction ( $p = 0.027$ ). The main effect of group was not statistically significant ( $p > 0.05$ ). Exploration of the interaction revealed that at the level of the condition experienced relaxation was significantly higher in the experimental group than in the control group for the stepped increase in tempo stimulus only ( $p = 0.010$ , see Figure 3.63). At the level of the control group, experienced relaxation was significantly higher at the end of baseline than at the end of the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli (all  $p < 0.001$ ). Also, experienced relaxation was significantly higher at the end of the recovery compared to at the end of the stable tempo ( $p = 0.001$ ), stepped increase in tempo ( $p < 0.001$ ) and stepped decrease in tempo ( $p = 0.032$ ) stimuli. At the level of the experimental group, experienced relaxation was significantly higher at the end of recovery than at the end of the stable tempo ( $p = 0.017$ ) and stepped increase in tempo ( $p = 0.009$ ) stimuli.

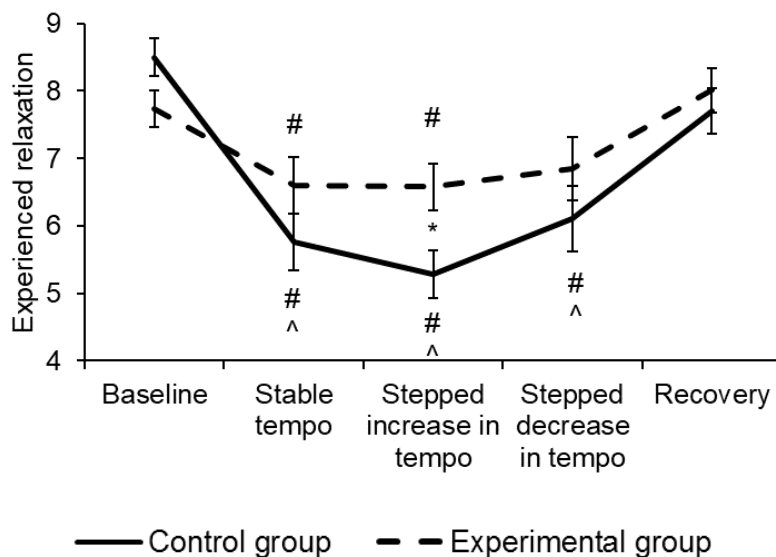


Figure 3.63: Experienced relaxation during the five conditions significantly interacted with group (control, experimental). Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two groups; # = significantly different to recovery; ^ = significantly different to baseline.

### 3.3.3.1.3 Stress

Although the main effect of condition reached significance ( $p < 0.001$ ), so did the interaction ( $p = 0.006$ , see Figure 3.64). Further analysis of the interaction revealed that at the level of the condition, experienced stress at the end of the stepped increase in tempo stimulus was significantly higher in the control group compared to the experimental group ( $p = 0.003$ ). At the level of the control group, experienced stress was significantly lower at the end of baseline compared to after the stable tempo ( $p = 0.004$ ) and stepped increase in tempo ( $p < 0.001$ ) stimuli. Also, experienced stress was significantly lower at the end of recovery than after the stable tempo ( $p = 0.001$ ) and stepped increase in tempo stimuli ( $p < 0.001$ ). There were no significant differences between any of the five recordings for the experimental group ( $p > 0.05$ ).

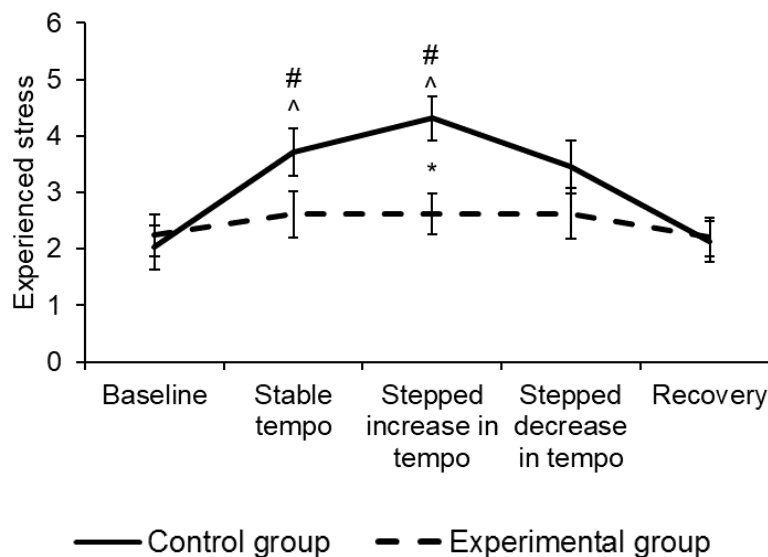


Figure 3.64: Experienced stress during the five conditions significantly interacted with group (control, experimental). Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two groups; # = significantly different to recovery; ^ = significantly different to baseline.

#### 3.3.3.1.4 Calm

Like stress, a significant main effect of condition ( $p < 0.001$ ) and interaction ( $p = 0.004$ ) emerged for experienced calm. As shown in Figure 3.65, calm was significantly lower during the stepped increase in tempo stimulus for the control group compared to the experimental group ( $p = 0.002$ ). Examination at the level of the control group revealed that experienced calm was significantly higher after baseline compared to at the end of the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p < 0.001$ ) and stepped decrease in tempo ( $p = 0.001$ ) stimuli. In addition, experienced calm was significantly higher after recovery than after the stable tempo ( $p = 0.003$ ) and stepped increase in tempo ( $p < 0.001$ ) stimuli. Experienced calm was also significantly lower at the end of the stepped increase in tempo stimulus compared to at the end of the stable tempo ( $p = 0.044$ ) and stepped decrease in tempo ( $p = 0.021$ ) stimuli. For the experimental group, experienced calm was significantly lower at the end of the stepped increase in tempo stimulus than at the end of baseline ( $p = 0.023$ ) and recovery ( $p = 0.018$ , see Figure 3.65).

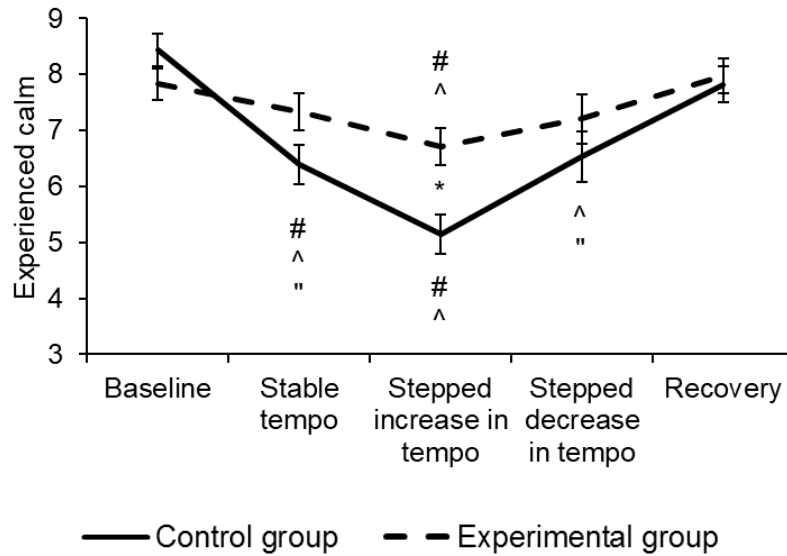


Figure 3.65: Experienced calm during the five conditions significantly interacted with group (control, experimental). Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two groups; # = significantly different to recovery; ^ = significantly different to baseline; " = significantly different to the stepped increase in tempo stimulus.

### 3.3.3.2 Experienced emotion: valence

#### 3.3.3.2.1 Happiness

The main effect of condition on experienced happiness reached significance ( $p < 0.001$ ). As shown in Figure 3.66, experienced happiness was significantly higher after baseline than after the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p = 0.025$ ) and stepped decrease in tempo stimuli ( $p = 0.011$ ). Also, experienced happiness was significantly higher at the end of recovery than after the stable tempo ( $p = 0.017$ ) and stepped decrease in tempo ( $p = 0.032$ ) stimuli.

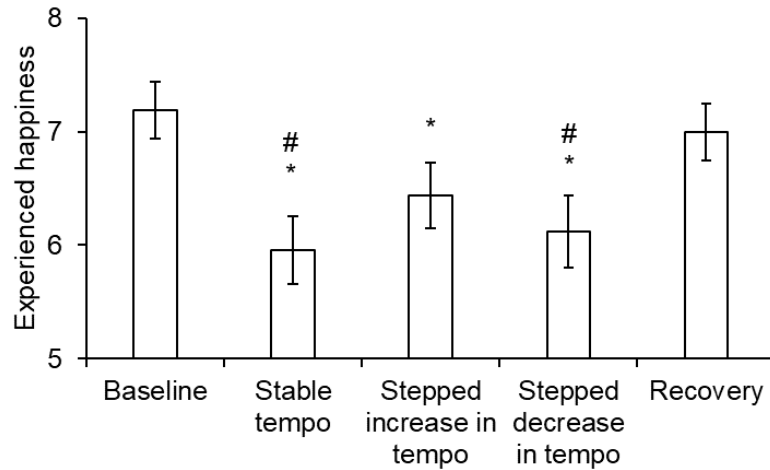


Figure 3.66: Experienced happiness significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = statistically significant different to baseline; # = significantly different to recovery.

### 3.3.3.2.2 Sadness

Although a statistically significant main effect of condition on experienced sadness emerged ( $p < 0.001$ ), sadness varied between different conditions compared to experienced happiness. Indeed, examination of Figure 3.67 shows that experienced sadness was significantly higher after the stepped decrease in tempo stimulus compared to after baseline ( $p = 0.008$ ), stepped increase in tempo ( $p = 0.046$ ) and recovery ( $p = 0.016$ ).

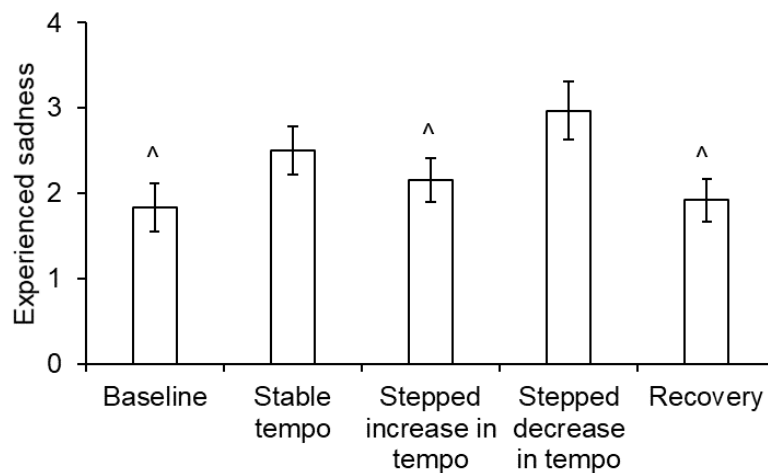


Figure 3.67: Experienced sadness significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. ^ = significantly different to the stepped decrease in tempo stimulus.

### 3.3.3.3 Perceived emotion: arousal

#### 3.3.3.3.1 Stimulation

A statistically significant main effect of condition on perceived stimulation transpired ( $p < 0.001$ ). As depicted in Figure 3.68, perceived stimulation was significantly lower for the baseline recording compared to the stable tempo and stepped increase in tempo (both  $p < 0.001$ ) stimuli. Furthermore, participants perceived the recovery condition as significantly less stimulating than all three auditory stimuli (all  $p < 0.001$ ). Finally, perceived stimulation was significantly higher for the stepped increase in tempo stimulus than for recovery ( $p < 0.001$ ) and for the stable tempo ( $p = 0.004$ ) and stepped decrease in tempo stimuli ( $p = 0.001$ ).

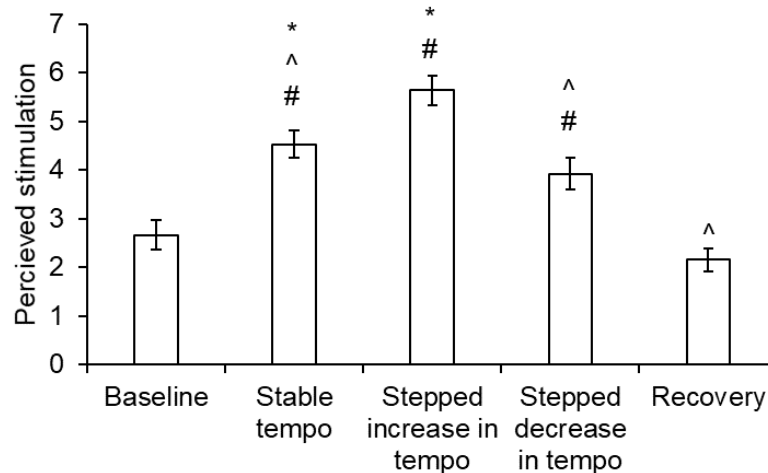


Figure 3.68: Perceived stimulation significantly differed between the five conditions. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery; ^ = significantly different to the stepped increase in tempo stimulus.

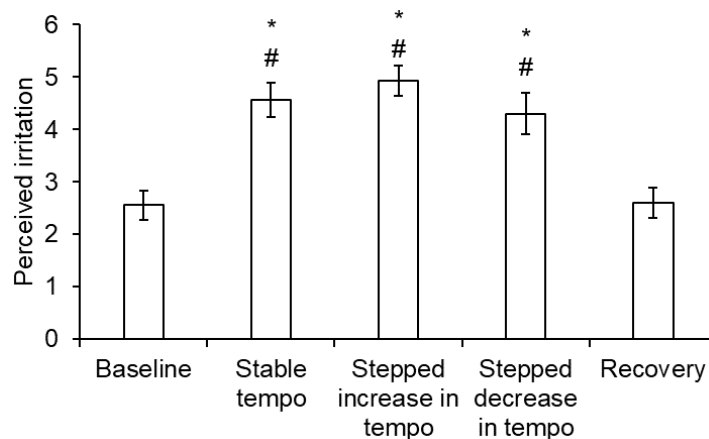
#### 3.3.3.3.2 Irritation

Main effects of condition and group on perceived irritation also reached significance ( $p < 0.001$  and  $p = 0.011$  respectively). As illustrated in Figure 3.69a, perceived irritation was significantly lower for baseline than for the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p < 0.001$ ) and stepped decrease in tempo ( $p = 0.004$ ) stimuli. The same pattern emerged for the recovery condition: perceived irritation was significantly lower for recovery than for the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p$



< 0.001) and stepped decrease in tempo ( $p = 0.003$ ) stimuli. Exploration of the main effect of group revealed that perceived irritation was significantly higher for the control group than for the experimental group ( $p = 0.011$ , see Figure 3.69b).

**a.**



**b.**

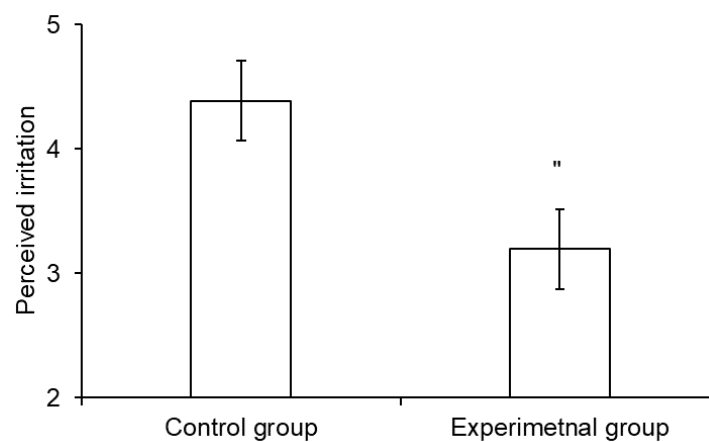


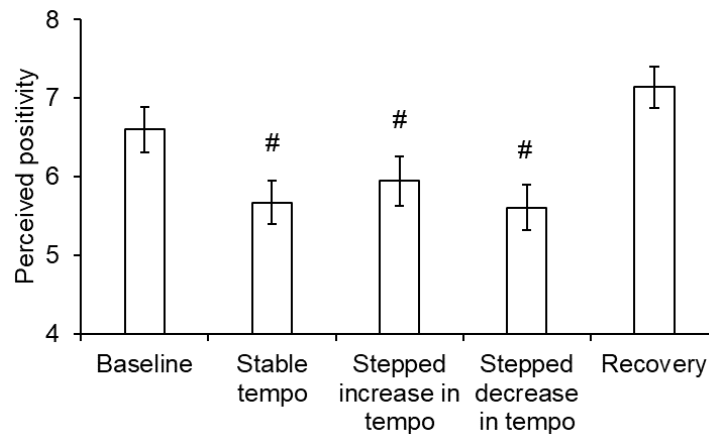
Figure 3.69: Perceived irritation significantly differed between the five conditions (a) and the two groups (b). Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery; ^ = significantly different to the stepped increase in tempo stimulus; " = statistically significant difference between the two groups.

### **3.3.4 Perceived emotion: valence**

#### **3.3.4.1.1 Positivity**

Despite the interaction failing to reach statistical significance, this was not the case for main effects of condition and group ( $p < 0.001$  and  $p = 0.005$  respectively). Analysis of the main effect of condition revealed that perceived positivity was significantly higher for recovery than for the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p = 0.017$ ) and stepped decrease in tempo stimuli ( $p = 0.001$ , see Figure 3.70a). Exploration of the main effect of group demonstrated that perceived positivity was significantly higher for the experimental group compared to the control group ( $p = 0.005$ , see Figure 3.70b).

a.



b.

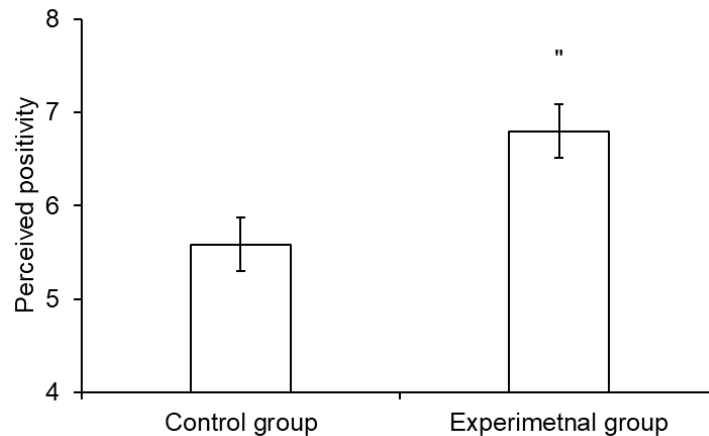


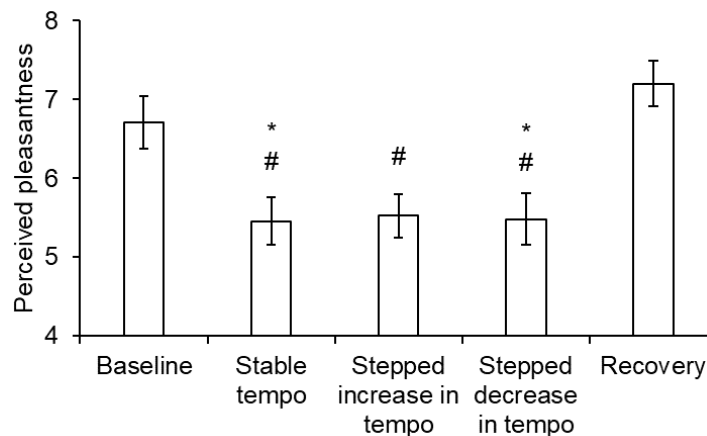
Figure 3.70: Perceived positivity significantly differed between the five conditions (a) and the two groups (b). Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery; " = statistically significant difference between the two groups.

### 3.3.4.1.2 Pleasantness

The main effects of condition and group were statistically significant for perceived pleasantness ( $p < 0.001$  and  $p = 0.030$  respectively). As shown in Figure 3.71a, perceived pleasantness was significantly higher for baseline than for the stable tempo ( $p = 0.013$ ) and stepped decrease in tempo ( $p = 0.046$ ) stimuli. Also, the perceived pleasantness of the recovery condition was significantly higher than that for the stable tempo ( $p < 0.001$ ), stepped increase in tempo ( $p < 0.001$ ) and stepped decrease in tempo ( $p = 0.001$ ) stimuli. Examination of the main effect of group revealed that perceived

pleasantness was significantly higher for the experimental group compared to the control group ( $p = 0.030$ , see Figure 3.71b).

a.



b.

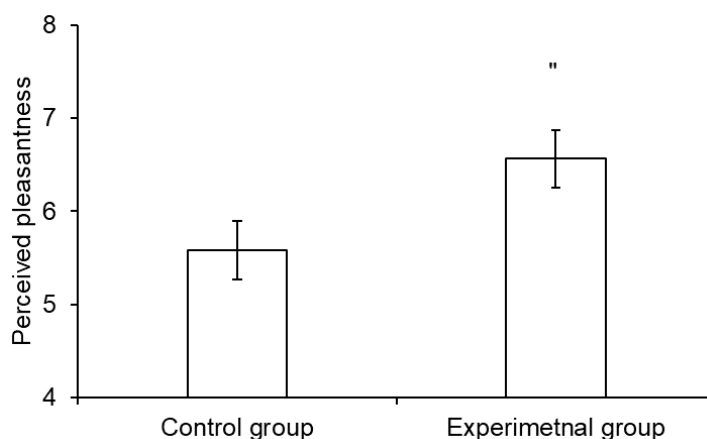
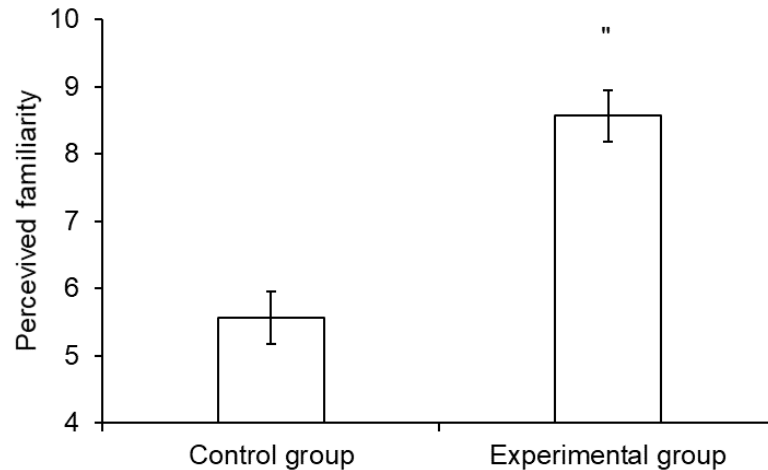


Figure 3.71: Perceived pleasantness significantly differed between the five conditions (a) and the two groups (b). Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery; " = statistically significant difference between the two groups.

### 3.3.4.2 Perceived familiarity

Although the main effect of condition and interaction failed to reach significance ( $p > 0.05$ ), this was not the case for the main effect of group ( $p < 0.001$ ). Indeed, familiarity was significantly higher for the experimental group than for the control group ( $p < 0.001$ , see Figure 3.72).



*Figure 3.72: Perceived familiarity differed between the control and experimental groups. Data presented as mean  $\pm$  1 SEM. " = statistically significant difference between the two groups.*

### **3.3.4.3 VAS factor analysis**

As similar patterns in some of the subjective measures were observed, factor analysis was performed to reduce the data to a smaller set of variables. Mean scores for the 10 subjective measures during the five conditions were calculated and exploratory factor analysis performed. This was performed on the six experienced musical emotions and four perceived musical emotions individually and combined. As the variables were theoretically related to one another and were found to be inter-correlated, oblique rotation rather than orthogonal (Varimax) rotation was performed. This resulted in implementing the Direct Oblimin method. Missing data were excluded pairwise and coefficients less than 0.4 were suppressed.

#### **3.3.4.3.1 Experienced musical emotion**

The data were checked to ensure the factor analysis was feasible. The p-values for the correlation matrix were statistically significant except for experienced stimulation. The determinant was 0.026, greater than the recommended value of 0.00001, demonstrating that multicollinearity was not a problem. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.765, above the commonly recommended value of 0.50 and Bartlett's test of sphericity was significant ( $p < 0.001$ ). The communalities were all

above 0.30 (see Table 3.9) further confirming that each item shared some common variance with other items. All items except experienced stimulation had anti-image correlations greater than 0.50. It was anticipated that two factors would be identified. One representing experienced valence and the other representing experienced arousal.

Initial Eigenvalues showed that the first and second factors explained 57.90% and 19.22% of the variance respectively. This variance dropped to 9.03% for the third factor. The two-factor solution was implemented as it accounted for 77.12% of the variance and the Eigenvalues levelled off on the scree plot from the third factor onwards. The factor loading matrix for this solution is depicted in Table 3.9.

*Table 3.9: Two-factor model for experienced emotions.*

	<b>Factor 1</b>	<b>Factor 2</b>	<b>Communalities</b>
Experienced stimulation		0.95	1.00
Experienced relaxation	0.91		1.00
Experienced stress	-0.81		1.00
Experienced calm	0.93		1.00
Experienced happiness	0.72		1.00
Experienced sadness	-0.76		1.00

As experienced stimulation had an anti-image correlation less than 0.50 and few significant correlations, the item was removed and the analysis re-run. This led to the identification of only one factor, upon which the items loaded  $< 0.40$ . No further analysis was performed.

### **3.3.4.3.2 Perceived musical emotion**

The same data checks were performed on the four perceived musical emotion variables. The data met all factor analysis requirements. The determinant was 0.021, KMO was 0.790 and Bartlett's test of sphericity was significant ( $p < 0.001$ ). All variables correlated at least 0.30 and no greater than 0.90. All anti-image correlations were  $> 0.50$  and all communalities were  $> 0.30$ . It was anticipated that the factor analysis would identify two factors: perceived valence and perceived arousal.

However, only one factor emerged from the Direct Oblimin rotation, upon which the items loaded  $< 0.40$ . As a result, no further analysis was performed.

### 3.3.4.3.3 Experienced and perceived musical emotion

The data were checked as previously. The determinant was 0.0000638, KMO was 0.859 and Bartlett's test of sphericity was significant ( $p < 0.001$ ). The  $p$ -values for the correlation matrix were statistically significant except for experienced stimulation. Also, all items except experienced stimulation had anti-image correlations greater than 0.50. It was anticipated that the factor analysis would identify two factors: valence and arousal; or experienced musical emotion and perceived musical emotion.

Initial Eigenvalues showed that the first and second factors explained 60.04% and 15.53% of the variance respectively. This variance dropped to 6.87% for the third factor. The two-factor solution was implemented as it accounted for 75.57% of the variance and the Eigenvalues levelled off on the scree plot from the third factor onwards. The factor loading matrix for this solution is depicted in Table 3.10.

*Table 3.10: Two-factor model for experienced and perceived emotions.*

	Factor 1	Factor 2	Communalities
Experienced stimulation		0.86	1.00
Experienced relaxation	0.82		1.00
Experienced stress	-0.75		1.00
Experienced calm	0.80		1.00
Experienced happiness	0.78		1.00
Experienced sadness	-0.79		1.00
Perceived stimulation	-0.44	0.73	1.00
Perceived irritation	-0.93		1.00
Perceived positivity	0.91		1.00
Perceived pleasantness	0.92		1.00

As experienced stimulation had an anti-image correlation less than 0.50 and few significant correlations, the item was removed and the analysis re-run. The determinant was 0.001, KMO was 0.889 and Bartlett's test of sphericity was significant ( $p < 0.001$ ). All anti-image correlations were  $> 0.50$  and the communalities were all 1.00.

The Direct Oblimin identified two factors. The first accounted for 66.49% and the second accounted for 11.27%. The cumulative percentage both factors explained was 77.75%. Inspection of the scree plot showed that the Eigenvalues levelled off from the third factor onwards. The factor loaded matrix for the solution is shown in Table 3.11.

*Table 3.11: Two-factor model for experienced and perceived emotions excluding experienced stimulation.*

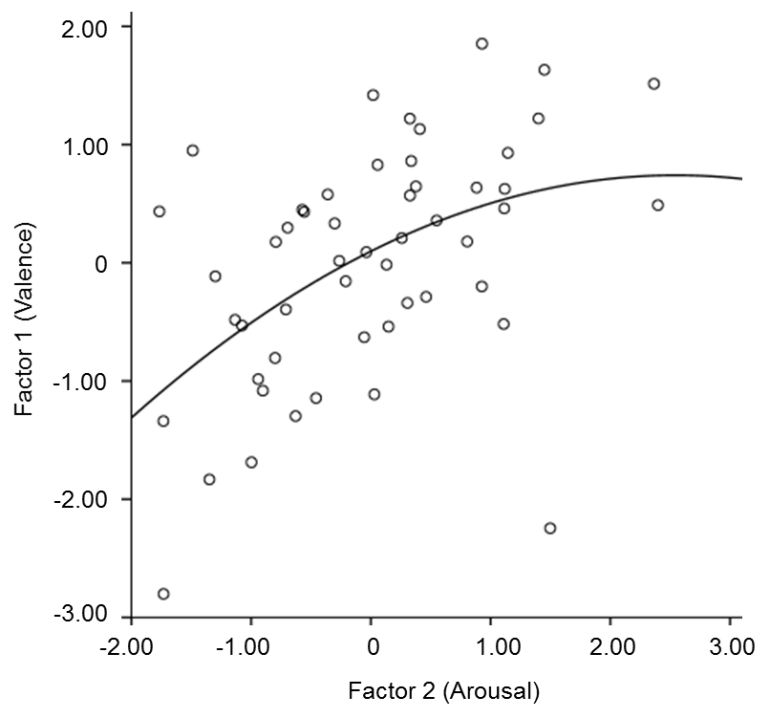
	<b>Factor 1</b>	<b>Factor 2</b>	<b>Communalities</b>
Experienced relaxation	0.48	0.60	1.00
Experienced stress	-0.81		1.00
Experienced calm	0.47	0.61	1.00
Experienced happiness	0.78		1.00
Experienced sadness	-0.89		1.00
Perceived stimulation		-0.97	1.00
Perceived irritation	-0.86		1.00
Perceived positivity	0.87		1.00
Perceived pleasantness	0.83		1.00

The design of the VAS questionnaires was based on the circumplex model of emotion: the affective space can be divided into two dimensions: one concerned with valence (positivity and negativity) and the other concerned with arousal (stimulation and relaxation) (Hevner, 1937; Russell, Ward & Pratt, 1981; Roberts & Wedell, 1994). Consistent with expectations, a two-factor solution was revealed in the factor analysis. In addition, item loading suggests that the circumplex model of emotion maps onto the two-factor solution. Therefore, the first factor can be interpreted to represent the valence dimension of the model and the second factor can be argued to represent the arousal dimension.

Liking of artistic stimuli and arousal potential have been shown to be related in the form of an inverted-U relationship. This was most prominently demonstrated by Berlyne who maintained that extremely low or high levels of arousal are associated with stimulus dislike, whilst moderate levels of arousal are associated with high levels of stimulus liking. As Berlyne's theory of aesthetic response and the circumplex model of emotion employ similar dimensions to explain emotional responses to music (valence/liking and arousal) curvilinear regression on the factor scores (derived using the



Bartlett method) saved from the factor analysis was performed. Although the linear model reached significance ( $n = 52$ ,  $R^2 = 0.230$ ,  $p < 0.001$ ), a greater percentage of the variance in scores was accounted by the quadratic model ( $n = 52$ ,  $R^2 = 0.245$ ,  $p = 0.001$ , see Figure 3.73). The regression equation of the quadratic model was:  $Y = 0.97 + 0.505x - 0.099x^2$ , demonstrating that the relationship between valence and arousal is best described by an inverted-U function. This finding is consistent with Berlyne's theory of aesthetic response.



*Figure 3.73: A quadratic curve emerged between the two factors identified from the factor analysis.*

#### **3.3.4.4 Interpretation of the stimuli**

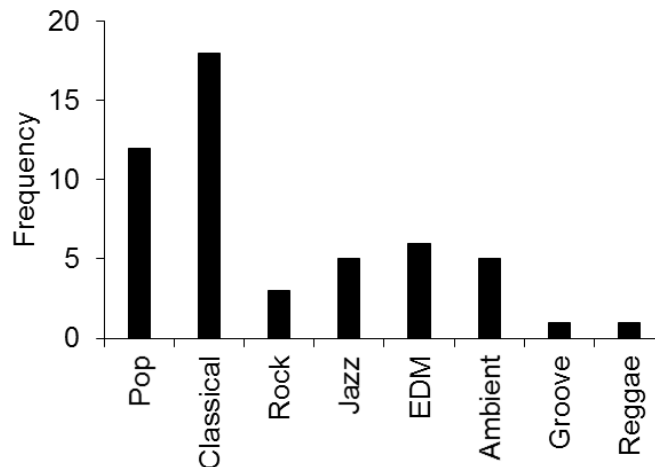
Chi-square tests of independence revealed that the distribution of participants who rated the three auditory stimuli as music did not significantly differ between the two groups ( $p > 0.05$ ). As illustrated in Table 3.12, similar percentages of participants in the control and experimental groups rated the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli as music.

*Table 3.12: Percentage of participants who interpreted the stimuli at music did not significantly differ between the control and experimental groups ( $p > 0.05$ ).*

	Control group (n = 25)	Experimental group (n = 27)	p-value
% of participants who interpreted the stable tempo stimulus as music	56.00	77.78	ns
% of participants who interpreted the stepped increase in tempo stimulus as music	64.00	85.19	ns
% of participants who interpreted the stepped decrease in tempo stimulus as music	60.00	81.48	ns

### 3.3.4.5 Preferred music genre for the study room

At the end of the music background questionnaire, participants were required to state which music genre they would most prefer to listen to in the study room. A chi-square goodness of fit test revealed that the observed distribution of music genre preferences significantly differed to expected ( $p < 0.001$ ). Inspection of Figure 3.74 shows that participants most preferred to listen to classical music in the study room. This was closely followed by pop.



*Figure 3.74: Distribution of preferred musical genres for the study room. Classical was the most popular genre of music participants preferred to listen to in the study room. This was followed by pop. Data presented as frequency.*

### 3.3.5 Correlations between the physiological and self-report measures

Correlations were performed on the full data set to explore the extent to which the VAS data were related to the measures of cardiac autonomic activity. It was anticipated that the self-report measures would be consistent with the physiological measures.

#### 3.3.5.1 Experienced arousal

Table 3.13 summarises the statistically significant correlations that emerged for experienced stimulation for each condition. It was expected that positive correlations between measures of sympathetic predominance (e.g. LF power, SD2, SD2/SD1) and experienced stimulation would emerge. For instance, if LF/HF increased it was anticipated that so would experienced stimulation. In contrast, negative correlations between measures of parasympathetic predominance (e.g. HF power, SD1, SD1/SD2) and experienced stimulation were expected.

Contrary to expectations, the significant correlations between the measures of cardiac autonomic activity and experienced stimulation did not emerge in the direction that was anticipated. This was the case for all five conditions. For example, in the baseline and stable tempo conditions, experienced stimulation increased with decreasing nuLF power. Similarly, experienced stimulation appeared to increase with a shift towards parasympathetic predominance, as indexed by the negative correlations for HF%, nuHF, LF/HF and SD2/SD1.

However, this was not the case for experienced relaxation. Indeed, inspection of Table 3.14 demonstrates two points:

1. Experienced relaxation correlated with no measures of cardiovascular autonomic function in baseline and only one variable (QRS interval) during the stable tempo stimulus.
2. The direction of the correlations for the remaining conditions were consistent with expectations. Indeed, experienced relaxation increased with longer RR intervals and increases in SD1/SD2.

The experienced stress correlation results (see Table 3.15) demonstrate that the correlations emerged in a direction contrary to expectations. Like the experienced stimulation results, stress was related to decreases in sympathetic predominance and increases in parasympathetic predominance. This was the case for all conditions, except stable tempo for which no correlations reached statistical significance ( $p > 0.05$ ).

Interestingly, no significant correlations emerged for experienced calm for the baseline and stable tempo conditions ( $p > 0.05$ ). Nevertheless, as shown in Table 3.16, the correlations that were statistically significant for the remaining conditions were in the direction that was anticipated. For instance, experienced calm increased with decreasing LF/HF and SD2/SD1. Whereas, positive correlations between experienced calm and HF%, SD1/SD2 and the RR interval measures transpired.

Table 3.13: Summary of correlations between the measures of autonomic function and experienced stimulation.

	Baseline			Stable tempo			Stepped increase in tempo			Stepped decrease in tempo			Recovery		
	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n
VLF%							0.293	0.035	52						
LF%	-0.304	0.028	52				-0.346	0.012	52						
HF%	0.283	0.042	52												
nuLF	-0.393	0.004	52	-0.391	0.005	52									
nuHF	3.940	0.004	52	0.377	0.006	52									
LF/HF	-0.396	0.004	52	-0.338	0.014	52									
CVRR										-0.353	0.010	52			
HR				-0.388	0.005	52									
SD HR				-0.325	0.019	52				-0.342	0.013	52			
SD2n										-0.374	0.006	52			
SD1/SD2				0.327	0.018	52									
SD2/SD1				-0.327	0.018	52									
Mode RR				0.390	0.004	52									
Min RR				0.343	0.013	52									
Q3 RR				0.366	0.008	52									
Max-Q3 RR										-0.295	0.034	52			
Q amplitude													-0.344	0.013	52
S amplitude							0.360	0.009	52						
SBP	-0.423	0.002	51							-0.377	0.006	51			
DBP	-0.285	0.043	52							-0.359	0.010	51			
MAP	-0.305	0.028	52							-0.433	0.002	51			
UP BRS				0.273	0.050	52									
DOWN BRS	0.445	0.001	51												
MEAN BRS	0.335	0.016	51	0.282	0.430	52									
Respiration rate										0.332	0.016	52			

Table 3.14: Summary of correlations between the measures of autonomic function and experienced relaxation.

	Baseline			Stable tempo			Stepped increase in tempo			Stepped decrease in tempo			Recovery		
	r or p	p-value	n	r or p	p-value	n	r or p	p-value	n	r or p	p-value	n	r or p	p-value	n
VLF%										-0.288	0.039	52			
HR										-0.341	0.013	52	-0.387	0.005	52
SD1/SD2							0.400	0.003	52	0.279	0.045	52			
SD2/SD1							-0.400	0.003	52	-0.279	0.045	52			
Mean RR										0.348	0.016	52	0.385	0.005	52
Median RR										0.331	0.016	52	0.394	0.004	52
Mode RR										0.354	0.010	52	0.374	0.006	52
Min RR													0.370	0.007	52
Max RR										0.332	0.016	52	0.322	0.020	52
Δ RR										0.284	0.042	52			
Q1 RR										0.342	0.013	52	0.406	0.003	52
Q3 RR										0.353	0.010	52	0.383	0.005	52
Q1-min RR										0.325	0.019	52			
Max-Q3 RR							-0.288	0.038	52						
QRS interval				0.283	0.042	52				0.364	0.008	52			
QT interval							0.280	0.045	52				0.304	0.029	52
Q amplitude							0.495	< 0.001	52				0.285	0.050	52
R amplitude							-0.474	< 0.001	52						
T amplitude							-0.316	0.022	52						
ST height							0.457	0.001	52						
Respiration rate							0.289	0.038	52						



Table 3.16: Summary of correlations between the measures of autonomic function and experienced calm.

	Baseline			Stable tempo			Stepped increase in tempo			Stepped decrease in tempo			Recovery		
	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n	r or $\rho$	p-value	n
VLF%							-0.336	0.015	52						
HF%							0.355	0.010	52						
LF/HF							-0.292	0.036	52						
HR													-0.287	0.039	52
SD1/SD2							0.447	0.001	52						
SD2/SD1							-0.447	0.001	52						
Mean RR													0.284	0.041	52
Median RR													0.288	0.038	52
Mode RR													0.278	0.460	52
Min RR													0.280	0.045	52
Q1 RR													0.307	0.027	52
Q3 RR													0.277	0.047	52
Q1-min RR										0.294	0.034	52			
QRS interval										0.309	0.026	52			
Q amplitude							-0.372	0.007	52				0.298	0.032	52
R amplitude							-0.372	0.007	52				-0.287	0.039	52
ST height							0.341	0.013	52						
UP BRS							0.285	0.040	52						
MEAN BRS							0.283	0.042	52						







### 3.3.6 Predicting physiological response to the three stimuli

The pilot study showed that it was possible to predict the direction and magnitude of an individual's change in LF% to the auditory stimuli based on baseline readings. As the protocol and stimuli were similar in the current study, the same analyses were performed to see whether the results could be replicated. Therefore, it was anticipated that baseline LF% would significantly predict percentage change in LF% between baseline and the three auditory stimuli.

Table 3.19 summarises all regressions performed on the full sample and their result.

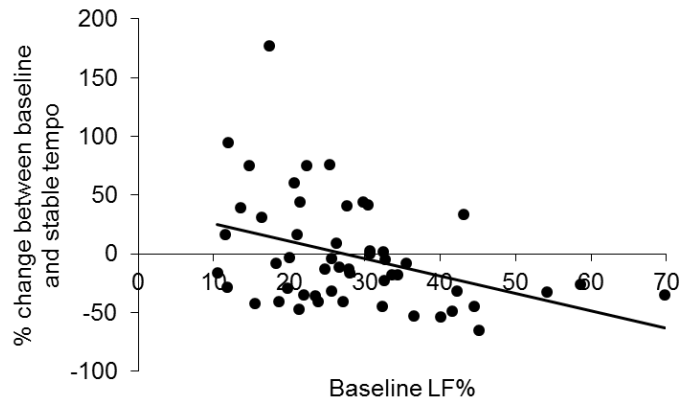
*Table 3.19: Summary of statistically significant linear regressions for the full sample.*

	Baseline → stable tempo		Baseline → tempo increase		Baseline → tempo decrease		Tempo increase → tempo decrease	
	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value
LF power	0.256	< 0.001	0.080	0.042	0.191	0.001		
HF power								
LF%	0.150	0.005	0.149	0.005	0.169	0.002		
HF%			0.085	0.036			0.085	0.036
nuLF	0.132	0.008	0.090	0.031				
nuHF	0.105	0.019	0.141	0.006			0.111	0.016
LF/HF								
SDRR	0.224	< 0.001	0.075	0.050	0.128	0.009		
SDSD								
RMSSD								
SD1								
nSD1								
SD2	0.296	< 0.001			0.111	0.016		
nSD2	0.292	< 0.001	0.080	0.042	0.148	0.005		
SD1/SD2			0.081	0.040				
SD2/SD1			0.096	0.026				
Mean RR	0.089	0.031	0.084	0.037				
Median RR	0.106	0.019	0.104	0.019				
Δ RR	0.252	< 0.001	0.141	0.006	0.106	0.018		
IQR RR	0.190	0.001	0.104	0.020	0.160	0.003		

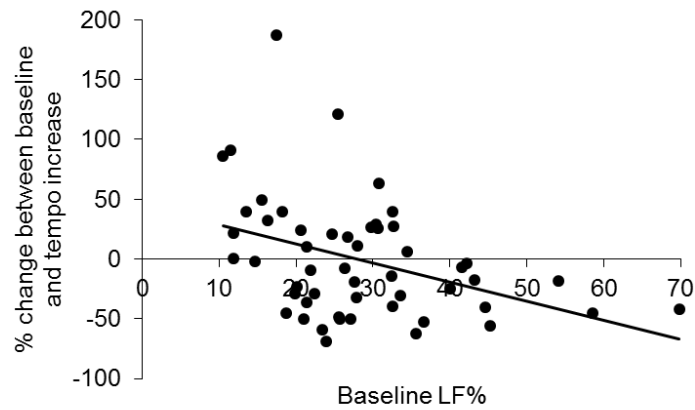
In support of this expectation baseline LF% significantly predicted percentage change between: baseline and stable tempo ( $R^2 = 0.150$ ,  $p = 0.005$ ); baseline and stepped increase in tempo ( $R^2 = 0.149$ ,  $p = 0.005$ ); and baseline and stepped decrease in tempo ( $R^2 = 0.169$ ,  $p = 0.002$ ). However,

the regression for percentage change in LF% between the stepped increase and decrease in tempo stimuli failed to reach significance ( $p > 0.05$ ). Indeed, Figure 3.75 shows that higher baseline LF% was associated with greater decreases in LF% for all three stimuli.

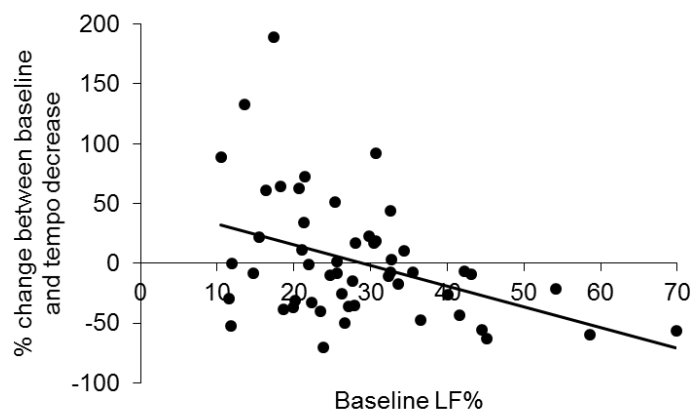
a.



b.



c.



*Figure 3.75: Baseline LF% significantly predicted change in LF% between baseline and the stimuli in the full sample. Reductions in LF% between baseline and: stable tempo (a), stepped increase in tempo (b) and stepped decrease in tempo (c) increased with higher baseline LF%.*

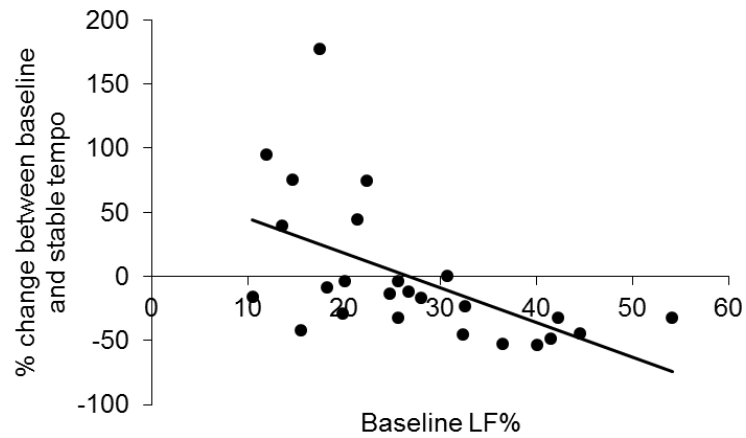
Although group did not significantly interact with physiological responses to the three stimuli, the same linear regression was performed on the control and experimental groups separately. The outcomes are summarised in Table 3.20 for the control group and Table 3.21 for the experimental group.

Baseline LF% was a significant predictor of percentage change between baseline and the three stimuli for the control group. Indeed, similar to when the two groups were combined, Figure 3.76 shows that the higher the baseline LF%, the greater the decrease in LF% for all three stimuli.

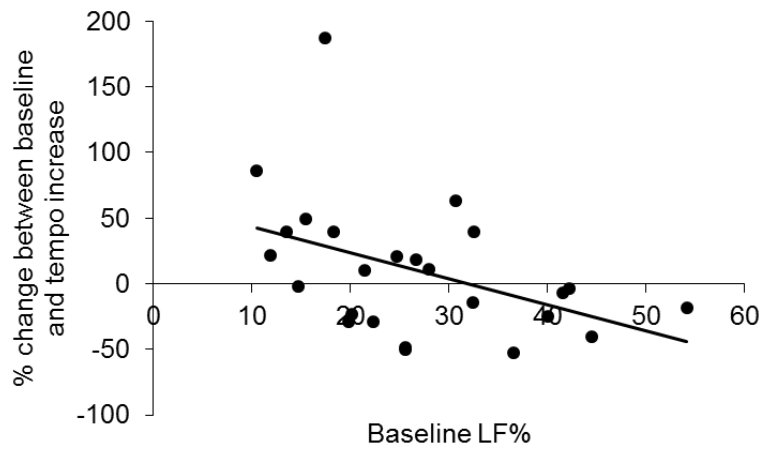
*Table 3.20: Summary of statistically significant linear regressions for the control group.*

	Baseline → stable tempo		Baseline → tempo increase		Baseline → tempo decrease		Tempo increase → tempo decrease	
	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value
LF power	0.250	0.011			0.172	0.039		
HF power								
LF%	0.310	0.004	0.187	0.031	0.222	0.017		
HF%								
nuLF	0.236	0.014						
nuHF								
LF/HF								
SDRR	0.186	0.032			0.215	0.019		
SDSD								
RMSSD								
SD1								
nSD1								
SD2	0.270	0.008			0.270	0.008		
nSD2	0.296	0.005	0.200	0.025	0.392	0.001		
SD1/SD2			0.279	0.007	0.163	0.046		
SD2/SD1			0.288	0.006				
Mean RR								
Median RR								
Δ RR	0.365	0.001	0.253	0.010				
IQR RR					0.232	0.015		

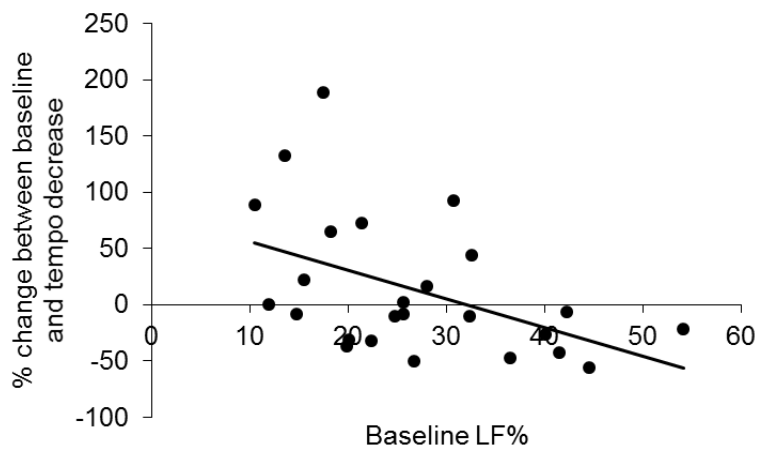
a.



b.



c.



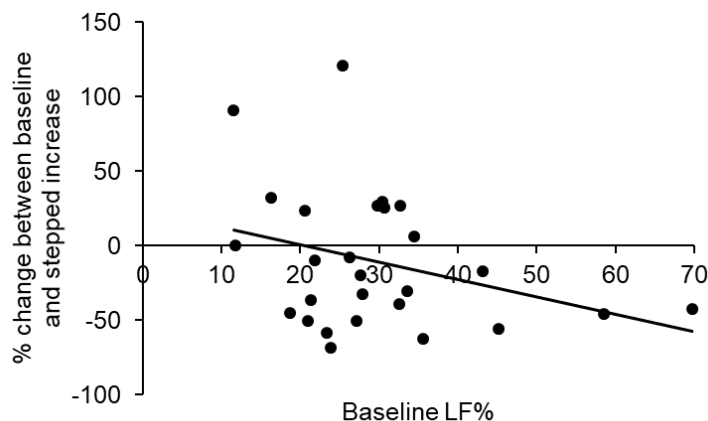
*Figure 3.76: Baseline LF% significantly predicted change in LF% between baseline and the stimuli in the control group. Reductions in LF% between baseline and the: stable tempo (a), stepped increase in tempo (b) and stepped decrease in tempo (c) increased with higher baseline LF%.*

Fewer significant predictions emerged in the experimental group. For instance, Table 3.21 shows that baseline LF% only significantly predicted change between baseline and the stepped increase in tempo stimulus ( $R^2 = 0.237$ ,  $p = 0.010$ ). Nonetheless, as shown in Figure 3.77, a similar pattern transpired: the higher the baseline LF%, the greater the decrease in LF% in the stepped increase in tempo stimulus.

*Table 3.21: Summary of statistically significant linear regressions for the experimental group.*

	Baseline → stable tempo		Baseline → tempo increase		Baseline → tempo decrease		Tempo increase → tempo decrease	
	$R^2$	p-value	$R^2$	p-value	$R^2$	p-value	$R^2$	p-value
LF power	0.317	0.002			0.206	0.017		
HF power								
LF%			0.237	0.010				
HF%							0.171	0.032
nuLF								
nuHF	0.171	0.032	0.312	0.002	0.201	0.019	0.190	0.023
LF/HF								
SDRR	0.260	0.007						
SDSD								
RMSSD								
SD1								
nSD1								
SD2	0.324	0.002						
nSD2	0.286	0.004						
SD1/SD2								
SD2/SD1								
Mean RR								
Median RR			0.155	0.042				
$\Delta$ RR								
IQR RR	0.262	0.006						





*Figure 3.77: Baseline LF% significantly predicted change in LF% between baseline and the stepped increase in tempo stimulus in the experimental group. Reductions in LF% between baseline and the stepped increase in tempo stimulus increased with higher baseline LF%.*

### 3.3.6.1 Defining responder types

Due to these significant relationships, participants were subsequently categorised based on percentage change in LF% between baseline and the three stimuli (this permitted changes in responder definition between the stimuli). Like the pilot study, two approaches were tested:

1. Two-level response definition:
  - a. Non-responder: < 20% change in LF%
  - b. Responder:  $\geq$  20% change in LF%
2. Three-level response definition:
  - a. Non-responder: < 20% change in LF%
  - b. Type 1 responder:  $\geq$  20% decrease in LF%
  - c. Type 2 responder:  $\geq$  20% increase in LF%

This involved examining the extent to which baseline LF% for each visit differed between the responder types.

The first approach revealed no significant differences in baseline LF% between non-responders and responders for any stimulus ( $p > 0.05$ ). In contrast, significant differences in baseline LF% emerged for the three-level definition: baseline to stable tempo ( $p = 0.021$ ), baseline to stepped increase in tempo ( $p = 0.032$ ) and baseline to stepped decrease in tempo ( $p = 0.026$ ).

Exploration of these effects revealed that for all three stimuli, type 1 responders (stable tempo:  $n = 21$ ; stepped increase in tempo:  $n = 21$ ; stepped decrease in tempo:  $n = 20$ ) had significantly higher baseline LF% than type 2 responders (stable tempo:  $n = 13$ ; stepped increase in tempo:  $n = 17$ ; stepped decrease in tempo:  $n = 13$ ) ( $p = 0.027$ ,  $p = 0.012$  and  $p = 0.022$  respectively). Although the pattern between the three responder types was similar for all three stimuli (see Figure 3.78a), no other significant differences emerged ( $p > 0.05$ ).

The same analyses were performed on stimulation LF%. For all stimuli, significant differences between the three responder types were identified (stable tempo:  $p = 0.001$ ; stepped increase in tempo:  $p < 0.001$ ; stepped decrease in tempo:  $p < 0.001$ ). As shown in Figure 3.78b, LF% during the stable tempo stimulus was significantly higher in type 2 responders than in non-responders ( $p = 0.013$ ) and type 1 responders ( $p < 0.001$ ). In addition, LF% was significantly higher for non-responders than type 1 responders ( $p = 0.043$ ). Similarly, type 1 responders had significantly lower LF% during the stepped increase and decrease in tempo stimuli compared to non-responders ( $p = 0.002$  and  $p = 0.003$ ) and type 2 responders (both  $p < 0.001$ ). Although the differences between non-responders and type 2 responder for the stepped increase and decrease in tempo stimuli were not statistically significant, Figure 3.78b illustrates that similar patterns emerged ( $p > 0.05$ ).

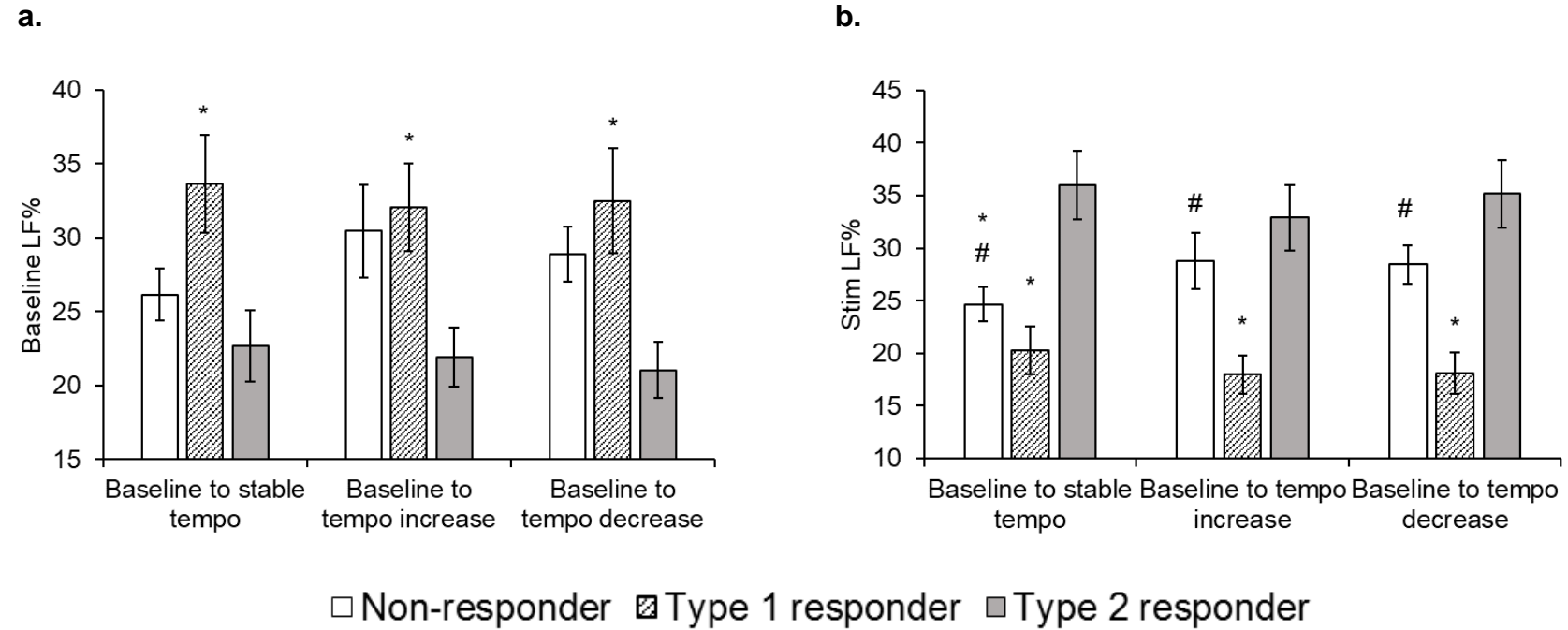


Figure 3.78: At baseline (a), type 1 responders had significantly higher LF% compared to type 2 responders. For all three stimuli (b), differences between the three responder types emerged. Data presented as mean  $\pm$  1 SEM. \* = significantly different to type 2 responder; # = significantly different to type 1 responder.

### **3.3.6.2 Differences in demographic characteristics and baseline measures between the three responder types**

As three auditory stimuli were employed (stable tempo, stepped increase in tempo and stepped decrease in tempo) differences in demographic characteristics and baseline measures between the three responder groups were explored for each stimulus. This involved performing: chi-square tests of independence, to examine whether responder type was associated with demographic group (e.g. gender, age, formal music training); and one-way ANOVAs (or Kruskal-Wallis tests for non-normally distributed data), to assess whether differences in demographic characteristics (e.g. age, exercise, hours spent undertaking music-related activities) and/or baseline measures differed between the three responder types (non-responders, type 1 responders, type 2 responders). Independent sample t-tests (or Mann-Whitney U tests for non-normally distributed data) were performed on the percentage change in LF% values to explore whether any differences emerged between different demographic groups. Finally, linear regressions were performed to examine whether percentage change values could be predicted by demographic variables (e.g. age, exercise and hours spent undertaking music-related activities). Statistics were performed on the full sample.

#### **3.3.6.2.1 Differences between the three responder types for the stable tempo stimulus**

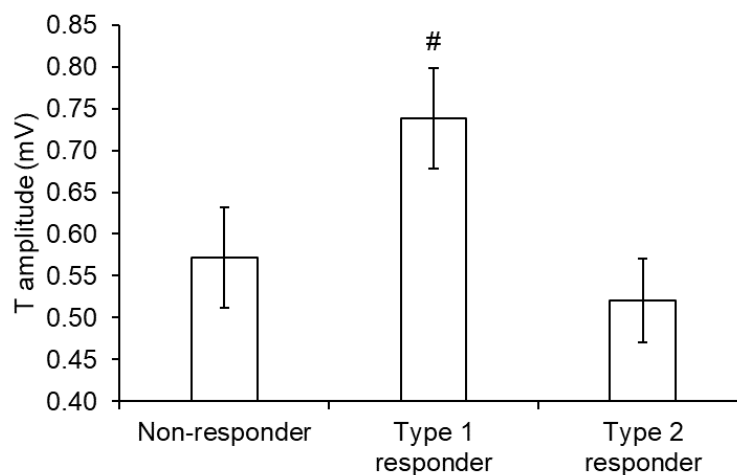
No statistically significant associations between responder type and demographic group emerged ( $p > 0.05$ ). In addition, none of the differences in baseline measures or percentage change in LF% values (between baseline and the stable tempo stimulus) reached statistical significance ( $p > 0.05$ ). No linear regressions emerged as statistically significant ( $p > 0.05$ ).

### **3.3.6.2.2 Differences between the three responder types for the stepped increase in tempo stimulus**

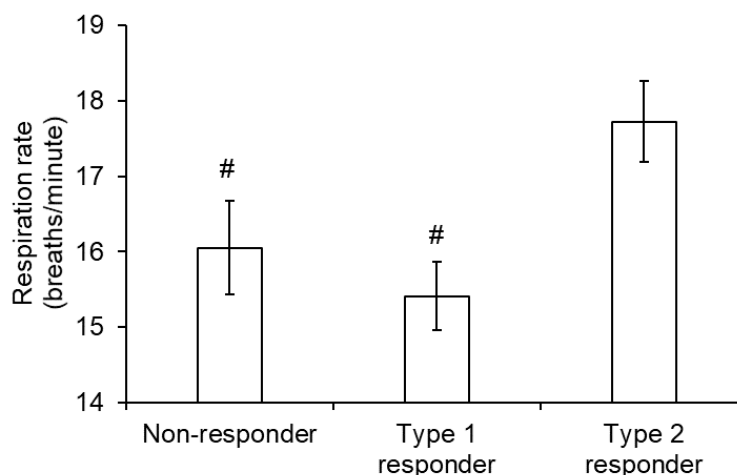
The three responder types were not significantly associated with any of the demographic groups ( $p > 0.05$ ).

However, baseline T amplitude and pre-baseline respiration rate significantly differed between the three responder types ( $p = 0.015$  and  $p = 0.006$  respectively). Inspection of Figure 3.79a demonstrates that type 1 responders ( $n = 21$ ) had significantly higher T amplitude at baseline compared to type 2 responders ( $n = 17$ ,  $p = 0.007$ ). Figure 3.79b also shows that type 2 responders had a significantly higher respiration rate at pre-baseline compared to non-responders ( $p = 0.014$ ) and type 1 responders ( $p = 0.003$ ).

a.

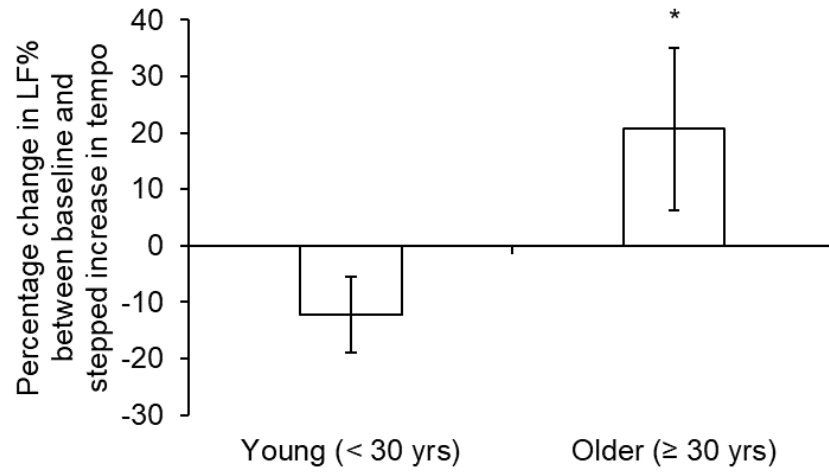


b.



*Figure 3.79: Differences in baseline T amplitude (a) and pre-baseline respiration rate (b) existed between the responder types for the stepped increase in tempo stimulus. Data presented as mean  $\pm$  1 SEM. # = significantly different to type 2 responders.*

A Mann-Whitney U test revealed that percentage change in LF% (between baseline and the stepped increase in tempo stimulus) significantly differed between young (aged < 30 years,  $n = 34$ ) and older participants (aged  $\geq 30$  years,  $n = 18$ ) ( $p = 0.028$ ). As depicted in Figure 3.80, older participants experienced increases in LF% (between baseline and the stepped increase in tempo stimulus), whilst young participants showed decreases.



*Figure 3.80: Young participants (< 30 yrs) showed decreases in LF% between baseline and the stimulus whereas older participants (≥ 30 yrs) showed increases in LF%. Data presented as mean  $\pm$  1 SEM. \* = significantly different to young participants.*

No linear regressions between percentage change in LF% and demographic variables reached statistical significance ( $p > 0.05$ ).

### 3.3.6.2.3 Differences between the three responder types for the stepped decrease in tempo stimulus

A chi-square test of independence revealed that responder type was significantly associated with age group ( $p = 0.047$ ). As illustrated in Figure 3.81, significantly more young participants (aged < 30 years) were either non-responders (< 20% increase or decrease in LF% between baseline and the stable tempo stimulus) or type 1 responders ( $\geq 20\%$  decrease in LF% between baseline and the stable tempo stimulus) compared to older participants (aged  $\geq 30$  years). Indeed, older participants tended to show responses that were characteristic of type 2 responders ( $\geq 20\%$  increase in LF% between baseline and the stable tempo stimulus).



*Figure 3.81: Non-responders and type 1 responders were associated with young participants (< 30 yrs) whilst type 2 responders were associated with older participants ( $\geq 30$  yrs). Data presented as frequency.*

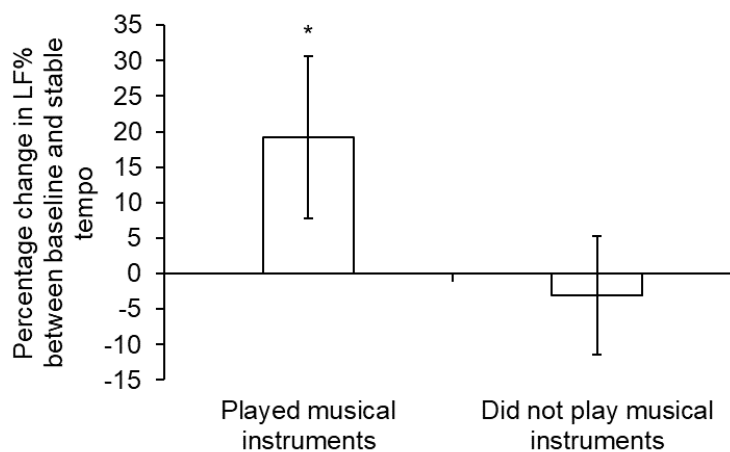
No significant differences in any baseline measures of cardiovascular autonomic function emerged between the three responder types ( $p > 0.05$ ). However, percentage change in LF% (between baseline and the stepped decrease in tempo stimulus) significantly differed between those who did and did not play instruments ( $p = 0.046$ ). As shown in Figure 3.82a, those who played musical instruments showed increases in LF% between baseline and the stable tempo stimulus. In contrast, participants who did not play



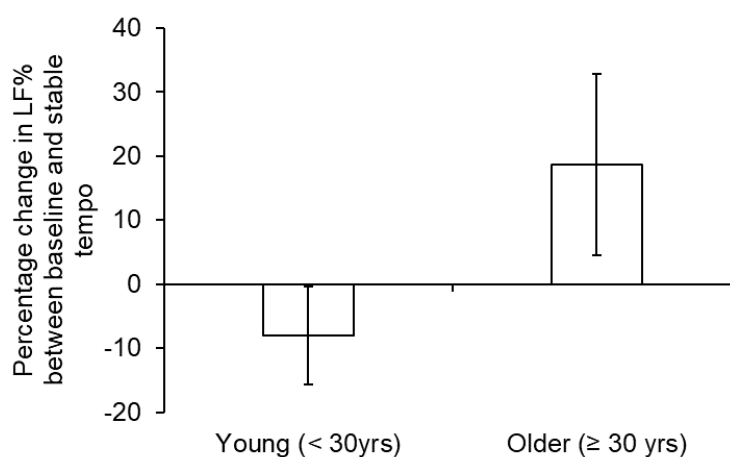
musical instruments showed a small decrease in LF% between the two conditions.

A difference in percentage change in LF% emerged between young and older participants, however it just failed to reach statistical significance ( $p = 0.054$ ). Nevertheless, inspection of Figure 3.82b demonstrates that older participants encountered increases in LF% whereas young participants showed decreases in LF% between the two conditions. This finding is consistent with that obtained for the stepped increase in tempo stimulus, as well as from the chi-square test of independence.

**a.**



**b.**

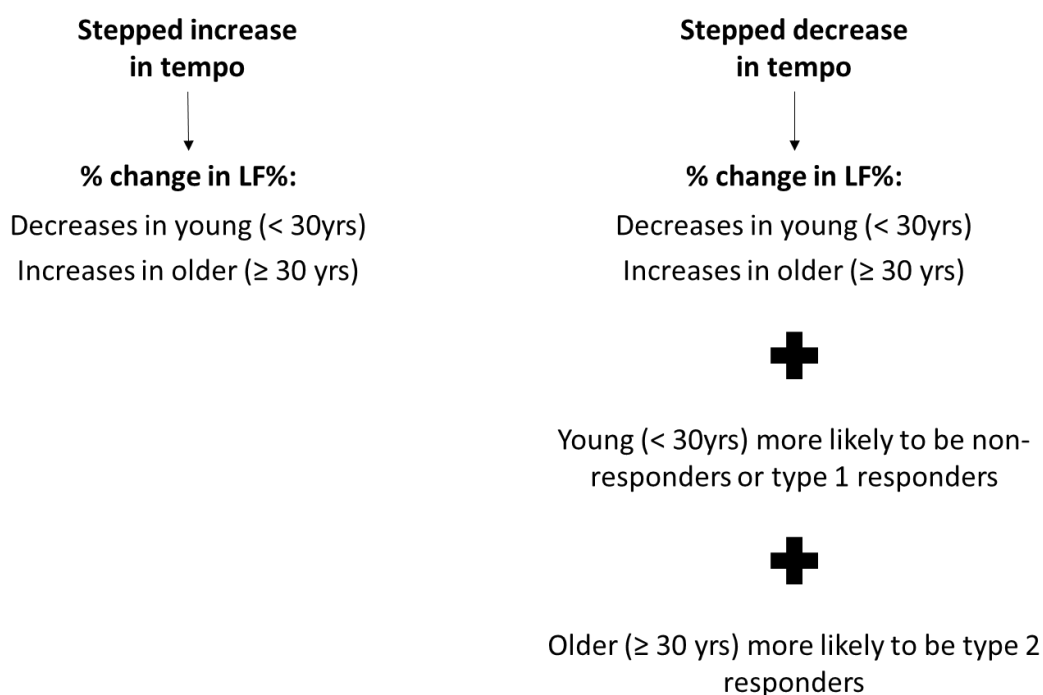


*Figure 3.82: Those who played musical instruments (a) and older participants ( $\geq 30$  yrs, b) showed increases in LF% between baseline and the stimulus. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between the two groups.*

No linear regressions between percentage change in LF% and demographic variables reached statistical significance ( $p > 0.05$ ).

### 3.3.6.2.4 Take-home message

Age was the common denominator when exploring differences in demographic information between the three responder types for the stepped increase and decrease in tempo stimuli (see Figure 3.83). Young participants ( $< 30$  yrs) showed decreases in percentage change in LF%, predisposing them to be type 1 responders or non-responders. In contrast, older participants ( $\geq 30$  yrs) showed increases in percentage change in LF%, leading them to be type 2 responders.



*Figure 3.83: Take-home message concerning responder types. Response type is associated with age whereby young participants (< 30yrs) respond in a way that is consistent with non-responders and/or type 1 responders. In contrast, older participants ( $\geq 30$ yrs) respond in a way that is characteristic of type 2 responders.*

### 3.3.6.3 Response consistency between the three auditory stimuli

Similar to the pilot, consistency in response type between the stimuli was investigated. Two definitions of response consistency were tested.

### 3.3.6.3.1 Initial response consistency definition

The first definition classified consistent responders as those who showed the same responses (non-responder, type 1 responder or type 2 responder) in all three stimuli. Inconsistent responders were those who showed different responses between the stimuli (e.g. a non-responder for the stable tempo and stepped increase in tempo stimuli but a type 1 responder for the stepped decrease in tempo stimulus).

Twenty participants (38.46%) were consistent responders and the remaining were inconsistent responders. Appendix B.11. summarises the characteristics of inconsistent and consistent responders. Analysis of these characteristics revealed no statistically significant differences between the two groups ( $p > 0.05$ ).

Nevertheless, mixed mode ANOVAs were performed to examine the extent to which response consistency (inconsistent, consistent: between-participants variable) interacted with:

- a) The five conditions: baseline (5-10 min period), stable tempo (full duration), stepped increase in tempo (full duration), stepped decrease in tempo (full duration) and recovery (5-10 min period). Within-participants variable.
- b) The five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm). Within-participants variable.
- c) The five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm). Within-participants variable.

As autonomic responses did not differ between the control and experimental groups, group was not added into the ANOVA. The results are as follows.

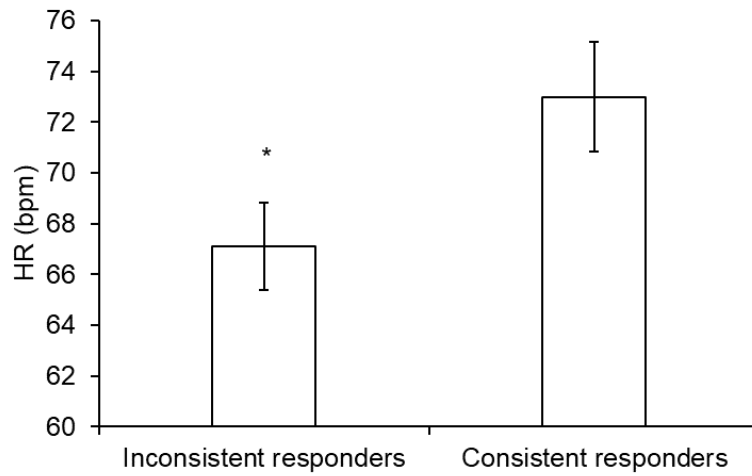
### **3.3.6.3.1.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)**

#### **3.3.6.3.1.1.1 *Time-domain HRV***

A statistically significant main effect of response consistency on HR emerged ( $p = 0.039$ ). As depicted in Figure 3.84a, consistent responders had significantly higher HR compared to inconsistent responders.

The interaction between condition and response consistency on SD HR was also statistically significant ( $p = 0.019$ ). However, no differences between consistent and inconsistent responders emerged when analysed at the level of the condition ( $p > 0.05$ ). Nonetheless, analysis at the level of response consistency revealed that SD HR was significantly lower during the stable tempo stimulus compared to recovery for consistent responders ( $p = 0.042$ , see Figure 3.84b). SD HR did not significantly differ between any of the conditions for inconsistent responders ( $p > 0.05$ ).

a.



b.

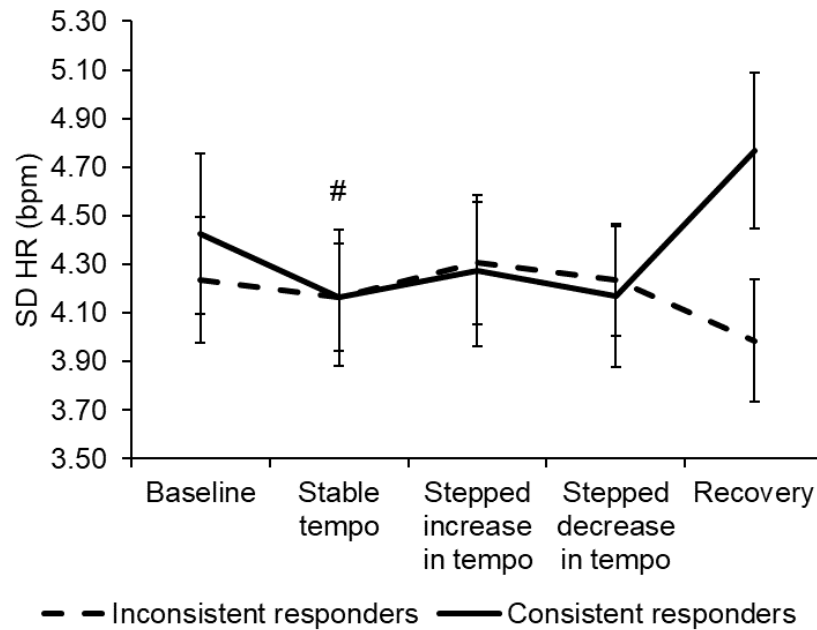


Figure 3.84: HR (a) was significantly lower in inconsistent responders and SD HR (b) significantly interacted with condition and response consistency. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; # = significantly different to recovery.

### 3.3.6.3.1.1.2 Frequency-domain HRV

A mixed mode ANOVA revealed a statistically significant interaction between condition and response consistency ( $p = 0.008$ ). Analysis at the level of the condition showed that inconsistent responders had significantly higher VLF% during baseline compared to consistent responders ( $p = 0.025$ ). In addition,

as shown in Figure 3.85, analysis at the level of response consistency revealed that VLF% was significantly higher during baseline than during the stable tempo stimulus ( $p = 0.044$ ) and recovery ( $p = 0.017$ ).

No other significant differences emerged ( $p > 0.05$ ).

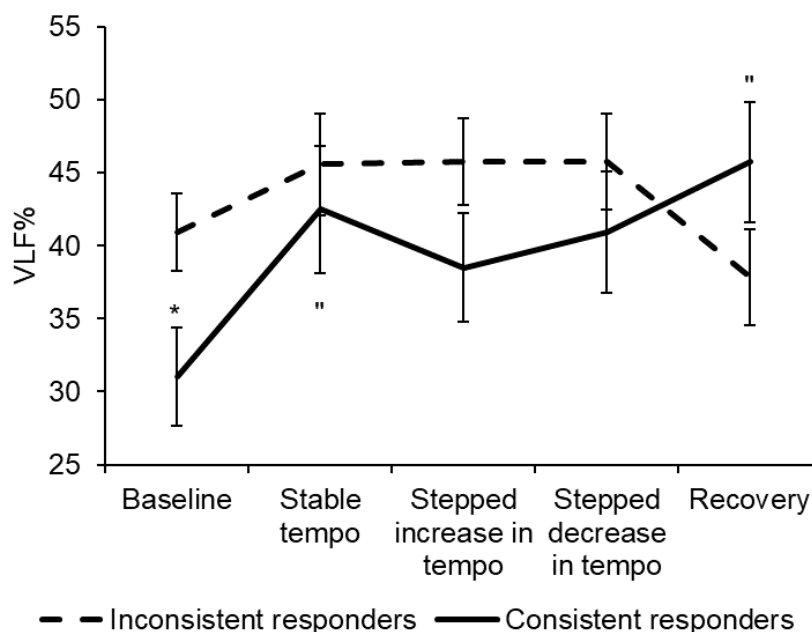


Figure 3.85: VLF% significantly interacted with condition and response consistency. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; " = significantly different to baseline.

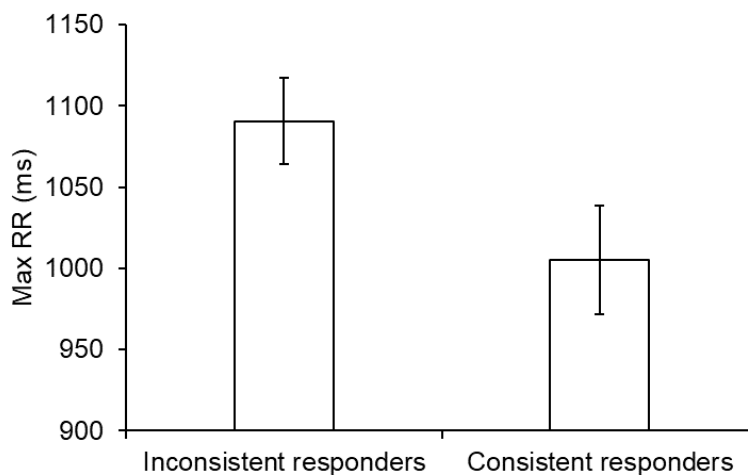
### 3.3.6.3.1.1.3 RR analysis

A main effect of response consistency on max RR was detected, however, this just failed to reach significance ( $p = 0.051$ ). Nevertheless, as shown in Figure 3.86a, max RR tended to be greater in inconsistent responders compared to consistent responders.

A statistically significant interaction between condition and response consistency on  $\Delta$  RR transpired ( $p = 0.026$ , see Figure 3.86b). Further analysis at the level of the conditions revealed that inconsistent responders had significantly greater  $\Delta$  RR than consistent responders during the stable tempo ( $p = 0.049$ ) and stepped increase in tempo ( $p = 0.020$ ) stimuli. Analysis at the level of response consistency demonstrated that for inconsistent responders only baseline  $\Delta$  RR was significantly lower compared to that during the stepped increase in tempo ( $p = 0.002$ ) and

stepped decrease in tempo ( $p = 0.036$ ) stimuli. In addition,  $\Delta$  RR during the stepped increase in tempo stimulus was significantly greater compared to that during the stable tempo stimulus ( $p = 0.020$ ). Finally,  $\Delta$  RR during recovery was significantly greater compared to  $\Delta$  RR during the stepped increase in tempo ( $p < 0.001$ ) and stepped decrease in tempo ( $p = 0.006$ ) stimuli.  $\Delta$  RR for consistent responders did not significantly differ between any of the five conditions ( $p > 0.05$ ).

a.



b.

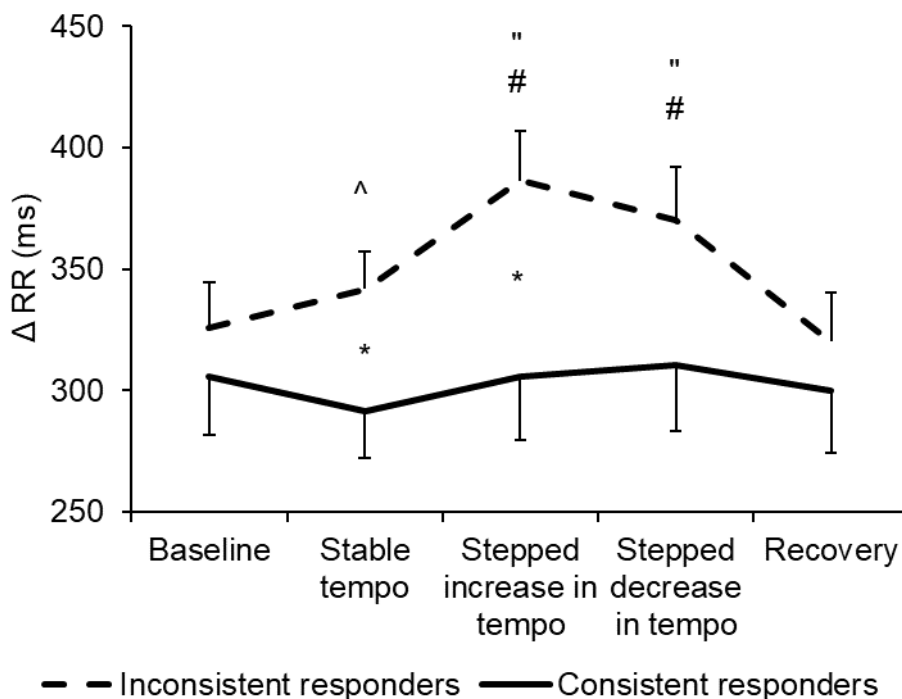


Figure 3.86: Max (a) and  $\Delta$  (b) RR intervals were impacted by response consistency. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; " = significantly different to baseline; ^ = significantly different to stepped increase in tempo; # = significantly different to recovery.

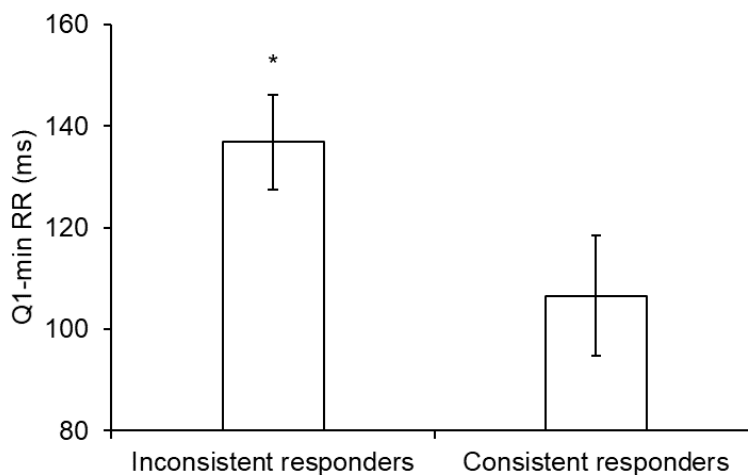
A main effect of response consistency on Q1-min RR also reached significance ( $p = 0.050$ ). As illustrated in Figure 3.87a, inconsistent



responders had significantly greater Q1-min RR compared to consistent responders.

Finally, a statistically significant interaction between condition and response consistency on max-Q3 RR emerged ( $p = 0.034$ , see Figure 3.87b). Analysis at the level of the condition failed to detect any significant differences between the two response types ( $p > 0.05$ ). However, analysis at the level of response consistency revealed that for inconsistent responders, max-Q3 RR during the stepped increase in tempo stimulus was significantly greater than during baseline ( $p = 0.001$ ), stable tempo ( $p = 0.014$ ) and recovery ( $p < 0.001$ ). Additionally, max-Q3 RR during the stepped decrease in tempo stimulus was significantly greater than that during recovery ( $p = 0.010$ ). Max-Q3 RR did not significantly differ between any of the conditions for consistent responders ( $p > 0.05$ ).

a.



b.

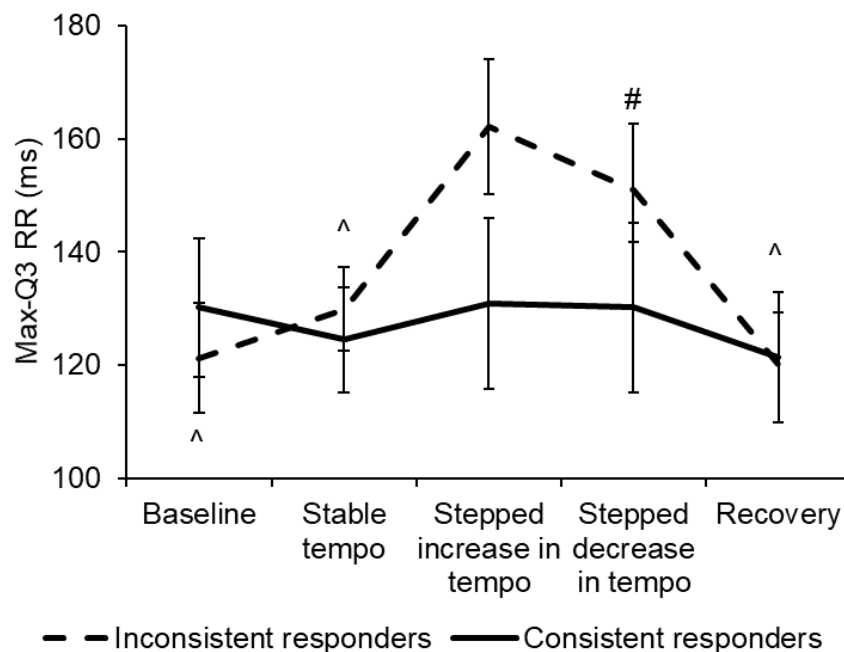
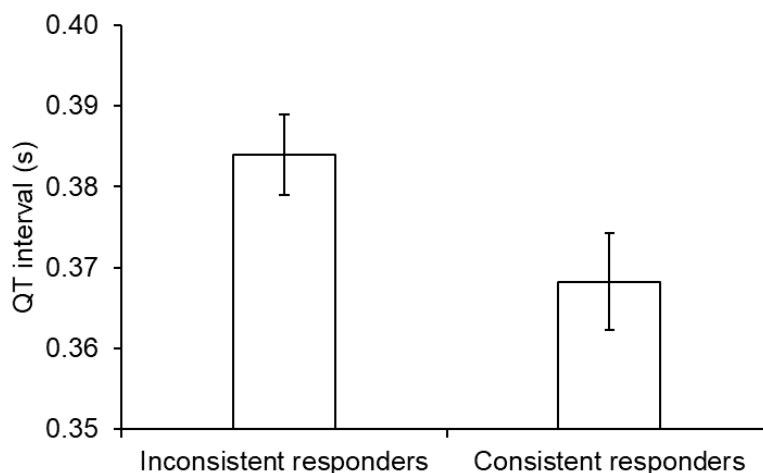


Figure 3.87: Q1-min (a) and max-Q3 (b) RR intervals were significantly impacted by response consistency. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; ^ = significantly different to stepped increase in tempo; # = significantly different to recovery.

#### 3.3.6.3.1.1.4 ECG analysis

A main effect of response consistency on QT interval was detected, however, this just failed to reach significance ( $p = 0.051$ ). Nonetheless, as

illustrated in Figure 3.88, QT interval tended to be longer in inconsistent responders compared to consistent responders.

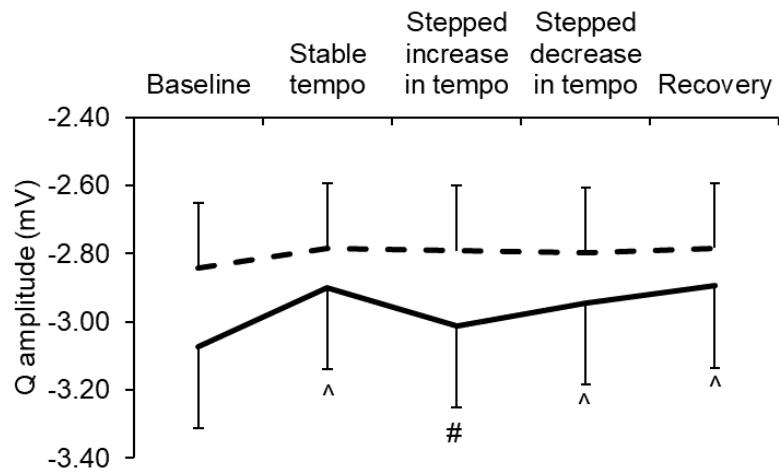


*Figure 3.88: QT interval tended to be higher in inconsistent responders than in consistent responders. Data presented as mean  $\pm$  1 SEM.*

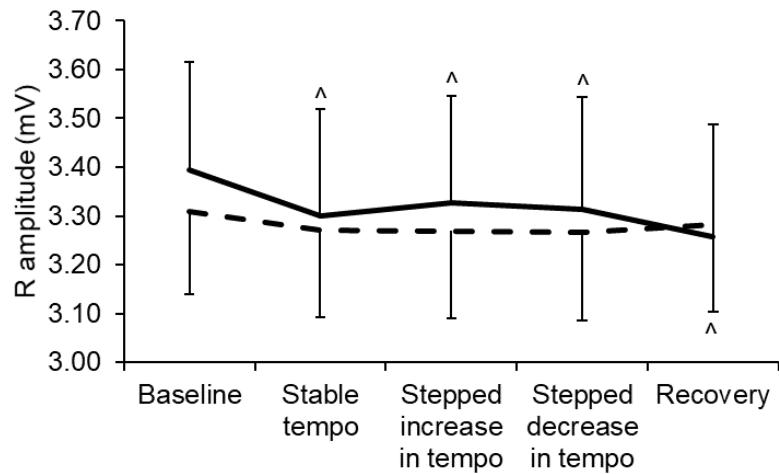
Interactions between condition and response consistency transpired for Q amplitude ( $p = 0.046$ ) and R amplitude ( $p = 0.045$ ). For Q amplitude, analysis at the level of the condition revealed no significant differences between consistent and inconsistent responders ( $p > 0.05$ ). However, analysis at the level of response consistency revealed that baseline Q amplitude was significantly lower compared to that during stable tempo ( $p = 0.011$ ), stepped decrease in tempo ( $p = 0.001$ ) and recovery ( $p < 0.001$ ) for consistent responders. Furthermore, Q amplitude during the stepped increase in tempo stimulus was significantly lower compared to that during recovery ( $p = 0.018$ , see Figure 3.89a).

For R amplitude (see Figure 3.89b), no significant differences between consistent and inconsistent responders emerged for any of the five conditions ( $p > 0.05$ ). However, for consistent responders, R amplitude was significantly greater during baseline compared to stable tempo ( $p = 0.020$ ), stepped increase in tempo ( $p = 0.008$ ), stepped decrease in tempo ( $p = 0.002$ ) and recovery ( $p = 0.005$ ). No significant differences between any of the conditions emerged for inconsistent responders ( $p > 0.05$ ).

a.



b.



— — Inconsistent responders — Consistent responders

Figure 3.89: There were interactions between condition and response consistency occurred for Q amplitude (a) and R amplitude (b). Data presented as mean  $\pm$  1 SEM. ^ = significantly different to baseline; # = significantly different to recovery.

### 3.3.6.3.1.2 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

#### 3.3.6.3.1.2.1 Time-domain HRV

A main effect of response consistency on HR was detected, however, this just failed to reach significance ( $p = 0.051$ ). Nonetheless, Figure 3.90 demonstrates that HR tended to be higher in consistent responders compared to those who showed inconsistent responses.

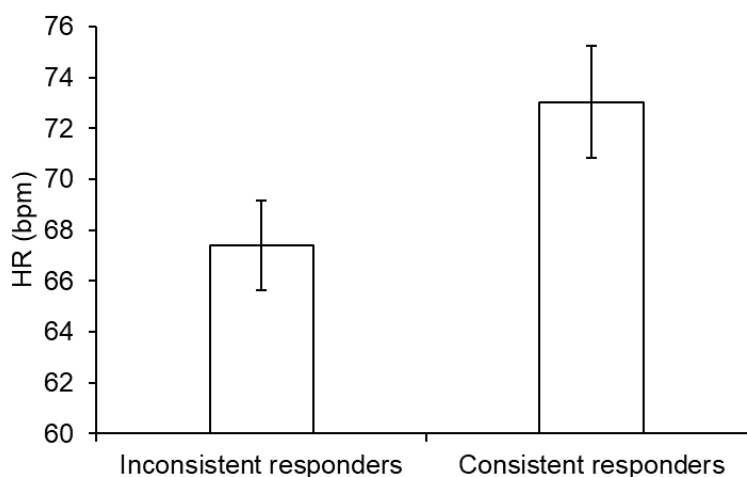


Figure 3.90: HR tended to be lower in inconsistent compared to consistent responders in the stepped increase in tempo stimulus. Data presented as mean  $\pm$  1 SEM.

#### 3.3.6.3.1.2.2 Frequency-domain HRV

The interaction between response consistency and tempi on VLF power reached statistical significance ( $p = 0.026$ ). Analysis at the level of the tempo revealed that inconsistent responders had significantly higher VLF power during 180bpm tempo compared to consistent responders ( $p = 0.017$ ). Analysis at the level of response consistency revealed that for inconsistent responders only, VLF power was significantly higher during 180bpm compared to during 90bpm ( $p = 0.033$ ), 120bpm ( $p = 0.004$ ) and 150bpm ( $p = 0.005$ , see Figure 3.91). No other significant differences emerged.

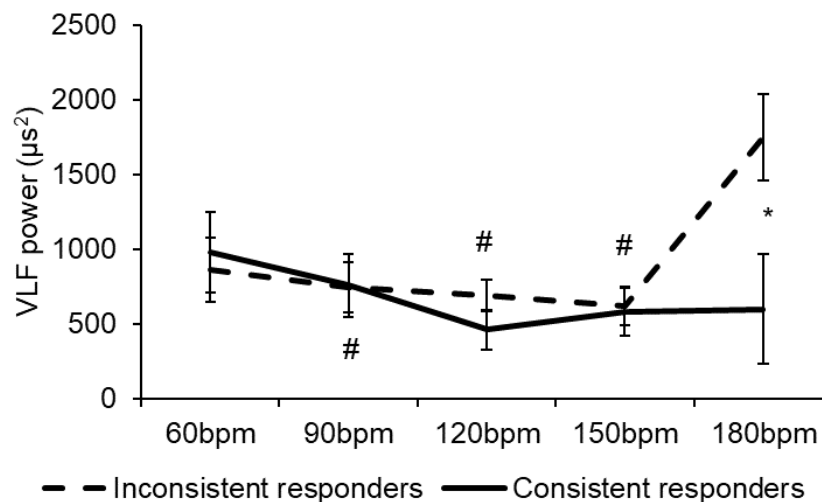


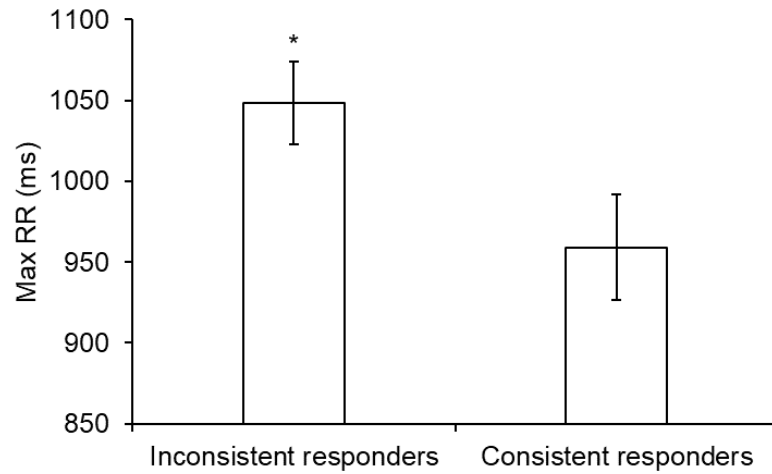
Figure 3.91: VLF power significantly interacted between tempo and response consistency for the stepped increase in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; # = significantly different to 180bpm.

### 3.3.6.3.1.2.3 RR analysis

A statistically significant main effect of response consistency transpired for max RR interval ( $p = 0.036$ ). As shown in Figure 3.92a, participants who showed inconsistent responses had significantly greater max RR intervals compared to those who responded in a consistent manner to all three stimuli.

A similar pattern emerged for Q1 RR interval (main effect of response consistency:  $p = 0.050$ ). Indeed, Figure 3.92b illustrates that inconsistent responders also had significantly greater Q1 RR intervals compared to consistent responders.

a.



b.

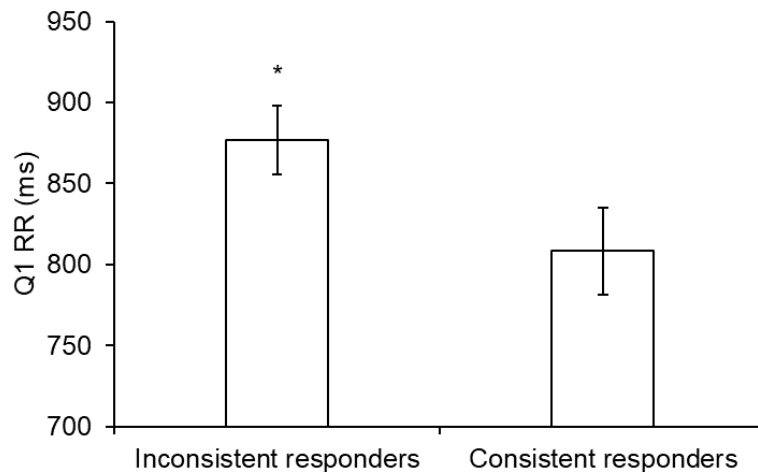
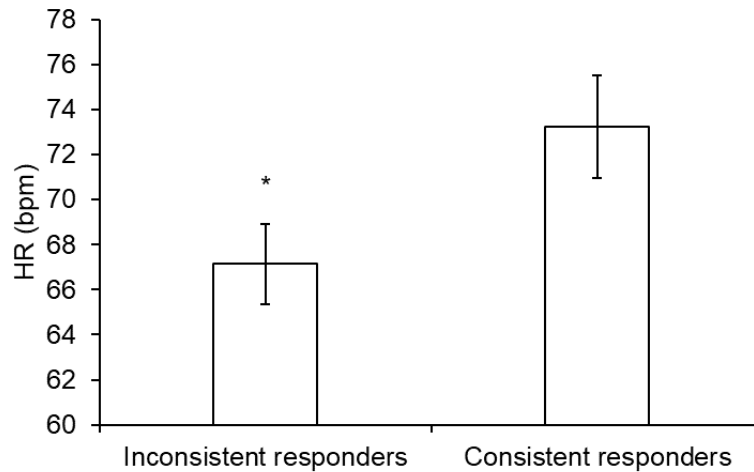


Figure 3.92: Max (a) and Q1 (b) RR intervals significantly differed between inconsistent and consistent responders in the stepped increase in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders.

### 3.3.6.3.2 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)

#### 3.3.6.3.2.1.1 Time-domain HRV

The main effect of response consistency on HR reached statistical significance ( $p = 0.040$ , see Figure 3.93). Bonferroni pairwise comparisons revealed that HR was significantly higher for consistent responders compared to inconsistent responders.



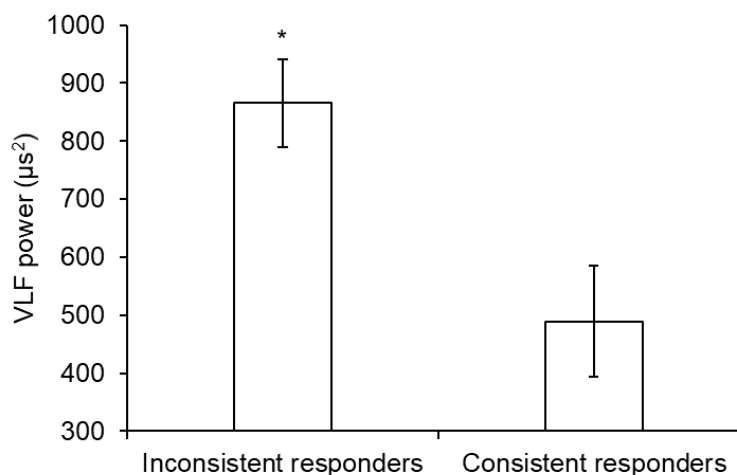
*Figure 3.93: HR significantly differed between inconsistent and consistent responders in the stepped decrease in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders.*

#### **3.3.6.3.2.1.2 Frequency-domain HRV**

A statistically significant main effect of response consistency on VLF power ( $p = 0.030$ , see Figure 3.94a) and LF power ( $p = 0.023$ , see Figure 3.94b) emerged. VLF and LF power were significantly higher in inconsistent responders compared to consistent responders. The interaction between tempi and response consistency failed to reach significance ( $p > 0.05$ ).



a.



b.

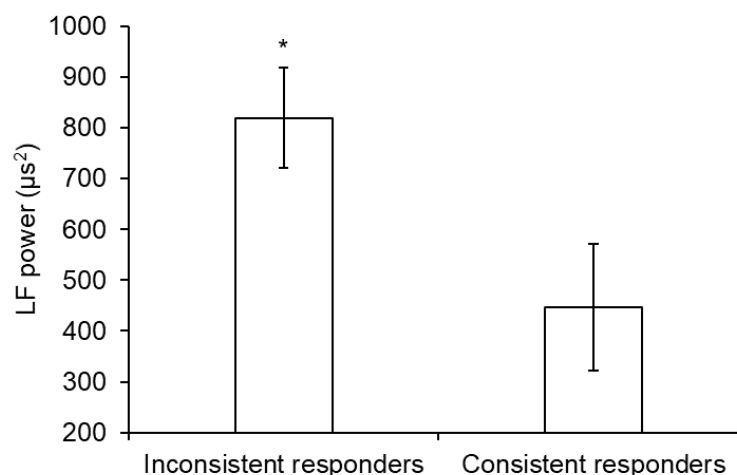


Figure 3.94: VLF power (a) and LF power (b) significantly differed between inconsistent and consistent responders in the stepped decrease in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders.

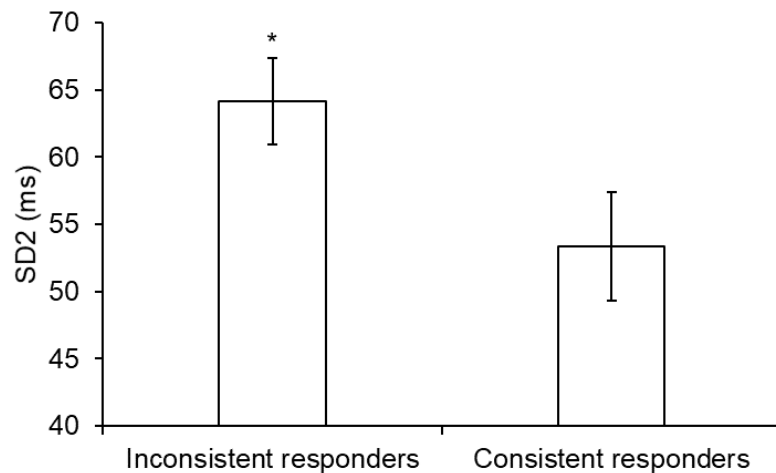
### 3.3.6.3.2.1.3 Non-linear HRV

A mixed mode ANOVA revealed a statistically significant main effect of response consistency on SD2 ( $p = 0.040$ ). Bonferroni pairwise comparisons demonstrated that SD2 was significantly higher in inconsistent responders compared to consistent responders (see Figure 3.95a).

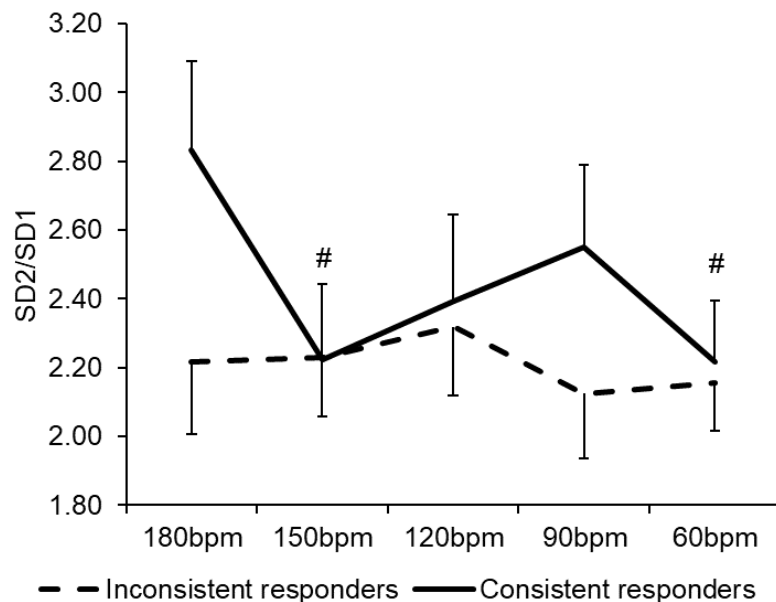
The interaction between tempo and response consistency on SD2/SD1 reached statistical significance ( $p = 0.041$ , see Figure 3.95b). At the level of

the tempi, no significant differences between consistent and inconsistent responders emerged for any tempo ( $p > 0.05$ ). However, analysis at the level of response consistency revealed that for consistent responders only, 180bpm had significantly higher SD2/SD1 compared to 150bpm ( $p = 0.021$ ) and 60bpm ( $p = 0.035$ ). No significant differences between any of the tempi transpired for the group of inconsistent responders ( $p > 0.05$ ).

**a.**



**b.**



*Figure 3.95: SD2 (a) and SD2/SD1 (b) for the stepped decrease in tempo stimulus were significantly impacted by response consistency. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders; # = significantly different to 180bpm.*

### 3.3.6.3.2.1.4 RR analysis

The only main effect of response consistency that emerged statistically significant was for max RR ( $p = 0.037$ ). As illustrated in Figure 3.96, max RR was significantly greater in inconsistent responders compared to consistent responders. No interactions reached statistical significance ( $p > 0.05$ ).

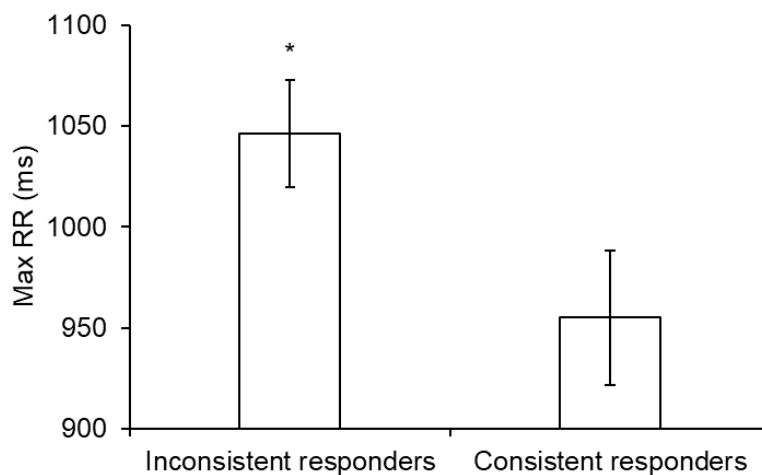


Figure 3.96: Max RR interval significantly differed between inconsistent and consistent responders in the stepped decrease in tempo stimulus. Data presented as mean  $\pm$  1 SEM. \* = significantly different to consistent responders.

### 3.3.6.3.3 Alternative response consistency definition

As a  $\geq 20\%$  change in LF% (regardless of direction) could be interpreted as a response, consistency was re-evaluated. This led to testing an alternative response consistency definition. This classified consistent responders as those who were responders (type 1 and/or type 2) across the three stimuli (e.g. a type 1 responder for the stable tempo and stepped increase in tempo stimuli and a type 2 responder for the stepped decrease in tempo stimulus). Inconsistent responders were classified in the same way as that used in the initial definition.

Twenty-one participants (40.38%) always responded (either type 1 or type 2 responder) whilst the remaining were inconsistent responders (non-responders, type 1 or type 2 responders in any given visit). Appendix B.12. summarises the characteristics of these two groups. No statistically

significant differences emerged when comparing the characteristics between the two groups ( $p > 0.05$ ).

Nonetheless, similar mixed mode ANOVAs were performed to examine whether response consistency (inconsistent, consistent: between-participants variable) interacted with:

- a) The five conditions: baseline (5-10 min period), stable tempo (full duration), stepped increase in tempo (full duration), stepped decrease in tempo (full duration) and recovery (5-10 min period). Within-participants variable.
- b) The five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm). Within-participants variable.
- c) The five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm). Within-participants variable.

### **3.3.6.3.3.1 Differences between the five conditions (baseline, stable tempo, stepped increase in tempo, stepped decrease in tempo and recovery)**

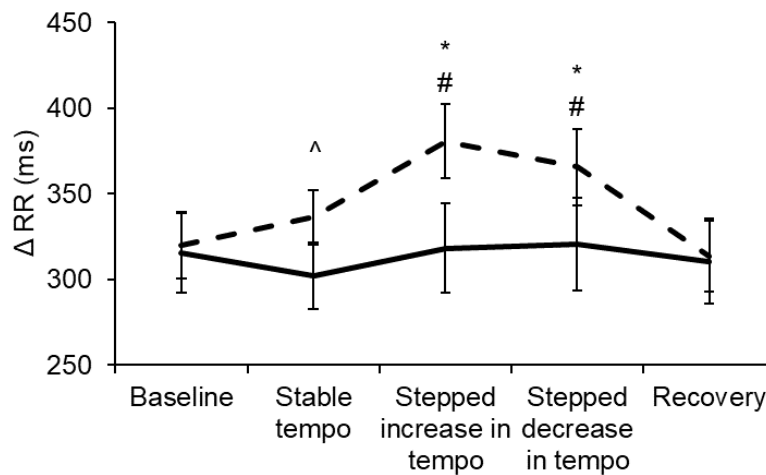
#### **3.3.6.3.3.1.1 RR analysis**

A mixed mode ANOVA revealed a statistically significant interaction between condition and response consistency on  $\Delta$  RR ( $p = 0.026$ ). Analysis at the level of the conditions revealed no significant differences between inconsistent and consistent responders ( $p > 0.05$ ). However, analysis at the level of response consistency demonstrated that for inconsistent responders,  $\Delta$  RR was significantly lower in baseline compared to the stable tempo ( $p = 0.003$ ) and stepped increase in tempo ( $p = 0.032$ ) stimuli. In addition,  $\Delta$  RR during the stepped increase in tempo stimulus was significantly higher compared to that during the stable tempo stimulus ( $p = 0.026$ ). Finally,  $\Delta$  RR was identified as being significantly lower during recovery compared to during the stepped increase ( $p < 0.001$ ) and decrease ( $p = 0.005$ ) in tempo stimuli (see Figure 3.97a).

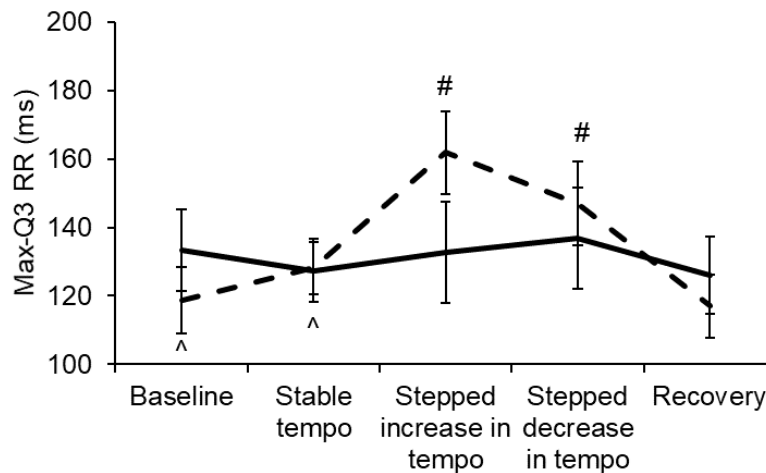
A significant interaction also emerged for max-Q3 RR ( $p = 0.019$ ). Similar to  $\Delta$  RR, no significant differences emerged when analysed at the level of the

condition ( $p > 0.05$ ). Nonetheless, differences did emerge in the group of inconsistent responders only. As illustrated in Figure 3.97b, max-Q3 RR was significantly higher during the stepped increase in tempo stimulus compared to baseline ( $p = 0.001$ ) and the stable tempo stimulus ( $p = 0.009$ ). In addition, recovery max-Q3 RR was significantly lower than that during the stepped increase ( $p < 0.001$ ) and decrease ( $p = 0.016$ ) in tempo stimuli.

a.



b.



— — Inconsistent responders — — Consistent responders

Figure 3.97: Significant interactions between condition and response consistency emerged for  $\Delta$  (a) and max-Q3 (b) RR intervals. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; ^ = significantly different to stepped increase in tempo; # = significantly different to recovery.

### 3.3.6.3.3.1.2 ECG analysis

Condition also interacted with response consistency for R amplitude ( $p = 0.049$ , see Figure 3.98). No statistically significant differences emerged between the inconsistent and consistent responders during any of the conditions ( $p > 0.05$ ). However, analysis at the level of response consistency revealed that for consistent responders, R amplitude during baseline was significantly higher compared to that during the stable tempo ( $p = 0.026$ ), stepped increase in tempo ( $p = 0.006$ ), stepped decrease in tempo ( $p = 0.001$ ) and recovery ( $p = 0.004$ ) conditions. None of the comparisons for the group of inconsistent responders reached statistical significance ( $p > 0.05$ ).

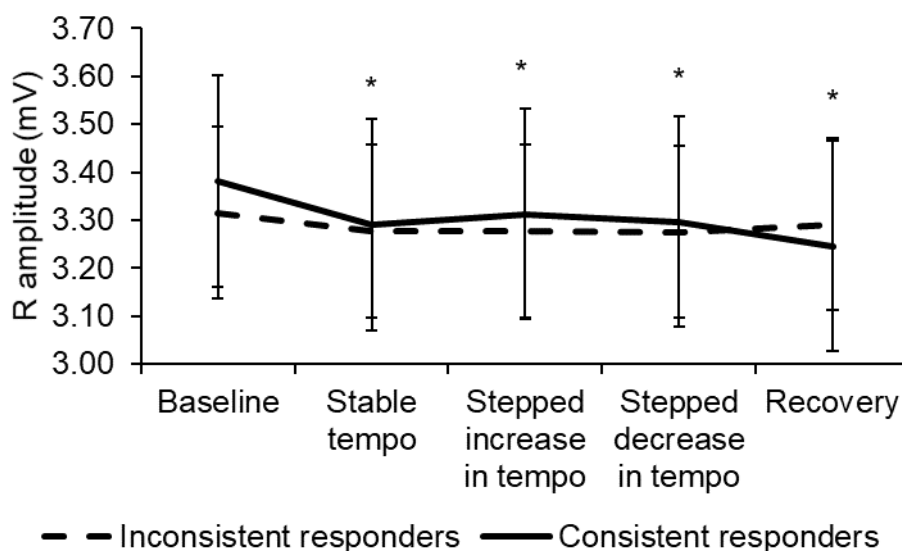


Figure 3.98: R amplitude was significantly higher in baseline compared to the other conditions for consistent responders only. Data presented as mean  $\pm 1$  SEM. \* = significantly different to baseline.

### 3.3.6.3.4 Differences between the five tempi of the stepped increase in tempo stimulus (60bpm, 90bpm, 120bpm, 150bpm, 180bpm)

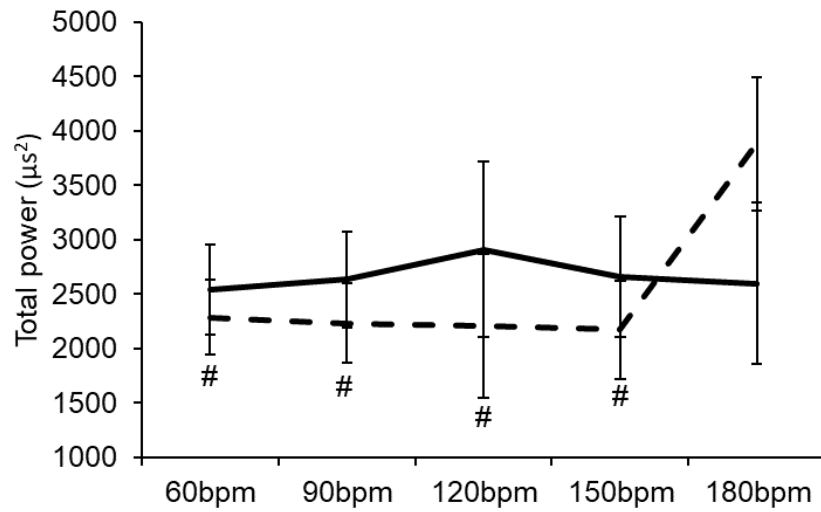
#### 3.3.6.3.4.1.1 Frequency-domain HRV

Statistically significant interactions between condition and response consistency emerged for total power ( $p = 0.042$ ) and VLF power ( $p = 0.008$ ). For total power, no significant differences between consistent and inconsistent responders were detected when analysing at the level of the condition ( $p > 0.05$ ). However, analysis at the level of response consistency

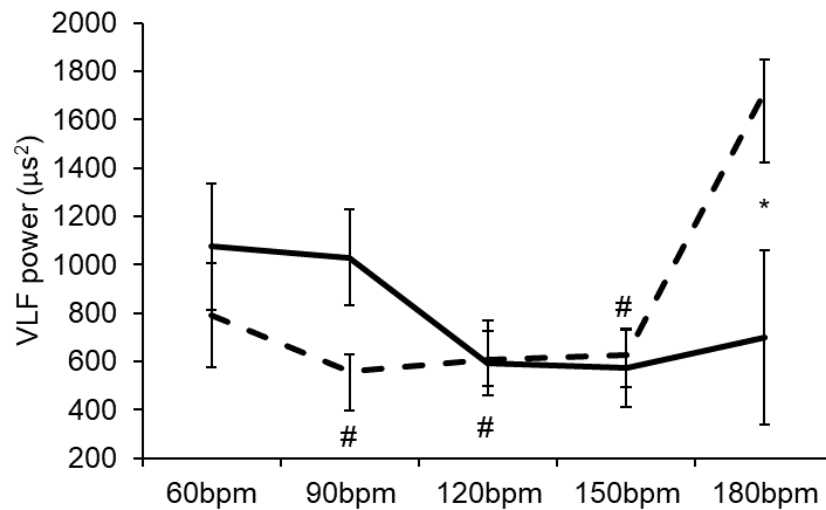
revealed that for inconsistent responders only, total power was significantly higher during recovery compared to baseline ( $p = 0.023$ ), stable tempo ( $p = 0.008$ ), stepped increase in tempo ( $p = 0.025$ ) and stepped decrease in tempo ( $p = 0.001$ , see Figure 3.99a).

Analysis of VLF power revealed that at the level of the condition, inconsistent responders had significantly higher VLF power during recovery compared to consistent responders ( $p = 0.034$ ). Pairwise-comparisons at the level of response consistency revealed that VLF power was significantly higher during recovery compared to the stable tempo ( $p = 0.007$ ), stepped increase in tempo ( $p = 0.003$ ) and stepped decrease in tempo ( $p = 0.010$ ) stimuli for the inconsistent responders only (see Figure 3.99b). None of the comparisons for the group of consistent responders emerged ( $p > 0.05$ ).

a.



b.



--- Inconsistent responders    — Consistent responders

Figure 3.99: Statistically significant interactions were observed for total power (a) and VLF power (b) between the five tempi of the stepped increase in tempo stimulus and response consistency. Data presented as mean  $\pm 1$  SEM. \* = statistically significant difference between inconsistent and consistent responders; # = significantly different to recovery.



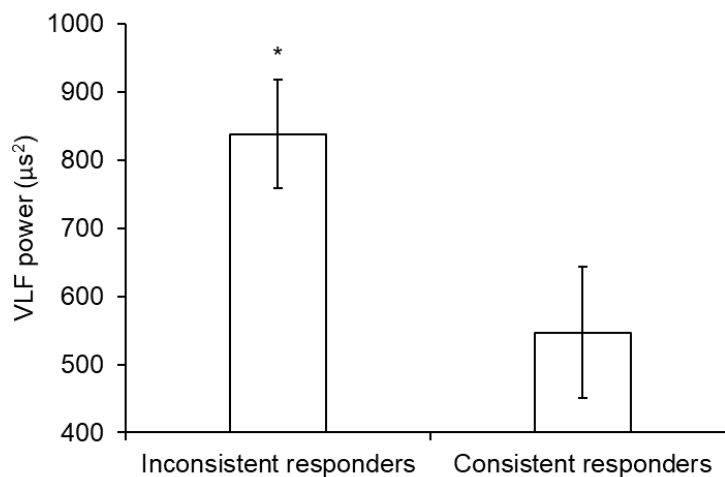
### **3.3.6.3.4.2 Differences between the five tempi of the stepped decrease in tempo stimulus (180bpm, 150bpm, 120bpm, 90bpm, 60bpm)**

#### **3.3.6.3.4.2.1 *Frequency-domain HRV***

A mixed mode ANOVA revealed a significant main effect of response consistency on VLF power ( $p = 0.024$ ). As illustrated in Figure 3.100a, VLF power was significantly higher in participants who showed inconsistent responses across the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli.

Similarly, further analysis of the main effect of response consistency on LF power ( $p = 0.033$ ), demonstrated that LF power was also significantly higher in inconsistent responders than in consistent responders (see Figure 3.100b).

a.



b.

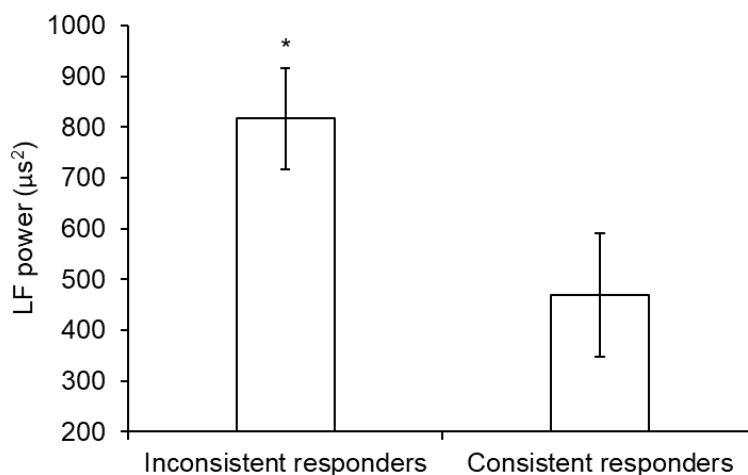


Figure 3.100: For the stepped decrease in tempo stimulus, VLF power (a) and LF power (b) were significantly higher in inconsistent responders compared to consistent responders. Data presented as mean  $\pm$  1 SEM. \* = statistically significant difference between inconsistent and consistent responders.

### 3.3.6.3.4.2.2 ECG analysis

Finally, an interaction between tempo and response consistency emerged for S amplitude ( $p = 0.008$ ). At the level of the condition, no significant differences emerged between consistent and inconsistent responder ( $p > 0.05$ ). However, when analysed at the level of response consistency, S amplitude was significantly higher during the 150bpm tempo compared to the 120bpm tempo for the consistent responders only ( $p = 0.018$ , see Figure

3.101). No differences between any of the five conditions reached statistical significance for the group of inconsistent responders ( $p > 0.05$ ).

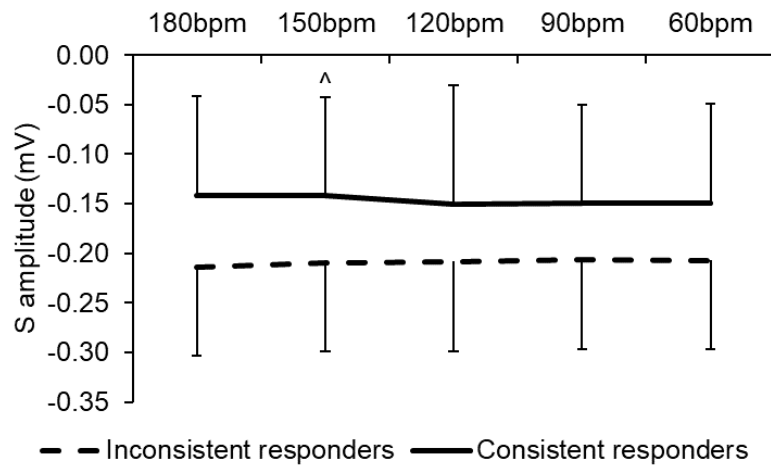


Figure 3.101: S amplitude significantly interacted with tempo and response consistency. Data presented as mean  $\pm$  1 SEM. ^ = significantly different to the 120bpm tempo.

#### 3.3.6.4 Predicting baseline LF%

It has been shown that baseline LF% significantly predicted response magnitude between the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli. Therefore, exploration of the possible variables that predicted baseline LF% was performed. This involved performing linear regressions with baseline LF% as the dependent variable and demographic information as the independent variable. Independent sample t tests (or Mann-Whitney U tests for non-normally distributed data) were also conducted. These latter analyses aimed to investigate whether differences in baseline LF% existed between demographic groups.

Linear regressions revealed that baseline LF% was significantly predicted by: height ( $R^2 = 0.319$ ,  $p = 0.021$ , Figure 3.102a); Godin moderate exercise score ( $R^2 = 0.374$ ,  $p = 0.032$ , Figure 3.102b); pre-baseline brachial SBP ( $R^2 = 0.485$ ,  $p < 0.001$ , Figure 3.102c) and pre-baseline respiration rate ( $R^2 = -0.384$ ,  $p = 0.005$ , Figure 3.103).

As shown in Figure 3.102, baseline LF% increased with increases in height, Godin moderate exercise score and pre-baseline brachial SBP.

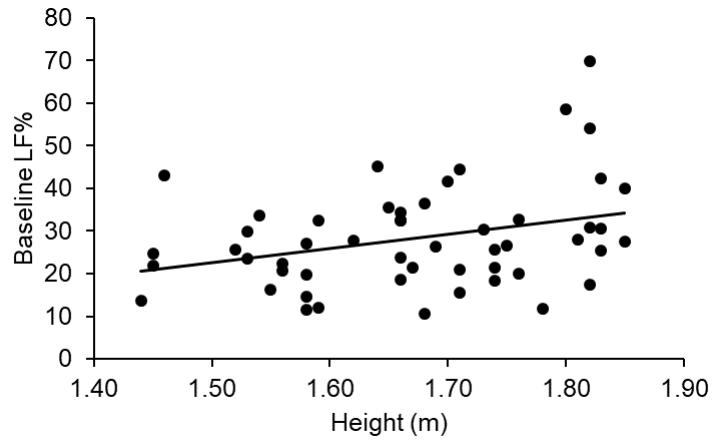
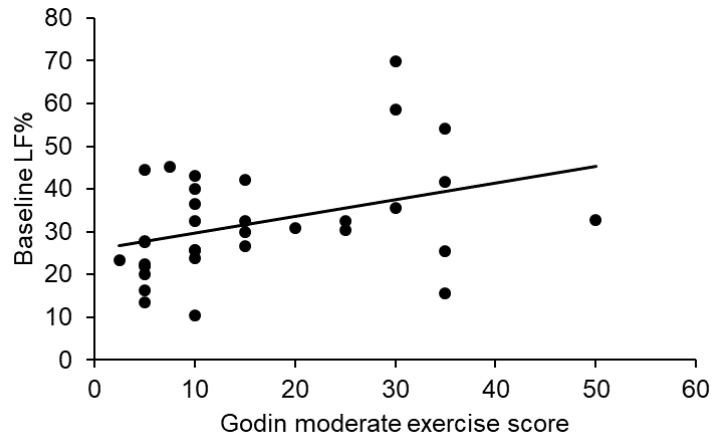
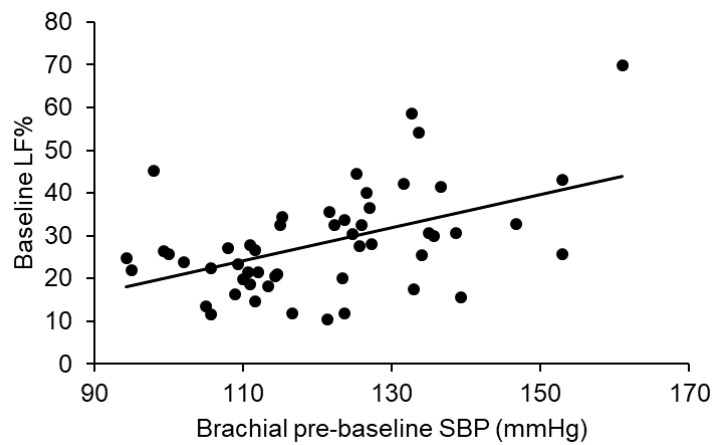
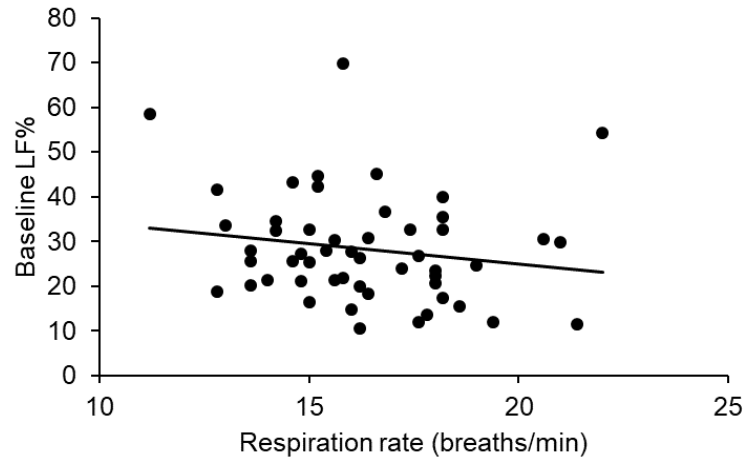
**a.****b.****c.**

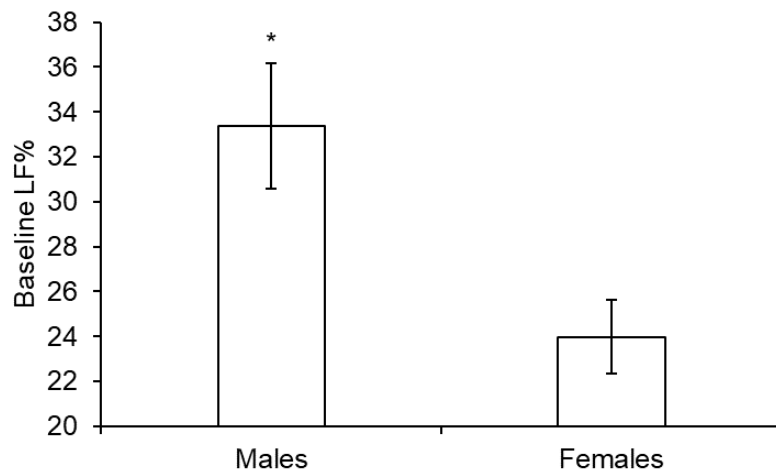
Figure 3.102: Baseline LF% was significantly predicted by height (a), Godin moderate exercise score (b) and pre-baseline brachial SBP (c).

In contrast, as illustrated in Figure 3.103, a negative relationship between baseline LF% and pre-baseline respiration rate emerged: baseline LF% increased with reductions in respiration rate.



*Figure 3.103: Baseline LF% was significantly predicted by pre-baseline respiration rate.*

In addition, as shown in Figure 3.104, baseline LF% was also found to be significantly higher in males compared to females.



*Figure 3.104: Baseline LF% was significantly higher in males than females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to females.*

## 3.4 Discussion

### 3.4.1 Main findings

This study examined the effects of stepped increases and decreases in tempo on cardiac autonomic function and self-reported emotion.

Recommendations from Camm et al. (1996) combined with potential confounding orienting responses in the pilot led to ascertaining which five-minute section, out of a 10-minute baseline and recovery recording, was the most valid measure of participants at rest. Although the results clearly showed that greatest vagal tone occurred during the last five minutes of baseline, this was not as clear for recovery. This may have been due to participant anticipatory responses during recovery (i.e. participants looking forward to the end of the study). Nevertheless, the final five minutes of the two recordings were used as the 'final' baseline and recovery conditions. Discounting the initial couple of minutes for data analysis is not uncommon in the literature. For instance, Watanabe et al. (2017) analysed the last three minutes of their five-minute conditions and Gomez and Danuser (2007) used the second half of the baseline and stimulus conditions (latter 15-seconds). However, it should be acknowledged that this type of analysis was not performed at a lower-level e.g. a rolling five-minute section for every minute or 30-seconds of the recording. Therefore, it was not clear whether autonomic activity fluctuated to a great extent within the 10-minute baseline and recovery recordings. This is particularly important for baseline because it could lead to over- or under-inflated response patterns and the misidentification of responder types. Therefore, the variation in autonomic measures in a 10-minute recording requires further exploration in future studies.

The stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli significantly impacted cardiac autonomic function. The stable tempo stimulus was associated with increases in VLF% and Q amplitude and decreases in nSD1, QT interval, QTc interval, JT interval and R amplitude. Although the same patterns emerged in the ECG parameters for

the stepped increase in tempo stimulus, it also elicited decreases in minimum RR intervals. Finally, the stepped decrease in tempo stimulus was associated with similar changes in the ECG parameters as the other stimuli, but provoked increases in  $\Delta$  RR. No significant changes in frequency-domain HRV or MSNA were observed, making interpretation of the observed results challenging. Nevertheless, the results suggest that the three stimuli did modify the electrocardiography characteristics of heart activity as well as the dispersion of RR intervals. This finding is not surprising given that Dousty et al. (2011) found that R amplitude differed between arousal and sedative music.

Unlike the between-condition analyses, clearer impacts on participant physiology occurred when comparing autonomic activity between the five tempi of the stepped increase in tempo stimulus. For instance, the 60bpm tempo in the stepped decrease in tempo stimulus was associated with shifts towards parasympathetic predominance. This was indexed by decreases in HR and T amplitude, and increases in HF power, RMSSD, pRR50, SD1, nSD1, up BRS, mean BRS, QT interval, JT interval, mean RR interval and  $\Delta$  RR interval. In contrast, these patterns did not manifest in the 60bpm tempo during the stepped increase in tempo stimulus. Instead, 180bpm elicited increases in the following measures of parasympathetic activity: SDSD, RMSSD and (n)SD1. However, as measures of overall variability increased (e.g. SDRR, CVRR, SD HR, total power, (n)SD2 and  $\Delta$  RR interval), this suggests that the greatest variability in heart activity occurred for the 180bpm in the stepped increase in tempo stimulus and was driven by increases in both sympathetic and parasympathetic activity.

Khalifa et al. (2008) showed that rhythm and melody interacted with the effects of tempo on cardiac autonomic activity. However, this pattern did not really manifest here. One significant impact of group (control and experimental) transpired for the physiological measures: SBP was higher in recovery compared to all conditions for the control group only. Initially this may suggest that removal of the melody resulted in heightened SBP. However, given that finometer BP measurements were significantly lower

compared brachial BP measurement, the reliability of the condition x group interaction is called into question. Despite this concern, clear differences in perceived musical emotion emerged between the two groups. Results were in line with anticipations: the control stimuli were perceived as more irritating and less pleasant and positive than the experimental stimuli. This finding is consistent with those of Khalifa et al. (2008) who showed that the rhythm only and melody only versions were rated significantly less positively than the original version (which had the rhythmic and melodic variations). However, no differences between the groups emerged for experienced emotion. Perhaps this could be interpreted to mean that the power of music exerting its effects on human physiology lies in tempo alterations. Interestingly, this conclusion does not diverge from that of Krumhansl (1997), Chuen et al. (2016), Gomez and Danuser (2007), Iwanaga (1995a, 1995b) Watanabe et al. (2017) and van Dyck et al. (2017). In addition, this finding implies that the self-report and physiological responses were consistent for the five conditions.

However, upon further reflection, this may not necessarily be the case. This is because the correlations between the physiological measures and experienced stimulation and stress were in the direction contrary to expectations. Correlations between physiological measures and affective responses to music have been reported elsewhere. For example, van der Zwaag et al. (2011) showed that increases in subjective arousal and tension coincided with increases in SCRs. In contrast, increases in RMSSD were related to decreases in arousal and tension. In addition, Gomez and Danuser (2004) revealed that inspiratory time, expiratory time and total breath duration decreased with increasing valence and increased with increasing arousal. Minute ventilation and SCL also increased with increasing valence and arousal. Finally, Khalifa et al. (2002) found that SCR positively correlated with fear and happiness, but not with sadness and peacefulness. However, these studies have used alternative self-report methods. For example, van der Zwaag et al. (2011) used 7-point Likert Scales, Gomez and Danuser (2004) used a 9-point self-assessment manikin, and Khalifa et al. (2002) used a combination of forced-choice scales



and 10-point Likert Scales. Nevertheless, the use of VASs in measuring experienced and perceived musical emotion is valid, given that the two-factor model of affective space (Russel & Carroll, 1999) and Berlyne's theory of aesthetic response was replicated.

Baseline LF% significantly predicted responses (based on percentage change in LF%) to the three auditory stimuli. This finding replicates the pilot which also showed that baseline LF% was a significant predictor for determining participant responses. This is an important finding for three reasons. Firstly, it suggests that even with more musically complex stimuli which include a control group and stimulus, it is possible to predict how a listener responds (i.e. this a real effect that is attributable to stimuli that manipulate musical tempo). Secondly, the finding reinforces the point that listeners do vary in how they respond to stimuli and that this can be determined by baseline readings. Finally, the finding lends support to the notion that it is possible to identify individuals who will respond to a stimulus (or intervention) from the outset. This has important consequences for intervention-style studies that look at the beneficial effects of music used as an alternative therapy.

Due to this replicated finding, further analysis was performed. This entailed exploring differences in demographic characteristics between non-responders, type 1 responders and type 2 responders. As three stimuli were employed, these analyses were conducted on the three responder types for each individual stimulus. Interestingly, not one demographic variable consistently differentiated between the three responder types across the stimuli. However, age group was significantly associated with responder type for the stepped decrease in tempo stimulus only, suggesting that young participants were more likely to be type 1 responders and older participants were more likely to be type 2 responders. This is counter-intuitive given that youngsters have lower sympathetic predominance at baseline than older individuals (Kuo et al., 1999; Stein et al., 1997), although baseline LF% did not differ between young and older participants here. Nevertheless, this result is consistent with the finding that percentage change in LF% from

baseline for the stepped increase and decrease in tempo stimuli differed between young and older participants. That is, older participants experienced increases in LF% whereas young participants exhibited decreases. Therefore, age appears to be an influential factor in determining the direction of change in LF% between baseline and stimuli that manipulate tempo. But it is still unclear whether this stems from other variables that influence baseline LF%, such as height, moderate exercise frequency, resting respiration rate and gender.

Unlike the pilot which showed that demographic differences existed between those who consistently responded and those who showed inconsistent responses, no such result emerged here. This was the case for both response consistency definitions. However, this could be due to differences between the two studies in how consistency was defined. Indeed, as the current study was interested in examining consistency in responses to the three stimuli, consistency was defined with respect to between-stimulus responses. In contrast, as the pilot was interested in exploring whether participants showed different responses in a second visit compared to an initial visit, consistency was defined with respect to between-visit responses (rather than between-stimulus responses). Nevertheless, these differences between the two studies do not negate the finding that response consistency impacted autonomic activity during the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli. Indeed, inconsistent responders showed greater overall variability (as indexed by higher total power, SD2 and  $\Delta$  RR) compared to consistent responders, suggesting that there may be an element of randomness in autonomic response between stimuli. This may result from day-to-day variation in sympathetic and parasympathetic activity introduced by external factors. The results also provide support for adopting the initial definition of response consistency. The reasons for this are two-fold. Firstly, the definition preserves the three-level distinction (between non-responders, type 1 responders and type 2 responders). Secondly, the initial definition is more sensitive to changes in autonomic activity that occur between the three stimuli. Indeed, it seems that the greatest parasympathetic predominance that occurred in the 60bpm

tempo of the stepped decrease in tempo stimulus was driven by the consistent responders.

As the main finding was that physiological and affective responses were significantly impacted by the auditory stimuli, this finding will be discussed in more detail below.

### **3.4.2 The tempo manipulations altered cardiac autonomic function and subjective emotion**

The pilot showed the stepped decrease in tempo stimulus promoted shifts towards parasympathetic predominance. This result was consistent with those reported elsewhere. For instance, van der Zwaag et al. (2011) found that RMSSD was significantly higher during slow music compared to fast music. Also, Iwanaga et al. (2005) revealed that HF power was significantly higher during sedative music compared to excitative music. Furthermore, Iwanaga (1995b) and van Dyck et al. (2017) showed that stimuli decreasing in tempo are more effective at boosting autonomic tone than those that increase in tempo.

Contrary to this past work, the stepped decrease in tempo stimulus employed here was not associated with a shift towards parasympathetic predominance. In fact, the results suggest that there were no meaningful differences in cardiovascular autonomic responses between the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli. For instance, all stimuli were associated with similar changes in ECG parameters. The only variables which differentiated the stimuli were: VLF% and nSD1 for the stable tempo stimulus; minimum RR interval for the stepped increase in tempo stimulus; and  $\Delta$  RR for the stepped decrease in tempo stimulus. Furthermore, as LF/HF and MSNA (the primary outcome measures) did not significantly differ between the three stimuli, it appears that autonomic activity was consistent across all stimuli.

Due to this lack of meaningful differences, it could be concluded that tempo was not as influential as proposed in the literature. This argument is in stark contrast to that of Bernardi et al. (2006) who found that HR, LF/HF and  $\alpha$

BRS increased with faster tempi. It is also contrary to Gomez and Danuser (2007) who showed that HR and respiration quickened as tempo increased, and da Silva et al. (2014) who discovered that faster music was associated with decreases in SD2. In addition, the between-stimuli patterns observed here contradict those of Iwanaga et al. (2005) who showed that HF power was significantly higher during sedative music than excitative music and van der Zwaag et al., (2011) who revealed that RMSSD increased during slow music compared to fast music. However, the robustness of the argument positing that tempo has little autonomic impact seems unlikely for two reasons. Firstly, there are methodological differences between previous work and the study conducted here. These include:

1. Differences in auditory stimuli used, including the inclusion of a control stimulus (and group).
2. Differences in the sample characteristics. For instance, the current study recruited both musicians and non-musicians (some research only focusses on one group e.g. non-musicians: da Silva et al., 2014); participants spanned a wide age range (in comparison to the narrow age-ranges previously employed e.g. 18-37 years: Gomez and Danuser, 2007; 19-22 years: Iwanaga, 1995a; 19-21 years: Iwanaga, 1995b; 19-27 years: Iwanaga et al., 2005; 18-30 years: da Silva et al., 2014; 19-31 years: van Dyck et al., 2017).
3. Differences in procedure. For instance, the following conditions were put in place to minimise the impact of confounding variables: abstinence from coffee, nicotine and alcohol 12 hours before the study; conducting the study between 14.00 and 18.00 and asking participants to empty their bladder immediately before testing. Inspection of the methodologies of previous work reveals either slight differences in the controls employed (e.g. conducting the study on a morning, between 09.00 and 11.00) or no details.

Secondly, differences in autonomic activity emerged when analysing the five tempi of the stepped increase and decrease in tempo stimuli. Indeed, the 60bpm tempo of the stepped decrease in tempo stimulus was associated with the greatest shifts towards parasympathetic predominance. Also, the

180bpm tempo of the stepped increase in tempo stimulus was associated with the greatest increases in total variability. This is consistent with the results of Iwanaga (1995b), Peretz et al. (1998) and van Dyck et al. (2017) who showed that direction of stepped change (increase or decrease) impacts autonomic responses to the tempi. Moreover, as 60bpm elicited the greatest vagal tone (as indexed by HF power, pRR50 and (n)SD1) it is reasonable to argue that this tempo may have driven the effect observed in the pilot. Therefore, this does provide some closure concerning the potential mechanism that was responsible for the association between the stepped decrease in tempo stimulus and the shift towards parasympathetic predominance in the pilot.

Perhaps the finding that the 60bpm tempo elicited the greatest parasympathetic activity is not completely unsurprising. This is because Iwanaga (1995a, 1995b) showed that preferred tempo is 1 or 1.5 times a participant's HR and baseline HR in this study ranged from 48.48-93.52bpm. Furthermore, Wang (1984) demonstrated that the accuracy of detecting changing tempi was greater for decreasing tempi compared to faster tempi, suggesting that the final decreasing tempo may have been detected and interpreted most favourably. As the self-report measures were not continuously recorded, it was not possible to fully ascertain whether this was the case. This is something to consider in future work.

Consistent with expectations the stable tempo, stepped increase in tempo and stepped decrease in tempo stimuli prompted a change in how participants felt. Indeed, all three stimuli were associated with increases in experienced and perceived arousal and decreases in perceived valence when compared to baseline and recovery. However, consistent with the measures of cardiac autonomic activity, few differences in these measures transpired between the three stimuli. That is, the stepped decrease in tempo stimulus was equally as stimulating as the stepped increase in tempo and stable tempo stimuli. Although these findings are contrary to those of Gomez and Danuser (2007), Peretz et al. (1998), Husain et al. (2002) and Gagnon

and Peretz (2003), they are consistent with Kamenetsky et al.'s (1997) failure to find an effect of tempo on subjective responses.

However, experienced sadness did emerge as significantly differing between the stepped increase and stepped decrease in tempo stimuli: sadness was significantly heightened for the stepped decrease in tempo stimulus compared to the stepped increase in tempo stimulus. Similar results have been reported in the literature. For instance, Gagnon and Peretz (2003) and Webster and Weir (2005) showed that music with slower tempi were rated as significantly sadder than faster tempi and silence. Therefore, the results substantiate the finding that slower tempi are not only perceived as being sadder, but they also elicit increases in the intensity of sadness experienced by listeners.

### **3.4.3 Conclusion**

The study examined the effect of tempo manipulations on cardiac autonomic control and self-reported emotion. This involved: using more musically sophisticated stimuli compared to the pilot, and incorporating a control group, control stimulus and MSNA. Unlike the pilot, the stepped decrease in tempo stimulus was not associated with a shift towards parasympathetic predominance. However, the 60bpm tempo of the stepped decrease in tempo stimulus elicited the greatest vagal tone, implying that this tempo out of the five tested was the most physiologically relaxing. A lack of differences in autonomic activity between the three stimuli also emerged in the self-report measures, initially suggesting that autonomic and affective responses were consistent. But, this was not always the case, thus reinforcing the argument for combining self-report with physiological measures. Despite differences in results between the pilot and current study, the predictive power of baseline LF% in determining how participants responded to the three stimuli was replicated, suggesting that this is a real effect. Further work exploring the application of responder types to other 'alternative' stimuli is required to fully explore its robustness. In addition, similar to the pilot, the methodology employed here worked and has important theoretical and applied impact for future music studies.

**Chapter 4. The effects of relaxation music  
combined with tVNS on autonomic function  
and subjective emotion**

## 4.1 Introduction

Modern-day life is fast-paced, stressful and pressurised. As a result, society is cognisant of the effects this type of lifestyle can have on both physical and mental health. In 2014, it was reported that 58% of females and 48% of males used prescription drugs, and that consumption was greatest (> 67.7%) in individuals aged 55 years and over<sup>34</sup>. In addition, prescription drug costs have increased over the last few years, from £7.65 in 2011 to the present-day £8.60 per item<sup>35</sup>. This has resulted in a need for alternative treatments that avoid the consumption of medications and are cheaper. As a consequence, multiple alternative therapies have emerged. These include: acupuncture, sound therapy, touch therapies (like Reiki) and non-invasive neuromodulatory techniques (e.g. transcutaneous vagal nerve stimulation: tVNS). In addition, this demand has prompted the rise of wearable technologies which monitor and record health-related indices, such as HR and physical activity. Wearables, music and tVNS have received a great deal of attention, due to their non-invasive characteristics, portability and easy to self-administer qualities. Therefore, this study aimed to explore how music and tVNS packaged as a wearable influenced cardiac autonomic activity and subjective emotion.

### 4.1.1 Relaxing music, autonomic activity and subjective responses

Relaxing music is a popular choice of music amongst many people, perhaps because in healthy volunteers it can effectively reduce HR, BP and respiration, and boost HRV (Chafin et al., 2004; Iwanaga et al., 2005; Bernardi et al., 2006; Labbé et al., 2007). In addition, subjective relaxation can be enhanced, and tension and stress attenuated (Davis & Thaut, 1989; Knight & Rickard, 2001; Burns et al., 2002; Iwanaga et al., 2005; Labbé et al., 2007; Jiang, Zhou, Rickson & Jiang, 2013). These positive findings have led to an acceptance in society that relaxing music is good for listeners. They have also prompted the drive to compose tracks that are highly

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<sup>34</sup> [http://ec.europa.eu/eurostat/statistics-explained/index.php/Medicine\\_use\\_statistics](http://ec.europa.eu/eurostat/statistics-explained/index.php/Medicine_use_statistics), accessed 18.09.2017.

<sup>35</sup> <https://www.gov.uk/government/news/nhs-charges-to-increase-from-april-2012>, accessed 18.09.2017.



effective at relaxing individuals. One example is 'Weightless', composed by Marconi Union in collaboration with sound therapists and Radox. The band claims that composition of the track was based on previous work investigating the musical qualities that induce maximum relaxation. This resulted in a piece of eight minutes in duration that had a slow tempo (60bpm), simple rhythms, narrow pitch range, legato notes and long, smooth phrasing.

A research study investigating the impact of relaxing music on HR, BP, respiration and brain activity was performed. This involved recruiting a sample of 40 female participants who were tasked with solving challenging puzzles as quickly as they could to increase stress. HR, BP, respiration and brain activity were recorded while participants were completing the puzzles. In addition, participants listened to different pieces of relaxation music, including Weightless, Enya's Watermark and Coldplay's Strawberry Swing, while solving the puzzles. The results reported that Weightless was 11% more relaxing compared to the other tracks used in the study. Additionally, Weightless was associated with a 65% reduction in anxiety, with anxiety being 35% lower than baseline levels<sup>36</sup>. Issue can be taken with the reliability of this study and its outcomes for the following reasons:

1. Details on how the sample was recruited and its characteristics are unavailable.
2. The experimental methodology (e.g. type of puzzles, control conditions, duration of recordings) are not reported.
3. The outcomes detailed in terms of 'anxiety' do not directly relate to the dependent variables (HR, BP, respiration and brain activity).
4. The study is reported in online news sources only (e.g. The Telegraph), no evidence of this study could be found in peer-reviewed journals.

Despite these ambiguities, Weightless was an interesting track to explore in a research study as it could potentially have a significant impact on human physiology and subjective emotion. Therefore, as Weightless is considered

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<sup>36</sup> <http://www.telegraph.co.uk/news/uknews/8830066/Band-creates-the-most-relaxing-tune-ever.html>, accessed 05.06.2017.

to be 'the most relaxing tune ever', one of the study aims sought to test whether this track boosted vagal tone and self-reported relaxation.

#### **4.1.2 Transcutaneous vagal nerve stimulation (tVNS)**

Electrical stimulation of the vagus nerve (VNS) is an approved intervention for patients with treatment-resistant depression (Rush et al., 2000) and epilepsy (Ben-Menachem et al., 1994). VNS in humans involves implanting a multiprogrammable pulse generator in the left chest wall (George et al., 2000). The electrode is wrapped around the left cervical vagus nerve in the neck and connected to the generator subcutaneously. The generator generates small electrical impulses which are targeted to the left vagus nerve via the electrodes (George et al., 2000). As this intervention requires surgical implantation and has associated side-effects (e.g. croakiness of voice, coughing, chest pain and infection (George et al., 2000; Kreuzer et al., 2012)), demand for a non-invasive method arose. This led to the emergence of transcutaneous vagal nerve stimulation (tVNS).

tVNS involves administering small electrical impulses across the skin (transcutaneously) on the outer ear. This can be achieved by using transcutaneous electrical nerve stimulation (TENS) machines which have traditionally been used to relieve pain (Johnson, 2007). TENS machines used to administer tVNS generate electrical impulses between two electrodes that are high-frequency (50-100 Hz), low-intensity (below sensory/pain threshold) and small pulse width (50-200 $\mu$ s) (Johnson, 2007). Despite a range of different tVNS stimulation parameters having been used, tVNS appears to effectively modulate autonomic activity towards parasympathetic dominance (Clancy et al., 2014; Antonino et al., 2017). In addition, tVNS has been associated with improved mood (Kraus et al., 2007) and associative memory (Jacobs, Riphagen, Razet, Wiese & Sack, 2015) and positive effects in patients with epilepsy (Bauer et al., 2016), depression (Hein et al., 2013; Fang et al., 2016) and tinnitus (Kreuzer et al., 2012, 2014).

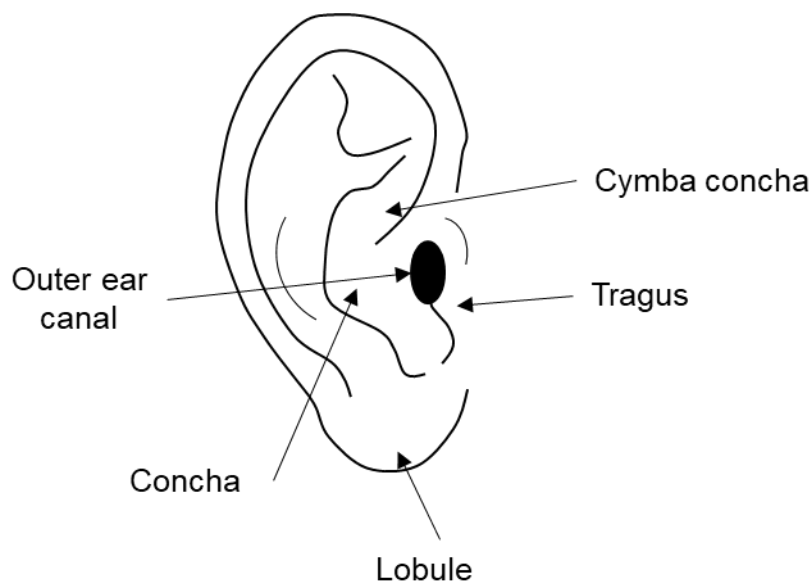
tVNS is administered to the outer ear because it is supplied by three sensory nerves: the auriculotemporal nerve (ATN); the great auricular nerve (GAN); and the auricular branch of the vagus nerve (ABVN) (Peucker & Filler, 2002).

On 14 human ears Peuker and Filler (2002) found that innervation differed between different parts of the ear. Table 4.1 provides an adapted summary of Peuker and Filler's (2002) results.

*Table 4.1: Summary of Peuker and Filler's (2002) findings.*

	ABVN	GAN	ATN
Tragus	45%	46%	9%
Cavity of concha	45%	55%	
Cymba concha	100%		
Lobule		100%	

Popular areas for targeting tVNS are the tragus (Clancy et al., 2014; Jacobs et al., 2015; Antonino et al., 2017); cymba concha (Kreuzer et al., 2012); concha (Bauer et al., 2016; Burger et al., 2016) and outer ear canal (Hasan et al., 2015). Figure 4.1 provides an illustration of the areas of the outer ear of interest.



*Figure 4.1: Diagram of the outer ear. The cymba concha, tragus, concha, lobule and outer ear canal are all parts of the outer ear that have received interest in projects investigating the impact of tVNS on human physiology.*

#### **4.1.3 Wearable technologies**

Wearable technologies are becoming increasingly popular in everyday life. Most serve a range of different functions, including: monitoring HR; recording physical activity; keeping track of sleep characteristics (e.g. sleep duration and disturbances) and providing bio and emotional feedback. In addition, there has been a surge of interest in more 'intelligent' devices that

generate vibroacoustic signals based on the physiological profile of wearers. In other words, devices that provide an intervention-style service are increasing in demand and popularity.

One example is the Doppel device which delivers on-demand, discrete, user-controlled vibrations applied via a wristband (Azevedo et al., 2017). The wristband is connected via Bluetooth to a smart phone app which permits the user to choose settings and monitor HR. The vibrations Doppel generates are supposed to resemble heartbeat-like stimuli. The rationale underpinning this feature appeals to entrainment research which shows that humans behaviourally and psychologically synchronise to rhythms in the external environment. Supporting evidence comes from the music psychology literature e.g. Vickhoff et al. (2013) found that the HR of singers in a choir synchronised so that all singers simultaneously showed increases and decreases in HR. In addition, the developmental psychology literature reports that foetal HR is influenced by maternal HR and anxiety levels (Monk et al., 2009), and mother-infant attachment can also influence HR synchrony. For instance, in secure dyads there is greater consistency in HR changes between mother and infant, whereas in insecure-resistant dyads there is less consistency (Zelenko et al., 2005).

Testing of the Doppel device in a validated anxiety-inducing paradigm (anticipation of giving a public speech) demonstrated that the device worked (Azevedo et al., 2017). For instance, the active treatment (device turned on administering vibrations) showed significantly lower SCL and state anxiety than the control group (device turned off with participants under the belief it was working). However, HR was not found to differ between the two groups.

Another device, HIRREM (high-resolution, relational, resonance based, electroencephalic mirroring) has also received research interest. HIRREM is a non-invasive neurotechnology that aims to reduce neural oscillatory disturbance and autonomic dysfunction (Gerdes, Gerdes, Lee & Tegeler, 2013). The technology uses sensors placed on the scalp to measure brain electrical activity and to generate feedback as audible tones. The tones produced are derived from a mathematical algorithm which translates the dominant EEG frequency into an audible tone. These tones are then

presented to users via standard earbud headphones. How and when the dominant EEG frequencies are selected for translating into an auditory tone is determined by the relationships among the parameters of an individual's own spectral EEG. Therefore, the tones produced by the device are unique to an individual. The idea that auditory musical tones can facilitate beneficial change in neural oscillation and autonomic function is based on the notion that musical tones can correspond to dominant frequencies in individual spectral EEG (Gerdes et al., 2013).

Positive effects of HIRREM have been found in a range of studies. For instance, in hypertensives SBP and DBP were significantly lower post-intervention, and SDRR and BRS were significantly higher post-intervention, consistent with an increased vagal and/or decreased sympathetic output to the heart (Shaltout, Tegeler & Tegeler, 2016). Additionally, in athletes with persistent symptoms following concussion (which present with decreased HRV), SDRR and BRS were significantly higher post-intervention, and physical activity levels increased and reaction time improved upon completion of the intervention (Tegeler et al., 2016).

A more recent addition to the wearable device market is the Nervana Headphones. These combine tVNS with music with the hope of helping users 'to feel relaxed, calm, and focused like never before'<sup>37</sup>. The device comprises a generator (resembling a TENS machine) and in-ear headphones. Consistent with their interpretation of tVNS recommendations, only left headphone generates the electrical impulses whilst both permit the playing of music. One of the major selling points of the Nervana headphones concerns its functionality: tVNS can be synchronised to music. Nervana LLC claim that the synchrony between the two stimuli facilitate enhanced relaxation effects<sup>38</sup>. Similar to the Doppel device, this claim is based on the entrainment literature. However, no empirical work has been performed examining the autonomic effects of this technology.

A more established device that has been tested in controlled studies is the Net-1000 device. It is a TENS micro-stimulator unit made by Auri-Stim

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<sup>37</sup> <https://experiencenervana.com/>, accessed 25.06.2017

<sup>38</sup> <https://experiencenervana.com/>, accessed 25.06.2017.

Medical, Inc. and has received a CE conformity certificate for the European Market. The device has been labelled as a nerve stimulator and classified as a Class II and IIa, low risk medical device. Clinical trials have shown that the tVNS generated by the Net-1000 can improve migraine headaches and smoking cessation, as ascertained via self-report measures<sup>39</sup>. In addition, Hein et al. (2013) demonstrated that the device can relieve depressive symptoms in patients with major depression. Interestingly, no improvements in the Hamilton Depression Rating Scale and Beck's Depression Inventory were observed for the sham group.

These findings illustrate that the Net-1000 has beneficial effects for individuals who experience migraines, have difficulty in stopping smoking or have major depression. In addition, no side-effects have been reported suggesting that the device is safe for self-administration. However, since these studies relied on self-report measures only, the impact of this device on cardiovascular autonomic function is unknown. Also, as none of these studies combined the tVNS with music, the viability of using the Net-1000 as a music therapy intervention is uncertain.

#### **4.1.4 Music combined with tVNS**

Music and tVNS are most commonly combined as a therapeutic intervention for patients with tinnitus. Tinnitus is considered to be caused by changes in the organisation of the auditory cortex and neuronal hyperactivity as a result of cochlea trauma (Engineer et al., 2011). This leads to individuals perceiving noises that have no external source. Type of noise can vary e.g. buzzing, whistling or ringing sounds; as can the duration (intermittent or constant) and volume. Tinnitus is a debilitating condition which requires treatment. Tinnitus treatments are numerous and depend on the anticipated cause e.g. hearing aids if the cause is postulated to be hearing loss related, or leaving the radio on if the tinnitus occurs in silence.

An alternative therapy is to pair VNS (or tVNS) with tones that are out of the tinnitus frequency range. This was first discovered in a rat-model of tinnitus by Engineer et al. (2011) who postulated that pairing of VNS with tones

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<sup>39</sup> <http://net1device.com/results.htm>, accessed 25.06.2017.

could drive neural plasticity that would reverse the behavioural characteristics of tinnitus. The study involved: 1) exposing normal-hearing rats to a single tone paired with VNS; and 2) presenting noise-exposed rats with a range of tone frequencies combined with VNS. The first part of the study revealed that VNS paired with single tones led to changes in the auditory cortex map. The second part of the study showed that repeatedly pairing tones (excluding the tinnitus frequency range) with VNS eliminated the neurological and behavioural aspects of tinnitus in noise-exposed rats. Similar results have been reported by Shetake, Engineer, Vrana, Wolf and Kilgard (2012) and Borland et al. (2016). Consequently, this finding has prompted investigations into the efficacy of combining VNS with tones in humans with tinnitus.

Results are promising: De Ridder, Kilgard, Engineer and Vanneste (2015) showed that repeatedly pairing VNS with tones significantly reduced tinnitus symptoms. Similarly, in studies using tVNS and paired tones, subjective tinnitus symptoms were improved (Lehtimäki et al., 2013; Mei et al., 2014; Shim et al., 2015) and neuronal hyperactivity in the primary auditory cortex was reduced (Mei et al., 2014). Together, these results demonstrate that (t)VNS combined with specific tones could be an effective tinnitus treatment.

Although this treatment appears to be effective in tinnitus patients, it is plausible that combining music with tVNS may lead to positive effects in non-patient samples. After all, relaxation music and tVNS have individually been shown to shift autonomic function towards parasympathetic dominance and promote improvements in mood. Furthermore, the popularity of the Nervana headphones and the existence of other wearable devices that use vibroacoustic signals to modulate human physiology would suggest that demand for this combination-intervention exists. Therefore, the current study aimed to investigate the effects of music combined with tVNS on autonomic function and self-reported emotion.

#### **4.1.5 Hypothesis**

Weightless is construed as being 'the most relaxing tune ever' and the Net-1000 has empirically been shown to benefit individuals with migraines and smoking addiction. Therefore, the study was premised on the notion that

combining Weightless with tVNS via the Net-1000 will enhance parasympathetic dominance and subjective relaxation than either stimulus alone or a sham (control) condition.

#### **4.1.6 Aims and objectives**

The study aimed to examine the effects of relaxing music in combination with tVNS on cardiac autonomic function and self-reported emotion.

The objectives were to:

1. Determine the extent to which measures of autonomic function and subjective emotion changed from baseline during four different stimulation periods (tVNS only, music only, tVNS + music, sham).  
Research question: Is there an effect of stimulation?
2. Investigate the extent to which change in cardiac autonomic function and subjective emotion from baseline to stimulation differed between the four types of stimulation. Research question: Does one type of stimulation provoke greater changes than the other types?
3. Explore how gender, age, musical background and physical activity impact physiological and subjective responses to the four stimuli.  
Research question: Are pre-existing individual differences (e.g. males/females, young/older, musicians/non-musicians) associated with different response patterns?
4. Examine the extent to which baseline measures predict response patterns to the four stimuli. Research question: Is it possible to predict response type based on baseline readings?



## **4.2 Method**

### **4.2.1 Participants**

Thirty-two participants were initially recruited for the study (15 males, mean age: 33.22 years, SD: 9.67 years, minimum: 22 years, maximum: 58 years). The inclusion criteria for participation in the study were healthy males and females aged between 18 and 60 years. The exclusion criteria were the same as used in the previous study (see Chapter 3). Appendix C.1. provides a summary of the characteristics of the initial group of 32 participants.

As for the previous two studies, similar controls were enforced: the study was conducted between 13:00 and 18:00; participants were asked to avoid caffeine, nicotine and strenuous exercise for 12 hours before each visit, to have a light breakfast and lunch, and to empty their bladder before the study commenced. The study was approved by the University of Leeds Ethics Committee (BIOSCI 16-001) and written informed consent was obtained from all participants prior to testing.

### **4.2.1 Materials**

#### **4.2.1.1 Musical stimulus**

The follow-up study (see Chapter 3) showed that participants most preferred to listen to music that had classical and ambient qualities in the study room. The study also identified that 60bpm was associated with greatest physiological relaxation. These findings informed the choice of music for the current study: Weightless by Marconi Union (downloaded as an mp3 from Amazon).

#### **4.2.1.2 Questionnaires**

Like the previous study, participants completed several questionnaires at specific time points during the study. The information obtained from these questionnaires aided with the analysis of the outcome measures. The health, Godin Leisure Time, and initial, baseline and recovery VAS questionnaires were the same as those employed in the previous study. The following questionnaires were modified:

1. Musical background questionnaire. Based on feedback from participants in the previous study, the music-making question was removed (due to lack of clarity) and three questions were added. These pertained to singing, music ensemble and music composition activities (see Appendix C.2.).
2. Stimuli VAS questionnaire. As the previous study comprised of musical stimuli only, the stimulus VAS questionnaire needed to be modified for each of the stimuli employed in the current study. This involved adding an extra perceived emotion variable: obtrusiveness, as feedback from previous participants indicated differences in how intrusive the stimuli were perceived. A scale which participants could rate how strongly they felt the tVNS was also included. This was based on pilot testing of the tVNS device which identified differences between volunteers in how strongly they felt the impulses. The stimuli VAS questionnaires for the tVNS only, music only and tVNS + music stimulation periods were the same (see Appendix C.3.). The questionnaire employed for the sham was modified by removing the questions pertaining to audio interpretation, familiarity and room suitability (see Appendix C.4.).

## **4.2.2 Apparatus**

### **4.2.2.1 Net-1000 device**

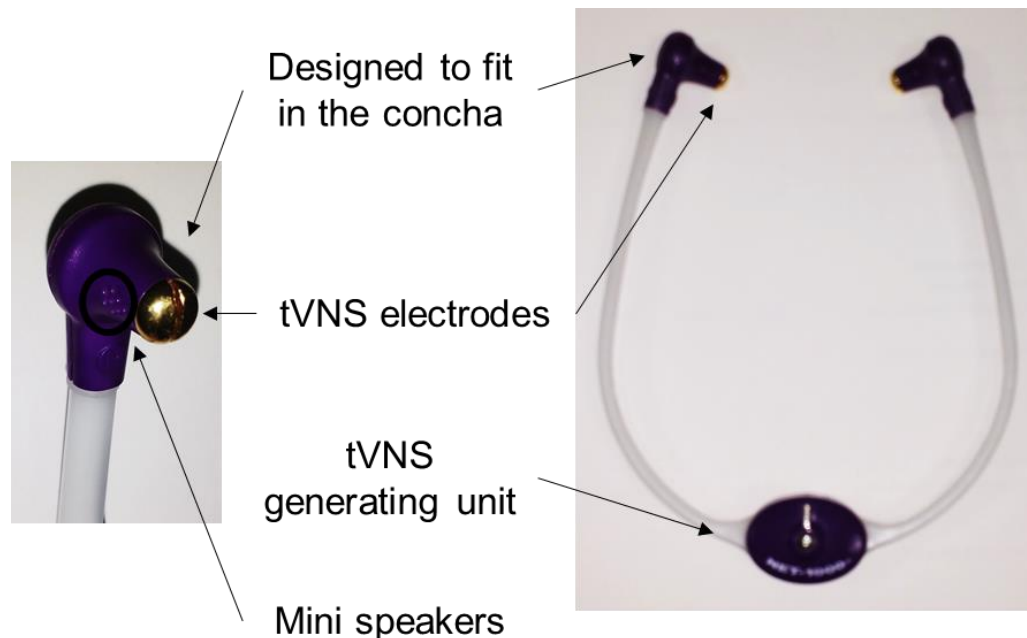
The Net-1000 provides tVNS via electrodes which are embedded into the 'ear-phone' part of the device (see Figure 4.2). These electrodes are placed in the outer ear, on the concha, as opposed to in the ear canal or on the tragus. The tVNS generating unit and battery are integrated into the device. The intensity of the tVNS ranges from 90 $\mu$ A to 130 $\mu$ A (below sensory threshold) and can be selected by the user. For the current study, an intensity of 130 $\mu$ A was selected. This rationale was three-fold:

1. The goal of the study was to determine whether it had any impact on cardiac autonomic function and subjective emotion. The goal of the study was not to determine the lowest current that elicited a response.
2. It was the highest available setting on the device.

3. The range of tVNS intensities available on the device was lower than those employed in other tVNS studies. Therefore, 130 $\mu$ A was selected so that the tVNS current was closer to that used in other studies.

The tVNS had a fixed stimulation frequency of 1.5Hz and was programmed to operate for 20-minute cycles with the device shutting off upon completion of the cycle. Pilot testing revealed that the cycle was short: each cycle lasted approximately 18 minutes. The tVNS stimulation was programmed to be accompanied by beeping sounds. These sounds cycled with the tVNS during activation, with one more pronounced beep occurring every 30 seconds. The sounds were presented to participants via micro-speakers which were located underneath the tVNS generating electrodes.

The device also functioned as a music therapy device: music could be presented via the micro-speakers in combination with the tVNS. This involved attaching a mini USB to 3.5mm male to male audio AUX cable from the stimulating/battery unit to an mp3 player. The sounds accompanying the tVNS continued when the device was connected to the mp3 player and playing music.



*Figure 4.2: The Net-1000 device. The Net-1000 device administers tVNS via the electrodes to the concha. It is controlled by the tVNS generating unit located at the bottom of the device. tVNS can be combined with music, which is presented via the mini speakers located in front of the electrodes.*

#### **4.2.2.2 MP3 player**

A SanDisk Sansa Fuse+ MP3 player was used to present the musical stimulus to participants.

#### **4.2.2.3 Physiology equipment**

##### **4.2.2.3.1 Heart rate**

A three-lead ECG was used to monitor and record HR (ADInstruments, UK). Like the previous studies, the electrode pads (Ambu, UK) were attached to the right collarbone and to either side of the bottom of the ribcage. An Omron upper-arm digital BP monitor was also used to obtain instantaneous measures of HR (and BP) at the end of each condition.

##### **4.2.2.3.2 Blood pressure**

A non-invasive BP device (ADInstruments, UK) was used to continuously monitor and record BP. This employed the same method as the Finometer machine, with the exemption of two differences:

1. Two small inflatable cuffs were attached onto the middle phalanx of the middle and fourth fingers on the right hand.
2. The device was set up in LabChart (ADInstruments, UK) to record from the middle finger and to change to the fourth finger after 30 minutes of continuous inflation. This was done to prevent loss of blood circulation in the middle finger.

##### **4.2.2.3.3 Respiration**

Respiration rate per minute was recorded using a piezo-electric transducer (ADInstruments, UK). This was placed in the same location as the previous two studies.

#### **4.2.3 Procedure**

Figure 4.3 summarises the procedure employed. Participants attended the University of Leeds on four occasions spaced at least two weeks apart. At the beginning of the first visit informed consent was obtained and the health, Godin Leisure Time, musical background and initial VAS questionnaires were administered. The height and weight of participants were obtained at

the beginning of every visit. Participants were then asked to lie in a semi-supine position on a couch with a memory foam mattress with pillows supporting the head and lower back. Physiological equipment which continuously recorded HR, BP and respiration were then attached to participants on each testing occasion. Participants underwent an adaptation period of approximately three minutes which allowed cardiovascular measures to stabilise before commencing the baseline recording. Data collection commenced following stabilisation of HR and BP.

In each visit, participants underwent three conditions: baseline (10 minutes: rested with the Net-1000 device in place but turned off); stimulation (15 minutes: tVNS only, music only, tVNS + music, or sham); and recovery (same as baseline). One stimulus was presented per visit and the order of stimulus presentation was randomised between participants (see Figure 4.3). HR, BP and respiration were continuously recorded during the three conditions. At the end of each condition, participants completed a VAS questionnaire and had their brachial HR and BP measured three times using the Omron BP monitor. Following this, an adaptation period of three minutes was implemented to allow HR and BP to stabilise before commencement of the subsequent condition.

Participants were asked to remain still, close their eyes and to refrain from talking and falling asleep during the recordings. The study room was kept at a constant temperature of  $21 \pm 2$  degrees Celsius.

Upon completion of the data collection, physiological recording equipment was detached from participants. Participants who completed all four visits received £30 reimbursement to cover costs incurred by travelling to the University.

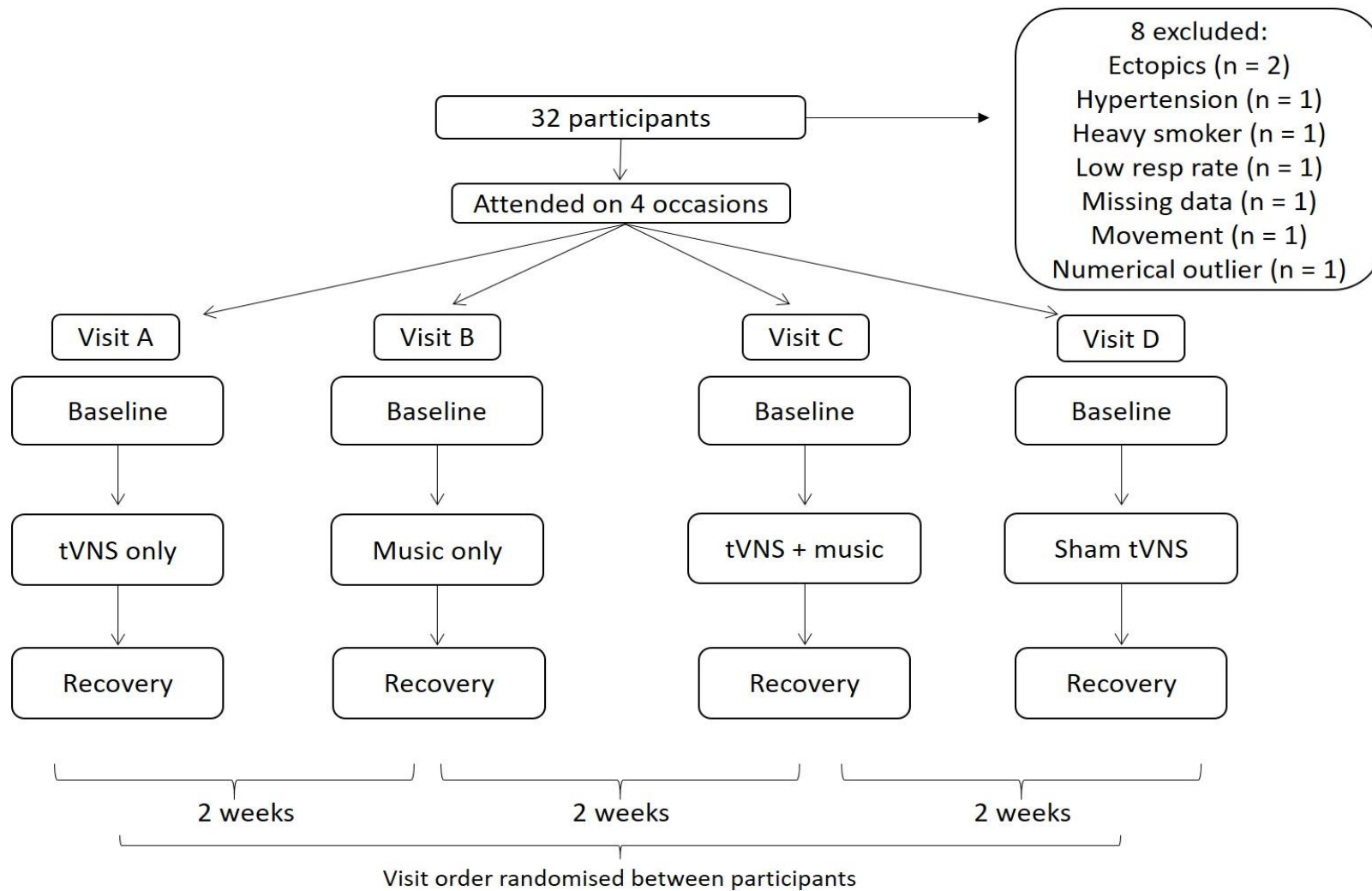


Figure 4.3: Procedure employed in the Net-1000 device study. Thirty-two participants attended on four occasions spaced at least two weeks apart. Over the four visits, participants encountered four different stimuli: tVNS only, music only, their combination (tVNS + music) and sham. Baseline and recovery conditions occurred in all visits.

#### 4.2.4 Data acquisition

ECG, BP, respiration and audio signals were split into four channels, fed into a data amplification system (PowerLab 8/35, ADInstruments, UK) and sampled at 12 kHz. These data were also digitised (PowerLab 8/35, ADInstruments, UK) and passed to a Dell computer to be visualised in real-time and continuously recorded in individual data channels in LabChart software. Each data channel was independently calibrated before digitisation of the incoming signals.

#### 4.2.5 Data analysis

Similar to the previous two studies HRV, HR, ECG, SBP, DBP, MAP, BRS and respiration rate were analysed offline in LabChart software. Values for each measure were derived for each condition in five-minute sections. The last five minutes of each condition (baseline, stimulation and recovery) were used in the statistical analyses.

The second study objective was to assess the extent to which cardiac autonomic function and self-reported emotion differed between the stimulation periods. A range of approaches were tested, these included:

1. Normalising stimulation values relative to baseline
2. Deriving percentage change values (between baseline and stimulation)
3. Calculating absolute change (between baseline and stimulation)

For some participants, some measures at baseline were zero. This was particularly the case for pRR50 and the BRS measures. Consequently, this resulted in using absolute change (between the last five minutes of the baseline and stimulation conditions) to examine objective two. Absolute change values were calculated for every measure in Excel.

To address the third objective the sample was split in to eight sub-groups: males (n = 12) and females, young (n = 10) and older participants, musicians (n = 7) and non-musicians, participants who detected (n = 14) and did not detect tVNS. Appendices C.5.-C.8. provide summaries of the characteristics of each sub-group. The seventh group comprised of

participants who provided a score  $\geq 5$  on the 'felt tVNS' VAS. The final group consisted of participants who gave a score  $< 5$ .

To address the final objective, percentage change values were derived for all measures. The following formula was used:

$$\left( \frac{(\text{new value} - \text{old value})}{\text{old value}} \right) \times 100$$

#### 4.2.6 Statistical analysis

Like the previous two studies, normality was tested for via the Shapiro-Wilk test. If data were not normally distributed non-parametric equivalents were implemented.

To fulfil the first and second objectives (is there an effect of stimulation and does one type of stimulation provoke greater changes than the other types?) repeated measure ANOVAs (or Friedman Tests) were performed. For the first objective, these tests were conducted on the raw values, where the within-participants variable was condition (baseline, stimulation, recovery). For the second objective, repeated measure ANOVAs (or Friedman Tests) were performed on the absolute change values, where the within-participants variable was visit (tVNS only, music only, tVNS + music, sham).

To examine the third objective (are pre-existing individual differences associated with different response patterns?) the data set was split accordingly and repeated measure ANOVAs (or Friedman tests) were performed: both on the raw (within-participants variable was condition) and absolute change (within-participants variable was visit) values.

To fulfil the fourth objective (is it possible to predict response type based on baseline readings?) linear regressions were performed between baseline and percentage change values. To examine differences between responder types, independent sample t-tests (or Mann-Whitney U) and one-way ANOVAs (or Kruskal-Wallis tests) were performed.

For statistically significant ANOVAs, Bonferroni pairwise comparisons were performed. The Greenhouse-Geisser correction was used when data did not meet sphericity.



For statistically significant Friedman tests, pairwise comparisons were performed using the Wilcoxon Signed-Ranks test. As the Wilcoxon did not automatically adjust the alpha level for multiple comparisons, the p-value was adjusted by dividing it by the number of pairwise comparisons made. So, in the case of three pairwise comparisons for a statistically significant Friedman test, the alpha level was set to 0.017. In the instance of six pairwise comparisons the alpha level was set to 0.008.

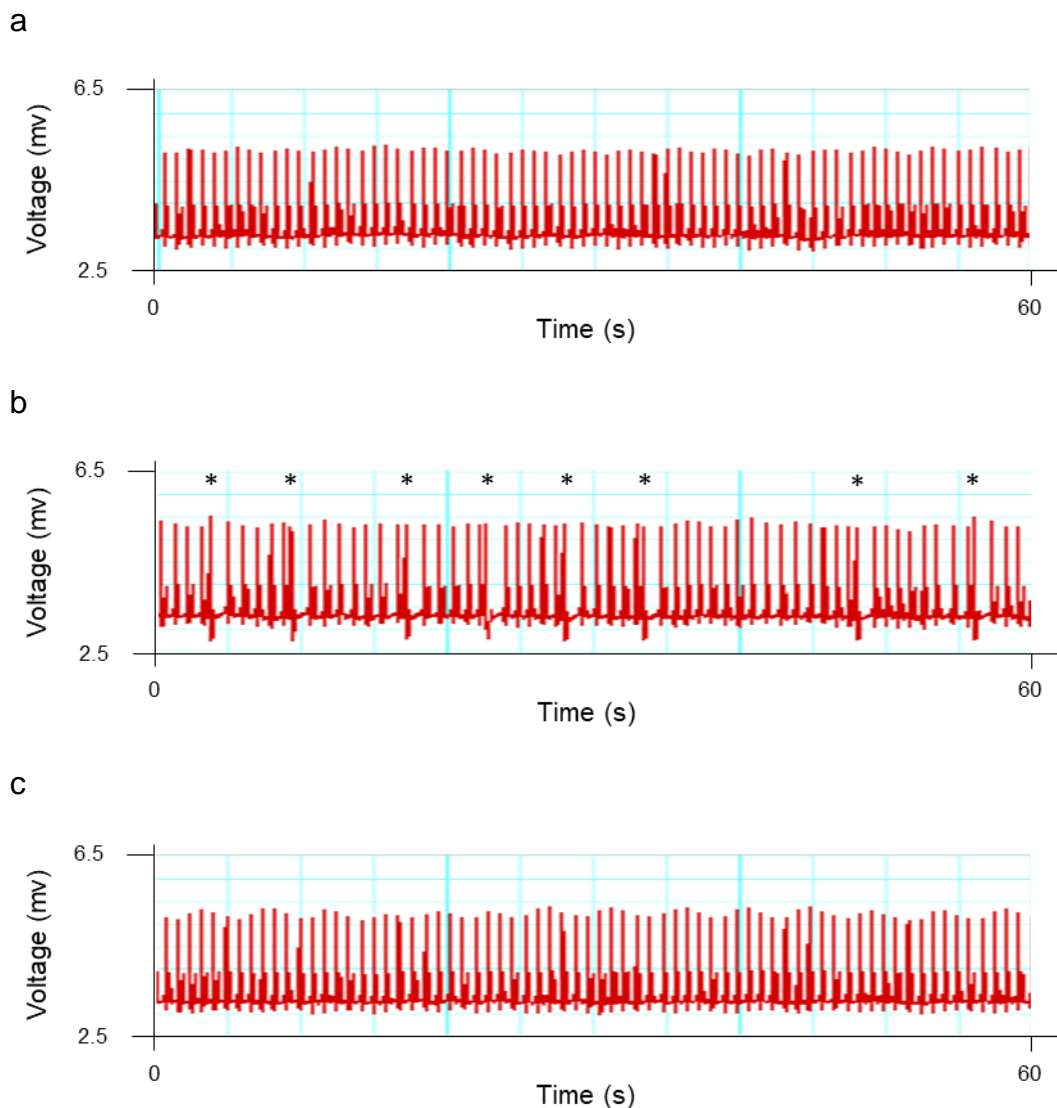
#### **4.2.7 Study failures**

In total, 32 volunteers took part in the study. Data from eight participants were excluded, the reason and their characteristics are presented in Table 4.2. Appendix C.9. provides a summary of the final sample of 24 participants (12 males).

Table 4.2: Net-1000 device study failures.

	<b>Gender</b>	<b>Age (yrs)</b>	<b>Physically active?</b>	<b>Formally music trained?</b>	<b>Reason</b>
1	F	46	N	Y	≥ two ectopic heartbeats in any given five-minute section
2	F	36	Y	Y	≥ two ectopic heartbeats in any given five-minute section
3	M	41	Y	Y	Hypertension
4	F	40	Y	Y	Respiration rate < 10 breaths per minute
5	M	24	Y	Y	Heavy smoker (10/day)
6	F	23	N	N	Fidgeted throughout
7	M	23	Y	Y	Extreme response during the last five minutes of the tVNS + music stimulus, which skewed the results
8	F	23	Y	N	Missing data due to BP cuff malfunction

The ectopic heartbeats that occurred in exclusion 1 showed an interesting pattern. During baseline in all four visits, there were minimal ectopic heartbeats (~one every five minutes). However, in the tVNS only and tVNS + music there were profound increases in the number of ectopic heartbeats (~20 in five minutes). Then in recovery, the frequency of ectopic heartbeats reduced to baseline values. Figure 4.4 provides examples of the change in the number of ectopic heartbeats that occurred in one-minute sections during baseline (a), stimulation (b) and recovery (c) in the tVNS only visit (identified with \*).



*Figure 4.4: Pattern of ectopic heart beats in exclusion 1. No ectopics occurred during baseline (a) or recovery (c). But when the tVNS was administered (b) the frequency of ectopics increased.*

Minimal side-effects of tVNS have been reported, including: skin irritation at the electrode site; local electrode pressure; spontaneous muscle contractions; headaches; dizziness and fatigue (Kreuzer et al., 2012). These side effects are normally transient, disappearing following tVNS termination. However, more adverse effects, like palpitations, have been noted (Kreuzer et al., 2012; Magis, Gérard & Schoenen, 2013). The likelihood of these adverse effects occurring are reportedly enhanced when tVNS is administered to the right and left ears. This is because the right ear has afferent vagal nerve fibres that travel to the NTS, with efferent vagal activity being sent to the SA node. Although left afferent vagal nerve fibres are also sent to the NTS, the subsequent efferent activity is sent to the AV node (George et al., 2000; Kreuzer et al., 2012).

## 4.3 Results

The study aimed to examine the effects of relaxing music in combination with tVNS on cardiac autonomic function and self-reported emotion. It was anticipated that each stimulus (tVNS only, music only and tVNS + music), but not the sham, would be associated with increases in parasympathetic predominance (LF/HF) and subjective relaxation compared to baseline. This was examined by looking at each visit separately.

Furthermore, it was expected that tVNS + music would be associated with greater parasympathetic predominance (LF/HF) and subjective relaxation than the other visits. This was explored by comparing the absolute change values (from baseline to stimulus) between the four visits (henceforth called between-visit comparisons).

The results section reports the physiological measure results followed by the self-report. The physiological measure results section is split into seven sub-sections, which address in turn the: 1) time-domain HRV; 2) frequency-domain HRV; 3) non-linear HRV; 4) RR; 5) ECG; 6) BP, BRS and respiration; and 7) brachial BP and HR analyses. In each sub-section, the effect of each stimulus and between-visit comparisons will be explored.

The self-report results section is split into two sub-sections which will address in turn the: 1) experienced emotion; and 2) perceived emotion analyses. Similar to the physiological measures, the effect of each stimulus and between-visit comparisons will be examined.

### 4.3.1 The effects of the four stimuli on cardiac autonomic control

First, differences in baseline measures between the four visits were ascertained. No significant differences in any of the measures of autonomic function for the full sample, males, females and older participants emerged ( $p > 0.05$ ). However, differences emerged in young participants, non-musicians, musicians and participants who detected tVNS. Table 4.3 provides a summary. As a result, the between-visit analyses needed to control for these differences in baseline measures. This was achieved by using the absolute change values.

Table 4.3: Statistically significant differences in baseline autonomic measures between the four visits.

Sample	Measure	ANOVA p-value	Pairwise comparison (p-value)	
			Comparison	p-value
Females	Brachial SBP	0.001	tVNS > tVNS + music	0.014
			tVNS > music	0.012
			tVNS > sham	0.023
	Brachial HR	0.05	tVNS > tVNS + music	0.045
Young	UP BRS	0.039	music > tVNS	0.042
	MEAN BRS	0.028	tVNS + music > tVNS	0.042
Non-musicians	MEAN BRS	0.010	sham > tVNS	0.038
Musicians	Mode RR	0.010	music > sham	0.040
	Min RR	0.006	music > tVNS	0.035
	Q1 RR	0.013	music > sham	0.041
Felt tVNS	QTc	0.034	sham > tVNS + music	0.031

#### 4.3.1.1 Time-domain HRV analysis

##### 4.3.1.1.1 tVNS only visit

tVNS had no significant effect on HR in the full sample, males, females, young and older participants, non-musicians, and those who did and did not detect tVNS ( $p > 0.05$ ). But, a significant effect of tVNS on HR emerged in musicians ( $p = 0.012$ ). As shown in Figure 4.5, HR was significantly lower in tVNS than baseline ( $p = 0.016$ ).

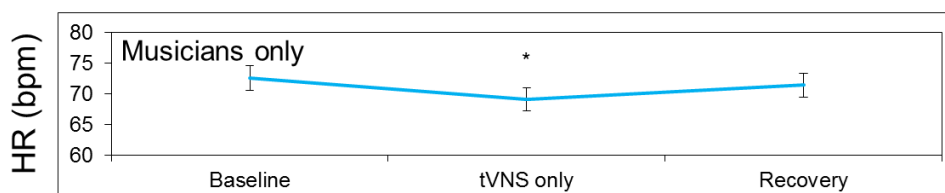


Figure 4.5: HR was significantly impacted by tVNS in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

A significant effect of tVNS for SDRR was identified in the full data set ( $p = 0.011$ ). SDRR was significantly higher during tVNS compared to recovery ( $p = 0.043$ , see Figure 4.6).

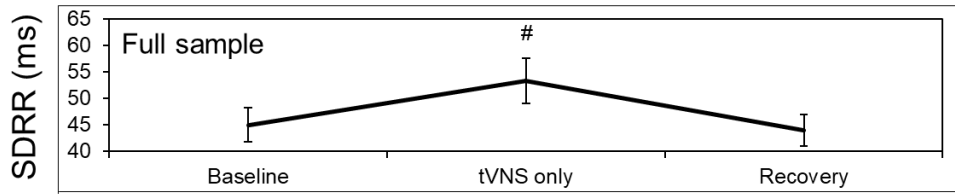


Figure 4.6: SDRR was significantly impacted by tVNS in the full sample. Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery.

tVNS had significant effects on SDRR in the full sample ( $p = 0.013$ , see Figure 4.7). Pairwise comparisons revealed that SDRR was significantly higher during tVNS than baseline ( $p = 0.037$ ). Musicians also showed significant changes in SDRR ( $p = 0.003$ , see Figure 4.7). Pairwise comparisons revealed that SDRR was significantly higher during tVNS compared to baseline ( $p = 0.015$ ) and recovery ( $p = 0.030$ ).

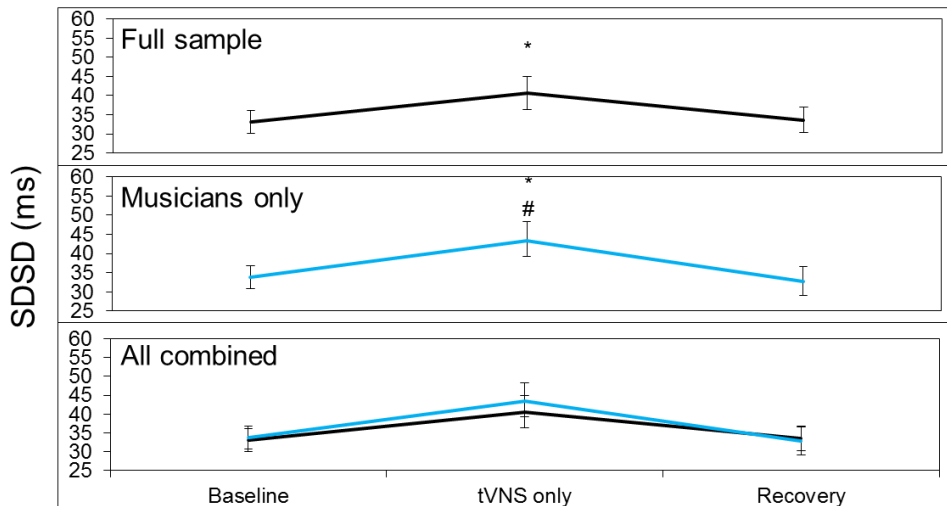


Figure 4.7: SDRR was significantly impacted by tVNS in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

RMSSD was also significantly impacted by tVNS in the full sample ( $p = 0.013$ , Figure 4.8). Pairwise comparisons revealed that RMSSD was significantly higher during tVNS than baseline ( $p = 0.037$ ). Additionally, musicians showed significant changes in RMSSD ( $p = 0.003$ , see Figure 4.8). Pairwise comparisons revealed that RMSSD was significantly higher during tVNS compared to baseline ( $p = 0.015$ ) and recovery ( $p = 0.030$ ).

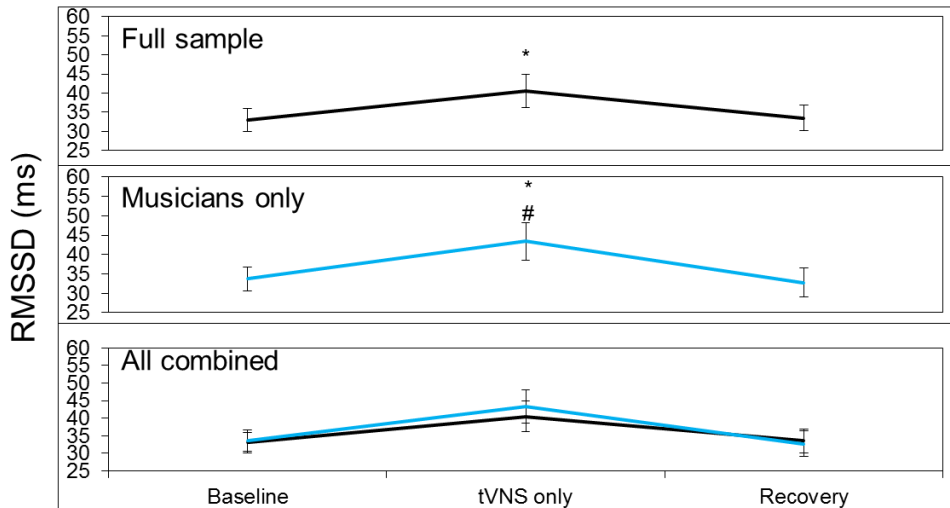


Figure 4.8: RMSSD was significantly impacted by tVNS in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

There were significant effects of tVNS on pRR50 in the full sample ( $p = 0.012$ , see Figure 4.9). Pairwise comparisons revealed that pRR50 was significantly higher during tVNS than baseline ( $p = 0.012$ ). Musicians also showed significant changes in pRR50 ( $p = 0.003$ , see Figure 4.9). Pairwise comparisons revealed that pRR50 was significantly higher during tVNS compared to baseline ( $p = 0.002$ ) and recovery ( $p = 0.006$ ).

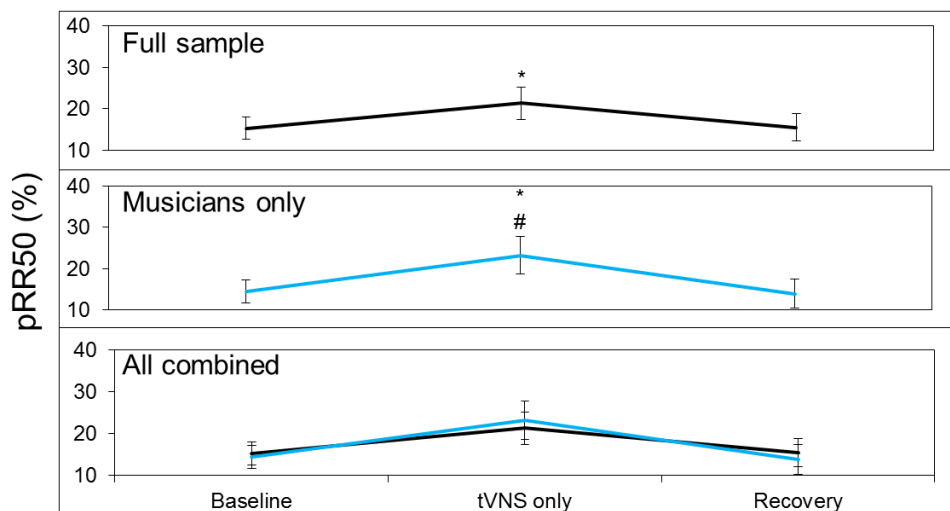


Figure 4.9: pRR50 was significantly impacted by tVNS in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.



#### 4.3.1.1.2 Music only visit

The effect of music on HR emerged as statistically significant for the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.001$ ), musicians ( $p = 0.010$ ) and those who detected tVNS ( $p = 0.014$ , see Figure 4.10). Pairwise comparisons revealed that HR was significantly lower during music compared to baseline for the full data set ( $p = 0.002$ ), females ( $p < 0.001$ ), older participants ( $p = 0.008$ ), musicians ( $p = 0.010$ ) and those who detected tVNS ( $p = 0.025$ ). Interestingly, recovery HR was significantly lower than baseline HR for females ( $p = 0.018$ ) and older participants ( $p = 0.022$ ).

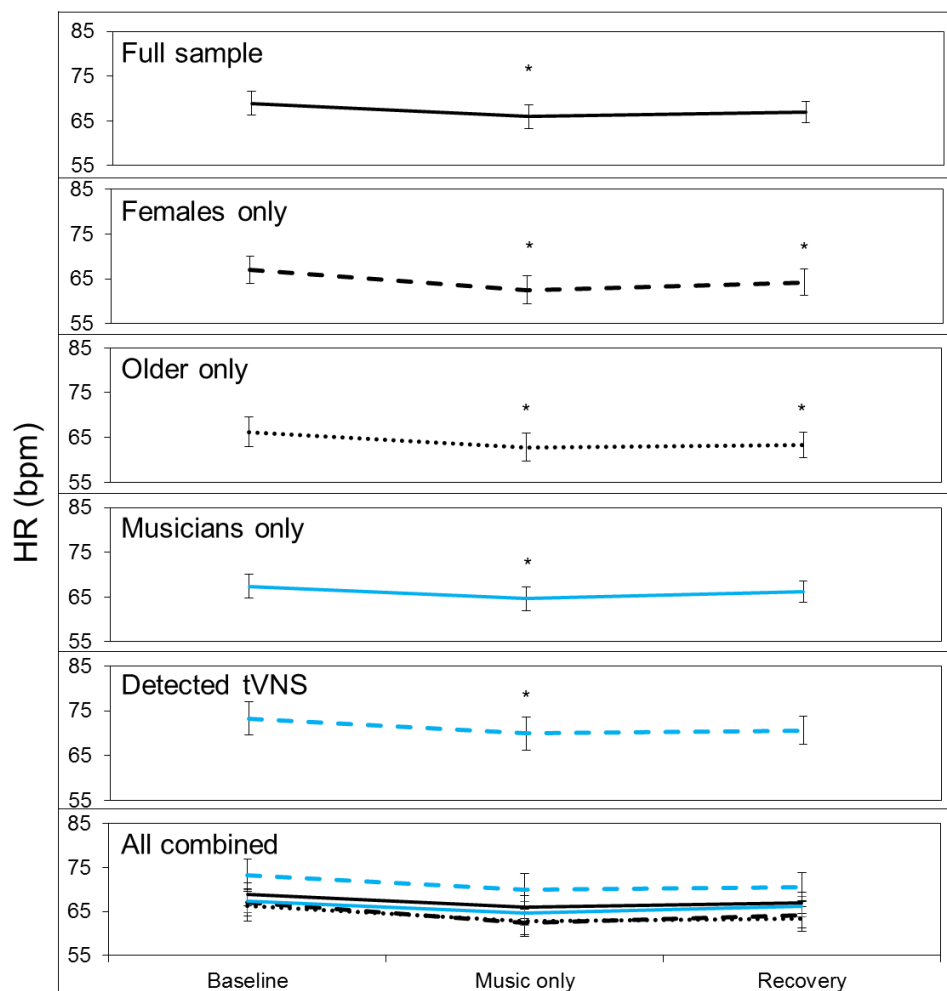
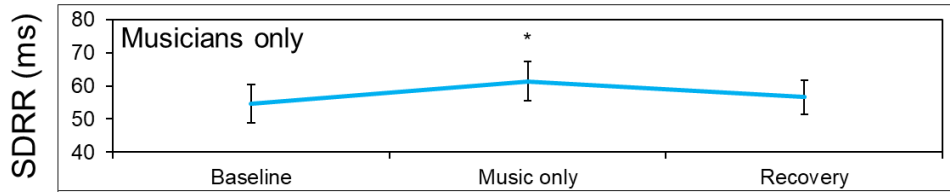


Figure 4.10: HR was significantly impacted by music in the full sample, females, older participants, musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Finally, an effect of stimulus on SDRR was only detected in the group of musicians ( $p = 0.047$ ). Bonferroni revealed that SDRR was significantly higher during music than baseline ( $p = 0.018$ , see Figure 4.11).



*Figure 4.11: SDRR was significantly impacted by music in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.*

The effect of music on SDSD was significant for the full sample ( $p = 0.006$ ); females ( $p = 0.006$ ), older participants ( $p = 0.002$ ), musicians ( $p = 0.023$ ) and those who detected tVNS ( $p = 0.020$ , see Figure 4.12). Further analysis identified that SDSD was significantly higher during music than during baseline (full data set:  $p = 0.002$ ; females:  $p = 0.010$ ; older participants:  $p = 0.004$ ; musicians:  $p = 0.009$ ; detected tVNS:  $p = 0.022$ ).

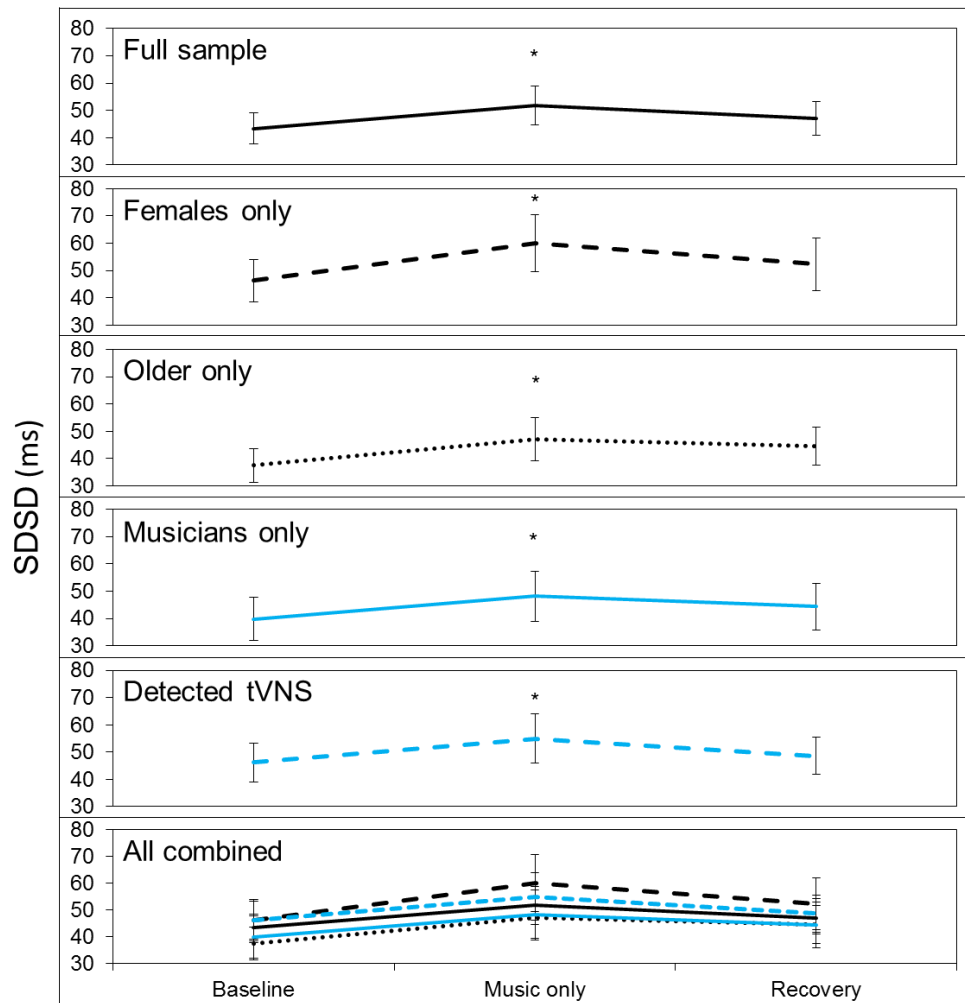


Figure 4.12: SDSD was significantly impacted by music in the full sample, females, older participants, musicians only and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Music only also significantly impacted RMSSD for the full sample ( $p = 0.006$ ); females ( $p = 0.006$ ), older participants ( $p = 0.002$ ), musicians ( $p = 0.023$ ) and those who detected tVNS ( $p = 0.020$ , see Figure 4.13). RMSSD was significantly higher during music than during baseline (full data set:  $p = 0.002$ ; females:  $p = 0.010$ ; older participants:  $p = 0.004$ ; musicians:  $p = 0.009$ ; detected tVNS:  $p = 0.022$ ).

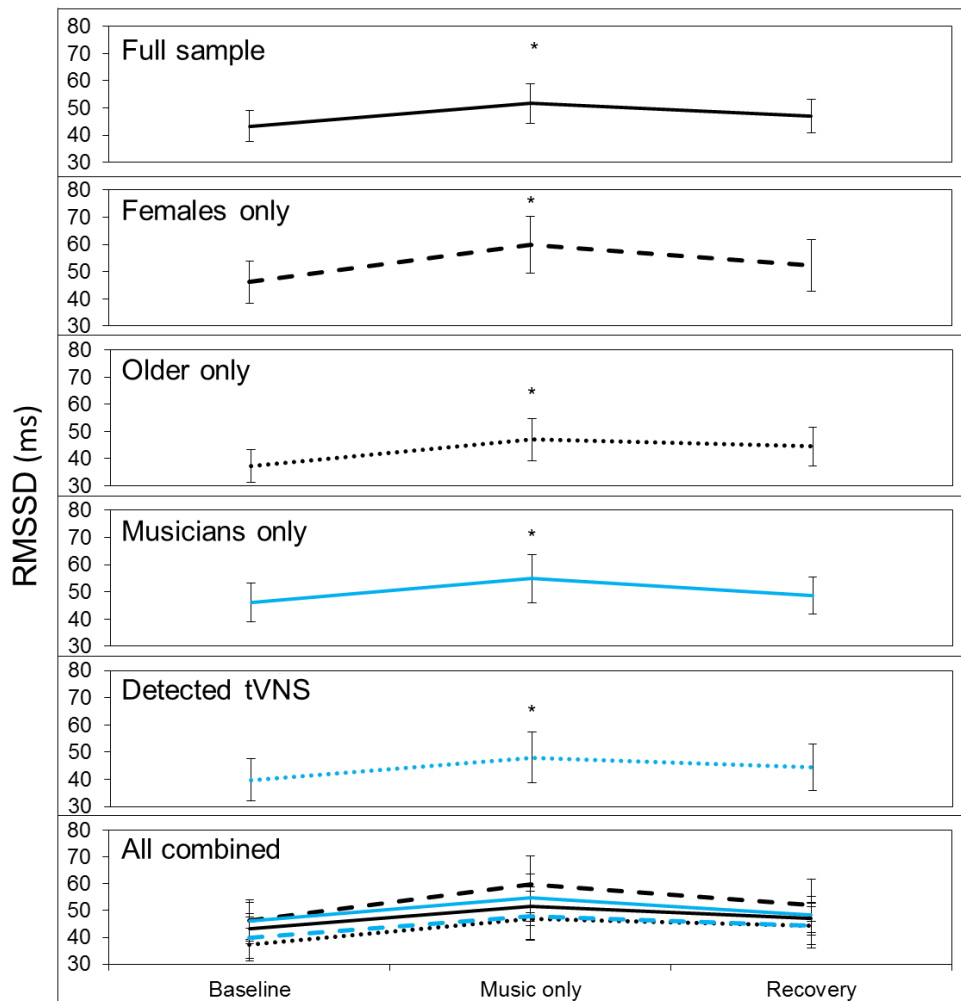


Figure 4.13: RMSSD was significantly impacted by music in the full sample, females, older participants, musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

pRR50 significantly differed over the course of the music visit (effect of music). This occurred in females ( $p = 0.011$ ) and musicians ( $p = 0.022$ , see Figure 4.14). Pairwise comparisons revealed that pRR50 was significantly higher during music compared to baseline (females:  $p = 0.014$ ; musicians:  $p = 0.010$ ).

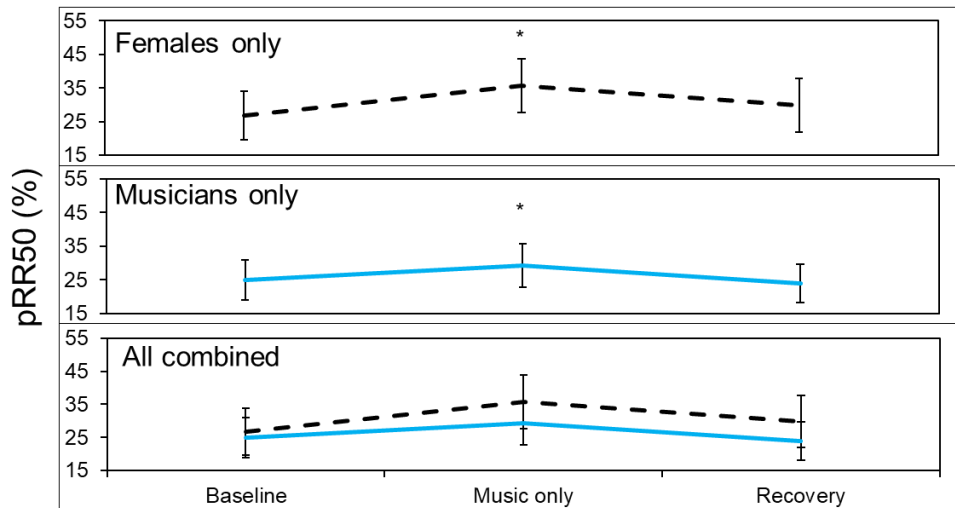


Figure 4.14: *pRR50* was significantly impacted by music in females and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.1.3 tVNS + music visit

There were statistically significant effects of tVNS + music on HR for the full sample ( $p = 0.011$ ), females ( $p = 0.001$ ) and those who detected tVNS ( $p = 0.009$ , see Figure 4.15). Pairwise comparisons revealed that HR was significantly lower during tVNS compared to baseline for the full data set ( $p = 0.005$ ), females ( $p = 0.002$ ) and those who detected tVNS ( $p = 0.047$ ). Surprisingly, those who detected tVNS showed a significant decrease in HR between baseline and recovery ( $p = 0.026$ ).

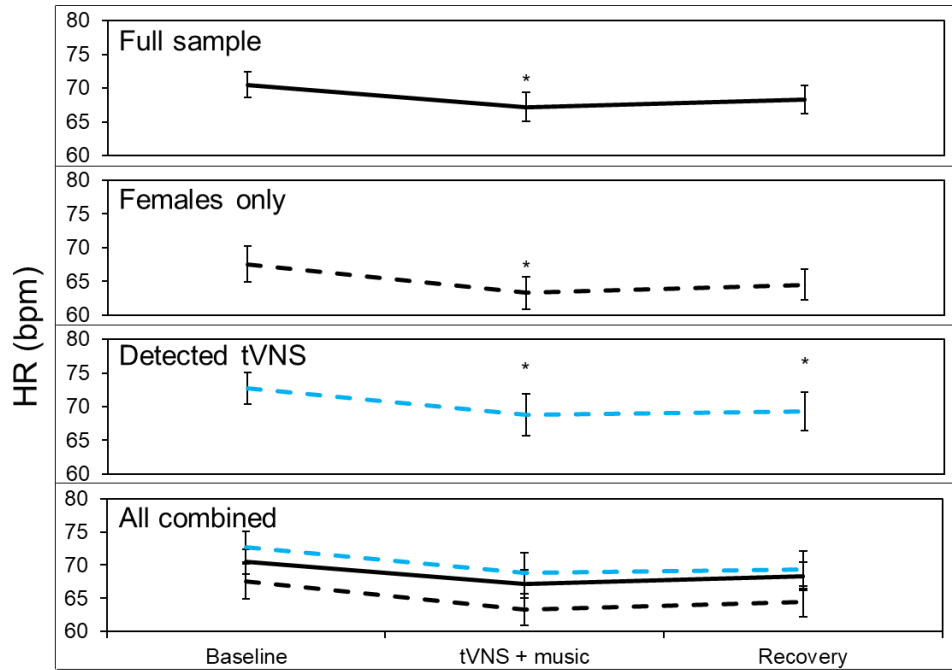
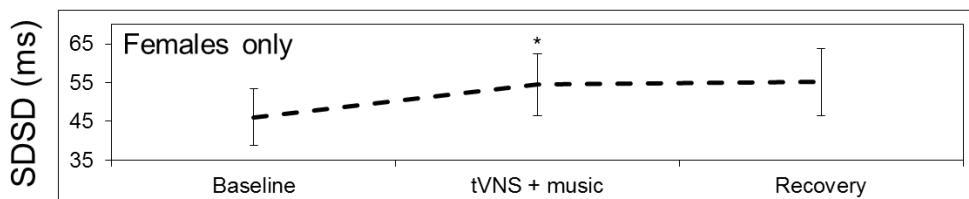


Figure 4.15: HR was significantly impacted by tVNS + music in the full sample, females and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

The effect of music on SDSD and RMSSD emerged only for females ( $p = 0.011$  for both variables). As shown in Figure 4.16a and b, SDSD and RMSSD were higher during tVNS + music than baseline ( $p = 0.030$ ).

a.



b.

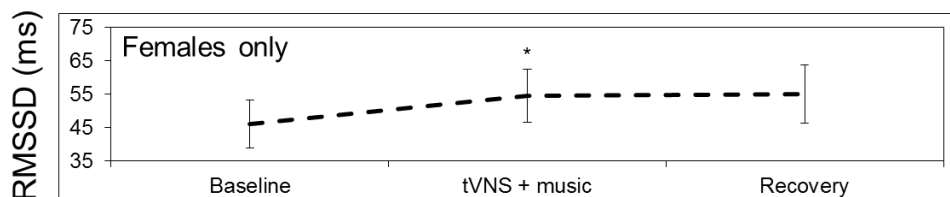


Figure 4.16: SDSD (a) and RMSSD (b) were significantly impacted by the tVNS + music stimulus in females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Nevertheless, pRR50 significantly differed for the full sample ( $p = 0.037$ ) and females ( $p = 0.008$ , see Figure 4.17). pRR50 was significantly higher during tVNS + music than baseline for the full sample ( $p = 0.008$ ) and females ( $p = 0.030$ ).

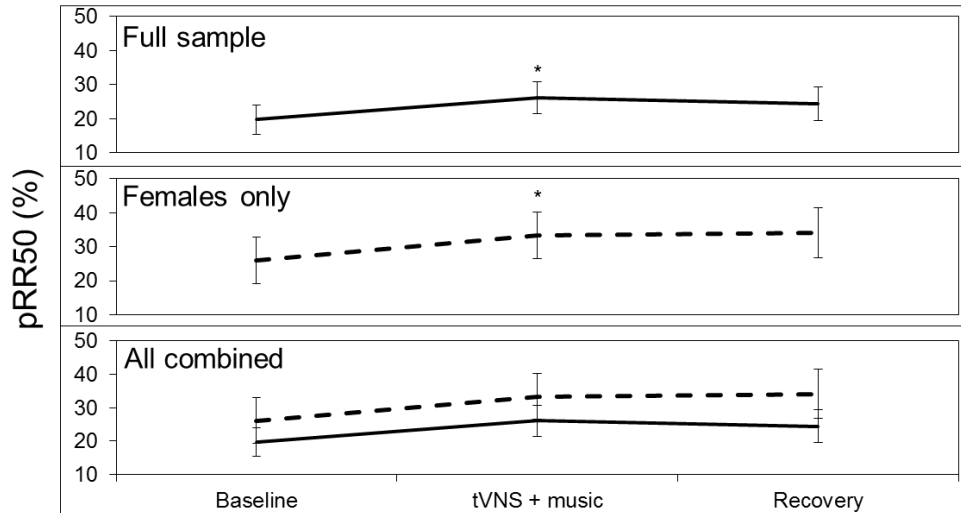


Figure 4.17: pRR50 was significantly impacted by tVNS + music in the full sample and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.1.4 Sham visit

Sham significantly impacted HR for the full sample ( $p < 0.001$ ), females ( $p = 0.017$ ), young participants ( $p = 0.006$ ), older participants ( $p = 0.002$ ), non-musicians ( $p = 0.005$ ), musicians ( $p = 0.001$ ), those who did ( $p < 0.001$ ) and did not detect tVNS ( $p = 0.006$ , see Figure 4.18). Pairwise comparisons showed that HR significantly decreased during the sham in all group analyses (full sample:  $p < 0.001$ ; females:  $p = 0.001$ ; older participants:  $p < 0.001$ ; younger participants:  $p = 0.005$ ; non-musicians:  $p = 0.007$ ; musicians:  $p < 0.001$ ; those who detected tVNS:  $p < 0.001$  and those who did not:  $p = 0.009$ ). Additionally, HR was significantly lower during sham compared to recovery for the full sample ( $p = 0.047$ ) and those who did not detect tVNS ( $p = 0.041$ ). Also, for participants who detected tVNS, HR was significantly lower during recovery than baseline ( $p = 0.005$ ).

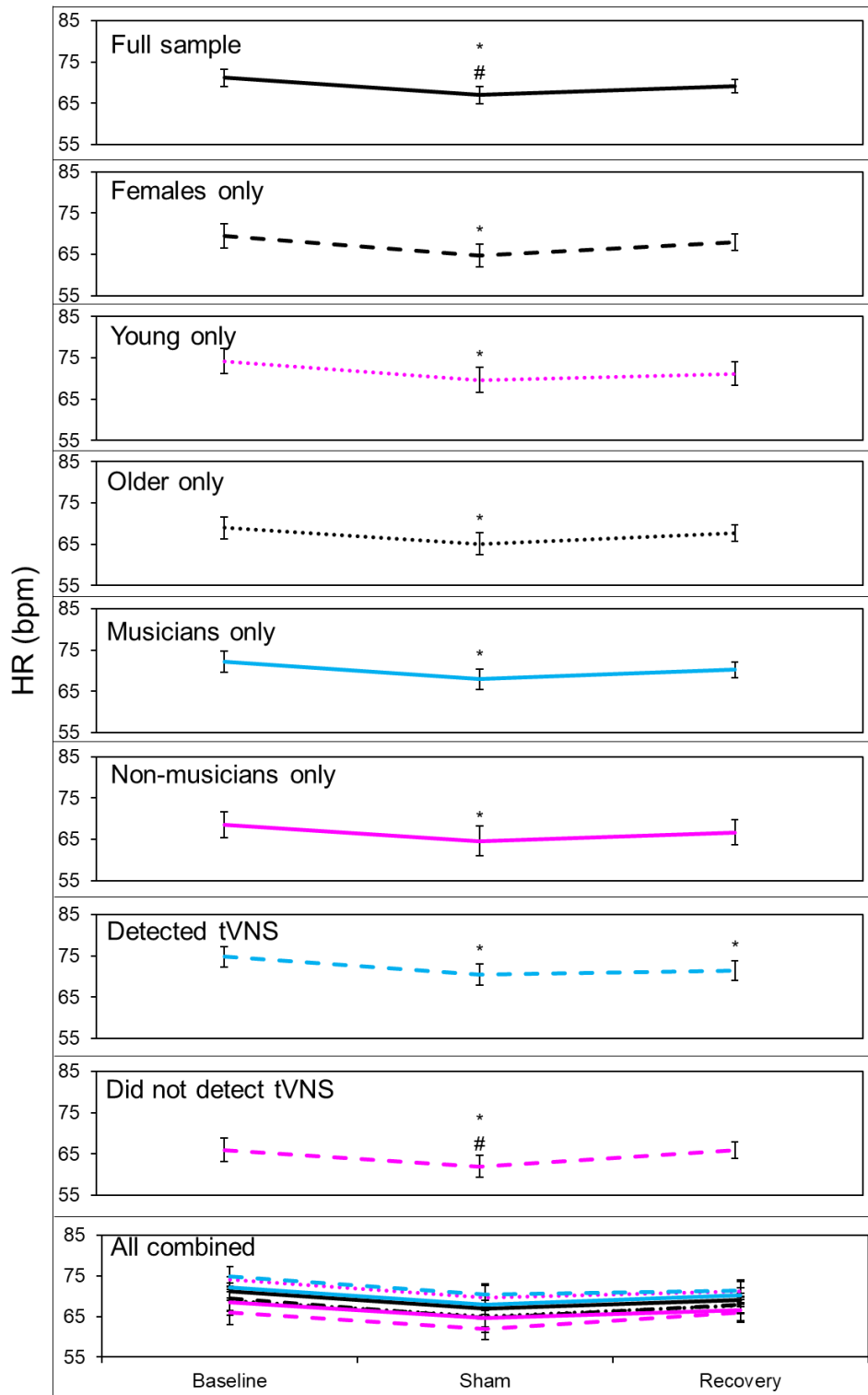


Figure 4.18: HR was significantly impacted by sham in all groups except males. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Figure 4.19 shows that a significant effect of sham on pRR50 was observed in the full sample ( $p = 0.002$ ), males ( $p = 0.028$ ), females ( $p = 0.046$ ), older



participants ( $p = 0.030$ ), musicians ( $p = 0.007$ ) and those who detected tVNS ( $p = 0.007$ ). pRR50 was significantly higher during sham than baseline for all group analyses (full sample:  $p < 0.001$ ; males:  $p = 0.006$ ; females:  $p = 0.012$ ; older participants:  $p = 0.004$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.003$ ). In addition, sham pRR50 was significantly lower than recovery pRR50 for the full sample ( $p = 0.004$ ) and females ( $p = 0.012$ ).

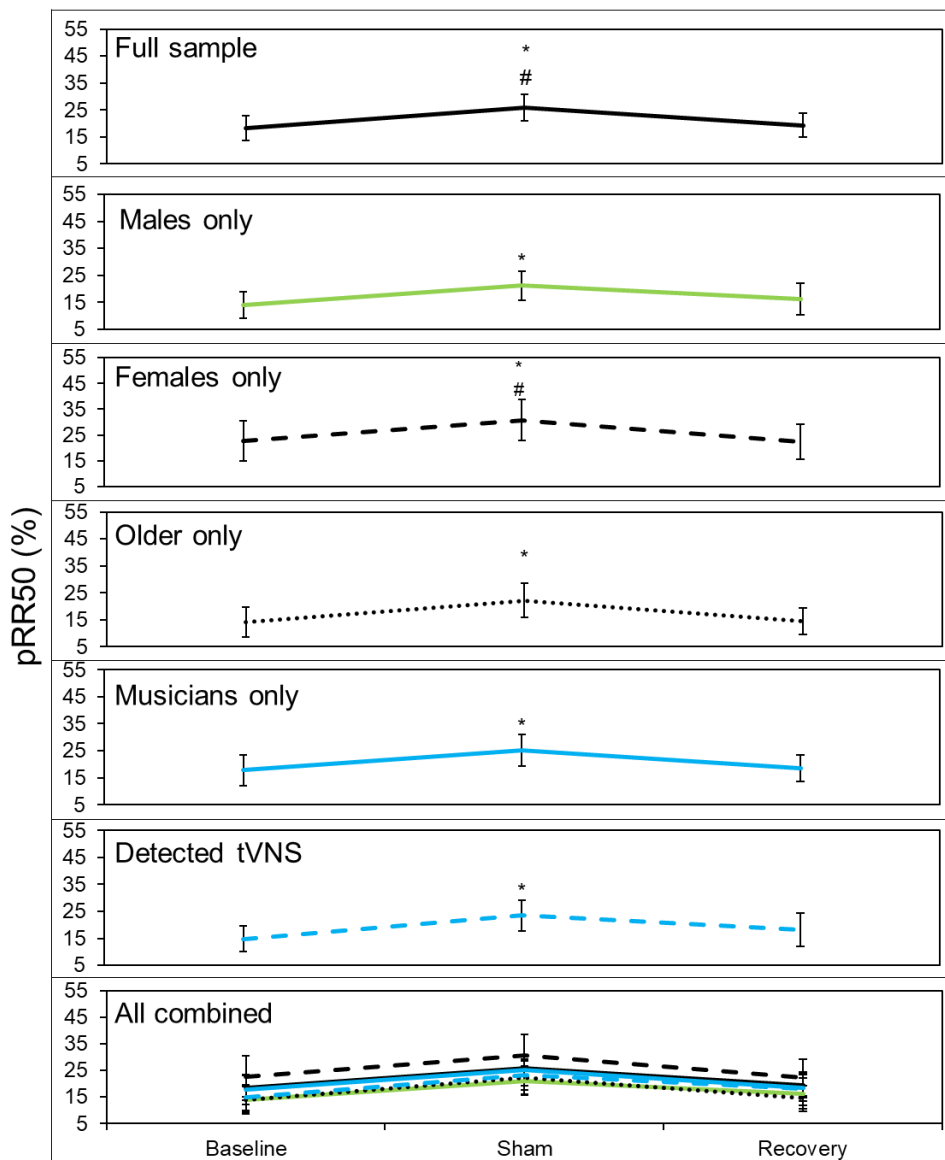
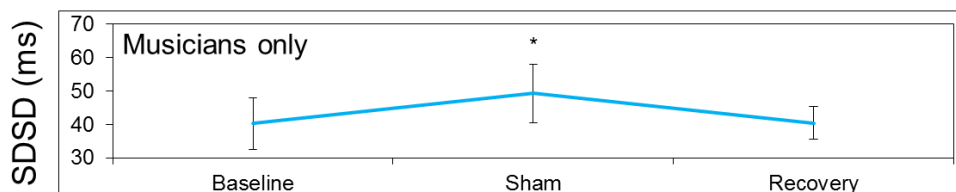


Figure 4.19: pRR50 was significantly impacted by sham in all groups except young participants, non-musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Significant effects of sham on SDSD and RMSSD were detected in the group of musicians (both  $p = 0.023$ , Figure 4.20a and b). SDSD and RMSSD were significantly greater in sham than baseline ( $p = 0.004$  for both variables).

a.



b.

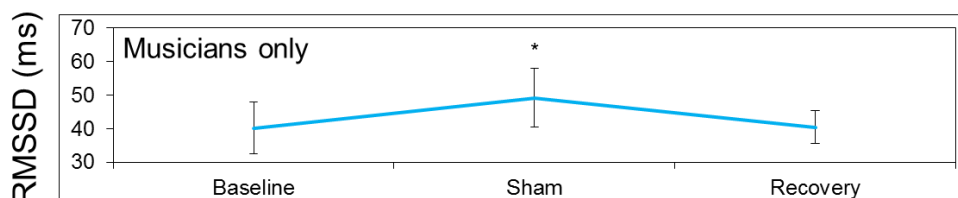


Figure 4.20: SDSD (a) and RMSSD (b) were significantly impacted by sham in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.1.5 Between-visit comparisons

For all group analyses, none of the time-domain HRV measures significantly differed between the four visits ( $p > 0.05$ ).

#### 4.3.1.2 Frequency-domain HRV analysis

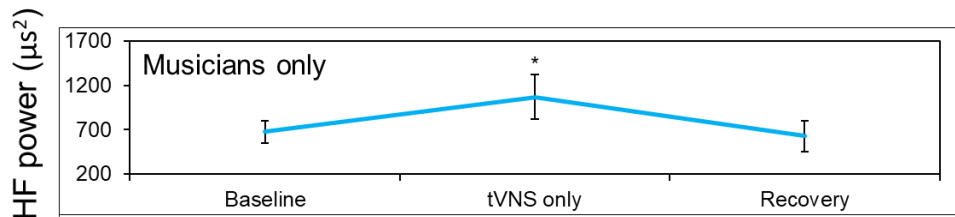
##### 4.3.1.2.1 tVNS only visit

Musicians showed a significant effect of tVNS on HF power ( $p = 0.010$ ). Pairwise comparisons revealed that vagal tone was significantly higher during tVNS compared to baseline ( $p = 0.013$ , see Figure 4.21a).

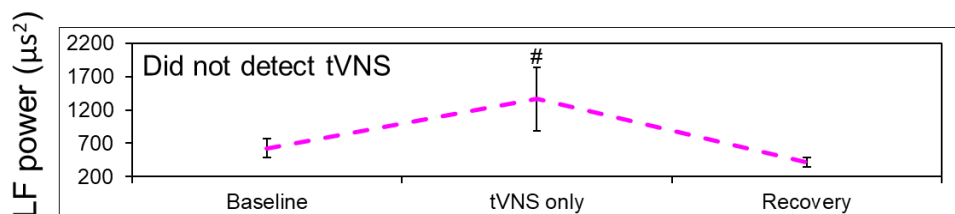
Interestingly, for participants who did not detect tVNS a significant effect of the stimulus on LF power emerged ( $p = 0.045$ ): LF power was significantly higher during tVNS compared to recovery ( $p = 0.013$ , see Figure 4.21b).

VLF% was also found to be impacted by the stimulation for musicians only ( $p = 0.025$ ). As shown in Figure 4.21c, VLF% significantly decreased between baseline and tVNS ( $p = 0.016$ ).

a.



b.



c.

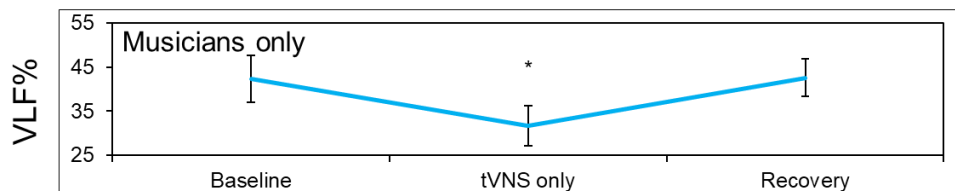


Figure 4.21: HF power (a), LF power (b) and VLF% (c) were significantly impacted by tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.1.2.2 Music only

There was a significant effect of music on VLF% power in the full sample ( $p = 0.009$ , see Figure 4.22). Further analysis showed that VLF% significantly decreased during music compared to baseline ( $p = 0.005$ ). In older participants, a significant effect of music on VLF% also emerged ( $p = 0.008$ ). Further analysis showed that VLF% was significantly lower during music than during baseline ( $p = 0.002$ ). Music also significantly impacted VLF% in

the group who detected tVNS ( $p = 0.013$ ). Pairwise comparisons revealed that VLF% was significantly lower during music than baseline ( $p = 0.011$ ).

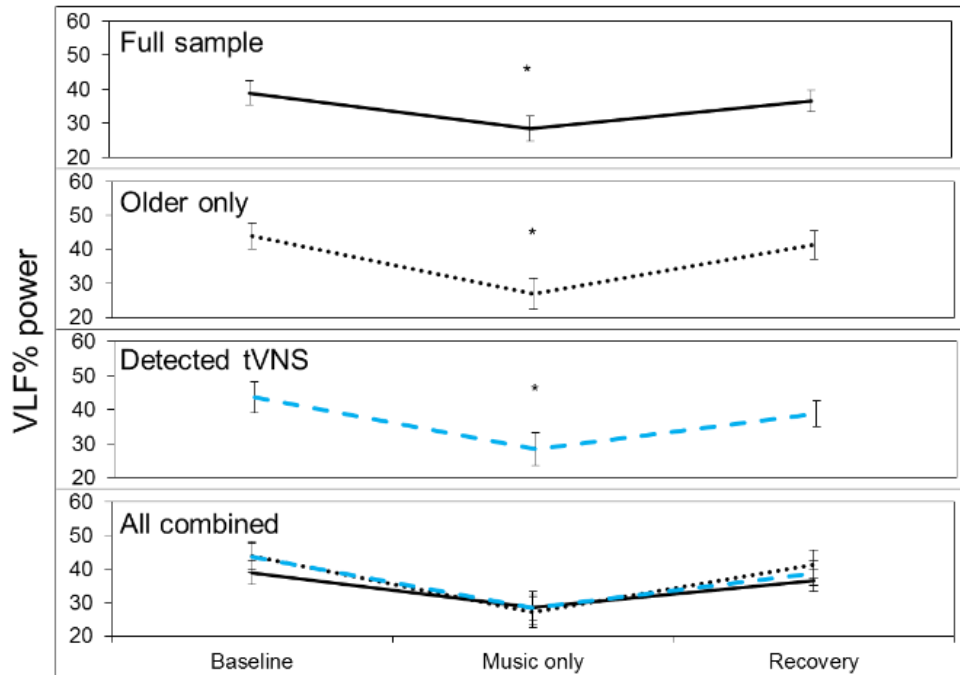


Figure 4.22: VLF% was significantly impacted by music in the full sample, older participants and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of music on LF power in older participants only ( $p = 0.017$ , see Figure 4.23). Further analysis showed that LF power was significantly higher during music than baseline ( $p = 0.016$ ),

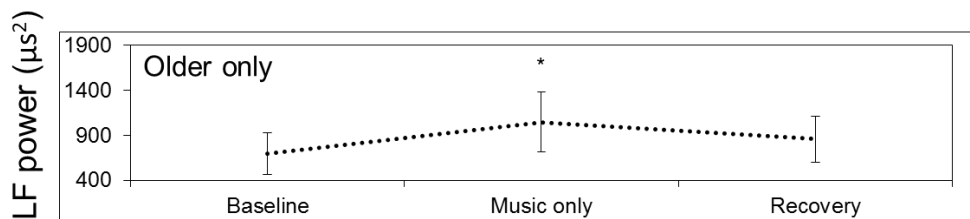
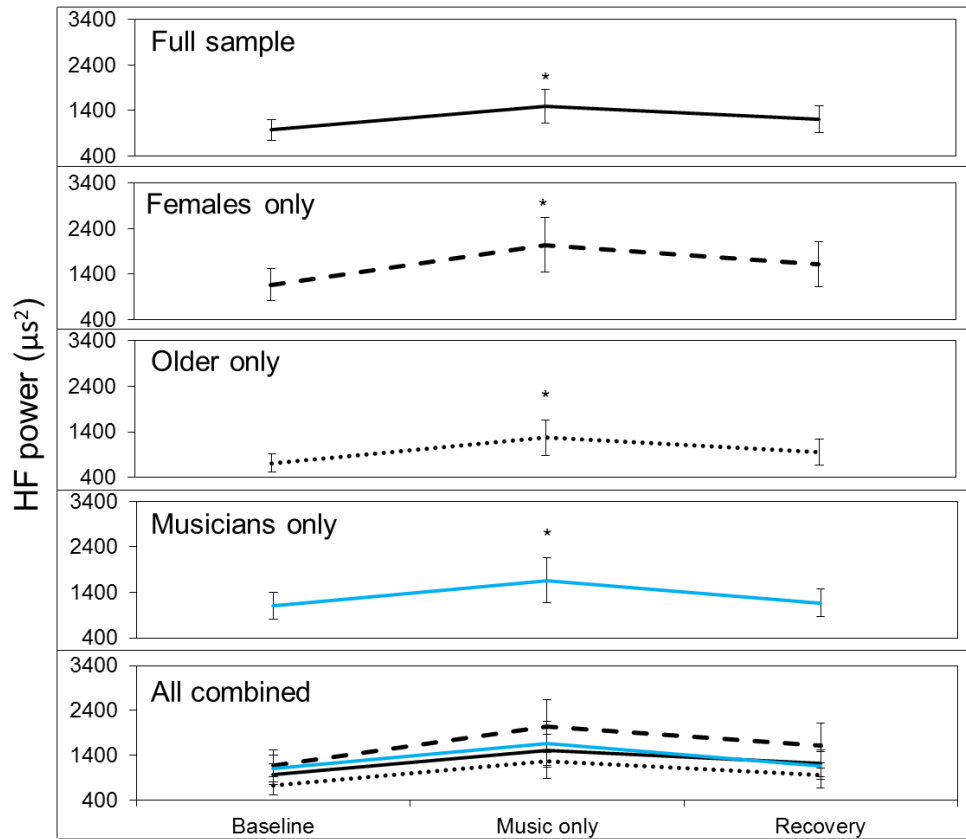


Figure 4.23: LF power was significantly impacted by music in older participants only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of music on HF power in the full sample ( $p = 0.001$ ), females ( $p = 0.001$ ), older participants ( $p = 0.003$ ) and musicians ( $p = 0.003$ , see Figure 4.24). HF power was significantly higher during music

compared to baseline (full sample:  $p = 0.002$ ; females:  $p = 0.002$ ; older participants:  $p = 0.016$ ; musicians:  $p = 0.007$ ).



*Figure 4.24: HF was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.*

A significant effect of music on HF% was also observed in the full sample ( $p = 0.010$ ), females ( $p = 0.008$ ) and those who detected tVNS ( $p = 0.024$ , see Figure 4.25). HF% was significantly higher during music compared to baseline (full sample:  $p = 0.008$ ; females:  $p = 0.018$ ; detected tVNS:  $p = 0.006$ ).

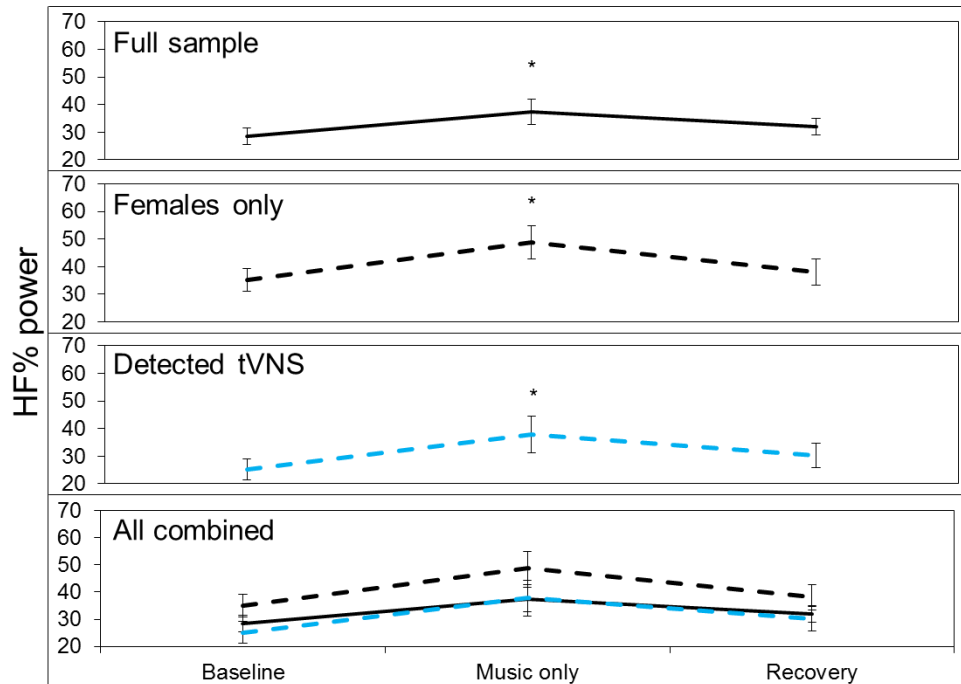


Figure 4.25: HF% was significantly impacted by music in the full sample, females and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.2.3 tVNS + music

For all group analyses, none of the frequency-domain HRV measures significantly differed within the visit ( $p > 0.05$ ).

#### 4.3.1.2.4 Sham visit

HF power significantly differed during the sham visit for the full sample ( $p = 0.011$ ), males ( $p = 0.050$ ) and musicians ( $p = 0.019$ , see Figure 4.26). In the full sample, HF power was significantly higher during sham compared to baseline ( $p = 0.005$ ) and recovery ( $p = 0.004$ ). Males and musicians showed a similar response: HF power was higher in sham compared to baseline (males:  $p = 0.012$ ; musicians:  $p = 0.003$ ).

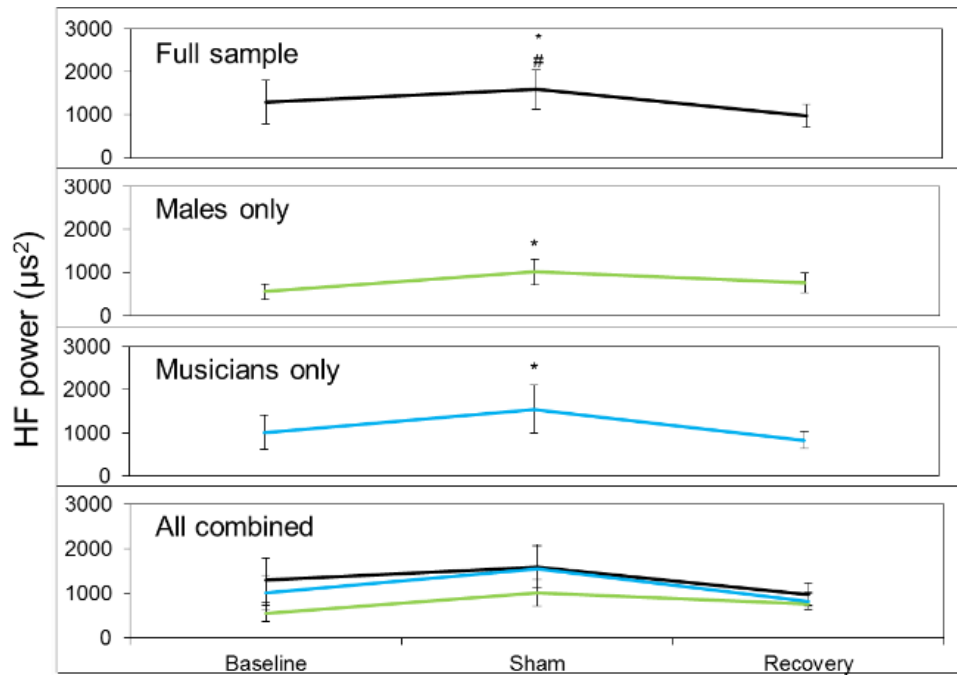


Figure 4.26: HF power was significantly impacted by sham in the full sample, males and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.1.2.5 Between-visit comparisons

The only significant differences that emerged were in non-musicians for VLF% ( $p = 0.009$ ) and HF% ( $p = 0.007$ ). As shown in Figure 4.27, VLF% increased in tVNS but decreased in music ( $p = 0.0017$ ) and HF% decreased in tVNS yet increased in sham ( $p = 0.028$ ).

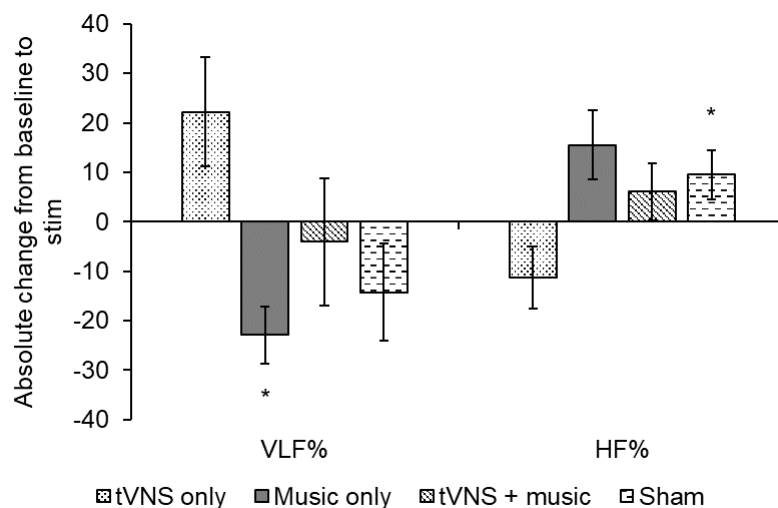
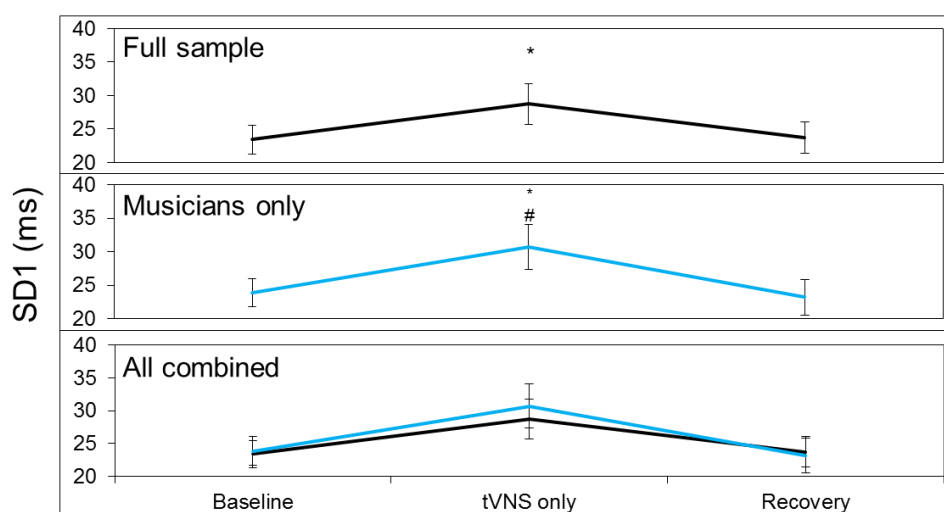


Figure 4.27: VLF% decreased in music but increased in tVNS. HF% increased in sham but decreased in tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to the tVNS only visit.

### 4.3.1.3 Non-linear HRV analysis

#### 4.3.1.3.1 tVNS only visit

tVNS had significant effects on SD1 in the full sample ( $p = 0.013$ ) and musicians ( $p = 0.003$ , see Figure 4.28). Further analysis revealed that SD1 was significantly higher during the stimulation than baseline in the full sample ( $p = 0.037$ ). Musicians showed responses consistent with those observed in the full sample: SD1 was significantly higher during tVNS than baseline ( $p = 0.015$ ). Additionally, tVNS values were significantly higher compared to recovery ( $p = 0.030$ ).



*Figure 4.28: SD1 was significantly impacted by tVNS in the full sample and group of musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.*

tVNS also had significant effects on nSD1 in the full sample ( $p = 0.014$ ) and musicians ( $p = 0.026$ , see Figure 4.29). Further analysis revealed that nSD1 in the full sample was significantly higher during the stimulation than baseline ( $p = 0.046$ ). Similar patterns were observed in musicians: nSD1 was significantly higher during tVNS than baseline ( $p = 0.026$ ) and recovery ( $p = 0.028$ ).



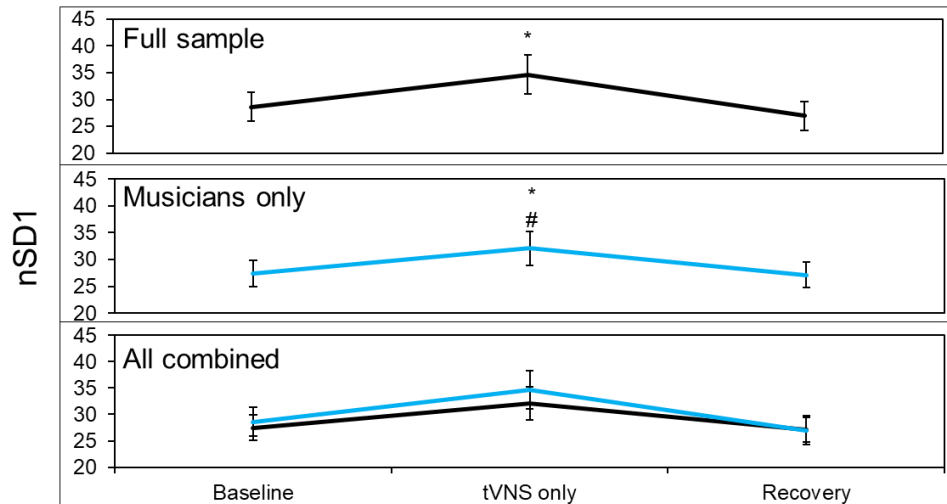


Figure 4.29: *nSD1* was significantly impacted by tVNS in the full sample and group of musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

S was significantly impacted by tVNS in the full sample ( $p = 0.011$ , see Figure 4.30). Further analysis showed that S was significantly higher during the stimulation than baseline ( $p = 0.003$ ). Furthermore, S during tVNS was significantly higher compared to recovery ( $p = 0.009$ ). When analysing females, there was a significant effect of tVNS on S ( $p = 0.018$ ): S was significantly higher during stimulation than at baseline ( $p = 0.012$ ). Similar changes in S were identified in musicians: S was significantly higher during tVNS than baseline (Friedman:  $p = 0.010$ ; pairwise comparison:  $p = 0.011$ ) and recovery ( $p = 0.010$ ).

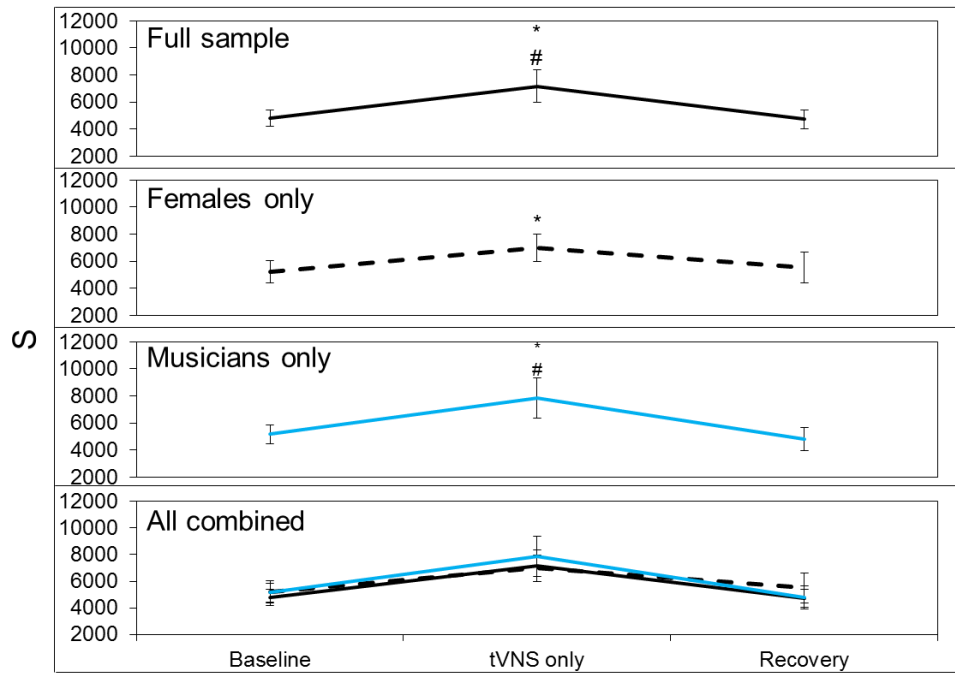


Figure 4.30:  $S$  was significantly impacted by tVNS in the full sample, females and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.1.3.2 Music only visit

A significant effect of music on SD1 occurred in the full sample ( $p = 0.006$ ), females ( $p = 0.006$ ), older participants ( $p = 0.002$ ), musicians ( $p = 0.023$ ) and those who detected tVNS ( $p = 0.020$ , see Figure 4.31). Further analysis showed that SD1 was significantly higher during music compared to baseline (full sample:  $p = 0.002$ ; females:  $p = 0.010$ ; older:  $p = 0.004$ ; musicians:  $p = 0.009$ ; detected tVNS:  $p = 0.022$ ).

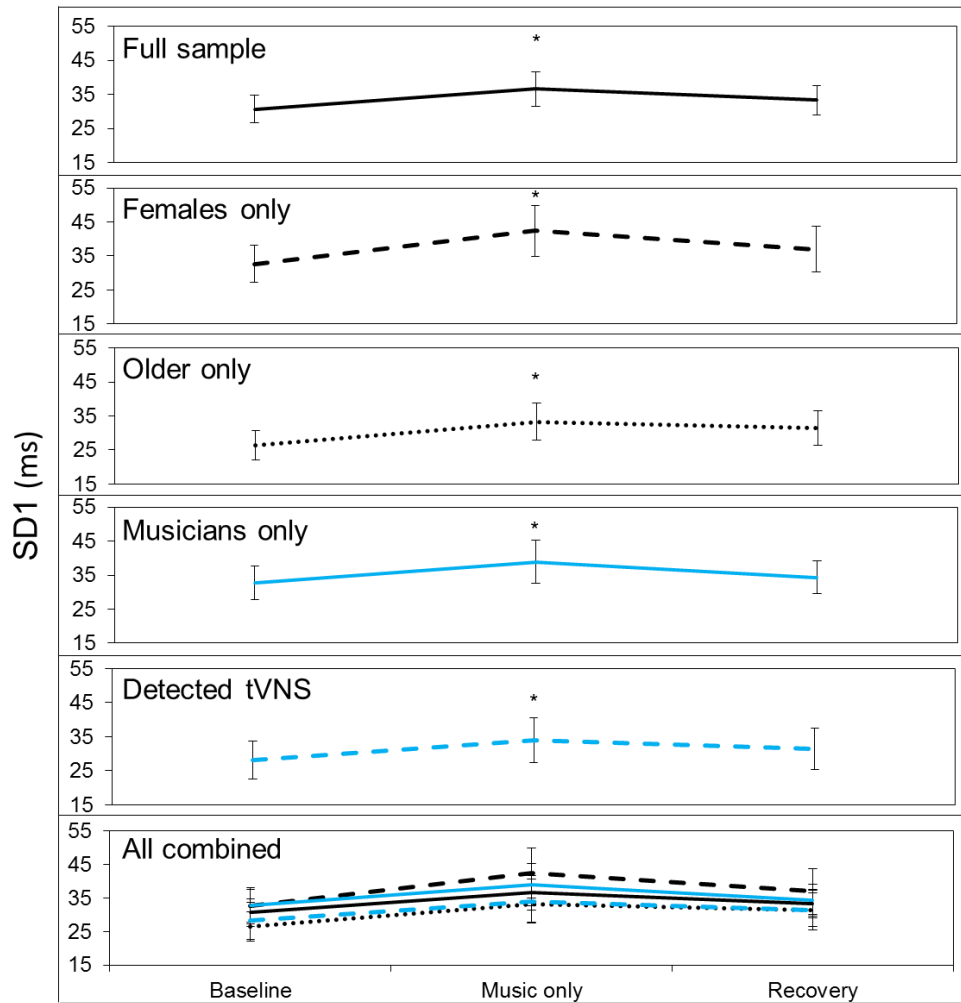


Figure 4.31: SD1 was significantly impacted by music in the full sample, females, older participants, musicians and the detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of music on nSD1 in the full sample ( $p = 0.025$ ), females ( $p = 0.016$ ), older participants ( $p = 0.013$ ) and those who detected tVNS ( $p = 0.016$ , see Figure 4.32). Further analysis revealed that nSD1 was significantly higher during music compared to baseline (full sample:  $p = 0.012$ ; females:  $p = 0.015$ ; older:  $p = 0.010$ ; detected tVNS:  $p = 0.011$ ).

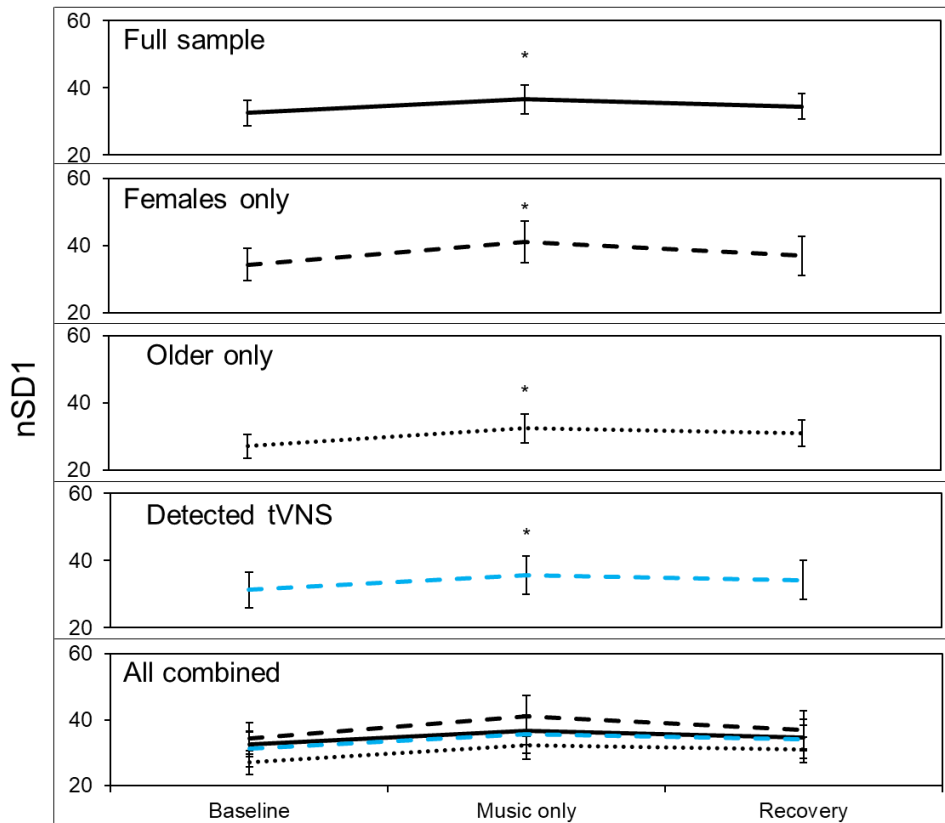


Figure 4.32: *nSD1* was significantly impacted by music in the full sample, females, older participants and the detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of music on S in musicians ( $p = 0.011$ ) and the group who detected tVNS ( $p = 0.010$ , see Figure 4.33). Pairwise comparisons revealed that S was significantly higher during music compared to baseline (musicians:  $p = 0.015$ ; detected tVNS:  $p = 0.011$ ).

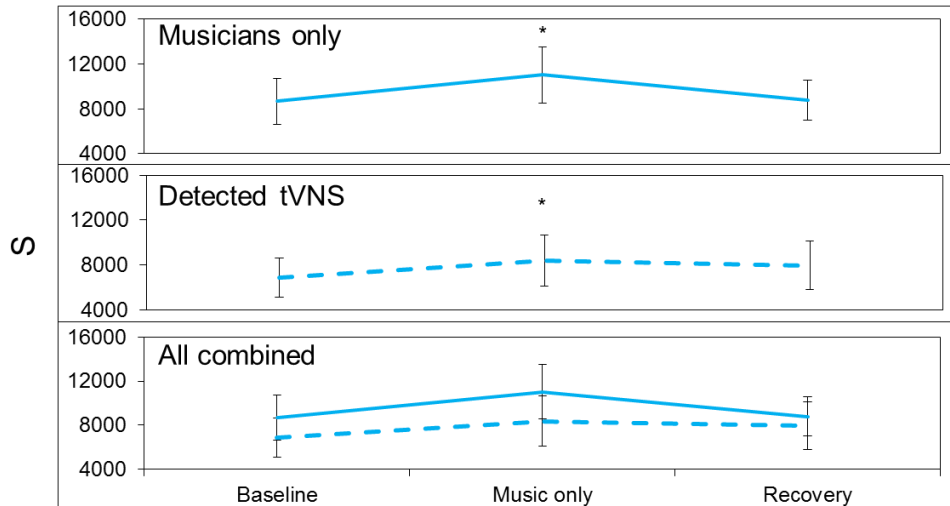


Figure 4.33: *S* was significantly impacted by music in musicians and the detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Significant effects on SD1/SD2 were detected in females ( $p = 0.008$ ), non-musicians ( $p = 0.017$ ) and those who detected tVNS ( $p = 0.015$ , see Figure 4.34). SD1/SD2 significantly increased between baseline and music for all groups (females:  $p = 0.017$ ; non-musicians:  $p = 0.043$ ; detected tVNS:  $p = 0.044$ ).

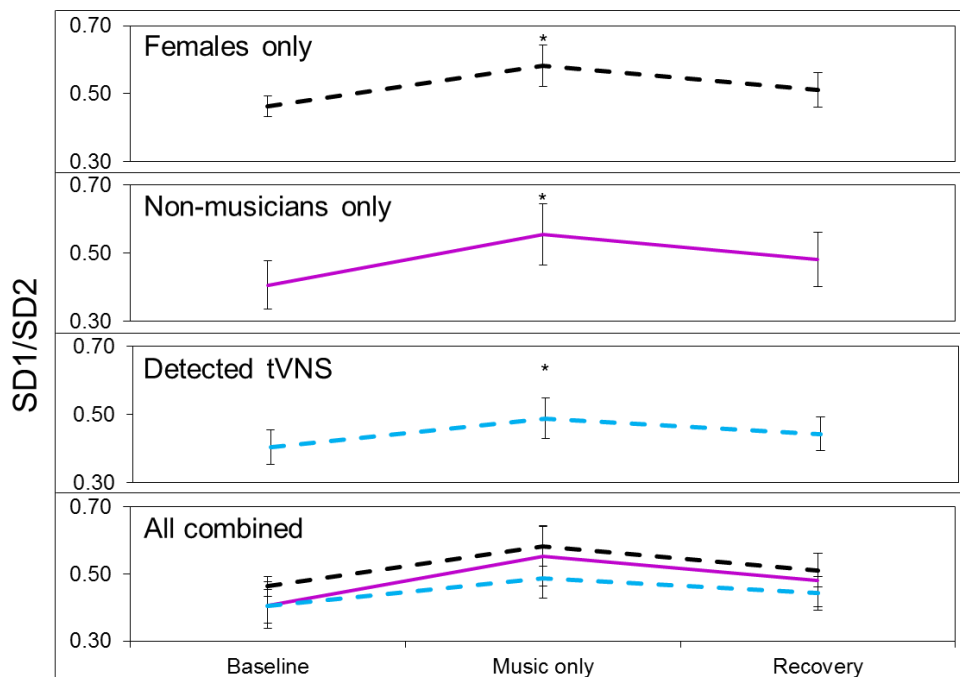


Figure 4.34: *SD1/SD2* was significantly impacted by music in females, non-musicians and the detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Finally, a significant effect on SD2/SD1 was identified in those who detected tVNS ( $p = 0.046$ , see Figure 4.35). As anticipated, SD2/SD1 during music was significantly lower than that during baseline ( $p = 0.008$ ).

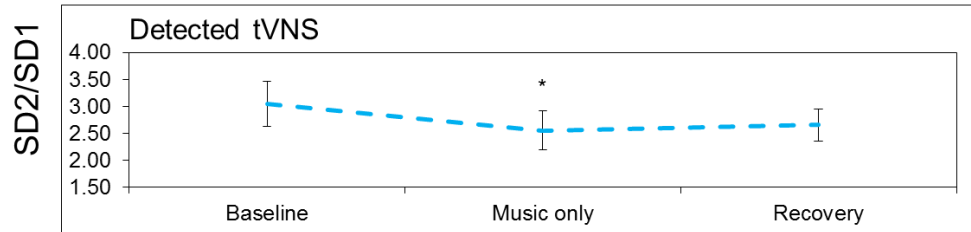


Figure 4.35: SD2/SD1 was significantly impacted by music in the detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.3.3 tVNS + music visit

Analysis of the tVNS + music visit revealed no significant changes in any of the non-linear HRV measures at the level of the entire group, males, young and older participants, non-musicians, musicians and those who did and did not detect tVNS ( $p > 0.05$ ).

But, for females, a significant effect on SD1 was identified ( $p = 0.011$ ). Further analysis showed that SD1 was significantly higher during tVNS + music than baseline ( $p = 0.030$ , see Figure 4.36).

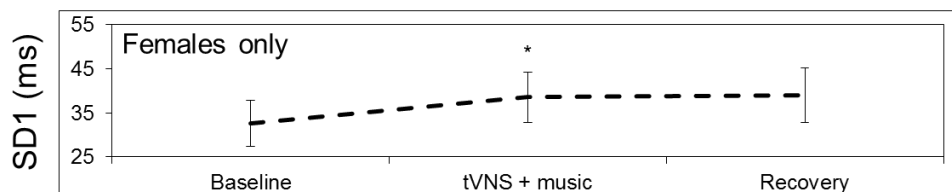


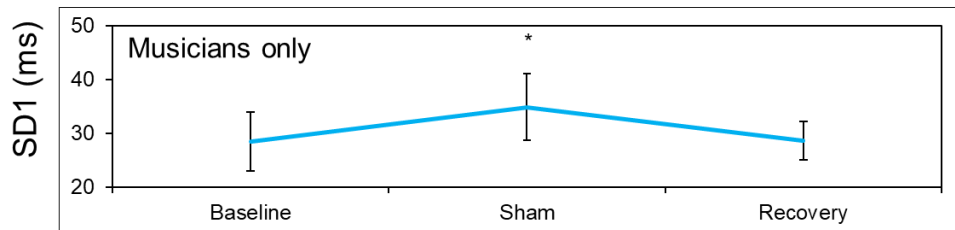
Figure 4.36: SD1 in females only was significantly higher during tVNS + music compared to baseline. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.3.4 Sham visit

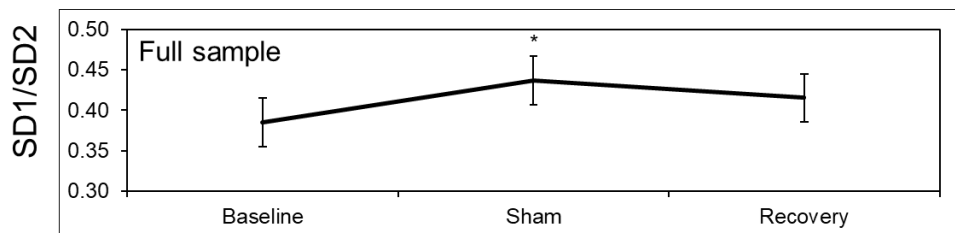
SD1 was significantly impacted by sham in musicians only ( $p = 0.023$ ). Further analysis showed that SD1 significantly increased between baseline and sham ( $p = 0.004$ , see Figure 4.37a). There was a significant effect of sham on SD1/SD2 for the full sample, ( $p = 0.048$ , see Figure 4.37b). Further

exploration revealed that SD1/SD2 was significantly higher during sham compared to baseline ( $p = 0.014$ ). Female SD2/SD1 was also significantly impacted during sham ( $p = 0.017$ , see Figure 4.37c): values were significantly lower during sham than baseline ( $p = 0.014$ ).

a.



b.



c.

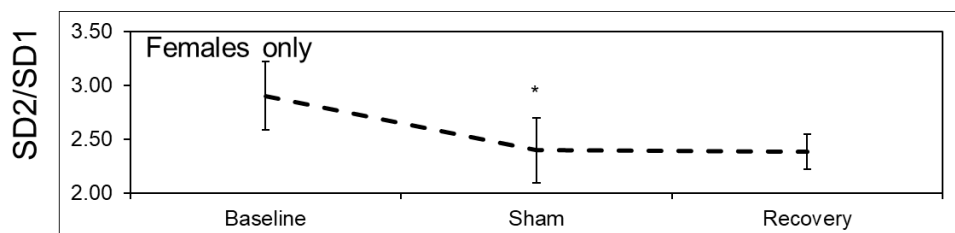


Figure 4.37: SD1 (a), SD1/SD2 (b) and SD2/SD1 (c) significantly changed during sham. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.3.5 Between-visit comparisons

For all group analyses, none of the non-linear HRV measures significantly differed between the four visits ( $p > 0.05$ ).

#### 4.3.1.4 RR analysis

##### 4.3.1.4.1 tVNS only visit

Mean RR interval was significantly impacted during tVNS in the full sample ( $p = 0.032$ ) and musicians ( $p = 0.012$ , see Figure 4.38). Further analysis revealed that mean RR intervals were significantly higher during tVNS compared to baseline (full sample,  $p = 0.048$ ; musicians:  $p = 0.015$ ).

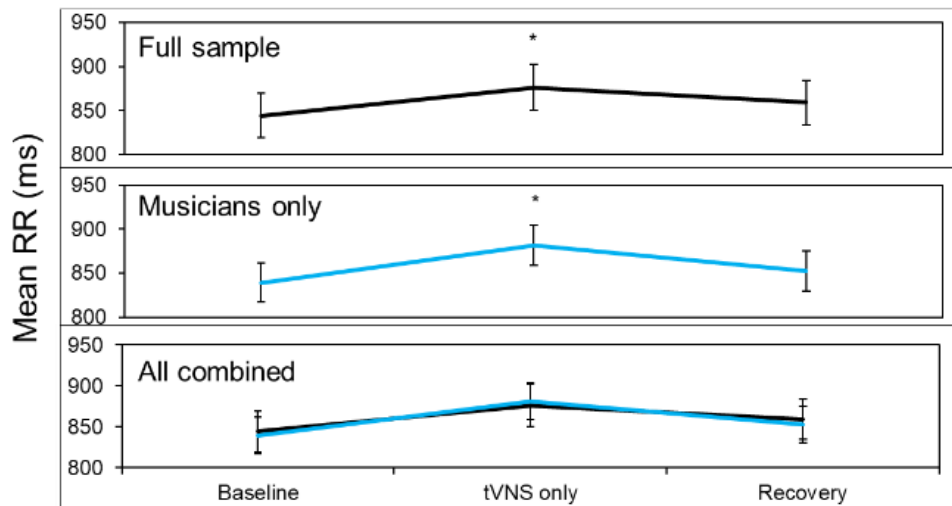


Figure 4.38: Mean RR interval was significantly impacted by tVNS in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS on median RR intervals in musicians only ( $p = 0.012$ , see Figure 4.39). Further analysis showed that median RR intervals were significantly longer during tVNS than baseline ( $p = 0.017$ ).

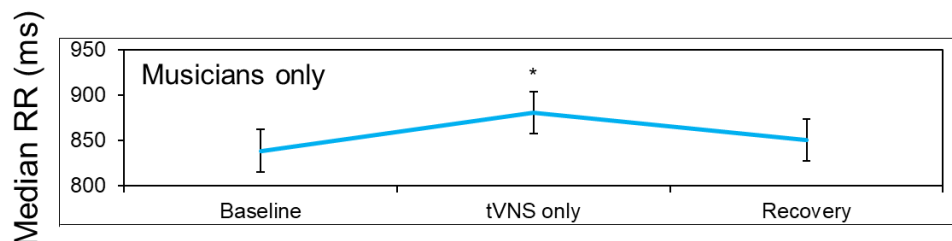


Figure 4.39: Median RR interval was significantly impacted by tVNS in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.



Max RR was significantly impacted by tVNS in the full sample ( $p = 0.022$ ), musicians ( $p = 0.007$ ) and those who did not detect tVNS ( $p = 0.014$ , see Figure 4.40). Further analysis demonstrated that max RR intervals were significantly greater during music than baseline in the full sample ( $p = 0.033$ ) and those who did not detect tVNS ( $p = 0.044$ ). Also, for musicians, max RR was significantly greater during tVNS compared to recovery ( $p = 0.017$ ).

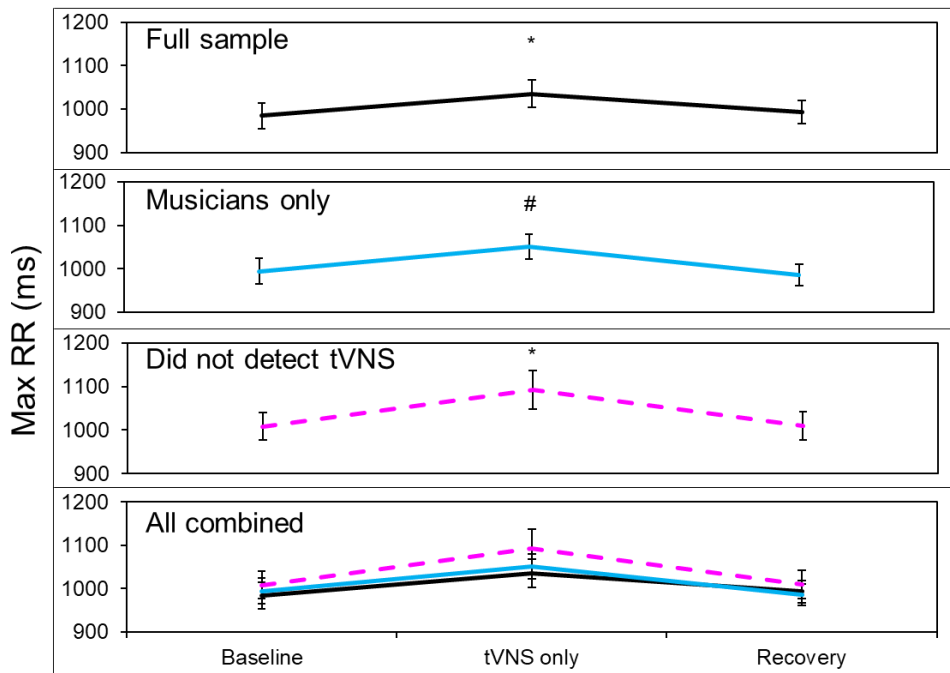


Figure 4.40: Max RR interval was significantly impacted by tVNS in the full sample, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

A significant effect of tVNS on  $\Delta$  RR ( $p = 0.010$ ) transpired for musicians only. Further analysis showed that  $\Delta$  RR was significantly higher in tVNS than recovery ( $p = 0.032$ , see Figure 4.41).

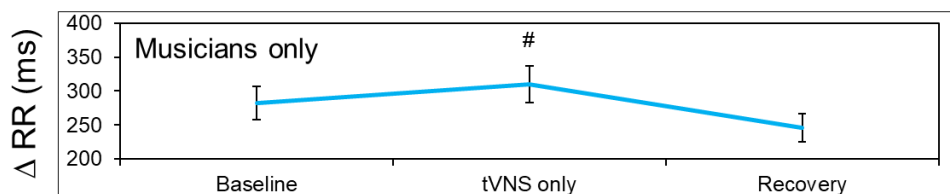


Figure 4.41:  $\Delta$  RR was significantly impacted by tVNS in musicians only. Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery.

Q1 RR was also significantly impacted by tVNS but for musicians only ( $p = 0.044$ ): values were significantly higher during tVNS compared to baseline. ( $p = 0.045$ , see Figure 4.42).

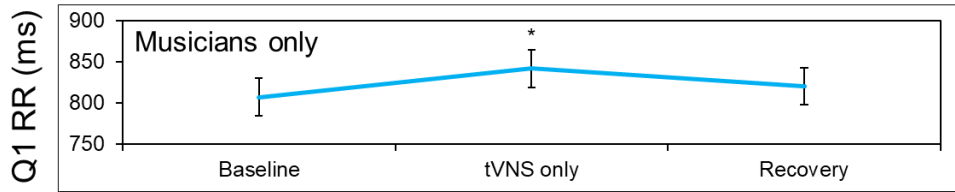


Figure 4.42: Q1 RR was significantly impacted by tVNS in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS on Q3 RR for the full sample ( $p = 0.010$ ), musicians ( $p = 0.006$ ) and those who did not detect tVNS ( $p = 0.044$ ). Further analysis revealed that Q3 RR intervals were significantly longer during tVNS than baseline in all three group comparisons (full sample:  $p = 0.018$ ; musicians:  $p = 0.011$ ; did not detect tVNS:  $p = 0.029$ , see Figure 4.43).

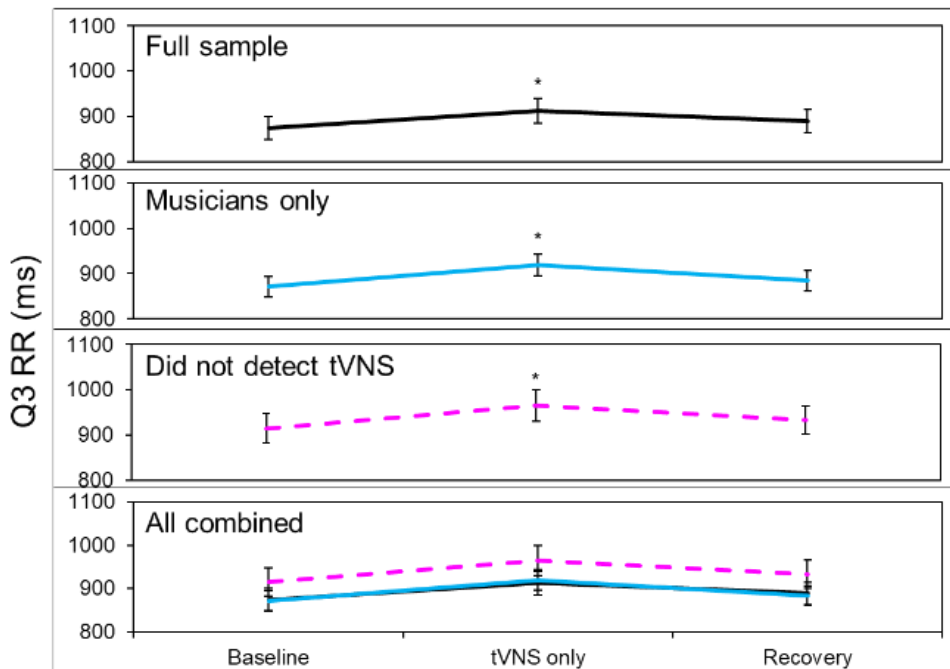


Figure 4.43: Q3 RR was significantly impacted by tVNS in the full sample, musicians and the group who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

IQR RR was significantly impacted by tVNS but in the full sample only ( $p = 0.012$ , see Figure 4.44). Further analysis revealed that IQR RR was significantly higher during tVNS compared to baseline ( $p = 0.024$ ).

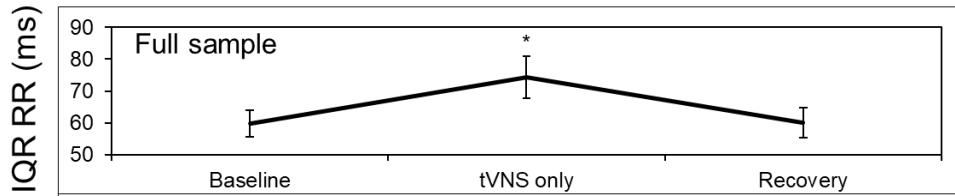


Figure 4.44: IQR RR was significantly impacted by tVNS in the full sample. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Finally, there was a significant effect of tVNS on Q3-max RR in musicians only ( $p = 0.028$ ). Further analysis showed that values were significantly lower during recovery than during baseline ( $p = 0.020$ , see Figure 4.45).

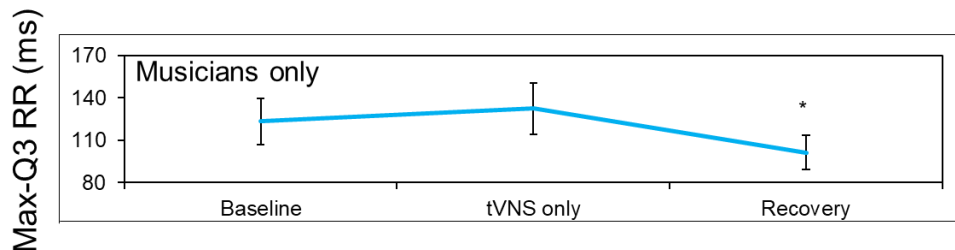


Figure 4.45: Max-Q3 RR intervals were significantly impacted by tVNS in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.4.2 Music only visit

A significant effect of music on mean RR interval was found in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.001$ ) and musicians ( $p = 0.005$ ). Pairwise comparisons showed that values were significantly larger during music compared to baseline (full sample:  $p = 0.004$ ; females:  $p < 0.001$ ; older:  $p = 0.015$ ; musicians:  $p = 0.013$ , see Figure 4.46). In addition, mean RR intervals for females and older participants were significantly higher during recovery compared to baseline ( $p = 0.014$  and  $p = 0.025$  respectively).

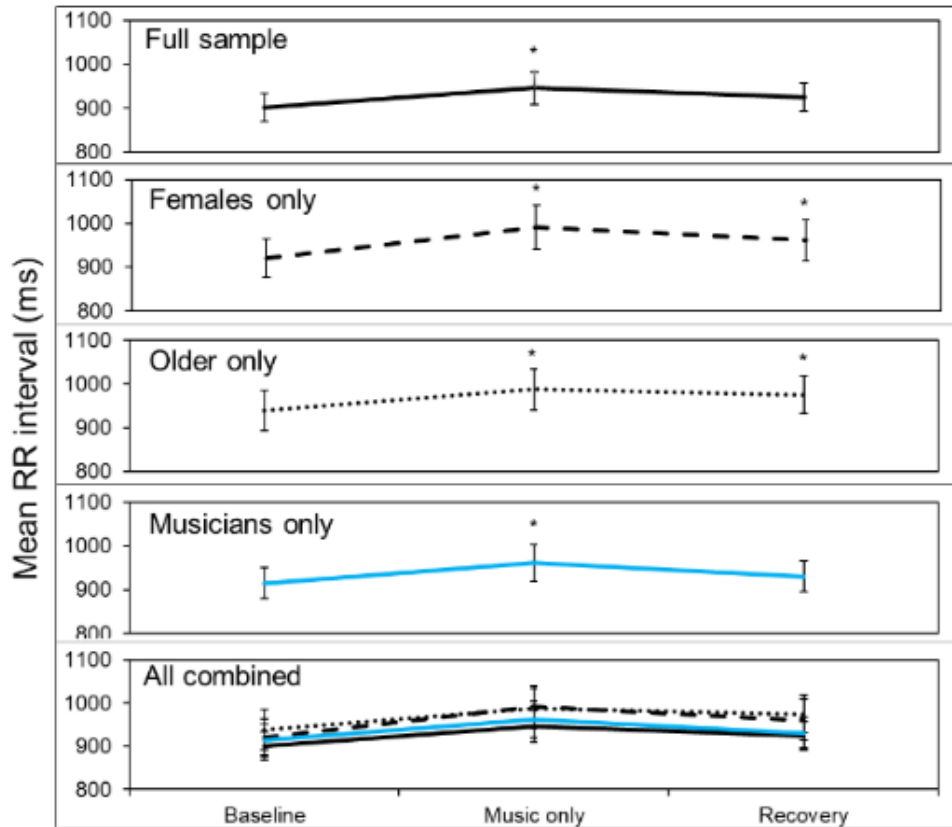


Figure 4.46: Mean RR interval was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm 1$  SEM. \* = significantly different to baseline.

There was also a significant effect of music on median RR interval in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.020$ ) and musicians ( $p = 0.016$ ). Pairwise comparisons showed that values were significantly greater during music than baseline (full sample:  $p = 0.006$ ; females:  $p < 0.001$ ; older:  $p = 0.020$ ; musicians:  $p = 0.016$ , see Figure 4.47). Additionally, median RR intervals in females and older participants were significantly greater during recovery than baseline ( $p = 0.007$  and  $p = 0.020$  respectively).

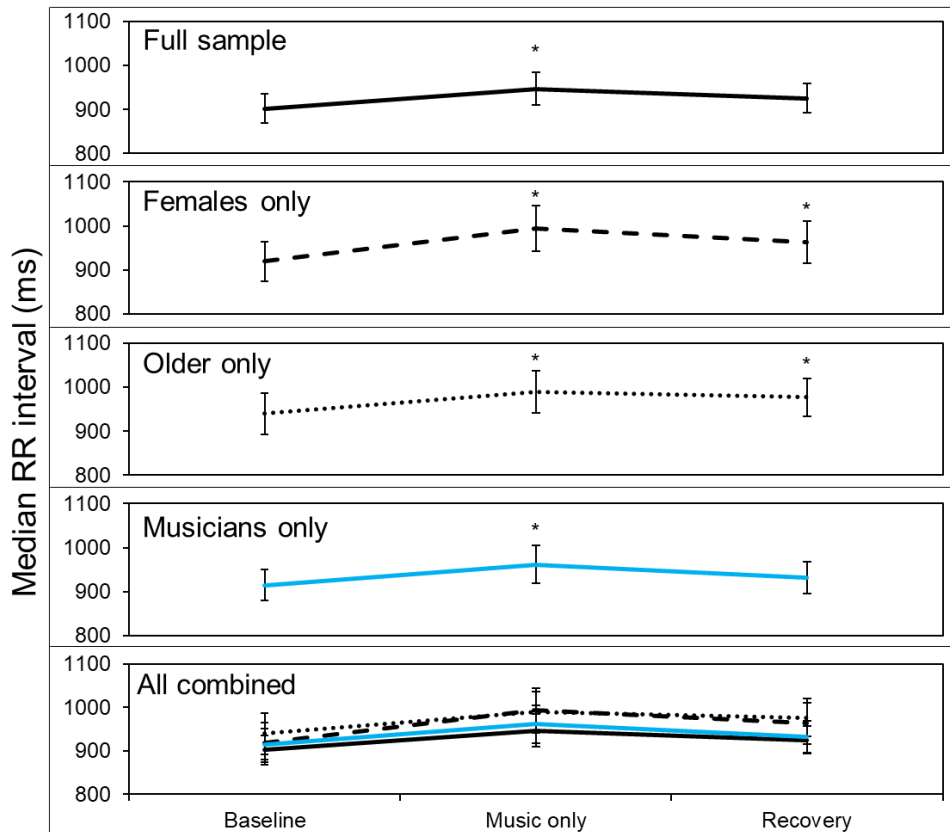


Figure 4.47: Median RR interval was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

A significant effect of music on mode RR interval emerged in the full sample ( $p = 0.022$ , see Figure 4.48): values were significantly greater during music than baseline ( $p = 0.039$ ). Mode RR was also significantly impacted in females ( $p = 0.001$ ): values during music were significantly greater than those during baseline ( $p < 0.001$ ). Also, mode RR during recovery was significantly higher than mode RR during baseline ( $p = 0.042$ ).

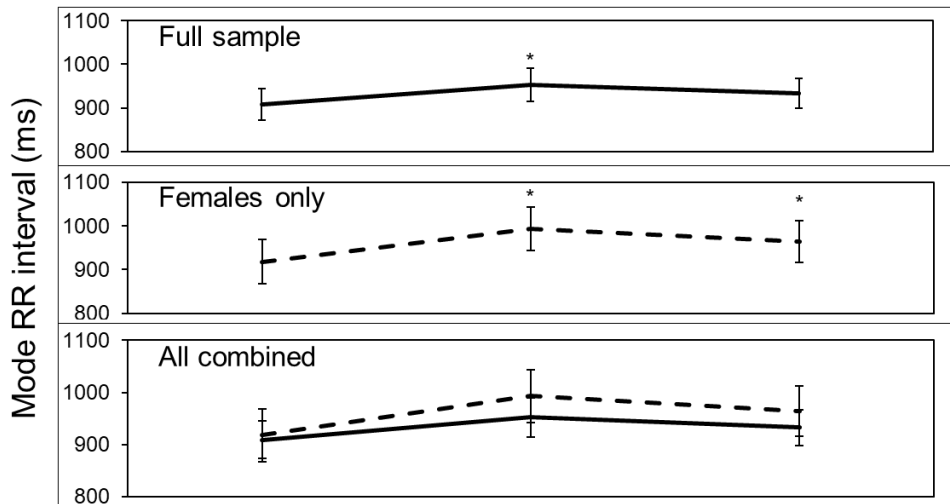


Figure 4.48: Mode RR interval was significantly impacted by music in the full sample and females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Significant effects of music on min RR interval was found in the full sample ( $p = 0.010$ ), females ( $p = 0.013$ ), older participants ( $p = 0.007$ ), non-musicians ( $p = 0.016$ ) and those who detected tVNS ( $p = 0.002$ ). Pairwise comparisons showed that min RR was significantly higher during music than baseline (full sample:  $p = 0.013$ ; females:  $p = 0.009$ ; older:  $p = 0.018$ ; detected tVNS:  $p = 0.005$ , see Figure 4.49). In addition, min RR interval was significantly greater during recovery than baseline for older participants ( $p = 0.017$ ), the detected tVNS group ( $p = 0.022$ ) and non-musicians ( $p = 0.038$ ).

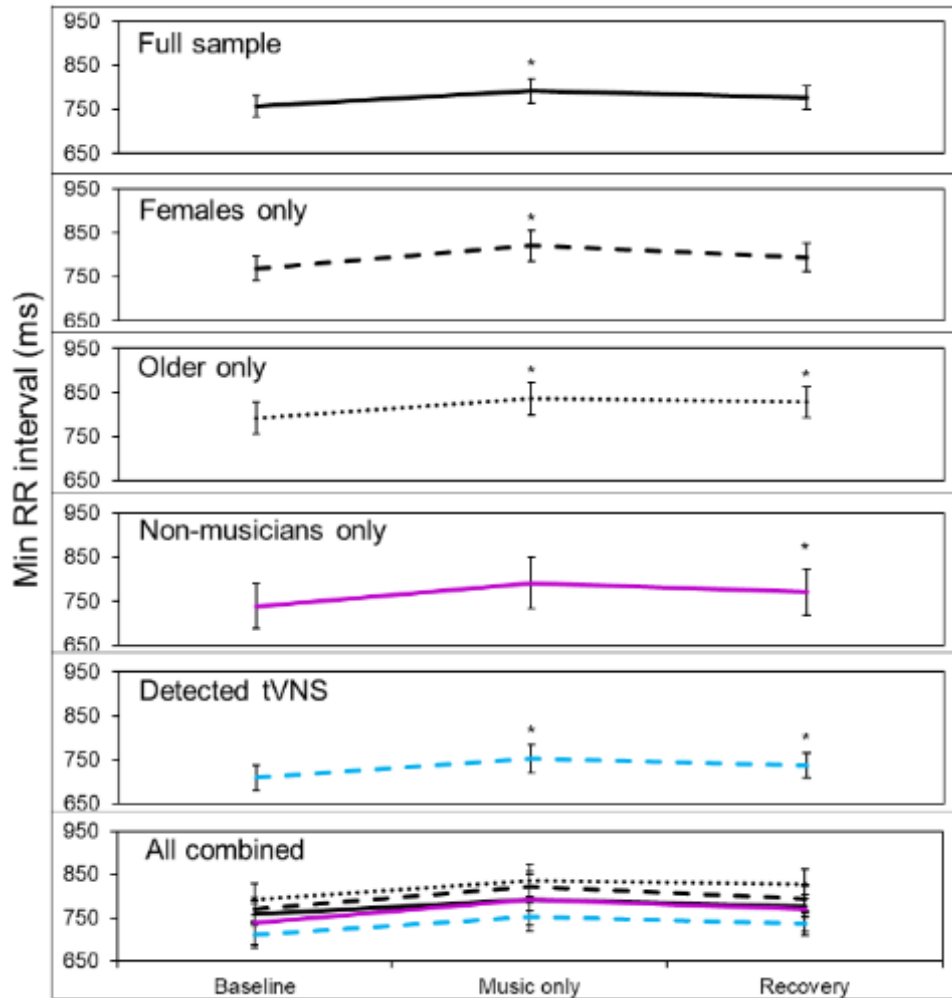


Figure 4.49: Min RR interval was significantly impacted by music in the full sample, females, older participants, non-musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant effects of music on max RR interval in the full sample ( $p = 0.001$ ), females ( $p = 0.001$ ), older participants ( $p = 0.010$ ), musicians ( $p = 0.001$ ) and those who did not detect tVNS ( $p = 0.007$ ). Further analysis showed that values were significantly higher during music compared to baseline (full sample:  $p = 0.003$ ; females:  $p = 0.050$ ; older:  $p = 0.033$ ; musicians:  $p = 0.003$ ; did not detect tVNS:  $p = 0.043$ , see Figure 4.50). Furthermore, in females only, max RR was significantly higher in recovery than baseline ( $p = 0.050$ ).

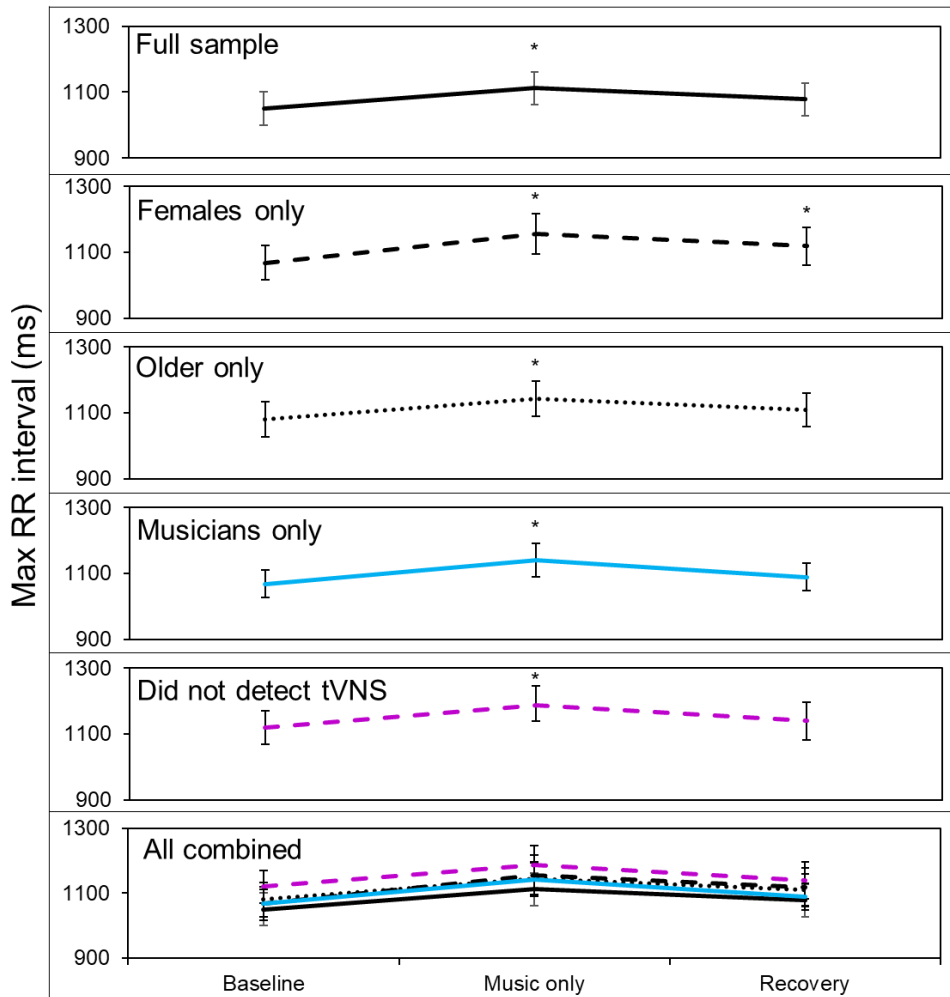


Figure 4.50: Max RR interval was significantly impacted by music in the full sample, females, older participants, musicians and did not detect tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

$\Delta$  RR interval was significantly impacted by the music in musicians only ( $p = 0.015$ ). As shown in Figure 4.51,  $\Delta$  RR was significantly greater during music than during baseline ( $p = 0.013$ ).

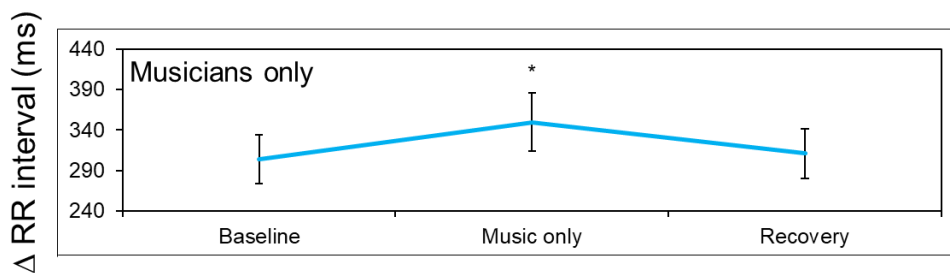


Figure 4.51:  $\Delta$  RR interval was significantly impacted by music in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.



Significant effects of music on Q1 RR were detected in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.004$ ) and musicians ( $p = 0.009$ ). Pairwise comparisons showed that Q1 RR was significantly higher during music than baseline (full sample:  $p = 0.006$ ; females:  $p = 0.016$ ; older:  $p = 0.029$ ; musicians:  $p = 0.021$ , see Figure 4.52). In addition, Q1 RR intervals were significantly greater during recovery than baseline for females ( $p = 0.016$ ) and older participants ( $p = 0.041$ ).

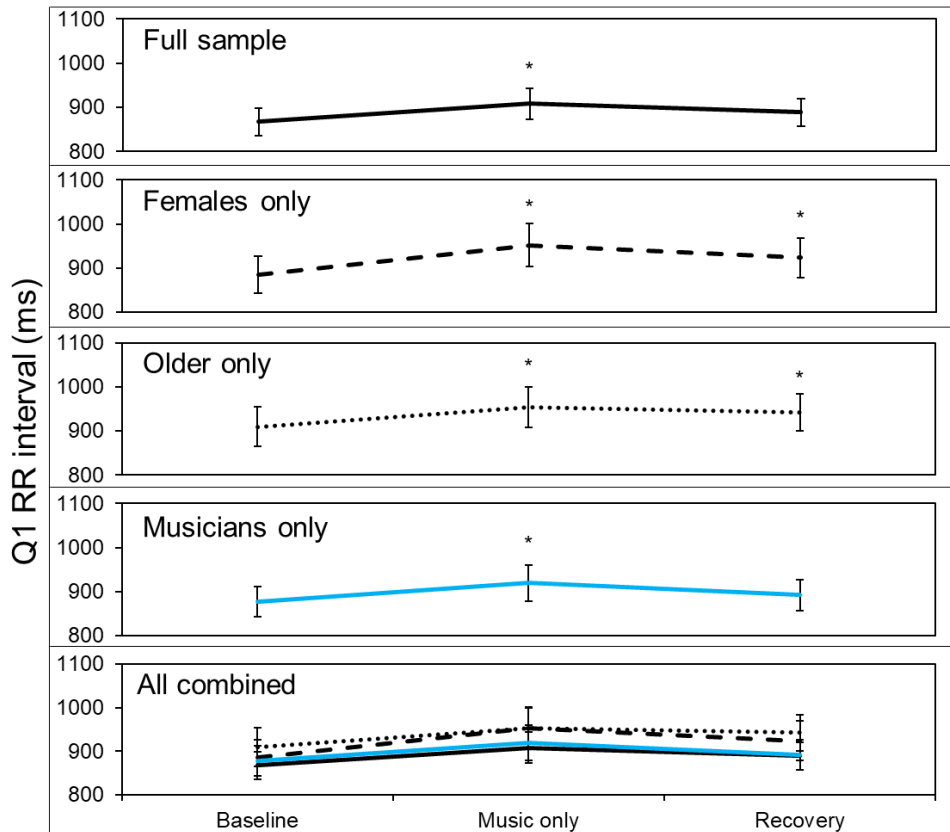


Figure 4.52: Q1 RR interval was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm 1$  SEM. \* = significantly different to baseline.

Finally, significant effects of music on Q3 RR emerged in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.001$ ) and musicians ( $p = 0.003$ , see Figure 4.53). Pairwise comparisons showed that Q3 RR was significantly higher during music than baseline (full sample:  $p = 0.004$ ; females:  $p < 0.001$ ; older:  $p = 0.016$ ; musicians:  $p = 0.008$ ). Moreover, Q3 RR during recovery was significantly greater than that during baseline in females ( $p = 0.012$ ) and older participants ( $p = 0.024$ ).

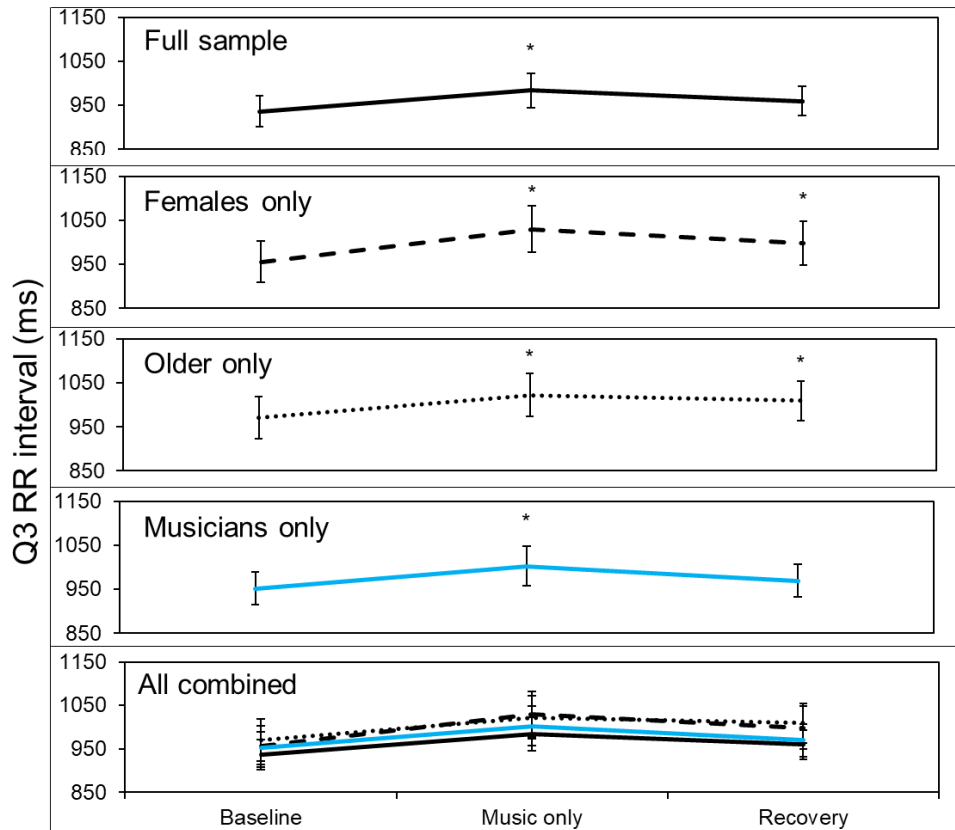


Figure 4.53: Q3 RR interval was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.4.3 tVNS + music visit

There was a significant effect of tVNS + music on mean RR interval in the full sample ( $p = 0.003$ ), females ( $p = 0.002$ ), musicians ( $p = 0.020$ ) and those who detected tVNS ( $p = 0.004$ ). Further analysis showed that mean RR interval was significantly greater during tVNS + music than baseline (full sample:  $p = 0.004$ ; females:  $p = 0.004$ ; musicians:  $p = 0.031$ ; detected tVNS:  $p = 0.022$ , see Figure 4.54). In addition, mean RR during recovery was significantly greater than that during baseline for those who detected tVNS ( $p = 0.030$ ).

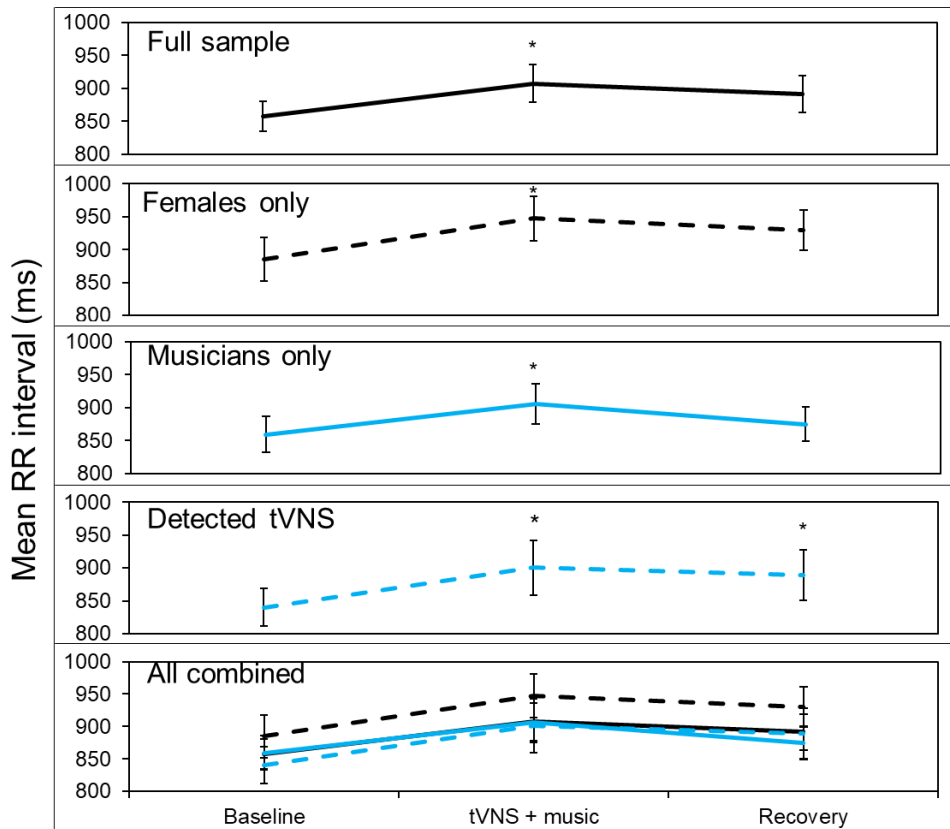


Figure 4.54: Mean RR interval was significantly impacted by tVNS + music in the full sample, females, musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Median RR interval was significantly impacted by tVNS + music in the full sample ( $p = 0.003$ ); females ( $p = 0.002$ ), musicians ( $p = 0.015$ ) and those who detected tVNS ( $p = 0.004$ ): values were significantly greater during stimulation than baseline (full sample:  $p = 0.004$ ; females:  $p = 0.003$ ; musicians:  $p = 0.026$ ; detected tVNS:  $p = 0.020$ , see Figure 4.55).

Furthermore, median RR during recovery was significantly greater than that during baseline for those who detected tVNS ( $p = 0.043$ ).

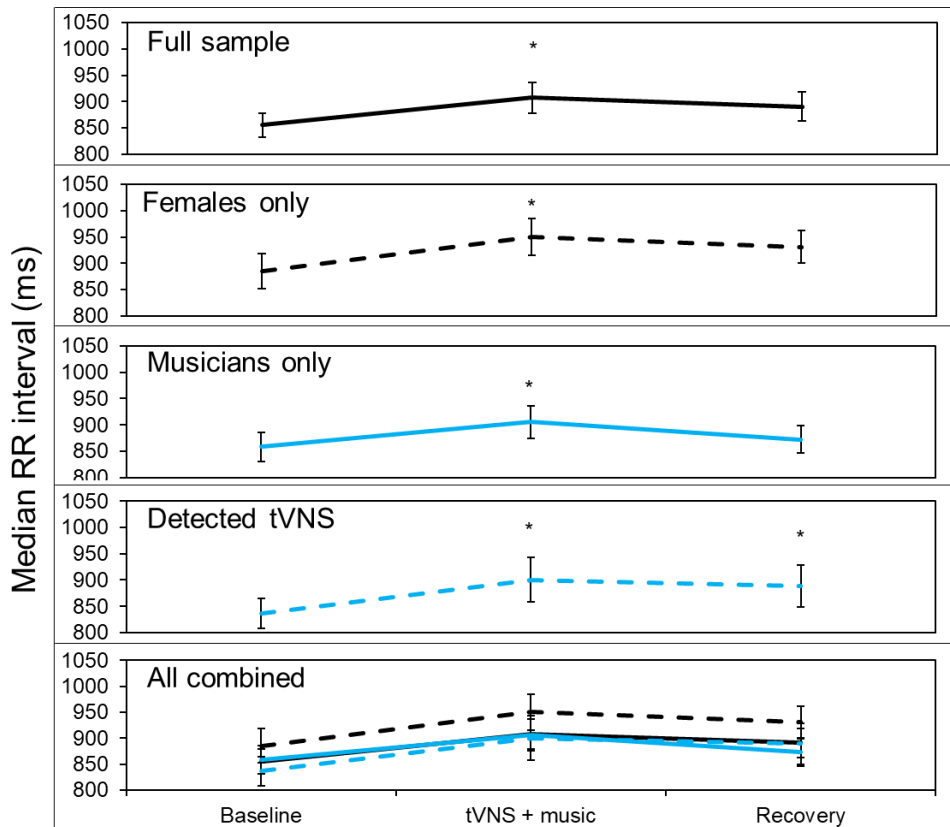


Figure 4.55: Median RR interval was significantly impacted by tVNS + music in the full sample, females, musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS + music on mode RR interval for the full sample ( $p = 0.004$ ); young ( $p = 0.020$ ); musicians ( $p = 0.007$ ) and those who detected tVNS ( $p = 0.001$ ). Mode RR was significantly greater during stimulation compared to baseline (full sample:  $p = 0.004$ ; young:  $p = 0.017$ ; musicians:  $p = 0.043$ ; detected tVNS:  $p = 0.011$ ) Also, mode RR was significantly greater during recovery than baseline (full sample:  $p = 0.002$ ; musicians:  $p = 0.029$ ; detected tVNS:  $p = 0.031$ , see Figure 4.56).

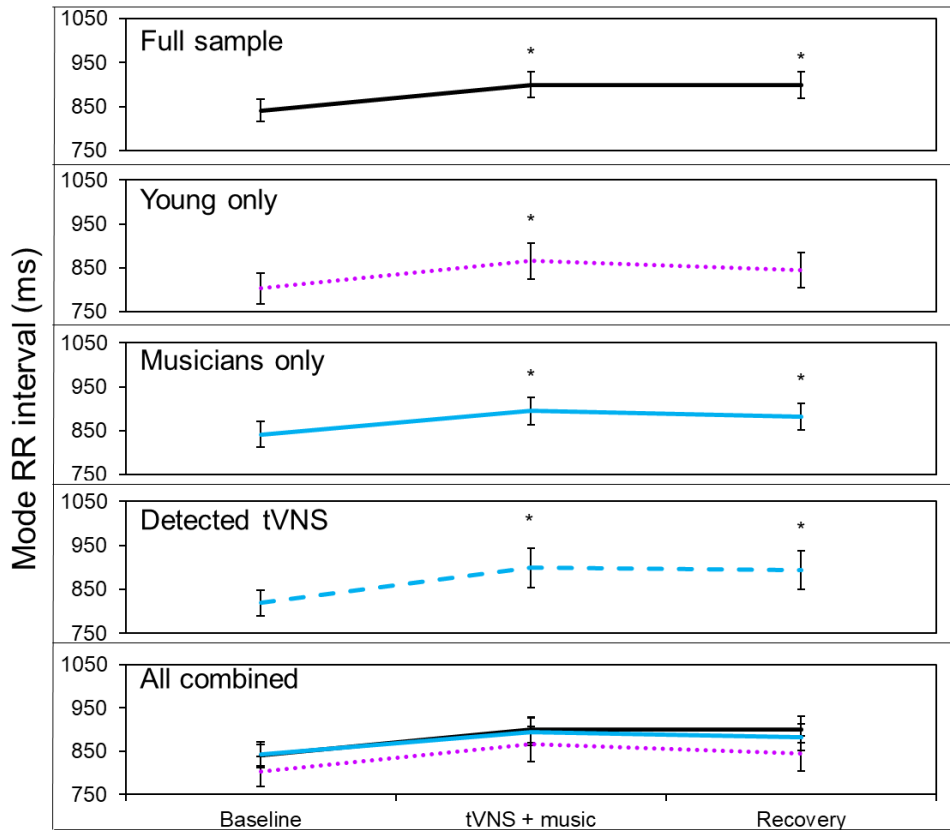


Figure 4.56: Mode RR interval was significantly impacted by tVNS + music in the full sample, young participants, musicians and detected tVNS group. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS + music on min RR interval for older participants only ( $p = 0.022$ ). Further analysis revealed that recovery min RR was significantly greater than baseline ( $p = 0.028$ , see Figure 4.57).

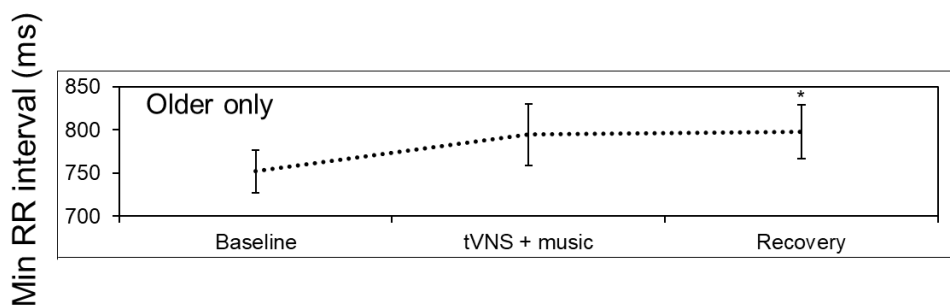


Figure 4.57: Min RR interval was significantly higher during recovery than baseline for older participants only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Max RR interval was significantly impacted by the tVNS + music stimulus in the group of females ( $p = 0.004$ ). Pairwise comparisons showed that max RR was significantly higher during tVNS + music compared to baseline ( $p = 0.014$ , see Figure 4.58).

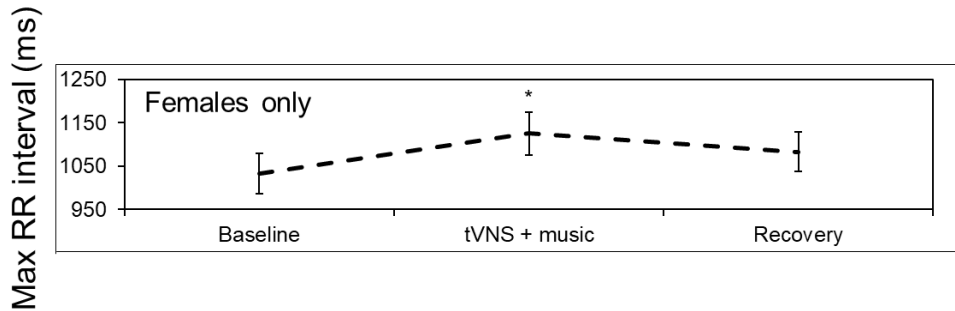


Figure 4.58: Max RR interval was significantly higher during tVNS + music than baseline for females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS + music on  $\Delta$  RR interval for females ( $p = 0.027$ , see Figure 4.59). Further analysis showed that  $\Delta$  RR was significantly higher during stimulation than during recovery ( $p = 0.038$ ). Young participants also showed significant changes in  $\Delta$  RR ( $p = 0.018$ ): values were significantly higher during tVNS + music compared to baseline ( $p = 0.014$ ).

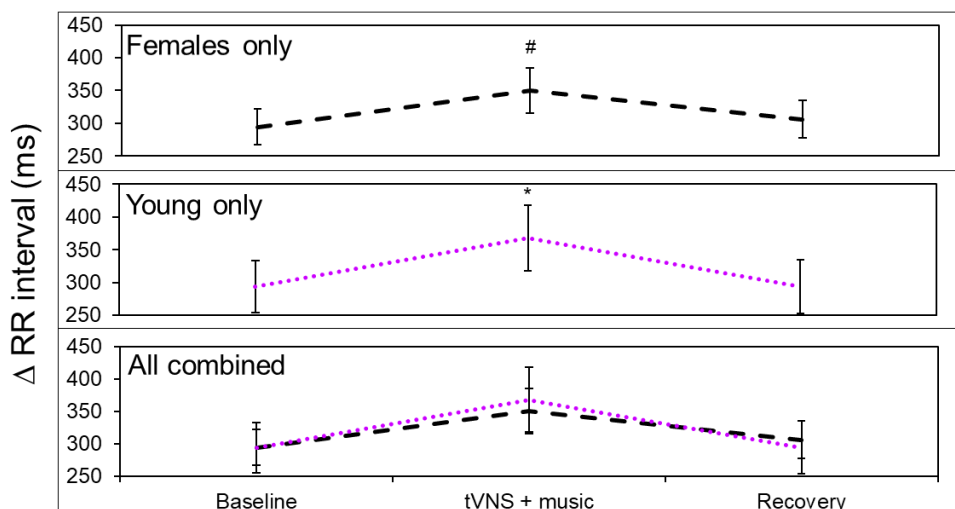


Figure 4.59:  $\Delta$  RR was significantly impacted during tVNS + music in females and young participants only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

tVNS + music significantly impacted Q1 RR interval in the full sample ( $p = 0.019$ ), females ( $p = 0.003$ ), musicians ( $p = 0.014$ ) and those who detected tVNS ( $p = 0.011$ ). Further analysis revealed that Q1 RR was significantly greater in tVNS + music compared to baseline (full sample:  $p = 0.003$ ; females:  $p = 0.008$ ; musicians:  $p = 0.030$ ; detected tVNS:  $p = 0.016$ , see Figure 4.60). Furthermore, Q1 RR was significantly higher during recovery than baseline in the group who detected tVNS ( $p = 0.006$ ).

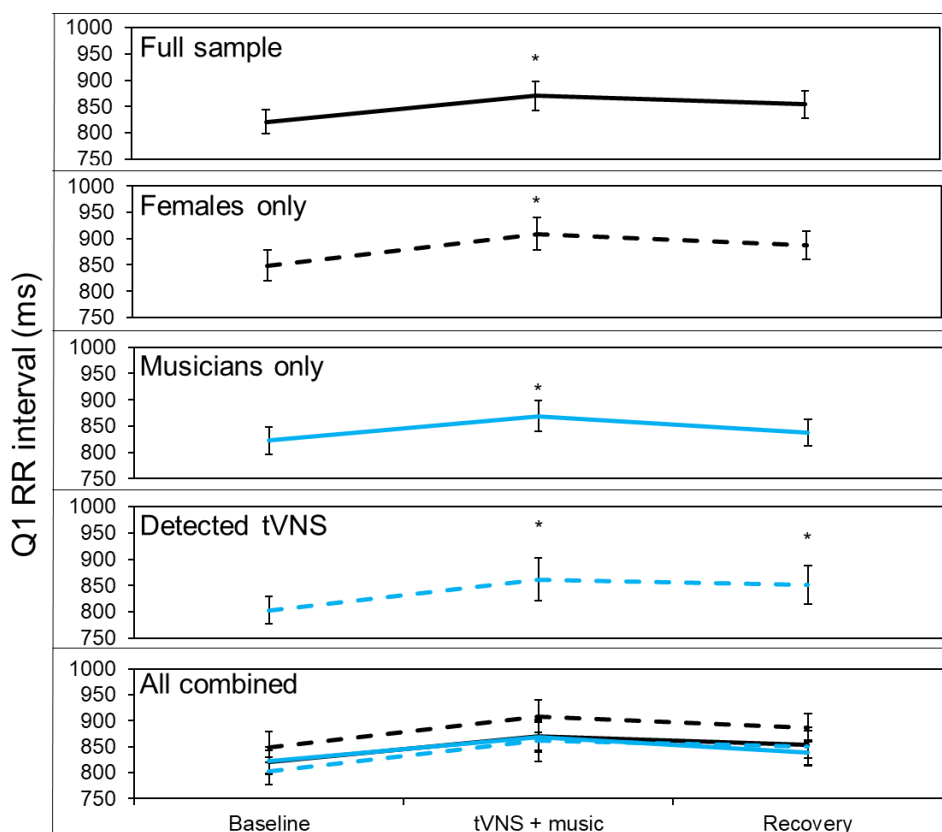


Figure 4.60: Q1 RR interval was significantly impacted by tVNS + music in the full sample, females, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant effects of tVNS + music on Q3 RR interval in the full sample ( $p = 0.004$ ), females ( $p = 0.002$ ), musicians ( $p = 0.023$ ) and those who detected tVNS ( $p = 0.005$ ). Further exploration showed that Q3 RR was significantly greater in tVNS + music compared to baseline (full sample:  $p = 0.004$ ; females:  $p = 0.014$ ; musicians:  $p = 0.027$ ; detected tVNS:  $p = 0.021$ , see Figure 4.61). In addition, Q3 RR was significantly higher during recovery than baseline in those who detected tVNS ( $p = 0.029$ , see Figure 4.61).

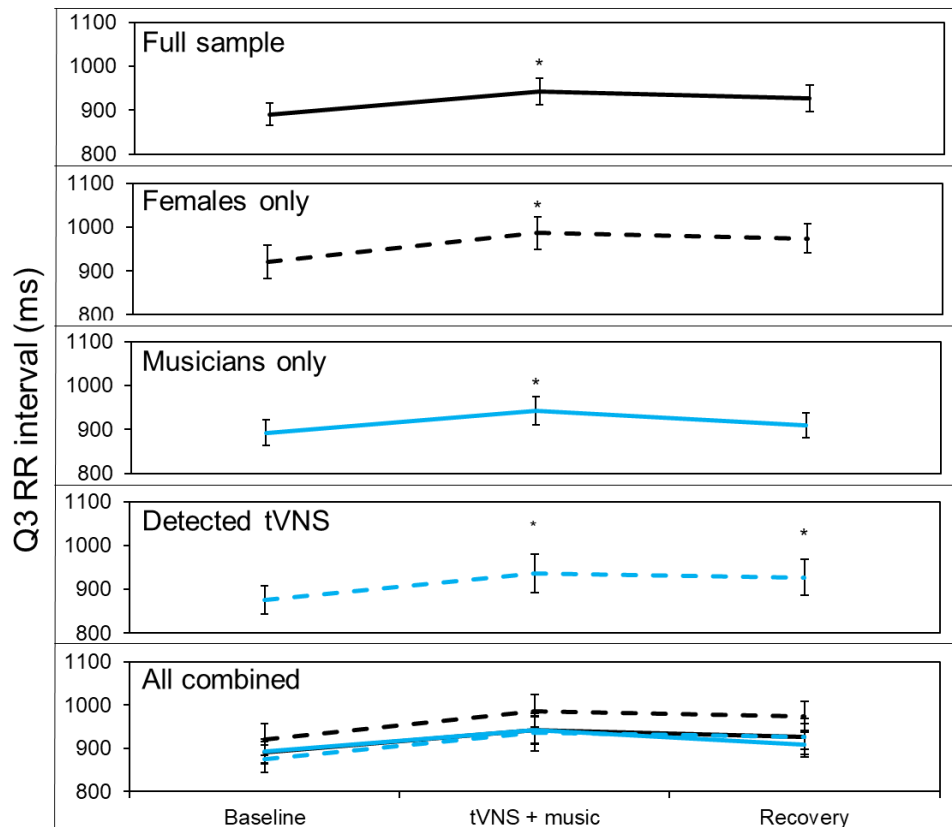


Figure 4.61: Q3 RR interval was significantly impacted by tVNS + music in the full sample, females, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.4.4 Sham visit

There was a significant effect of sham on mean RR interval in the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.005$ ), young participants ( $p = 0.006$ ), musicians ( $p = 0.002$ ), non-musicians ( $p = 0.006$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.007$ ). Further analysis revealed that mean RR interval was significantly higher during sham compared to baseline (full sample:  $p < 0.001$ ; males:  $p = 0.001$ ; females:  $p = 0.005$ ; young:  $p = 0.005$ ; older:  $p < 0.001$ ; musicians:  $p < 0.001$ ; non-musicians:  $p = 0.014$ ; detected tVNS:  $p < 0.001$ ; did not detect tVNS ( $p < 0.001$ , see Figure 4.62). Also, in the full sample and those who did not detect tVNS, mean RR intervals were significantly longer during sham than recovery ( $p = 0.0017$  and  $p = 0.038$  respectively). Furthermore, values were significantly greater during recovery than baseline in males and those who detected tVNS ( $p = 0.046$  and  $p = 0.005$  respectively).



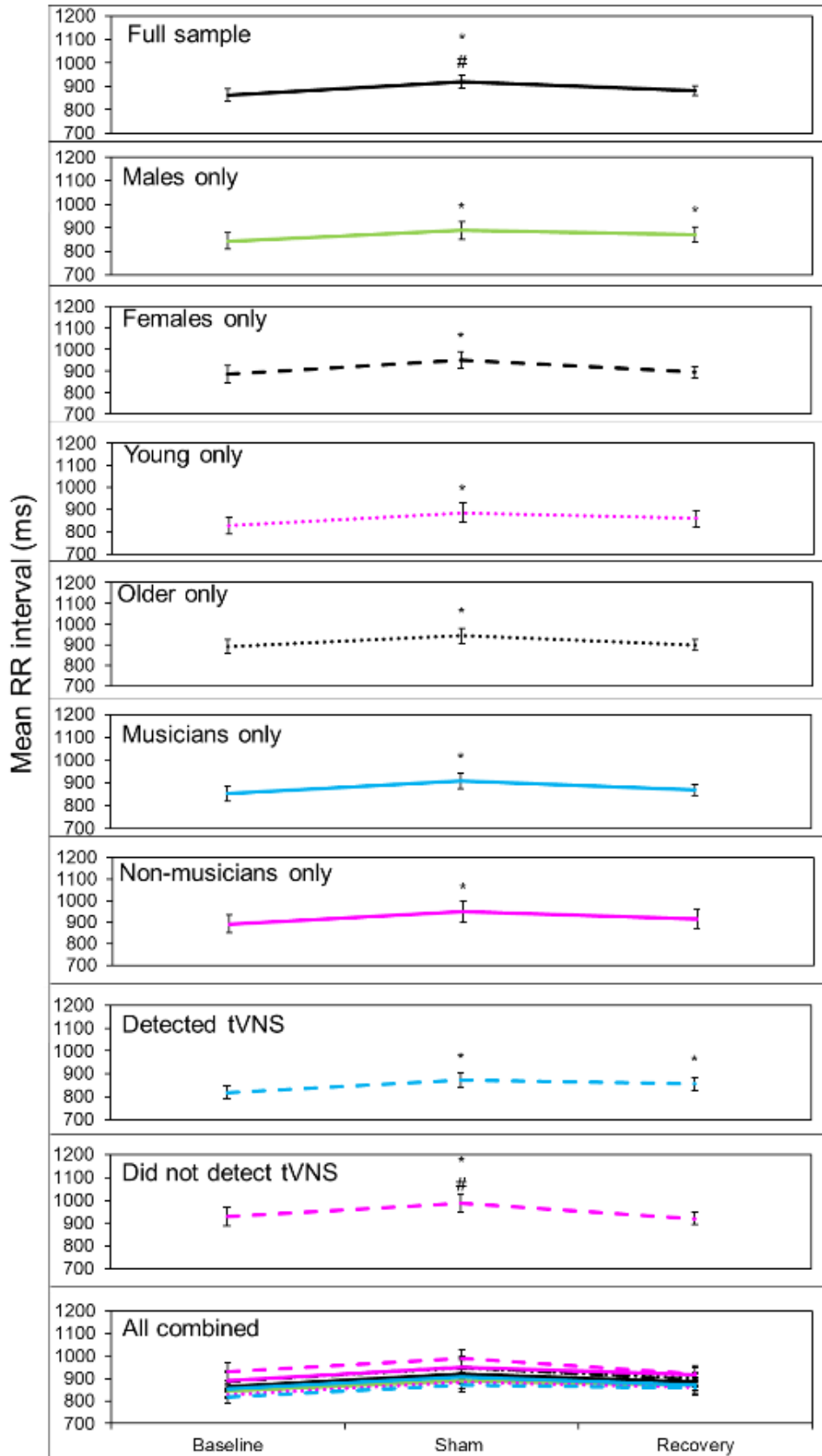


Figure 4.62: Mean RR interval was significantly impacted by sham in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Sham significantly impacted median RR interval in the full sample ( $p < 0.001$ ), females ( $p = 0.008$ ), males ( $p < 0.001$ ), young participants ( $p = 0.006$ ), musicians ( $p = 0.002$ ), non-musicians ( $p = 0.008$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.007$ ). Further analysis showed that median RR was significantly higher during sham compared to baseline (full sample:  $p < 0.001$ ; females:  $p = 0.008$ ; males:  $p = 0.001$ ; older:  $p = 0.001$ ; musicians:  $p < 0.001$ ; non-musicians:  $p = 0.013$ ; detected tVNS:  $p < 0.001$ ; did not detect tVNS:  $p = 0.021$ , see Figure 4.63). In addition, median RR intervals were significantly longer during recovery compared to baseline for young participants ( $p = 0.013$ ) and those who detected tVNS ( $p = 0.005$ ). Also, values were significantly higher during sham compared to recovery for the full sample ( $p = 0.013$ ), older participants ( $p = 0.040$ ), musicians ( $p = 0.047$ ) and those who did not detect tVNS ( $p = 0.032$ ).

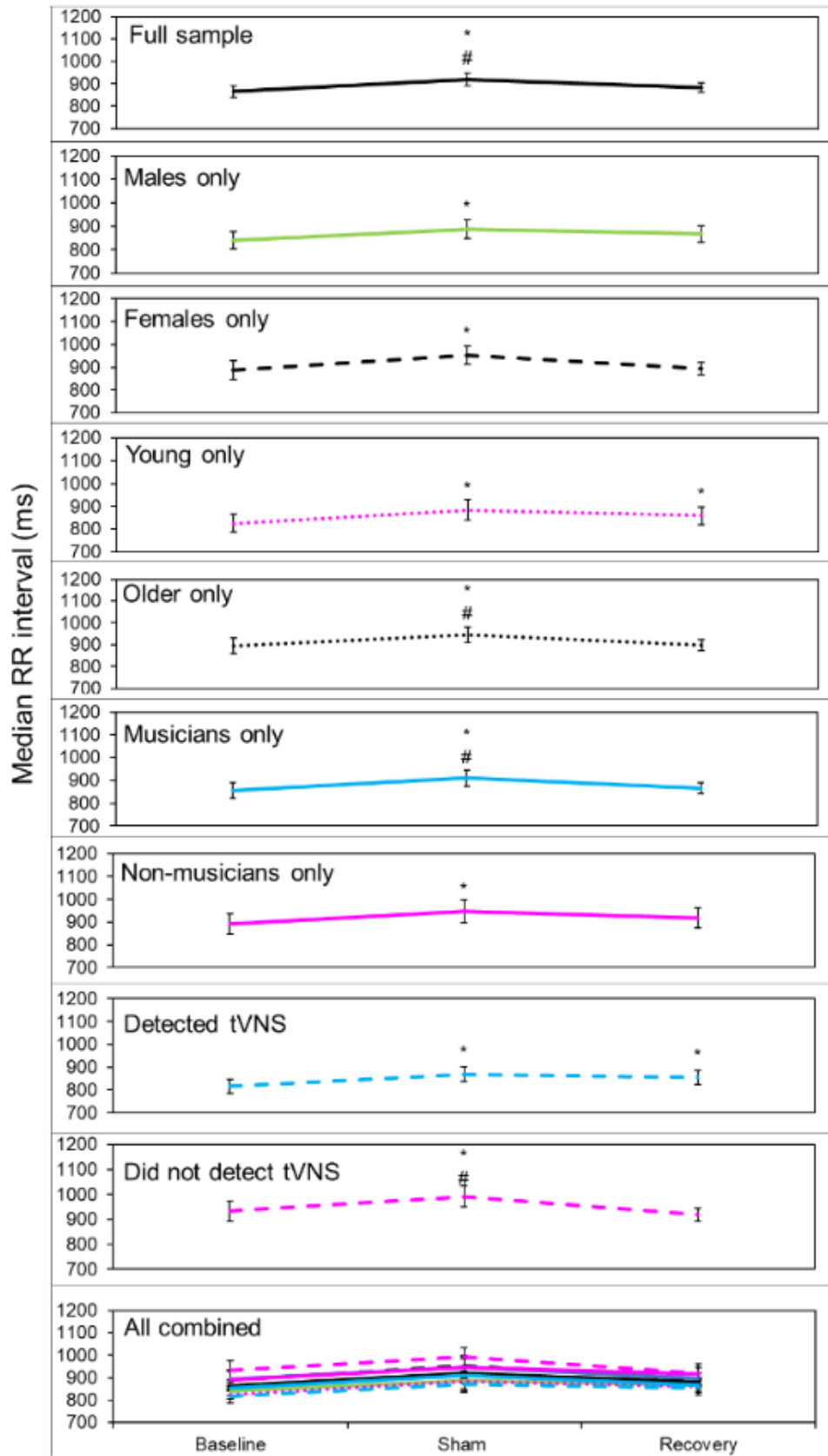
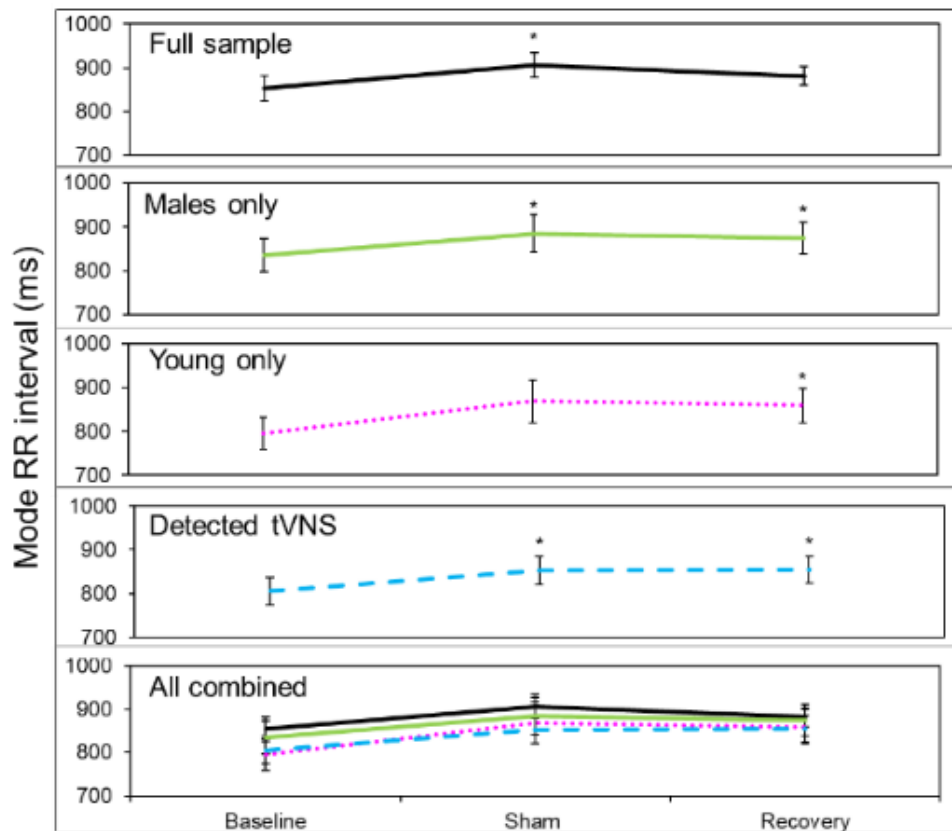


Figure 4.63: Median RR interval was significantly impacted by sham in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

There were significant effects of sham on mode RR interval in the full sample ( $p = 0.008$ ), males ( $p = 0.002$ ), young participants ( $p = 0.037$ ) and those who detected tVNS ( $p = 0.003$ , see Figure 4.64). Further analysis revealed that mode RR was significantly higher during sham than baseline (full sample:  $p = 0.011$ ; males:  $p = 0.025$ ; detected tVNS:  $p = 0.007$ ). In addition, recovery mode RR was significantly higher than that during baseline for young participants ( $p < 0.001$ ), males ( $p = 0.002$ ) and those who detected tVNS ( $p = 0.008$ ).



*Figure 4.64: Mode RR interval was significantly impacted by sham in the full sample, males, young participants and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.*

There was a significant effect of sham on min RR interval in the full sample ( $p = 0.016$ ) and older participants ( $p = 0.024$ ). Further exploration identified that min RR intervals were significantly greater during sham than baseline in the full sample ( $p = 0.007$ ) and older participants ( $p = 0.020$ , see Figure 4.65).

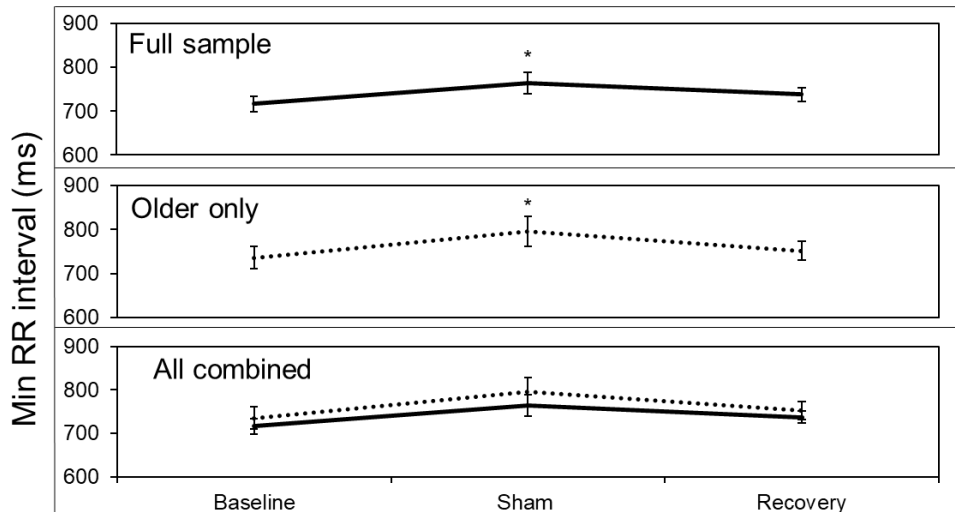


Figure 4.65: Min RR interval was significantly impacted by sham in the full sample and older participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

A significant effect of sham on max RR interval transpired in the full sample ( $p < 0.001$ ), males ( $p = 0.021$ ), females ( $p = 0.003$ ), young participants ( $p = 0.021$ ), older participants ( $p = 0.009$ ), musicians ( $p = 0.005$ ), those detected tVNS ( $p = 0.004$ ) and those who did not detect tVNS ( $p = 0.014$ , see Figure 4.66). Further analysis showed that values were significantly greater during sham than baseline (full sample:  $p < 0.001$ ; males:  $p = 0.049$ ; females:  $p = 0.002$ ; young:  $p = 0.009$ ; older:  $p = 0.009$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.002$ ; did not detect tVNS:  $p = 0.021$ ). In addition, max RR was significantly higher during sham than recovery in the full sample ( $p = 0.013$ ) and females ( $p = 0.025$ ). Also, values were significantly higher during recovery than baseline for those who detected tVNS ( $p = 0.002$ ).

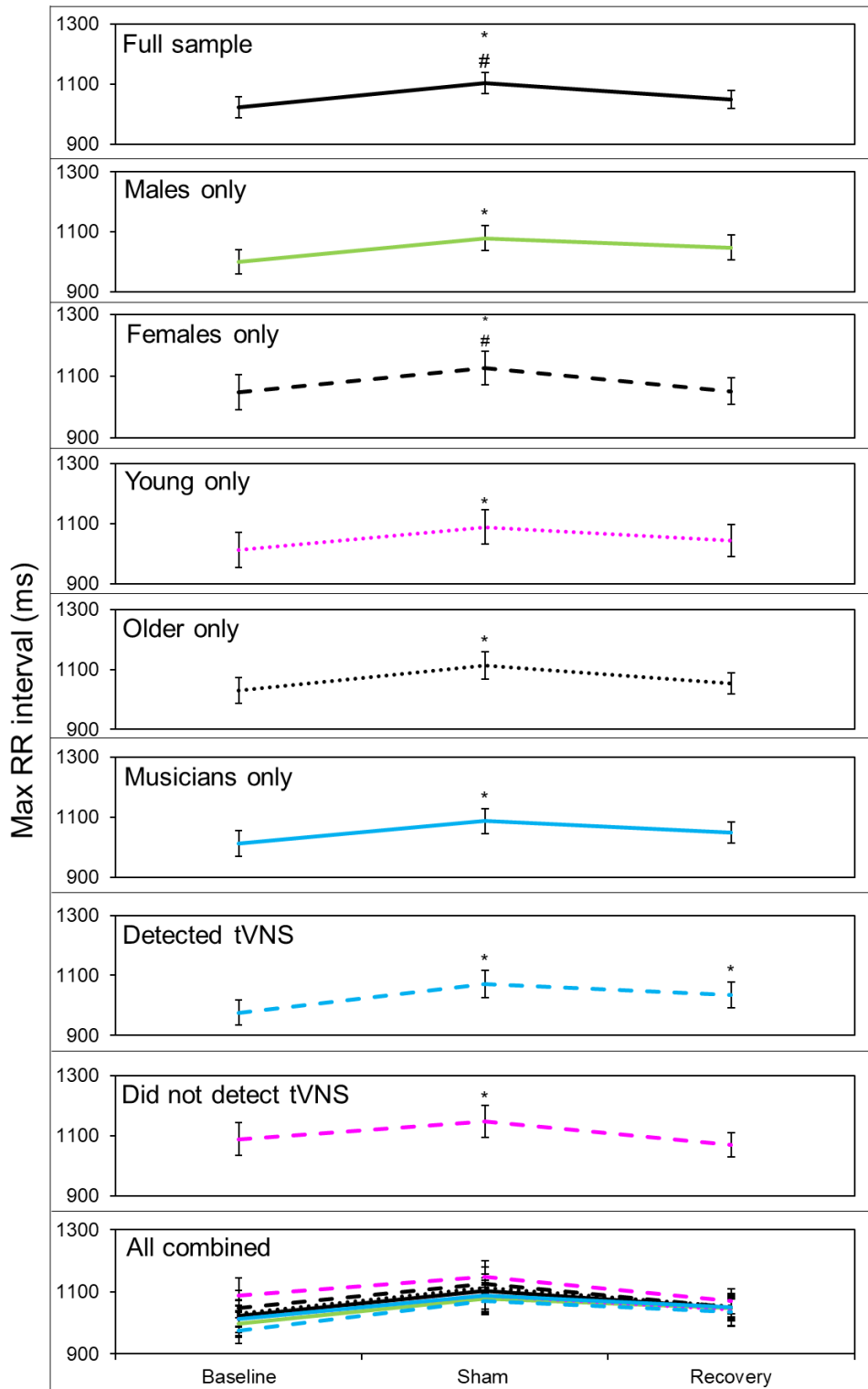


Figure 4.66: Max RR interval was significantly impacted by sham for all groups except non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly differed to recovery.

There was a significant effect of sham on Q1 RR interval in the full sample ( $p < 0.001$ ), males ( $p = 0.001$ ), females ( $p = 0.005$ ), young participants ( $p =$

0.006), older participants ( $p = 0.005$ ), musicians ( $p = 0.002$ ), non-musicians ( $p = 0.004$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.008$ ). Further analysis revealed that Q1 RR was significantly higher during sham than baseline (full sample:  $p < 0.001$ ; males:  $p = 0.005$ ; females:  $p = 0.005$ ; young:  $p = 0.005$ ; older:  $p = 0.002$ ; musicians:  $p = 0.002$ ; non-musicians:  $p = 0.016$ ; detected tVNS:  $p = 0.001$ ; did not detect tVNS:  $p = 0.013$ , see Figure 4.67). In addition, Q1 RR was significantly greater during recovery than baseline for males ( $p = 0.005$ ) and young participants ( $p = 0.013$ ). Finally, Q1 RR during sham was significantly higher than that during recovery for the full sample ( $p = 0.005$ ).

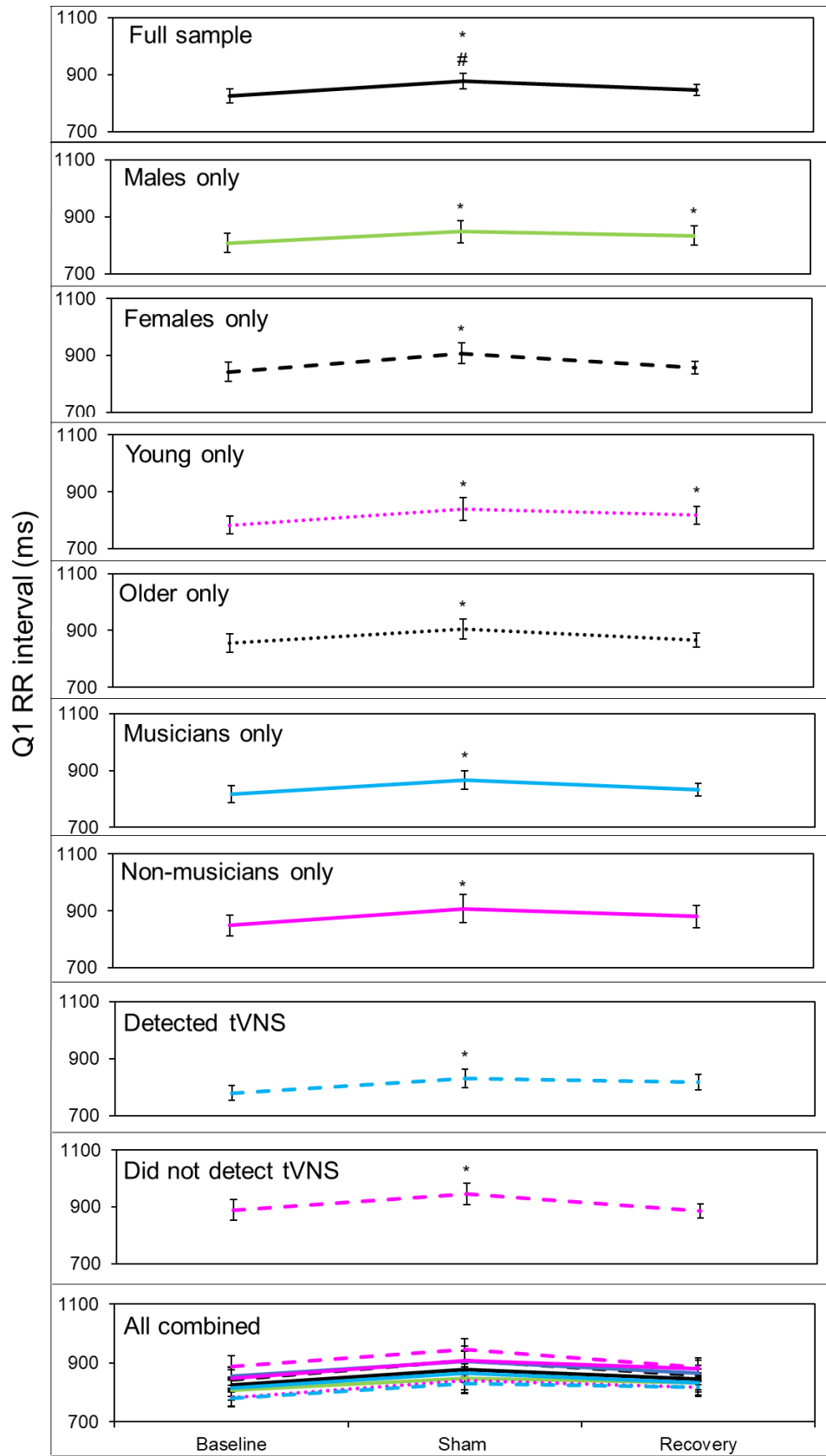


Figure 4.67: Q1 RR interval was significantly impacted by sham in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.



There were significant effects on Q3 RR interval in the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.002$ ), young participants ( $p = 0.006$ ), older participants ( $p = 0.016$ ), musicians ( $p = 0.006$ ), non-musicians ( $p = 0.016$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.008$ ). Values were significantly higher during sham compared to baseline (full sample:  $p < 0.001$ ; males:  $p < 0.001$ ; females:  $p = 0.009$ ; young:  $p = 0.005$ ; older:  $p < 0.001$ ; musicians:  $p < 0.001$ ; non-musicians:  $p = 0.027$ ; detected tVNS:  $p < 0.001$ ; did not detect tVNS:  $p = 0.010$ , see Figure 4.68). Values were also significantly higher during sham compared to recovery in the full sample ( $p = 0.014$ ) and those who did not detect tVNS ( $p = 0.041$ ); and during recovery compared to baseline for those who detected tVNS ( $p = 0.010$ ).

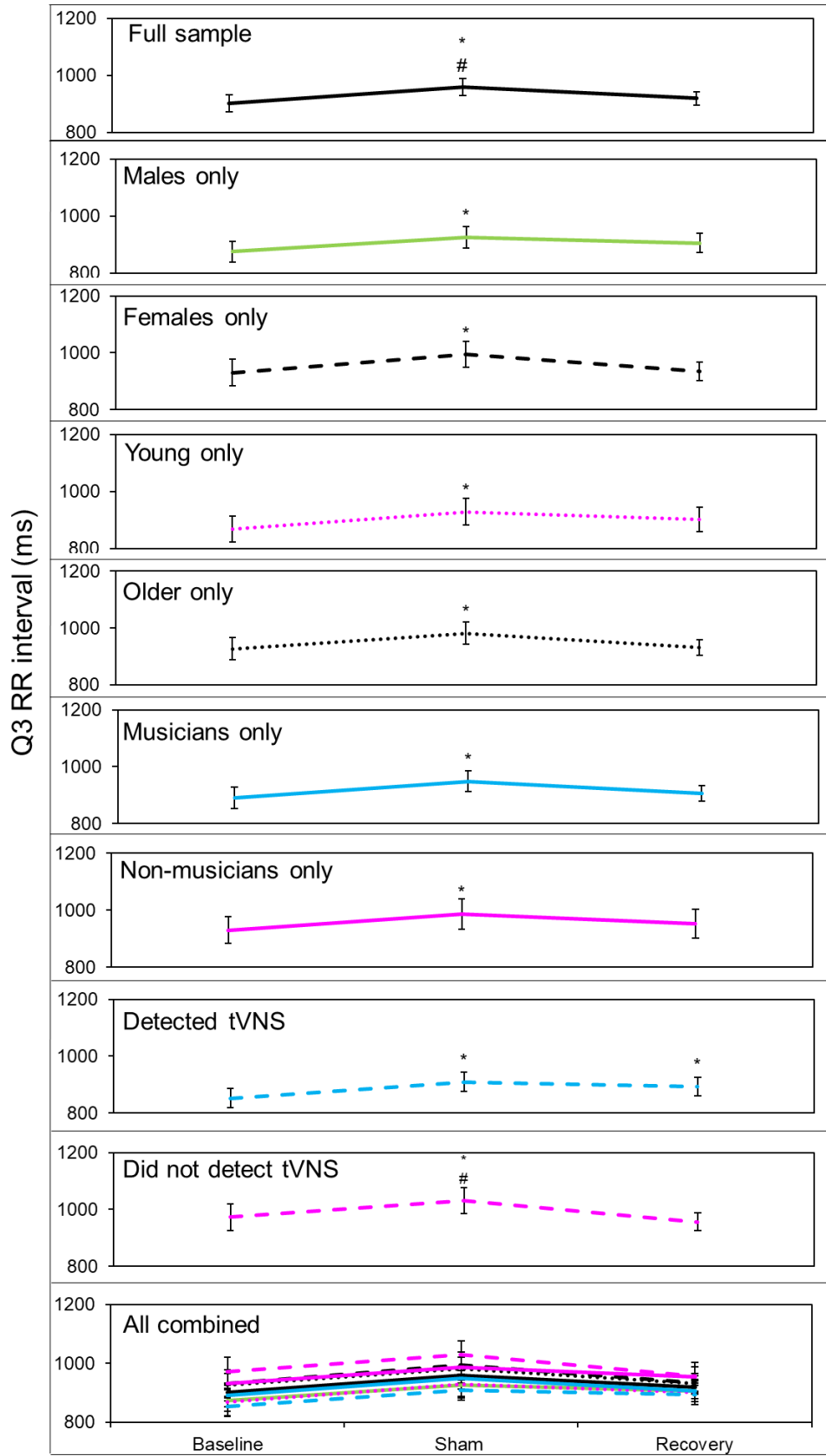


Figure 4.68: Q3 RR interval was significantly impacted by sham in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

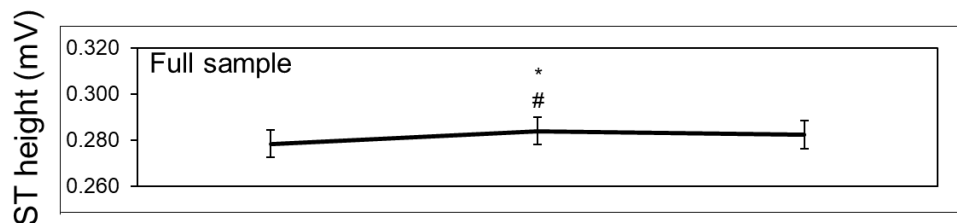
#### 4.3.1.4.5 Between-visit comparisons

Despite the emergence of many significant differences in the RR measures, none significantly differed between the four visits for any of the group analyses ( $p > 0.05$ ).

#### 4.3.1.5 ECG analysis

##### 4.3.1.5.1 tVNS only visit

There were significant effects of tVNS on ST height in the full sample ( $p = 0.014$ ). Further analysis revealed that ST height was significantly higher during tVNS compared to baseline ( $p = 0.039$ ) and recovery ( $p = 0.039$ , see Figure 4.69).



*Figure 4.69: ST height was significantly impacted by tVNS in the full sample. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.*

T amplitude was significantly impacted by tVNS in the full sample ( $p = 0.030$ ). Further exploration demonstrated that T amplitude was significantly lower during tVNS than during baseline ( $p = 0.026$ ) and recovery ( $p = 0.011$ , see Figure 4.70). Young participants also encountered a significant effect of tVNS on T amplitude ( $p = 0.014$ ): values were significantly lower during sham than baseline ( $p = 0.005$ ).

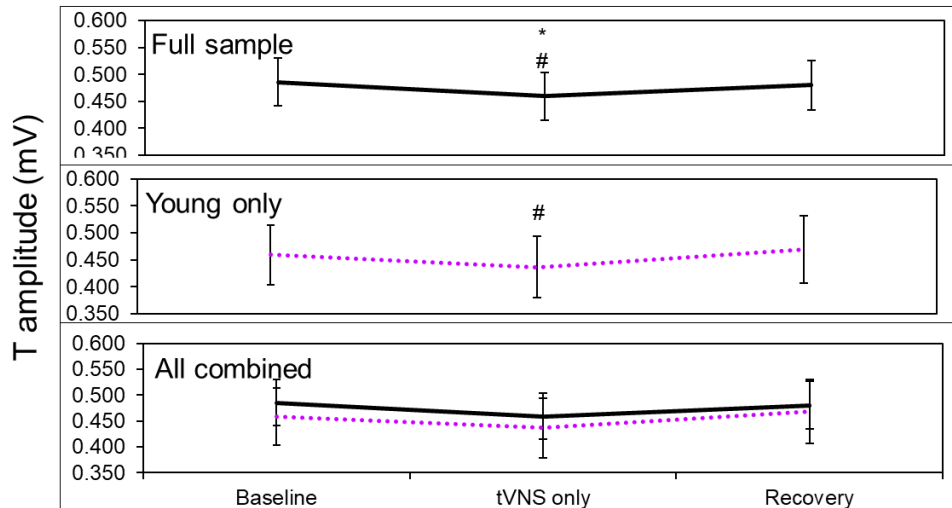


Figure 4.70: T amplitude was significantly impacted by tVNS in the full sample and young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline, # = significantly different to recovery.

A significant effect of tVNS on the QT interval was observed in the full sample ( $p = 0.010$ ), females ( $p = 0.021$ ) and musicians ( $p = 0.022$ , see Figure 4.71). Values were significantly higher in tVNS compared to baseline (full sample:  $p = 0.002$ ; females:  $p = 0.015$ ; musicians:  $p = 0.002$ ).

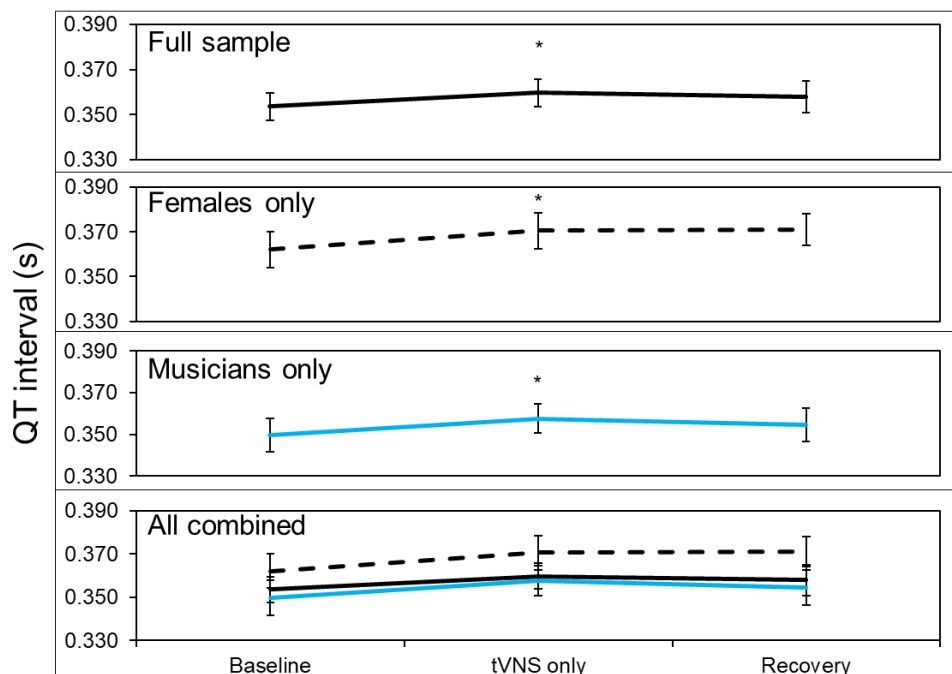


Figure 4.71: QT interval was significantly impacted by tVNS in the full sample, females and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant impacts of tVNS on the JT interval in the full sample ( $p = 0.012$ ), females ( $p = 0.006$ ) and musicians ( $p = 0.032$ ): values were significantly higher in tVNS compared to baseline (full sample:  $p = 0.005$ ; females:  $p = 0.022$ ; musicians:  $p = 0.004$ , see Figure 4.72).

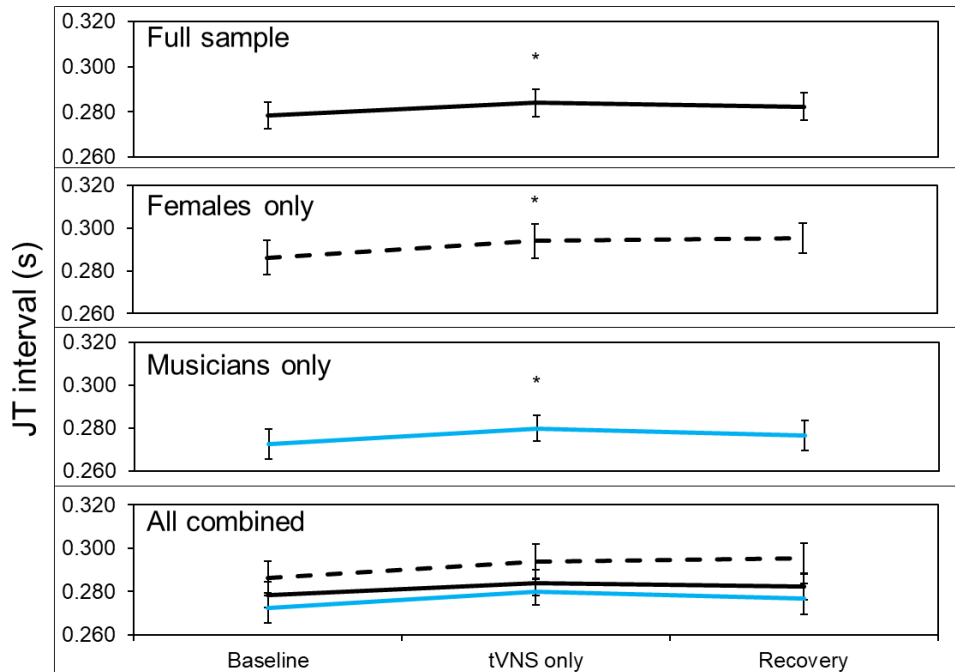


Figure 4.72: JT interval was significantly impacted by tVNS in the full sample, females and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.5.2 Music only visit

There was a significant effect of music on the QRS interval in the full sample ( $p = 0.005$ ), males ( $p = 0.006$ ), young participants ( $p = 0.005$ ), musicians ( $p = 0.013$ ) and those who detected tVNS ( $p = 0.002$ ). Further analysis showed that QRS intervals were significantly longer during recovery compared to baseline (full sample:  $p = 0.012$ ; males:  $p = 0.039$ ; young:  $p = 0.017$ ; musicians:  $p = 0.021$ ; detected tVNS:  $p = 0.012$ , see Figure 4.73).

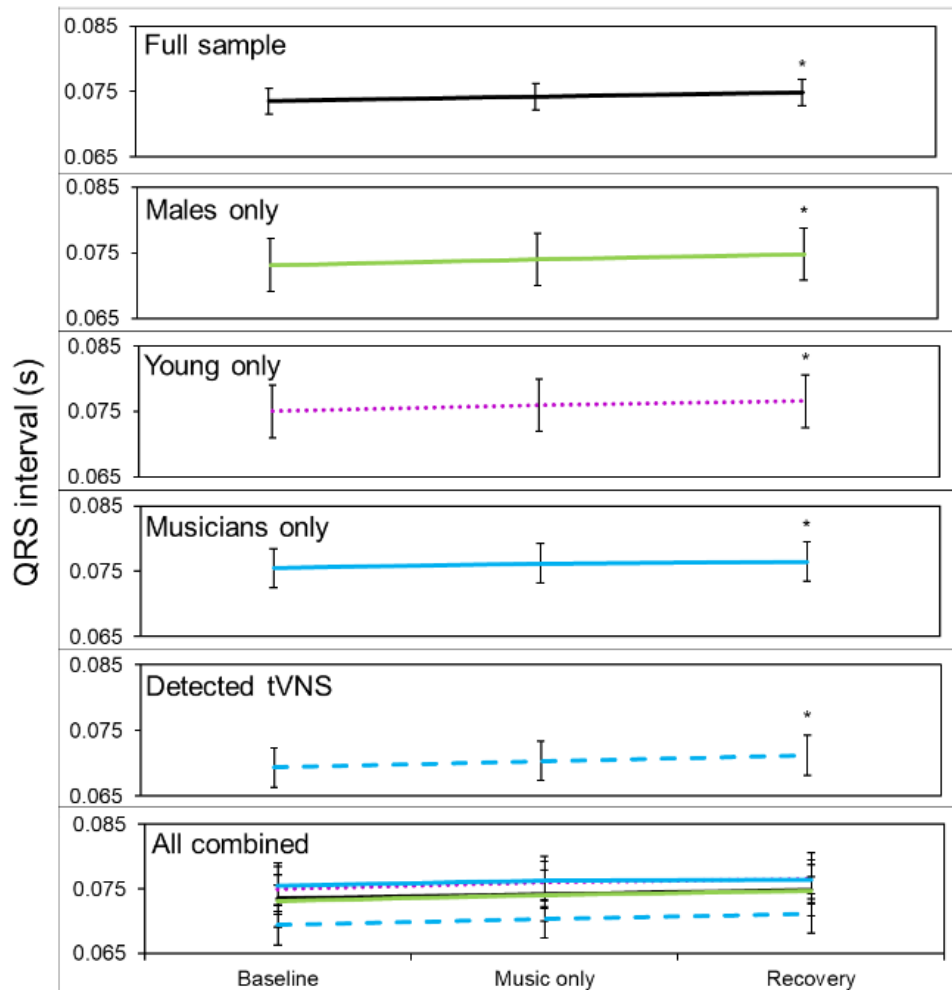


Figure 4.73: The QRS interval was significantly impacted by music in the full sample, males, young participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

Significant effects of music on QT intervals emerged for the full sample ( $p < 0.001$ ), females ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p = 0.001$ ) and those who detected tVNS ( $p = 0.002$ ). Further exploration demonstrated that QT intervals were significantly longer during music and recovery compared to baseline (full sample:  $p = 0.001$  and  $p < 0.001$  respectively; females:  $p < 0.0001$  and  $p = 0.001$  respectively; older:  $p < 0.001$  for both; musicians:  $p = 0.006$  and  $p = 0.009$  respectively; detected tVNS:  $p = 0.015$  and  $p = 0.009$  respectively, see Figure 4.74).

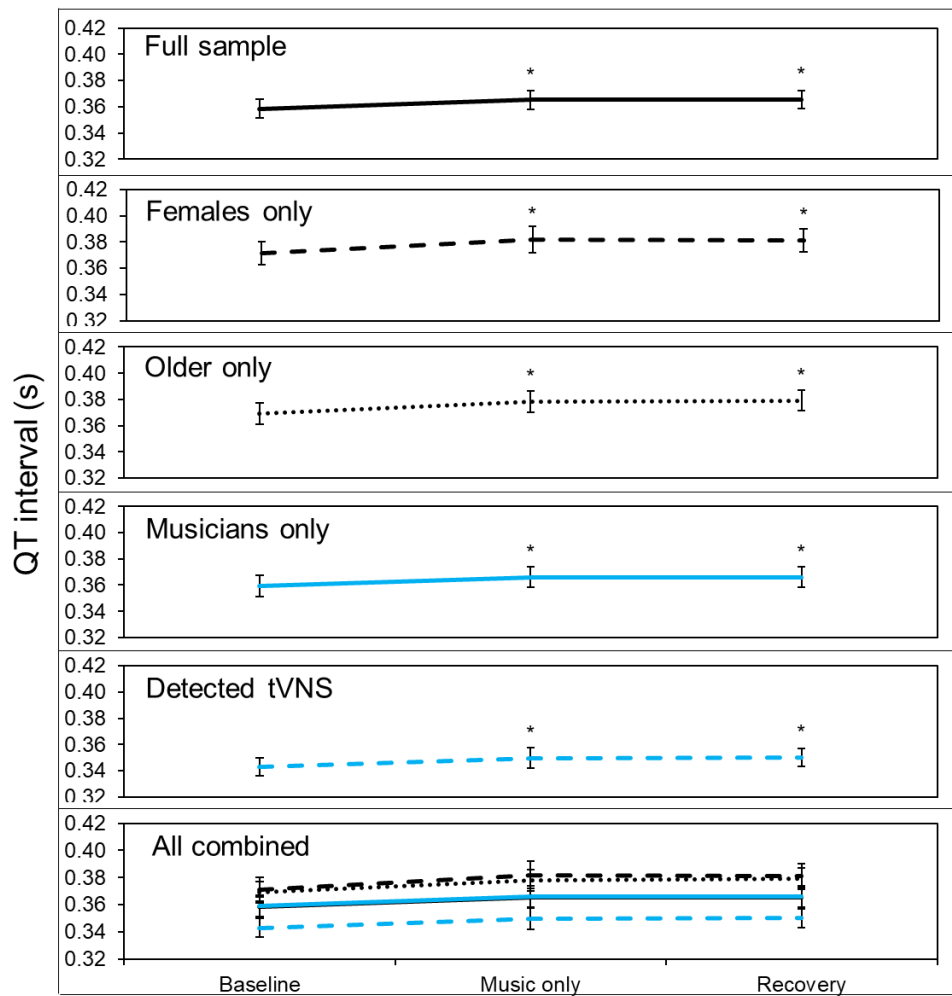


Figure 4.74: QT intervals were significantly impacted by music in the full sample, females, older participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant effects of music on the QTc interval in the full sample ( $p = 0.041$ ), females ( $p = 0.046$ ) and musicians ( $p = 0.002$ ). QTc intervals were significantly longer during recovery compared to baseline (full sample:  $p = 0.001$ ; females:  $p = 0.001$ ; musicians:  $p = 0.028$ , see Figure 4.75). Furthermore, QTc during music was significantly higher than that during recovery in musicians ( $p = 0.001$ ).

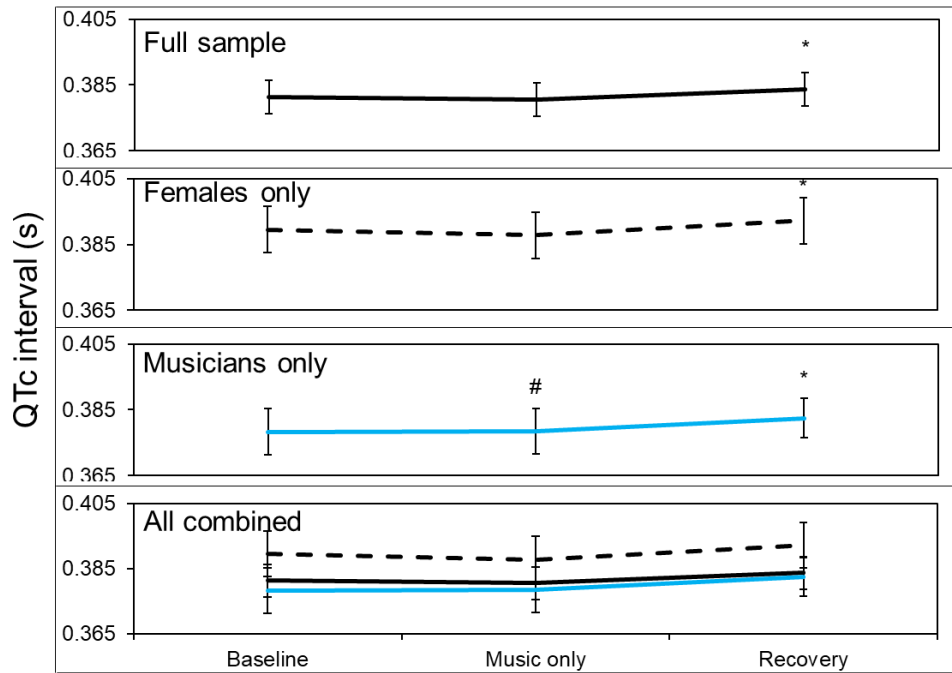


Figure 4.75: QTc intervals were significantly impacted by the music in the full sample, females and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

JT intervals were significantly impacted by music in the full sample ( $p < 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.001$ ), musicians ( $p = 0.013$ ) and those who detected tVNS ( $p = 0.006$ ). Further exploration demonstrated that intervals were significantly longer during music and recovery compared to baseline (full sample:  $p = 0.004$  and  $p = 0.005$  respectively; females:  $p < 0.001$  and  $p = 0.005$  respectively; older:  $p = 0.001$  and  $p < 0.001$  respectively; musicians:  $p = 0.013$  and  $p = 0.025$  respectively; detected tVNS:  $p = 0.041$  and  $p = 0.045$  respectively, see Figure 4.76).



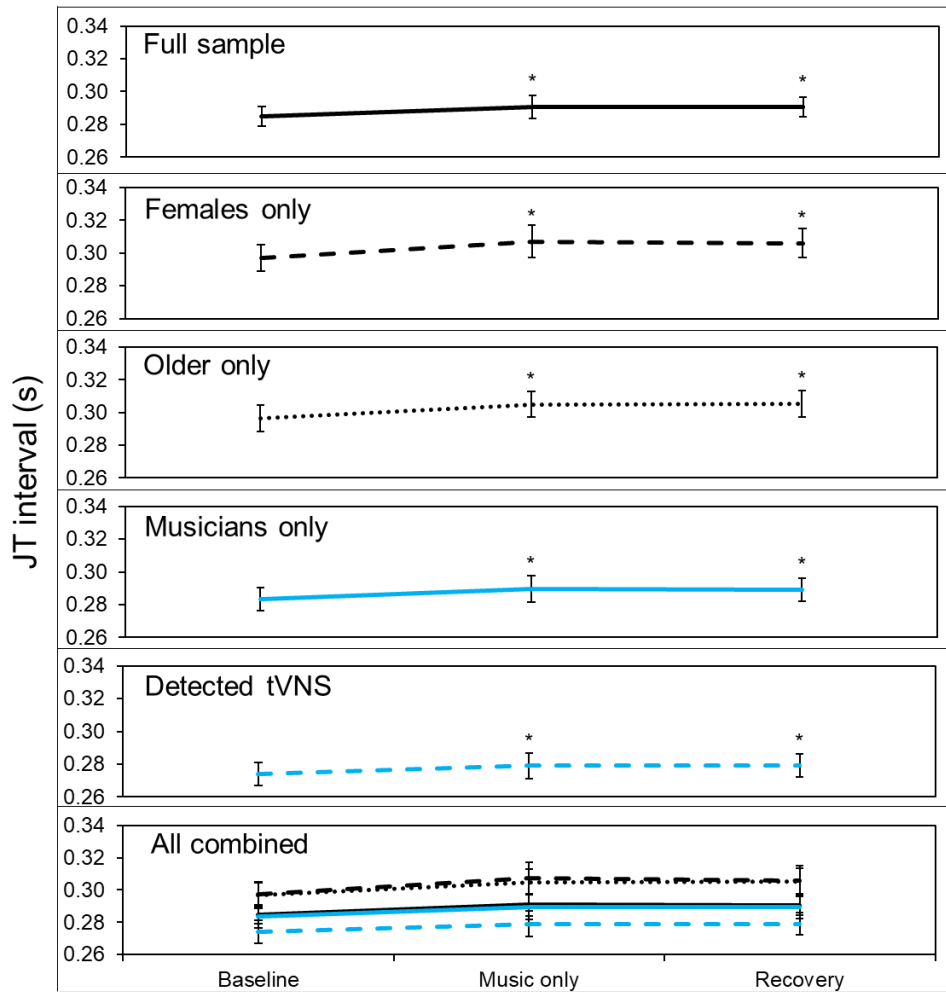


Figure 4.76: JT intervals were significantly impacted by the music in the full sample, females, older participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.5.3 tVNS + music visit

There was a significant effect of tVNS + music on Q amplitude in young participants only ( $p = 0.023$ ). Further analysis revealed that Q amplitudes significantly decreased during stimulation compared to baseline ( $p = 0.025$ , see Figure 4.77).

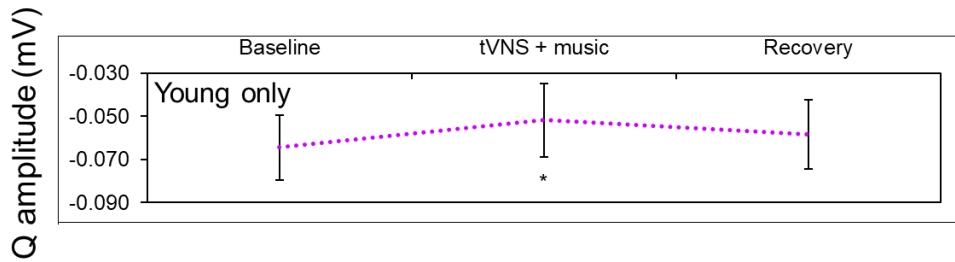


Figure 4.77: Q amplitudes were significantly impacted by tVNS + music in young participants only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

R amplitudes were also significantly impacted by the tVNS + music in the full sample ( $p = 0.008$ ), females ( $p = 0.020$ ), young participants ( $p = 0.011$ ) and musicians ( $p = 0.045$ ). Further exploration demonstrated that R amplitudes were significantly smaller in recovery compared to baseline (full sample:  $p = 0.049$ ; females:  $p = 0.026$ ; young:  $p = 0.025$ ; musicians:  $p = 0.016$ , see Figure 4.78).

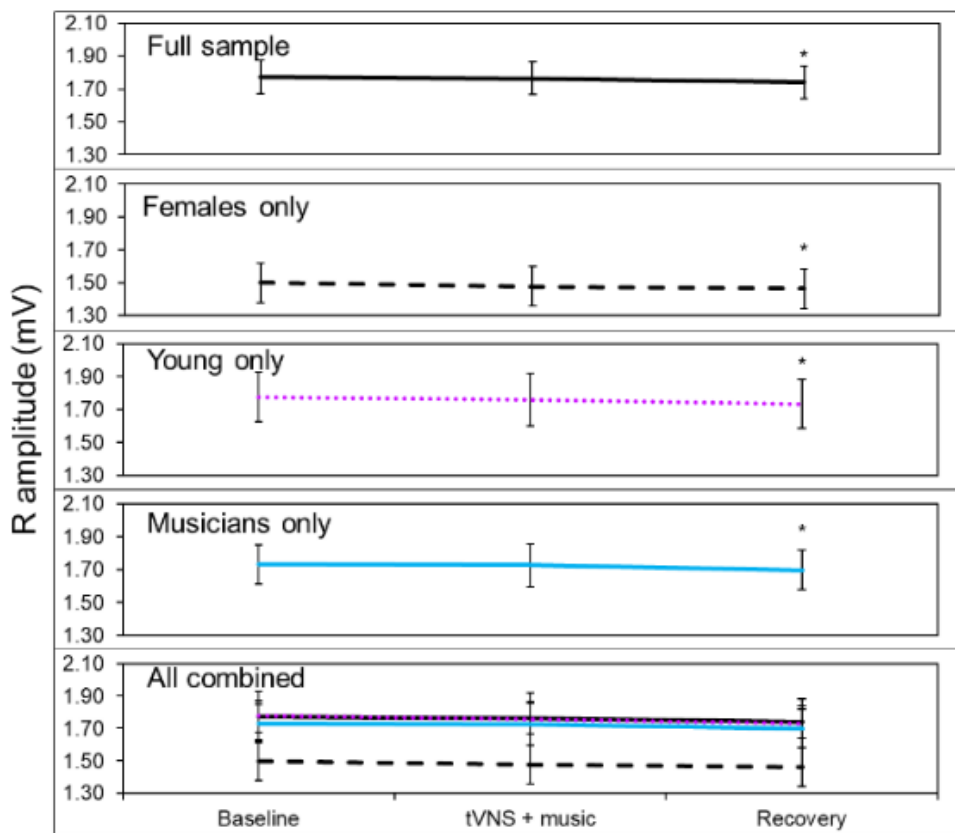


Figure 4.78: R amplitudes were significantly impacted by tVNS + music in the full sample, females, young participants and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant effects of tVNS + music on S amplitudes in the full sample ( $p = 0.042$ ). Further analysis revealed that S amplitudes were significantly smaller in recovery compared to baseline ( $p = 0.002$ , see Figure 4.79). S amplitudes in young participants were also impacted ( $p = 0.024$ ). Pairwise comparisons revealed that S amplitudes were significantly lower during tVNS + music and recovery compared to baseline ( $p = 0.024$  and  $p = 0.014$  respectively).

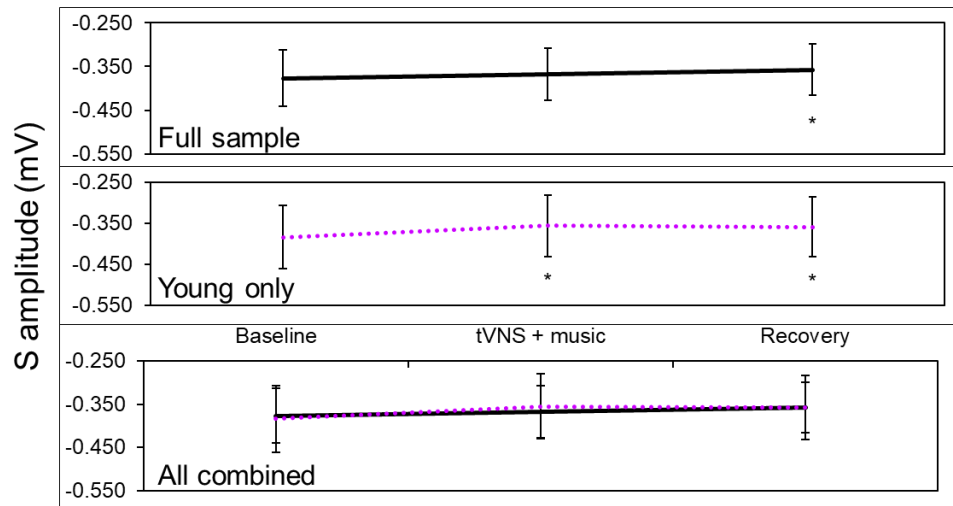


Figure 4.79: S amplitudes were significantly impacted by tVNS + music in the full sample and young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There was a significant effect of tVNS + music on QRS interval in females only ( $p = 0.017$ ). Further analysis revealed that QRS intervals significantly increased between recovery and baseline ( $p = 0.024$ , see Figure 4.80).

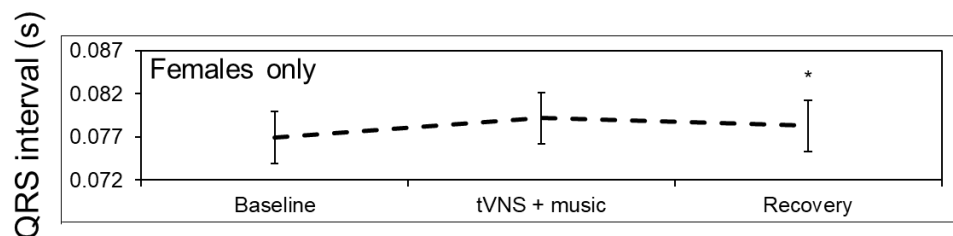


Figure 4.80: QRS intervals were significantly impacted by tVNS + music in females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

The QT interval was significantly impacted by the tVNS + music in the full sample ( $p = 0.004$ ), females ( $p = 0.001$ ), older participants ( $p = 0.011$ ), musicians ( $p = 0.013$ ) and those who detected tVNS ( $p = 0.002$ , see Figure 4.81). Bonferroni revealed that QT intervals were significantly higher in tVNS + music than baseline (full sample:  $p = 0.004$ ; females:  $p = 0.002$ ; older:  $p = 0.025$ ; musicians:  $p = 0.017$ ; detected tVNS:  $p = 0.022$ ). In addition, QT intervals were significantly longer during recovery than baseline in females ( $p = 0.045$ ) and those who detected tVNS ( $p = 0.016$ ).

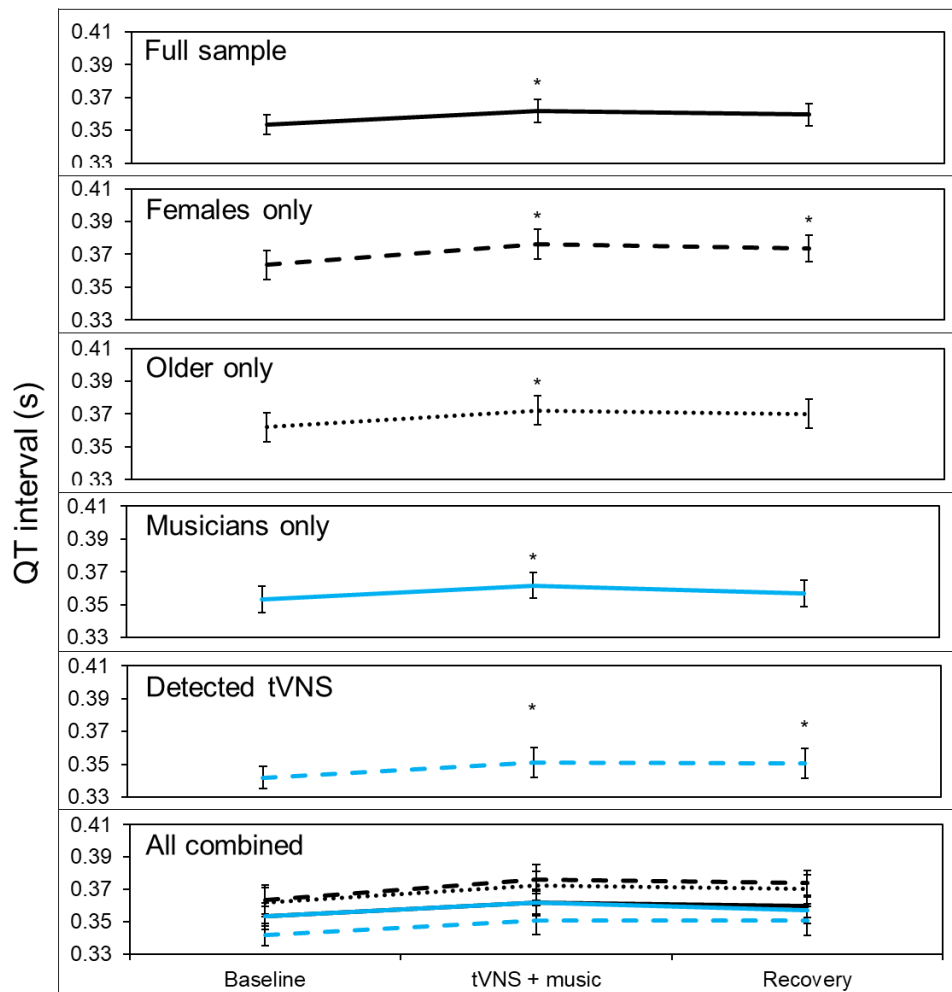


Figure 4.81: QT intervals were significantly impacted by tVNS + music in the full sample, females, older participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

There were significant effects of tVNS + music on JT intervals in the full sample ( $p = 0.018$ ), females ( $p = 0.003$ ) and those who detected tVNS ( $p = 0.017$ ). Further exploration of this effect demonstrated that JT intervals were

significantly longer during tVNS + music than baseline (full sample:  $p = 0.029$ ; females:  $p = 0.005$ , see Figure 4.82). Also, JT intervals for those who detected tVNS were significantly longer during recovery than baseline ( $p = 0.012$ ).

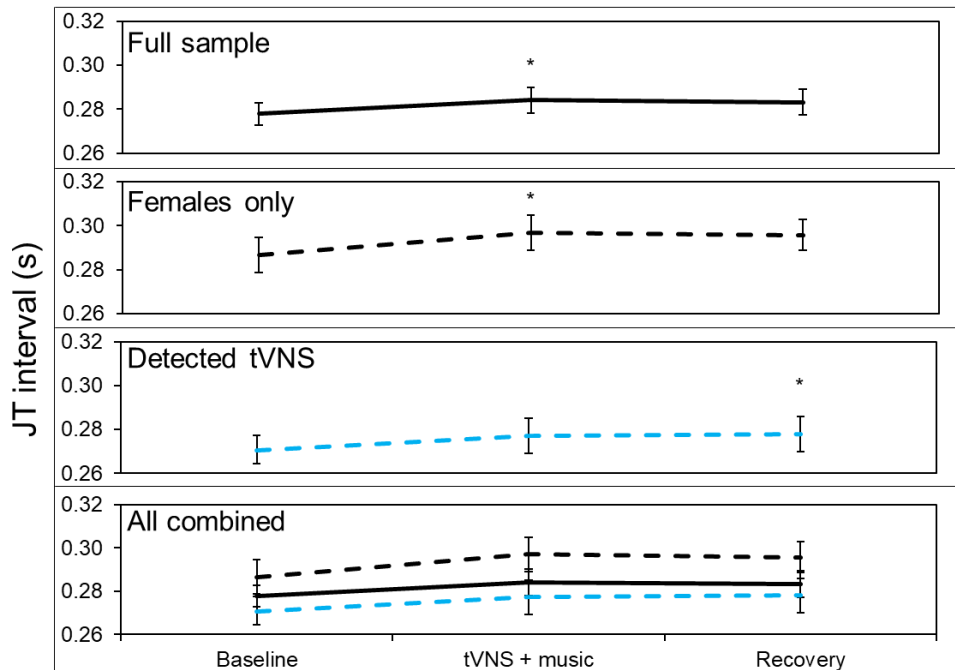


Figure 4.82: JT intervals were significantly impacted by tVNS + music in the full sample, females and those who detected tVNS. Data presented as mean  $\pm 1$  SEM. \* = significantly different to baseline.

#### 4.3.1.5.4 Sham visit

There was a significant effect of tVNS + music on Q amplitude in those who detected tVNS ( $p = 0.016$ ). Further analysis revealed that Q amplitude during sham was not low as that during baseline ( $p = 0.008$ , see Figure 4.83).

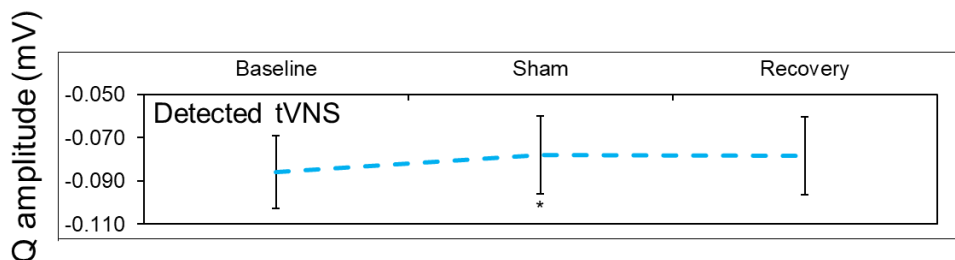
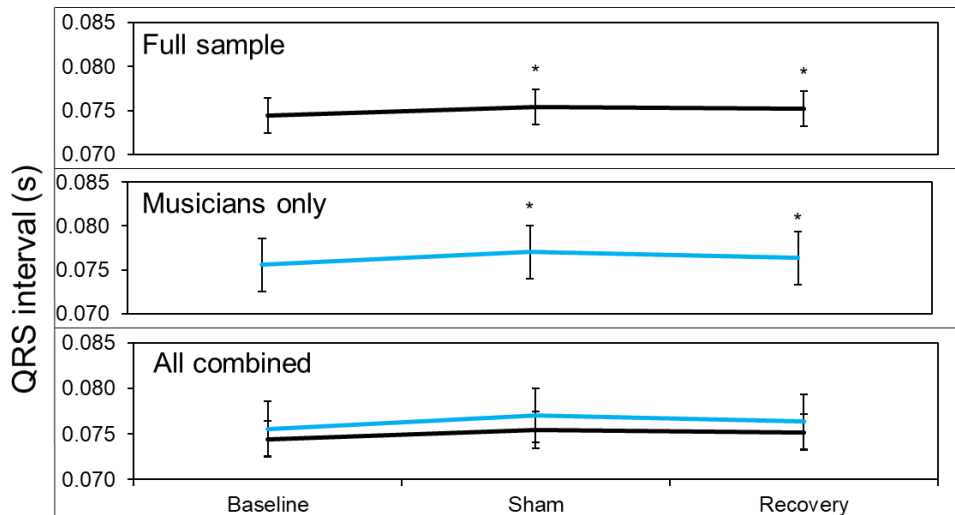


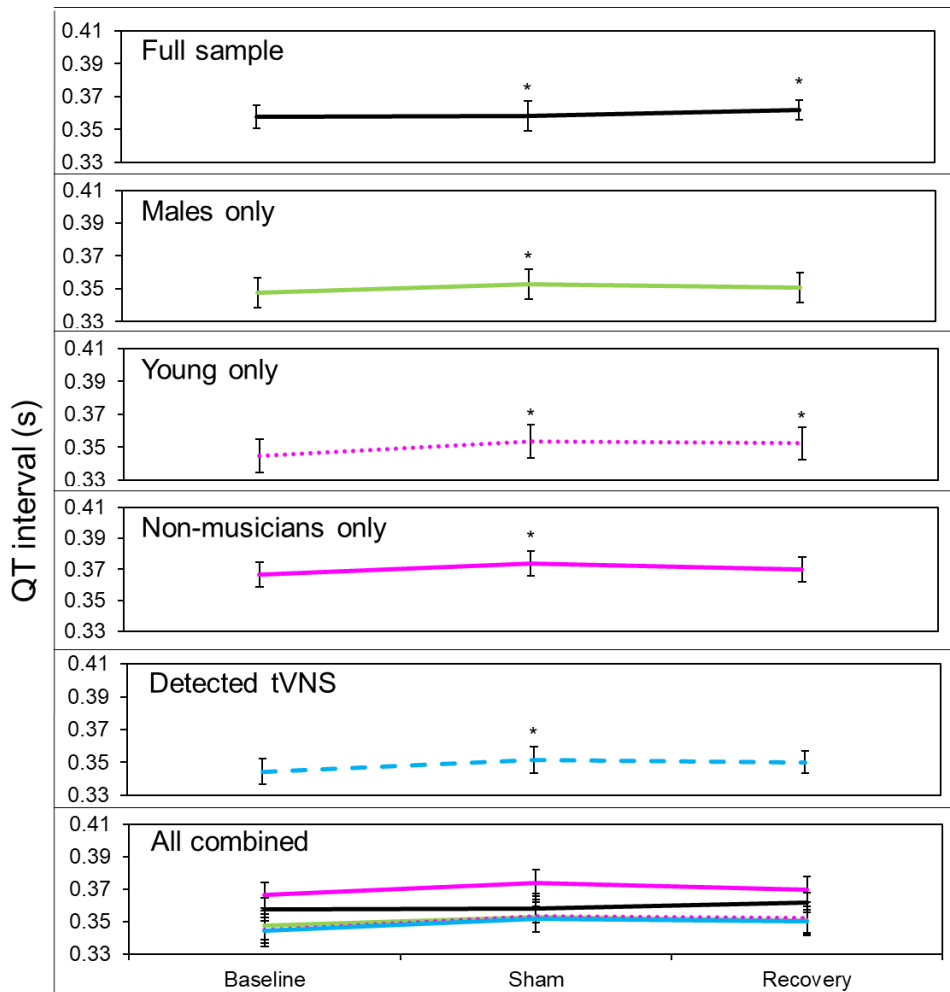
Figure 4.83: Q amplitude was significantly impacted by sham in the group who detected tVNS. Data presented as mean  $\pm 1$  SEM. \* = significantly different to baseline.

QRS interval was significantly impacted by sham in the full sample ( $p = 0.012$ ) and musicians ( $p = 0.002$ ). Further analysis demonstrated that QRS intervals significantly increased between baseline and sham (full sample:  $p = 0.041$ ; musicians:  $p = 0.012$ ) and between baseline and recovery (full sample:  $p = 0.016$ ; musicians:  $p = 0.038$ , see Figure 4.84).



*Figure 4.84: QRS intervals were significantly impacted by sham in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.*

There was a significant effect of sham on QT intervals in the full sample ( $p = 0.001$ ), males ( $p = 0.029$ ), young participants ( $p = 0.002$ ), non-musicians ( $p = 0.025$ ) and those who detected tVNS ( $p = 0.002$ ). Further exploration of this effect showed that the QT intervals significantly increased between baseline and sham (full sample:  $p = 0.001$ ; males:  $p = 0.017$ ; young:  $p = 0.004$ ; non-musicians:  $p = 0.039$ ; detected tVNS:  $p = 0.002$ , see Figure 4.85). Additionally, QT intervals were significantly longer during recovery than baseline for the full sample ( $p = 0.016$ ) and young participants ( $p = 0.050$ ).



*Figure 4.85: QT intervals were significantly impacted by sham in the full sample, males, young participants, non-musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.*

QTc intervals were also impacted by sham in the full sample ( $p = 0.011$ ), males ( $p = 0.010$ ), females ( $p = 0.039$ ), older participants ( $p = 0.013$ ), musicians ( $p = 0.010$ ), those who detected tVNS ( $p = 0.036$ ) and those who did not detect tVNS ( $p = 0.003$ ). Further analysis demonstrated that QTc intervals were significantly shorter during sham compared to baseline (full sample:  $p = 0.001$ ; males:  $p = 0.021$ ; musicians:  $p = 0.004$ ; detected tVNS:  $p = 0.015$ , see Figure 4.86). In addition, QTc intervals during sham were significantly shorter than those during recovery for the full sample ( $p = 0.006$ ), females ( $p = 0.010$ ), older participants ( $p = 0.011$ ), musicians ( $p = 0.003$ ) and those who did not detect tVNS ( $p = 0.005$ ).

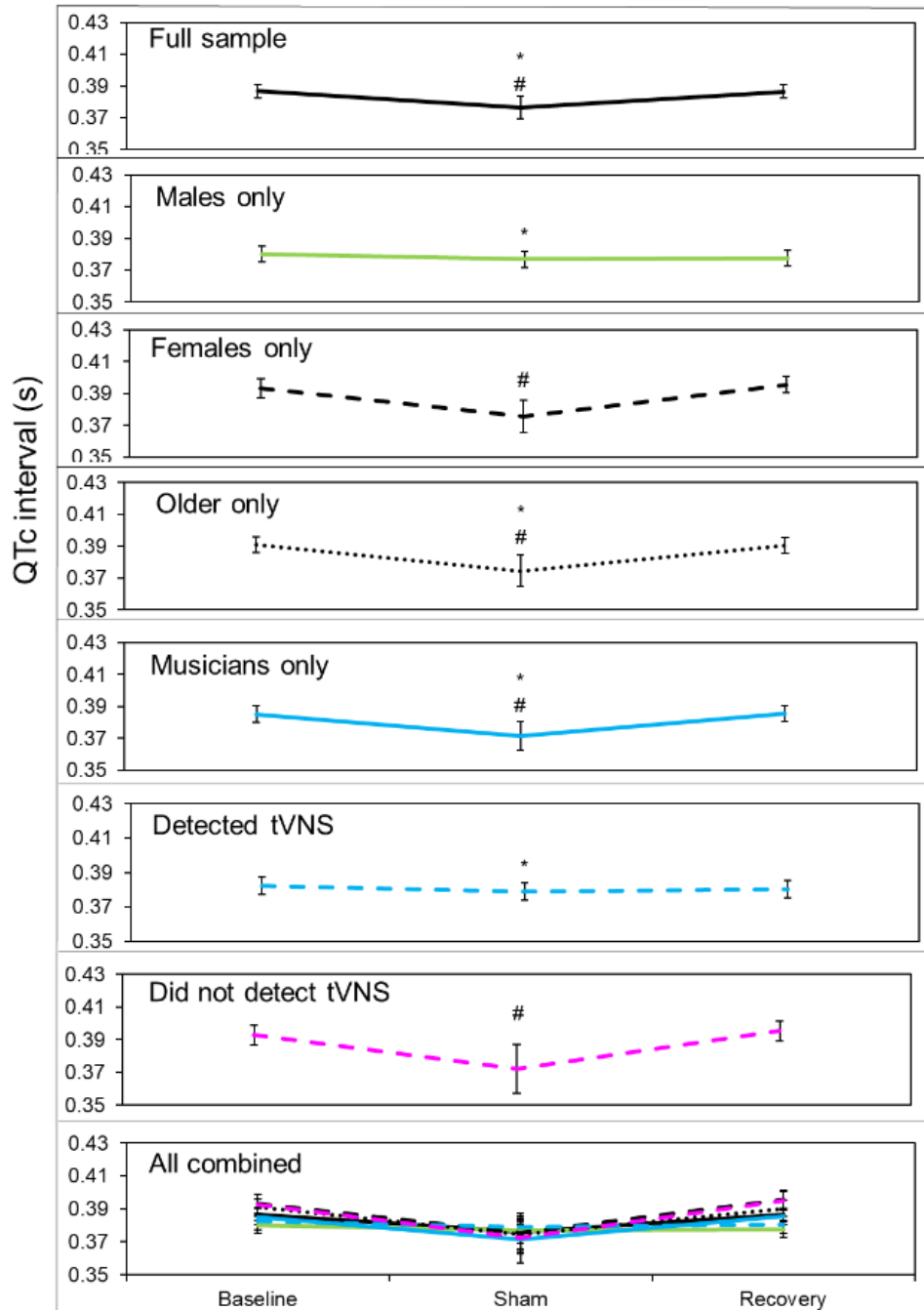


Figure 4.86: QTc intervals were significantly impacted by sham in all groups except young participants and non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Finally, JT intervals were significantly impacted by sham in the full sample ( $p = 0.002$ ), young participants ( $p = 0.006$ ) and those who detected tVNS ( $p = 0.005$ ). Further analysis of this effect revealed that JT intervals significantly decreased between baseline and sham (full sample:  $p = 0.002$ , young:  $p = 0.011$ ; detected tVNS:  $p = 0.005$ , see Figure 4.87).



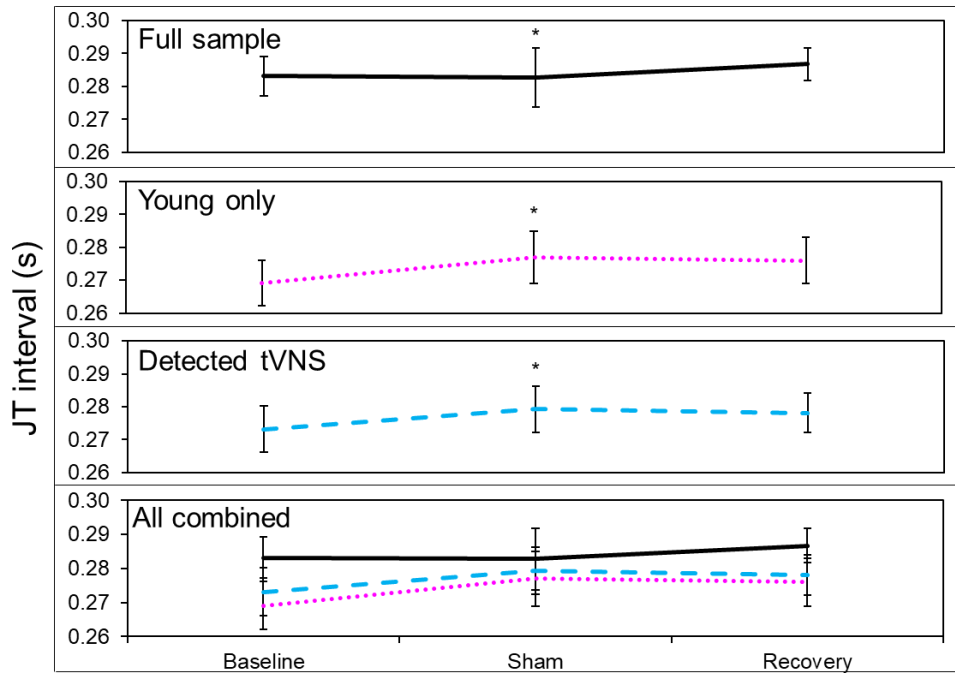


Figure 4.87: JT intervals were significantly impacted during sham in the full sample, young participants and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.5.5 Between-visit comparisons

Contrary to expectations, the only significant difference that emerged was in S amplitude for the young participants ( $p < 0.001$ ). As shown in Figure 4.88, there was a significantly greater increase in S amplitude in the tVNS + music visit, than in the music only ( $p = 0.016$ ) and sham ( $p = 0.012$ ) visits.

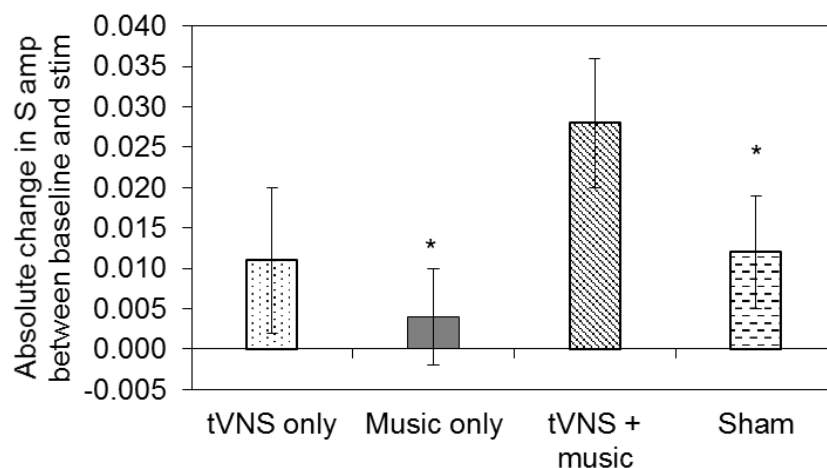


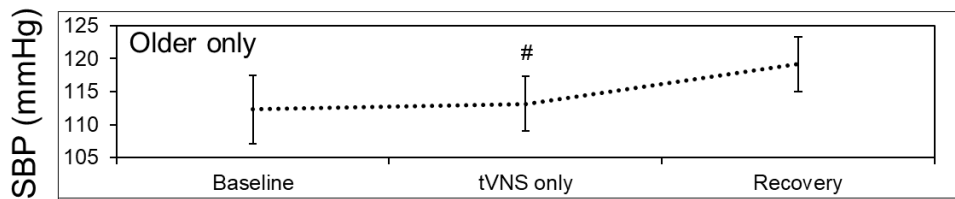
Figure 4.88: S amplitude was significantly lower during sham than during tVNS + music in young participants only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to the tVNS + music visit.

### 4.3.1.6 BP, BRS and respiration

#### 4.3.1.6.1 tVNS only visit

There were significant effects of tVNS on SBP and MAP in older participants only ( $p = 0.011$  and  $p = 0.013$  respectively). Further analysis revealed that SBP and MAP were significantly lower during tVNS than recovery ( $p = 0.028$  and  $p = 0.023$  respectively). Figure 4.89 summarises these outcomes.

a.



b.

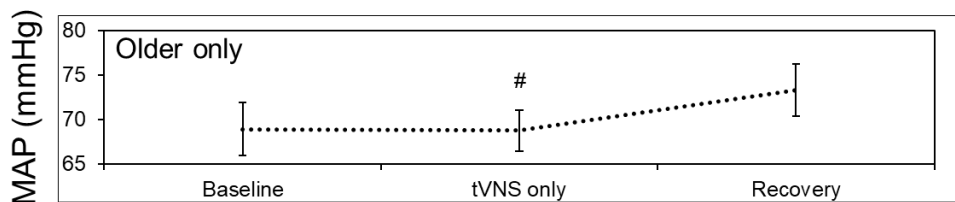
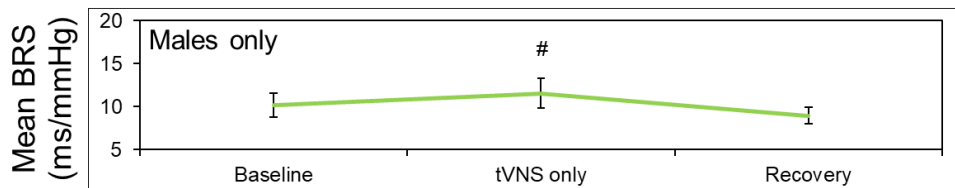


Figure 4.89: SBP (a) and MAP (b) were significantly impacted by tVNS in older participants only. Data presented as mean  $\pm$  1 SEM. # = significantly different to recovery.

A significant effect of tVNS on mean BRS in males was identified ( $p = 0.010$ , see Figure 4.90a). Further analysis demonstrated that mean BRS during tVNS was significantly higher than recovery ( $p = 0.040$ ). A significant effect on respiration rate was also observed in males only ( $p = 0.006$ , see Figure 4.90b): respiration rate was significantly lower during tVNS than baseline ( $p = 0.045$ ) and recovery ( $p = 0.017$ ).

a.



b.

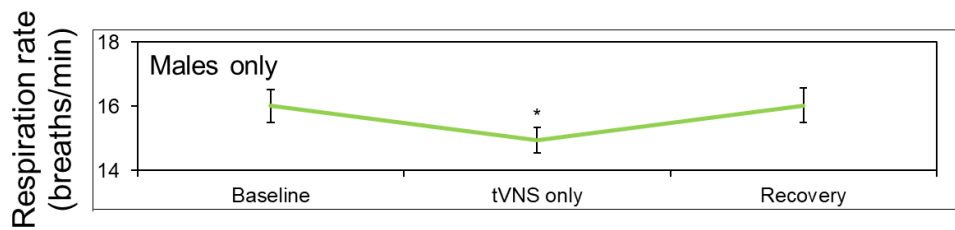


Figure 4.90: Mean BRS (a) and respiration rate (b) were significantly impacted by tVNS in males only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.1.6.2 Music only visit

A significant effect of music on respiration rate transpired for the full sample ( $p = 0.005$ ): respiration rate was significantly lower during music than baseline ( $p = 0.007$ , see Figure 4.91). A similar pattern in respiration rate emerged for females ( $p = 0.008$ ). Indeed, as shown in Figure 4.91, respiration rate was significantly lower during music than baseline ( $p = 0.005$ ).

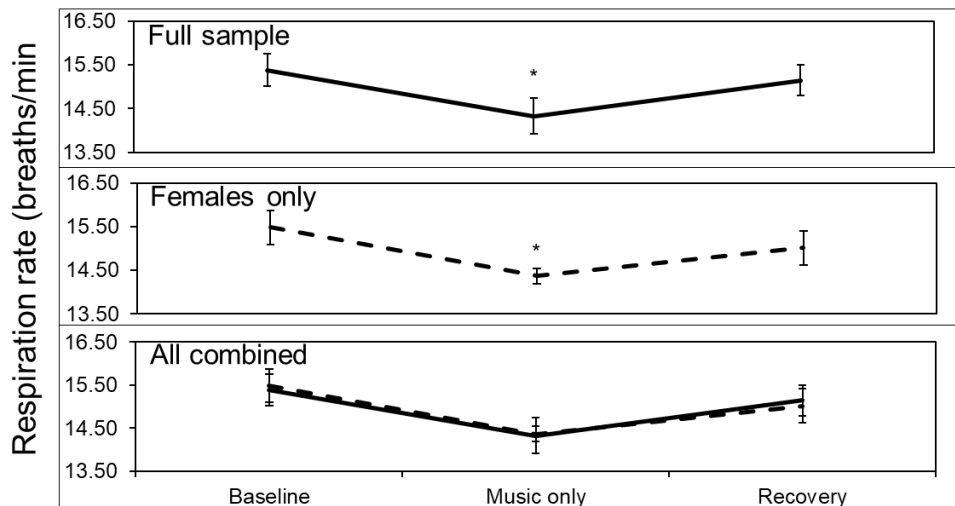


Figure 4.91: Respiration rate was significantly impacted by the music in the full sample and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.6.3 tVNS + music visit

A significant effect of tVNS + music on SBP emerged in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), musicians ( $p < 0.001$ ) and those who did not detect tVNS ( $p < 0.001$ ). SBP was significantly higher in recovery than baseline (full sample:  $p = 0.004$ ; females:  $p = 0.004$ ; musicians:  $p = 0.004$ ; did not detect tVNS:  $p = 0.004$ ) and tVNS + music (full sample:  $p = 0.043$ ; females:  $p = 0.002$ ; musicians:  $p = 0.002$ ; did not detect tVNS:  $p = 0.002$ , see Figure 4.92). In addition, SBP in young participants significantly changed ( $p = 0.005$ ): baseline SBP was significantly lower than SBP during tVNS + music ( $p = 0.017$ ) and recovery SBP ( $p = 0.037$ ).

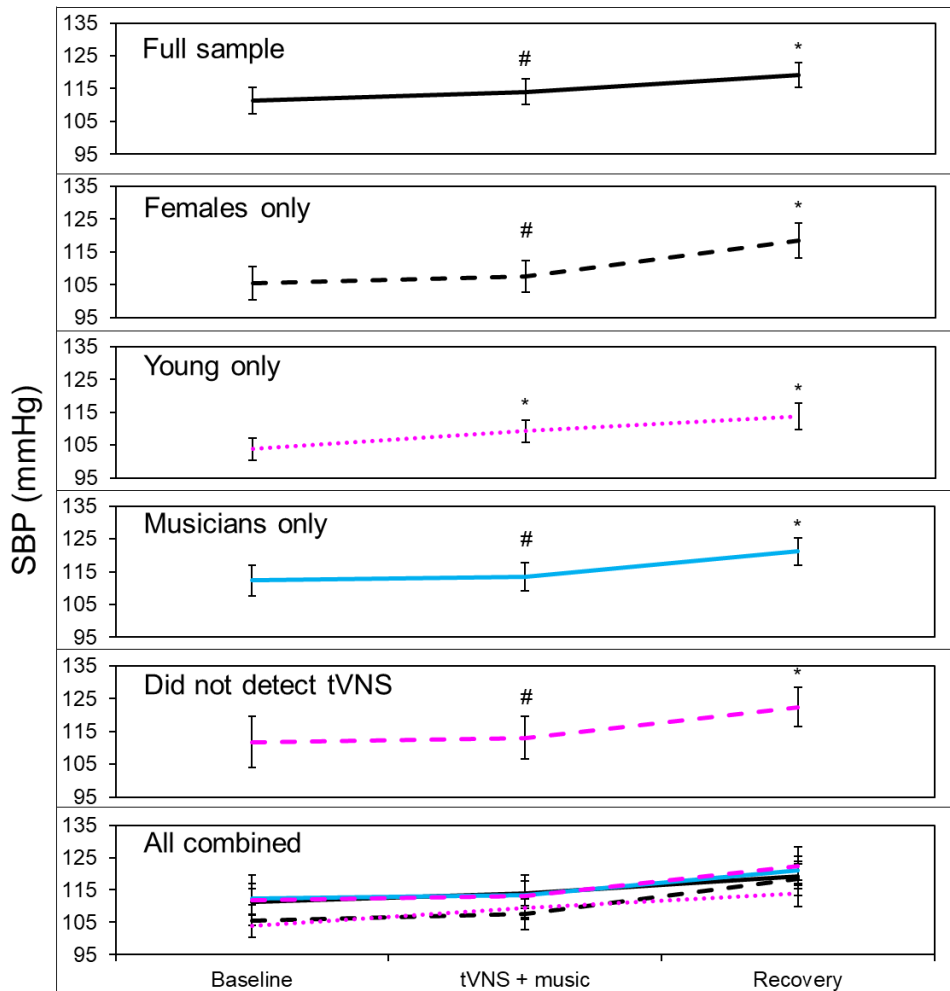


Figure 4.92: SBP was significantly impacted by tVNS + music in the full sample, females, young participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Significant effects of tVNS + music on DBP transpired in the full sample DBP ( $p = 0.001$ ), females ( $p < 0.001$ ) and musicians ( $p = 0.002$ ). DBP was significantly higher in recovery than baseline (full sample:  $p = 0.008$ ; females:  $p = 0.005$ ; musicians:  $p = 0.015$ ) and stimulation (full sample:  $p = 0.011$ ; females:  $p = 0.009$ ; musicians:  $p = 0.017$ , see Figure 4.93). Furthermore, DBP was significantly impacted by the tVNS + music in those who did not detect tVNS ( $p = 0.008$ ): DBP was significantly higher during recovery than baseline ( $p = 0.033$ ).

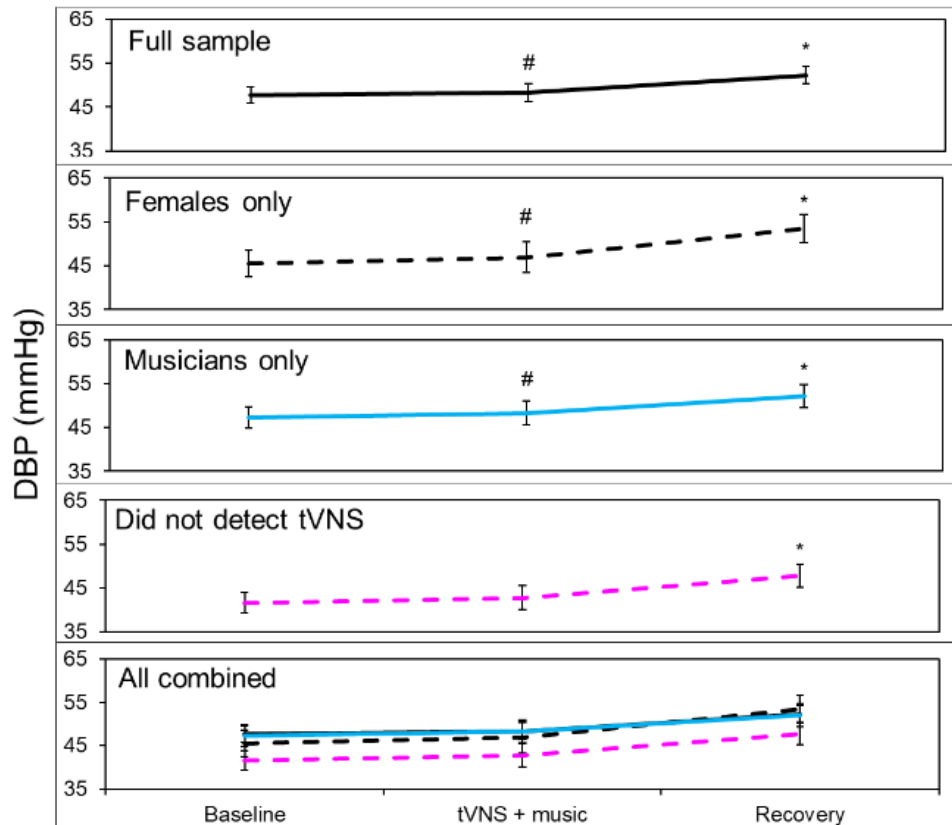
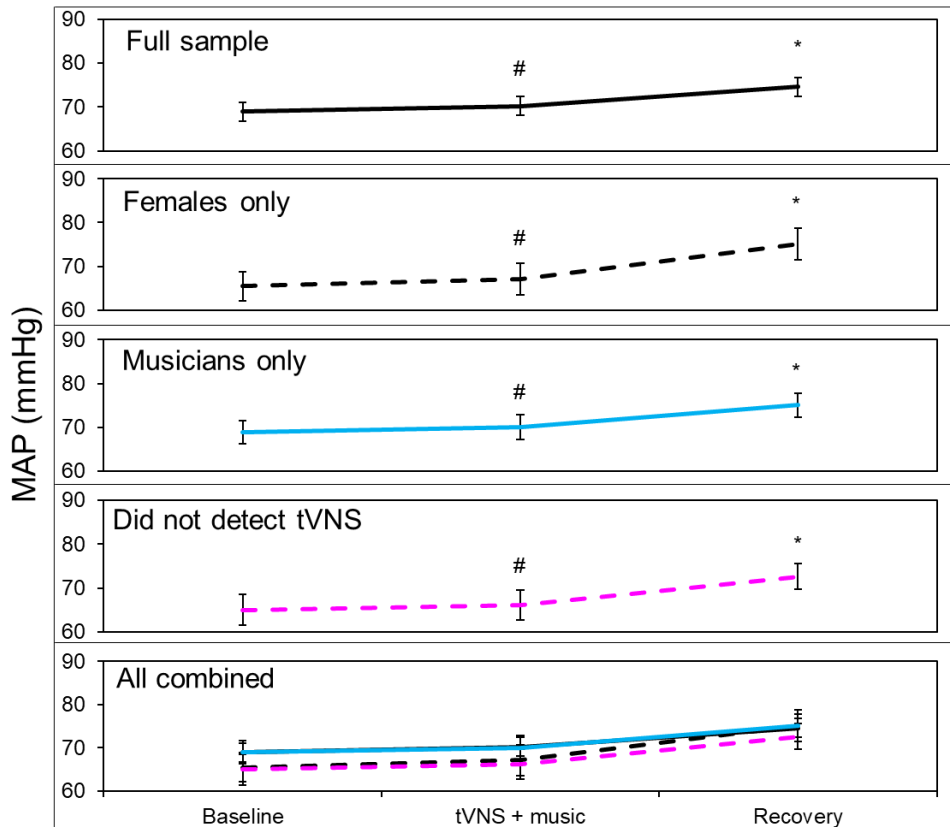


Figure 4.93: DBP was significantly impacted by tVNS + music in the full sample, females, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Consistent with the SBP and DBP results, there were significant effects of tVNS + music on MAP (see Figure 4.94). For the full sample ( $p = 0.001$ ), MAP was significantly higher in recovery than baseline ( $p = 0.004$ ) and stimulation ( $p = 0.009$ ). Also, for females ( $p < 0.001$ ), MAP was significantly higher in recovery compared to baseline ( $p = 0.003$ ) and tVNS + music ( $p = 0.002$ ). In musicians ( $p < 0.001$ ), values were significantly higher during recovery than: baseline ( $p = 0.008$ ) and tVNS + music ( $p = 0.002$ ). These patterns also emerged for those who did not detect tVNS (ANOVA:  $p = 0.003$ ; Bonferroni:  $p = 0.024$  for both).



*Figure 4.94: MAP was significantly impacted by tVNS + music in the full sample, females, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.*

Finally, down BRS was significantly impacted by the stimulation in the full sample ( $p = 0.022$ ) and those who detected tVNS ( $p = 0.040$ , see Figure 4.95). In both groups, down BRS was significantly higher during tVNS + music than baseline (full sample:  $p = 0.002$ ; detected tVNS:  $p = 0.003$ ).

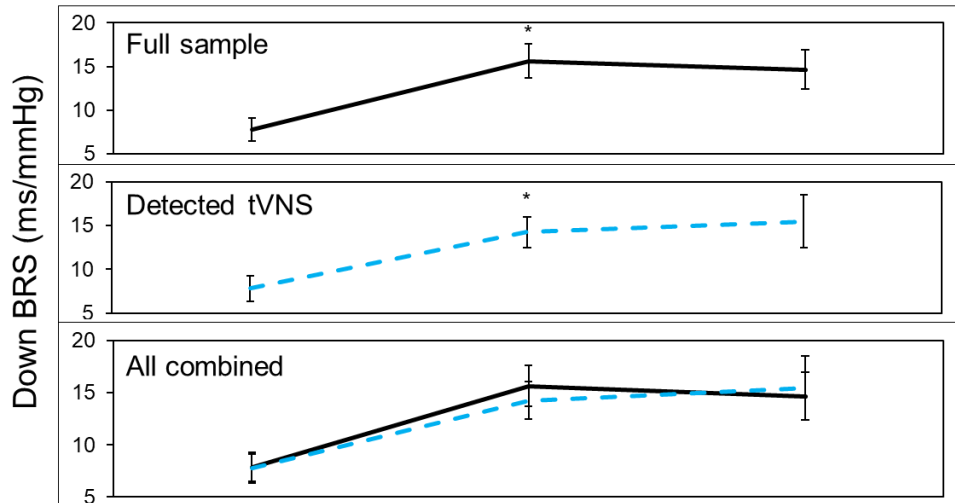


Figure 4.95: Down BRS was significantly impacted by tVNS + music in the full sample and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.1.6.4 Sham visit

There were significant effects of sham on SBP in the full sample ( $p = 0.004$ ), females ( $p = 0.029$ ) and those who detected tVNS ( $p = 0.021$ ). Exploration of these effects demonstrated that SBP was significantly higher during recovery than: baseline (full sample:  $p = 0.030$ ) and stimulation (full sample:  $p = 0.020$ ; females:  $p = 0.009$ ; detected tVNS:  $p = 0.012$ , see Figure 4.96).



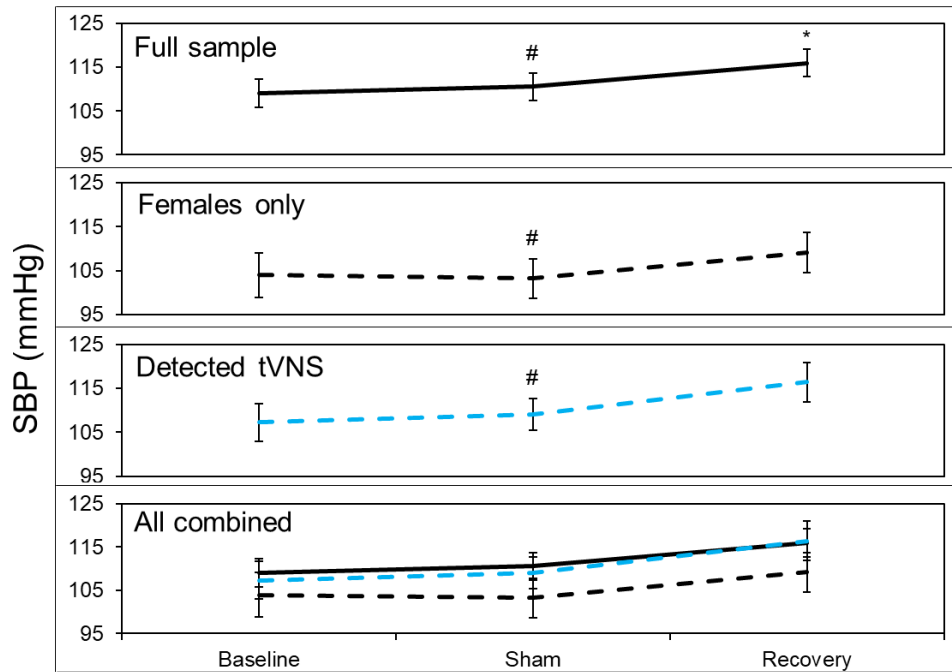


Figure 4.96: SBP was significantly impacted by sham in the full sample, females and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

DBP was also significantly impacted by sham when analysing the full sample ( $p = 0.003$ ), non-musicians ( $p = 0.002$ ) and those who detected tVNS ( $p = 0.005$ ). Exploration of these effects revealed that DBP was significantly higher in recovery than sham (full sample:  $p = 0.002$ ; non-musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.036$ ) and baseline (non-musicians:  $p = 0.034$ ; detected tVNS:  $p = 0.036$ , see Figure 4.97).

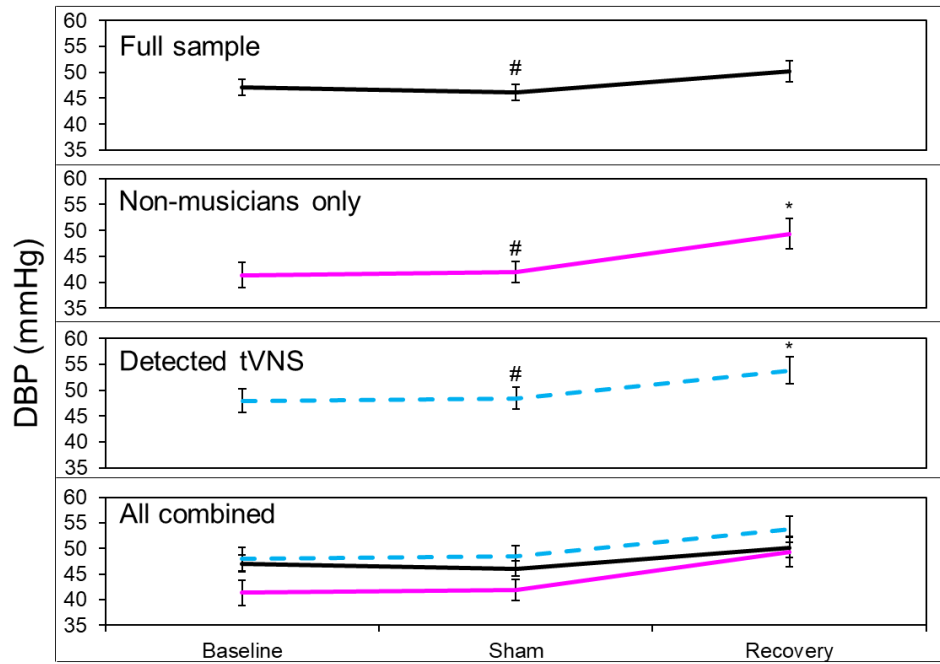


Figure 4.97: DBP was significantly impacted by sham in the full sample, non-musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Consistent with the SBP and DBP results, there was a significant effect of sham on MAP in the full sample ( $p = 0.005$ ), non-musicians ( $p = 0.005$ ) and those who detected tVNS ( $p = 0.008$ ). Further examination of these effects revealed that MAP was significantly higher during recovery than: baseline (full sample:  $p = 0.048$ ; detected tVNS:  $p = 0.045$ ) and sham (full sample:  $p = 0.003$ ; non-musicians:  $p = 0.012$ ; detected tVNS:  $p = 0.003$ , see Figure 4.98).

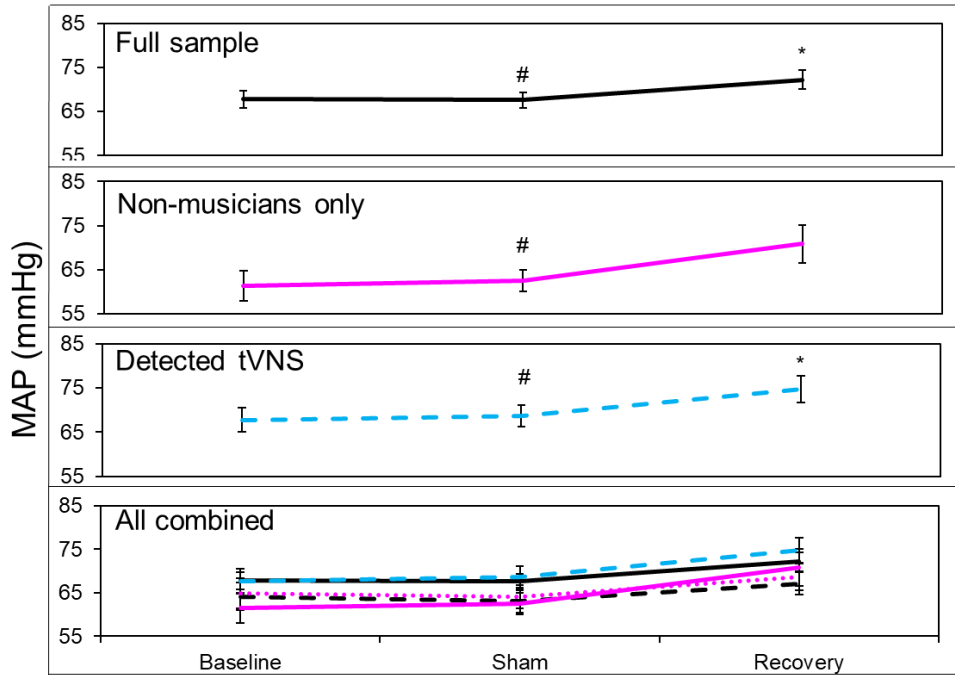


Figure 4.98: MAP was significantly impacted by sham in the full sample, non-musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Finally, a significant effect of sham on mean BRS emerged for females only ( $p = 0.006$ ): baseline mean BRS was significantly higher than that in recovery ( $p = 0.015$ , see Figure 4.99).

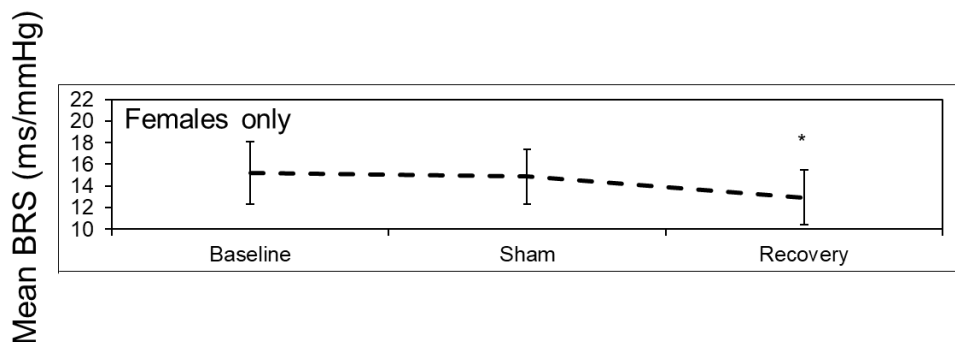


Figure 4.99: Mean BRS was significantly impacted by sham in females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.1.6.5 Between-visit comparisons

The only significant difference that emerged concerned female respiration rate ( $p < 0.001$ ). There was a significant difference in change in respiration rate between the tVNS visit and: music only ( $p = 0.014$ ) and tVNS + music visits ( $p = 0.003$ ). As illustrated in Figure 4.100, respiration rate increased in tVNS but decreased in music and tVNS + music.

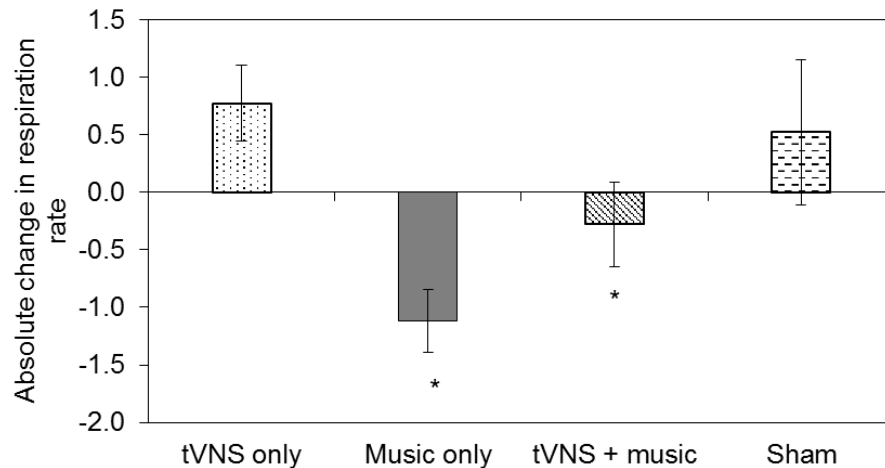


Figure 4.100: Absolute change in respiration rate significantly differed between the four visits. Data presented as mean  $\pm$  1 SEM. \* = significantly different to the tVNS only visit.

#### 4.3.1.7 Brachial BP and HR

Brachial BP and HR were obtained at four time points (before baseline, after baseline, after stimulation and after recovery). Therefore, ANOVAs (or Friedman tests) were performed to determine whether brachial SBP, DBP, MAP and HR significantly differed between any of these four time points. It was anticipated that for all visits, pre-baseline measures would be significantly higher than those obtained after baseline, stimulation and recovery.

##### 4.3.1.7.1 tVNS only visit

Significant effects of tVNS on brachial SBP were observed in the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.003$ ), young participants ( $p = 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p = 0.012$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p < 0.001$ ). Consistent with expectations, pre-baseline SBP

was significantly higher when compared with baseline (full sample:  $p < 0.001$ ; males:  $p = 0.001$ ; females:  $p = 0.045$ ; young:  $p = 0.020$ ; older:  $p = 0.007$ ; musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.010$ ; did not detect tVNS:  $p = 0.002$ ), tVNS (full sample:  $p < 0.001$ ; males:  $p < 0.001$ ; females:  $p = 0.011$ ; young:  $p = 0.003$ ; older:  $p < 0.001$ ; non-musicians:  $p = 0.045$ ; musicians:  $p < 0.001$ ; detected tVNS:  $p < 0.001$ ; did not detect tVNS:  $p = 0.002$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.007$ ; females:  $p = 0.042$ ; older:  $p = 0.004$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.011$ ; did not detect tVNS:  $p = 0.026$ ). Furthermore, SBP in older participants was significantly lower after tVNS than after baseline ( $p = 0.005$ , see Figure 4.101).

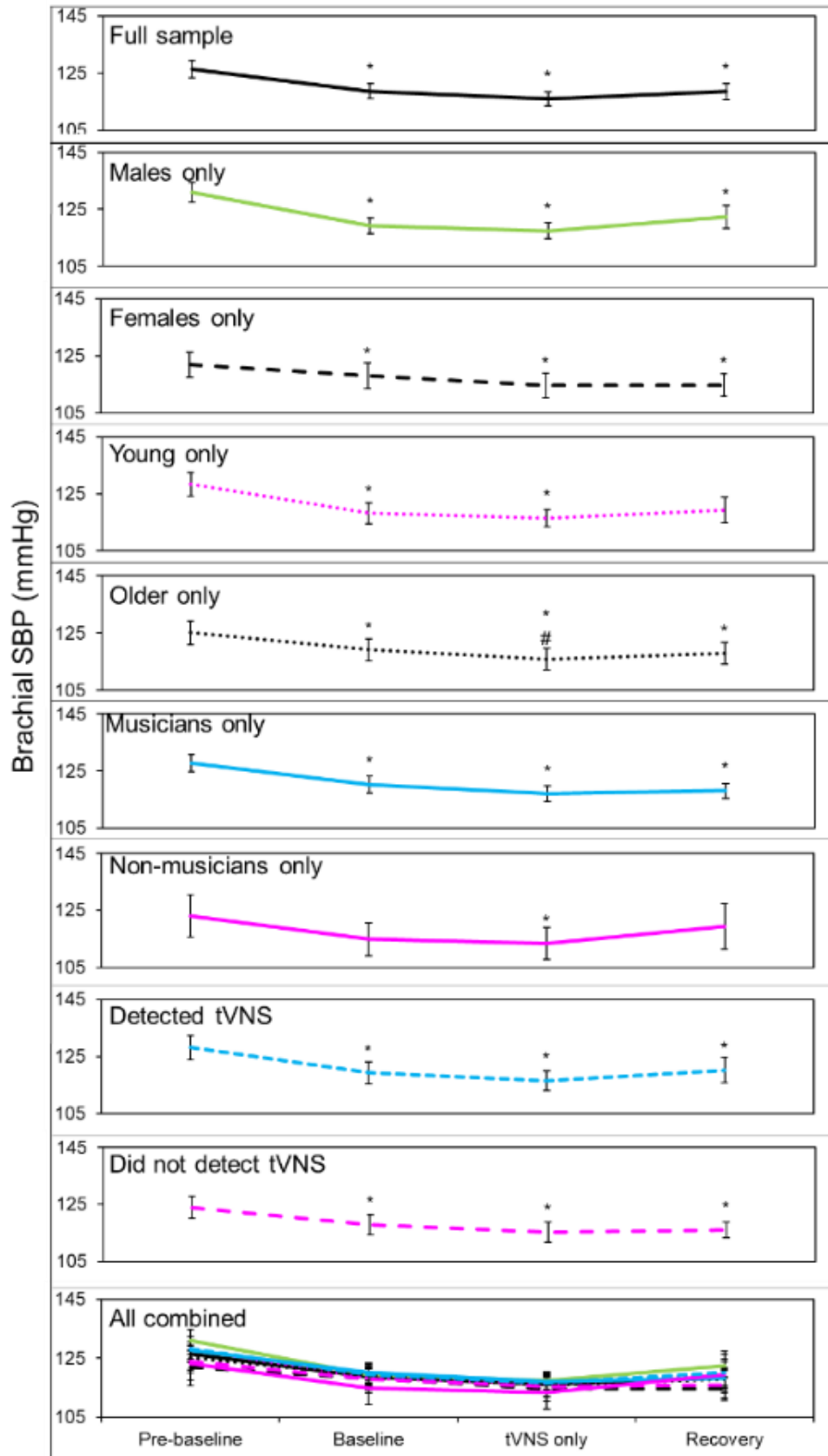
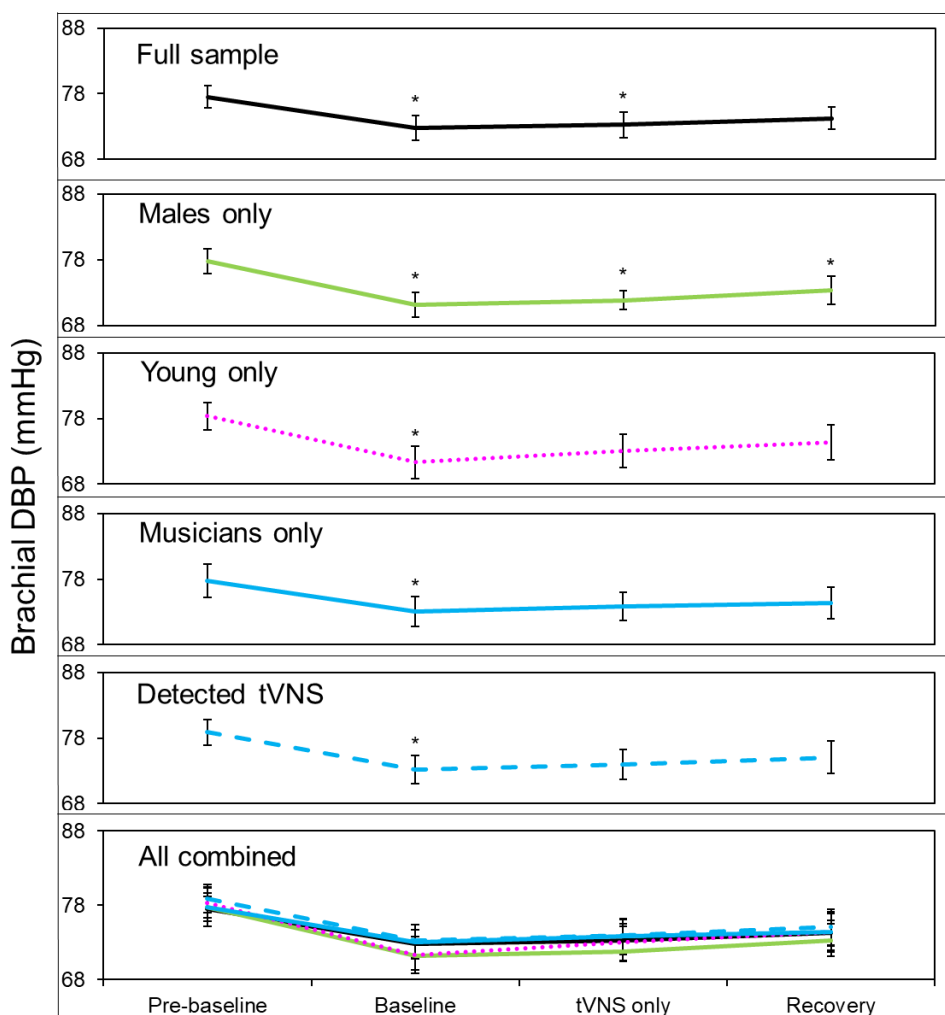


Figure 4.101: Brachial SBP significantly differed within the tVNS only visit in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to baseline.

There was a significant effect of tVNS on brachial DBP in the full sample ( $p = 0.002$ ), males ( $p < 0.001$ ), young participants ( $p = 0.001$ ), musicians ( $p = 0.004$ ) and those who detected tVNS ( $p = 0.002$ ). Pre-baseline DBP was significantly higher when compared with baseline (full sample:  $p = 0.001$ ; males:  $p = 0.002$ ; young:  $p = 0.004$ ; musicians:  $p = 0.014$ ; detected tVNS:  $p = 0.002$ ), tVNS (full sample:  $p = 0.003$ ; males:  $p < 0.001$ ) and recovery (males:  $p = 0.048$ ). Figure 4.102 summarises these findings.



*Figure 4.102: Brachial DBP significantly differed within the tVNS only visit in the full sample, males, young participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.*

A significant effect of tVNS on brachial MAP also emerged in the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.050$ ), young participants ( $p = 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ) and those who detected tVNS ( $p < 0.001$ ). Further analysis revealed that pre-baseline MAP

was significantly higher when compared with baseline (full sample:  $p < 0.001$ ; males:  $p = 0.001$ ; females:  $p = 0.037$ ; young:  $p = 0.006$ ; older:  $p = 0.013$ ; musicians:  $p = 0.002$ ; detected tVNS:  $p = 0.002$ ), tVNS (full sample:  $p < 0.001$ ; males:  $p < 0.001$ ; young:  $p = 0.030$ ; older:  $p = 0.007$ ; musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.003$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.009$ ; older:  $p = 0.031$ ; musicians:  $p = 0.006$ ;  $p = 0.035$ ). Figure 4.103 summarises these findings.

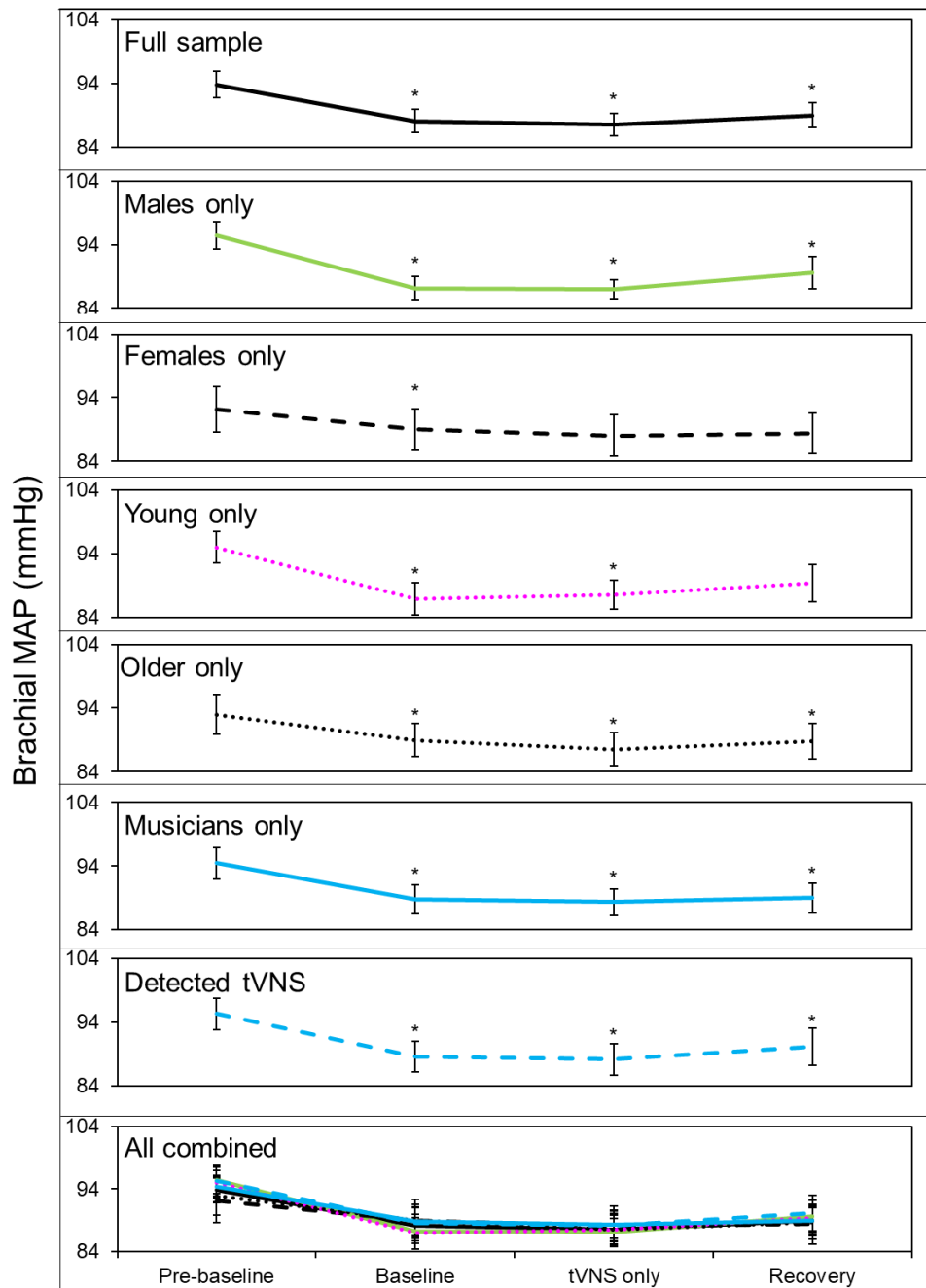


Figure 4.103: Brachial MAP significantly differed within the tVNS only visit in all groups except non-musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.



Finally, there was a significant effect of tVNS on brachial HR for the full sample ( $p = 0.003$ ), young participants ( $p = 0.026$ ) and those who detected tVNS ( $p = 0.024$ ). Further exploration of these effects demonstrated that pre-baseline HR was significantly higher than that after baseline (full sample:  $p = 0.019$ ; young:  $p = 0.010$ ; detected tVNS:  $p = 0.036$ ) and recovery (full sample:  $p = 0.022$ ). Figure 4.104 diagrammatically illustrates these findings.

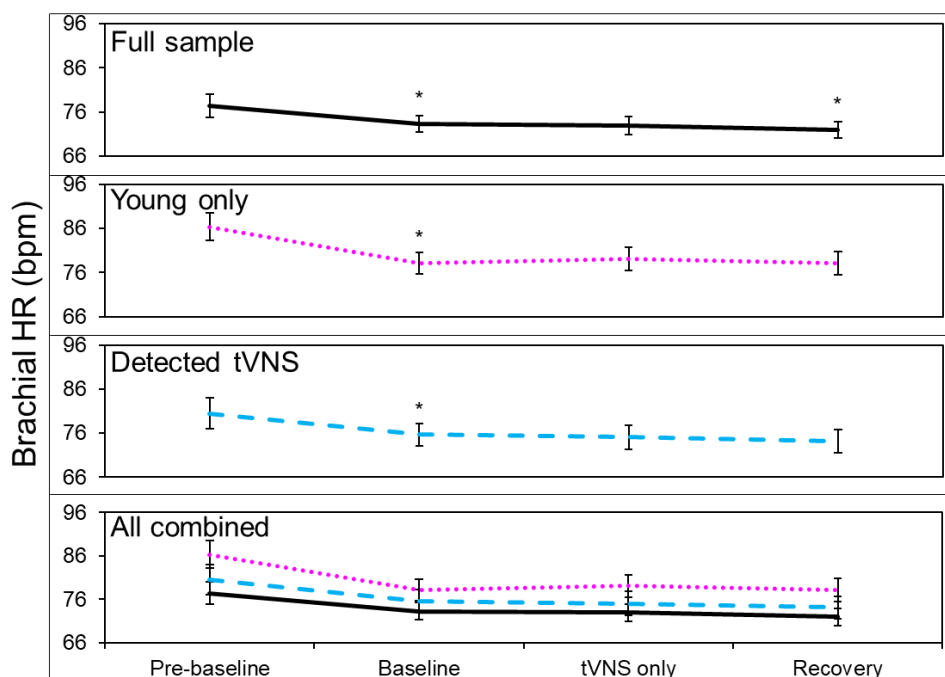


Figure 4.104: Brachial HR significantly differed within the tVNS only visit for the full sample, young participants and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.1.7.2 Music only visit

There was a significant effect of music on brachial SBP. This occurred in the full sample ( $p < 0.001$ ), males ( $p = 0.009$ ), females ( $p = 0.003$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p = 0.011$ ) and those who did not detect tVNS ( $p = 0.002$ ). Further analysis revealed that brachial SBP was significantly higher pre-baseline than after baseline (full sample:  $p < 0.001$ ; males:  $p < 0.001$ ; females:  $p = 0.046$ ; older:  $p = 0.001$ ; musicians:  $p < 0.001$ ; detected tVNS:  $p = 0.001$ ; did not detect tVNS:  $p = 0.029$ ), music (full sample  $p < 0.001$ ; males:  $p = 0.045$ ; females:  $p = 0.004$ ; older:  $p = 0.001$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.020$ ; did not detect tVNS:  $p = 0.008$ ) and recovery (full sample:  $p = 0.003$ ; males:  $p = 0.033$ ; older:  $p = 0.006$ ; musicians:  $p = 0.003$ ). Additionally, SBP after

music was significantly lower than that after recovery ( $p = 0.035$ ). Figure 4.105 summarises these findings.

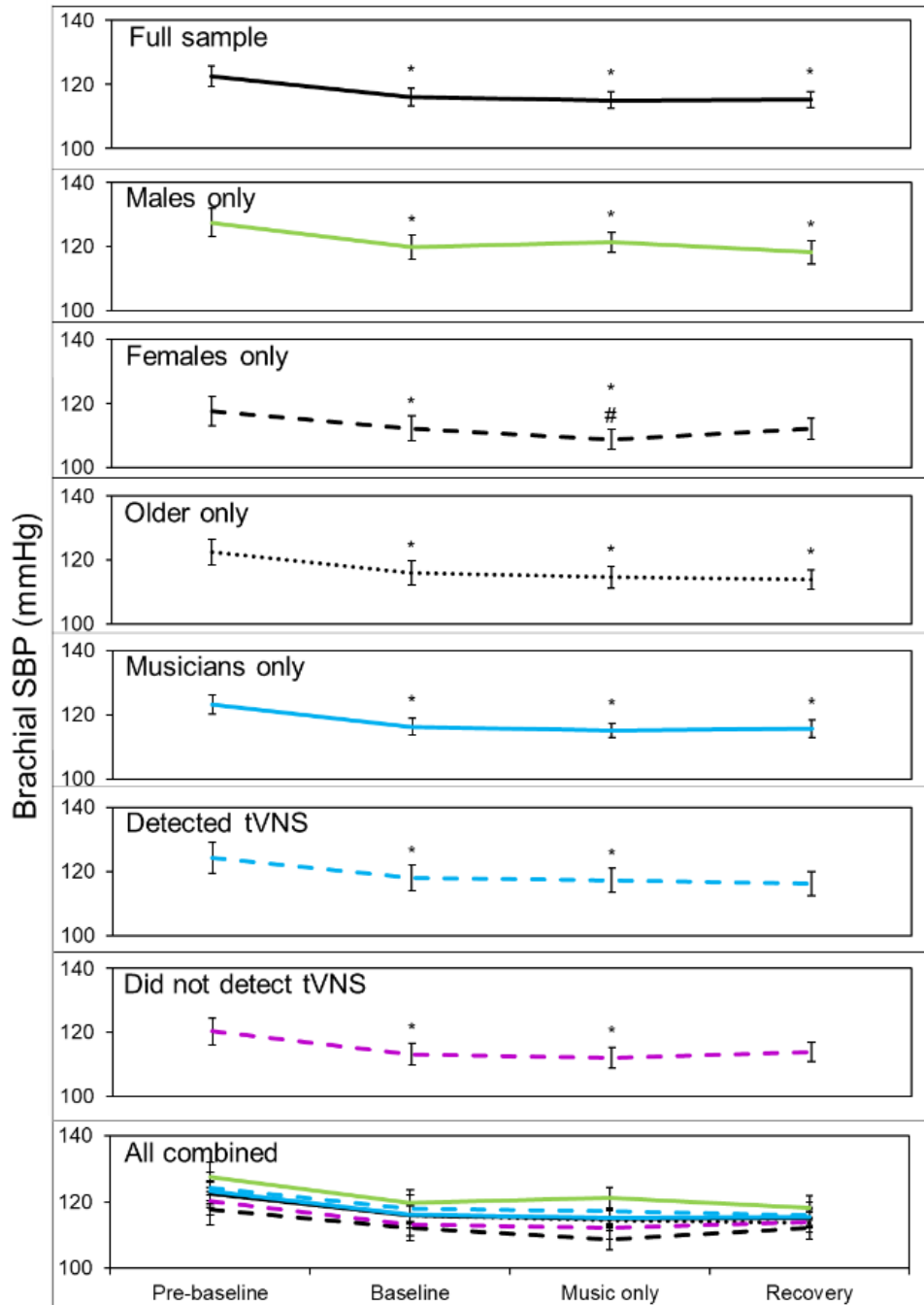
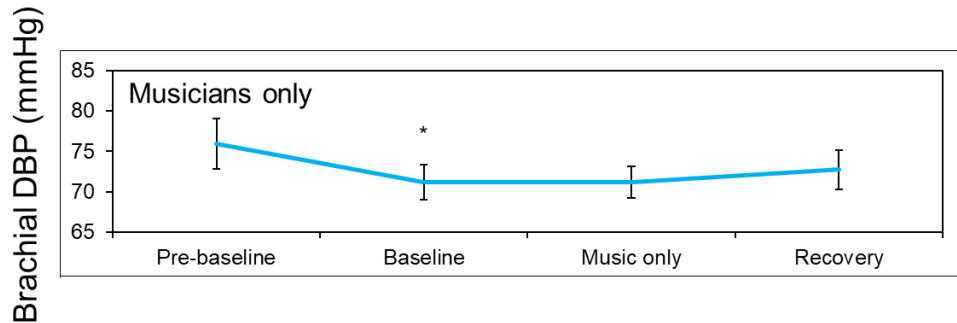


Figure 4.105: Brachial SBP was significantly impacted by music in all groups apart from young participants and non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to recovery.

A significant effect of music on brachial DBP also emerged but for musicians only ( $p = 0.025$ ). Further exploration showed that pre-baseline DBP was significantly higher than that after baseline ( $p = 0.032$ , see Figure 4.106).



*Figure 4.106: Brachial DBP was significantly impacted by music in musicians only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.*

There was a significant effect of music on brachial MAP in the full sample ( $p = 0.001$ ), males ( $p = 0.034$ ), older participants ( $p = 0.001$ ), musicians ( $p = 0.002$ ) and those who did not detect tVNS ( $p = 0.001$ ). Further examination of this effect revealed that pre-baseline MAP was significantly higher than that after baseline (full sample:  $p = 0.001$ ; males:  $p = 0.008$ ; older:  $p = 0.016$ ; musicians:  $p = 0.001$ ; did not detect tVNS:  $p = 0.010$ ), music (full sample:  $p = 0.007$ ; musicians:  $p = 0.010$ ; did not detect tVNS:  $p = 0.042$ ) and recovery (full sample:  $p = 0.039$ ; older:  $p = 0.045$ ). Figure 4.107 summarises these findings.

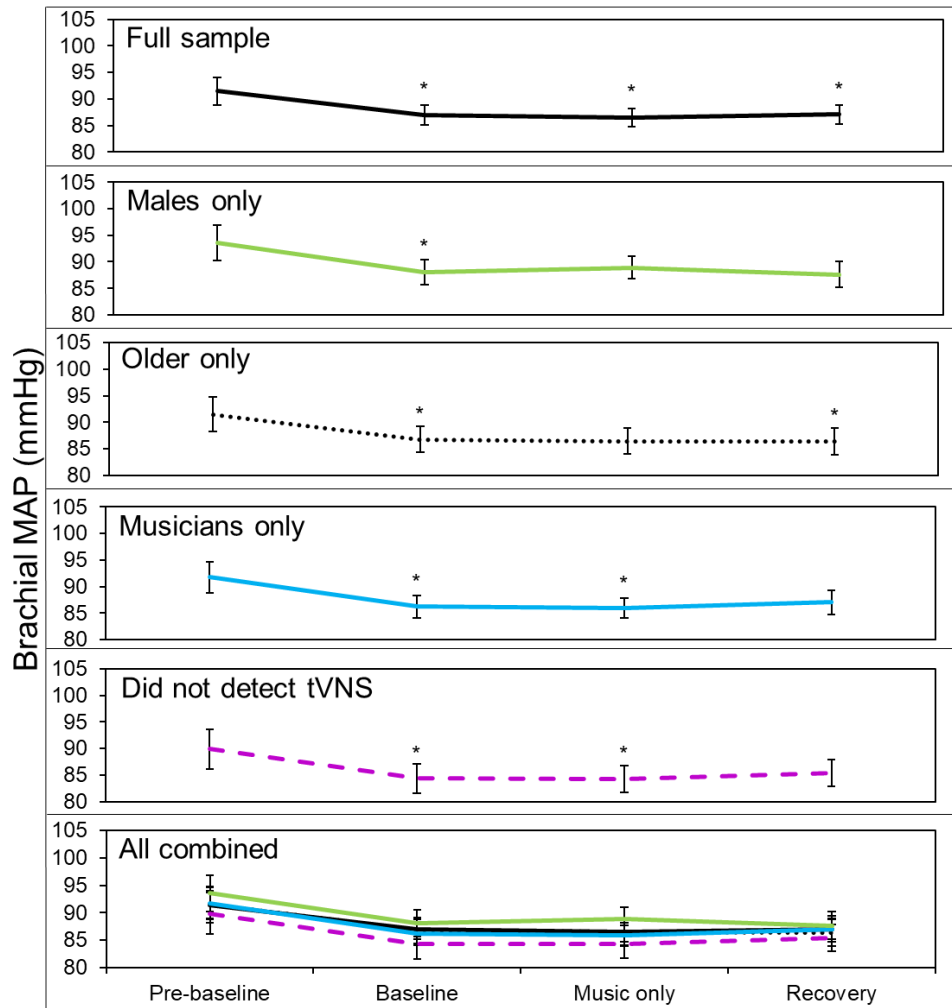


Figure 4.107: Brachial MAP was significantly impacted by music in the full sample, males, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Finally, brachial HR was significantly impacted by the music. This was apparent in the full sample ( $p = 0.001$ ), males ( $p = 0.014$ ) and females ( $p = 0.003$ ). Pairwise comparisons revealed that pre-baseline brachial HR was significantly higher than that after baseline (full sample:  $p = 0.026$ ; males:  $p = 0.010$ ), music (full sample:  $p = 0.032$ ; females:  $p = 0.043$ ) and recovery (full sample:  $p = 0.010$ ; females:  $p = 0.035$ ). Figure 4.108 diagrammatically illustrates these findings.

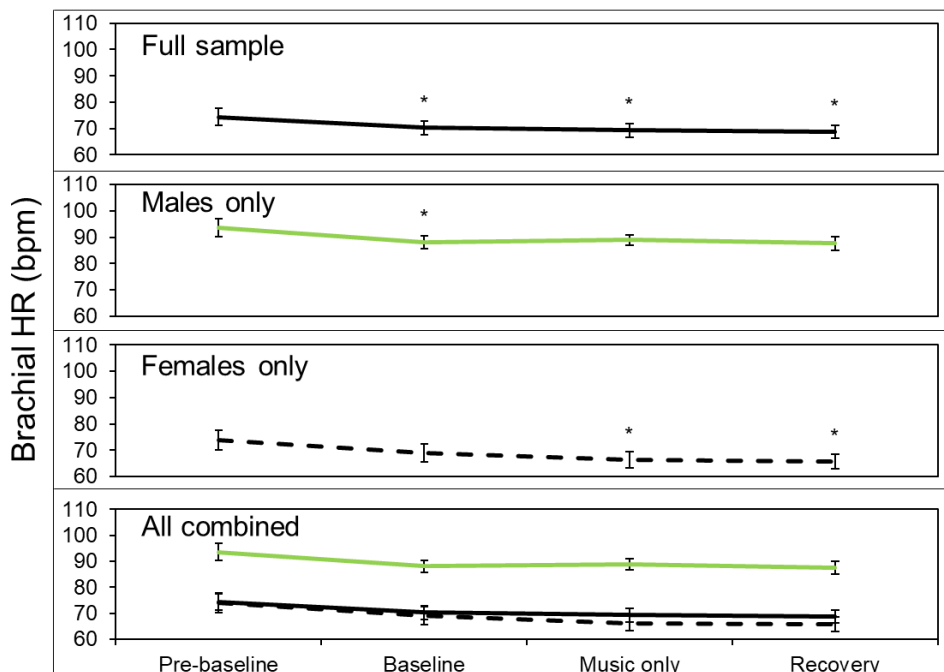


Figure 4.108: Brachial HR was significantly impacted by music in the full sample, males and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to recovery.

#### 4.3.1.7.3 tVNS + music visit

There was a significant effect of tVNS + music on brachial SBP in the full sample ( $p < 0.001$ ), males ( $p = 0.002$ ), females ( $p < 0.001$ ), young participants ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p = 0.010$ ) and those who did not detect tVNS ( $p < 0.001$ ). Further analysis showed that brachial SBP at pre-baseline was significantly higher than that after baseline (full sample:  $p = 0.001$ ; young:  $p = 0.002$ ; musicians:  $p = 0.004$ ; did not detect tVNS:  $p = 0.015$ ), tVNS + music (full sample:  $p < 0.001$ ; males:  $p = 0.005$ ; females:  $p = 0.002$ ; young:  $p = 0.028$ ; older:  $p = 0.001$ ; musicians:  $p < 0.001$ ; did not detect tVNS:  $p = 0.001$ ) and sham (full sample:  $p < 0.001$ ; females:  $p = 0.012$ ; young:  $p = 0.003$ ; musicians:  $p = 0.001$ ; did not detect tVNS:  $p = 0.009$ ). Figure 4.109 summarises these findings.

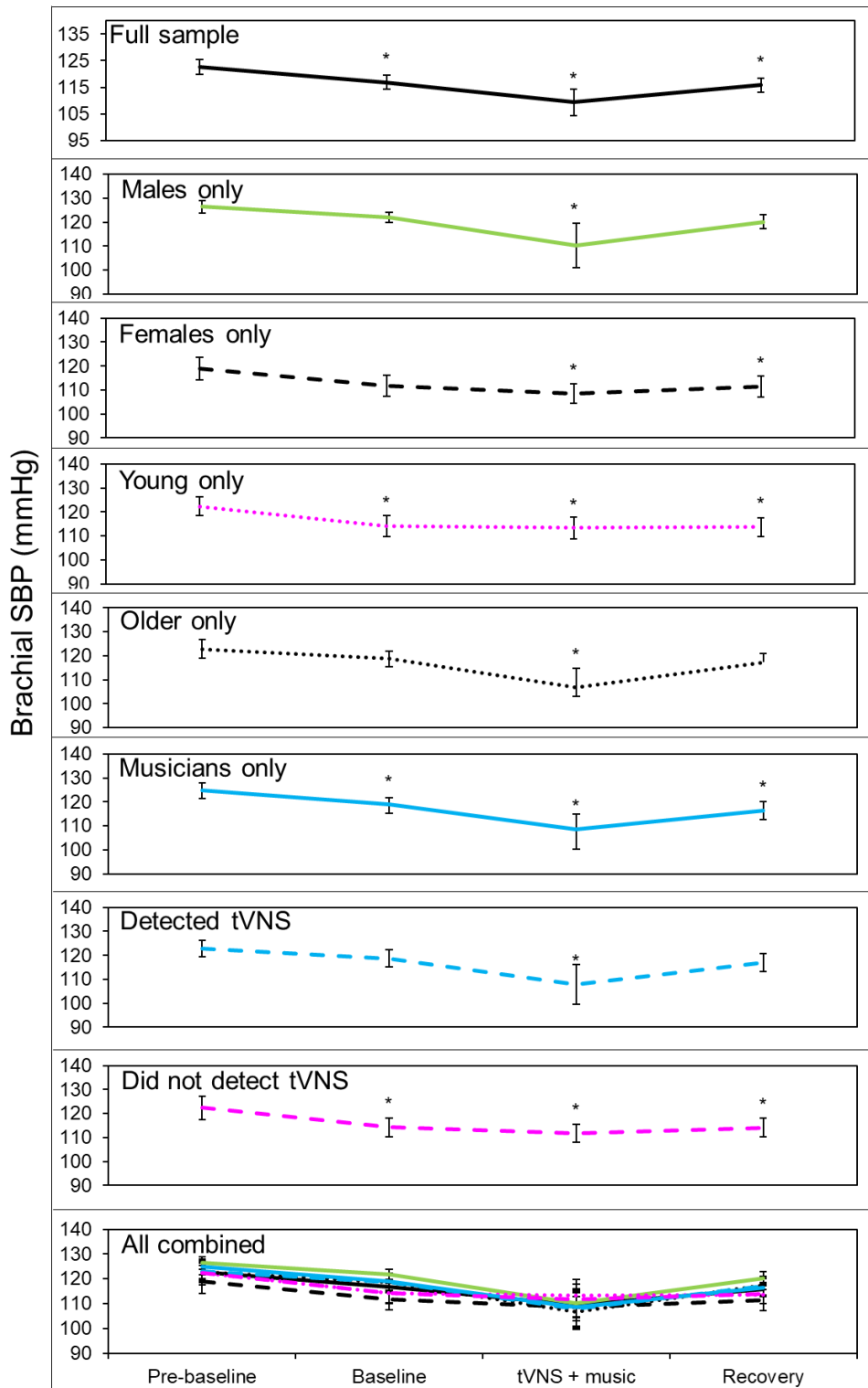


Figure 4.109: Brachial SBP was significantly impacted by tVNS + music in all groups except non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Although there was no impact of tVNS + music on brachial DBP, there was for brachial MAP. This was the case for young participants ( $p = 0.001$ ), older

participants ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.004$ ). Further exploration demonstrated that brachial MAP at pre-baseline was significantly higher than that after baseline (young:  $p = 0.020$ ; older:  $p = 0.013$ ; did not detect tVNS:  $p = 0.030$ ), tVNS + music (young:  $p = 0.043$ ; older:  $p = 0.031$ ) and recovery (young:  $p = 0.015$ ; older:  $p = 0.031$ , see Figure 4.110).

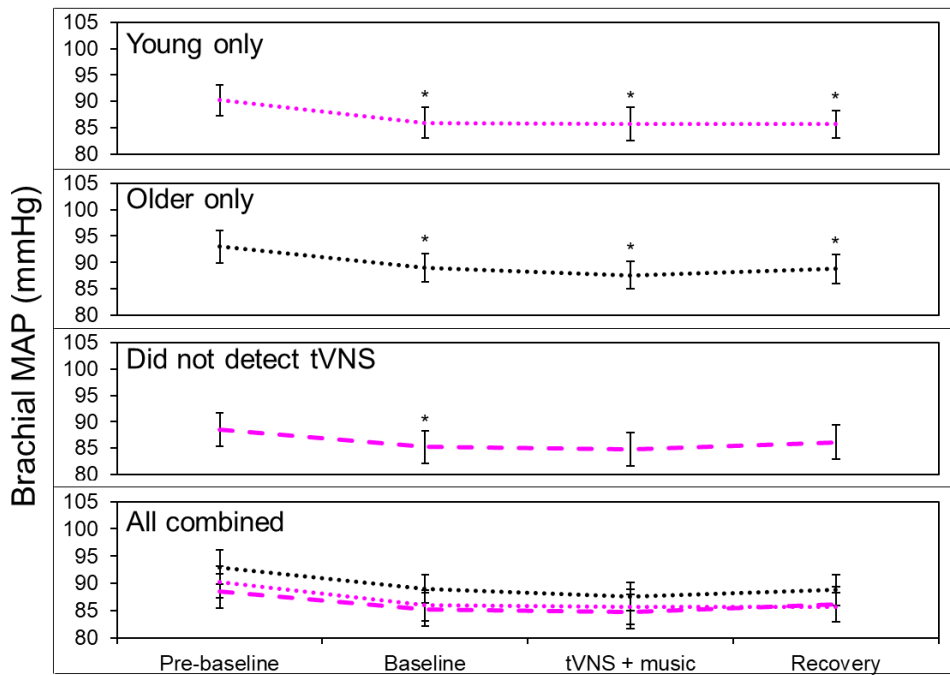


Figure 4.110: Brachial MAP was significantly impacted by tVNS + music in young participants, older participants and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

A significant effect of tVNS + music on brachial HR emerged for the full sample ( $p = 0.005$ ). Further exploration demonstrated that pre-baseline brachial HR was significantly higher than that after tVNS + music ( $p = 0.028$ , see Figure 4.111).

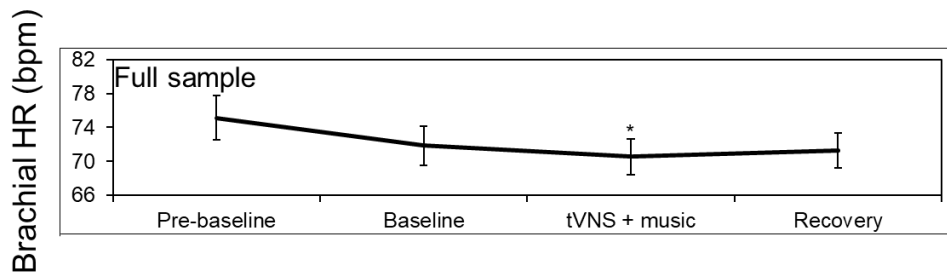


Figure 4.111: Brachial HR was significantly impacted by tVNS + music in the full sample only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.1.7.4 Sham visit

There was a significant effect of sham on brachial SBP. This transpired in the full sample ( $p < 0.001$ ), males ( $p = 0.009$ ), females ( $p = 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.002$ ). Further analysis revealed that pre-baseline brachial SBP was significantly higher compared to that after baseline (full sample:  $p < 0.001$ ; males:  $p = 0.005$ ; females:  $p = 0.013$ ; older:  $p = 0.001$ ; musicians:  $p < 0.001$ ; detected tVNS:  $p = 0.003$ ; did not detect tVNS:  $p = 0.025$ ), sham (full sample:  $p < 0.001$ ; males:  $p = 0.006$ ; females:  $p = 0.003$ ; older:  $p < 0.001$ ; musicians:  $p < 0.001$ ; detected tVNS:  $p = 0.001$ ; did not detect tVNS:  $p = 0.025$ ) and recovery (full sample:  $p = 0.002$ ; older:  $p = 0.019$ ; musicians:  $p = 0.001$ ; did not detect tVNS:  $p = 0.027$ ). Figure 4.112 summarises these findings.



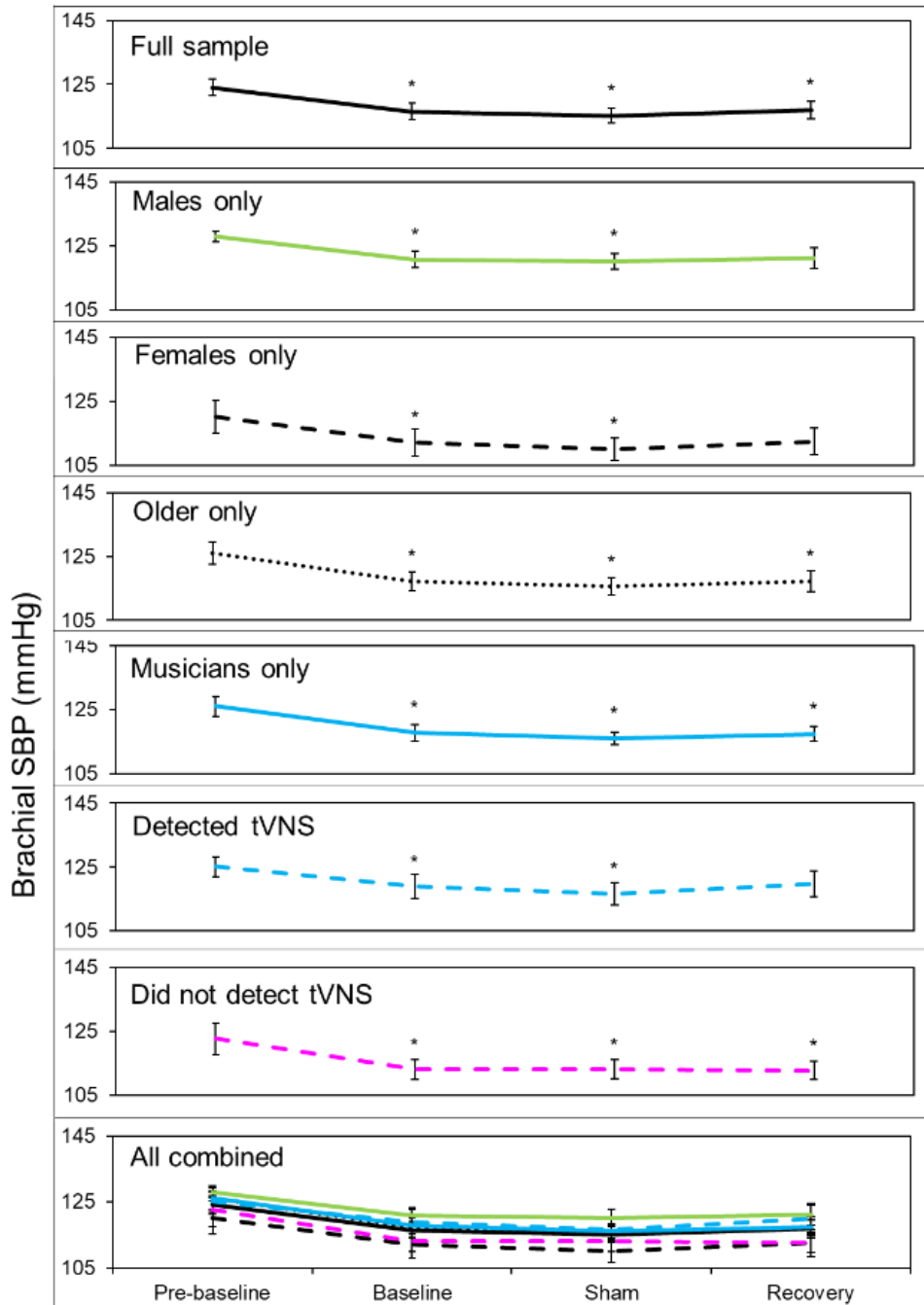


Figure 4.112: Brachial SBP was significantly impacted by sham in all groups except young participants and non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

A significant effect of sham on brachial DBP also occurred in the full sample ( $p = 0.008$ ) and musicians ( $p = 0.003$ ). Further exploration revealed that pre-baseline brachial DBP was significantly higher than that at sham (full sample:  $p = 0.028$ ; musicians:  $p = 0.007$ , see Figure 4.113).

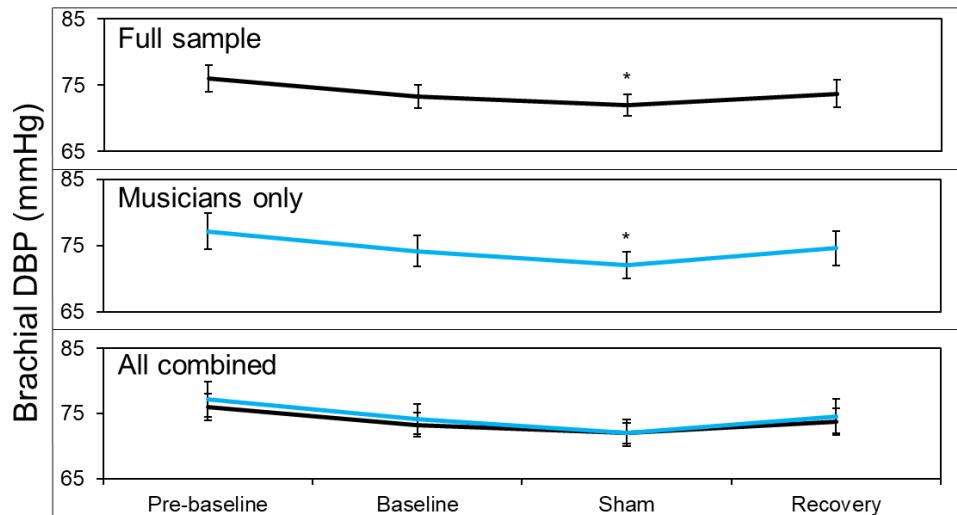


Figure 4.113: Brachial DBP was significantly impacted by sham in the full sample and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Brachial MAP was significantly impacted by sham in the full sample ( $p < 0.001$ ), females ( $p = 0.001$ ), older participants ( $p = 0.002$ ), musicians ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.001$ ). Further analysis showed that pre-baseline brachial MAP was significantly higher than that baseline (full sample:  $p = 0.005$ ; older:  $p = 0.038$ ; musicians:  $p = 0.012$ ), sham (full sample:  $p < 0.001$ ; females:  $p = 0.015$ ; older:  $p = 0.011$ ; musicians:  $p < 0.001$ ; did not detect tVNS:  $p = 0.006$ ) and recovery (full sample:  $p = 0.039$ ; musicians:  $p = 0.018$ ; did not detect tVNS:  $p = 0.017$ ). Figure 4.114 summarises these findings.

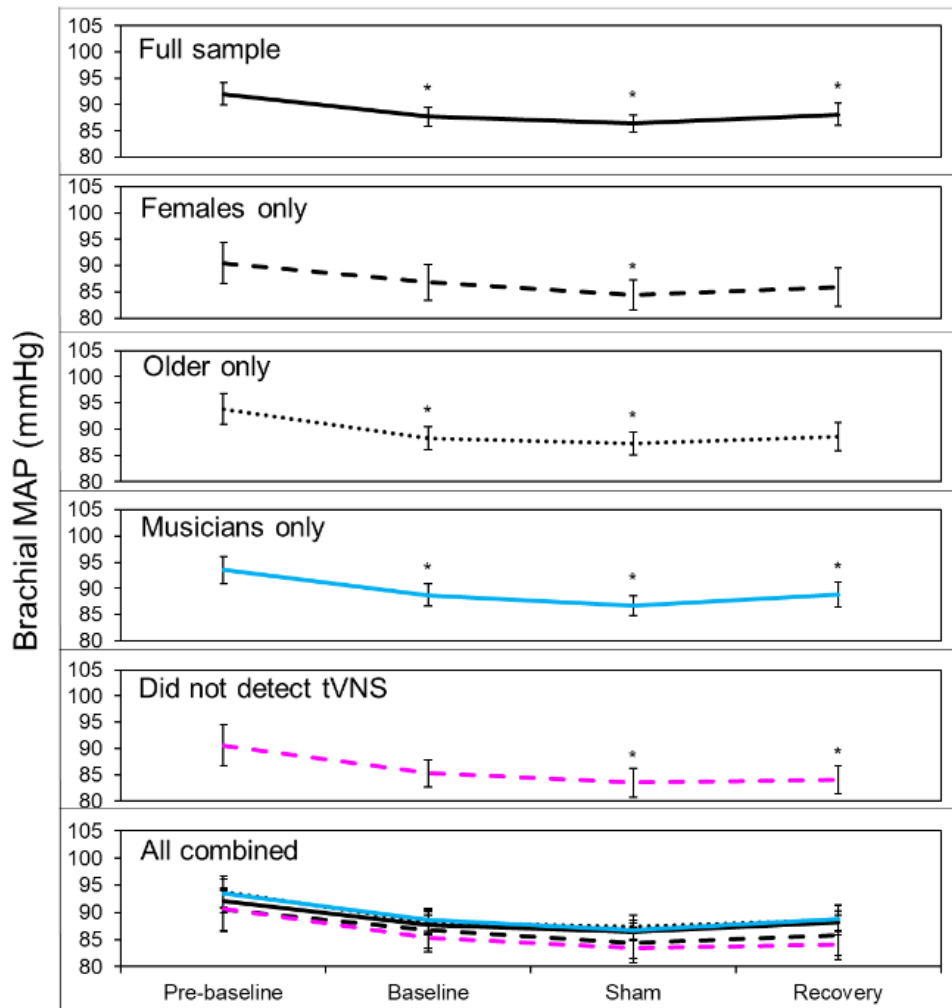


Figure 4.114: Brachial MAP was significantly impacted by sham in the full sample, females, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Finally, there was a significant effect of sham on brachial HR. This effect emerged in the full sample ( $p = 0.001$ ), females ( $p = 0.005$ ), young participants ( $p = 0.001$ ) and musicians ( $p = 0.002$ ). Further exploration revealed that brachial HR at pre-baseline was significantly greater than after baseline (full sample:  $p = 0.007$ ; musicians:  $p = 0.004$ ), sham (full sample:  $p = 0.003$ ; females:  $p = 0.006$ ; young:  $p = 0.018$ ; musicians:  $p = 0.004$ ) and recovery (full sample:  $p = 0.006$ ; young:  $p = 0.021$ ). Figure 4.115 summarises these findings.

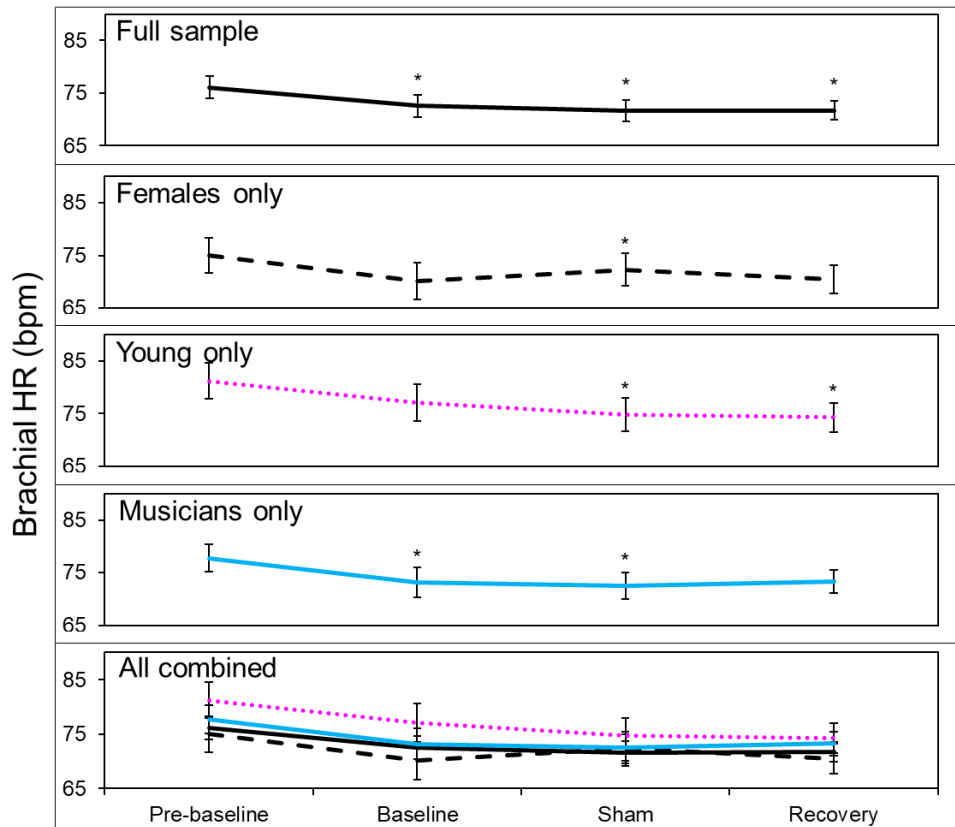


Figure 4.115: Brachial HR was significantly by sham in the full sample, females, young participants and musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.1.7.5 Between-visit comparisons

Despite the emergence of many significant differences when looking at each visit separately, no significant differences emerged between the four visits for any of the group analyses ( $p > 0.05$ ).

#### 4.3.2 The effects of the four types of stimulation on self-reported emotion

Similar to the physiology measures, differences in baseline self-report measures were ascertained. Significant differences emerged (see Table 4.4). This provided further support for using absolute change values to explore differences between visits.

*Table 4.4: Statistically significant differences in baseline self-report measures between the four visits.*

Sample	Measure	ANOVA p-value	Pairwise comparison (p-value)	
Full sample	Calm	0.036	tVNS > music	0.037
	Happy	0.001	music < tVNS	0.005
			music < tVNS + music	< 0.001
	Sad	0.007	music < tVNS	0.001
Males	Happy	0.037	music < tVNS	0.003
Females	Relaxation	0.027	music < tVNS	0.042
	Happy	0.004	music < sham	0.021
Older	Happy	0.018	music < tVNS + music	0.007
Musicians	Happy	0.019	music < tVNS + music	< 0.001
Felt tVNS	Calm	0.012	music < tVNS	0.041
Did not feel tVNS	Sad	0.006	music > tVNS	0.008
Felt tVNS	Pleasantness	0.034	sham < tVNS	0.049
	Obtrusiveness	0.009	tVNS < tVNS + music	0.043

#### 4.3.2.1 Experienced emotion

##### 4.3.2.1.1 tVNS only visit

There was a significant effect of tVNS on experienced stimulation when analysing the full sample ( $p < 0.001$ ), females ( $p < 0.001$ ), older participants ( $p = 0.003$ ), musicians ( $p = 0.003$ ) and those who did not detect tVNS ( $p < 0.001$ ). Further analysis highlighted that experienced stimulation at pre-baseline was significantly higher than that after baseline (full sample:  $p = 0.003$ ; females:  $p = 0.008$ ; older:  $p = 0.040$ ; musicians:  $p = 0.002$ ; did not detect tVNS:  $p < 0.001$ ), tVNS (full sample:  $p = 0.018$ ; musicians:  $p = 0.005$ ; did not detect tVNS:  $p = 0.011$ ) and recovery (full sample:  $p = 0.003$ ; females:  $p = 0.040$ ; musicians:  $p = 0.002$ ; did not detect tVNS:  $p < 0.001$ ). Figure 4.116 summarises these findings.

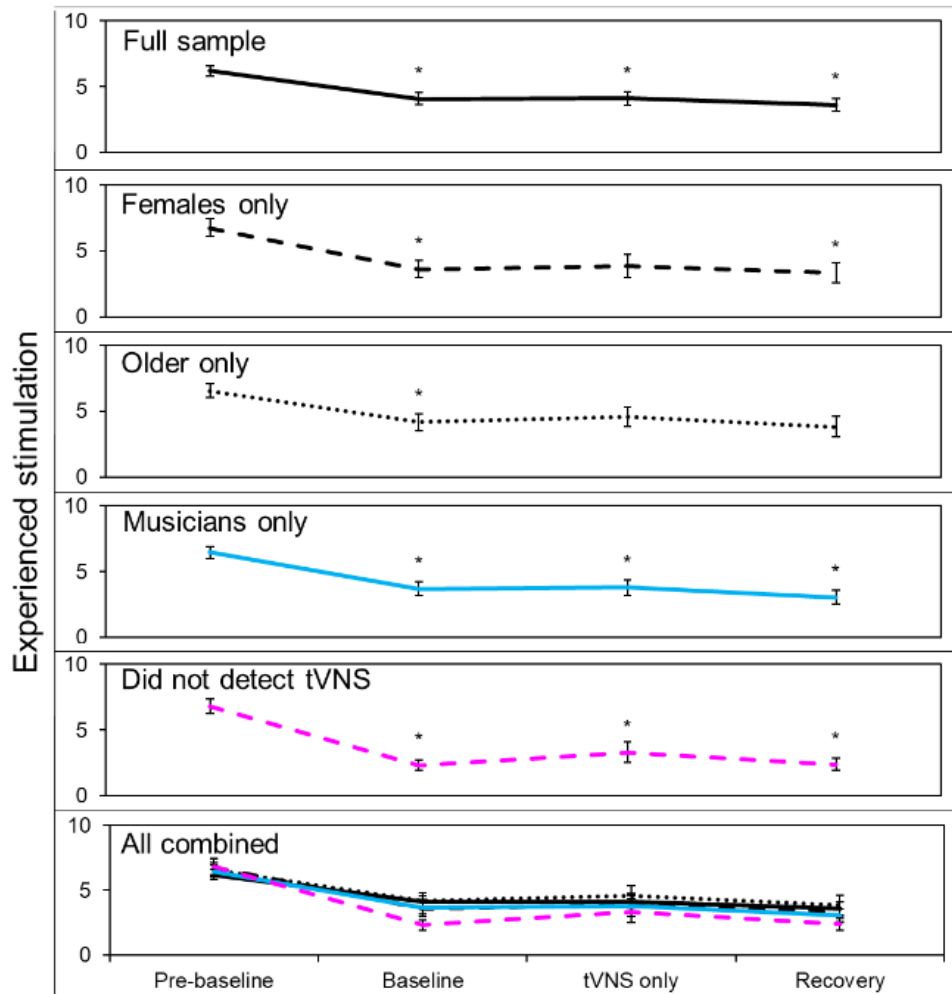


Figure 4.116: Experienced stimulation was significantly impacted by tVNS in the full sample, females, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Experienced relaxation was significantly impacted by tVNS. This was particularly the case in the full sample ( $p < 0.001$ ), females ( $p < 0.001$ ), young participants ( $p = 0.026$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p = 0.001$ ) and those who did not detect tVNS ( $p = 0.007$ ). Experienced relaxation was significantly lower at pre-baseline than after baseline (full sample:  $p < 0.001$ ; females:  $p = 0.001$ ; older:  $p = 0.005$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.032$ ; did not detect tVNS:  $p = 0.001$ ), tVNS (full sample:  $p = 0.049$ ; females:  $p = 0.026$ ) and recovery (full sample:  $p < 0.001$ ; females:  $p = 0.001$ ; young:  $p = 0.024$ ; older:  $p = 0.002$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.024$ ; did not detect tVNS:  $p = 0.006$ ). Figure 4.117 summarises these findings.

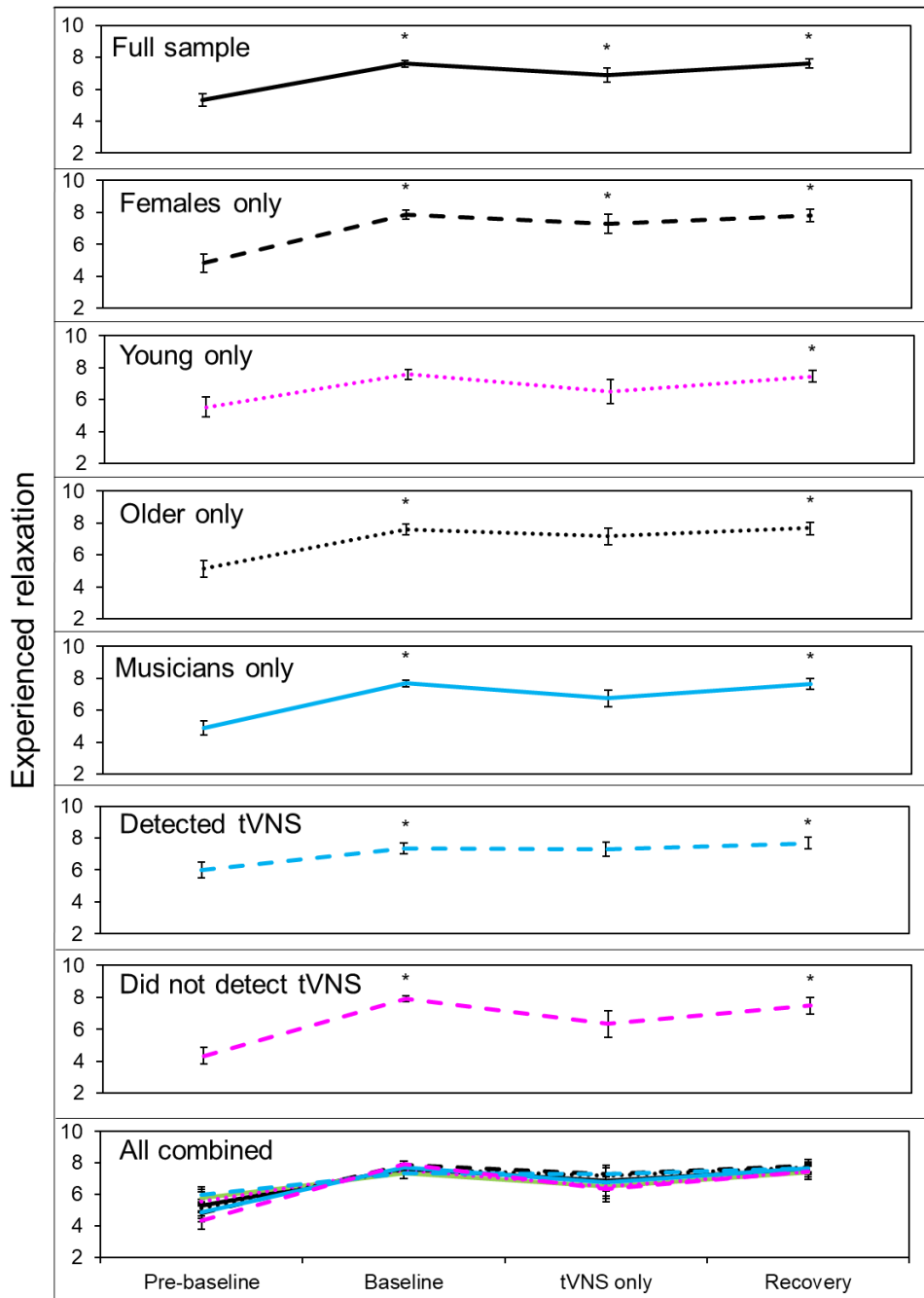


Figure 4.117: Experienced relaxation was significantly impacted by tVNS in all groups except males and non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

There was a significant effect of tVNS on experienced stress in the full sample ( $p = 0.001$ ), females ( $p = 0.009$ ), older participants ( $p = 0.024$ ), musicians ( $p = 0.003$ ) and those who detected tVNS ( $p = 0.010$ ). Exploration revealed that pre-baseline experienced stress was significantly higher than that after baseline (older:  $p = 0.015$ ), tVNS (detected tVNS:  $p = 0.003$ ) and

recovery (full sample:  $p = 0.001$ ; females:  $p = 0.040$ ; musicians:  $p = 0.002$ ; detected tVNS:  $p = 0.008$ , see Figure 4.118).

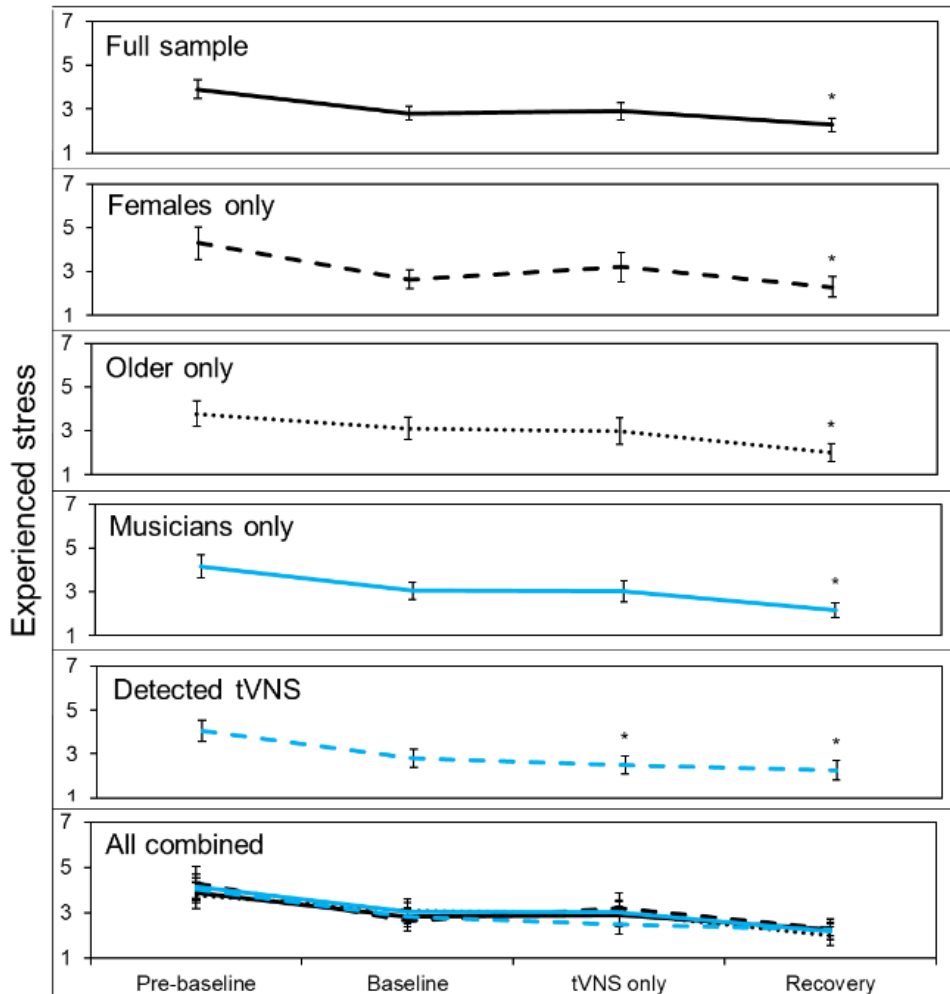


Figure 4.118: Experienced stress was significantly impacted by tVNS in the full sample, females, older participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Finally, experienced calm was significantly impacted by tVNS in the full sample ( $p < 0.001$ ), females ( $p = 0.002$ ), older participants ( $p = 0.009$ ), musicians ( $p = 0.001$ ), those who detected tVNS ( $p = 0.003$ ) and those who did not detect tVNS ( $p = 0.026$ ). Further analysis showed that pre-baseline experienced calm was significantly lower than that after baseline (full sample:  $p = 0.001$ ; females:  $p = 0.008$ ; older:  $p = 0.014$ ; musicians:  $p = 0.002$ ; detected tVNS:  $p = 0.015$ ; did not detect tVNS:  $p = 0.001$ ) and recovery (full sample:  $p = 0.001$ ;  $p = 0.002$ ; older:  $p = 0.005$ ; musicians:  $p =$



0.001; did not detect tVNS:  $p = 0.033$ ). Figure 4.119 summarises these patterns.

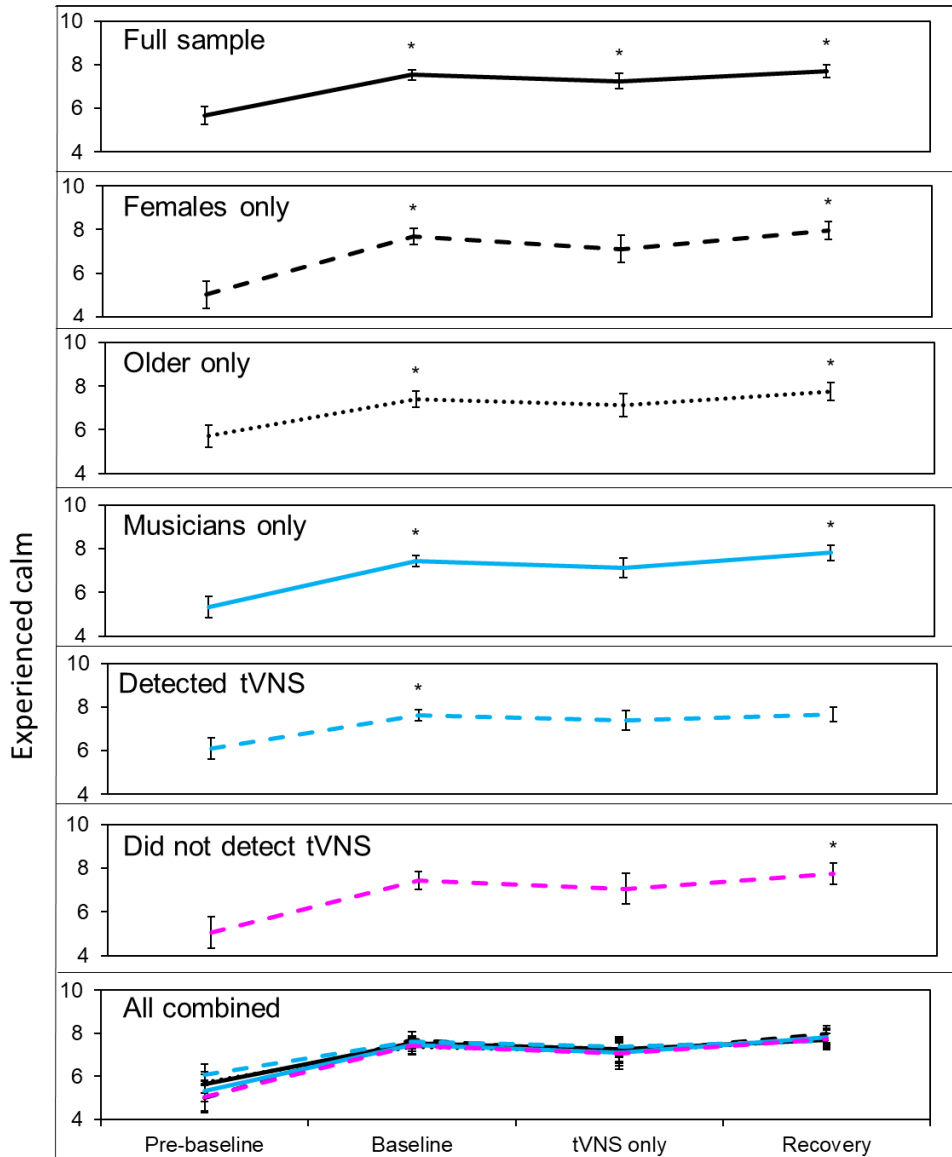


Figure 4.119: Experienced calm was significantly impacted by tVNS in all groups except males, young participants and non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.2.1.2 Music only visit

There was a significant effect of music on experienced stimulation in the full sample ( $p = 0.035$ ), males ( $p = 0.031$ ), females ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.004$ ). Further analysis revealed that pre-baseline experienced stimulation was significantly higher than after baseline (females:  $p = 0.015$ ; did not detect tVNS:  $p = 0.010$ ), music (females:  $p = 0.017$ ) and recovery (full sample:  $p = 0.010$ ; females:  $p = 0.025$ ; did not detect tVNS:  $p =$

0.032). Also, in males experienced stimulation was significantly higher post-music than post-baseline ( $p = 0.026$ ). Figure 4.120 summarises these findings.

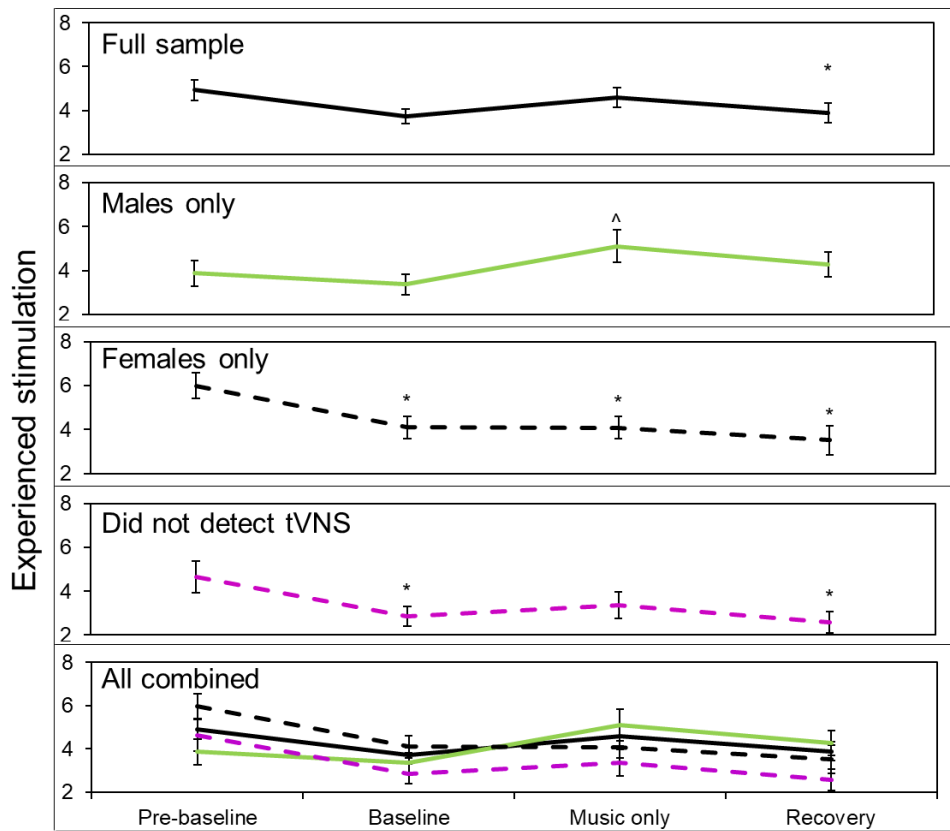


Figure 4.120: Experienced stimulation was significantly impacted by music in the full sample, males, females and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; ^ = significantly different to baseline.

A significant effect of music on experienced relaxation emerged in the full sample ( $p < 0.001$ ), females ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p = 0.001$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p < 0.001$ ). Further exploration showed that pre-baseline experienced relaxation was significantly lower than after baseline (full sample:  $p = 0.002$ ; females:  $p = 0.008$ ; musicians:  $p = 0.006$ ; did not detect tVNS:  $p = 0.005$ ), music (full sample:  $p < 0.001$ ; females:  $p = 0.002$ ; older:  $p = 0.004$ ; musicians:  $p = 0.001$ ; non-musicians:  $p = 0.009$ ; detected tVNS:  $p = 0.005$ ; did not detect tVNS:  $p = 0.006$ ) and recovery (full sample:  $p = 0.001$ ; females:  $p = 0.025$ ;

older:  $p = 0.011$ ; musicians:  $p = 0.008$ ; non-musicians:  $p = 0.048$ ; did not detect tVNS:  $p = 0.014$ ). Figure 4.121 summarises these findings.

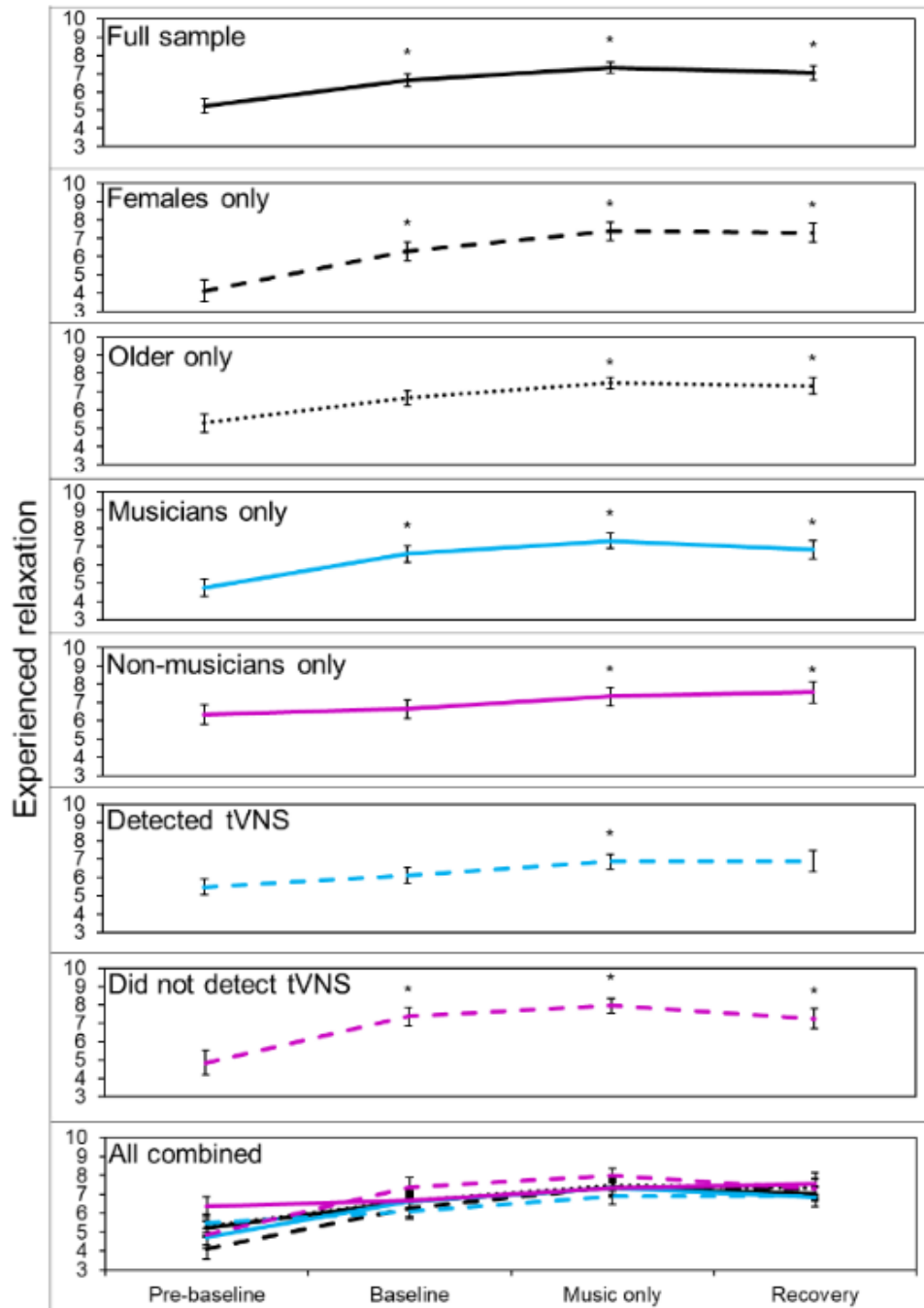


Figure 4.121: Experienced relaxation was significantly impacted by music in all groups except males and young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

The effect of music on experienced stress also reached statistical significance in the full sample ( $p = 0.003$ ), females ( $p < 0.001$ ), musicians ( $p = 0.015$ ) and those who did not detect tVNS ( $p = 0.018$ ). Analysis

investigating this effect revealed that experienced stress at pre-baseline was significantly higher than that after baseline (females:  $p = 0.003$ ), music (full sample:  $p = 0.004$ ; females:  $p = 0.003$ ; musicians:  $p = 0.012$ ; did not detect tVNS:  $p = 0.021$ ) and recovery (females:  $p = 0.002$ ). Also, for the full sample, stress after music was significantly lower than that for baseline ( $p = 0.049$ ). Figure 4.122 illustrates these findings.

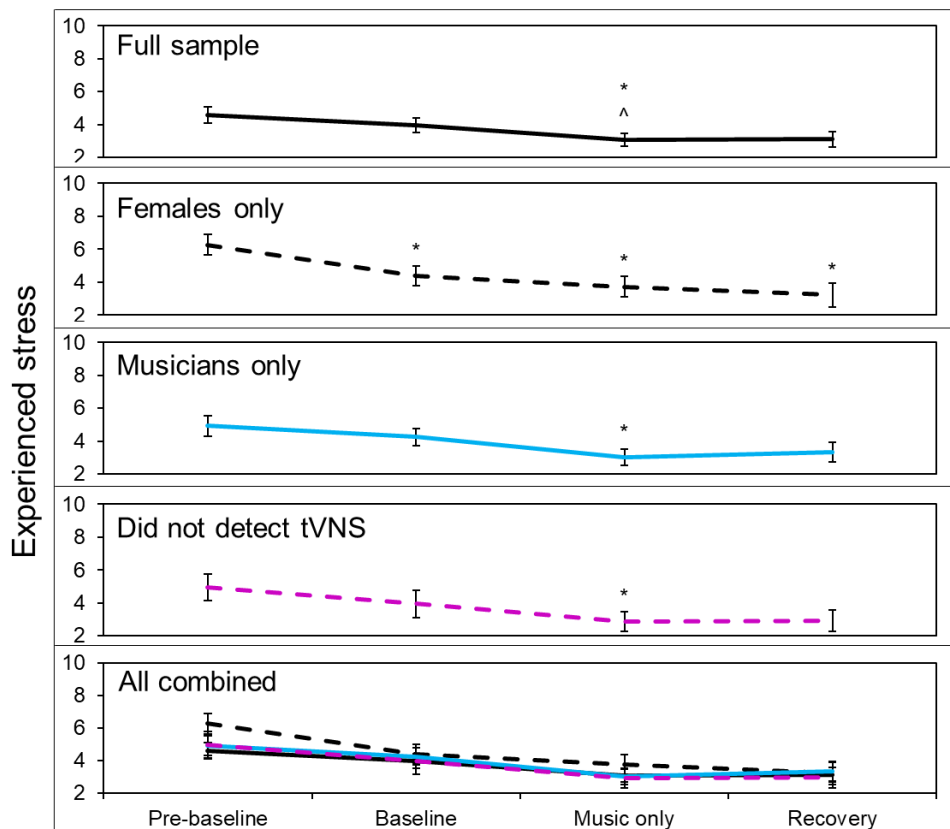


Figure 4.122: Experienced stress was significantly impacted by music in the full sample, females, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; ^ = significantly different to baseline.

There was a significant effect of music on experienced calm which emerged in the full sample ( $p < 0.001$ ), males ( $p = 0.026$ ), females ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p = 0.004$ ), those who detected tVNS ( $p = 0.005$ ) and those who did not detect tVNS ( $p < 0.001$ ). Analysis exploring this effect revealed that experienced calm at pre-baseline was significantly lower than that after baseline (full sample:  $p = 0.008$ ; males:  $p = 0.044$ ; females:  $p = 0.014$ ), music (full sample:  $p < 0.001$ ; females:  $p = 0.001$ ; older:  $p < 0.001$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p$

= 0.022; did not detect tVNS:  $p = 0.001$ ) and recovery (full sample:  $p < 0.001$ ; females:  $p < 0.001$ ; older:  $p = 0.005$ ; musicians:  $p = 0.004$ ; non-musicians:  $p = 0.047$ ; did not detect tVNS:  $p = 0.008$ ). Also, for musicians and those who detected tVNS, experienced calm after music was significantly higher than that after baseline ( $p = 0.002$  and  $p = 0.007$  respectively). Figure 4.123 summarises these findings.

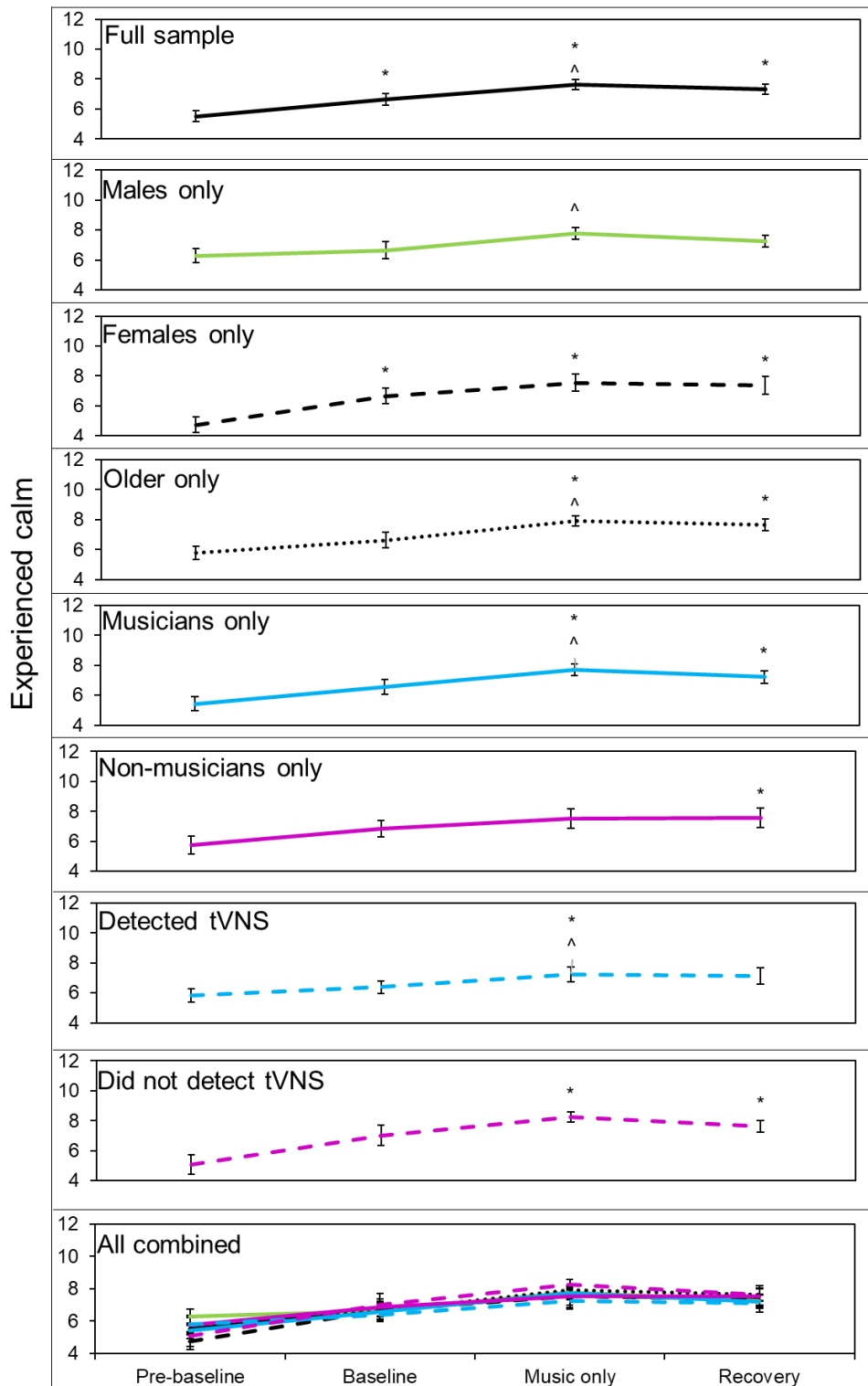


Figure 4.123: Experienced calm was significantly impacted by music in all groups except young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; ^ = significantly different to baseline.

Finally, there was a significant effect of music on experienced happiness in the full sample ( $p = 0.001$ ), females ( $p < 0.001$ ), older participants ( $p =$

0.009) and musicians ( $p = 0.011$ ). Further analysis revealed that in the full sample participants were significantly happier after music than baseline ( $p = 0.006$ ) and after recovery than baseline ( $p = 0.002$ ). In females, happiness was significantly greater post-music compared to post-baseline ( $p = 0.006$ ) and post-recovery than post-baseline ( $p = 0.002$ ). In older participants, happiness post-baseline was significantly greater than that post-music ( $p = 0.006$ ) and post-recovery ( $p = 0.025$ ). For musicians, experienced happiness was significantly higher after recovery than after baseline ( $p = 0.004$ ). Figure 4.124 summarises the findings

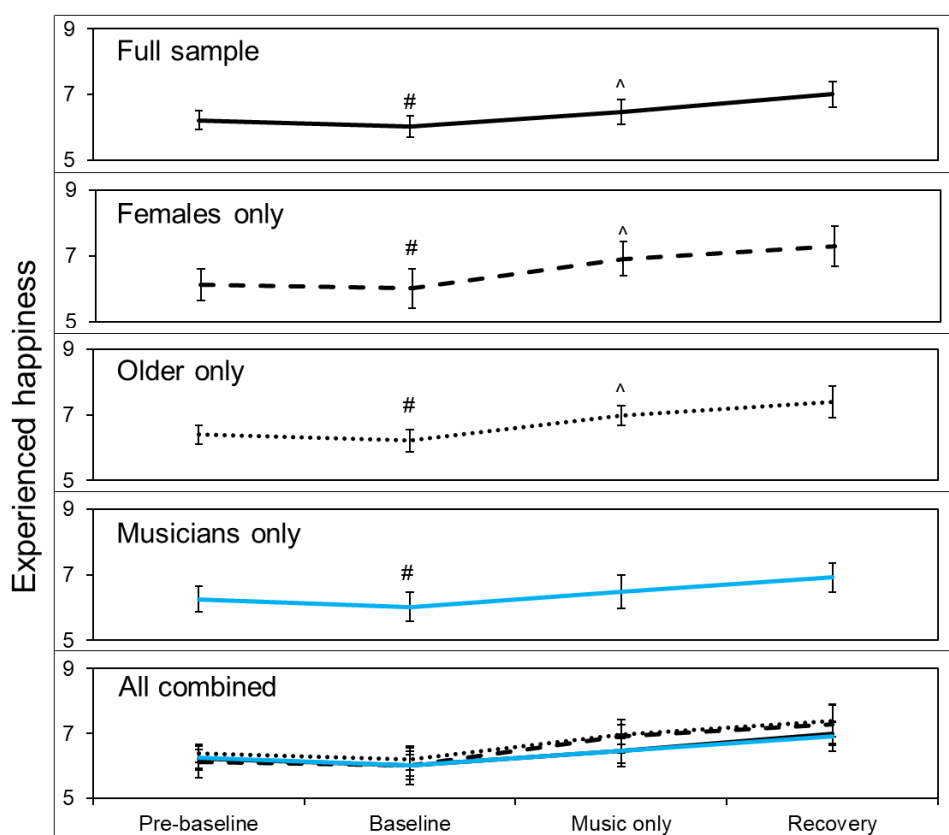


Figure 4.124: Experienced happiness was significantly impacted by music in the full sample, females, older participants and musicians. Data presented as mean  $\pm$  1 SEM. ^ = significantly different to baseline; # = significantly different to recovery.

#### 4.3.2.1.3 tVNS + music visit

There was a significant effect of tVNS + music on experienced stimulation for the full sample ( $p = 0.004$ ), females ( $p = 0.050$ ), musicians ( $p = 0.001$ ) and those who did not feel tVNS ( $p < 0.001$ ). Further analysis showed that pre-baseline experienced stimulation was significantly greater than that after baseline (full sample:  $p = 0.008$ ; musicians:  $p = 0.022$ ; did not detect tVNS:  $p < 0.001$ ), tVNS + music (did not detect tVNS:  $p = 0.001$ ) and recovery (full sample:  $p = 0.003$ ; females:  $p = 0.005$ ; musicians:  $p = 0.027$ ; did not detect tVNS  $p < 0.001$ ). Figure 4.125 summarises these findings.

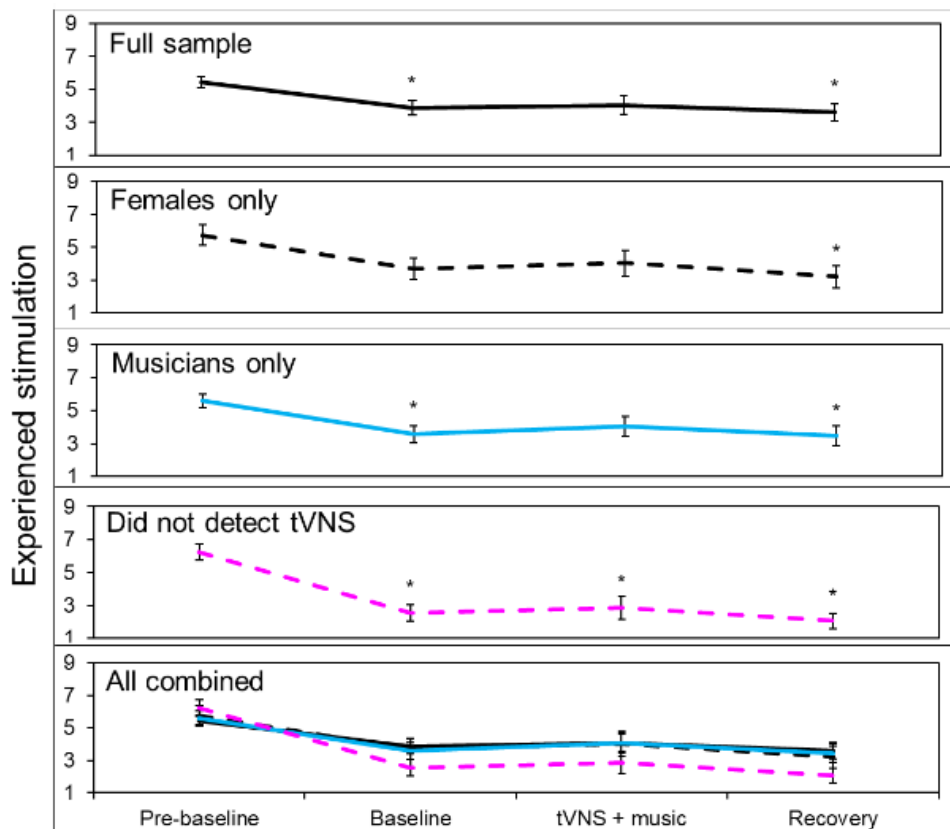


Figure 4.125: Experienced stimulation was significantly impacted by tVNS + music in the full sample, females, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

A significant effect of tVNS + music on experienced relaxation also occurred, but for the full sample ( $p < 0.001$ ), males ( $p = 0.001$ ), females ( $p = 0.001$ ), young participants ( $p = 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p < 0.001$ ), those who detected tVNS ( $p = 0.001$ ) and



those who did not detect tVNS ( $p = 0.001$ ). Further analysis showed that pre-baseline experienced relaxation was significantly lower than that after baseline (full sample:  $p < 0.001$ ; males:  $p = 0.008$ ; females:  $p = 0.008$ ; young:  $p = 0.029$ ; musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.006$ ; did not detect tVNS:  $p = 0.008$ ), tVNS + music (full sample:  $p < 0.001$ ; older:  $p = 0.004$ ; musicians:  $p = 0.006$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.005$ ; females:  $p = 0.006$ ; young:  $p = 0.028$ ; older:  $p = 0.005$ ; musicians:  $p = 0.002$ ; non-musicians:  $p = 0.050$ ; detected tVNS:  $p = 0.005$ ; did not detect tVNS:  $p = 0.007$ ). Moreover, for the full sample, females and older participants, experienced relaxation was significantly higher after recovery than after baseline ( $p = 0.001$ ,  $p = 0.005$  and  $p = 0.002$  respectively). Additionally, in the full sample stress was significantly higher after baseline than after tVNS + music ( $p = 0.001$ ) and recovery ( $p = 0.004$ ). Figure 4.126 summarises these findings.

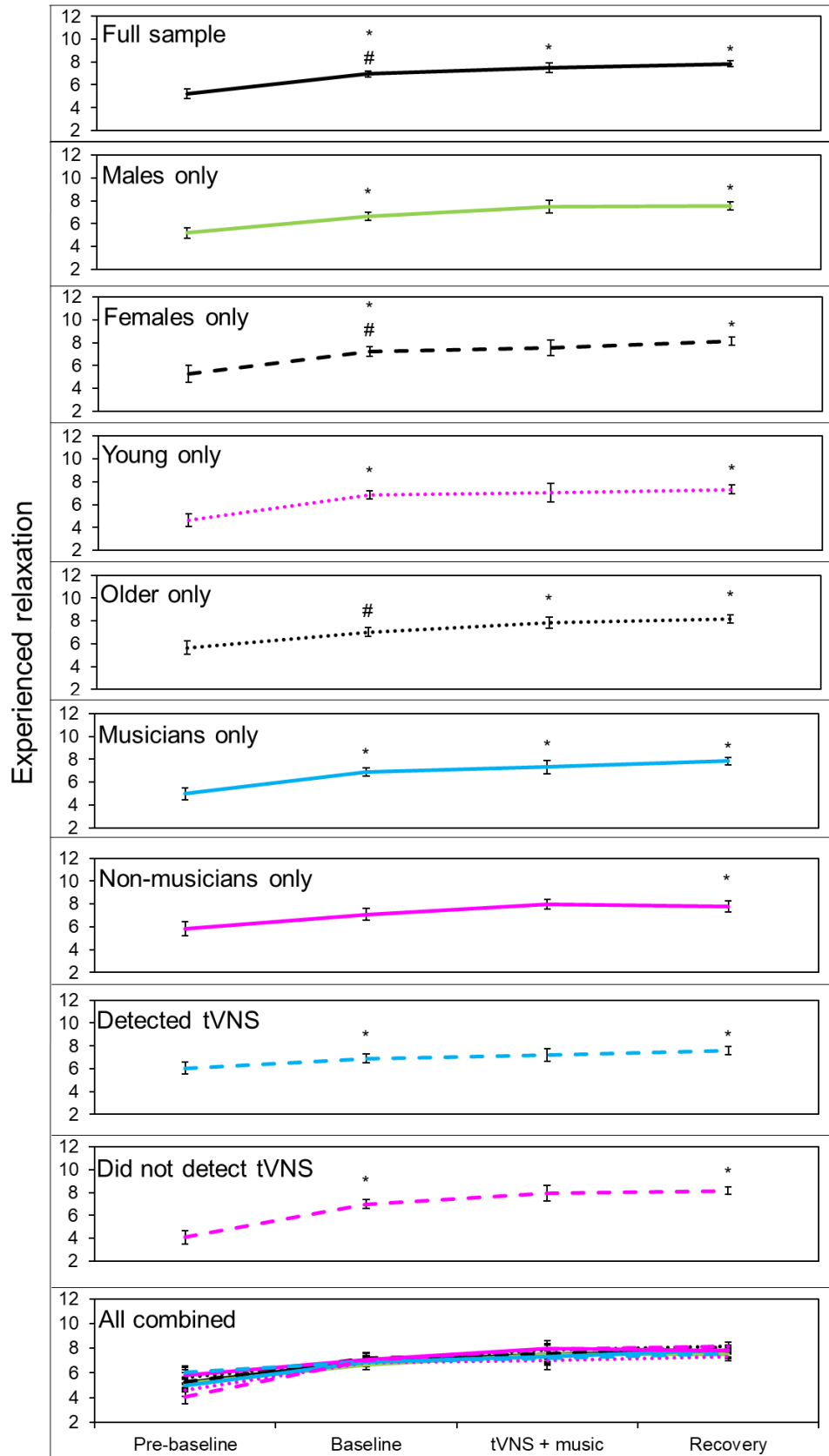


Figure 4.126: Experienced relaxation was significantly impacted by tVNS + music in all groups. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to recovery.

Experienced stress was significantly impacted by tVNS + music in the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.011$ ), young participants ( $p < 0.001$ ), older participants ( $p = 0.014$ ), musicians ( $p < 0.001$ ) and those who detected tVNS ( $p = 0.001$ ). Experienced stress at pre-baseline was significantly higher than that after baseline (full sample:  $p = 0.003$ ; females:  $p = 0.041$ ), tVNS + music (full sample:  $p < 0.001$ ; males:  $p = 0.002$ ; young:  $p = 0.013$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.002$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.002$ ; females:  $p = 0.021$ ; young:  $p = 0.001$ ; older:  $p = 0.004$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.002$ ). In addition, experienced stress in musicians was also higher post-baseline than post-tVNS + music ( $p = 0.005$ ). Figure 4.127 illustrates these findings.

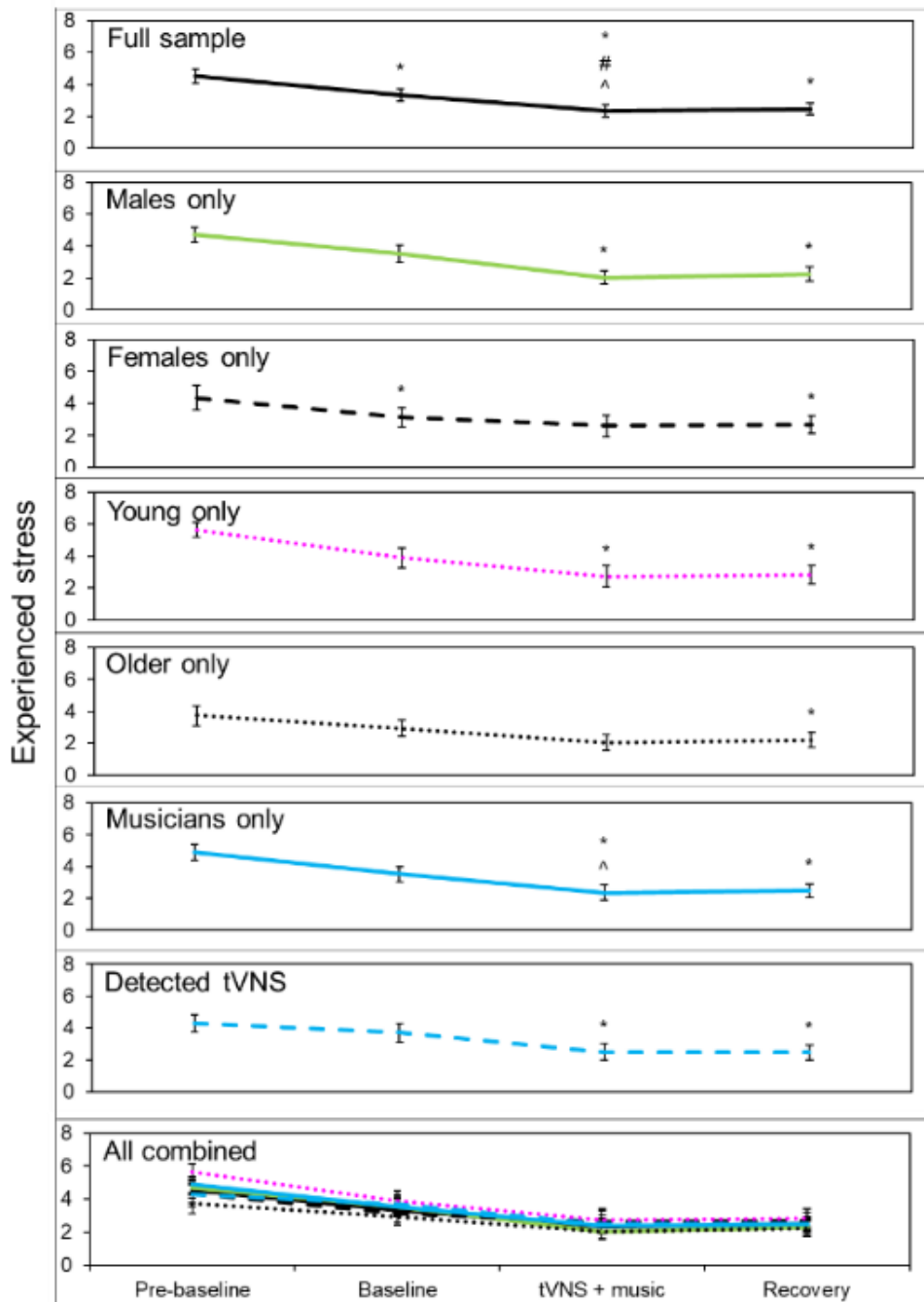


Figure 4.127: Experienced stress was significantly impacted by tVNS + music in all groups except non-musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to recovery; ^ = significantly different to baseline.

There was a significant effect of tVNS + music on experienced calm when analysing the full sample ( $p < 0.001$ ), males ( $p = 0.002$ ), females ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ) and those who detected tVNS ( $p < 0.001$ ). Pre-baseline experienced calm was significantly lower

than that after baseline (full sample:  $p < 0.001$ ; females:  $p = 0.002$ ; older:  $p = 0.004$ ; musicians:  $p = 0.001$ ), tVNS + music (full sample:  $p = 0.001$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.006$ ; females:  $p = 0.002$ ; older:  $p = 0.001$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.003$ ). Additionally, in the full sample, older participants and those who detected tVNS, experienced calm was significantly higher after recovery than after baseline ( $p = 0.010$ ,  $p = 0.004$  and  $p = 0.005$  respectively). Figure 4.128 summarises these findings.

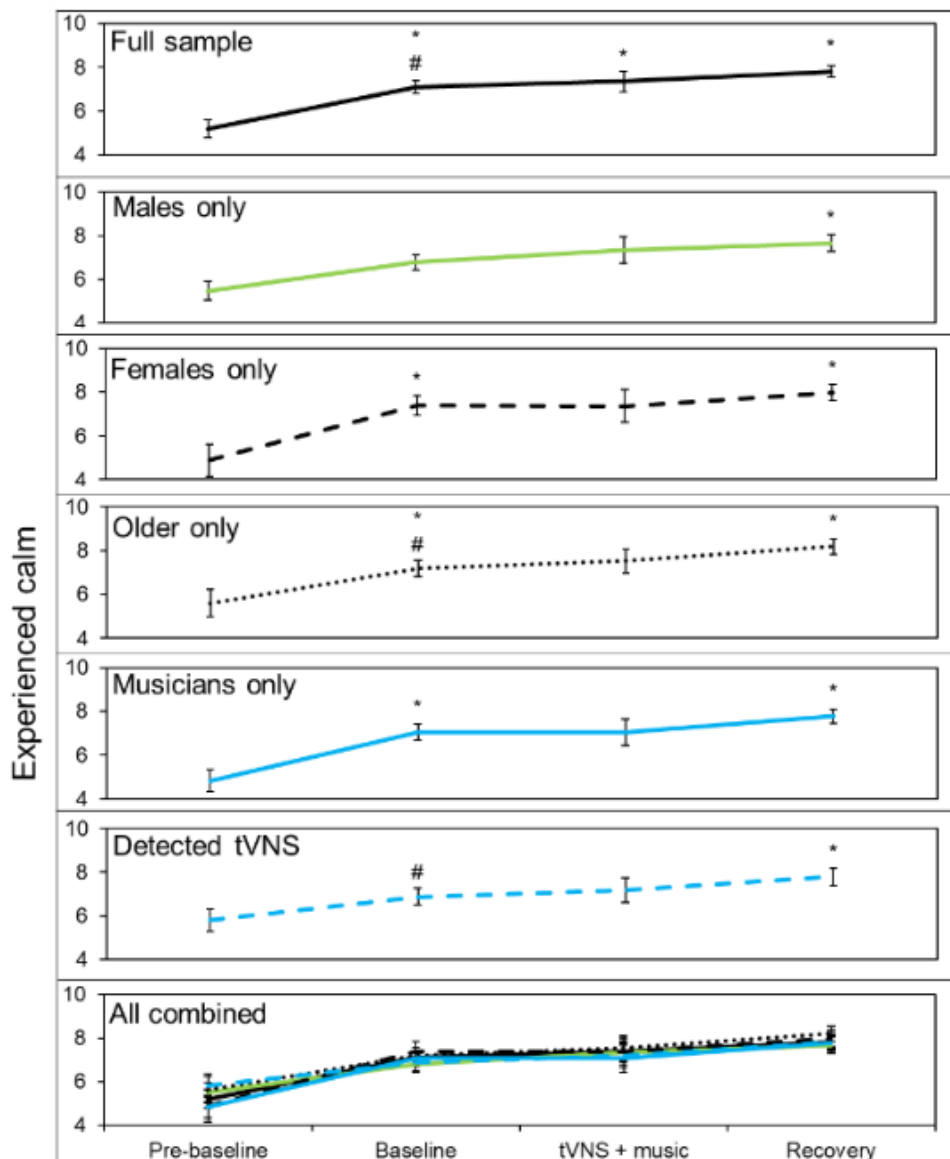


Figure 4.128: Experienced calm was significantly impacted by tVNS + music in the full sample, males, females, older participants, musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; # = significantly different to recovery.

Finally, a significant effect of tVNS + music on experienced happiness emerged in young participants only ( $p = 0.002$ ). Further analysis revealed that experienced happiness increased between pre-baseline and post-recovery ( $p = 0.037$ , see Figure 4.129).

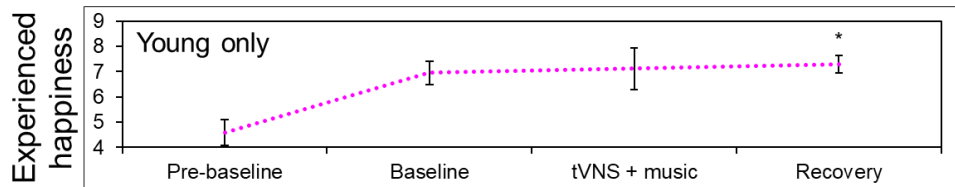


Figure 4.129: Experienced happiness was significantly impacted by tVNS + music in the young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.2.1.4 Sham visit

There was a significant effect of sham on experienced stimulation in the full sample ( $p = 0.012$ ) and those who did not detect tVNS ( $p = 0.002$ ). Further analysis revealed that pre-baseline experienced stimulation was significantly higher than that after sham (full sample:  $p = 0.034$ ; did not detect tVNS:  $p = 0.005$ ) and recovery (did not detect tVNS:  $p = 0.007$ , see Figure 4.130).

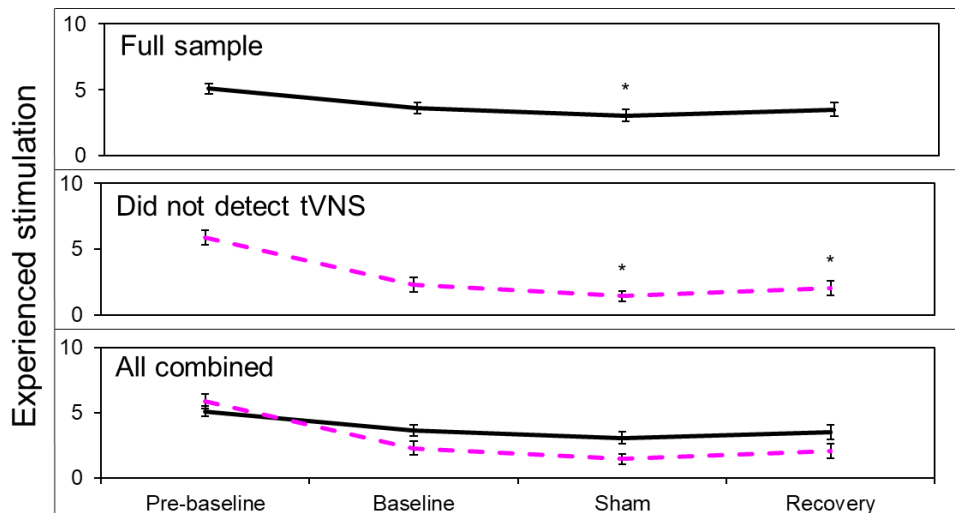


Figure 4.130: Experienced stimulation was significantly impacted by sham in the full sample and group who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

A significant effect of sham on experienced relaxation emerged when analysing the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p = 0.002$ ),

young participants ( $p = 0.008$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.004$ ). Pre-baseline values were significantly lower than those after baseline (full sample:  $p < 0.001$ ; females:  $p = 0.006$ ; older:  $p = 0.038$ ; musicians:  $p = 0.005$ ; detected tVNS:  $p = 0.002$ ), sham (full sample:  $p < 0.001$ ; males:  $p = 0.002$ ; females:  $p = 0.005$ ; young:  $p = 0.007$ ; older:  $p = 0.003$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.001$ ; did not detect tVNS:  $p = 0.008$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.005$ ; young:  $p = 0.007$ ; older:  $p = 0.042$ ; musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.002$ ). Relaxation in musicians was also identified as being significantly higher post-sham than post-baseline ( $p = 0.005$ ). Figure 4.131 summarises these findings.

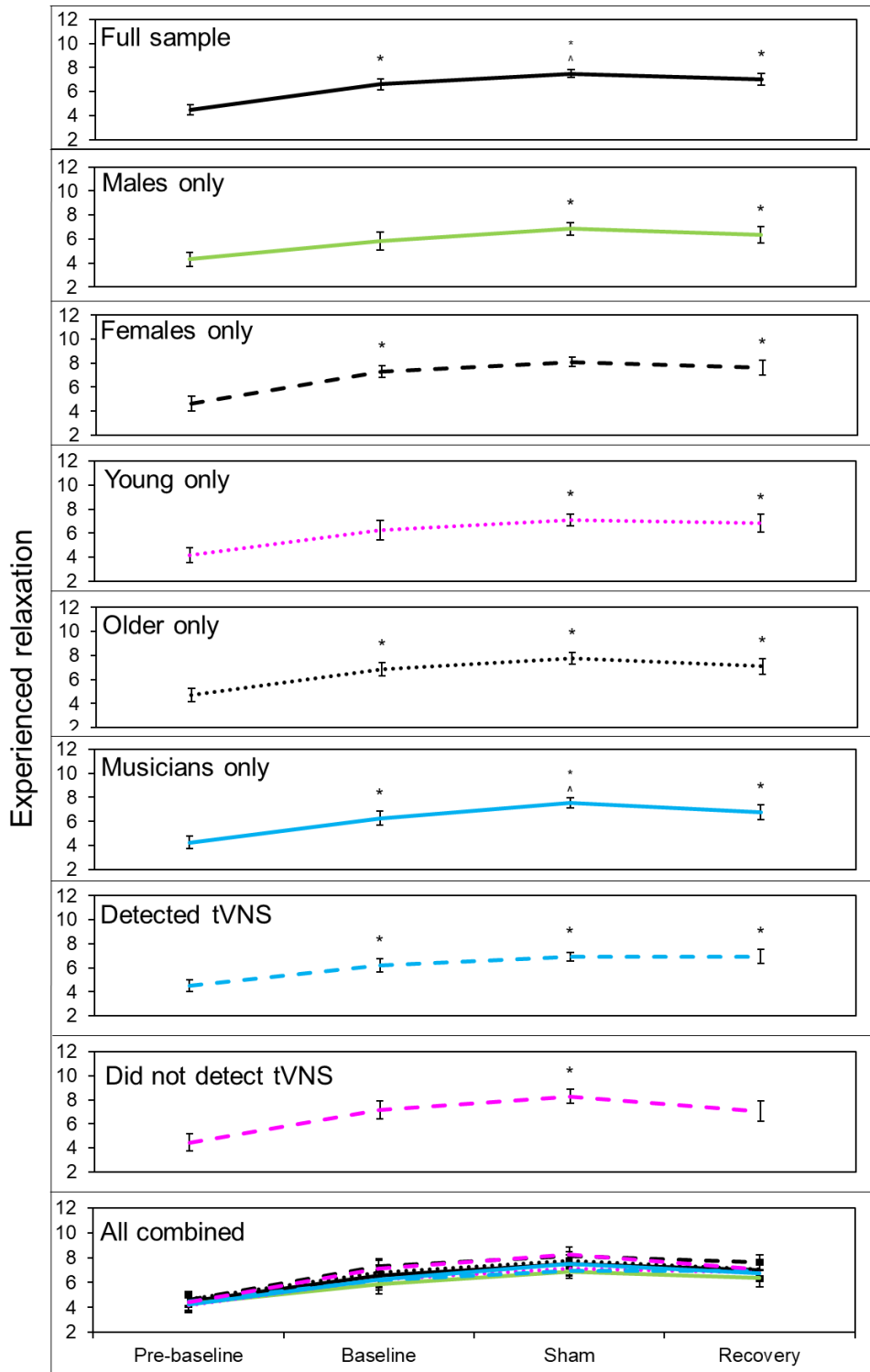


Figure 4.131: Experienced relaxation was significantly impacted by sham in all groups except non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline; ^ = significantly different to baseline.

A significant effect of sham also emerged on experienced stress in the full sample ( $p < 0.001$ ), females ( $p = 0.010$ ), older participants ( $p = 0.003$ ),



musicians ( $p = 0.001$ ) and those who did not detect tVNS ( $p = 0.003$ ). Experienced stress at pre-baseline was significantly higher than that after sham (full sample:  $p < 0.001$ ; older:  $p = 0.010$ ; musicians:  $p = 0.002$ ; did not detect tVNS:  $p = 0.005$ ) and recovery (full sample:  $p = 0.001$ ; females:  $p = 0.034$ ; older:  $p = 0.018$ ; musicians:  $p = 0.004$ ; did not detect tVNS:  $p = 0.007$ ). Figure 4.132 illustrates these findings.

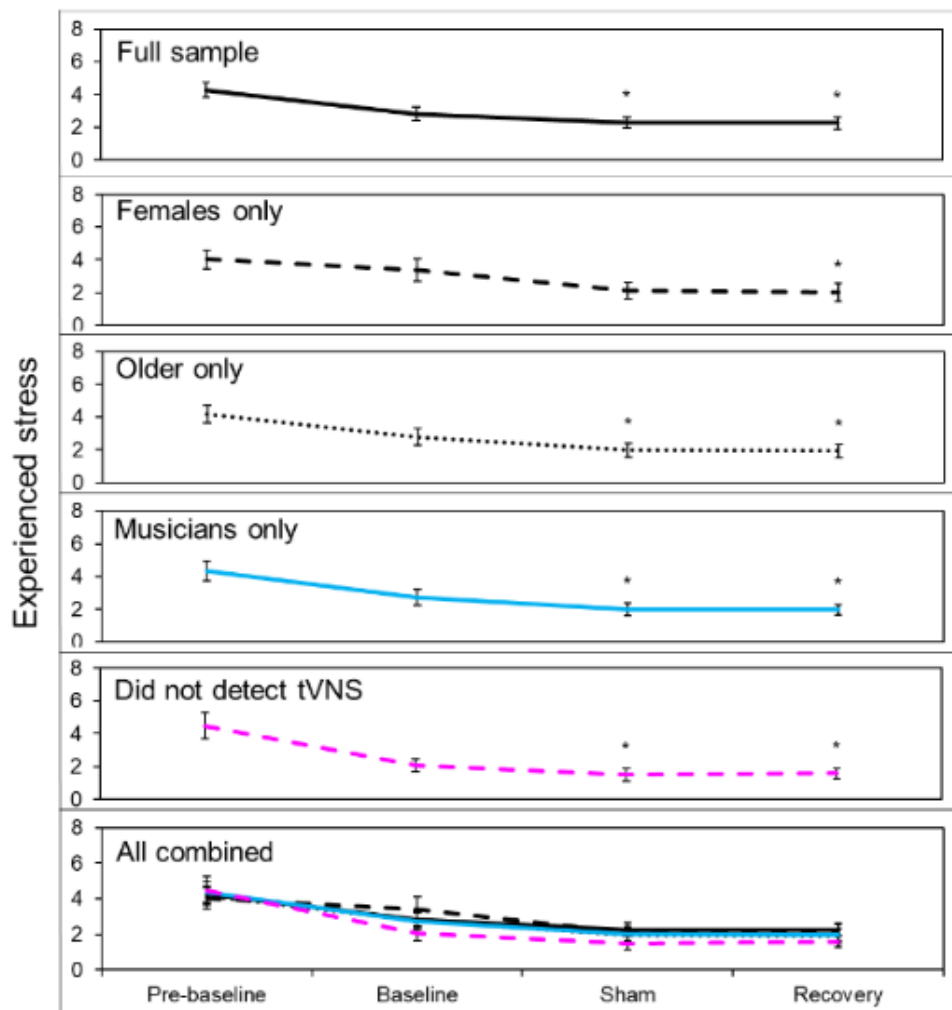


Figure 4.132: Experienced stress was significantly impacted by sham in the full sample, females, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Experienced calm was also significantly impacted by sham when analysing the full sample ( $p < 0.001$ ), males ( $p < 0.001$ ), females ( $p < 0.001$ ), young participants ( $p < 0.001$ ), older participants ( $p = 0.002$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.007$ ). Pre-baseline experienced calm was significantly lower than that

after baseline (full sample:  $p < 0.001$ ; males:  $p = 0.004$ ; females:  $p = 0.028$ ; older:  $p = 0.001$ ; musicians:  $p = 0.001$ ; detected tVNS:  $p = 0.011$ ; did not detect tVNS:  $p = 0.007$ ), sham (full sample:  $p < 0.001$ ; females:  $p = 0.009$ ; young:  $p = 0.014$ ; older:  $p = 0.029$ ; musicians:  $p = 0.002$ ; detected tVNS:  $p = 0.004$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.021$ ; females:  $p = 0.003$ ; young:  $p < 0.001$ ; older:  $p = 0.038$ ; musicians:  $p < 0.001$ ; detected tVNS:  $p = 0.001$ ). Figure 4.133 summarises these findings.

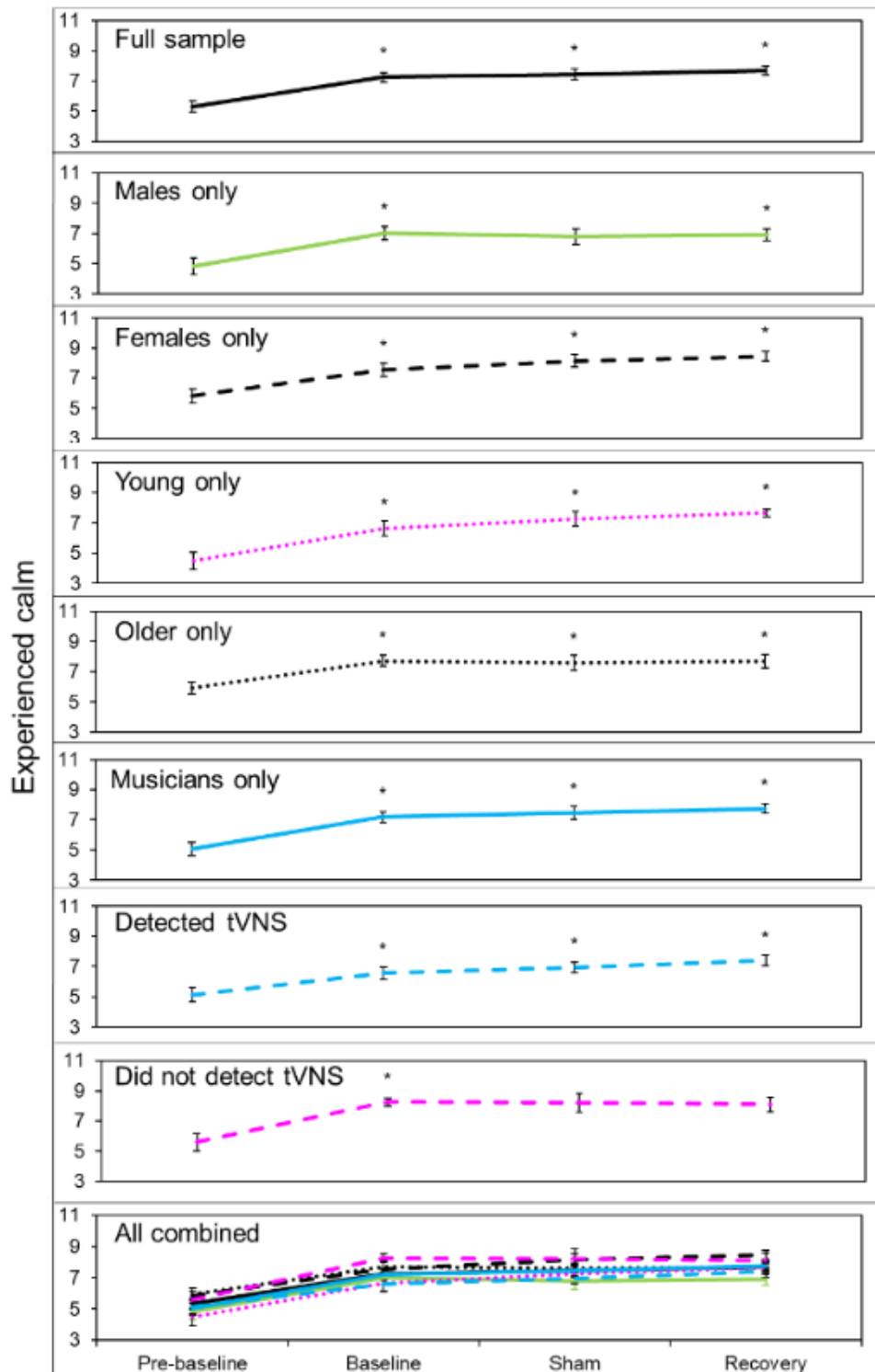


Figure 4.133: Experienced calm was significantly impacted by sham in all groups except non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

Finally, there was a significant effect of sham on experienced happiness in the full sample ( $p = 0.020$ ). Pre-baseline happiness was significantly lower

than that at after baseline ( $p = 0.008$ ) and sham ( $p = 0.006$ , see Figure 4.134).

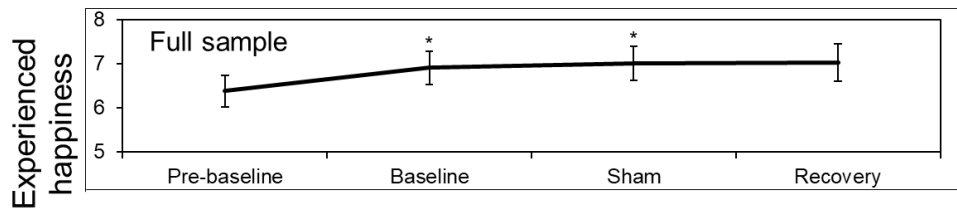


Figure 4.134: Experienced happiness was significantly impacted by sham in the full sample. Data presented as mean  $\pm$  1 SEM. \* = significantly different to pre-baseline.

#### 4.3.2.1.5 Between-visit analysis

There were significant differences in experienced stimulation between the four visits in the full sample ( $p = 0.028$ ) and those who did not detect tVNS ( $p = 0.020$ ). In the full sample, absolute change in stimulation increased for the music only visit and decreased in sham ( $p = 0.007$ ). For the group who did not detect tVNS, experienced stimulation increased in tVNS but decreased in sham ( $p = 0.007$ ). Figure 4.135 summarises these findings.

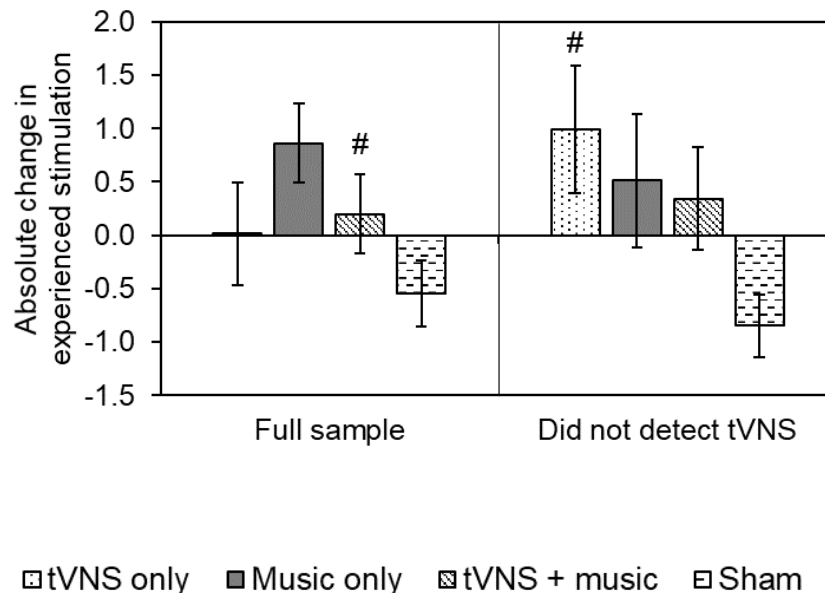
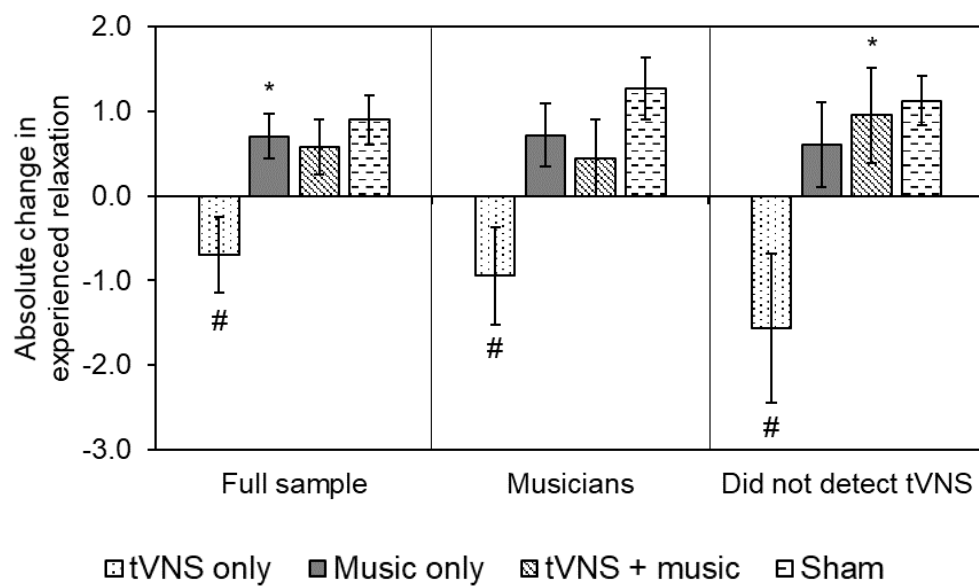


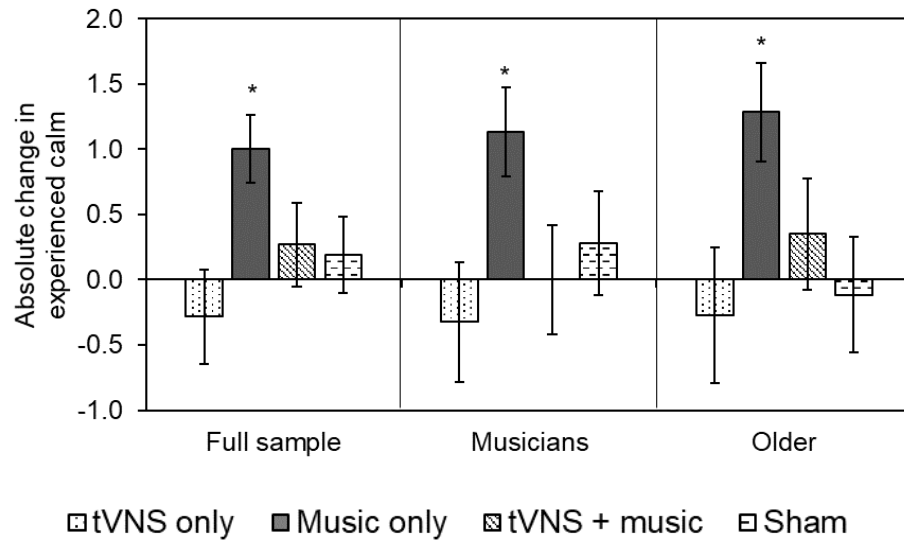
Figure 4.135: Absolute change in experienced stimulation significantly differed between the visits in the full sample and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. # = significantly different to sham.

Significant differences between the visits also emerged for experienced relaxation in the full sample ( $p = 0.019$ ), musicians ( $p = 0.004$ ) and those who did not detect tVNS ( $p = 0.024$ ). Further analysis revealed that in the full sample, absolute change in relaxation decreased in the tVNS only visit and increased in the music only ( $p = 0.007$ ) and sham visits ( $p = 0.022$ ). In musicians, experienced relaxation decreased in the tVNS only visit but increased in sham ( $p = 0.001$ ). Finally, for those who did not detect tVNS, relaxation decreased in tVNS yet increased in tVNS + music and sham (both  $p = 0.007$ , see Figure 4.136).



*Figure 4.136: Absolute change in experienced relaxation significantly differed between the visits in the full sample, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only; # = significantly different to sham.*

Significant differences between the visits transpired for experienced calm in the full sample ( $p = 0.005$ ), older participants ( $p = 0.021$ ) and musicians ( $p = 0.003$ ). In the full sample there was a decrease in calm for the tVNS only visit and an increase in the music only visit ( $p = 0.001$ ). In older participants, calm increased in the music visit but decreased in the tVNS visit ( $p = 0.005$ ). Finally, in musicians calm decreased in tVNS yet increased in the music only visit ( $p = 0.001$ ). Figure 4.137 summarises these findings.



*Figure 4.137: Absolute change in experienced calm significantly differed between the visits in the full sample, musicians and older participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only.*

Experienced stress, happiness and sadness did not significantly differ between the four visits for any group comparison ( $p > 0.05$ ).

#### 4.3.2.2 Perceived emotion

##### 4.3.2.2.1 tVNS only visit

There was a significant effect of tVNS on perceived positivity in the full sample ( $p < 0.001$ ), females ( $p = 0.013$ ), young participants ( $p = 0.023$ ), older participants ( $p = 0.004$ ), musicians ( $p < 0.001$ ) and those who did not detect tVNS ( $p = 0.002$ ). Perceived positivity was significantly lower during tVNS compared to baseline (full sample:  $p = 0.018$ ; older:  $p = 0.049$ ; musicians:  $p = 0.023$ ; did not detect tVNS:  $p = 0.008$ ) and recovery (full sample:  $p = 0.001$ ; females:  $p = 0.031$ ; young:  $p = 0.008$ ; older:  $p = 0.022$ ; musicians:  $p = 0.003$ ; did not detect tVNS:  $p = 0.022$ ). Figure 4.138 summarises these findings.

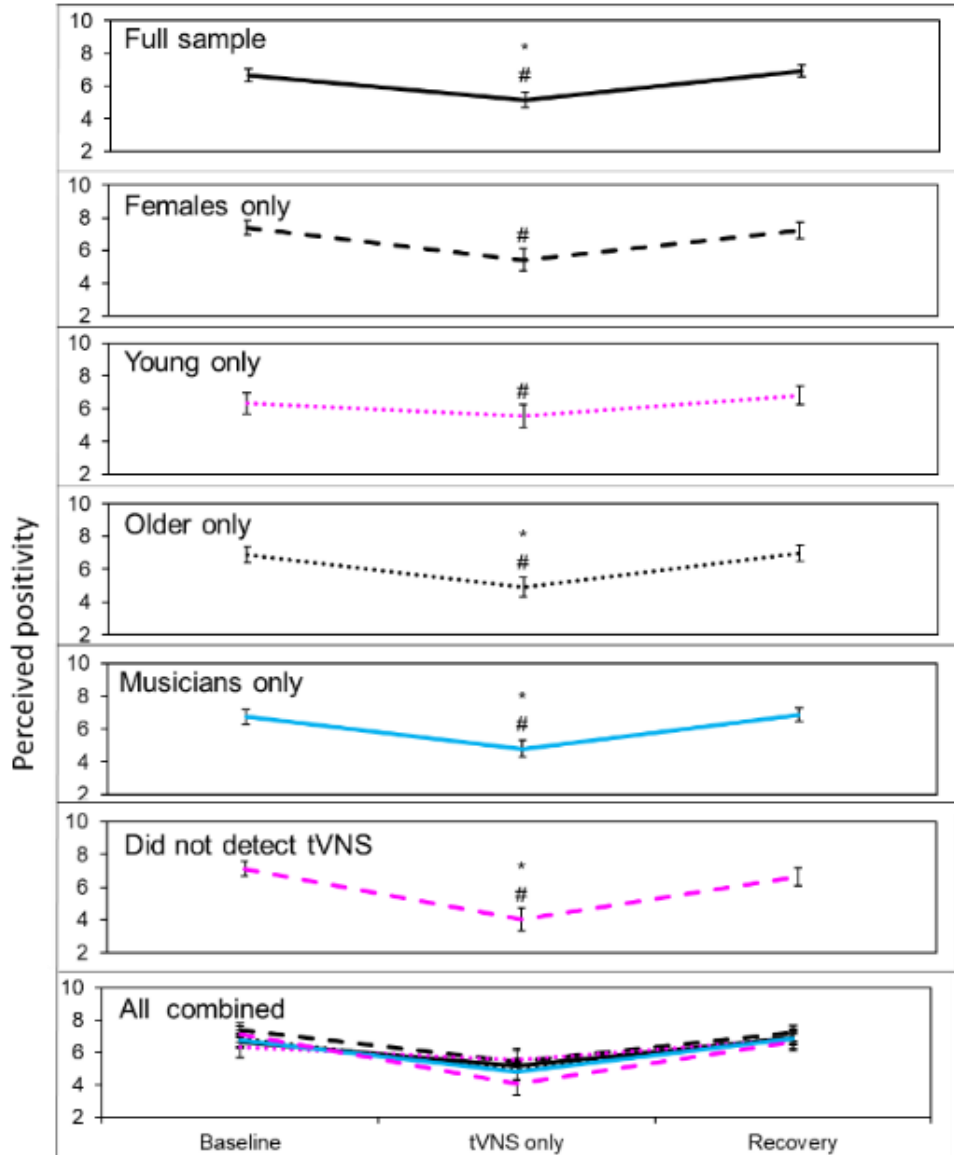


Figure 4.138: Perceived positivity was significantly impacted by tVNS in all groups except males, non-musicians and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

Perceived stimulation was also significantly impacted by tVNS in the full sample ( $p < 0.001$ ), males ( $p = 0.004$ ), females ( $p < 0.001$ ), young participants ( $p = 0.007$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), those who detected tVNS ( $p = 0.003$ ) and those who did not detect tVNS ( $p < 0.001$ ). Further analysis showed that perceived stimulation was significantly higher during tVNS than baseline (full sample:  $p < 0.001$ ; females:  $p = 0.008$ ; older:  $p = 0.001$ ; musicians:  $p = 0.008$ ; did not detect tVNS:  $p = 0.007$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.031$ ;

females:  $p = 0.002$ ; young:  $p = 0.038$ ; older:  $p = 0.005$ ; musicians:  $p = 0.003$ ; detected tVNS:  $p = 0.027$ ; did not detect tVNS:  $p = 0.004$ ). Figure 4.139 summarises these findings.

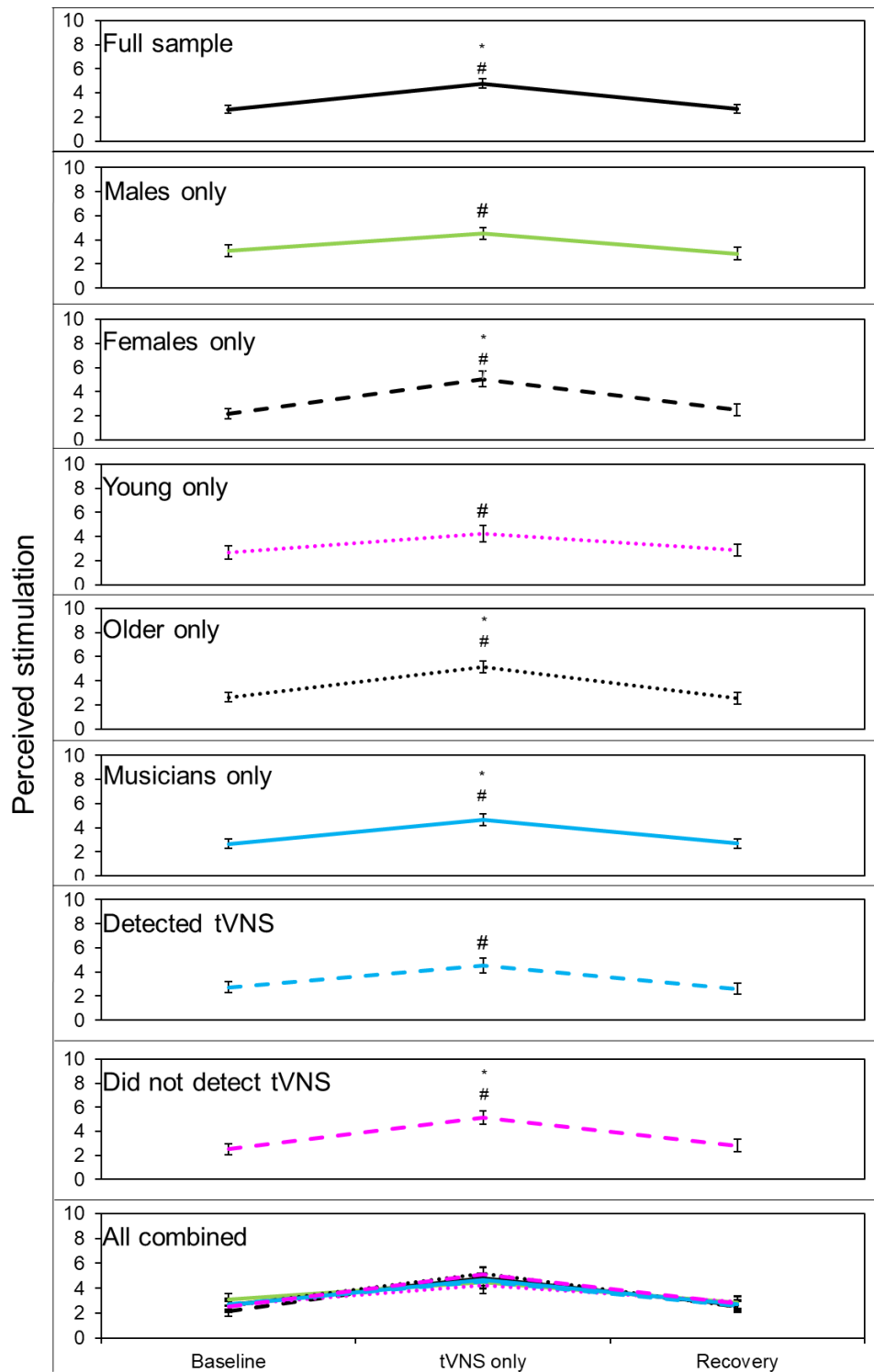


Figure 4.139: Perceived stimulation was significantly impacted by tVNS in all groups except non-musicians. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.



A significant effect of tVNS on perceived pleasantness emerged in the full sample ( $p < 0.001$ ), males ( $p = 0.002$ ), females ( $p = 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p = 0.004$ ) and those who did not detect tVNS ( $p < 0.001$ ). Further exploration demonstrated that perceived pleasantness was significantly lower during tVNS compared to baseline (full sample:  $p < 0.001$ ; males:  $p = 0.035$ ; females:  $p = 0.009$ ; older:  $p = 0.001$ ; musicians:  $p = 0.005$ ; non-musicians:  $p = 0.020$ ; did not detect tVNS:  $p = 0.001$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.031$ ; females:  $p = 0.010$ ; older:  $p = 0.003$ ; musicians:  $p = 0.001$ ; did not detect tVNS:  $p = 0.001$ ). Figure 4.140 illustrates these findings.

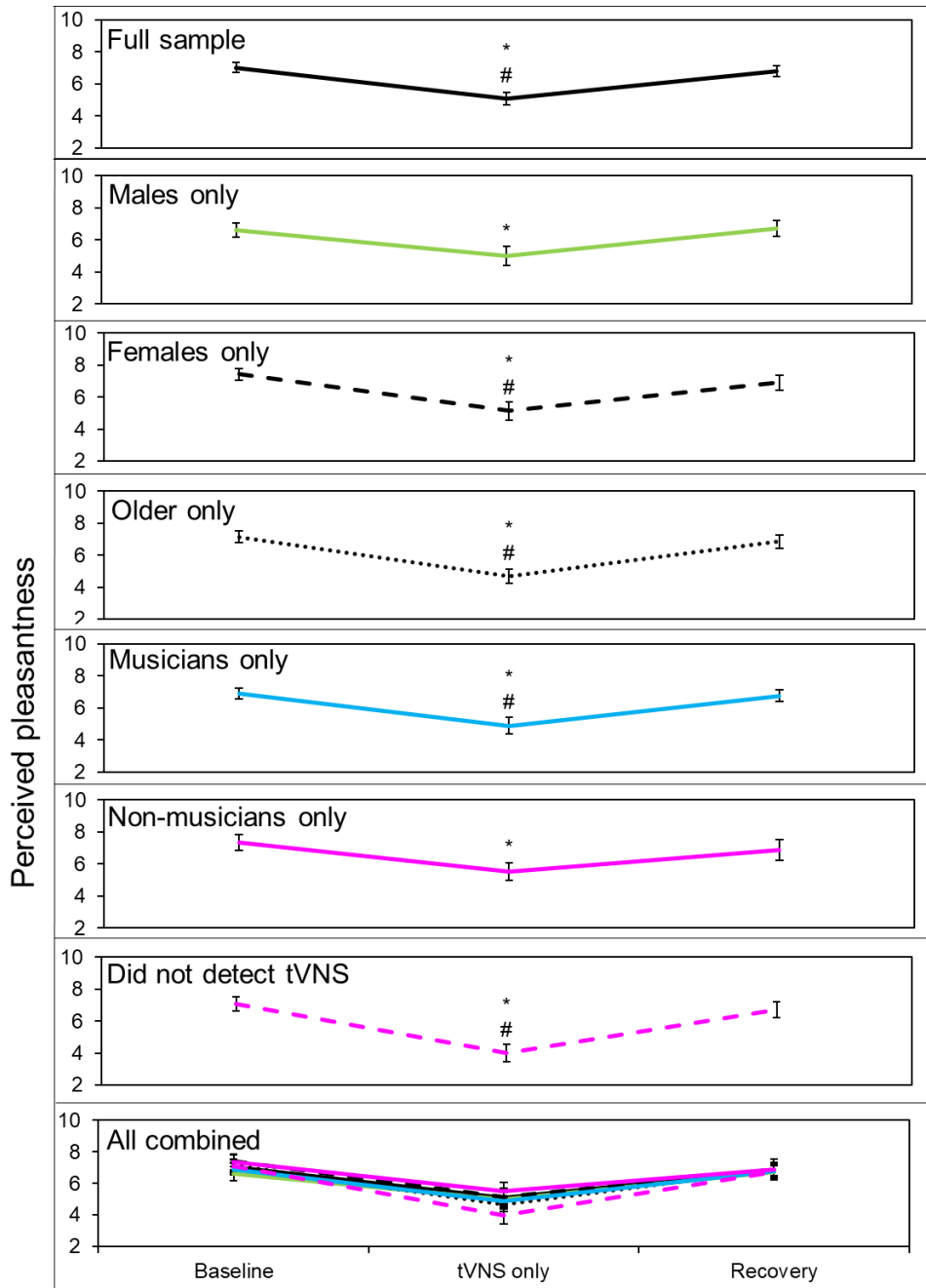


Figure 4.140: Perceived pleasantness was significantly impacted by tVNS in all groups except young participants and those who detected tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

There was a significant effect of tVNS on perceived irritation in the full sample ( $p < 0.001$ ), males ( $p = 0.010$ ), females ( $p < 0.001$ ), older participants ( $p < 0.001$ ), musicians ( $p = 0.010$ ), non-musicians ( $p = 0.006$ ), those who detected tVNS ( $p = 0.002$ ) and those who did not detect tVNS ( $p < 0.001$ ). Perceived irritation was significantly higher during tVNS than

during baseline (full sample:  $p < 0.001$ ; females:  $p = 0.002$ ; older:  $p = 0.002$ ; musicians:  $p = 0.004$ ; non-musicians:  $p = 0.018$ ; detected tVNS:  $p = 0.027$ ; did not detect tVNS:  $p = 0.002$ ) and recovery (full sample:  $p < 0.001$ ; males:  $p = 0.025$ ; females:  $p = 0.004$ ; older:  $p = 0.002$ ; musicians:  $p = 0.002$ ; detected tVNS:  $p = 0.019$ ; did not detect tVNS:  $p = 0.002$ ). Figure 4.141 summarises these findings.

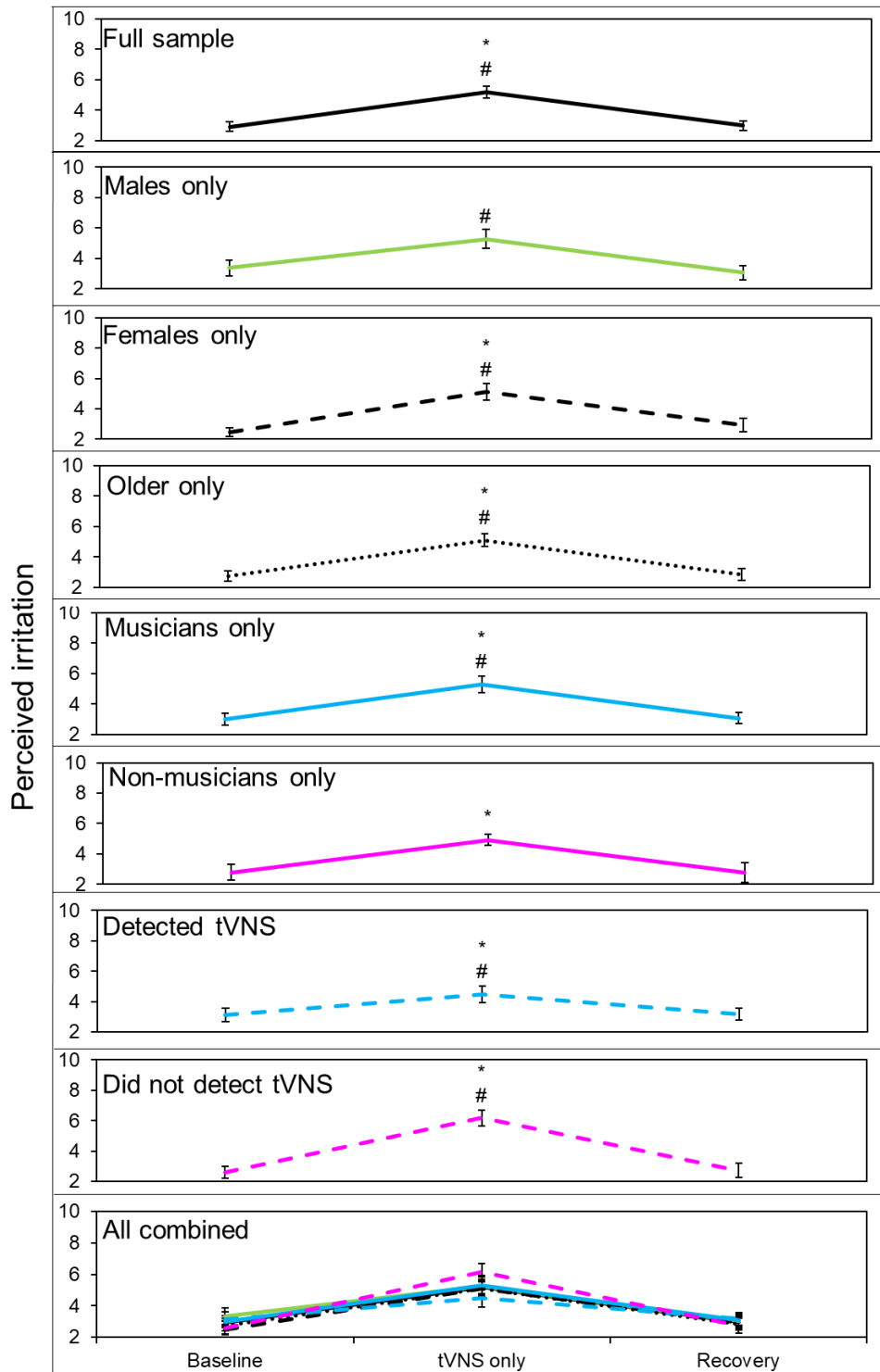


Figure 4.141: Perceived irritation was significantly impacted by tVNS in all groups except young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

There was a significant effect of tVNS on perceived obtrusiveness in the full sample ( $p < 0.001$ ), males ( $p = 0.008$ ), females ( $p < 0.001$ ), older participants ( $p = 0.001$ ), musicians ( $p < 0.001$ ), non-musicians ( $p = 0.005$ ),

those who detected tVNS ( $p = 0.023$ ) and those who did not detect tVNS ( $p < 0.001$ ). Perceived obtrusiveness was significantly higher during tVNS compared to baseline (full sample:  $p < 0.001$ ; males:  $p = 0.025$ ; females:  $p = 0.002$ ; older:  $p = 0.001$ ; musicians:  $p < 0.001$ ; non-musicians:  $p = 0.020$ ; detected tVNS:  $p = 0.009$ ; did not detect tVNS:  $p < 0.001$ ) and recovery (full sample:  $p < 0.001$ ; females:  $p = 0.002$ ; older:  $p = 0.005$ ; musicians:  $p = 0.001$ ; did not detect tVNS:  $p = 0.001$ ). Figure 4.142 summarises these findings.

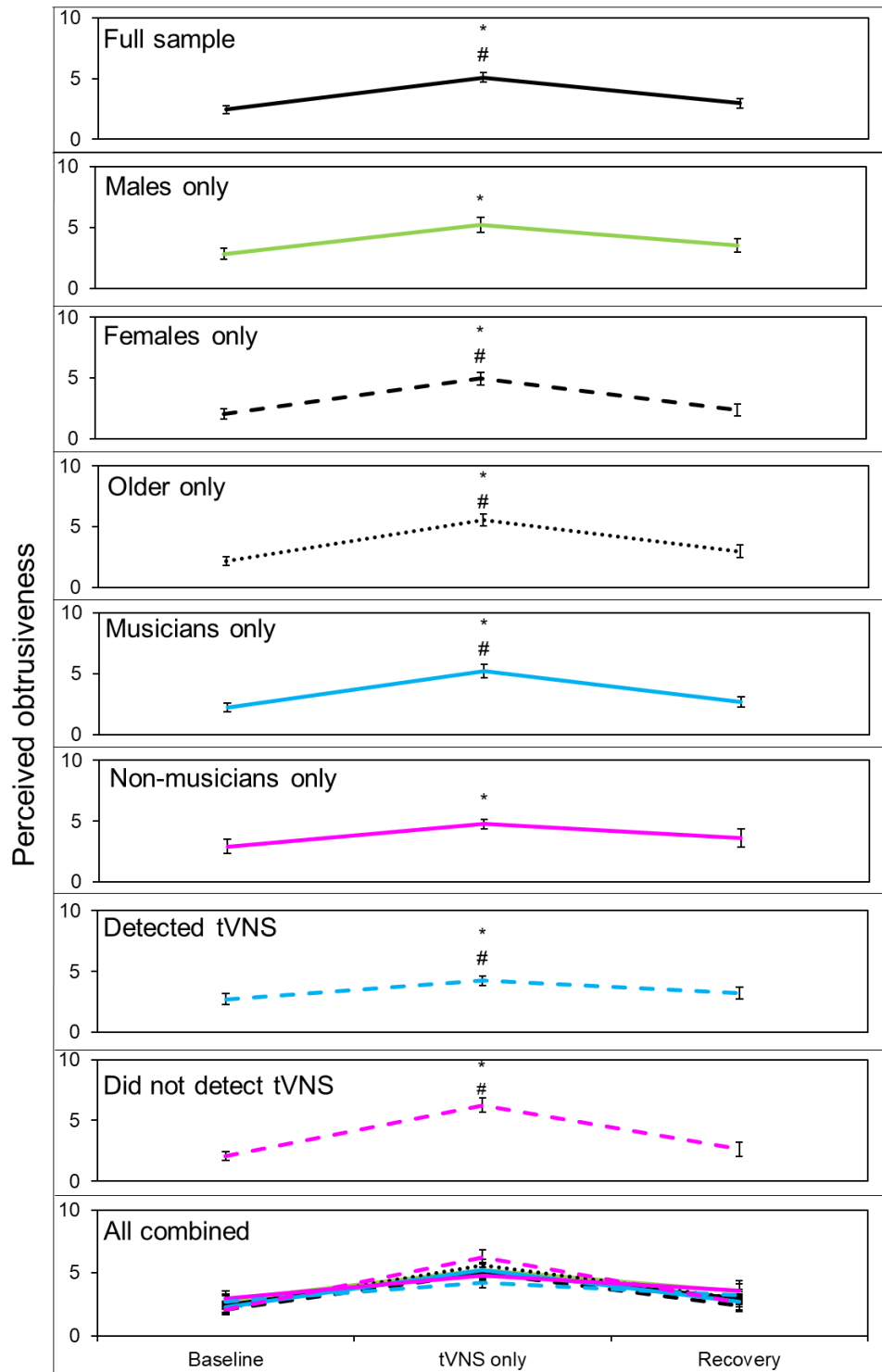


Figure 4.142: Perceived obtrusiveness was significantly impacted by tVNS in all groups except young participants. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline; # = significantly different to recovery.

#### 4.3.2.2.2 Music only visit

There were no significant effects of music on perceived emotion for any group analyses ( $p > 0.05$ ).

#### 4.3.2.2.3 tVNS + music visit

There were no significant effects of tVNS + music on perceived emotion for any group analyses except females ( $p = 0.002$ ): females perceived the tVNS + music stimulus as significantly more obtrusive than baseline ( $p = 0.010$ , see Figure 4.143).

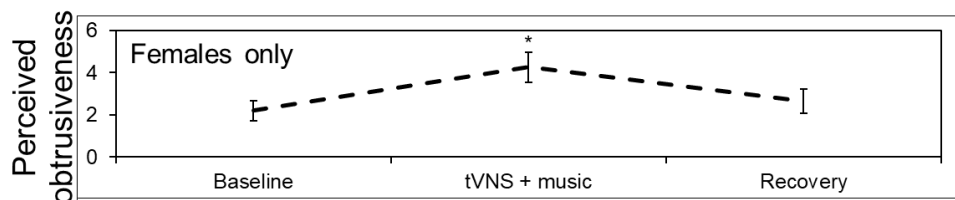


Figure 4.143: Perceived obtrusiveness was significantly impacted by tVNS + music in females only. Data presented as mean  $\pm$  1 SEM. \* = significantly different to baseline.

#### 4.3.2.2.4 Sham visit

There were no significant effects of sham on perceived emotion for any group analyses ( $p > 0.05$ ).

#### 4.3.2.2.5 Between-visit comparisons

There were significant differences in perceived stimulation between the four visits when analysing the full sample ( $p < 0.001$ ), females ( $p = 0.006$ ), older participants ( $p < 0.001$ ), musicians ( $p = 0.013$ ) and those who did not detect tVNS ( $p = 0.020$ ). In the full sample, absolute change in perceived stimulation increased in the tVNS only visit and decreased in the tVNS + music ( $p = 0.005$ ) and sham visits ( $p < 0.001$ ). In females, perceived stimulation increased during the tVNS visit and decreased during sham ( $p = 0.013$ ). In older participants, stimulation increased in the tVNS only visit but decreased in the sham visit ( $p < 0.001$ ). In musicians, perceived stimulation decreased in the sham visit but increased in the tVNS only ( $p = 0.004$ ) and music only ( $p = 0.007$ ) visits. Finally, for the group who did not detect tVNS, perceived stimulation increased in the tVNS visit and decrease in the sham visit ( $p = 0.009$ ). Figure 4.144 summarises these findings.

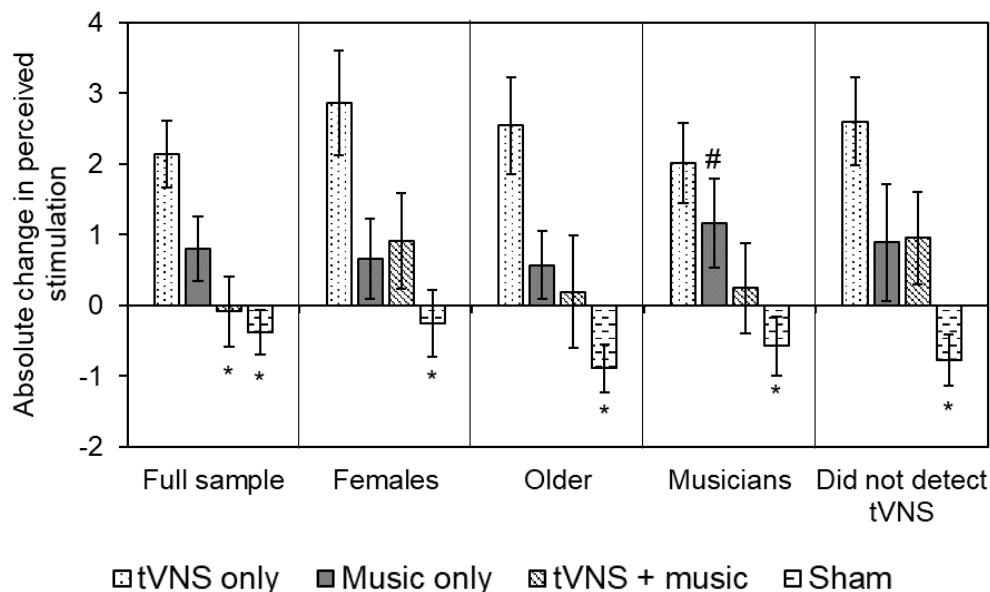
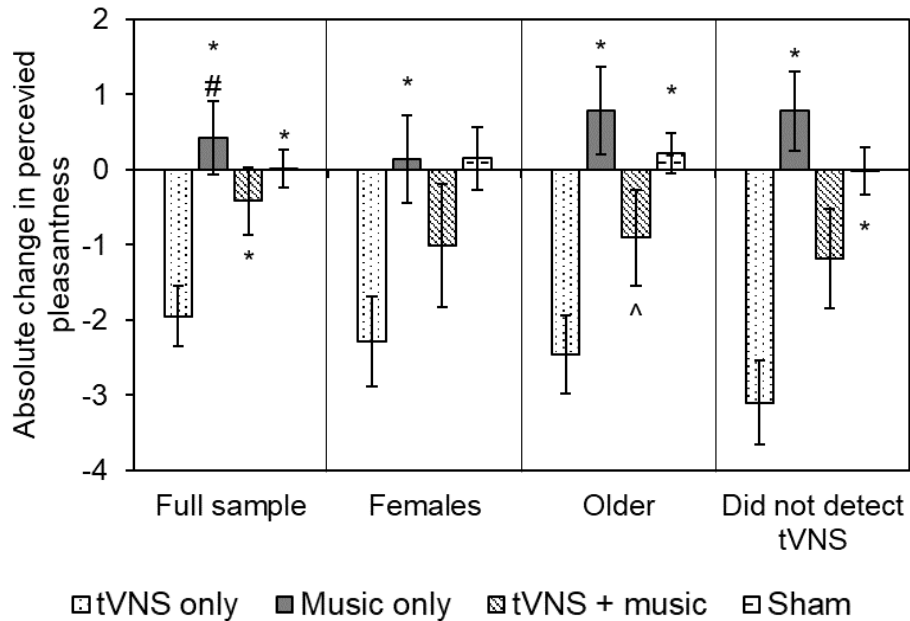


Figure 4.144: Change in perceived stimulation significantly differed between the visits in the full sample, females, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only; # = significantly different to sham.

Absolute change in perceived pleasantness also significantly differed between the four visits in the full sample ( $p < 0.001$ ), females ( $p = 0.011$ ), older participants ( $p < 0.001$ ) and those who did not detect tVNS ( $p < 0.001$ ). In the full sample, there was a significantly greater decrease in pleasantness for tVNS than tVNS + music ( $p = 0.005$ ) and a significantly greater increase in music than sham ( $p < 0.001$ ). Also, pleasantness decreased in tVNS only and increased in music only ( $p = 0.002$ ) and sham ( $p = 0.003$ ). In females, pleasantness decreased in the tVNS visit but increased in the music visit ( $p = 0.031$ ). In older participants, perceived pleasantness decreased in the tVNS only visit but increased in the music only ( $p = 0.001$ ) and sham visit ( $p = 0.002$ ). Furthermore, pleasantness decreased in the tVNS + music visit but increased in the music only visit ( $p = 0.003$ ). Finally, in the group who did not detect tVNS, pleasantness decreased in tVNS yet increased in music ( $p = 0.005$ ), and the decrease in pleasantness in tVNS was significantly greater than the decrease that occurred in sham ( $p = 0.005$ ). Figure 4.145 illustrates these findings.





*Figure 4.145: Change in perceived pleasantness significantly differed between the visits in the full sample, females, older participants and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only; # = significantly different to sham; ^ = significantly different to music only.*

Perceived irritation also significantly differed between the four visits in the full sample ( $p = 0.004$ ), males ( $p = 0.034$ ), females ( $p < 0.001$ ), young participants ( $p = 0.034$ ), older participants ( $p = 0.001$ ), musicians ( $p = 0.005$ ) and those who did not detect tVNS ( $p < 0.001$ ). For the full sample, the increase in perceived irritation for the tVNS only visit was significantly greater than the increase observed in the tVNS + music visit ( $p = 0.005$ ). Additionally, perceived irritation increased in the tVNS only visit but decreased for the music only ( $p = 0.001$ ) and sham visits ( $p < 0.001$ ). In the group of males, perceived irritation increased in the tVNS only visit and decreased in the music only visit ( $p = 0.003$ ). In females, irritation increased during tVNS but decreased during music ( $p = 0.014$ ) and sham ( $p = 0.005$ ). Perceived irritation increased in the tVNS only visit and decreased in the music only visit ( $p = 0.003$ ) in young participants, with irritation increasing in tVNS only and decreasing in music only ( $p = 0.004$ ) and sham ( $p = 0.005$ ) in the older cohort. In musicians, perceived irritation increased in tVNS but decreased in music ( $p = 0.046$ ), the increase observed in the tVNS only visit was significantly greater than that observed in the sham visit ( $p = 0.031$ ).

Finally, for those who did not detect tVNS, perceived irritation increased in the tVNS only visit and decreased in the music only visit ( $p = 0.003$ ). Also, the increase in irritation that emerged in the tVNS only visit was significantly greater than that which occurred in sham ( $p = 0.030$ ). Figure 4.146 summarises these findings.

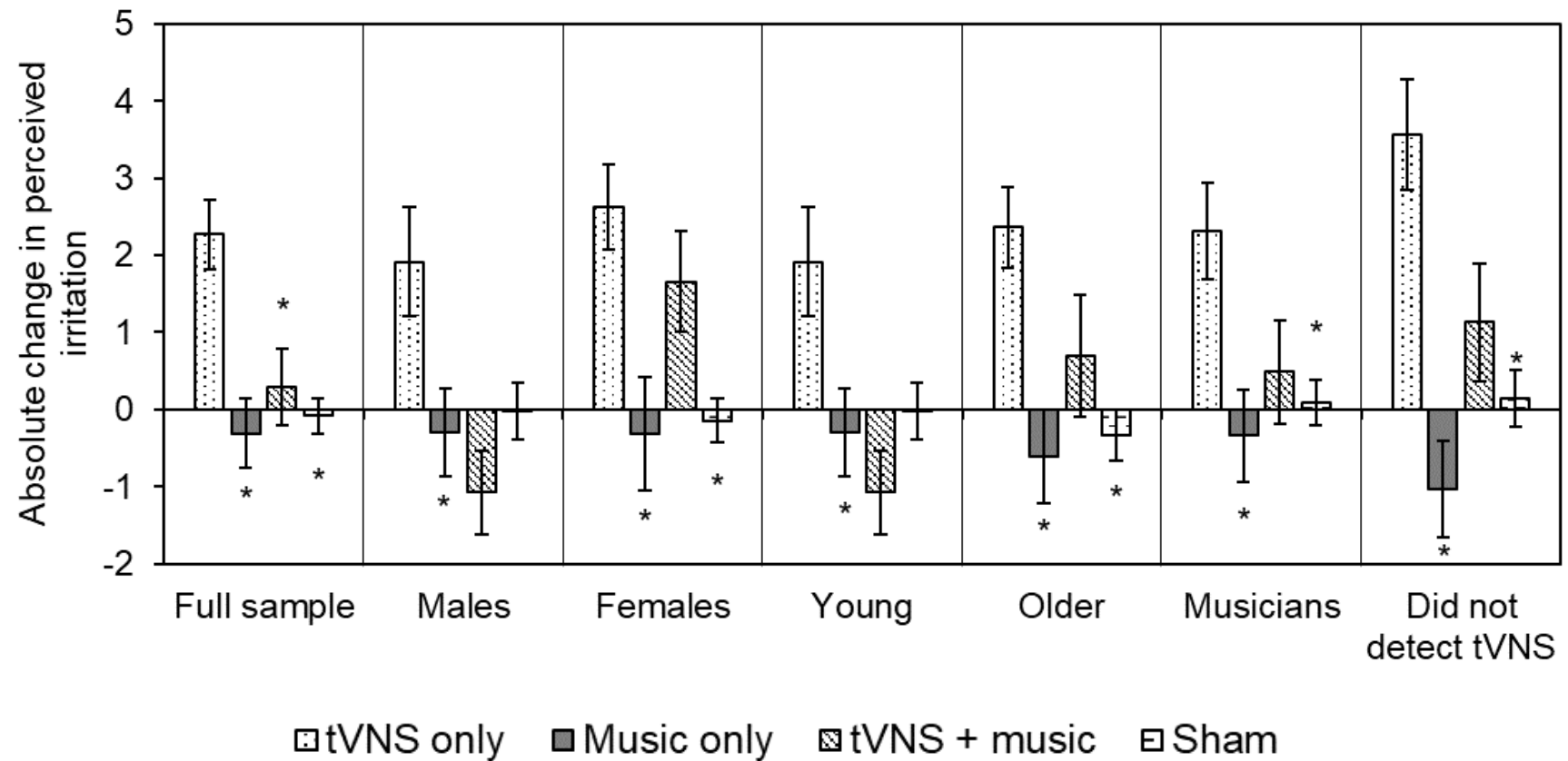
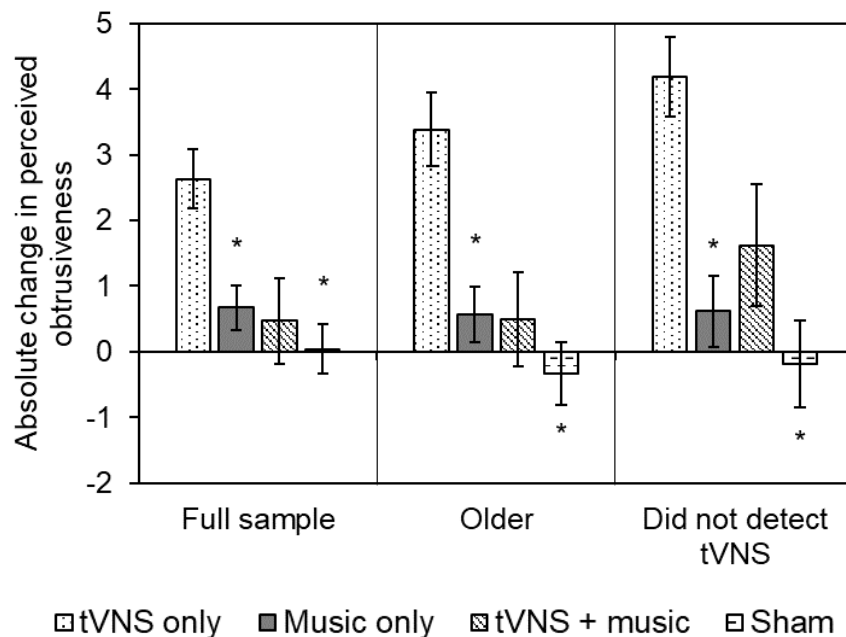


Figure 4.146: Change in perceived irritation significantly differed between the visits in the full sample, males, females, young participants, older participants, musicians and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only.

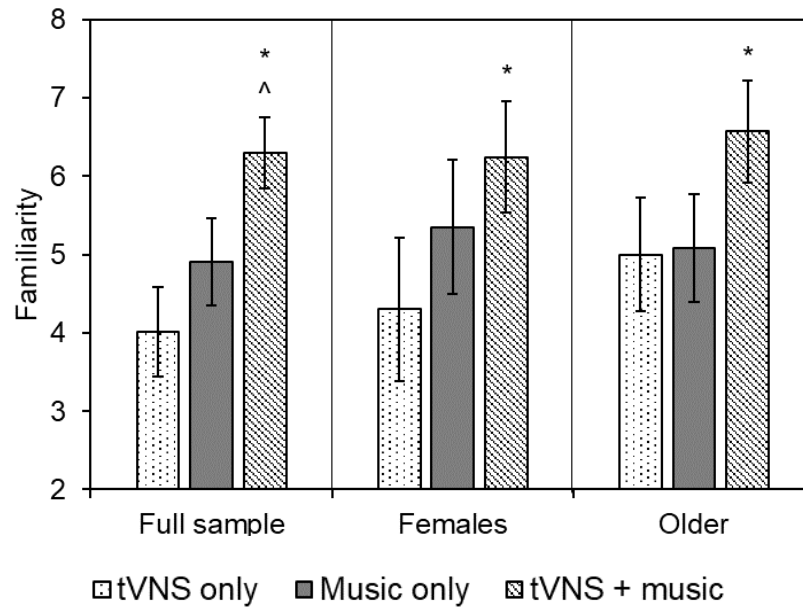
Change in perceived obtrusiveness significantly differed between the four visits when analysing the full sample ( $p = 0.012$ ), older participants ( $p = 0.001$ ) and those who did not detect tVNS ( $p = 0.002$ ). For the full sample, significantly greater increases in perceived obtrusiveness occurred for the tVNS only visit than for the music only ( $p = 0.003$ ) and sham visits ( $p < 0.001$ ). For the group of older participants, the increase in obtrusiveness in the tVNS only visit was significantly greater than that in the music only visit ( $p = 0.003$ ). Also, perceived obtrusiveness in tVNS increased but decreased in sham ( $p = 0.002$ ) in this older cohort. A similar pattern emerged for those who did not detect tVNS: obtrusiveness decreased in sham but increased in tVNS ( $p = 0.005$ ) with the increases that occurred in the tVNS only visit being significantly greater than the increase that emerged in the music only visit ( $p = 0.006$ ). Figure 4.147 illustrates these findings.



*Figure 4.147: Change in perceived obtrusiveness significantly differed between the visits in the full sample, older participants and those who did not detect tVNS. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only.*

Perceived familiarity of the stimuli significantly differed between the visits in the full sample ( $p = 0.035$ ), females ( $p = 0.021$ ) and older participants ( $p = 0.020$ ). In the full sample, familiarity was significantly higher for the tVNS + music stimulus than for tVNS ( $p < 0.001$ ) and music ( $p = 0.014$ ). Also, in

females and older participants, familiarity was significantly higher in tVNS + music than tVNS only ( $p = 0.023$  and  $p = 0.032$  respectively). Figure 4.148 summarises these patterns.



*Figure 4.148: Perceived familiarity of the stimuli significantly differed between tVNS only, music only and tVNS + music. Data presented as mean  $\pm$  1 SEM. \* = significantly different to tVNS only; ^ = significantly different to music only.*

Suitability of the auditory stimulus to the study room significantly differed between tVNS only, music only and tVNS + music for the non-musicians only ( $p = 0.008$ ). As shown in Figure 4.149, suitability was significantly higher for tVNS + music than tVNS only ( $p = 0.034$ ).

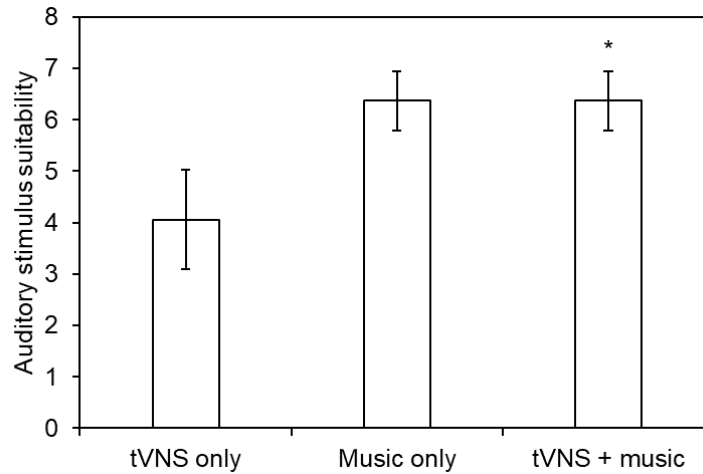


Figure 4.149: Suitability of the stimuli to the study room significantly differed between tVNS only, music only and tVNS + music. Data presented as mean  $\pm$  1 SEM. \* = significantly differed to tVNS only.

Perceived intensity of the tVNS significantly differed between the four stimuli for the full sample ( $p = 0.005$ ), females ( $p = 0.010$ ) and those who detected tVNS ( $p = 0.002$ ). For all three group comparisons, perceived intensity of the tVNS was significantly higher during tVNS + music compared to music (full sample:  $p = 0.001$ ; females:  $p = 0.007$ ; detected tVNS:  $p = 0.001$ ). Figure 4.150 summarises these findings.

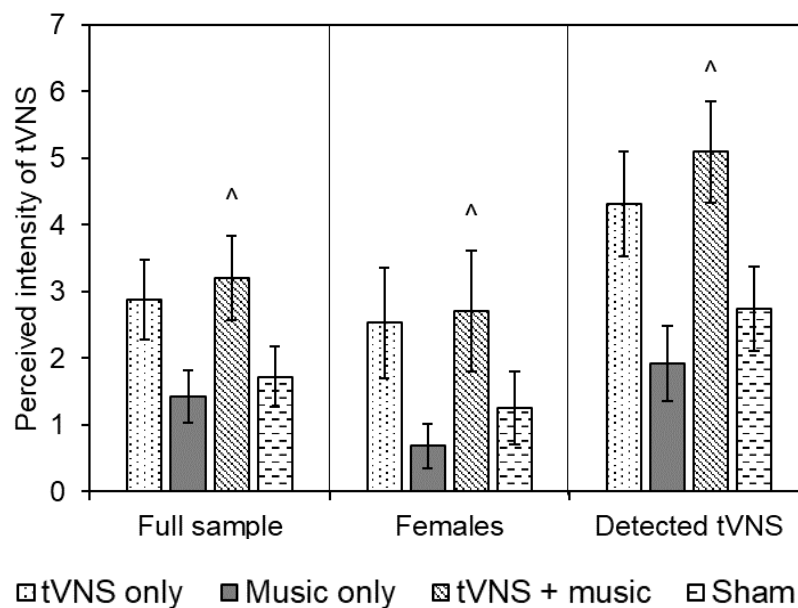


Figure 4.150: Perceived intensity of tVNS significantly varied between the four visits. Data presented as mean  $\pm$  1 SEM. ^ = significantly different to music only.

### 4.3.3 Predicting physiological response to the four stimuli

Work by Clancy et al. (2014) showed that baseline LF/HF predicted response to tVNS. Although the Net-1000 tVNS stimulation parameters were different to those used by Clancy et al. (2014), it was anticipated that the same prediction would emerge here. In support of this expectation, baseline LF/HF was found to significantly predict percentage change in LF/HF (between baseline and tVNS LF/HF:  $R^2 = 0.219$ ,  $p = 0.021$ ). Indeed, Figure 4.151 illustrates that higher baseline LF/HF was associated with greater decreases in LF/HF in the tVNS only visit.

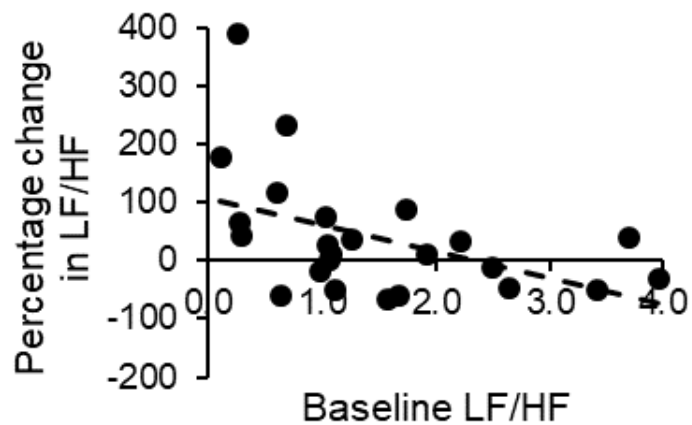


Figure 4.151: Baseline LF/HF significantly predicted change in LF/HF between baseline and tVNS ( $R^2 = 0.219$ ,  $p = 0.021$ ).

However, baseline LF/HF did not significantly predict change in LF/HF for the remaining stimuli. This prompted exploration of alternative measures which may have predicted response to all four stimuli. Table 4.5 summarises the measures and regression outcomes that were considered.

Table 4.5: Summary of statistically significant linear regressions for the full sample in all four visits.

	tVNS only		Music only		tVNS + music		Sham	
	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value	R <sup>2</sup>	p-value
LF power								
HF power								
LF%	0.455	< 0.001	0.256	0.012	0.201	0.028	0.436	< 0.001
HF%								
nuLF	0.455	< 0.001						
nuHF	0.190	0.033					0.236	0.016
LF/HF	0.219	0.021						
SDRR								
SDSD								
RMSSD								
SD1								
SD1n								
SD2			0.247	0.014			0.199	0.029
SD2n	0.197	0.030	0.217	0.022				
SD1/SD2								
SD2/SD1								
Mean RR								
Median RR								
Range RR	0.239	0.015						
IQR RR							0.325	0.004

Table 4.5 demonstrates that the only measure that significantly predicted percentage change for all four stimuli was LF%. Indeed, the scatter plots (see Figure 4.152a-d) demonstrate that higher baseline LF% was associated with greater decreases in LF% in all four visits.



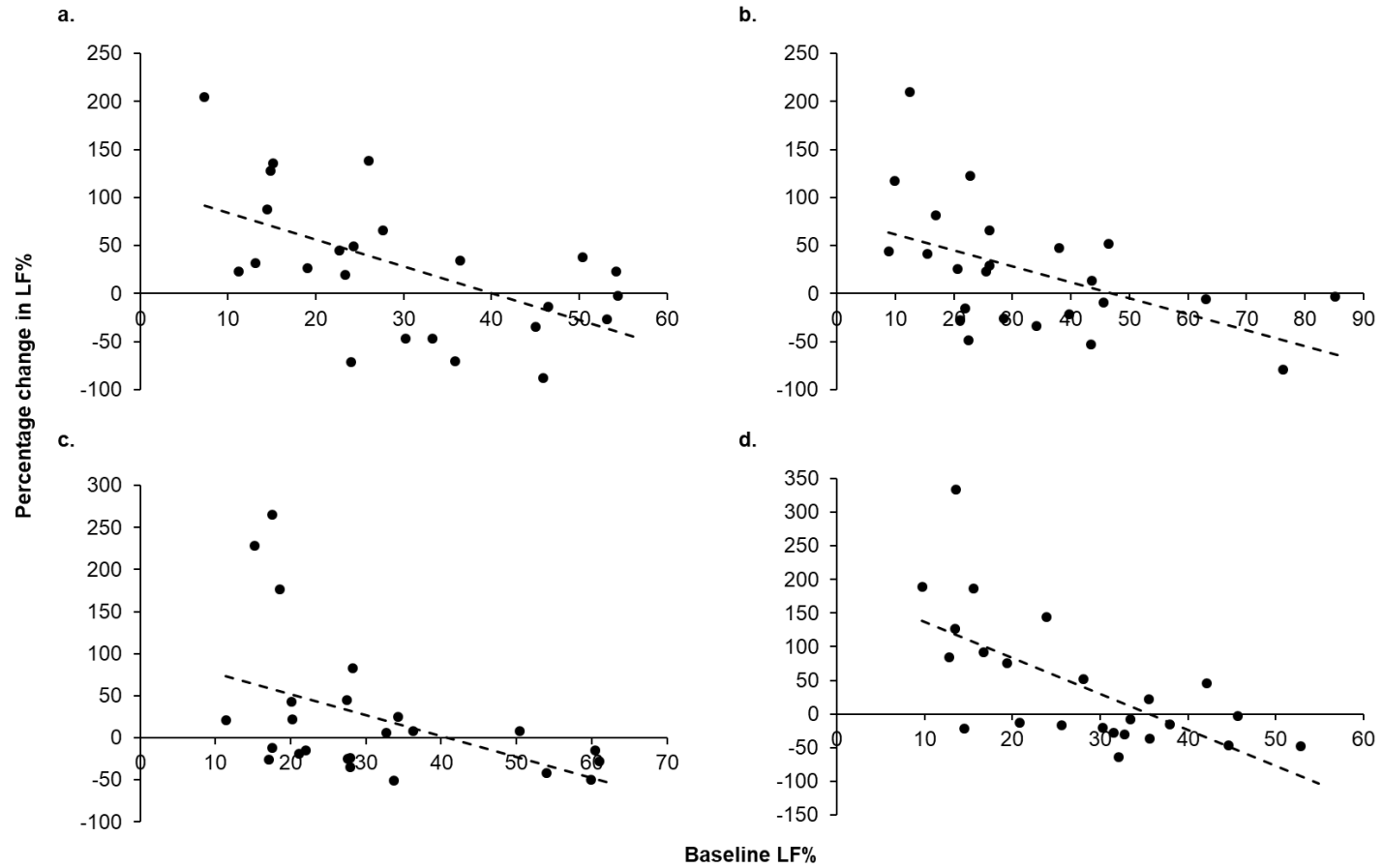


Figure 4.152: Baseline LF% significantly predicted change in LF% between *t*VNS only (a), music only (b), *t*VNS + music (c) and sham (d). All  $p < 0.05$ .

#### 4.3.3.1 Defining responder types

Due to these significant relationships, participants were subsequently categorised based on percentage change in LF% for each visit (this permitted changes in responder definition between the visits). Two approaches were tested:

1. Two-level response definition:
  - a. Non-responder: < 20% change in LF%
  - b. Responder:  $\geq$  20% change in LF%
2. Three-level response definition:
  - a. Non-responder: < 20% change in LF%
  - b. Type 1 responder:  $\geq$  20% decrease in LF%
  - c. Type 2 responder:  $\geq$  20% increase in LF%

This involved examining the extent to which baseline LF% for each visit differed between the responder types.

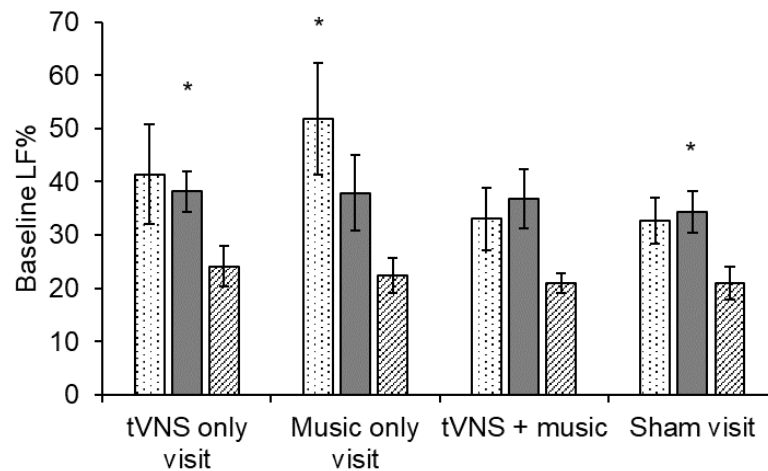
The first approach revealed no significant differences in baseline LF% between non-responders and responders for any visit ( $p > 0.05$ ). In contrast, significant differences in LF% emerged for the three-level definition: tVNS only visit ( $p = 0.047$ ), music only visit ( $p = 0.009$ ) and sham visit ( $p = 0.026$ ). The Kruskal-Wallis for the tVNS + music visit failed to reach significance ( $p > 0.05$ ).

Exploration of these effects revealed that in the tVNS only and sham visits, type 1 responders ( $n = 7$  and  $8$  respectively) had significantly higher baseline LF% than type 2 responders ( $n = 14$  and  $11$ ) ( $p = 0.030$  and  $p = 0.039$  respectively). But in the music only visit, non-responders ( $n = 5$ ) had significantly higher baseline LF% than type 2 responders ( $n = 12$ ,  $p = 0.009$ ). Although the difference between type 1 ( $n = 9$ ) and type 2 responders ( $n = 7$ ) did not reach significance ( $p > 0.05$ ), Figure 4.153a shows similar patterns emerged.

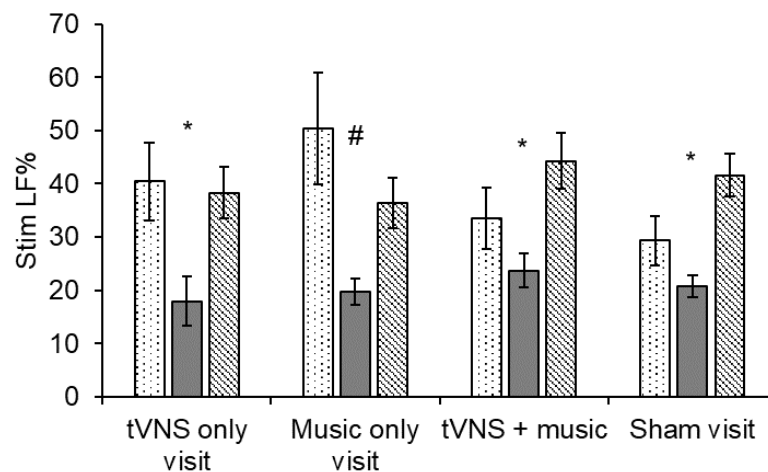
The same analyses were performed on stimulation LF%. For all visits, significant differences between the three responder types were identified (tVNS only:  $p = 0.030$ ; music only:  $p = 0.012$ ; tVNS + music:  $p = 0.022$ ; sham:  $p = 0.003$ ). As shown in Figure 4.153b, LF% during tVNS was

significantly higher in type 2 responders than type 1 responders ( $p = 0.037$ ). An identical pattern emerged for the tVNS + music ( $p = 0.019$ ) and sham visits ( $p < 0.001$ ). However, in the music visit this difference did not transpire ( $p > 0.05$ ), rather non-responders had significantly higher LF% during music than type 1 responders ( $p = 0.011$ ). Nevertheless, Figure 4.153b shows there was heightened LF% in type 2 responders compared to type 1 responders.

**a.**



**b.**



□ Non-responder   ■ Type 1 responder   ▨ Type 2 responder

*Figure 4.153: Baseline LF% (a) and stimulus LF% (b) significantly differed between non-responders, type 1 responders and type 2 responders. Data presented as mean  $\pm$  1 SEM. \* = significantly different to type 2 responders; # = significantly different to non-responders.*

#### **4.3.3.2 Differences in demographic characteristics and baseline measures between the three responder types**

As four stimuli were employed (tVNS only, music only, tVNS + music and sham) differences in demographic characteristics and baseline measures between the three responder groups were explored for each stimulus. This involved performing similar statistical tests as those used in the follow-up study (Chapter 3), including:

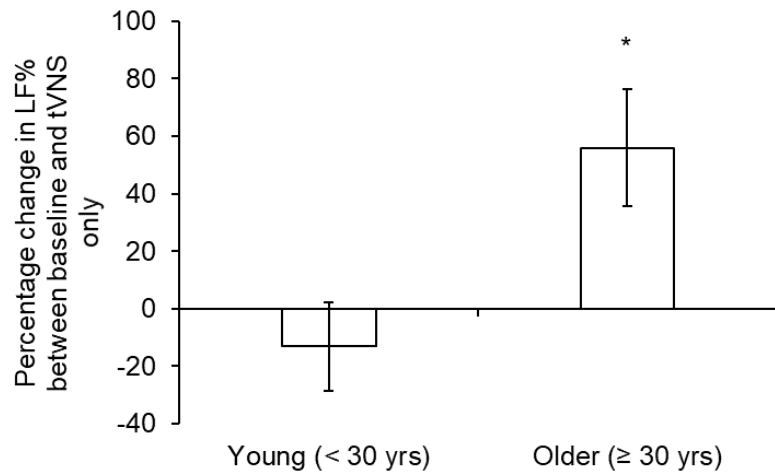
- a) Chi-square tests of independence; to examine whether responder type was associated with demographic group (e.g. gender, age group and formal music training).
- b) One-way ANOVAs (or Kruskal-Wallis tests for non-normally distributed data); to assess whether differences in demographic characteristics (e.g. age, exercise and hours spent undertaking music-related activities) and/or baseline measures differed between the three responder types (between-subject variable: non-responders, type 1 responders, type 2 responders).
- c) Independent sample t-tests (or Mann-Whitney U tests for non-normally distributed data) on the percentage change in LF% values; to explore whether any differences emerged between different demographic groups (e.g. males vs. females; active vs. insufficiently active; musician vs. non-musician).
- d) Linear regression; to examine whether percentage change values could be predicted by demographic variables (e.g. age, exercise and hours spent undertaking music-related activities).

##### **4.3.3.2.1 Differences between the three responder types for tVNS only**

For the tVNS only visit, no statistically significant associations (ascertained from chi-square tests of independence) or differences in demographic characteristics or baseline autonomic function emerged between the three responder types ( $p > 0.05$ ).

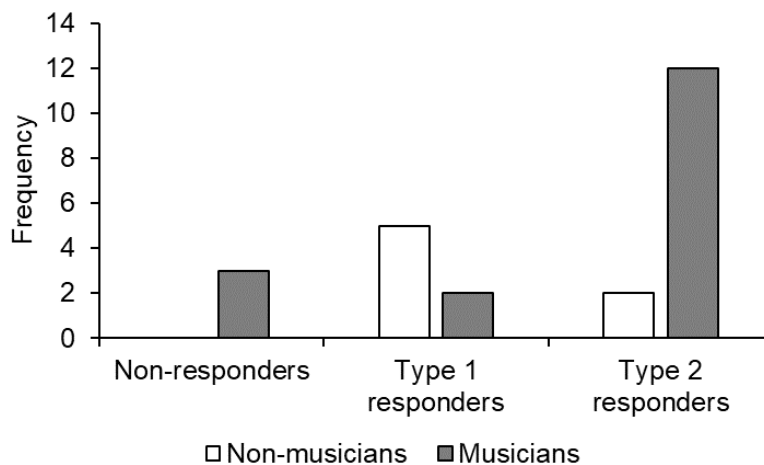
However, percentage change in LF% (between baseline and tVNS only) significantly differed between young ( $< 30$  yrs) and older ( $\geq 30$  yrs) participants ( $p = 0.019$ ). As shown in Figure 4.154, young participants

exhibited decreases in LF% between the two conditions whereas older participants exhibited increases.



*Figure 4.154: Percentage change in LF% significantly differed between young (< 30 yrs) and older (≥ 30 yrs) participants in the tVNS only visit. Data presented as mean ± 1 SEM. \* = significantly different to young participants.*

A chi-square test of independence revealed that music background (musicians vs. non-musicians) was significantly associated with responder type ( $p = 0.012$ ). Inspection of Figure 4.155 demonstrates that more non-musicians were type 1 responders, whilst more musicians tended to be type 2 responders.

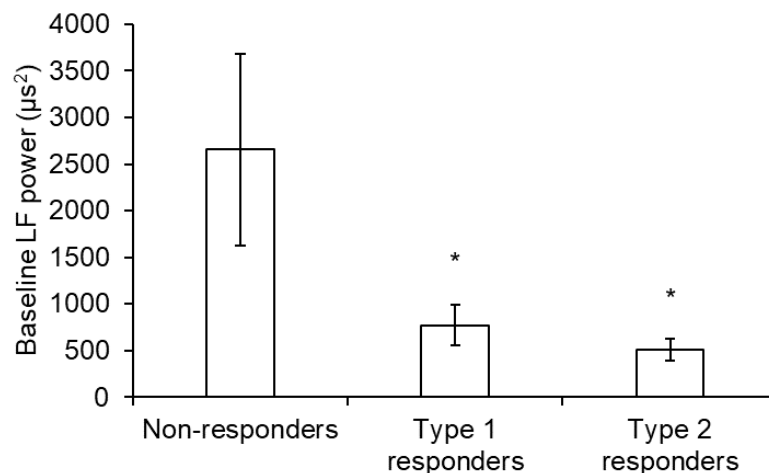


*Figure 4.155: Musicians responded in a way characteristic of type 2 responders, whilst response patterns of non-musicians resembled those of type 1 responders in the tVNS only visit. Data presented as frequency.*

#### 4.3.3.2.2 Differences between the three responder types for music only

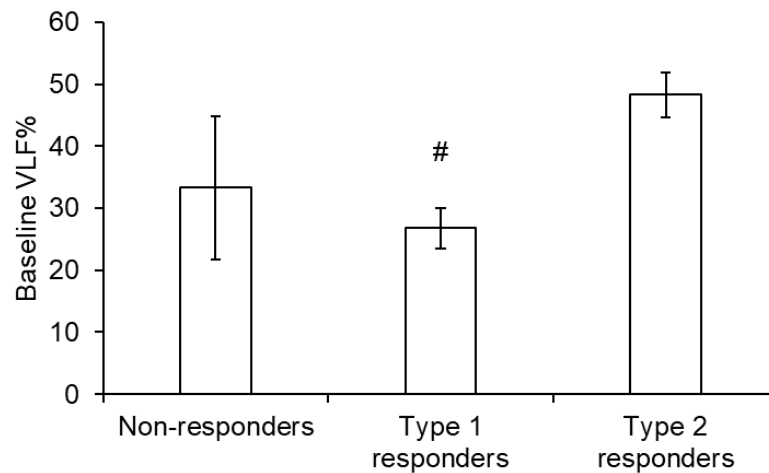
For the music only visit, no statistically significant associations (ascertained from chi-square tests of independence) or differences in demographic characteristics emerged between the three responder types ( $p > 0.05$ ). In addition, percentage change in LF% (between baseline and music only) did not significantly differ between demographic groups ( $p > 0.05$ ).

However, differences in baseline physiological measures (other than LF%) between the three responder types transpired. Indeed, a one-way ANOVA demonstrated that LF power was significantly impacted by responder type ( $p = 0.004$ ). Bonferroni pairwise comparisons revealed that non-responders had significantly higher baseline LF power compared to type 1 responders ( $p = 0.023$ ) and type 2 responders ( $p = 0.004$ , see Figure 4.156).



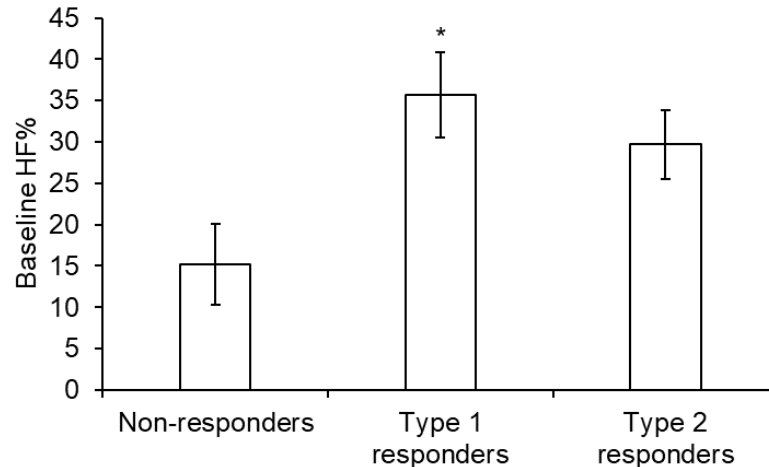
*Figure 4.156: Baseline LF power significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.*

VLF% also significantly differed between the responder types ( $p = 0.019$ ). Inspection of Figure 4.157 demonstrates that type 2 responders had significantly higher VLF% at baseline than type 1 responders ( $p = 0.022$ ).



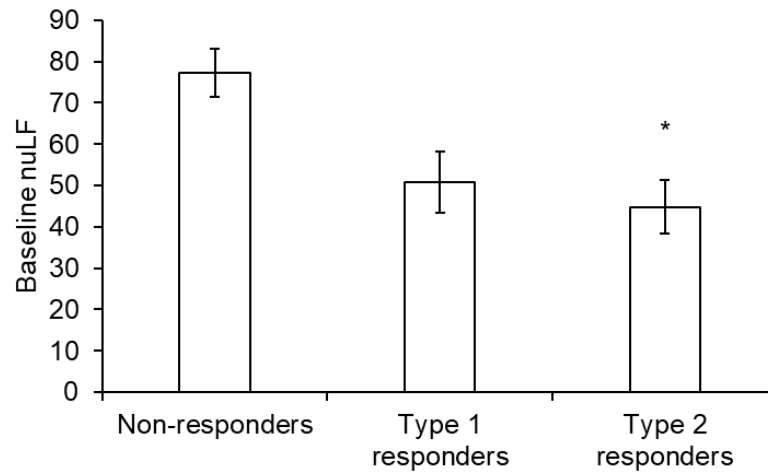
*Figure 4.157: Baseline VLF% significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. # = significantly different to type 2 responders.*

Baseline HF% also significantly differed between the three responder types ( $p = 0.046$ ). As illustrated in Figure 4.158, baseline HF% was significantly higher in type 1 responders compared to non-responders ( $p = 0.019$ ).



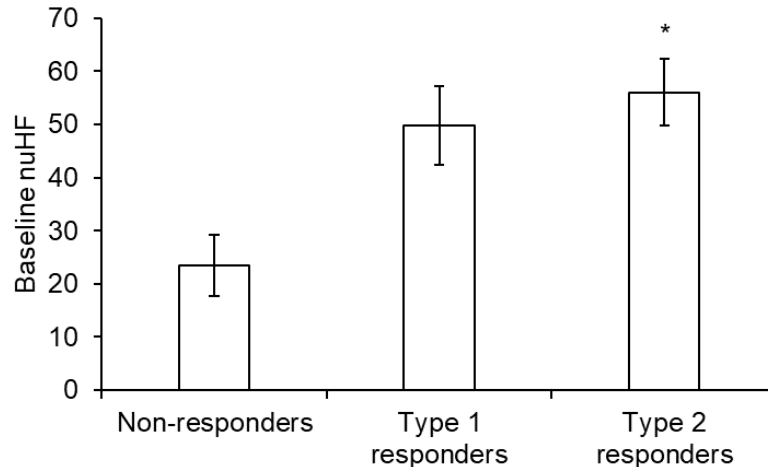
*Figure 4.158: Baseline HF% significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.*

nuLF power was significantly impacted by responder type ( $p = 0.021$ ). Bonferroni pairwise comparisons demonstrated that non-responders had significantly higher nuLF at baseline compared to type 2 responders ( $p = 0.019$ , see Figure 4.159).



*Figure 4.159: Baseline nuLF significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.*

In addition, nuHF significantly differed between the three responder types ( $p = 0.018$ ). As depicted in Figure 4.160, baseline nuHF was significantly higher in type 2 responders than in non-responders ( $p = 0.016$ ).

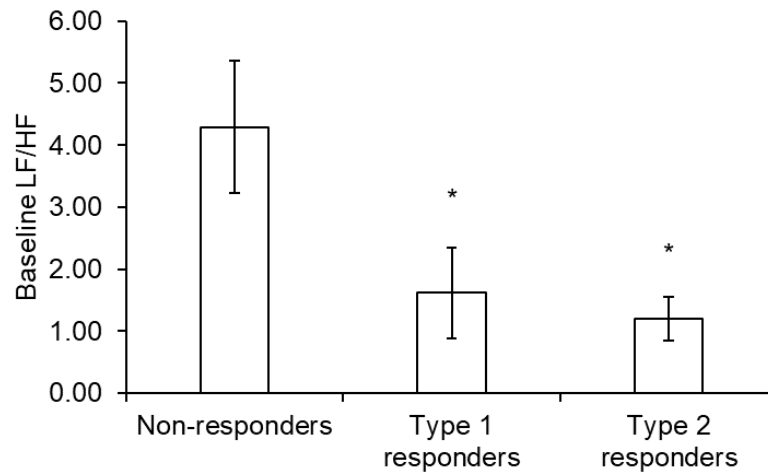


*Figure 4.160: Baseline nuHF significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.*

As both LF and HF power in absolute and normalised values significantly differed between the responder types, it comes as little surprise to find that LF/HF was also significantly impacted by responder type ( $p = 0.028$ ). Figure



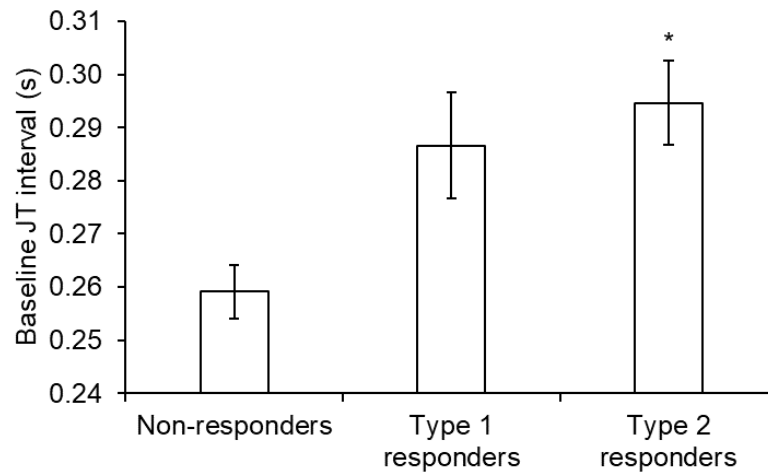
4.161 shows that baseline LF/HF was significantly higher in non-responders compared to type 1 ( $p = 0.042$ ) and type 2 responders ( $p = 0.011$ ).



*Figure 4.161: Baseline LF/HF significantly differed between the three responder types in the music only visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.*

One-way ANOVAs revealed that baseline JT interval and ST height significantly different between the responder types ( $p = 0.044$  and  $p = 0.023$  respectively). As shown in Figure 4.162, baseline JT interval was significantly higher in type 2 responders compared to non-responders ( $p = 0.042$ ), and baseline ST height was significantly lower in type 1 responders compared to non-responders ( $p = 0.022$ ).

a.



b.

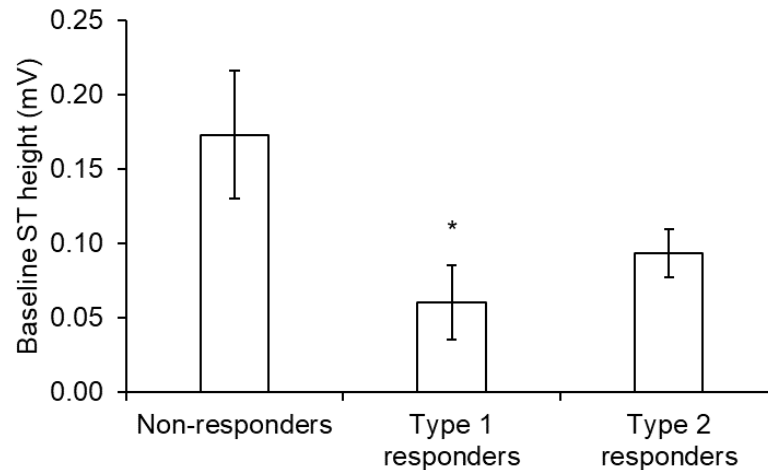


Figure 4.162: Baseline JT interval (a) and ST height (b) significantly differed between the three response types. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders.

#### 4.3.3.2.3 Differences between the three responder types for tVNS + music

For the tVNS + music visit, no statistically significant associations (ascertained from chi-square tests of independence) or differences in demographic characteristics emerged between the three responder types ( $p > 0.05$ ). Similarly, percentage change in LF% (between baseline and tVNS + music) did not significantly differ between or was significantly predicted by demographic characteristics.

But, a one-way ANOVA revealed that baseline VLF% significantly differed between responder types ( $p = 0.029$ ). As shown in Figure 4.163, baseline VLF% was significantly higher in type 2 responders compared to type 1 responders ( $p = 0.035$ ). This replicates the finding that was observed in the music only visit.

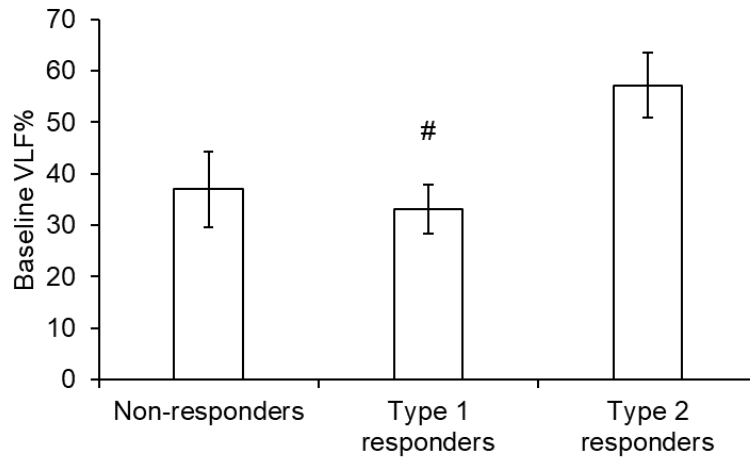


Figure 4.163: Baseline VLF% significantly differed between the three responder types in the tVNS + music visit. Data presented as mean  $\pm$  1 SEM. # = significantly different to type 2 responders.

S amplitude was also significantly impacted by responder type ( $p < 0.001$ ). Indeed, Bonferroni pairwise comparisons revealed that S amplitude in type 1 responders was significantly smaller than that in non-responders ( $p = 0.004$ ) and type 2 responders ( $p < 0.001$ , see Figure 4.164).

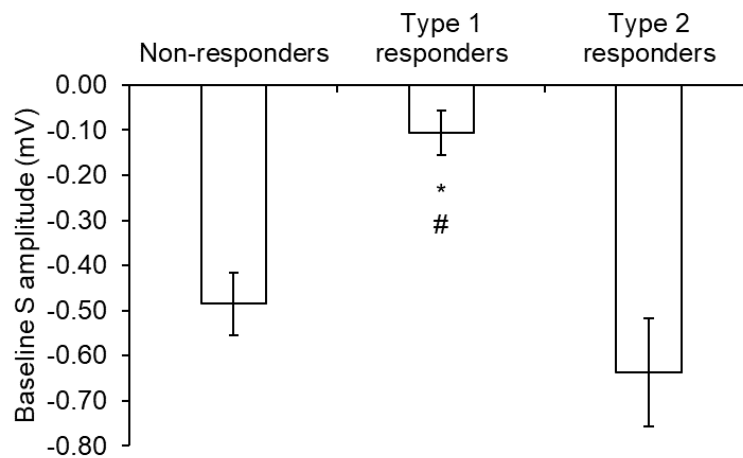
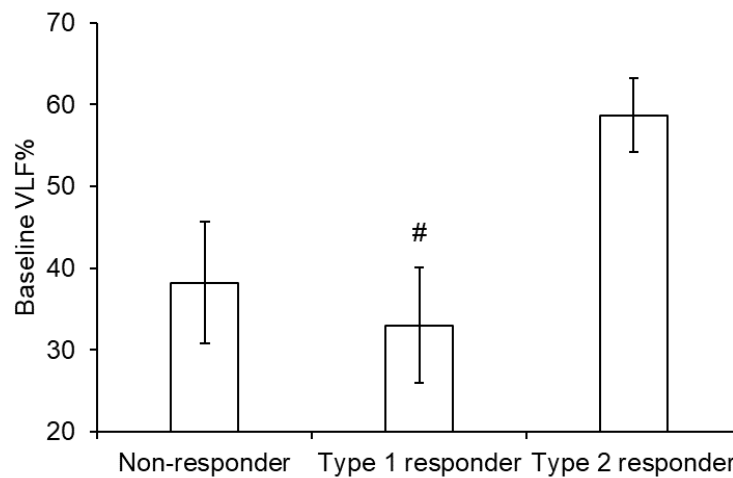


Figure 4.164: Baseline S amplitude significantly differed between the three responder types in the tVNS + music visit. Data presented as mean  $\pm$  1 SEM. \* = significantly different to non-responders; # = significantly different to type 2 responders.

#### 4.3.3.2.4 Differences between the three responder types for sham

For the sham visit, no statistically significant associations (ascertained from chi-square tests of independence) or differences in demographic characteristics emerged between the three responder types ( $p > 0.05$ ). However, baseline VLF% significantly differed between the three responder types ( $p = 0.036$ ). As depicted in Figure 4.165 baseline VLF% was significantly lower in type 1 responders compared to type 2 responders ( $p = 0.017$ ). No other differences reached statistical significance ( $p > 0.05$ ).



*Figure 4.165: Baseline VLF% significantly differed between the three responder types in the sham visit. Data presented as mean  $\pm$  1 SEM. # = significantly different to type 2 responders.*

Percentage change in LF% (between baseline and sham) did not significantly differ between demographic groups ( $p > 0.05$ ). However, a linear regression revealed that percentage change in LF% was significantly predicted by age ( $R^2 = 0.480$ ,  $p = 0.018$ ). Inspection of Figure 4.166 demonstrates that as age increases, so does percentage change in LF%.

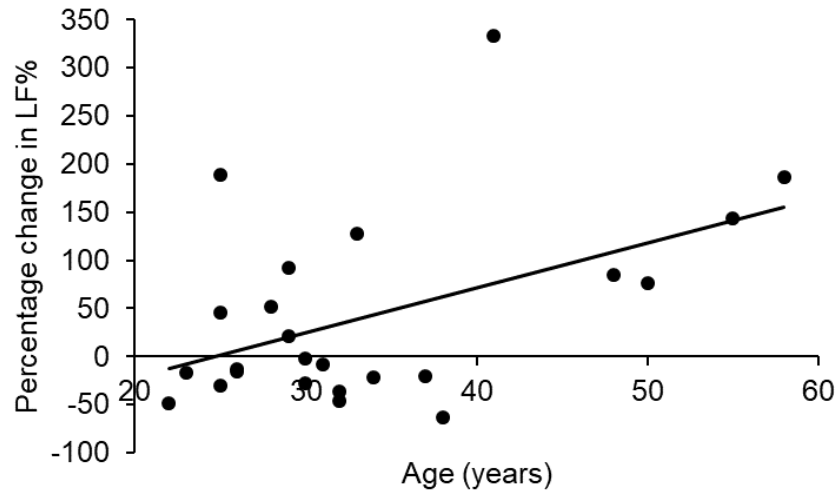
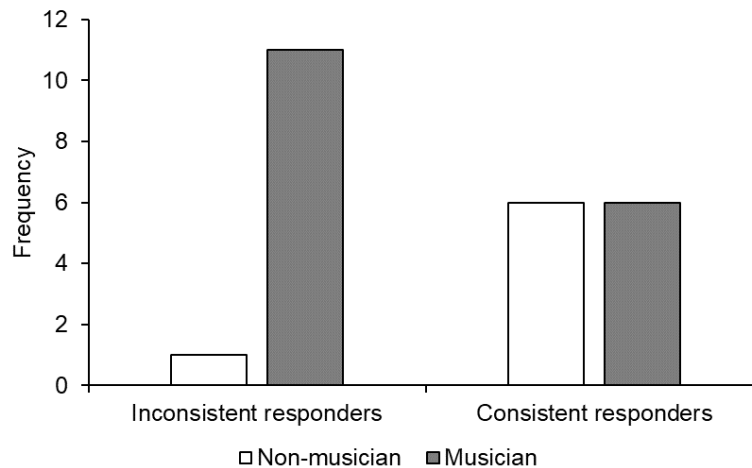


Figure 4.166: Percentage change in LF% between baseline and sham was significantly predicted by age ( $R^2 = 0.480$ ,  $p = 0.018$ ).

#### 4.3.3.3 Response consistency between the four auditory stimuli

Consistency in response type between the visits was investigated. Only one participant showed consistent responses (type 1 responder) in all four visits. As a  $\geq 20\%$  change in LF% (regardless of direction) could be interpreted as a response, consistency was re-evaluated. 50% of the sample always responded (either type 1 or type 2 responder) and the remaining 50% were inconsistent responders (non-responders, type 1 or type 2 responders in any given visit). Appendix C.10. summarises the characteristics of these two groups. Analysis of the characteristics between these two groups revealed that consistent responders ( $n = 12$ , mean = 37.92, SEM = 3.46) were significantly older than inconsistent responders (mean = 29.33, SEM = 1.49,  $p = 0.038$ ). In addition, a chi-square test revealed that response type was significantly associated with formal music training ( $p = 0.025$ ). As shown in Figure 4.167 a greater number of inconsistent responders were musicians.



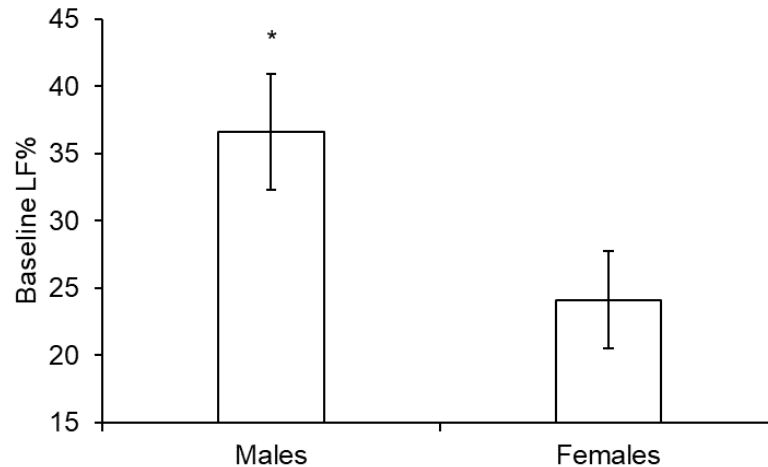
*Figure 4.167: Response consistency was significantly associated with musical training. Musicians showed responses characteristic of inconsistent responders. Similar numbers of musicians and non-musicians showed responses characteristic of consistent responders. Data presented as frequency.*

#### **4.3.3.4 Predicting baseline LF%**

Baseline LF% significantly predicted change between baseline and the four stimuli. In addition, the follow-up revealed that baseline LF% can be predicted by demographic information. Therefore, predictors of baseline LF% were explored here. Like the follow-up, this involved performing linear regressions with baseline LF% as the dependent variable and demographic information as the independent variable. Independent sample t tests (or Mann-Whitney U tests for non-normally distributed data) were also conducted to investigate whether differences in baseline LF% existed between demographic groups. Since participants attended on four occasions these analyses were performed on the four baseline readings.

##### **4.3.3.4.1 tVNS only baseline LF%**

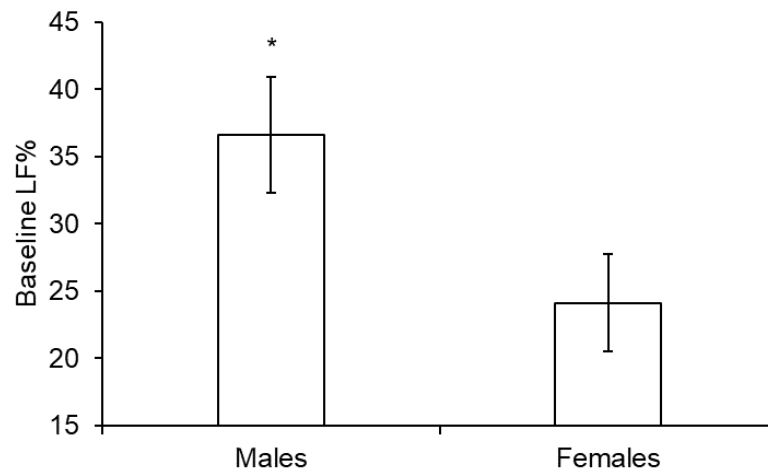
Baseline LF% was not significantly predicted by any demographic variable ( $p > 0.05$ ). But, like the follow-up study, males had significantly higher baseline LF% compared to females ( $p = 0.037$ , see Figure 4.168). No other differences reached statistical significance ( $p > 0.05$ ).



*Figure 4.168: Baseline LF% in the tVNS only visit significantly differed between males and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to females.*

#### **4.3.3.4.2 Music only baseline LF%**

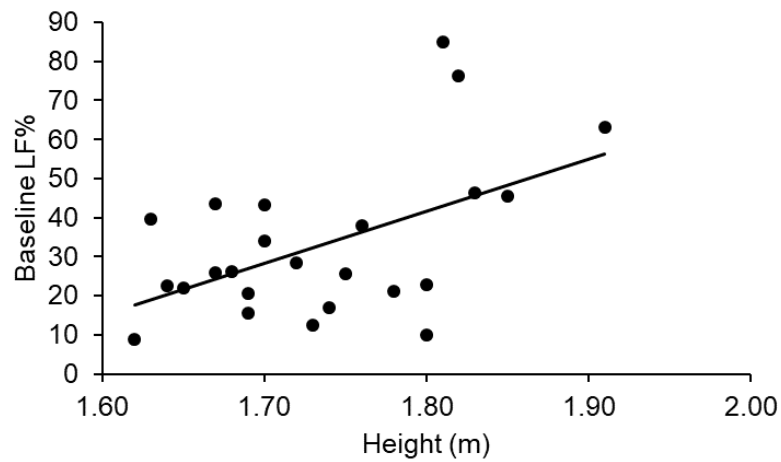
The difference between males and females in the tVNS only visit was replicated in the music only visit: baseline LF% was significantly higher in males compared to females ( $p = 0.037$ , see Figure 4.169).



*Figure 4.169: Baseline LF% in the music only visit significantly differed between males and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to females.*

Similar to the follow-up study, linear regressions revealed that height was a significant predictor of baseline LF% ( $R^2 = 0.516$ ,  $p = 0.010$ ). In addition, inspection of Figure 4.170 demonstrates that baseline LF% was positively

correlated with height: the taller the participants, the greater the baseline LF%.

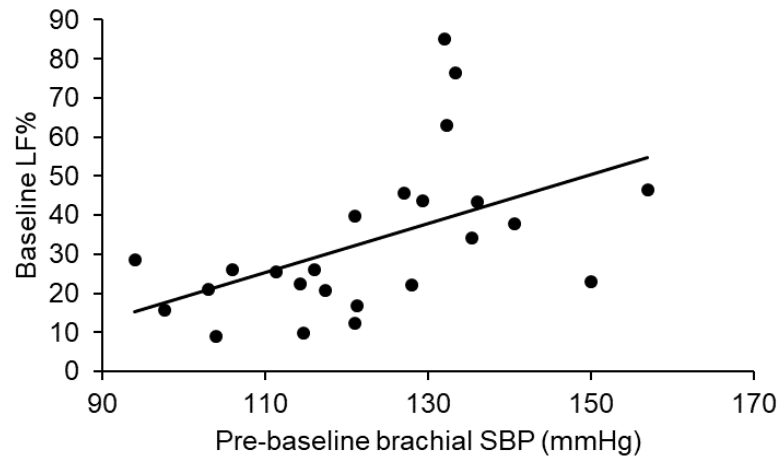


*Figure 4.170: Baseline LF% in the music only visit was significantly predicted by height ( $R^2 = 0.516$ ,  $p = 0.010$ ).*

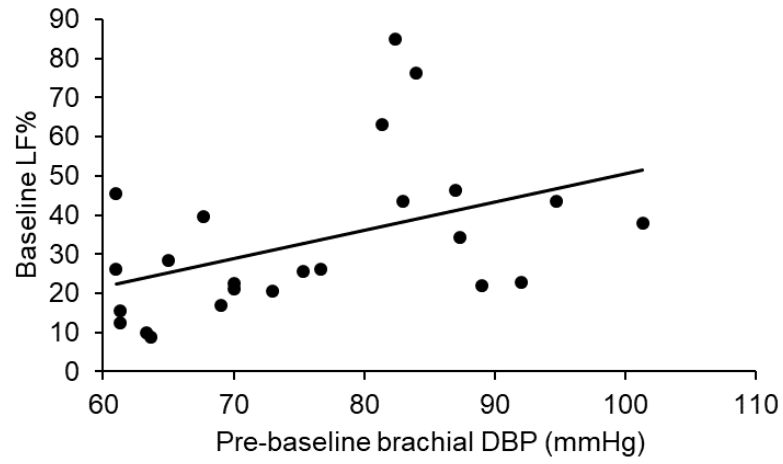
Pre-baseline brachial SBP, DBP and MAP were also found to significantly predict baseline LF% ( $R^2 = 0.500$ ,  $p = 0.013$ ;  $R^2 = 0.441$ ,  $p = 0.031$ ;  $R^2 = 0.494$ ,  $p = 0.014$ ). As shown in Figure 4.171a-c, higher pre-baseline brachial SBP, DBP and MAP were related to higher baseline LF%.



a.



b.



c.

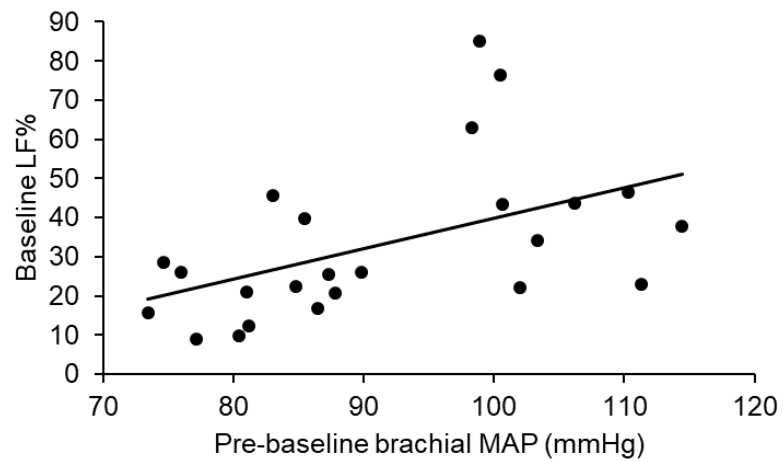


Figure 4.171: Pre-baseline brachial SBP (a), DBP (b) and MAP (c) significantly predicted baseline LF% in the music only visit;  $p < 0.05$ .

#### 4.3.3.4.3 tVNS + music baseline LF%

Similar to the follow-up and the tVNS only and music only visits, baseline LF% for the tVNS + music visit was impacted by gender ( $p = 0.009$ ). As shown in Figure 4.172, males had significantly higher baseline LF% compared to females.

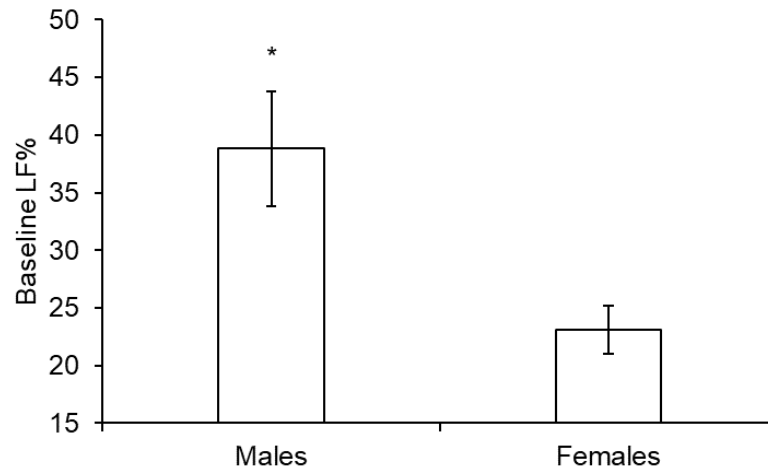


Figure 4.172: Baseline LF% in the tVNS + music visit significantly differed between males and females. Data presented as mean  $\pm$  1 SEM. \* = significantly different to females.

Baseline LF% was also found to be significantly predicted by height ( $R^2 = 0.513$ ,  $p = 0.010$ ), where increases in height were associated with increases in baseline LF% (see Figure 4.173).

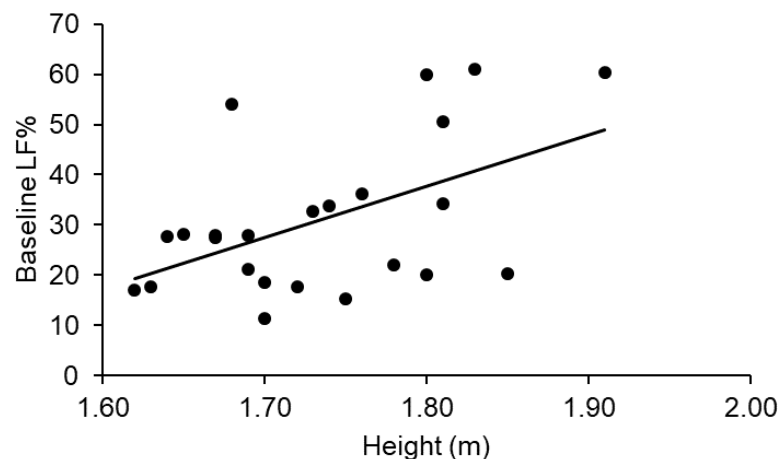
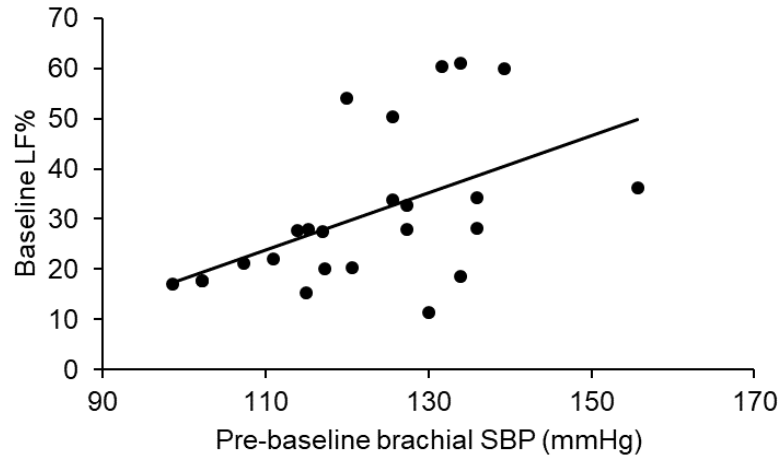


Figure 4.173: Baseline LF% in the tVNS + music visit was significantly predicted by height ( $R^2 = 0.513$ ,  $p = 0.010$ ).

Similar to the music only visit, pre-baseline brachial SBP ( $R^2 = 0.502$ ,  $p = 0.012$ ) and pre-baseline brachial MAP ( $R^2 = 0.451$ ,  $p = 0.027$ ) also significantly predicted baseline LF%. As shown in Figure 4.174, baseline LF% increased with increases in pre-baseline brachial SBP and MAP.

a.



b.

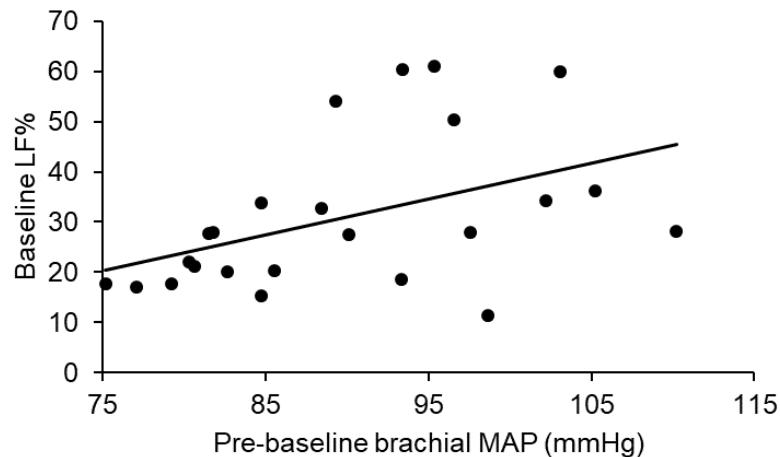


Figure 4.174: Pre-baseline brachial SBP (a) and MAP (b) significantly predicted baseline LF% in the tVNS + music visit;  $p < 0.05$ .

The final variable which significantly predicted baseline LF% was hours spent listening to music/week ( $R^2 = 0.408$ ,  $p = 0.048$ ). As illustrated in Figure 4.175, baseline LF% decreased with increases in time spent listening to music.

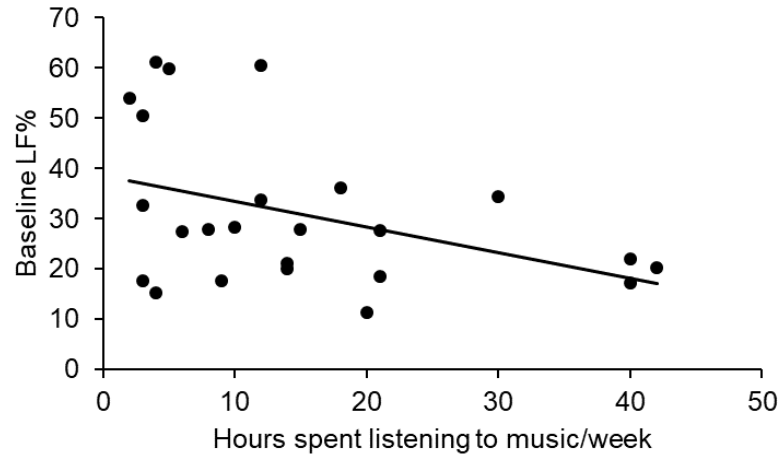


Figure 4.175: Hours spent listening to music per week significantly predicted baseline LF% in the tVNS + music visit ( $R^2 = 0.408$ ,  $p = 0.048$ ).

#### 4.3.3.4.4 Sham baseline LF%

Sham baseline LF% was not found to significantly differ between any demographic groups ( $p > 0.05$ ). Nevertheless, baseline LF% was significantly predicted by age ( $R^2 = 0.446$ ,  $p = 0.029$ ), height ( $R^2 = 0.420$ ,  $p = 0.041$ ) and weight ( $R^2 = 0.427$ ,  $p = 0.038$ ). Contrary to expectations and past research, baseline LF% decreased with increasing age (see Figure 4.176a). But as shown in Figure 4.176b and c, baseline LF% increased with increasing height and weight.

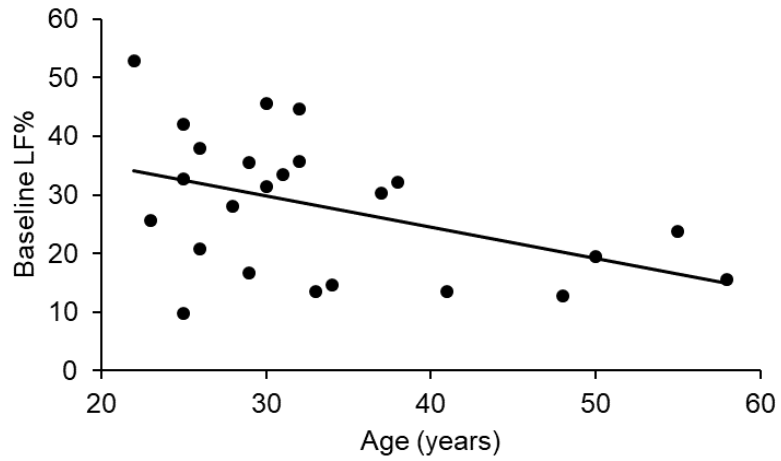
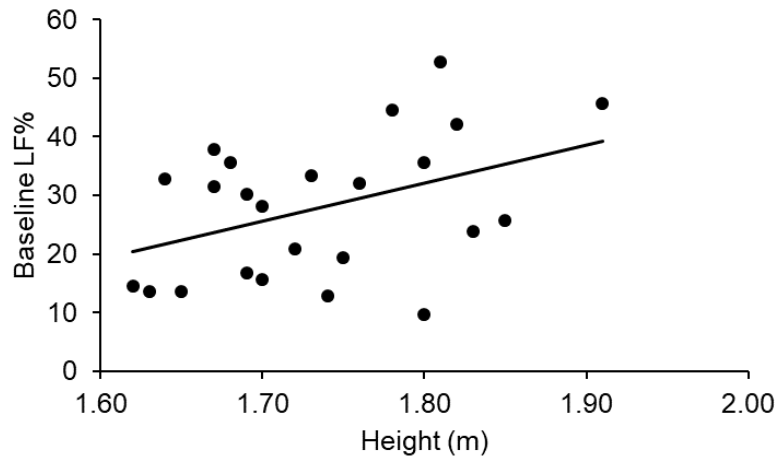
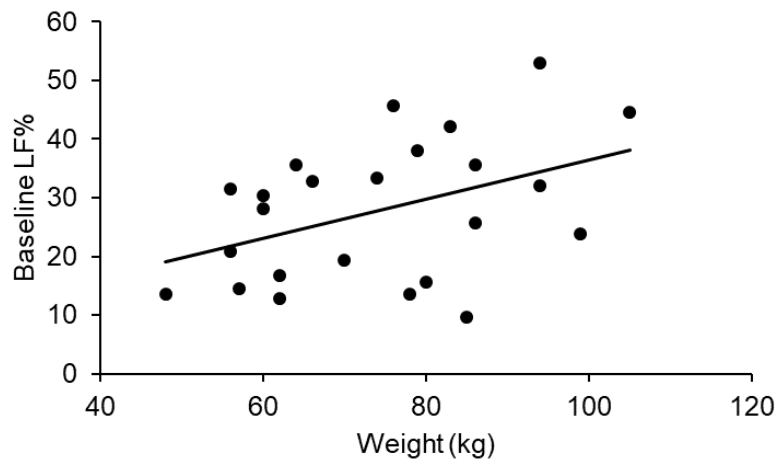
**a.****b.****c.**

Figure 4.176: Sham baseline LF% was predicted by age (a), height (b) and weight (c); all  $p < 0.05$ .

## 4.4 Discussion

### 4.4.1 Main findings

This study examined the effects of relaxing music in combination with tVNS on cardiac autonomic function and self-reported emotion. Significant changes in both physiological and subjective measures occurred within each visit. These changes differed between: males and females; young and older participants; and musicians and non-musicians. Indeed, it could be argued that females, older participants and musicians showed the greatest physiological and subjective responses. In contrast, no meaningful differences in response patterns emerged between participants who detected tVNS and those who did not. This may have been due to the subjective and post-hoc nature of the measure (participants reported on a scale at the end of each stimulation period how intensely they felt the tVNS).

Despite the changes that emerged within each visit, no significant differences in cardiac autonomic function between the four visits were found at the level of the entire group. This means that the sham was equally as effective as modulating physiology as tVNS, music and their combination. This claim is partly supported by the self-report measures which showed that participants felt most relaxed and calm during the sham condition and music stimulus. In addition, these two stimuli were perceived as being more pleasant and less stimulating, irritating and obtrusive than tVNS. But surprisingly, familiarity and tVNS intensity were greatest for tVNS + music. This may have been due to a cumulative effect.

Nevertheless, in the sub-group analyses, some differences between the visits occurred. For instance, females had lower respiration rate in music and tVNS + music than tVNS; young participants had higher S amplitude in tVNS + music than music and sham; and non-musicians had higher HF% in sham than tVNS only. Consequently, there is some evidence to suggest that the music and sham were most effective in these groups at facilitating physiological relaxation. Therefore, Weightless could be argued to be relaxing in a more complex way than it has been advertised. Interestingly, females, young participants and non-musicians reported similar changes in

experienced emotion despite the changes in physiology. Rather, older participants, musicians and those who did not detect tVNS reported higher relaxation and calm during music and sham than tVNS. Perhaps these discrepancies were due to a dissociation between physiological and subjective changes.

Clancy et al.'s (2014) relationship between baseline LF/HF and change in LF/HF was found for tVNS only. This suggests that despite differences in tVNS stimulation site and parameters, it is possible to predict how individuals respond to this form of neuromodulation. However, this relationship failed to persist for the other stimuli. Instead, baseline LF% emerged as a significant predictor for all four stimuli. This finding has not been reported in the literature and is important because it suggests that it is plausible to identify groups of people who may respond to non-invasive interventions similar to the ones employed here.

The predictive power of baseline LF% also emerged in the pilot and follow-up studies, suggesting that this may be a robust effect. Furthermore, baseline LF% was consistently higher in males compared to females and height was a significant predictor of baseline LF%. As these variables were also implicated in the follow-up, this suggests that basic demographic information may aid with identifying individuals who will respond to the types of stimuli employed here. This argument also applies to baseline VLF%, as it was consistently higher in type 2 compared to type 1 responders (in the music only, tVNS + music and sham visits). In addition, age was also associated with response: older participants ( $\geq 30$  yrs) showed increases in LF% between baseline and stimulus whilst young participants ( $< 30$  yrs) showed decreases. This finding was also observed in the follow-up. Therefore, even without deriving frequency-domain HRV, it appears plausible to ascertain, based on demographic information, how individuals are most likely to respond. Indeed, it could be argued that, young female participants, who are not tall and who have low baseline VLF% and LF% are most likely to be type 1 responders. In contrast, older male participants who are tall and have high baseline VLF% and LF% are most likely to be type 2 responders.

As the main finding was that physiological responses to music, tVNS and their combination were no different to the sham, this finding will be discussed in more detail below.

#### **4.4.2 Placebo effects**

The effectiveness of tVNS is normally compared to a control condition. This generally involves passing no current whilst participants believe they are receiving the intervention. Unfortunately, there is inconsistency in the literature regarding the effectiveness of active (t)VNS compared to sham. For instance, Clancy et al. (2014) reported active tVNS as having greater decreases in LF/HF than sham. However, they also disclosed that decreases in HR occurred in both interventions. In studies investigating the impact of VNS in participants with major depression disorder, there is also conflicting evidence. For instance, Rush et al. (2005a) found no significant difference in change in medication use and clinical inventory outcomes between active and sham VNS groups. Indeed, similar numbers of adverse effects were reported in both groups (active: 16; sham: 14). However, in their 2005b paper, improvements in the Hamilton Depression-Rating Scale were significantly higher in active VNS compared to sham. In a different study looking at the impact of VNS in patients with depression on pain perception, Brockardt, Kozel, Anderson, Walker and George (2005) discovered that sham VNS was associated with greater tolerance to heat than active VNS. Furthermore, in schizophrenia patients, no significant differences in improvement of any outcome measures emerged between active and sham tVNS (Hasan et al., 2015). These results, as well as those reported in the current study, suggest that a placebo effect may have occurred.

The definition of a placebo is widely contested. It tends to refer to an inert treatment that is administered as though it is an active ('real') treatment. The placebo is considered to have no physical mechanisms of action on the physiological or biochemical status of the user (Touwen & Engberts, 2012). They are also traditionally conceptualised as a form of medication (e.g. a sugar pill), but can also be a therapeutic sham procedure (e.g. non-penetrating acupuncture needles or naïve Reiki practitioners), similar to the one employed in the current study. Furthermore, placebos can be pure



(using an inert substance or method) or impure (substances or methods which have known physiological effects but which would have no impact on the variables of concern). In the case of the current study, the placebo was pure, as the Net-1000 was turned off. Therefore, placebo effects (positive effects of placebos) demonstrate that the belief of receiving real treatments is a healing power in and of itself (van Deventer, 2008).

The debate concerning the effectiveness of placebos is particularly prevalent in the alternative treatment literature (van Deventer, 2008), with particular focus on acupuncture and reiki. This is based on the assumption that they tend to have elaborate set-up and treatment rituals. For instance, acupuncture involves inserting small needles into specific sites located on the body with the belief of reducing pain (Zheng, Yuan & Liu, 2014). Whereas, Reiki involves channelling energy from practitioners hands to the patient's body via direct or indirect touch (Mackay, Hansen & McFarlane, 2004). A review of acupuncture randomised placebo (sham)-controlled clinical trials revealed no significant differences in outcome measures between real and sham acupuncture (Zheng et al., 2014). This led to the conclusion that the benefits associated with acupuncture are only attributable to robust placebo effects. Given the premise of Reiki (the transference of energy by placing hands on or above the body), the power of placebo effects come into force. Although most evidence supporting the efficacy of Reiki has been anecdotal, Reiki has also been found to significantly lower HR and BP (Mackay et al., 2004), reduce perceived pain (Olson & Hanson, 1997; Olson, Hanson & Michaud, 2003) and improve quality of life (Olson et al., 2003). As the physiological mechanism responsible for these changes remains unclear, this leaves the observed benefits to be virtue of placebo effects.

A similar argument could be applied to wearable technologies, including those on the mass-market e.g. FitBits, Garmin Forerunners, TomTom Sparks and the Nervana headphones; more clinically-related devices e.g. Doppel device and HIRREM; and tracking mobile apps that track HR, as well as physical activity (e.g. Nike+ and Runtastic). Although these technologies allow users to record and monitor a range of health-related variables, they do not intrinsically improve health. That is, they are not an active

intervention, despite claims of enhancements in health and well-being. This is interesting, given their popularity, and could be due to heightened feelings of control (which has been shown to reduce perceived pain: Mitchell et al., 2006) and placebo effects. As the Net-1000 is also a wearable technology, a similar pro-placebo effect argument could be applied. This argument is particularly pertinent as like other wearables, the Net-1000 looked professional, has already been shown to have beneficial effects and seems to have high face validity, leading to users believing that it must work. Therefore, as the sham was equally as effective as the tVNS only, music only, and tVNS + music, it appears that the Net-1000 device's effectiveness lies in its use as a fake treatment (placebo). This is particularly noteworthy given that participants who were cognisant of receiving the sham still showed a shift towards parasympathetic dominance and subjective relaxation. This points to the possibility of there being a meta-placebo effect, whereby the beneficial effects are due to held beliefs that placebo's have healing effects (van Deventer, 2008). This has practical and moral implications as it suggests that deception may not be necessary to facilitate beneficial effects associated with fake treatments.

Therefore, initially it appears that a placebo effect was occurring in this study. However, it is challenging to firmly come to this conclusion. This is because the sounds which accompanied the tVNS were absent in the sham and music only conditions. This creates a confound and suggests that the buzzing and beeps occurring with the tVNS may have stimulated individuals. Indeed, this claim is consistent with the experienced emotion results. In addition, the changes that emerged could have been due to:

1. Lying on the couch for an hour
2. Experiencing pressure on the concha
3. Slight inconsistencies in experimenter behaviour between the visits
4. Experimenter-participant interaction

The most feasible way of fully unpicking this 'placebo effect' would be to run a double-blind placebo controlled study, with the buzzing and beeps removed and a sham condition that involved targeting the lobule (as the lobule is not innervated by the ABVN and tVNS administered to the lobule is not associated with changes physiological changes (Kraus et al., 2007)).

This would also help determine the effectiveness of 'Weightless' at promoting greater vagal tone. This is because the effects of this stimulus are confounded by similar variables as well as the knowledge that this piece has already been shown to work. Therefore, the relaxation effects of 'Weightless', and perhaps other relaxation-type pieces of music, may be more complex than originally thought.

#### **4.4.3 Conclusion**

This study was the first to examine the effect of music combined with tVNS on cardiac autonomic control and subjective emotion in healthy volunteers. A key finding was that the sham visit was equally as effective at modulating autonomic activity as music, tVNS and their combination. This provides evidence of a placebo effect, suggesting that the strength of the Net-1000 device lies in using it as a fake treatment. Similarly, the effects of 'Weightless' on cardiovascular autonomic activity and subjective emotion may not be as simple as first thought. The belief held by participants that the music works may have driven these results. Indeed, it is plausible that the effectiveness of relaxation music is due to meta-placebo effects.

Females, older participants and musicians appeared to respond better than their counterparts. This suggests that these populations may benefit most from music used in tandem with tVNS. Finally, it is possible to predict responses to the four stimulation periods based on resting LF%. As placebo effects, like the one here, are complex, it would be worthwhile further exploring why the sham was so effective. Indeed, it appears that by having a professional-looking medical device, improvements in autonomic activity and emotional status can be made. This finding has implications when considering the efficacy and popularity of wearable devices.

## **Chapter 5. General discussion**

## **5.1 Four key findings**

Four main outcomes were identified as a result of the three research studies (Chapters 2-4). Firstly, in Chapter 3 the 60bpm tempo of the stepped decrease in tempo stimulus was associated with the greatest shifts towards parasympathetic predominance. In addition, the follow-up study data identified dissociations between the physiological measures and self-reported experienced stress and stimulation. Chapter 4 revealed that the sham stimulus was equally as effective as tVNS only, music only and their combination at modulating human physiology. This, therefore, implicates placebo effects in music, tVNS and wearable technologies. Finally, the thesis consistently found that response to all stimuli employed was predicted by baseline LF%.

These four key finding will now be discussed.

### **5.1.1 Greatest vagal tone for 60bpm in a decreasing tempo stimulus**

The pilot study (Chapter 2) revealed that the stepped decrease in tempo stimulus was associated with shifts in autonomic activity towards parasympathetic predominance. This came as little surprise given that previous work indicated that slower tempi are associated with greater vagal tone. This finding is also consistent with research looking at tempo detection. For instance, early work by Wang (1984) demonstrated that the accuracy of detecting changing tempi is higher for decelerating tempi compared to accelerating tempi. Therefore, the finding that the stepped decrease in tempo stimulus in the pilot was associated with greater shifts towards parasympathetic predominance than the stepped increase in tempo stimulus may have been due to the former being more salient for participants than the latter. This is intriguing because the range of tempi employed in the stepped increase and decrease in tempo stimuli were exactly the same. The only aspect that differed between the two stimuli was the order of tempo presentation.

However, the strength of the tempo detection argument in accounting for the finding that the stepped decrease in tempo stimulus was associated with the greatest shifts towards parasympathetic predominance is weakened. This is

because mixed results in research investigating tempo detectability have been reported. For example, Wang (1983) showed that for Bach's *Solfeggietto*, tempo increases were detected more accurately than tempo decreases. In addition, in a study exploring the relationship between direction of tempo change and tempo preference, LeBlanc and McCrary (1983) demonstrated that participants showed greater preference for excerpts that had a faster tempo over those that had a slower tempo. Similar results were reported by Geringer and Madsen (2003). But, it appears that these inconsistencies may have been due to the interference of other variables. For instance, Wang and Salzberg's (1984) revealed that musical style significantly contributed to how participants perceived the different tempi. Also, Sheldon (1994) revealed that for a musical stimulus decreasing in tempo, the latency and accuracy of responses of non-music majors were not significantly different to those of music majors. In contrast, music majors showed significantly quicker and more accurate responses to the tempo increase stimulus. In addition, Wang (1983) demonstrated that rhythm, texture and beat emphasis also influence the perception of tempo changes. As well as the direction of tempo change, type of tempo manipulation has also been shown to impact the perception of changes in tempo. For instance, Wang (1983) demonstrated that tempo detection is easier for stepped changes compared to gradual changes. Also, Geringer and Madsen (2003) showed that no participants accurately identified the direction of tempo change for music that gradually increased or decreased by 20%. This is intriguing given that the just noticeable difference in detecting changes in the speed of speech is 5% (Quené, 2007). Furthermore, Grondin and Laforest (2004) showed that non-musicians (but not musically trained participants) were more accurate at detecting the direction of tempo change for abrupt tempo changes compared to stimuli that gradually increased or decreased tempo.

Nevertheless, the greater vagal tone that emerged for the stepped decrease in tempo stimulus in the pilot failed to transpire in the follow-up study (Chapter 3). Perhaps, this discrepancy between the two studies replicates the inconsistencies detailed above. Moreover, the discrepancy between the pilot and follow-up studies could be due to a change in the stimuli. This

seems a plausible rationale given that the follow-up increased the musical complexity of the stimuli (i.e. included rhythm and melody for the experimental group and rhythm only for the control group) and perception of tempo changes has been shown to be influenced by other variables (Wang, 1983; Sheldon, 1994; Geringer & Madsen, 2003; Grondin & Laforest, 2004).

Despite these inconsistencies, the follow-up revealed that the 60bpm tempo in the stepped decrease in tempo stimulus was associated with significantly greater physiological relaxation compared to the other four tempi. This was evidenced by the increases in SDSD, RMSSD, pRR50, HF power and (n)SD1. Two alternative explanations may account for this finding. The first appeals to the notion of preferred tempo. Early research looking at preferred musical tempo was pioneered by Fraise (1982). This typically adopted motor-related paradigms, which required participants to tap at spontaneous, optimal or natural speeds. This work resulted in the recommendation that preferred (or natural) tempo was around 100bpm. Initially, Fraise's (1982) finding is difficult to reconcile with the result identified in the follow-up.

Indeed, the tempo closest to Fraise's (1982) 'special' tempo was the 90bpm of the stepped decrease in tempo stimulus. But although 90bpm was associated with similar increases in measures of parasympathetic activity as 60bpm (see Table 3.5), there were fewer changes in the remaining measures (see Table 3.8). Therefore, it is unlikely that the 90bpm tempo of the stepped decrease in tempo stimulus was more preferred (as indexed by increases in measures of parasympathetic activity and decreases in sympathetic predominance) than the 60bpm tempo. However, more recent research exploring preferred tempo suggests that 100bpm may not be as accurate as first thought.

For example, Iwanaga (1995a, 1995b) showed that preferred tempo is in a similar range to that of HR. As healthy HRs typically range from 60-100bpm, the studies imply that preferred tempo is variable and tailored to the individual. This coupling between HR and tempo has interesting consequences: as HR is controlled by respiration one could postulate that as respiration changes so does preferred tempo. This means that contrary to Iwanaga (1995a, 1995b) it may be respiration, as opposed to HR, that determines tempo preference. Perhaps this line of enquiry requires further

examination. Nonetheless, an alternative perspective on preferred tempo exists. Indeed, contrary to both Fraisse (1982) and Iwanaga (1995a, 1995b), Moelants (2002) maintains that preferred tempo is much faster than that previously proposed. This postulation is based on evidence from two studies. Firstly, in a spontaneous tapping paradigm Moelants (2002) revealed that peak frequency occurred between 450 and 500ms. Secondly, the distribution of tempi used in current musical pieces ranges from 80 to 160bpm, with the largest peak occurring between 120 and 140bpm. As a result, Moelants (2002) suggests that preferred tempo is between 120 and 130bpm.

However, an issue associated with this view concerns metrical interpretation. For instance, a crotchet played at 120bpm is the same as a quaver<sup>40</sup> played at 60bpm. As Moelants (2002) does not provide any indication of the hierarchical level of the beats participants were tracking, it is possible that these findings are in fact consistent with those of Fraisse (1982) and Iwanaga (1995a, 1995b). Moreover, Moelants' (2002) results are also compatible with the follow-up's main finding that the 60bpm tempo of the stepped decrease in tempo stimulus was associated with the greatest physiological relaxation. Perhaps this argument also applies to the 180bpm tempo of the stepped increase in tempo stimulus, which elicited the most increases in measures of cardiac vagal tone. That is, participants may have been interpreting the notes as quavers that were played at 90bpm, as opposed to crotchets played at 180bpm. As the metrical interpretation participants employed in the follow-up (and pilot) was not ascertained, it is not feasible to fully determine how participants were tracking the beat.

Due to these conflicting perspectives, it is difficult to provide a reason for why the 60bpm tempo of the stepped decrease in tempo stimulus elicited the greatest shifts towards parasympathetic predominance. But, an alternative explanation could be provided which concerns musical expectations. The notion that music generates expectations that can either be violated or fulfilled was first introduced by Meyer (1956). Meyer maintained that the power of music rests in its ability to generate expectations in listeners,

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<sup>40</sup> Quaver: also known as an eighth note, it is played for half the duration of a crotchet.



regardless of the familiarity of a piece of music. These expectations are normally culturally-informed, automatic (difficult to suppress) and rely on memory. Meyer also believed that musical expectations are intrinsically linked with the ANS. That is, low probability musical events provoke a shift towards sympathetic predominance which results in increases in HR and BP. These changes are then consciously detected and interpreted by listeners in an emotionally-salient manner. Therefore, the changes that occur as a result of listening to music are due to the interaction between different musical parameters. In turn, this gives rise to cognitive expectations and changes in autonomic activity when the expectations are fulfilled or violated. The autonomic alterations are then followed by the experiencing of emotions.

Meyer's theory of musical expectations has received a great deal of research interest and validation. Indeed, Steinbeis, Koelsch and Sloboda (2006) investigated the effects of harmonic expectancy violations on HR, EDA<sup>41</sup> and brain activity along with self-reported tension and emotionality. Three versions of six Bach chorales were presented to 24 participants. The way in which the stimuli differed were with regards to one chord. It was either 'expected', 'unexpected' or 'very unexpected'. Increasing unexpectedness of the chords was positively related to EDA and self-reported emotionality. In addition, analysis of the brain imaging data revealed that early right anterior negativities<sup>42</sup> also increased with unexpectedness. Also, using a different paradigm, Egermann, Pearce, Wiggins and McAdams (2013) demonstrated that musical expectation violations in a live performance alter the real-time emotional and autonomic responses of audience members. Indeed, subjective arousal increased and subjective valence decreased with increasingly unlikely events. Furthermore, highly unlikely events elicited increases in SCR and decreases in HR. Also,

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<sup>41</sup> Electrodermal activity: Measures the electrical changes that occur at the skin's surface. Based on the premise that skin conductivity increases with shifts towards sympathetic predominance. Hence it is typically interpreted as a non-invasive measure of sympathetic activity.

<sup>42</sup> Early right anterior negativities refer to event-related potentials (peaks and troughs in an electroencephalogram) that are associated with a violation occurring in a stimulus. They also represent an increase in effort needed to process and integrate the information (Steinbeis et al., 2006).

facial activity in the corrugator and zygomaticus muscles were significantly lower for the unlikely events compared to the likely ones.

Therefore, Meyer's musical expectations may also account for the finding that the 60bpm tempo of the stepped decrease in tempo stimulus of the follow-up elicited greatest vagal activity. The reasons for this are two-fold. Firstly, participants were aware from the outset that they would hear stimuli that altered the tempo of a nursery rhyme. As a consequence, participants were already primed to expect a stimulus with different temporal attributes before hearing the stimuli. Then upon hearing the stimuli and the first couple of tempo changes, they may have begun to anticipate the subsequent tempo modifications. This would not be particularly demanding given that the changes were sudden (stepped) and of a magnitude greater than 5%. Therefore, the shift towards parasympathetic predominance may have been driven by the expectation that the tempo manipulations would cease at 60bpm. As they did, this facilitated a shift towards greater parasympathetic predominance. This line of reason is particularly persuasive given that the final tempo of the stepped increase in tempo stimulus (180bpm) was also associated with the highest parasympathetic predominance.

Consequently, contrary to initial interpretations which implied that the result was due to 60bpm being around preferred tempo and/or similar to participant HR, it could be argued that there was nothing intrinsically special about 60bpm. Instead, participant expectation that this was the final tempo produced a physiological relaxation effect. This conclusion is difficult to dispute given that a similar pattern also emerged for 180bpm in the stepped increase in tempo stimulus. Nonetheless, the only way to fully determine which of these two accounts is valid would be to randomise the order of presentation of the five tempi across participants. Furthermore, data pertaining to the metrical interpretation employed participants would further elucidate the role beat tracking has on cardiovascular autonomic responses to tempo manipulations. This would not only uncover the true reason underpinning the observed patterns between the five tempi, but it would also provide novel results regarding the effects of unpredictable changes in musical tempo.

### **5.1.2 Correlations between autonomic and subjective measures**

The follow-up study (Chapter 3) revealed that the measures of experienced arousal (stimulation, relaxation, stress, calm) and valence (happiness, sadness) significantly correlated with some measures of autonomic activity. These findings are consistent with previous work by van der Zwaag et al. (2011) and Gomez and Danuser (2004). In addition, high arousal stimuli have been associated with significantly higher HR and blood volume pulse amplitude, and positively valenced stimuli associated with enhanced LF power and NN50 (Ogg, Sears, Marin & McAdams, 2017). However, inspection of the direction of the correlations demonstrated that those for stimulation and stress were in the direction opposite to that which was anticipated. For instance, as experienced stimulation increased so did HF power (parasympathetic activity) and RR interval. Interestingly, the same pattern occurred for stress. However, for their 'opposites' (relaxation and calm respectively) the correlations were in the anticipated direction (HF power and RR interval increased relaxation and calm).

As each adjective had its own visual analogue scale this allowed participants to provide ratings for each individual variable. This was implemented because stimulation and relaxation were not perceived to be absolute antonyms or linearly related. The same argument also applied to: stress and calm, and happiness and sadness. As a result of using these separate scales, it was feasible for participants to indicate high levels of stimulation and low levels of relaxation (or vice versa), as well as high levels of stimulation and relaxation (or vice versa). It should be noted that all of these combinations occurred in the study. Although most participants showed linear relationships between stimulation and relaxation (and stress and calm), there was a sub-set of participants who indicated that they felt highly stimulated and highly relaxed.

It is up for debate whether the data from these participants should have been excluded. However, these responses were considered valid. This is based on research concerning flow. Flow is defined as 'the subjective experience of effortless attention, reduced self-awareness, and enjoyment that typically occurs during optimal task performance' (Harmat et al., 2015:

pg. 1). Therefore, it is typically characterised by intense feelings of enjoyment, positivity and rewards at the expense of undertaking tasks that may be redundant or lack importance to other individuals (Csikszentmihalyi, 1990; de Manzano, Theroell, Harmat & Ullén, 2010). Flow normally occurs when individuals are deeply and actively involved in a task (Csikszentmihalyi, 1990). These high levels of task absorption lead to individuals performing the tasks to the best of their ability and at a higher efficiency than individuals not experiencing flow (de Manzano et al., 2010). Therefore, research investigating the physiological aspects of flow suggests that the phenomenon is associated with heightened autonomic activity, including the activation of both sympathetic and parasympathetic nervous systems (Harmat et al., 2015).

For instance, de Manzano et al. (2015) found that increased flow was associated with decreases in RR interval and RSA, and increases in LF/HF, total HRV power and respiration depth. In addition, LF power and respiration depth were found to be significantly related to flow: both variables were significantly higher during flow states compared to non-flow states. This suggests that activation of the sympathetic branch of the ANS is particularly involved in the experience of flow. However, opposite patterns have also emerged: flow has been associated with higher HF power, elongated RR intervals and a higher respiration rate (Ullén, de Manzano, Theorell & Harmat, 2010). Indeed, it appears that flow proneness may explain these discrepancies. Nevertheless, as the psychological correlates of flow, are increased experienced arousal and valence (Manzano et al., 2015) combined with a shift towards parasympathetic predominance, it is plausible that a similar effect occurred in the follow-up study. Although this seems unlikely given that participants were passively engaged with the stimuli, it provides evidence of dissociations between self-report and physiological measures.

Counterintuitive findings regarding the treatment of Attention-deficit/hyperactivity disorder (ADHD) also suggest that the relationship between autonomic activation and experienced stimulation may not be straightforward. ADHD is one of the most common childhood neurobehavioral disorders and is characterised by a lack of

attention/concentration, impulsivity, and excessive motor activity (Wilens et al., 2003). Untreated, the condition can impair the academic performance of children and have debilitating consequences in terms of the child's social capacities both in childhood and in later adult life. Exploration of the autonomic correlates of ADHD demonstrate that the condition is associated with greater vagal tone when compared to age-matched controls (Stifter, Dollar & Cipriano, 2011; Musser et al., 2011, Rash & Aguirre-Camacho, 2012). As a result, this has led to the treatment of ADHD with sympathetic-stimulating drugs, such as Methylphenidate (Wilens & Spencer, 2000). As a consequence, this research into ADHD demonstrates that under activity of the sympathetic nervous system can lead to behaviour that is outwardly characterised as overly aroused.

A similar dissociation between subjective and autonomic measures occurs in individuals experiencing grief. This is characterised by high levels of autonomic activation accompanied with negative emotional responses. For instance, Bonanno, Keltner, Holen and Horowitz (1995) reported decreases in HR were associated with heightened negative emotion. In addition, contrary to expectations, they also showed that this negative relationship was stronger in those reporting minimal grief compared to those who had recovered or were experiencing prolonged grief. A dissociation between SCR and negative emotions was also reported by Coifman, Bonanno, Ray and Gross (2007). Whereby, more intense negative emotions were associated with lower SCRs. Using RSA to explore the relationship between vagal tone and grief intensity, O'Connor, Gündel, McRae and Lane (2007) identified a positive relationship between the two variables. Therefore, these results suggest that in grief, shifts towards parasympathetic predominance occur whilst experiencing intense negative emotions. Additionally, the more negative the emotions experienced, the greater the shift towards vagal tone. Although it is unlikely that the participants in the follow-up study (Chapter 3) had ADHD, grief and/or experienced flow, these two examples illustrate that it is possible for a state characterised as stimulating or intense sadness to be accompanied with a shift towards greater parasympathetic predominance. Therefore, the initially counterintuitive correlations between autonomic activity and: stimulation and stress, could be accounted for by

appealing to altered psychophysiological states. In addition, as previous research has tended to position bipolar adjective pairs at either end of (Likert Scales or VASs) scales, this may explain why the current results are inconsistent with those of Gomez and Danuser (2004) and van der Zwaag et al. (2011). Therefore, participants may not have actually provided erroneous subjective measures. Instead, the VAS may have afforded participants with the opportunity to provide self-report measures that had greater specificity. That is, the stimulation and stress correlations were driven by participants responding highly to all four measures of experienced arousal.

### **5.1.3 Music and placebos**

The final study demonstrated that the sham (control) visit was equally as effective as the music only, tVNS only and music + tVNS visits. That is, contrary to expectations the combination of the two stimuli were not more effective than either stimulus alone or the control. This is surprising given that there is growing evidence that suggests that, individually, music and tVNS confer physiological benefits. For instance, singing, particularly in a group setting such as a choir, can promote health benefits, including: increases in positive affect (Clift & Hancox, 2001; Clift et al., 2010; Kreutz, Bongard, Rohrmann, Hodapp & Grebe, 2004); increases in secretory immunoglobulin<sup>43</sup> (Kreutz et al., 2004); decreases in negative affect (Clift & Hancox, 2001; Clift et al., 2010; Coulton, Clift, Skingley & Rodriguez, 2015; Kreutz et al., 2004); improvements in lung function, posture, stamina (Clift & Hancox, 2001; Clift et al., 2010; Skingley, Martin & Clift, 2016); and improvements in the ability to cope with aches and pains (Skingley et al., 2016). In turn, this has led to a surge in the number of individuals joining choirs. However, despite some choir-goers having a genuine drive in developing singing technique, other individuals are only interested in experiencing the health benefits associated with choral singing. In turn, this can result in vocal impairments, including hoarseness of the voice and vocal

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<sup>43</sup> Secretory immunoglobulin: Marker of immune-system competence that fights against viral and bacterial infections in the upper respiratory tract. As the ANS influences immune-system function, measurement of secretory immunoglobulin can provide some indication of how autonomic function changes, as well as the impact of these changes on immune functions. Decreases in secretory immunoglobulin are associated with increases in sympathetic predominance (Kreutz et al., 2004).

fatigue (Coelho, Daroz, Silvério & Brasolotto, 2013). Consequently, this undermines the value of music in conferring health benefits.

Unlike music which is generally maintained as promoting good health, greater scepticism surrounds tVNS. Nonetheless, tVNS has been shown to reduce sympathetic predominance (Clancy et al., 2014; Brock et al., 2017; Frøkjær et al., 2016; Juel et al., 2017; Antonino et al., 2017); improve mood (Kraus et al., 2007) and associative memory (Jacobs et al., 2015) and relieve symptoms in patients with epilepsy (Bauer et al., 2016), depression (Hein et al., 2013; Fang et al., 2016) and tinnitus (Kreuzer et al., 2012, 2014). However, mixed findings have been reported, particularly for pain. For instance, Usichenko, Laqua, Leutzow and Lotze (2017) showed that tVNS reduced activity in pain brain regions (including the right thalamus and the anterior cingulate cortex) as induced by a contact heat-potential stimulator. However, these reductions in brain activity were not observed in participants' self-reported pain ratings. Similarly, Frøkjær et al. (2016) showed that although tVNS increased pain thresholds for pressure on the tibia bone, this finding was not paralleled in muscle pressure pain thresholds. In addition, Laqua, Leutzow, Wendt and Usichenko (2014) revealed that similar pain thresholds were observed for tVNS and placebo, and HR and BP did not significantly differ between the two stimuli. Therefore, although positive effects of tVNS have been documented, there remains controversy concerning the effectiveness of tVNS when compared to a placebo. This issue was raised in the Net-1000 device study and findings implied that the observed changes in cardiovascular autonomic function may have been driven by a placebo effect.

A similar argument may also apply to music: the positive effects of music on autonomic function and subjective emotion may be driven by the widely held belief that music makes listeners better. These perhaps overstated effects of music are problematic. This is because it makes it difficult to determine whether it is the music itself or listener's beliefs that the music will confer health benefits, which are responsible for the observed effects. This issue has not fully been addressed in the music literature. This may be due to two reasons. Firstly, much music research has failed to employ a control (group or stimulus) other than silence. Consequently, this renders it difficult in

ascertaining the extent to which the alterations in autonomic activity and subjective emotion are a result of music per se. Secondly, failure to address the possible placebo effects of music may have arisen from challenges in determining what constitutes an appropriate sham stimulus for musical stimuli. For instance, in research that has employed control stimuli, this has consisted of: white noise (Sakamoto et al., 2002; Sokhadze, 2007; O'Kelly et al., 2013; Nakajima et al., 2016) and natural sounds (Gomez & Danuser, 2004, 2007). However, the validity of comparing autonomic responses to music to those elicited by noises and/or natural sounds is dubious, seeing that music is comprised of many features that do not always occur in these 'control' stimuli. Therefore, it is possible that some aspects of autonomic and subjective responses to music may be a placebo, whilst others may not. That is, music (and tVNS) in their entirety may not be placebos. Rather specific elements may be more susceptible to placebo-like effects than others. This leads to an important question: how do we distinguish between real and music-related placebo effects? This may involve: manipulating individual musical parameters, similar to that done in the pilot and follow-up studies; or combining music with another 'alternative treatment' for which a sham stimulus has been validated.

Placebos are well-established phenomena. However, some argue that placebo controls are ethically dubious given that they typically involve some element of deception. However, as noted by van Deventer (2008), even participant knowledge of consuming a placebo can lead to positive health outcomes. Therefore, the deception concerning placebos may not be as big an ethical dilemma as originally thought. This comes as unfortunate news for the big pharmaceutical companies and for the founders of wearable technologies and health tracking apps. As some participants in the final study showed larger reductions in LF% during the sham visit, an additional issue arises concerning the prescription of fake or placebo treatments.

In addition to improvements in subjective outcome measures, placebos have also been shown to induce significant changes in neuronal activity. For instance, neuronal discharge in the sub-thalamic nucleus, whose functioning is impacted in Parkinson disease, significantly differed between those who did and did not respond to a placebo (saline) (Benedetti et al., 2004). That is,



neuronal frequency discharge was significantly lower post-placebo than pre-placebo in placebo responders compared to non-responders and the no-treatment group. Placebo responders also occur in healthy samples. For example, Elsenbruch et al. (2012) demonstrated that placebo responders had significantly greater decreases in activation in the somatosensory cortex, posterior cingulate cortex and thalamus during pain induction (rectal distensions) compared to non-responders. Therefore, placebos are associated with changes in both subjective and neural activity and the likelihood of responding to placebos differs between individuals.

The finding that some individuals respond to placebos and others do not raises the issue of whether responders should be prescribed placebos rather than active treatments. Furthermore, the discontinuation of placebos for responders in clinical trials is questionable, especially if the return to original medication or no medication results in a relapse and the reoccurrence of original symptoms. These issues also apply to Marconi Union's 'Weightless', the tVNS generated by the Net-1000 device and wearable technologies that are available to consumers. Therefore, the findings from the Net-1000 study not only have practical implications, but they also have ethical implications that need to be considered. This is particularly pertinent given the abundance of health-related apps and wearables.

#### **5.1.4 Responses to stimuli can be predicted by baseline LF%**

In the music literature, differences in response patterns between stimuli have been observed. For instance, Siddle and Heron (1976) acknowledged that not all participants showed changes in SCR, HR or finger pulse volume during the presentation of a different tone frequency following a habituation paradigm. However, despite acknowledging these differences, further analysis into exploring this effect was not performed. Personality characteristics have been proposed to influence responses to music (Chamorro-Premuzic & Furnham, 2007; Rawlings & Leow, 2008), as has age (Hilz et al., 2014), gender (Nater et al., 2006); musical background (Bernardi et al., 2006; Bernardi et al., 2009; Proverbio et al., 2015) and musical preferences (Kreutz, Ott, Teichmann, Osawa & Vaitl, 2008). But due to the subjective nature of these variables, one of the project aims was to

ascertain whether any measures of autonomic activity accounted for these response differences.

This aim primarily came from work by Clancy et al. (2014) who showed that baseline LF/HF significantly predicted response to tVNS in healthy volunteers. But other work in patient samples has showed that treatment response can be ascertained by other measures. For instance, SDRR is a predictor of sudden death in progressive heart failure (Nolan et al., 1998). Similarly, Ponikowski et al. (1997) showed that SDRR, SDANN and LF power significantly predicted survival in patients with congestive heart failure. SDRR and BRS also significantly predict mortality in patients following myocardial infarction (la Rovere et al., 1998). Also, baseline MAP significantly predicted reduction on MAP between pre- and post-intervention in hypertensives (Rosenthal, Alter, Peleg & Gavish, 2001). Similarly, Viskoper et al. (2003) showed that resistant hypertensives with higher baseline office and home SBP and DBP, and home MAP, showed greater reductions in their corresponding post-intervention readings than those with lower baseline readings.

Therefore, one of the most crucial and novel findings of the project concerns the predictive power of baseline LF%. Indeed, across all three studies, baseline LF% was a significant predictor in determining response (change in LF% between baseline and stimulation) to every stimulus (stepped increase in tempo, stepped decrease in tempo, stable tempo, Marconi Union's Weightless, tVNS only, Weightless combined with tVNS, and sham). Further analysis of response type revealed that change in LF% was associated with age, where young participants showed greater decreases in LF% than older participants. This implied that young participants responded more positively to stimuli than older individuals. This is counter-intuitive given that youth is associated with lower baseline sympathetic activity than older age. Indeed, the robust aging pattern that has reported in the literature led to the anticipation that older participants would more likely be type 1 responders and young participants type 2 responders or non-responders (i.e. the latter exhibiting a flooring effect). However, this did not occur, suggesting that the target audience for the stimuli employed in all three studies was adults aged between 18 and 30 years.

Baseline LF% was consistently found to be higher in males than females in all three studies. This finding replicates those reported elsewhere (Antelmi et al., 2004; Kuo et al., 1999; Matsukawa et al., 1998; Ng et al., 1993; Stein et al., 1997; Umetani et al., 1998). As a higher baseline LF% was associated with increases in LF% between baseline and stimulation, this gender difference suggests that females would have been more likely to be type 1 responders or non-responders compared to males. However, gender was not found to be significantly associated with responder type. This may have been due to the data reduction that occurred when classifying individuals as either non-responders, type 1 responders or type 2 responders. The unexpected prediction of height on baseline LF% warrants discussion. The positive relationship between the two variables was initially thought to be spurious when observed in the follow-up. But as it was also seen in the Net-1000 device data for three out of the four visits, perhaps this was a robust effect. It is possible that the increases in baseline LF% that were associated with increases in height may be reflected in other HRV frequency components. This requires further exploration. Nonetheless, as baseline LF% predicted magnitude and direction of response to the stimuli, this finding suggests that height may have been an influential factor. This not only implies that responses to non-invasive stimuli can be pre-empted based on resting autonomic activity and basic demographic information, but it also demonstrates that the predictive power of baseline LF% is a real and robust effect.

This is important for two reasons. Firstly, it is clear from the reviewed literature investigating the autonomic effects of music, that the potential for music to be used as an alternative treatment is being realised. In addition, its reported beneficial effects are being harnessed at individual and group levels. This comes in many forms, including community music initiatives, music and health mobile applications and wearable technologies, music therapy sessions (live music generated in an interactive manner with a therapist) and music medicine (e.g. passive listening to music in dental clinics, operating theatres and on hospital wards). Therefore, being able to identify a priori who is likely to respond to a music-based intervention is in the interest of both patients and practitioners. As the three studies

demonstrate that pre-intervention identification of responders is possible, this sets the scene for more effective use of music as a method for restoring autonomic balance.

The second reason underlying the importance of this finding relates to the derivation of LF%. Some research studies looking at the impact of treatments on patient groups require the measurement of brain activity. For instance, pre-treatment activity in the anterior cingulate cortex predicts reduction in anxiety and worry symptoms in patients with generalised anxiety disorder (Nitschke et al., 2009). Similarly, treatment improvement in post-traumatic stress disorder (PTSD) is determined by lower activity in the amygdala and anterior cingulate cortex (Bryant et al., 2008). Also, metabolism in the rostral anterior cingulate predicts response type in patients with depression: responders show greater metabolism in this brain region compared to non-responders (Mayberg et al., 1997). Activity in the dorsal and ventral occipitotemporal cortex has also been shown predict change in social anxiety in patients with social anxiety disorder (Doehrmann et al., 2013). Interestingly, as most of these brain imaging studies have focussed on anxiety disorders which negatively impact autonomic tone, deriving LF% may also act as a predictor of treatment response, especially if music is used as a means to reduce anxiety. Indeed, due to a restricted range of stimuli used and a sample of healthy participants, it would be worthwhile exploring the reproducibility of these findings with other forms of relaxation-inducing stimuli and in patient groups, such as those with mood disorders.

An additional outcome from the linear regressions concerns the consistent classifications of non-responders, type 1 responders and type 2 responders across the three studies. Although the more traditional response definition was tested (the two-level definition<sup>44</sup>), this did not seem to apply in these cases. Indeed, all the studies mentioned so far looking at treatment response have used this two-level definition. However, there is a case to be

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<sup>44</sup> The two-level definition was as follows:

Non-responders: < 20% change or a  $\geq 20\%$  increase in LF% between baseline and stim

Responders:  $\geq 20\%$  decrease in LF% between baseline and stim

made for preferring the three-way classification<sup>45</sup> over the two-level definition. Firstly, although a decrease in LF% power was preferred (since music should reduce sympathetic predominance), an increase was also considered to be a valid response, as opposed to a non-response. This was done to achieve greater specificity in order to fully appreciate the subtleties in between-participant autonomic responses. This argument has also been employed by previous work in the music literature which has noted that listeners often respond in different directions, as well as of different magnitudes.

A few comments should also be made concerning the 20% threshold that was used to differentiate between non-responders and type 1/type 2 responders. A vast array of thresholds has been used in the literature. These include 10% (Gurbel et al., 2011), 20% (Clancy et al., 2014), 30% (Walker, Sheather-Reid, Carmody, Day & Vial, 1997), 45% (Mayberg et al., 1997) and 50% (Bryant et al., 2008; de Wildt et al., 1995). As the 10% threshold was considered too low and the 50% threshold too conservative, the intermediate thresholds were considered. Since the three studies were not performed on patient samples and work in the lab has previously shown that a 20% threshold effectively differentiates between non-responders and responders, the 20% threshold was implemented. An awareness of falsely classifying individuals as type 1 or 2 responders was persistent. However, the three-level definition resulted in differences between the responder types at baseline and during stimulation across all studies. Therefore, this provides validation of the 20% threshold in differentiating between non-responders and type 1/type 2 responders. If inconsistencies between the studies ensued, an alternative threshold would have been tested.

Despite the debates surrounding responder type definition and response threshold, these studies provide the first evidence of being able to reliably predict response to musical stimuli based on a measure of combined sympathetic and parasympathetic activity (LF%). These results are

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<sup>45</sup> The three-way classification was as follows:

Non-responders: < 20% change in LF% between baseline and stim

Type 1 responders: ≥20% decrease in LF% between baseline and stim

Type 2 responders: ≥20% increase in LF% between baseline and stim

promising for studies in both healthy controls and patient samples. For instance, change in LF% may prove useful in determining the diagnosis and/or prognosis of patients with disorders of consciousness. This would be pertinent given that evidence suggests that these patients physiologically respond to music. In addition, powering studies based on detecting a 20% change in LF% may account for between-participant differences in how autonomic tone in healthy volunteers changes during tempo (and other musical parameters) manipulations.

## **5.2 Study limitations**

In the pilot study, the stepped increase and decrease in tempo stimuli were presented to participants consecutively. That is, there was no time period separating the two types of stimuli. As a result, it is possible that the effects incurred during the first stimulus carried over into the second. The follow-up study sought to remedy this limitation, by inserting a small wash-out period between the three stimuli (stable tempo, stepped increase and stepped decrease in tempo stimuli). However, this wash-out period may have been insufficient, given that music has been shown to have long-lasting effects on human physiology (Chuang, Han, Li, Song & Young, 2011). Therefore, it may have been preferable to have implemented a study design similar to that used in the final study (see Chapter 4). That is, the three stimuli could have been presented over three visits. This would have entailed participants attending on three occasions with the order of the visits (stable tempo, stepped increase in tempo, stepped decrease in tempo) randomised across participants. In turn, this would have eliminated the potential confound of carry-over effects that occurred here. This aspect should be considered in future studies looking at the impact of musical parameter manipulations on autonomic and affective responses.

In the follow-up study, the two-factor model of affective space emerged from the factor analysis, as did Berlyne's theory of aesthetic response. However, interpretation of the two-factor model is difficult given that items which were expected to load onto arousal (e.g. stress) did not do so. Moreover, as experienced stimulation needed to be protracted from the model, this suggests that there were issues with some of the items. Indeed, this may

have provoked interpretation difficulties for participants (some did require clarification of the meaning of 'stimulation'), perhaps leading to the dissociation that occurred between experienced stimulation and autonomic activation. Therefore, careful interpretation of the self-report results is required. In addition, it could be argued that the questionnaires used should have undergone further pre-testing and development.

Respiration is the driving force of modifications in autonomic activity. Research by Bernardi et al (2006), Ellis and Brighthouse (1952), Etzel et al. (2006), Mikutta et al. (2013), Sakaguchi and Aiba (2016) and Vickhoff et al. (2013) have shown that musical parameters play an important role in altering the characteristics of breathing. Moreover, work on music-guided breathing has demonstrated that respiratory entrainment with music can induce short- and long-term changes in cardiovascular autonomic function. Therefore, there is the potential for alterations in respiration rate to have contributed to the patterns observed in all three studies. Experiments have tried to control for the confound of respiration by requiring participants to undertake paced breathing (e.g. Watanabe et al., 2017 and van Dyck et al., 2017). This typically involves having a set breathing pattern, as directed by a metronome on a screen (e.g. Watanabe et al., 2017). In contrast to spontaneous breathing (which was employed here), this form of breathing enables an easier assessment to be made of the impact of specific musical parameters or other alternative treatments (like tVNS) on autonomic tone. Although, the variable breathing that occurred here may be a limitation, it also allowed participants to breathe as they would naturally. However, it could be argued that paced breathing would overcome the confounds that occurred in the current studies. But as it could be argued to be an additional stimulus, this would need to be controlled for.

A major limitation of the final study concerned the Net-1000 device itself. Administration of the tVNS was accompanied with buzzing and beeping sounds that were even audible during the music + tVNS visit. In contrast, these sounds were absent in the music only and sham visits, as no tVNS was being generated. This slight change in the auditory environment may well have confounded the results, especially as some participants commented on its annoying nature. This aspect was brought to awareness

during initial testing of the device. The buzzing and beeps could have been turned off by tampering with the device. However, it was decided to refrain from doing this because it was unknown what impact, if any, these sounds would have.

A further limitation with the Net-1000 device study concerns the lack of including microneurography. Although no significant differences were detected in MSNA in the follow-up, it would have been preferable to have included this measure in the Net-1000 device study. This is because the within-visit analyses pointed to a change in sympathetic and/or parasympathetic activity during stimulation compared to baseline. Indeed, due to the approximate nature of the measures employed, it is plausible that one visit may have elicited greater reductions in sympathetic nerve activity.

### **5.3 Future studies**

The follow-up and final studies obtained subjective measures from participants via VAS. This form of measurement is rare in the literature. Indeed, self-report methods that are typically used include: Likert Scales (Etzel et al., 2006; Khalfa et al., 2002, 2008; Kellaris & Kent, 1993; Holbrook & Anand, 1990; Iwanaga & Moroki, 1999; van der Zwaag et al., 2011), continuous rating scales (Chuen et al., 2013; Etzel et al., 2006; Krumhansl, 1997), the self-assessment manikin (Gomez & Danuser, 2004, 2007) and forced-choice questions (Khalifa et al., 2002, 2008). It could be argued that employing continuous rating scales along with continuous measures of autonomic activity, would allow for more sensitive analysis into the relationship between fluctuations in autonomic and affective responses. This is particularly important given that the physiological measures employed here were obtained during the conditions whereas the self-report were obtained upon their completion. Indeed, incorporating this method into a study may even aid with understanding the dissociations that occurred.

Nonetheless, this would require careful consideration of the methodology that would be employed. Indeed, this type of study could increase the probability of incurring movement artifacts, associated with moving a dial, joy-stick or slider. This could cause issues when deriving HRV and calculating sequence BRS. Although the physical demands on participants



would be minimal, they would nonetheless be marginally more than those incurred in the current studies. Mention should also be made of the cognitive differences that this study design would entail. As participants in the present studies were asked to relax and close their eyes, these differences would also need to be accounted for. However, by requiring participants to undertake a task whilst listening to music, this would reduce the risk of participants falling asleep, which was one of the largest risks in the studies. The Net-1000 device was designed to administer tVNS to the concha at a very low intensity (130 $\mu$ A: maximum current that could be generated). Perhaps the current was not strong enough to elicit greater shifts towards parasympathetic predominance. Therefore, it may be worthwhile to alter the Net-1000 device and test whether higher intensity tVNS administered to the concha provokes greater changes in autonomic function compared to a sham. Indeed, the data suggested that the tVNS was no more effective than a sham control. Although Peuker and Filler (2002) found that in 45% of cases, innervation of the concha was by the ABVN, they also revealed that the cymba concha was innervated by the ABVN in 100% of cases. The effects of administering tVNS to the cymba concha have previously been investigated. For instance, Kreuzer et al. (2012) revealed that tVNS administered to the cymba concha is safe, resulting in no adverse cardiac effects. Additionally, in an fMRI study Yakunina, Kim and Nam (2016) discovered that cymba concha tVNS elicited significantly greater activation in the NTS and Locus Coeruleus<sup>46</sup>. These results were consistent with those of Frangos, Ellrich and Komisaruk (2015) who showed that cymba concha stimulation resulted in significantly greater activation of the NTS compared to baseline and earlobe stimulation. As this evidence suggests that cymba concha stimulation may lead to greater vagal activation than stimulation at other sites, it would be worthwhile investigating the effects of cymba concha tVNS combined with relaxation music on autonomic activity. This should control for differences in the auditory environment (i.e. no untoward beeping or buzzing sounds), include microneurography and be administered to the

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<sup>46</sup> Locus Coeruleus: brainstem network that receives direct input from the NTS (Yakunina et al., 2016).

left ear only. This latter point is particularly important given the sudden occurrence of ectopic heartbeats in two participants.

An alternative device which also administers music in combination with tVNS is available on the market. This is called the Nervana headphones (see Chapter 4). Unlike the Net-1000 device, the tVNS generated by the Nervana headphones is administered to the left ear only and can be modulated based on the characteristics of the music. For instance, for slow, legato, relaxation-type music, tVNS intensity and frequency can be enhanced to facilitate greater vagal tone and subjective relaxation. Consequently, it would be worthwhile testing the effectiveness of the Nervana headphones, and comparing the results of 'modulated' tVNS with 'unmodulated' tVNS.

As the final study revealed that the Net-1000 device was effective as a fake treatment, future studies looking at the effects of the Nervana headphones (and other wearables) should further explore placebo effects. This is pertinent given that the observed effects of music may also be driven by a placebo (we are culturally primed to think that music confers health benefits). Although it would be challenging to fully disentangle the contribution placebos have (if any) to the effects of music, several strategies could be employed. For instance, the autonomic and affective effects of lying down on a couch should be explored. Indeed, many participants in all three studies commented that lying on a couch for an hour once a week should be prescribed. In addition, the effects of the experimenter on participants' autonomic and affective responses should be explored. This is because preliminary data in the lab suggests that the experimenter may influence how participants respond. As well as measuring participant physiology and psychology, it would be worthwhile to include EEG, as placebos have also been shown to modulate neuronal activity (see above).

Responses to musical stimuli, tVNS and a sham condition can be predicted by baseline LF%. As the range of stimuli employed in the thesis was narrow, it would be worthwhile further testing the replicability of the predictive power of baseline LF%. Furthermore, as these stimuli have the potential to be used as alternative therapies, examining the reproducibility of LF% as a treatment response predictor is pertinent. These recommendations also extend to the

response consistency analyses that were performed. This is because stability of response could aid with identifying from the outset who will and will not respond over the long-term. Thus, to identify and characterise the long-term response patterns of individuals, a study examining the chronic effects of music (and/or tVNS) in a sample over an extended period is timely. This could involve repeated exposure of a stimulus at specific times of day over a four-week period and measuring pre- and post-intervention cardiac autonomic activity.

#### **5.4 Concluding remarks**

This thesis has shown that it is possible to modulate cardiovascular autonomic function using tempo manipulations, relaxation music, tVNS and a sham condition. Also, response type and magnitude to all stimuli employed can be predicted by baseline LF%. This is a highly novel finding, particularly in the music literature and sets the precedence for using these non-invasive stimuli as adjunctive or alternative therapies that aim to restore imbalance between the SNS and PNS. Music (and tVNS) are relatively inexpensive stimuli that avoid the ingestion of medications and untoward side-effects. Based on the results detailed in this thesis, tempo manipulations appear to impact autonomic activity, but not necessarily in the way anticipated. This merits further investigation, preferably by developing the musical complexity and sophistication of the stimuli employed. The thesis also implicates the power of placebos in modulating physiological and psychological responses to stimuli presented via a health-related wearable device. This effect also requires further investigation by employing comprehensive methodologies. Finally, despite the potential incurrence of placebo effects and other confounds throughout the thesis, exploration of the impact of the stimuli employed in conditions that exhibit autonomic dysfunction would be worthwhile (e.g. stress, anxiety-related disorders, disorders of consciousness and hypertension). This may aid with further understanding the power of belief and tempo in contributing to these effects.

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## Appendix A: Chapter 2

### A.1.

Characteristics of the initial sample of 28 participants (before exclusions).

	Mean	SEM	Freq
Males			12
Average age (over the two visits, yrs)	25.68	0.73	
< 30 yrs			4
Godin strenuous	8.68	2.19	
Godin moderate	8.25	2.29	
Godin light	6.11	1.56	
Godin weekly	23.04	3.55	
Physically active			12
Hours spent listening to music/week	16.07	2.49	
Formally music trained			24
Age started studying music (yrs)	10.04	0.94	
Average height (over the two visits, m)	1.71	0.02	
Average weight (over the two visits, kg)	70.13	2.95	
Average BMI (over the two visits, kg/m <sup>2</sup> )	23.86	0.84	

**A.2.**

## Health questionnaire.

**Health questionnaire**

**We would be grateful if you could please complete this questionnaire. This information will aid the analysis of the results of this study. All information will be strictly confidential and will be made anonymous before being analysed.**

**Name:**..... **Gender:** Male / Female  
**D.o.B:** ..... **Age:** .....  
**Height:**.....(cm) **Weight:** .....(kg)

**Medical history**

Have you been diagnosed with any of the following? (please circle)

Heart Disease                      Yes              No

Diabetes                              Yes              No

High Blood Pressure              Yes              No

Severe Auditory Impairments      Yes              No

If yes, are you currently taking any medication for this condition?    Yes              No

**Lifestyle**

Do you smoke?                      Yes              No

If yes, how many do you smoke a day?.....

Do you take regular exercise (20 minutes or more per session)?    Yes              No

If yes, please detail what type of exercise (*walking, running, football etc*) and how often (*number of times per week*)

.....  
 .....  
 .....

**Thank you**

**A.3.**

Music background questionnaire.

**Music background questionnaire**

We would be grateful if you could please complete this questionnaire. This information will aid the analysis of the results of this study. All information will be strictly confidential and will be made anonymous before being analysed.

**Name:** ..... **Gender:** Male      Female

**D.o.B:** ..... **Age:** .....

**Ethnicity (please circle):**    White British      White Irish      White other

   Black Caribbean    Black African      Black British      Black Asian

   Any other background.....

1. How many hours a week do you spend listening to music? .....

2. What genres of music do you listen to? *(please list)*  
 .....  
 .....

3. Do you play any instruments? *(please circle)*      Yes      No

4. If yes, please list the instruments you play and the number of years you have been playing each instrument

<b>Instrument</b>	<b>Years playing</b>
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

5. Have you ever had any formal music training? If you are a self-taught musician, please answer yes. *(please circle)*

- a. Yes, I had formal musical training
- b. Yes, I am a self-taught musician
- c. No

6. If yes, at what age did you start studying music? .....

**Thank you**

**A.4.**

Characteristics of the sample of 24 participants.

	Mean	SEM	Freq
Males			10
Average age (over the two visits, yrs)	25.71	0.72	
< 30 yrs			3
Godin strenuous	9.00	2.48	
Godin moderate	6.71	2.39	
Godin light	6.25	1.65	
Godin weekly	21.96	3.89	
Physically active			10
Hours spent listening to music/week	15.17	2.67	
Formally music trained			22
Age started studying music (yrs)	10.32	1.01	
Average height (over the two visits, m)	1.71	0.02	
Average weight (over the two visits, kg)	69.58	3.35	
Average BMI (over the two visits, kg/m <sup>2</sup> )	23.74	0.97	

**A.5.**

Characteristics of consistent (n = 9) and inconsistent responses for percentage change in LF% between baseline and the stepped increase in tempo stimulus (initial definition).

	Inconsistent response			Consistent response		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			6			4
Average age (over the two visits, yrs)	25.60	0.79		25.89	1.48	
< 30 yrs			2			1
Godin strenuous	9.00	3.62		9.00	3.00	
Godin moderate	6.07	3.21		7.78	3.64	
Godin light	5.00	1.93		8.33	3.03	
Godin weekly	20.07	5.59		25.11	4.81	
Physically active			5			5
Hours spent listening to music/week	15.40	4.10		14.78	2.39	
Formally music trained			13			9
Age started studying music (yrs)	9.85	1.33		11.00	1.60	
Average height (over the two visits, m)	1.72	0.02		1.68	0.03	
Average weight (over the two visits, kg)	73.03	4.79		63.83	3.55	
Average BMI (over the two visits, kg/m <sup>2</sup> )	24.47	1.42		22.51	1.01	



**A.6.**

Characteristics of consistent (n = 6) and inconsistent responses for percentage change in LF% between baseline and the stepped increase in tempo stimulus (alternative definition).

	Inconsistent response			Consistent response		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			8			2
Average age (over the two visits, yrs)	25.33	0.69		26.83	2.12	
< 30 yrs			2			1
Godin strenuous	10.00	3.08		6.00	3.79	
Godin moderate	6.72	2.80		6.67	4.94	
Godin light	6.50	2.05		5.50	2.73	
Godin weekly	23.22	4.76		18.17	6.61	
Physically active			7			3
Hours spent listening to music/week	15.78	3.43		13.33	3.26	
Formally music trained			16			6
Age started studying music (yrs)	10.00	1.18		11.17	2.06	
Average height (over the two visits, m)	1.72	0.02		1.67	0.03	
Average weight (over the two visits, kg)	71.61	4.24		63.50	3.71	
Average BMI (over the two visits, kg/m <sup>2</sup> )	24.07	1.21		22.75	1.44	

**A.7.**

Characteristics of consistent (n = 9) and inconsistent responses for percentage change in LF% between baseline and the stepped decrease in tempo stimulus (initial definition).

	Inconsistent response			Consistent response		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			6			4
Average age (over the two visits, yrs)	26.33	0.64		24.67	1.61	
< 30 yrs			2			1
Godin strenuous	10.80	3.54		6.00	3.00	
Godin moderate	6.33	3.10		7.33	3.93	
Godin light	3.40	1.85		11.00	2.55	
Godin weekly	20.53	5.36		24.33	5.61	
Physically active			6			4
Hours spent listening to music/week	15.13	4.09		15.22	2.44	
Formally music trained			13			9
Age started studying music (yrs)	9.85	1.27		11.00	1.70	
Average height (over the two visits, m)	1.73	0.02		1.67	0.03	
Average weight (over the two visits, kg)	74.87	4.49		60.78	3.46	
Average BMI (over the two visits, kg/m <sup>2</sup> )	24.85	1.35		21.87	1.08	

**A.8.**

Characteristics of consistent (n = 13) and inconsistent responses for percentage change in LF% between baseline and the stepped decrease in tempo stimulus (alternative definition).

	Inconsistent response			Consistent response		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			6			4
Average age (over the two visits, yrs)	26.09	0.73		25.38	1.21	
< 30 yrs			1			2
Godin strenuous	10.64	3.99		7.62	3.20	
Godin moderate	6.82	3.95		6.62	3.02	
Godin light	3.00	1.81		9.00	2.45	
Godin weekly	20.46	6.18		23.23	5.14	
Physically active			4			6
Hours spent listening to music/week	16.36	5.63		14.15	1.65	
Formally music trained			9			13
Age started studying music (yrs)	11.22	1.66		9.69	1.28	
Average height (over the two visits, m)	1.73	0.02		1.69	0.03	
Average weight (over the two visits, kg)	73.00	4.86		66.69	4.65	
Average BMI (over the two visits, kg/m <sup>2</sup> )	24.42	1.52		23.16	1.28	

## Appendix B: Chapter 3

### B.1.

Characteristics of the initial sample of 58 participants (before exclusions)

	Mean	SEM	Freq
Males			27
Age (yrs)	32.92	1.68	
< 30 yrs			23
Post-menopausal			4
Godin strenuous	14.74	2.21	
Godin moderate	10.09	1.63	
Godin light	7.40	1.07	
Godin weekly	32.22	3.18	
Physically active			34
Hours spent listening to music/week	21.52	2.70	
Formally music trained			30
Age started studying music (yrs)	11.93	1.35	
Currently play instruments			13
Number of instruments currently play	1.46	0.18	
Currently compose			4
Hours spent composing music/week	3.00	1.00	
Currently take part in musical ensemble activities			7
Hours spent taking part in musical ensemble activities/week	7.14	1.72	
Mean height (kg)	1.67	0.01	
Average weight (kg)	72.71	2.54	
Average BMI (kg/m <sup>2</sup> )	25.81	0.68	
Experimental group			29



**B.3.**

## Godin Leisure Time Exercise Questionnaire.

**Godin Leisure Time Exercise Questionnaire**

During a typical week, how many times on average do you do the following kinds of exercise for more than 15 minutes during your free time (write on each line the appropriate number).

	Times per week
a). Strenuous exercise (heart beats rapidly) (e.g. running, jogging, hockey, football, rugby, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance cycling)	_____
b). Moderate exercise (not exhausting) (e.g. fast walking, baseball, tennis, easy cycling, volleyball, badminton, easy swimming, alpine skiing, dancing)	_____
c). Mild exercise (minimal effort) (e.g. yoga, archery, fishing, bowling, golf, easy walking)	_____

**Thank you**

**B.4.**

**Music background questionnaire.**

Music background questionnaire

We would be grateful if you could please complete this questionnaire. This information will aid with the analysis of the results of this study. All information will be strictly confidential and will be made anonymous before being analysed.

1. How many hours a week do you spend listening to music? .....

2. What are your five most preferred genres of music? (please list in order of preference where 1 is most preferred)

- 1) .....
- 2) .....
- 3) .....
- 4) .....
- 5) .....

3. Do you currently play any instruments? (please circle)    Yes    No

4. If yes, please list the instruments you play, the number of years you have been playing each instrument and the highest grade attained in a music examination for each instrument

Instrument	Years playing	Highest grade attained
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

5. Do you currently take part in music-making activities? (please circle)    Yes    No

6. If yes, what music-making activities do you take part in and how many hours a week do you spend taking part in these activities?

Music-making activity	Hours per week
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

7. Have you ever had any formal music training? (please circle)

Formal music training is defined as receiving music lessons in which you learn about melodic and rhythmic concepts and how to play an instrument.

If you are a self-taught musician, please answer yes.

- a. Yes, I had formal musical training
- b. Yes, I am a self-taught musician
- c. No

8. If yes, at what age did you start your music training and on what instrument did you start your music training?

Age	Instrument
.....	.....

9. What type of music would you most prefer to listen to in this environment?

.....

.....

.....

.....

Thank you

**B.5.**

Initial VAS questionnaire.

Initial questionnaire

1. How do you feel right now? Please place a vertical mark on the line below to indicate how you feel.

Not stimulated |-----| Very stimulated

Not relaxed |-----| Very relaxed

Not stressed |-----| Very stressed

Not calm |-----| Very calm

Not happy |-----| Very happy

Not sad |-----| Very sad

Thank you

**B.6.****Baseline and recovery condition VAS questionnaire.**

1. How do you feel right now? Please place a vertical mark on the line below to indicate how you feel.

Not stimulated | \_\_\_\_\_ | Very stimulated

Not relaxed | \_\_\_\_\_ | Very relaxed

Not stressed | \_\_\_\_\_ | Very stressed

Not calm | \_\_\_\_\_ | Very calm

Not happy | \_\_\_\_\_ | Very happy

Not sad | \_\_\_\_\_ | Very sad

2. What were your reactions to the silence? Please place a vertical mark on the line below to indicate the extent to which the silence was:

Very negative | \_\_\_\_\_ | Very positive

Very relaxing | \_\_\_\_\_ | Very stimulating

Very unpleasant | \_\_\_\_\_ | Very pleasant

Very soothing | \_\_\_\_\_ | Very irritating

Thank you



**B.7.**

Stimulus VAS questionnaire.

Stim post-condition questionnaire

1. How do you feel right now? Please place a vertical mark on the line below to indicate how you feel.

Not stimulated \_\_\_\_\_ Very stimulated

Not relaxed \_\_\_\_\_ Very relaxed

Not stressed \_\_\_\_\_ Very stressed

Not calm \_\_\_\_\_ Very calm

Not happy \_\_\_\_\_ Very happy

Not sad \_\_\_\_\_ Very sad

2. What were your reactions to the audio? Please place a vertical mark on the line below to indicate the extent to which the audio was:

Very negative \_\_\_\_\_ Very positive

Very relaxing \_\_\_\_\_ Very stimulating

Very unpleasant \_\_\_\_\_ Very pleasant

Very soothing \_\_\_\_\_ Very irritating

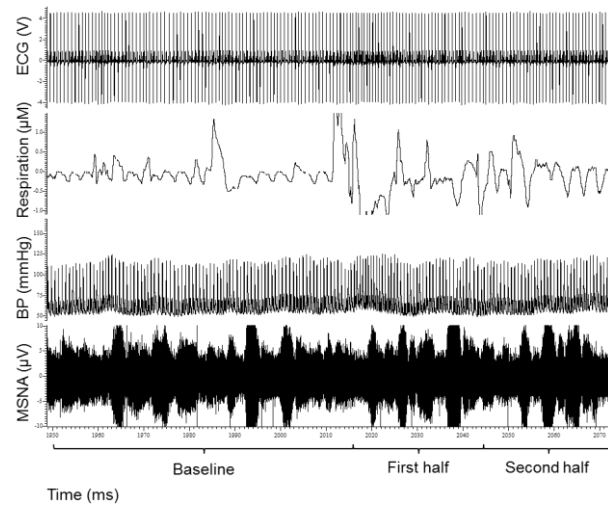
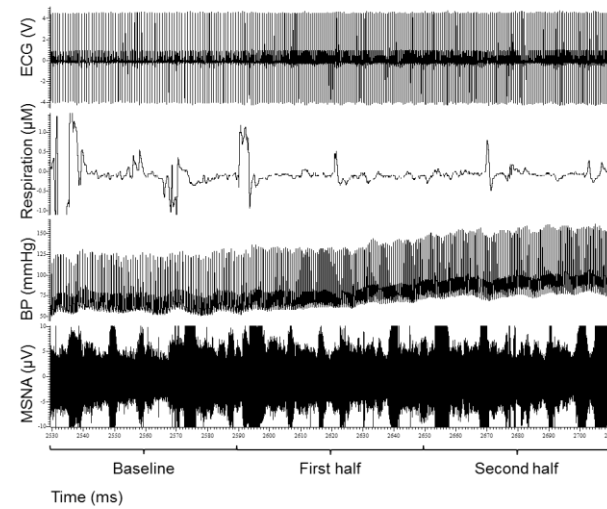
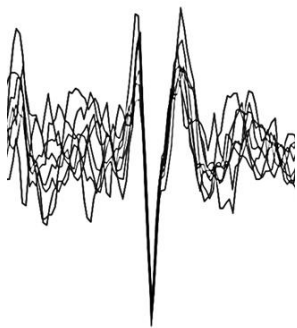
3. How did you interpret the audio? (please circle one statement)

- a) I interpreted the audio as music
- b) I did not interpret the audio as music

4. To what extent did you find the audio familiar? Please place a vertical mark on the line below to indicate how familiar you found the audio.

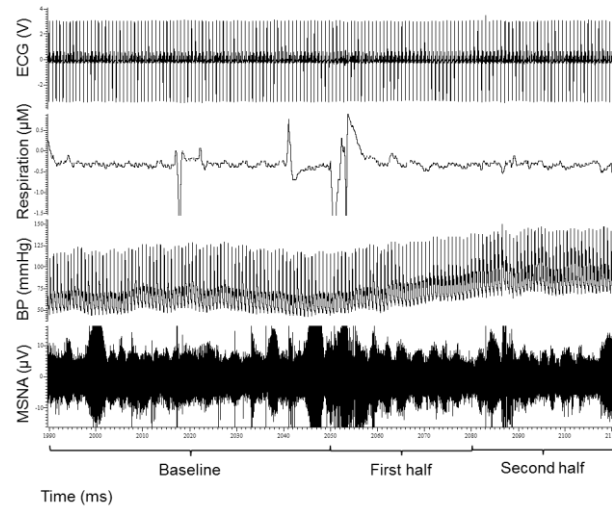
Not familiar \_\_\_\_\_ Very familiar

Thank you

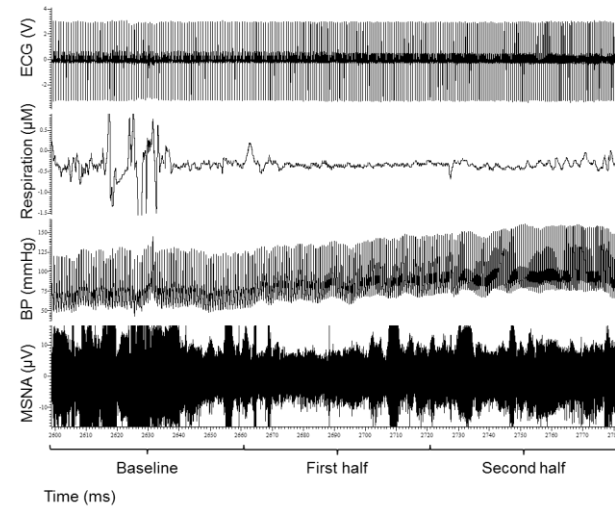
**B.8.****Confirmed MSNA unit 1****Cold pressor test****Hand grip test****Overlaid action potentials**

## Confirmed MSNA unit 2

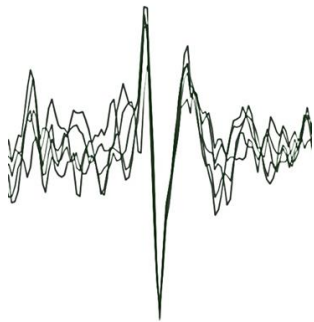
### Cold pressor test



### Hand grip test

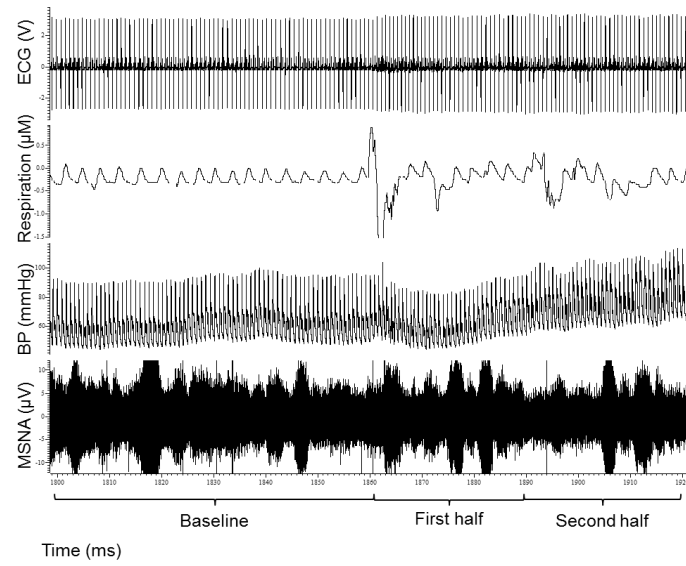


### Overlaid action potentials

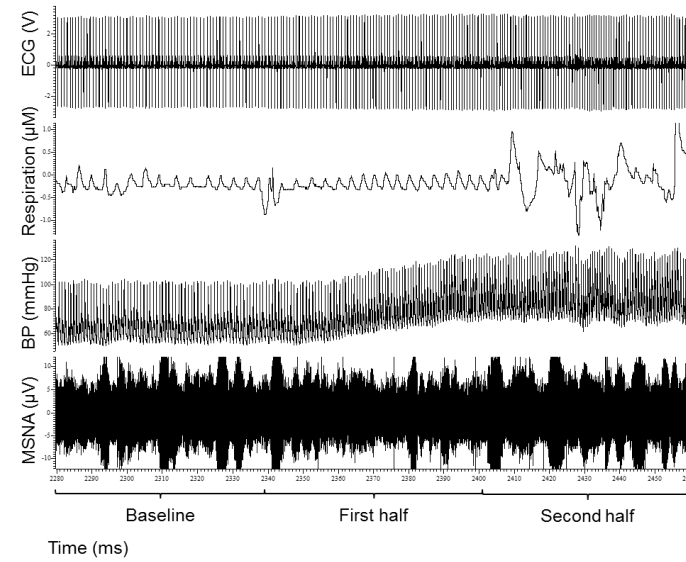


## Confirmed MSNA unit 3

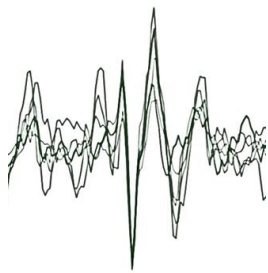
### Cold pressor test



### Hand grip test

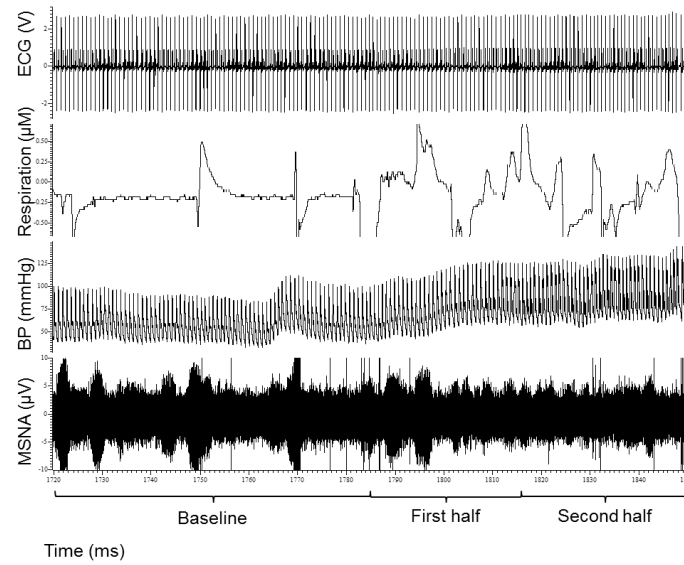


### Overlaid action potentials

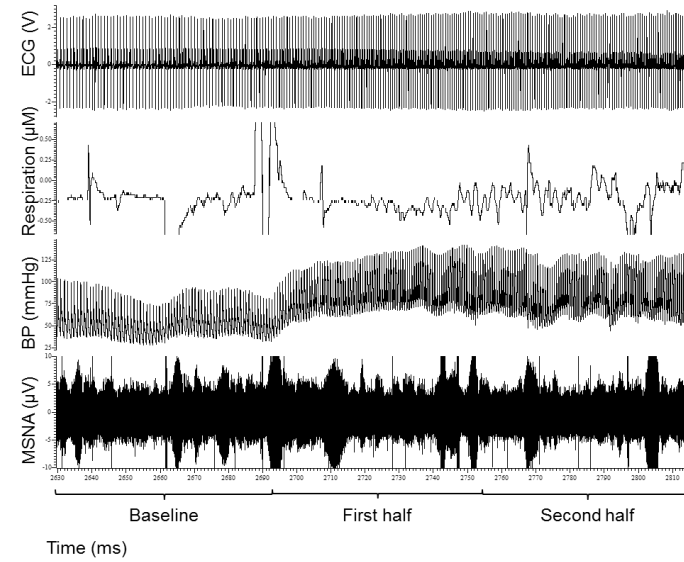


## Confirmed MSNA unit 4

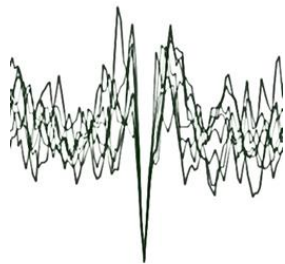
### Cold pressor test

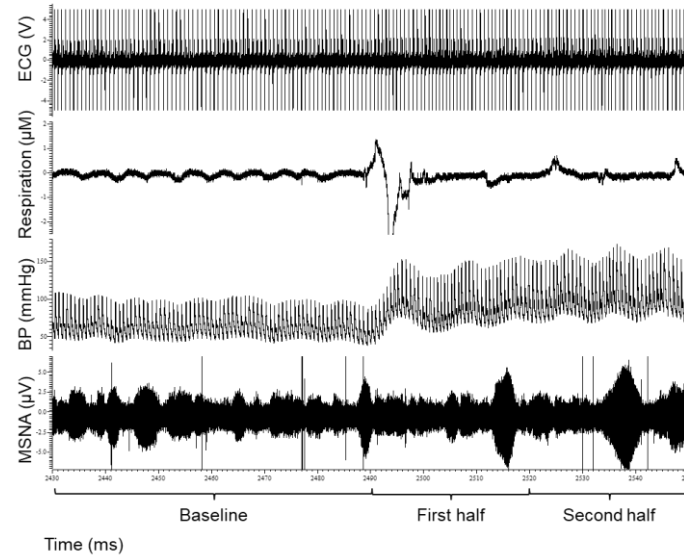


### Hand grip test



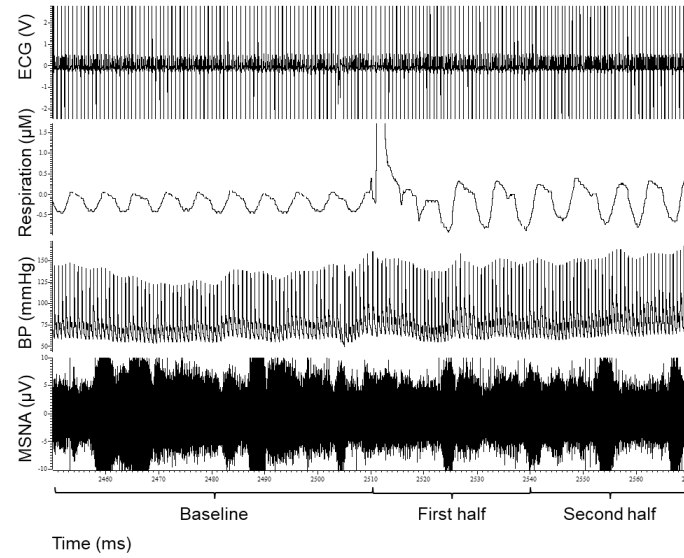
### Overlaid action potentials



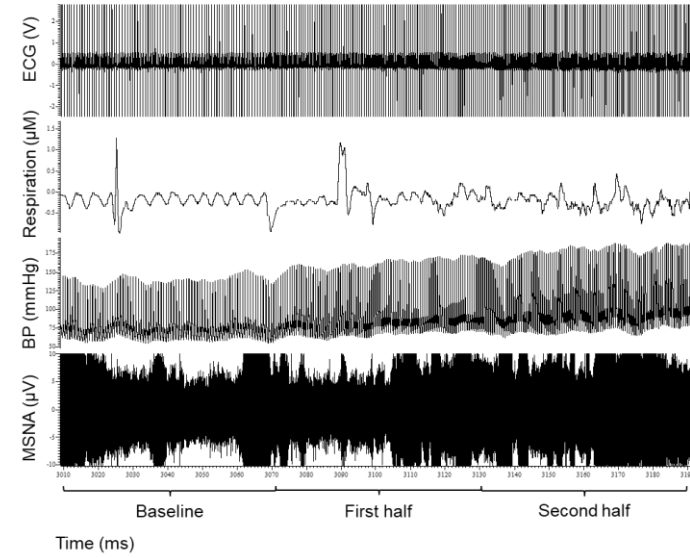
**Confirmed MSNA unit 5****Cold pressor test**

## Confirmed MSNA unit 6

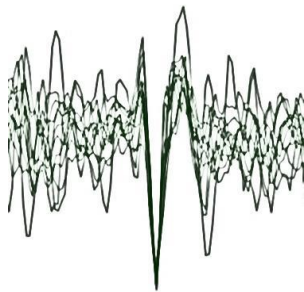
### Cold pressor test



### Hand grip test

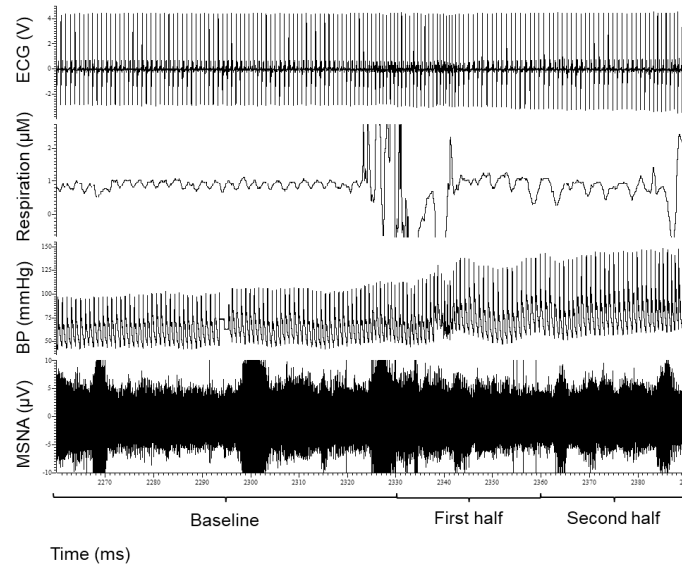


### Overlaid action potentials

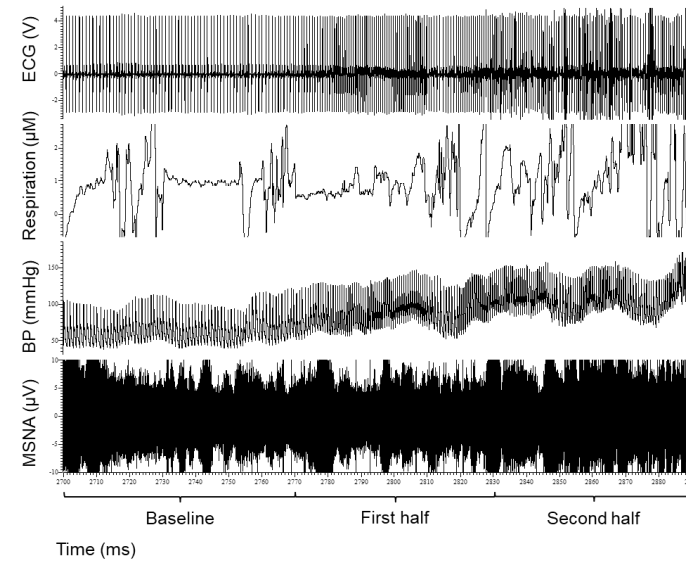


## Confirmed MSNA unit 7

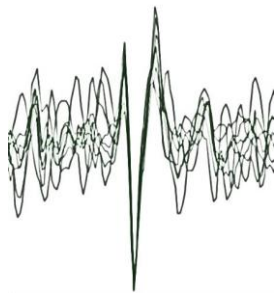
### Cold pressor test



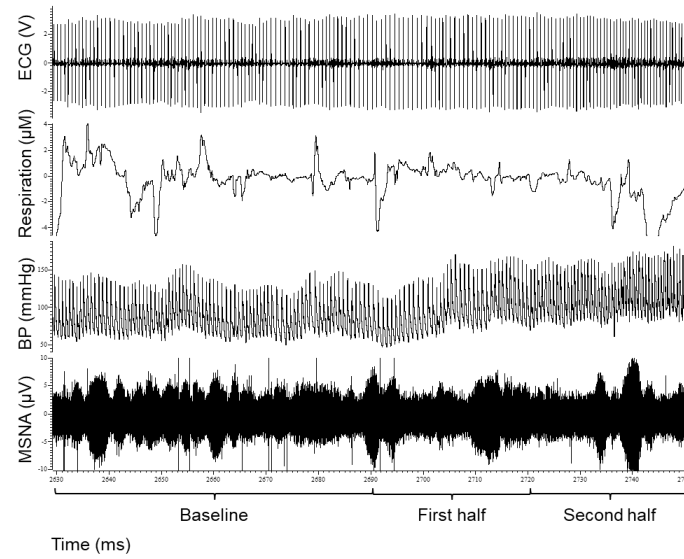
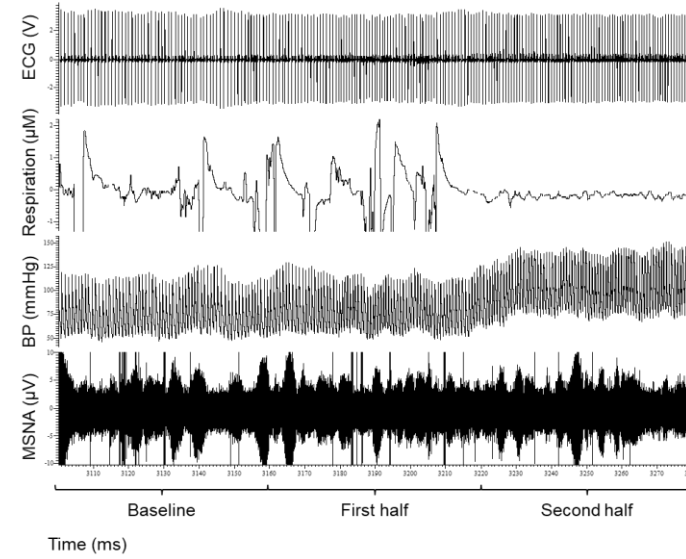
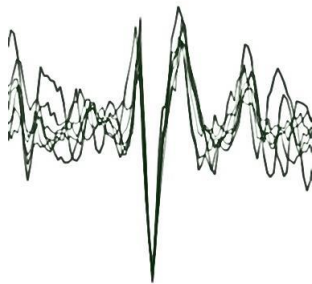
### Hand grip test

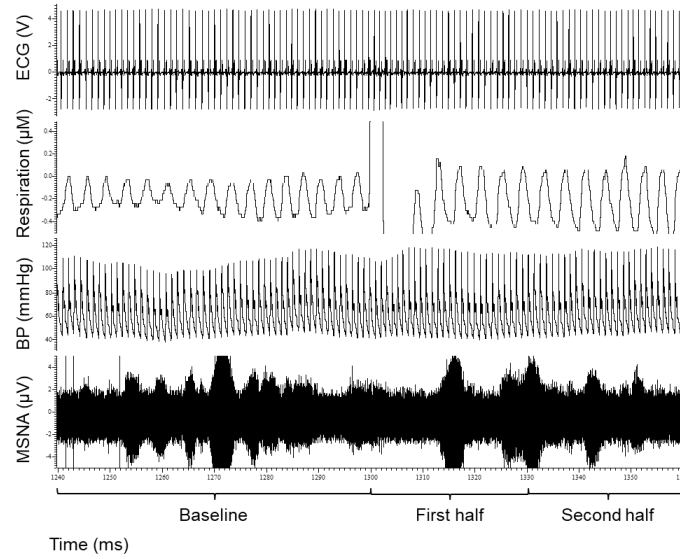
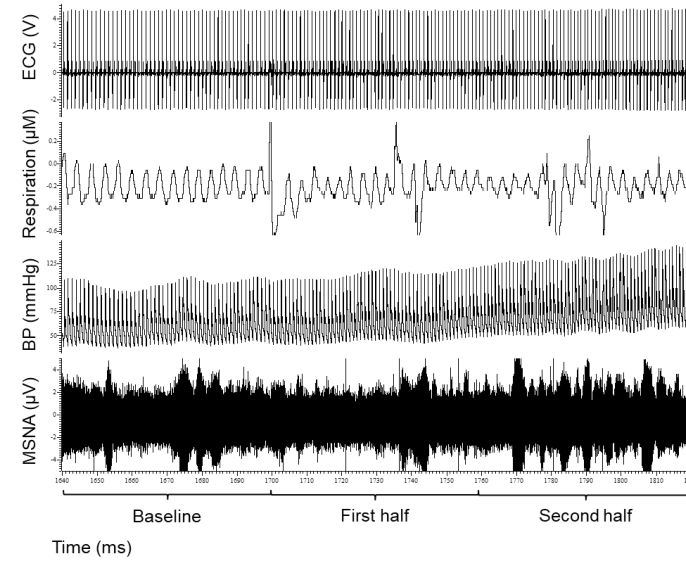
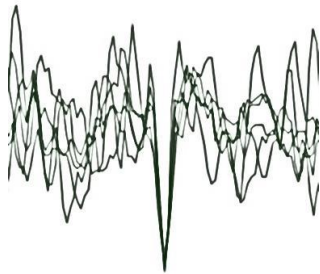


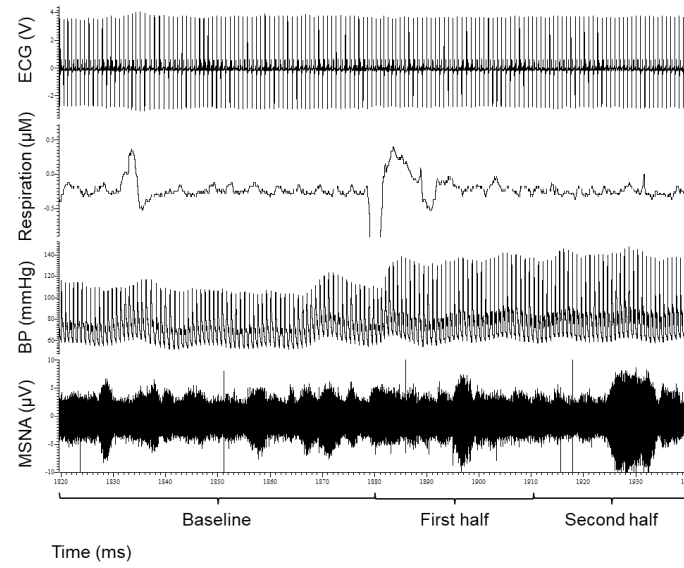
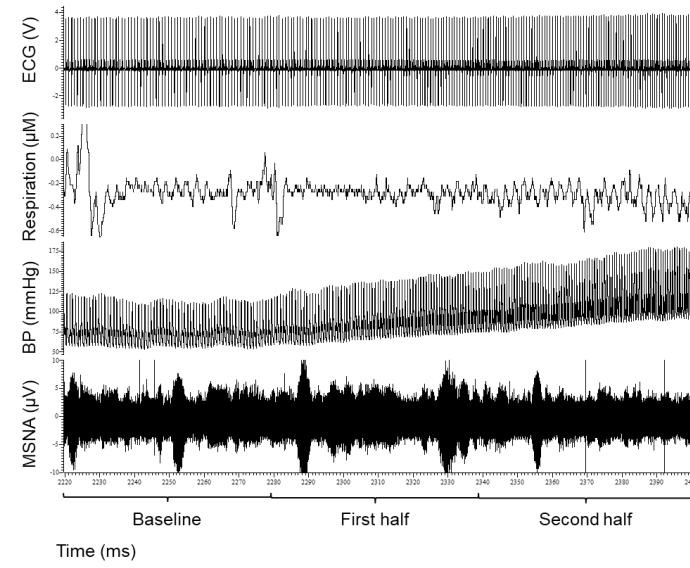
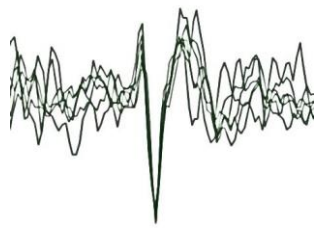
### Overlaid action potentials





**Confirmed MSNA unit 8****Cold pressor test****Hand grip test****Overlaid action potentials**

**Confirmed MSNA unit 9****Cold pressor test****Hand grip test****Overlaid action potentials**

**Confirmed MSNA unit 10****Cold pressor test****Hand grip test****Overlaid action potentials**

**B.9.**

Characteristics of the final sample of 52 participants.

	Mean	SEM	Freq
Males			24
Age	31.98	1.74	
< 30 yrs			34
Post-menopausal			2
Godin strenuous exercise score	14.71	2.30	
Godin moderate exercise score	10.39	1.75	
Godin light exercise score	6.87	1.14	
Godin weekly activity level score	31.96	3.18	
Physically active			31
Hours spent listening to music/week	21.08	2.85	
Formally music trained			26
Age started studying music (yrs)	12.15	1.54	
Currently play music			10
Number of instruments currently play	1.60	0.22	
Currently composes			4
Hours spent composing music/week	3.00	1.00	
Currently take part in musical ensemble activities			5
Hours spent taking part in musical ensemble activities/week	5.20	1.71	
Average height (m)	1.67	0.02	
Average weight (kg)	72.06	2.63	
Average BMI (kg/m <sup>2</sup> )	25.45	0.60	
Experimental group			27

**B.10.**

Characteristics of the final sample of 52 participants split by group (control, experimental).

	Control group (n = 25)			Experimental group		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			14			10
Age	31.76	2.56		32.19	2.42	
< 30 yrs			17			17
Post-menopausal			1			1
Godin strenuous exercise score	16.56	3.02		13.00	3.47	
Godin moderate exercise score	10.40	2.29		10.37	2.66	
Godin light exercise score	7.08	1.70		6.67	1.56	
Godin weekly activity level score	34.04	4.74		30.04	4.33	
Physically active			15			16
Hours spent listening to music/week	20.20	3.59		21.89	4.42	
Formally music trained			16			10
Age started studying music (yrs)	12.31	2.23		11.90	1.96	
Currently play music			5			5
Number of instruments currently play	1.40	0.24		1.80	0.37	
Currently composes			2			2
Hours spent composing music/week	2.00	0.00		4.00	2.00	
Currently take part in musical ensemble activities			4			1
Hours spent taking part in musical ensemble activities/week	6.00	1.96		2.00	0.00	
Average height (m)	1.68	0.02		1.66	0.02	
Average weight (kg)	72.36	3.77		71.78	3.73	
Average BMI (kg/m <sup>2</sup> )	25.31	0.93		25.58	0.80	

**B.11.**

Characteristics of inconsistent (n = 32) and consistent responses for percentage change in LF% between baseline and the three auditory stimuli.

	Inconsistent response (n = 32)			Consistent response		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			15			9
Age (yrs)	33.59	2.44		29.40	2.26	
< 30 yrs			19			15
Post-menopausal			2			0
Godin strenuous exercise score	18.00	3.31		9.45	2.46	
Godin moderate exercise score	10.94	2.37		9.50	2.57	
Godin light exercise score	7.31	1.54		6.15	1.71	
Godin weekly activity level score	36.25	4.48		25.10	3.79	
Physically active			22			9
Hours spent listening to music/week	20.63	3.48		21.80	5.00	
Formally music trained			15			11
Age started studying music (yrs)	13.07	2.53		10.91	1.25	
Currently play instruments			6			4
Number of instruments currently play	0.31	0.13		0.30	0.15	
Currently compose music			2			2
Hours spent composing music/week	0.25	0.20		0.20	0.14	
Currently take part in ensemble music activities			5			0
Hours spent taking part in ensemble music activities/week	0.81	0.42				
Height (m)	1.68	0.02		1.67	0.03	
Weight (kg)	74.31	3.55		68.45	3.77	
BMI (kg/m <sup>2</sup> )	26.09	0.84		24.41	0.78	
Experimental group			15			12

**B.12.**

Characteristics of inconsistent (n = 31) and consistently a (type 1 or type 2) responder

	Inconsistent response (n = 31)			Consistently a (type 1 or type 2) responder		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			15			9
Age (yrs)	31.97	2.34		32.00	2.66	
< 30 yrs			20			14
Post-menopausal			1			1
Godin strenuous exercise score	17.13	3.19		11.14	3.15	
Godin moderate exercise score	10.00	2.33		10.95	2.69	
Godin light exercise score	6.97	1.51		6.71	1.79	
Godin weekly activity level score	34.10	3.89		28.81	5.44	
Physically active			21			10
Hours spent listening to music/week	21.23	3.53		20.86	4.86	
Formally music trained			14			12
Age started studying music (yrs)	12.71	2.72		11.50	1.21	
Currently play instruments			7			3
Number of instruments currently play	0.39	0.14		0.19	0.11	
Currently compose			3			1
Hours spent composing music/week	0.32	0.21		0.10	0.10	
Currently take part in music ensemble activities			4			1
Hours spent taking part in ensemble music activities/week	0.68	0.41		0.24	0.24	
Height (m)	1.68	0.02		1.66	0.03	
Weight (kg)	74.23	3.67		68.86	3.57	
BMI (kg/m <sup>2</sup> )	25.92	0.88		24.75	0.75	
Experimental group			15			12

## **Appendix C: Chapter 4**

C.1.

Music background questionnaire.

**Music background questionnaire**

We would be grateful if you could please complete this questionnaire. This information will aid with the analysis of the results of this study. All information will be strictly confidential and will be made anonymous before being analysed.

1. How many hours a week do you spend listening to music? .....

2. What are your five most preferred genres of music? (please list in order of preference where 1 is most preferred)

- 1) .....
- 2) .....
- 3) .....
- 4) .....
- 5) .....

3. Have you ever had any formal music training? (please circle)

Formal music training is defined as receiving music lessons in which you learn about melodic and rhythmic concepts and learn how to play an instrument and/or sing.

If you are a self-taught musician, please answer yes.

- a. Yes, I had formal musical training
- b. Yes, I am a self-taught musician
- c. No

4. If yes, at what age did you start your music training and on what instrument did you start your music training?

<b>Age</b>	<b>Instrument</b>
.....	.....

**Please turn over**

5. Do you currently play any instruments? (please circle)    Yes    No

6. If yes, please list the instruments you play, how many hours per week you spend playing each instrument and the number of years you have been playing each instrument

Instrument	Hours per week	Years playing
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

7. Do you currently take part in any singing? (please circle)    Yes    No

8. If yes, how many hours per week do you spend singing and how many years have you been singing?

Hours per week	Years singing
.....	.....

9. Do you currently take part in any music composition activities? (Please circle)    Yes    No

10. If yes, what music composition activities do you take part in and how many hours a week do you spend taking part in these activities?

Music composition activity	Hours per week
.....	.....
.....	.....
.....	.....
.....	.....

11. Do you currently take part in any music ensemble activities? (please circle)    Yes    No

12. If yes, what music ensemble activities do you take part in and how many hours a week do you spend taking part in these activities?

Music ensemble activity	Hours per week
.....	.....
.....	.....
.....	.....
.....	.....

**Thank you**



**C.2.**

VAS questionnaire for the tVNS only, music only and tVNS + music stimuli.

1. How do you feel right now? Please place a vertical mark on the line below to indicate how you feel.

Not stimulated | \_\_\_\_\_ | Very stimulated

Not relaxed | \_\_\_\_\_ | Very relaxed

Not stressed | \_\_\_\_\_ | Very stressed

Not calm | \_\_\_\_\_ | Very calm

Not happy | \_\_\_\_\_ | Very happy

Not sad | \_\_\_\_\_ | Very sad

2. How did you perceive the audio? Please place a vertical mark on the line below to indicate the extent to which the audio was:

Very negative | \_\_\_\_\_ | Very positive

Very relaxing | \_\_\_\_\_ | Very stimulating

Very unpleasant | \_\_\_\_\_ | Very pleasant

Very soothing | \_\_\_\_\_ | Very irritating

Very unobtrusive | \_\_\_\_\_ | Very obtrusive

3. How did you interpret the audio? (please circle one statement)

a) I interpreted the audio as music

b) I did not interpret the audio as music

4. To what extent did you find the audio familiar? Please place a vertical mark on the line below to indicate how familiar you found the audio.

Not familiar | \_\_\_\_\_ | Very familiar

5. To what extent did you find the audio suited the room you are in? Please place a vertical mark on the line below to indicate how well you found the audio suited the current room.

Did not suit | \_\_\_\_\_ | Suited very well

6. To what extent did you feel electrical impulses in your ear? Please place a vertical mark on the line below to indicate the extent to which you felt electrical impulses in your ear.

Did not feel | \_\_\_\_\_ | Felt intensely

Thank you

**C.3.**

**VAS questionnaire for the sham condition.**

1. How do you feel right now? Please place a vertical mark on the line below to indicate how you feel.

Not stimulated | \_\_\_\_\_ | Very stimulated

Not relaxed | \_\_\_\_\_ | Very relaxed

Not stressed | \_\_\_\_\_ | Very stressed

Not calm | \_\_\_\_\_ | Very calm

Not happy | \_\_\_\_\_ | Very happy

Not sad | \_\_\_\_\_ | Very sad

2. How did you perceive the silence? Please place a vertical mark on the line below to indicate the extent to which the silence was:

Very negative | \_\_\_\_\_ | Very positive

Very relaxing | \_\_\_\_\_ | Very stimulating

Very unpleasant | \_\_\_\_\_ | Very pleasant

Very soothing | \_\_\_\_\_ | Very irritating

Very unobtrusive | \_\_\_\_\_ | Very obtrusive

3. To what extent did you feel electrical impulses in your ear? Please place a vertical mark on the line below to indicate the extent to which you felt electrical impulses in your ear.

Did not feel | \_\_\_\_\_ | Felt intensely

**Thank you**

**C.4.**

Characteristics of the initial sample of 32 participants (before exclusions).

	<b>Mean</b>	<b>SEM</b>	<b>Freq</b>
Males			15
Age (yrs)	22.50	0.50	
< 30 yrs			14
Post-menopausal			1
Godin strenuous	22.50	22.50	
Godin moderate	30.00	20.00	
Godin light	15.00	15.00	
Godin weekly	67.50	12.50	
Physically active			22
Hours spent listening to music/week	17.50	12.50	
Formally music trained			23
Age started studying music (yrs)	8.50	2.50	
Currently play instruments			11
Number of instruments currently play	2.50	1.50	
Hours spent playing/week	7.50	2.50	
Currently sing			11
Hours spent singing/week	5.00	1.00	
Currently compose			6
Hours spent composing music/week	3.00	1.00	
Mean height (kg)	1.72	0.02	
Average weight (kg)	72.99	3.03	
Average BMI (kg/m <sup>2</sup> )	24.32	0.73	

**C.5.**

Characteristics of males (n = 12) and females (n = 12) (following eight exclusions).

	Males (n = 12)			Females		
	Mean	SEM	Freq	Mean	SEM	Freq
Age	34.08	3.23		33.17	2.66	
< 30			5			5
Post-menopausal						1
Godin strenuous exercise score	12.33	3.31		13.50	4.22	
Godin moderate exercise score	16.88	4.56		11.67	4.14	
Godin light exercise score	12.25	4.04		6.50	2.44	
Godin weekly activity level score	41.46	7.66		31.67	6.42	
Physically active			8			8
Hours spent listening to music/week	12.17	3.49		17.50	3.57	
Formally music trained			9			8
Age started studying music (yrs)	10.44	1.18		9.75	1.67	
Number instruments currently play	1.00	0.00				4
Currently play instruments			4	1.25	0.25	
Hours spent playing/week	4.00	1.22		4.88	1.88	
Currently sing			5			3
Hours spend singing/week	3.20	0.97		4.33	1.86	
Currently compose			3			1
Hours spent composing music/week	3.33	0.67		10.00	0.00	
Average height (m)	1.78	0.02		1.69	0.01	
Average weight (kg)	78.75	3.64		69.94	4.92	
Average BMI (kg/m <sup>2</sup> )	24.88	0.94		24.24	1.38	

**C.6.**

Characteristics of young (n = 10) and older participants (n = 14) (following eight exclusions).

	Young (n = 10)			Older		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			5			7
Age	25.80	0.74		39.21	2.58	
Post-menopausal						1
Godin strenuous exercise score	6.30	2.70		17.64	3.63	
Godin moderate exercise score	15.75	5.32		13.21	3.77	
Godin light exercise score	8.70	3.33		9.86	3.42	
Godin weekly activity level score	30.75	8.55		40.71	6.03	
Physically active			5			11
Hours spent listening to	17.40	3.73		13.00	3.40	
Formally music trained			7			10
Age started studying music	9.29	1.02		10.70	1.51	
Currently play instruments			4			4
Number of instruments currently	1.00	0.00		1.25	0.25	
Hours spent playing/week	6.00	1.68		2.88	0.83	
Currently sings			4			4
Hours spend singing/week	3.00	1.08		4.25	1.44	
Currently compose			3			1
Hours spent composing	6.00	2.00		2.00	0.00	
Average height (m)	1.75	0.02		1.72	0.02	
Average weight (kg)	75.65	4.22		73.41	4.55	
Average BMI (kg/m <sup>2</sup> )	24.60	1.04		24.53	1.23	

**C.7.**

Characteristics of non-musicians (n = 7) and musicians (n = 17) (following eight exclusions).

	Non-musicians (n = 7)			Musicians		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			3			9
Age	35.57	4.54		32.82	2.28	
< 30 yrs			3			7
Post-menopausal						1
Godin strenuous exercise score	14.71	5.66		12.18	2.98	
Godin moderate exercise score	7.86	4.06		16.91	3.87	
Godin light exercise score	4.71	2.69		11.29	3.10	
Godin weekly activity level score	27.29	9.93		40.38	5.68	
Physically active			4			12
Hours spent listening to music/week	12.71	5.08		15.71	2.93	
Age started studying music				10.12	0.97	
Currently play instruments						8
Number of instruments currently play				1.13	0.13	
Hours spent playing/week				4.44	1.05	
Currently sing			2			6
Hours spend singing/week	1.50	0.50		4.33	0.99	
Currently compose						4
Hours spent composing music/week				5.00	1.73	
Average height (m)	1.72	0.03		1.74	0.02	
Average weight (kg)	70.39	6.41		75.97	3.60	
Average BMI (kg/m <sup>2</sup> )	23.59	1.43		24.96	1.01	

**C.8.**

Characteristics of participants who felt tVNS (n = 14) and those who did not (n = 10) (following eight exclusions).

	Felt tVNS (n = 14)			Did not feel tVNS		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			8			4
Age	33.86	3.11		33.30	2.46	
< 30 yrs			7			3
Post-menopausal			1			0
Godin strenuous exercise score	13.14	3.43		12.60	4.28	
Godin moderate exercise score	10.36	3.08		19.75	5.71	
Godin light exercise score	5.14	1.79		15.30	4.66	
Godin weekly activity level score	28.64	5.76		47.65	7.91	
Physically active			7			9
Hours spent listening to music/week	15.64	3.34		13.70	3.96	
Formally music trained			8			9
Age started studying music	10.75	1.25		9.56	1.51	
Currently play instruments			3			5
Number of instruments currently play	1.00	0.00		1.20	0.20	
Hours spent playing/week	6.33	2.33		3.30	0.77	
Currently sing			5			3
Hours spend singing/week	3.20	1.24		4.33	1.20	
Currently compose			2			2
Hours spent composing music/week	6.00	4.00		4.00	0.00	
Average height (m)	1.74	0.02		1.73	0.02	
Average weight (kg)	74.52	3.38		74.10	6.08	
Average BMI (kg/m <sup>2</sup> )	24.65	0.91		24.44	1.57	

**C.9.**

Characteristics of the (final) sample of 24 participants (following eight exclusions).

	<b>Mean</b>	<b>SEM</b>	<b>Freq</b>
Males			12
Age	33.63	2.05	
< 30 yrs			10
Post-menopausal			1
Godin strenuous exercise score	12.92	2.62	
Godin moderate exercise score	14.27	3.06	
Godin light exercise score	9.38	2.38	
Godin weekly activity level score	36.56	4.99	
Physically active			16
Hours spent listening to music/week	14.83	2.50	
Formally music trained			17
Age started studying music (yrs)	10.12	0.97	
Currently play music			8
Number of instruments currently play	1.13	0.13	
Hours spent playing/week	4.44	1.05	
Currently sing			8
Hours spent singing/week	3.63	0.86	
Currently composes			4
Hours spent composing music/week	5.00	1.73	
Average height (m)	1.73	0.02	
Average weight (kg)	74.34	3.13	
Average BMI (kg/m <sup>2</sup> )	24.56	0.82	



**C.10.**

Characteristics of inconsistent (n = 12) and consistent responders (n = 12).

	Inconsistent response (n = 12)			Consistently a (type 1 or 2) responder		
	Mean	SEM	Freq	Mean	SEM	Freq
Males			6			6
Age	29.33	1.49		37.92	3.46	
< 30 yrs			6			4
Post-menopausal			0			1
Godin strenuous exercise score	11.25	3.53		14.58	3.98	
Godin moderate exercise score	13.54	4.57		15.00	4.26	
Godin light exercise score	11.25	3.00		7.50	3.76	
Godin weekly activity level score	36.04	6.92		37.08	7.51	
Physically active			9			7
Hours spent listening to music/week	17.00	3.84		12.67	3.26	
Formally music trained			11			6
Age started studying music	9.91	1.31		10.50	1.50	
Currently play instruments			6			2
Number of instruments currently play	1.17	0.17		1.00	0.00	
Hours spent playing/week	4.58	1.20		4.00	3.00	
Currently sing			3			5
Hours spend singing/week	3.33	1.33		3.80	1.24	
Currently compose			3			1
Hours spent composing music/week	6.00	2.00		2.00	0.00	
Average height (m)	1.76	0.02		1.71	0.02	
Average weight (kg)	78.77	4.36		69.92	4.29	
Average BMI (kg/m <sup>2</sup> )	25.51	1.30		23.61	0.98	

## Appendix D: Ethics approvals

Performance, Governance and Operations  
 Research & Innovation Service  
 Charles Thackrah Building  
 101 Clarendon Road  
 Leeds LS2 9LJ Tel: 0113 343 4873  
 Email: [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk)



UNIVERSITY OF LEEDS

Beatrice Bretherton  
 School of Music  
 University of Leeds  
 Leeds, LS2 9JT

**PVAC & Arts Joint Faculty Research Ethics Committee**  
**University of Leeds**

17 February 2015

Dear Beatrice

**Title of study** A pilot study investigating the effects of stepped increases and decreases in tempo on autonomic nervous control of the heart

**Ethics reference** PVAR 14-050

I am pleased to inform you that the above research application has been reviewed by the Arts and PVAC (PVAR) Faculty Research Ethics Committee and following receipt of your response to the Committee's initial comments, I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	Version	Date
PVAR 14-050 Final ethics application form.pdf	1	28/01/15
PVAR 14-050 declaration.pdf	1	28/01/15
PVAR 14-050 Recruitment material.docx	1	28/01/15
PVAR 14-050 Information Sheet 02.docx	2	17/02/15
PVAR 14-050 Consent form 02.docx	2	17/02/15
PVAR 14-050 Health questionnaires.docx	1	28/01/15
PVAR 14-050 Music background questionnaires.docx	1	28/01/15

Please notify the committee if you intend to make any amendments to the original research as submitted at date of this approval as all changes must receive ethical approval prior to implementation. The amendment form is available at <http://ris.leeds.ac.uk/EthicsAmendment>. Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited. There is a checklist listing examples of documents to be kept which is available at <http://ris.leeds.ac.uk/EthicsAudits>.

We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk).

Yours sincerely

Jennifer Blaikie  
 Senior Research Ethics Administrator, Research & Innovation Service  
 On behalf of Dr William Rea, Chair, [PVAR FREC](#)

CC: Student's supervisor(s)

Performance, Governance and Operations  
 Research & Innovation Service  
 Charles Thackrah Building  
 101 Clarendon Road  
 Leeds LS2 9LJ Tel: 0113 343 4873  
 Email: [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk)



UNIVERSITY OF LEEDS

Biological Sciences Faculty Research Ethics Committee  
 University of Leeds

Beatrice Bretherton  
 School of Music  
 University of Leeds  
 LS2 9JT

12 February 2016

Dear Beatrice

**Title of study:** A comparison of the effects of stepped increases and decreases in tempo on autonomic control of the heart  
**Ethics reference:** BIOSCI 15-014, response 2

I am pleased to inform you that the above research application has been reviewed by the Faculty of Biological Sciences Research Ethics Committee and following receipt of your response to the Committee's comments, I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	Version	Date
BIOSCI 15-014 Follow-up study 1 ethics form final - amended one visit.doc	3	04/02/16
BIOSCI 15-014 Recruitment material - amended one visit.docx	2	26/01/16
BIOSCI 15-014 Study poster 01 - amended one visit.docx	2	26/01/16
BIOSCI 15-014 Information sheet 02 - amended one visit.docx	3	04/02/16
BIOSCI 15-014 Consent form 03 - amended.docx	3	04/02/16
BIOSCI 15-014 Health questionnaire 02 - amended.docx	3	04/02/16
BIOSCI 15-014 Music background questionnaire - amended.docx	2	26/01/16
BIOSCI 15-014 Risk assessment.doc	1	26/01/16
BIOSCI 15-014 Follow-up study 1 ethics form final.pdf	1	17/12/15
BIOSCI 15-014 Declaration.pdf	1	17/12/15
BIOSCI 15-014 Baseline postcondition questionnaire (VAS)03.docx	1	17/12/15
BIOSCI 15-014 Increase and decrease postcondition questionnaire (VAS)05.docx	1	17/12/15
BIOSCI 15-014 Recovery postcondition questionnaire (VAS)03.docx	1	17/12/15
BIOSCI 15-014 Stable postcondition questionnaire (VAS)03.docx	1	17/12/15
BIOSCI 15-014 Starting questionnaire (VAS)05.docx	1	17/12/15

Please notify the committee if you intend to make any amendments to the original research as submitted at date of this approval, including changes to recruitment methodology. All changes must receive ethical approval prior to implementation. The amendment form is available at <http://ris.leeds.ac.uk/EthicsAmendment>

Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be

audited. There is a checklist listing examples of documents to be kept which is available at <http://ris.leeds.ac.uk/EthicsAudits>

We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk).

Yours sincerely

Jennifer Blaikie  
 Senior Research Ethics Administrator, Research & Innovation Service  
 On behalf of Prof Edward White, Chair, [BIOSCI Faculty Research Ethics Committee](mailto:BIOSCI.Faculty.Research.Ethics.Committee)

CC: Student's supervisor(s)

Research & Innovation Service  
Level 11, Wortley Building  
University of Leeds  
Leeds, LS2 9JL  
Tel: 0113 343 4873  
Email: [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk)



UNIVERSITY OF LEEDS

Biological Sciences Faculty Research Ethics Committee  
University of Leeds

Beatrice Bretherton  
School of Music  
University of Leeds  
LS2 9JT

11 October 2016

Dear Beatrice

**Title of study:** An investigation into the effects of music and transcutaneous vagal nerve stimulation on autonomic control of the heart  
**Ethics reference:** BIOSCI 16-001

I am pleased to inform you that the above research application has been reviewed by the Faculty of Biological Sciences Research Ethics Committee and following receipt of your response to the Committee's comments, I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	Version	Date
BIOSCI 16-001 Committee Provisional.doc	1	03/10/16
BIOSCI 16-001 Net-1000 device study ethics form amended02.doc	3	03/10/16
BIOSCI 16-001 Information sheet amended02.docx	3	03/10/16
BIOSCI 16-001 Consent form amended02.docx	3	03/10/16
BIOSCI 16-001 Committee Provisional Response.doc	1	13/09/16
BIOSCI 16-001 Recruitment material amended.docx	2	13/09/16
BIOSCI 16-001 Study poster amended.docx	2	13/09/16
BIOSCI 16-001 Declaration.pdf	1	24/08/16
BIOSCI 16-001 sham postcondition VAS questionnaire.docx	1	24/08/16
BIOSCI 16-001 TMS only, music only, TMS+music and sham postcondition VAS questionnaires.docx	1	24/08/16
BIOSCI 16-001 Baseline and recovery postcondition VAS questionnaires.docx	1	24/08/16
BIOSCI 16-001 Health questionnaire.docx	1	24/08/16
BIOSCI 16-001 Music background questionnaire.docx	1	24/08/16
BIOSCI 16-001 Sharing VAS questionnaire.docx	1	24/08/16
BIOSCI 16-001 Risk assessment.docx	1	24/08/16

Please notify the committee if you intend to make any amendments to the information in your ethics application as submitted at date of this approval as all changes must receive ethical approval prior to implementation. The amendment form is available at <http://ris.leeds.ac.uk/EthicsAmendment>.

Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be

audited. There is a checklist listing examples of documents to be kept which is available at <http://ris.leeds.ac.uk/EthicsAudit>.

We welcome feedback on your experience of the ethical review process and suggestions for improvement. Please email any comments to [ResearchEthics@leeds.ac.uk](mailto:ResearchEthics@leeds.ac.uk).

Yours sincerely

Jennifer Blaikie  
Senior Research Ethics Administrator, Research & Innovation Service  
On behalf of Prof Edward White, Chair, [BIOSCI Faculty Research Ethics Committee](mailto:BIOSCI@leeds.ac.uk)

CC: Student's supervisor(s)