CHAPTER 3
BACKGROUND TO HRV AND TECHNIQUE EVALUATION

CHAPTER CONTENTS:

A. HEART RATE VARIABILITY

1. Introduction

1.1 The definition of heart rate variability

Blood circulation is a periodic process. The phasic nature of circulation is caused by the cyclic activity of the heart. As a heart period is the length of a heart cycle, heart rate is inversely proportional to the heart period. Instantaneous heart rate (HR) is not steady, but demonstrates continuous small fluctuations. Heart rate variability (HRV) describes the variations in the oscillations between consecutive heartbeats (RR-intervals) as well as the oscillations between consecutive instantaneous heart rates (1).

Sometimes heart rate does not change from cycle to cycle - a negative clinical sign described as a pendulum-like rhythm (2). The loss of HRV therefore serves as a prognostic marker for cardiovascular disease such as diabetic autonomic neuropathy, hypertension, myocardial infarction, and heart failure and can also be an indication of psychological illness (3).

In the first part of this chapter (section A), the physiology of HRV is discussed with regards to the mechanisms involved in the regulation of heart rate. This fundamental discussion is followed by an explanation of the origins, mathematics and different types of HRV analyses.

The second part of Chapter 3 (section B) discusses the technique evaluation of HRV analysis to be utilised in the fibromyalgia study.

2. The physiology of heart rate variability

The sinoartrial node, located at the posterior wall of the right atrium of the heart, initiates each heart beat. Spontaneous action potentials arise in the adapted myocytes in this region due to the unstable membrane potential of these cells. The physiological regulation of heart rate is complex, involving several overlapping control mechanisms, all influencing the autorhythmicity of the sinoartrial node (both directly and indirectly). The principle behind this regulation is to maintain homeostasis. That is, because various influences constantly

act on the heart, heart rate has to change in an effort to achieve and preserve stability. This ability to maintain stability through change is also referred to as allostasis (3,4).

2.1. Factors involved in the modulation of heart rate variability

Various pacemaker tissues control the intrinsic rate of cardiac contraction, which is further regulated by extrinsic factors. Examples of extrinsic influences on heart rate are changes in activity, posture, mental stress (state of arousal) and emotional stress. Intrinsic periodic factors include respiratory sinus arrhythmia, baroreceptor reflex activity, thermoregulation, neuroendocrine secretion and circadian rhythms (5). All the factors modulating the rhythm of the sinoartrial node, add variability to the heart rate signal at different frequencies (Figure 2.1)(3).

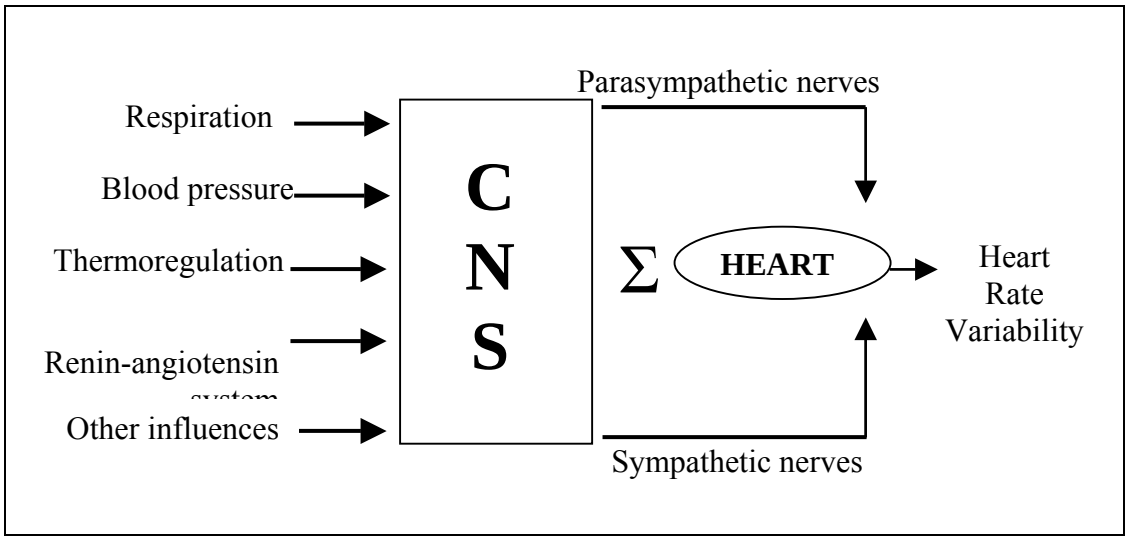


Figure 2.1. Factors affecting heart rate. **Abbreviations**: CNS, central nervous system; Σ, sum. Figure adapted from *Ori, Z./ Cardiology Clinics 1992;10:499-533 (5).*

These intrinsic and extrinsic factors are communicated to the heart mainly via the two branches of the autonomic nervous system, the sympathetic and the parasympathetic nervous system (SNS and PSN) (3). Neurohumoral regulation is especially involved in this process (6).

2.1.1. Autonomic control of the heart

The autonomic nervous system (ANS) controls the functioning of the visceral organs, blood and lymphatic vessels and smooth muscles. It interacts with the somatic nervous system and is, as illustrated in Figure 1, under the control of the central nervous system (CNS) (6).

The small fluctuations in heart rate are largely attributable to changes in autonomic input to the sinoartrial node (4), modulated by the antagonistic interaction between the SNS and PNS with each other. Both the SNS and PSN modify heart rate by altering the activity of the cyclic AMP second-messenger system in the innervated cardiac cells (7).

The two divisions of the autonomic nervous system differ with regards to their anatomic structure, functional effects and neuromediators released from the postganglionic nervous terminals. In effect, it has been suggested that the differentiation in the characteristics of sympathetically and vagally mediated heart rate fluctuations are mainly attributable to the different 'response properties of the nodal tissue to the respective neurotransmitters' (Berger, 1989) (8).

Sympathetic stimulation gives rise to increases in conductivity and contractility of the heart. These positive inotropic (force of cardiac contraction) and chronotropic (impulses increasing heart rate) effects redouble the sympathetic intensity though a process of syntaxis. It is well known that heart rate is also indirectly influenced by the sympathetic system through the release of adrenomedullary catecholamines (9). Parasympathetic stimulation results in a decreased conductivity and weaker atrial contraction (6).

2.1.1.1. Parasympathetic nervous system (PNS)

The postganglionic fibers of the PNS insert into the sinoartrial node, the atrio-ventricular node, the atrial musculature, the ventricular musculature and the coronary vessels (parasympathetic innervation of the ventricles is sparse, though) (7). Acetylcholine, released from the vagus nerve, mediates the parasympathetic influence on heart rate. Acetylcholine slows the rate of sinoartrial node depolarization and discharge by binding to muscarinic receptors, activating an inhibitory G protein that reduces activity of the cyclic AMP pathway, and in so doing, decreasing heart rate (1,6,9). Vagal stimulation is followed by a rapid response from the heart, with its maximum effect at approximately 0.5 seconds, a return to baseline within one second, followed by a slower rebound in the direction of decreasing R-R intervals (9).

2.1.1.2. Sympathetic nervous system (SNS)

The efferent nerve fibers of the SNS insert into a number of different structures within the heart e.g. the sinoartrial node, the conduction system, the atria, the ventricles and the

coronary vessels. Sympathetic influence on heart rate is mediated by the release of adrenalin and noradrenalin. Noradrenalin speeds the sinoartrial rhythm via a beta1-receptor–mediated second messenger cascade of intracellular signals (9). The result of an acceleration of the beta-adrenergic receptors is an increase in the rate of slow diastolic depolarization, accelerating heart rate (1). These impulses from the noradrenergic sympathetic nerves also inhibit the parasympathetic nervous system through the release of neuropeptide Y (a co-transmitter in the sympathetic nerve terminals) (6). Sympathetic stimulation is followed by a slower response from the heart (in comparison to parasympathetic stimulation), typified by a pure time delay of approximately one second, a maximum decrease in R-R intervals in four seconds and a return to baseline in 20 seconds (7,9).

2.1.1.3. The reciprocal action of the efferent innervation of the heart

As mentioned previously, vagal and sympathetic activity constantly interacts in the regulation of heart rate (10). In other words: at any given moment, heart rate will be determined by the balance between the inhibitory effects of the PSN and the stimulating effects of the SNS. Despite the fact that acetylcholine is rapidly hydrolysed (because of the sinus node's richness in acetylcholinesterase), and that the effect of any vagal impulse is therefore concise, vagal tone dominates and variations in heart period are, under resting conditions, largely dependent on vagal modulation (11). Parasympathetic influences most likely surpass sympathetic effects via two independent cholinergically induced mechanisms: a decrease of noradrenalin release in response to sympathetic activity, and an attenuation of the response to an adrenergic stimulus (1,6). The activity of these two branches of the ANS is coordinated by the cardiovascular control centre in the brain stem (7). It is important to note that the two branches are not always reciprocally controlled, and that they are able to vary independently, and can demonstrate coactivation and coinhibition (12).

Because the control of the heart rate is largely attributable to autonomic innervation, HRV offers a valuable tool for the assessment of autonomic nervous system function (3,13), granting information on both the sympathetic and parasympathetic nervous system as well as 'autonomic balance'.

2.1.2. Heart rate modulation by the higher control centres in the brain

The higher control centres in the brain involved in the regulation of the heart include the thalamus, the hypothalamus, the cerebral cortex, the cortical and diencephalic (innerbrain) centres, and the medulla oblongata. These centres mostly modulate heart rate, the heart rhythm and the contractility of the heart (6).

Stimulation of the thalamus results in tachycardia, which is an increase in heart rate (2). The hypothalamus is associated with the cerebral cortex and autonomic centres in the brainstem and spinal cord. It controls unconditional and conditional reflexes of vitally important functions such as breathing, circulation and metabolism. It is therefore expected that stimulation of the hypothalamus will produce variations in heart rate. Furthermore, the hypothalamus has reciprocal connections with the vasomotor centre, increasing blood pressure in response to emotions like anger (6). The paraventricular nucleus of the hypothalamus also appears to have a central role in mediating the circadian rhythm of the ANS (3). Cerebral cortex areas that have an effect on cardiac function are the anterior temporal lobe, the pre-motor and motor cortex, the cingulate gyrus, the orbital cortex, the insula and the frontal lobe. The cortical and diencephalic centers initiate cardiac reactions in response to emotional states like excitement or anxiety (6).

The vasomotor centre, comprising of the vasodilator and vasoconstrictor areas, is situated in the medulla oblongata. These areas exert their effects through the sympathetic and vagal innervation of the heart. The depressor area of the vasodilator area decreases heart rate by reducing both muscle contractility (lessening stroke volume) through vagal stimulation, and by reducing peripheral resistance (13). The vasoconstrictor area houses the pressor area, which produces a reciprocal effect to the depressor area through increased activity of the sympathetic neurons to the heart. The increased sympathetic discharge is accompanied by a decrease in the tonic activity of the vagal fibres (6).

2.1.3. Reflex control of heart rate

2.1.3.1. Respiratory sinus arrhythmia

Respiratory sinus arrhythmia (RSA) reflects the coupling between breathing and autonomic neural outflow. RSA is predominantly mediated by respiration-driven gating of parasympathetic efferent activity to the heart. Vagal efferent traffic to the sinus node occurs primarily in phase with expiration and is absent or attenuated during inspiration. The end

result is that RSA will fluctuate with the phase of respiration, e.g. cardio-acceleration during inspiration, and cardio-deceleration during expiration. Both sympathetic and parasympathetic nerve traffic fluctuate with respiration, but the time constant for changes in the sympathetic nervous system tone to affect heart rate is too long to affect heart rate at normal breathing frequencies (9). Because RSA is predominately mediated by the fluctuations in vagal nerve traffic, the respiratory frequency band, which ranges from 0.15 Hz to 0.4 Hz, can be used as an index for vagal activity (14) (the implications for HRV analysis will be discussed later).

2.1.3.2. Baroreceptor reflex

Baroreceptors are stretch receptors in the walls of the heart and the blood vessels that respond to stretching and distension. Their afferent fibres travel via the aortic and carotid sinus nerves to the medulla. The frequency at which action potentials are generated in the baroreceptor, are proportional to the changing pressure in the structure in which they are located (13). Increased baroreceptor discharge reduces the tonic discharge of the vasoconstrictor nerves and stimulates the vagal innervation of the heart, creating bradycardia (6).

In the frequency domain of power spectral analysis of HRV, baroreceptor activity is associated with the low frequency band (15). Chronic corticosterone treatment is one of the factors known to reduce baroreceptor reflex-mediated HRV (3). One can therefore perhaps expect high psychological or high physiological stress induced cortisol levels to have a similar effect.

2.1.4. Endocrine influences

Quantitative data on the time domain or frequency domain responses of heart rate to hormonal modulation is limited. However, it has been shown that thyroxine, reproductive hormones, the renin-angiotensin system, steroids and other endocrine factors have an affect on HRV (3,13). Evidence of non-autonomic control of heart rate in the time-domain or frequency domain is best surmised from HRV recordings on heart transplant patients (before sympathetic reinnervation occur). Results obtained in the time-domain on these patients suggest that hormonal heart rate control is only active at frequencies below 0.03 Hz (9). Therefore there is no need to be concerned that results obtained from HRV analysis could be biased by hormonal influences.

2.1.5. Thermoregulation

Cooling of the heart, a method used in heart surgery, causes bradycardia. Conversely, heart rate is increased by fever. Heart rate increases with 18 beats per minute per ºC increase in body temperature. Heart rate slows down in response to decreasing temperatures, until body temperatures of 15.5 ºC to 21.2 ºC are reached. At temperatures like these, the heart beats only at a few beats per minute and death as a result of hypothermia may results (6). Fluctuation in temperature is thus a significant source of changes in heart rate (3).

The effect of thermoregulation on HRV is not only achieved through ANS function. Studies have shown that both direct effects of temperature on the pacemaker activity of the sinus node, as well as indirect effects through the ANS, mediate temperature effects on HRV (3). The effects of temperature regulation on HRV should always be taken into account when the experimental set up (conditions) in which HRV experiments are to be conducted, are planned.

Applications of measures of HRV range from investigations into autonomic balance, to evaluations of cognitive development and clinical risk, to studies of fundamental links between psychological processes and physiological functions (1,16).

3. Analysis of heart rate variability data

Physicians recognized the importance of cardiac rhythms long before the emergence of modern constructs of HRV. Consequently they have been monitoring heart sounds and rhythms and noted beat-to-beat rhythms shifts related to aging, illness, and psychological states. Initially, the method for studying heart rate patterns was limited to auscultation. Yet, the technology for the quantification of the electrical activity of the heart progressed from the galvanometer, to the kymograph, to the polygraph, to electrocardiograms and now to digital signal processing systems (9).

During the last two decades researchers and clinicians started to recognise the significant relationship between alterations in autonomic nervous system activity and cardiovascular mortality. In the search for experimental data to confirm this observation, HRV analysis proved to be the most promising of all the quantitative markers for autonomic activity (1).

The R-wave in the electrocardiogram central waveform (QRS-complex) is the easiest to detect and is used to derive the HRV signal. Originally, HRV was assessed manually from calculating the mean R-R interval and its standard deviation measured over short-term (five minutes) electrocardiograms (Figure 3.a) (9). The smaller the standard deviation in R-R intervals, the lower is the HRV (16). At the present time, heart rate monitors able to record the R-wave digitally, are used (Figure 3.b).

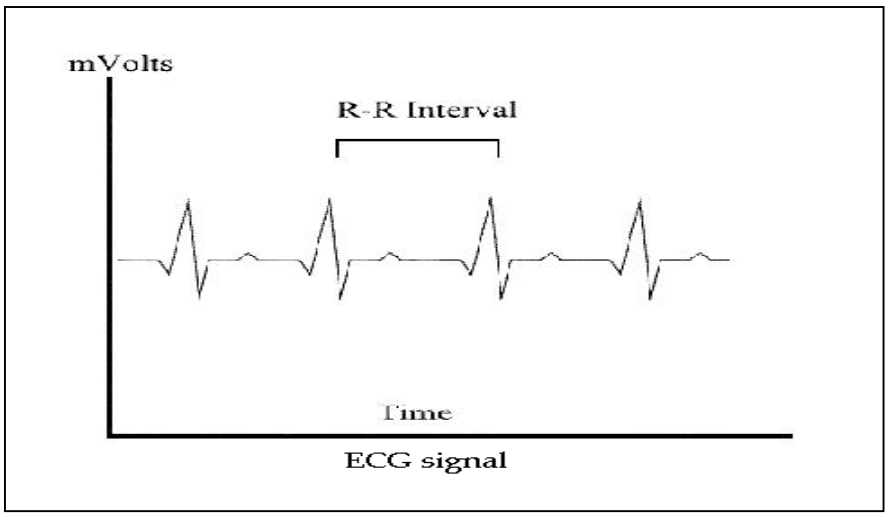


Figure 3.a. The RR-interval is derived from the electrocardiogram signal's QRS-complex

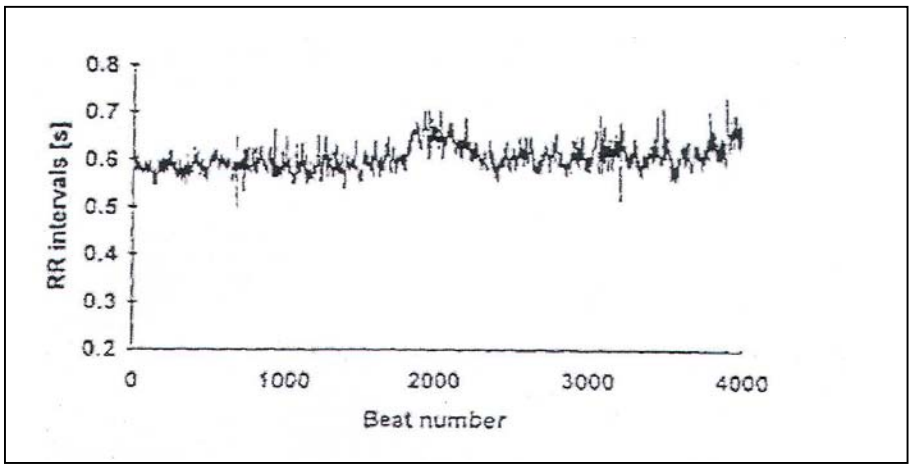


Figure 3.b. Heart rate monitors that record the RR-interval digitally produces a tachogram when the data are downloaded to a computer.

A 1996 report of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology presented a citation serving as an important step towards the standardisation of the field of HRV analysis (1). These guidelines were followed in HRV analysis in the technique evaluation and the fibromyalgia study.

3.1. Time domain analyses of heart rate variability

The time-domain parameters are the simplest of the HRV factors to calculate and includes statistical as well as geometric methods of calculation. Time-domain measures are computed from the raw R-R interval time series. In these calculations, either the heart rate at a certain point in time, or the intervals between successive normal R-R intervals are determined. Basically, this parameter measures the amount of variability. To date, over 26 different types of arithmetic manipulations of R-R intervals have been used in the literature to represent HRV (17). Table 3.1 summarises the most frequently used time domain measures.

Table 3.1. *Different time-domain measures*

Variable	Unit	Description
Statistical measures		
Mean & STD RR	s	Mean and standard deviation of the selected RR interval series (similar to SDNN)
Mean & STD HR	hr/min	Mean and standard deviation of the selected heart rate series (similar to SDANN)
RMSSD index	ms	The root-mean square of the difference of successive R-R intervals
NN50	count	Number of consecutive RR intervals that differ more than 50ms in the entire recording
pNN50	%	Percentage value of consecutive RR intervals that differ more than 50 ms
Geometric measures		
HRV triangular index		Base of the triangular area under the main peak of the R-R interval frequency distribution diagram
TINN	ms	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals
Differential index	ms	Difference between the widths of the histogram of differences between adjacent NN intervals measured at selected heights
Logarithmic index		Coefficient of the negative exponential curve which is the best approximation of the histogram of absolute differences between adjacent NN intervals

Table adapted from *Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology./, Circulation 1996;93:1043-55 (1).*

Since many of the time-domain measures correlate closely with others, the Task Force of the European Society of Cardiology recommended the use the following time-domain measures:

- SDNN (standard deviation of all normal RR intervals)
  - for the estimation of overall HRV
- HRV triangular index
  - to estimate the overall HRV
- SDANN (standard deviation of the average normal RR intervals)
  - for the of the long-term components of HRV
- RMSSD (square root of the mean differences between successive RR intervals)
  - to estimate the short-term components of HRV (1,18).

3.2. Frequency Domain Analyses

The total variance in heart rate is partitioned into underlying rhythms that occur at different frequencies. In other words: HRV has a propensity to aggregate into different frequency bands, which can be associated with the different underlying intrinsic rhythms involved in the regulation of heart rate (9,13). Although HRV in the frequency domain fails to provide full information on autonomic tone, it is still able to grant valuable information regarding autonomic function. Underlying rhythms, the physiological process represented by these rhythms and the power of each of these underlying rhythms can be determined by frequency domain analyses (1,3).

The underlying rhythms in the heart rate signal are (Figure 3.2):

- High frequency bands (HF) – at respiratory frequencies (9-24 cycles/minute)/ 0.15-0.40 Hz.
- Low frequency bands (LF) – at approximately every 8-10 seconds/0.04-015 Hz.
- Very low frequency bands (VLF) – at approximately every 20 seconds to every 5-minute frequency.
- Ultra low frequency power band (ULF) – at less than every 5 minutes to once in 24 hours (10,13,14).

Figure 3.2.a. illustrates the number of oscillations for the three major rhythms of the heart and Figure 3.2.b the combined effect of these individual rhythms.

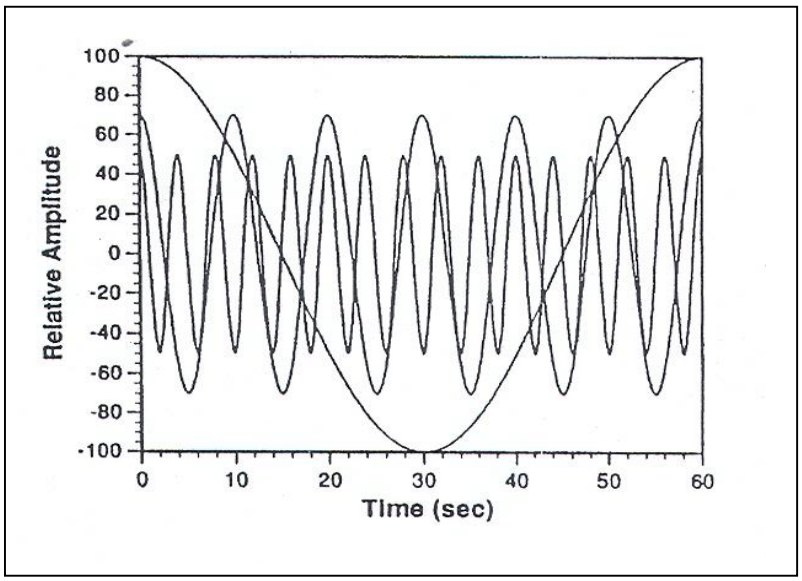


Figure 3.2.a. The number of oscillations for the three major rhythms of the heart: HF (0.25 Hz; 15 cycles/min), LF (0.1 Hz; 6 cycles/min), VLF (0.016 Hz (1cycle/min). **Abbreviations**: HF, high frequency; LF, low frequency; VLF, very low frequency; ULF, ultra low frequency. Figure taken from *Akselrod, S./ Science 1981;213:220-2 (13).*

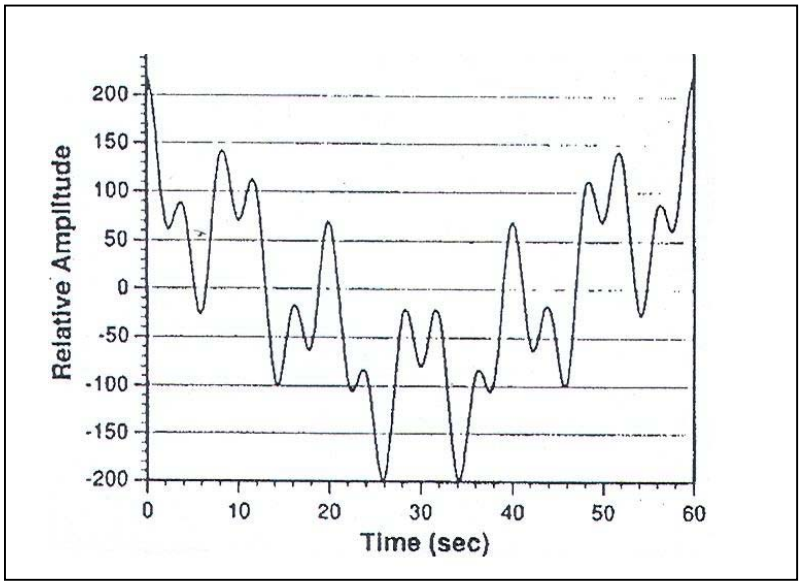


Figure 3.2.b. The combined effect of the different rhythms of the heart. Figure taken from *Akselrod, S./ Science 1981;213:220-2 (13).*

Power spectral density (PSD) is a traditional spectral practice, which provides information about power (variance) distribution as a function of frequency (1). In a PSD graph, the power of the respective spectral components is represented by the area (ms^2) under the relevant frequency curve as seen in Figure 3.2.c.

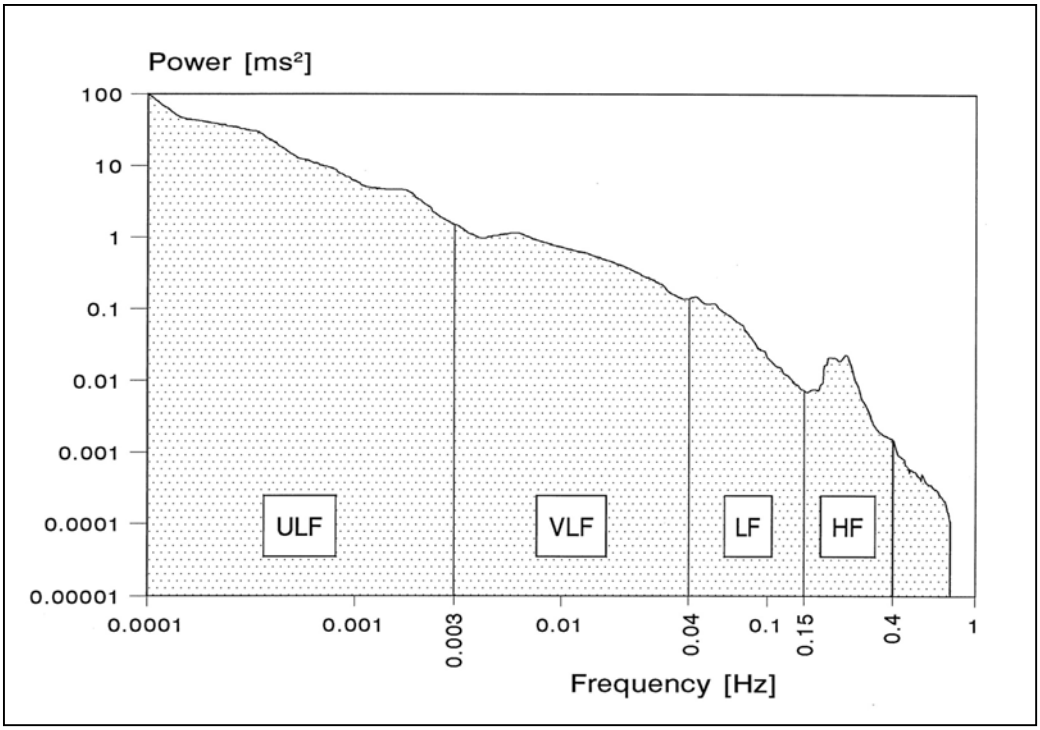


Figure 3.2.c. Power spectral density graph showing the different frequency bands. Note the frequency ranges for each frequency band: HF (0.15 - 0.4 Hz), LF (0.04 - 0.15 Hz), VLF (0.003 - 0.04 Hz), and ULF (0.0001 – 0.003 Hz). **Abbreviations**: HF, high frequency; LF, low frequency; VLF, very low frequency; ULF, ultra low frequency. Figure taken from *Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology./ Circulation 1996;93:1043-65 (1).*

Methods for PSD calculation, is non-parametric (based on fast Fourier transformations (FFT)); and parametric (based on autoregressive (AR) time series modeling) (Figure 3.2.d). The advantages and disadvantages of these methods are presented in Table 3.2.

Table 3.2. *The advantages and disadvantages of different PSD spectra*

Parametric spectrum	**Non-parametric spectrum**
Advantages Smooth spectral components Frequency bands are easy to distinguish Unproblematic post-processing Accurate PSD calculation **Disadvantage** The suitability of the chosen model order	**Advantages** Uncomplicated algorithm High processing speed (1).

needs to be verified (1).	

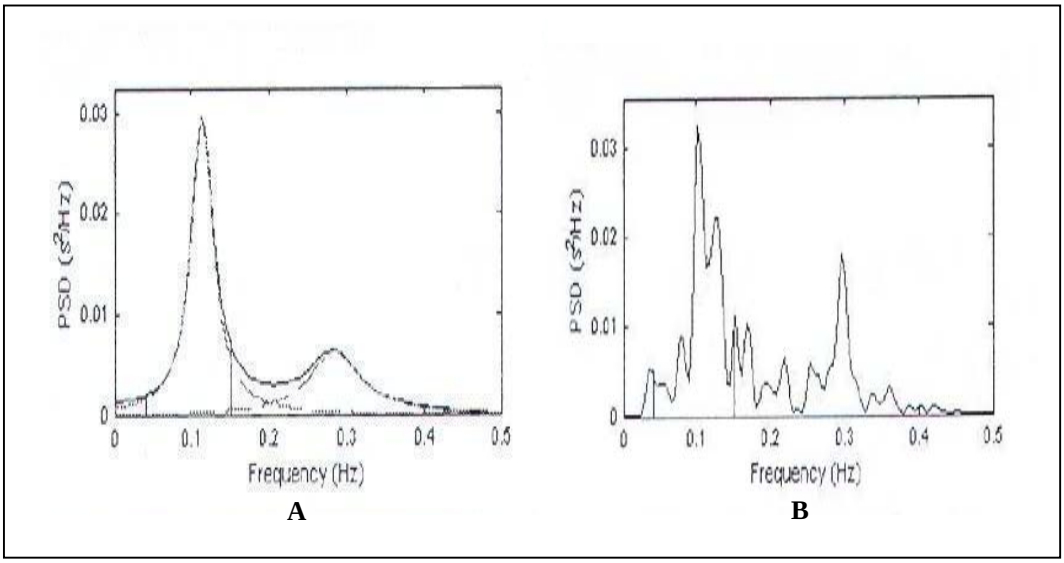


Figure 3.2.d. Power spectral density (PSD) calculated using parametric (A) and non-parametric methods (B)

3.2.1. The High Frequency Band

Spectral power in the high frequency (HF) solely reflects parasympathetic outflow (4,19) and, as mentioned before, enumerates RSA. The PNS responds over a wide frequency range, whereas the SNS only responds at frequencies below 0.1 Hz. This implies that the amount of power at high frequencies solely reflects vagal modulation of HR (amount of vagal-cardiac nerve traffic) (3).

The amount of power in the HF band is affected by the breathing rate. Figure 3.2.1. demonstrates how different breathing rates influences the maximum spectral power. Nonetheless, mean RR-intervals are nearly constant across the different breathing frequencies (refer to the right of Figure 3.2.1.). The consistency of the RR-intervals can be taken as evidence for the steadiness of vagal-cardiac traffic (20). It is thus important to note that the HF component in spectral analysis does not reflect vagal tone (3). If changes in HF power reflected changes in vagal tone, heart rate should also be affected. However, changes in HF power at different breathing rates do not affect heart rate (10). On the other hand, because HRV is derived from the electrocardiogram, it is not possible to distinguish reduced central vagal activity (in the vagal centres of the brain) from reduced peripheral activity (the contribution of the sinus node or the afferent/efferent pathways conducting the neural impulses to/from the brain) (20).

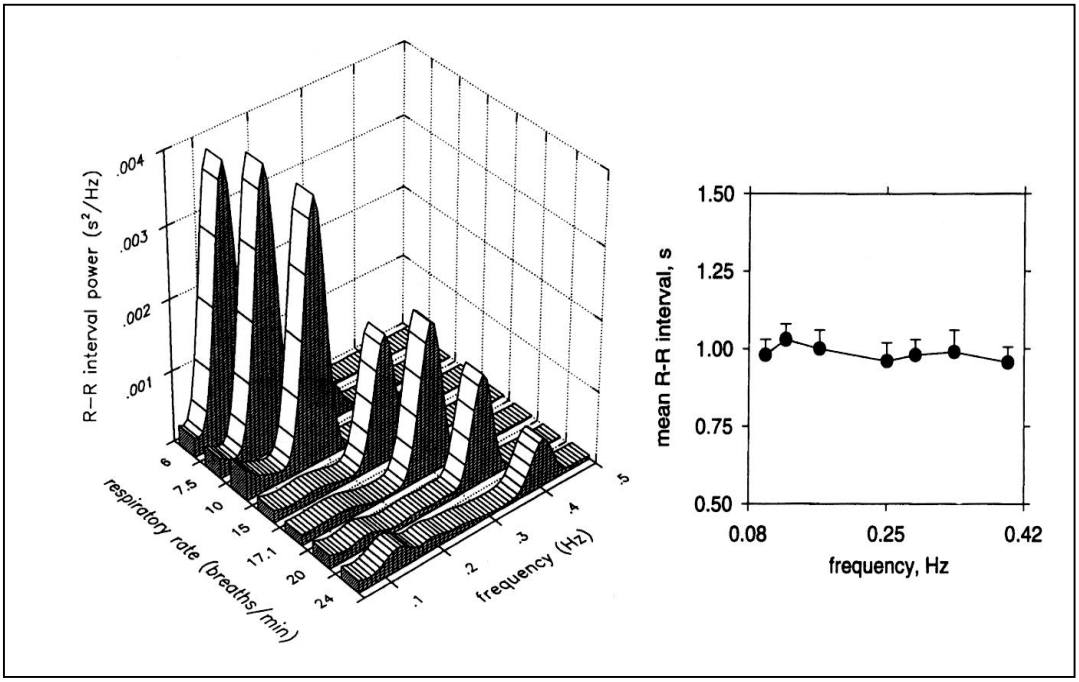


Figure 3.2.1. Average RR-interval spectral power and RR-intervals from 10 healthy supine subjects breathing at seven breathing rates. Note how the HF component changes with different breathing rates, whilst the mean R-R interval stays the same. Figure adapted from *Eckberg, D./ Circulation 1997;96:3224-32 (10).*

3.2.2. The Low Frequency band

The low frequency component (also called the mid-frequency) gives information regarding the sympathetic activity but with notable influence from the parasympathetic nervous system, baroreceptor feedback and centrally generated brainstem rhythms (3,10,13). Some researchers propose that, in order to obtain a reliable index for sympathetic activity, it is necessary to normalize the LF component (normalization are discussed in 2.2.3). They suggest that this frequency band can be used as an index of sympathetic modulation of HR with the requirement that the normalized HF power should be subtracted from the normalized LF power to exclude the parasympathetic influence from the HF component (19). However, according to the standardisation criteria set out by the Task Force of the European society of Cardiology, the LF component of HRV reflects the sympathetic activity directly (1). In this study, the LF is used as the index for sympathetic activity, like the Task Force proposed.

3.2.3. The low frequency / high frequency ratio

Since physiological intervals incite reciprocal changes in sympathetic and vagal neural outflow, it was necessary to find an index to reflect the balance between the opposing neural mechanisms. The LF/HF ratio provides a measure of sympathovagal balance, where an

increase in the LF/HF ratio reflects a predominance of sympathetic over parasympathetic activity. A decrease in LF/HF ratio would be interpreted as a shift of sympathovagal balance towards parasympathetic predominance (19). The use of this ratio as an index for sympathovagal balance remains controversial though, because of a lack of complete understanding of the low frequency component by researchers (1,10).

Power in normalized units (nu, also called relative power), is power centered at the frequency of interest (LF or HF) divided by the total power less the VLF power. Thus LF nu = LF/(LF + HF) and HF nu = HF/(LF + HF). Now, because calculations of normalized LF and HF powers involve the rearrangement of the same LF and HF terms, a change of normalized LF power must necessarily be associated with a change of normalized HF power. Sympathovagal balance (in dimensionless units) is then the ratio between the absolute LF to absolute HF power (10).

3.2.4. The very low frequency (VLF) band

VLF fluctuations are linked to changing vasomotor tone in response to localized needs (thermoregulatory or metabolic needs), activity, periodic breathing and thermogenesis. The effect of thermoregulation on the VLF HRV is already touched upon earlier in this chapter. The VL frequencies are also affected by hormonal systems, the action of the renin-angiotensin system has a dampening effect of the VLF band (3). When parasympathetic activity is blocked, VLF fades away. For this reason decreased VLF can be used as an indicator for parasympathetic abnormalities (13).

3.2.5. Ultra Low Frequency Band (ULF)

Circadian rhythms, reflected by ULF band oscillations, are significantly influenced by the ANS (21). A decrease in ULF is a strong predictor of mortality (9). The VLF and ULF rhythms of heart rate may have clinical applications and psychophysiological correlates, but these mechanisms are ambiguous (22).

3.3. Correlations and dissimilarities between time and frequency domain parameters

There are strong correlations between several time and frequency domain variables when RR-intervals are recorded over a 24-hour period (1). Although the recordings in the technique evaluation and the fibromyalgia study are rather short (30 min recordings divided

into 5 min intervals), the strong similarities in long-term recordings grant information on the mathematical and physiological interpretation of these variables. The next table (Table 3.3) compares some of these variables.

Table 3.3. *Correlations between time and frequency domain variables*

Time domain variable	**Appropriate frequency variable**
HRV triangular index	Total power
TINN	Total power
RMSSD	HF
NN50 count	HF
pNN50	HF
Differential index	HF
Logarithmic index	HF (1)

3.4. Non-linear Analyses

Poincaré plots are the simplest technique to describe non-linear components of HRV. Non-linear measures, also called 'return maps', quantify complexity and self-similarity by describing the relationship between successive samples of a time series. This relationship is described by graphing each R-R interval against the next R-R interval. The transversal axis of the poincaré plot is an indicator of the short-term variability (SD1) as the vagal induced RR-interval develops faster than those sympathetically. The longitudinal axis reflects global variability as an inverse function of sympathetic modulation (SD2) (23,24).

Figure 3.4. demonstrates how the poincaré plot pattern, time signal and power spectrum changes with five different manoeuvres: supine, controlled breathing, standing, exercise and a recovery period. The characteristic poincaré plot change pattern with each bodily manoeuvre is apparent. According to visual analysis it is firstly clear that the poincaré plot of a healthy subject is marked by an elliptic pattern (23). This pattern is larger for the supine bodily position, more scattered with controlled breathing, narrower in the standing position, and significantly smaller (reduced) during exercise. During the recovery phase, the pattern is similar to the standing pattern (24).

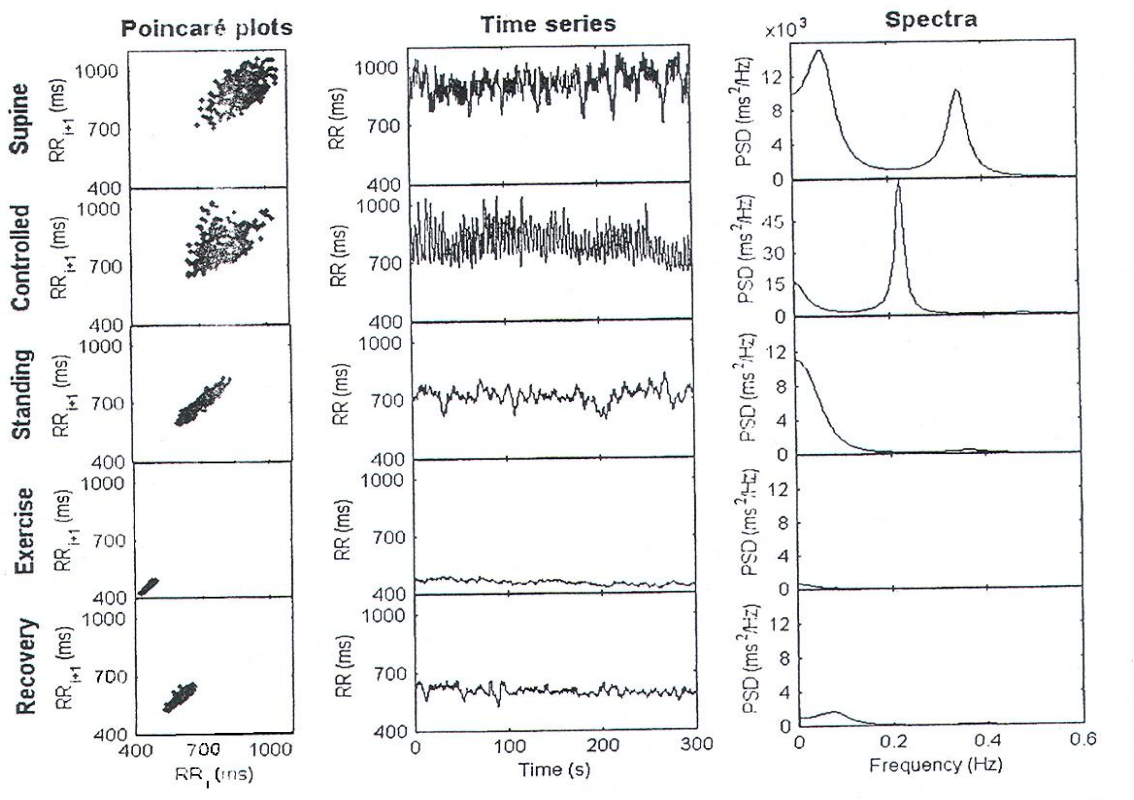


Figure 3.4. Poincaré plots, time series and spectra throughout five different manoeuvres for a representative subject. Note how the pattern is larger for the supine bodily position and recovery phase, more scattered with controlled breathing, narrower in the standing position, and significantly smaller (reduced) during exercise. Figure taken from *Woo, M.A./ Journal of the American College of Cardiology 1994;23:565-9 (24).*

Keeping in mind that the transversal axis represents the vagal, and the longitudinal axis the sympathetically induced R-R intervals, the physiological interpretation of these characteristic patterns is straightforward. When vagal and sympathetic activity are balanced (during supine position), the Poincaré plot's elliptic pattern is relatively scattered, whereas the pattern become even more dispersed when vagal modulation dominates (during controlled breathing). The elliptic pattern has a smaller dispersion with greater sympathetic influence (during exercise), with the minimum dispersion when vagal influence is suppressed in the midst of sympathetic activation (during exercise) (24).

In summary it can be said that there is a clear connection between the shape of the poincaré plot and the ANS activity. The narrower the pattern, the larger the sympathetic activity. A more scattered the plot, is indicative of increased vagal activity (23).

3.5. Filtering

Adequate removal of artifacts is critical for reasonably unbiased analysis. Most typical artifacts in HRV analysis are missed beats. The removal of missed beats does not result in any loss of information, but restores the original R-R series. The elimination of ectopic or abnormal sinus beats is more problematical though, as it involve the loss of information. To solve this problem, upper and lower bounds are placed regarding the changes in heart rate, so that R-R intervals are not allowed to vary more than 20% from their previous intervals. This can be done using an autonomic filter or RR statistics. It is thus possible to exclude outliners from analysis based on accumulated estimation of the RR probability distributions (4,19).

Tarvainen, Ranta-aho and Karjalainen (the same authors that developed the software for Advanced HRV Analysis) offered a detrending method to apply in the process of HRV analysis. This method is based on smoothness priors approach and operates like a time-varying FIR high pass filter. Using this approach, it is possible to adjust the frequency response to different situations by changing one single regularisation parameter (λ). The soothing parameter λ should be selected in such a way that the detrending does not affect the spectral components of interest significantly. For instance: if λ is adjusted properly, RSA can be successfully quantified through separation from the other frequency components of HRV (17).

Using this method, the distortion of data end points is also avoided, because the filtering effect is attenuated in the beginning and end of the data (17). The effect of detrending on time and frequency domain analysis of HRV will be demonstrated in the second part of this chapter, namely Section B.

3.6. Confounders and limitations in the interpretation of HRV and autonomic function

The significance and meaning of many of the HRV measures are rather complex. If researchers and clinicians do not recognise this, there is a potential for incorrect conclusions and unfounded extrapolations (1). Some of these pitfalls, identified through the technique evaluation for this study (section B), as well as by others workers in the field, are discussed next.

I. HRV determinations may not be meaningful in patients with a high degree of non-respiratory sinus arrhythmia (erratic sinus rhythm) (1). These cases are associated with abnormal-looking, blurred power spectral plots and exaggerated HRV.

II. HRV are affected by extrinsic factors like physical and mental activity, talking, emotions, disturbing thoughts and environmental temperatures (5). Great care needs to be taken to see to it that subjects do exactly as they are told when HRV recordings are performed so that these factors can be minimised.

III. The computation of HRV from data with more than 20% ectopic beats is ill-advised for it will not grant reliable results (1).

IV. It is important to keep in mind that a small RSA persists after combined pharmacologic cardiac sympathetic blockade and after cardiac transplantation (before autonomic reinnervation). These findings are indicative of a part of RSA not resulting from vagal activity, but possibly from an intracardiac origin (9). It is for instance known that baroreceptor reflexes contributes to RSA (12). Therefore researchers should be cautious in interpreting RSA results.

B. TECHNIQUE EVALUATION

1. Aim

The purpose of the technique evaluation was to establish whether the recording of R-R intervals with the Polar Vantage heart rate monitor and analysis of the intervals with the HRV Analysis Software 1.1 represent a reliable assessment tool for HRV to be used in this study. The technique was evaluated by determining the following:

- Technique Reproducibility (direct and indirect measures)
- Interpersonal Variation
- Intrapersonal Variation
- Sensitivity and response to stressors

Technique reproducibility was evaluated by comparing direct and indirect recordings, interpersonal and intrapersonal variation by determining the variation in HRV measures between subjects and within the same individual, and sensitivity and response to stressors by evaluating the effect of music on HRV.

2. Materials and Methods

2.1. Data collection

The R-R intervals were recorded using the Polar S810 heart rate monitor (indirect) and the Polar Advantage interface system (direct) simultaneously. HRV data recordings were made over short periods of time (20-30 min), with interventions, under carefully controlled laboratory conditions. Refer to Chapter 2 for a detailed explanation on how the Polar heart rate monitors are operated. At the end of each session, the Polar Precision Performance computer program was used to download the indirect R-R interval recordings the computer.

2.2. Experimental design

Nine healthy volunteers participated in the study. Baseline recordings, 20 minutes in duration, were obtained from each of the nine volunteers on six consecutive days. The final two recordings for each subject (day 5 and 6) consisted of a 20 minutes baseline recording followed by ten minutes of either raucous or soothing music. Participants were expected to remain in the supine position for the duration of every recording. Recordings were made on the same time of day to exclude the possible effect of circadian rhythms.

Schematic representation of technique evaluation study design
(every experimental volunteer took part for 6 days):

Day 1: → Baseline recording (20 min)
Day 2: → Baseline recording (20 min)
Day 3: → Baseline recording (20 min)
Day 4: → Baseline recording (20 min)
Day 5: → Baseline recording (20 min) + Soothing Music (10 min)
Day 6: → Baseline recording (20 min) + Raucous Music (10 min)

2.3. Analysis of the data

The process involved in the analysis of R-R intervals is set out in a flow diagram (Figure 2.3) and involves the following processes:

2.3.1. Polar Precision Performance

As soon as the R-R interval data has been downloaded, Polar Precision Performance displays the instantaneous heart rate (HR) in beats per second (bps) across time (in seconds). The first step is to change the curve properties to R-R intervals, transforming the data to a tachogram, displaying R-R intervals (in milliseconds) across time.

The importance of error correction in the analysis of HRV data has been explained in 3.5., section A (p. 3.19). Missed beats are filtered in Polar Precision with a low filter power and a minimum protection zone of 20 bpm. Data are then saved as a 'HRM'-file, which can be read by HRV Analysis Software.

2.3.2. HRV Analysis Software 1.1.

Time- and frequency domain parameters were calculated over the 20-minute baseline and 10-minute intervention-recording period using a FFT algorithm. Only the FFT based

spectrum is used in the analysis of the technique evaluation and fibromyalgia study as it gives more robust results for all data, whereas the AR spectrum, according to Tarvainen (2003), 'sometimes suffers from numerical problems and give unreasonable results' (25). HRV Analysis Software 1.1 applies the Welch's method in FFT analysis (17).

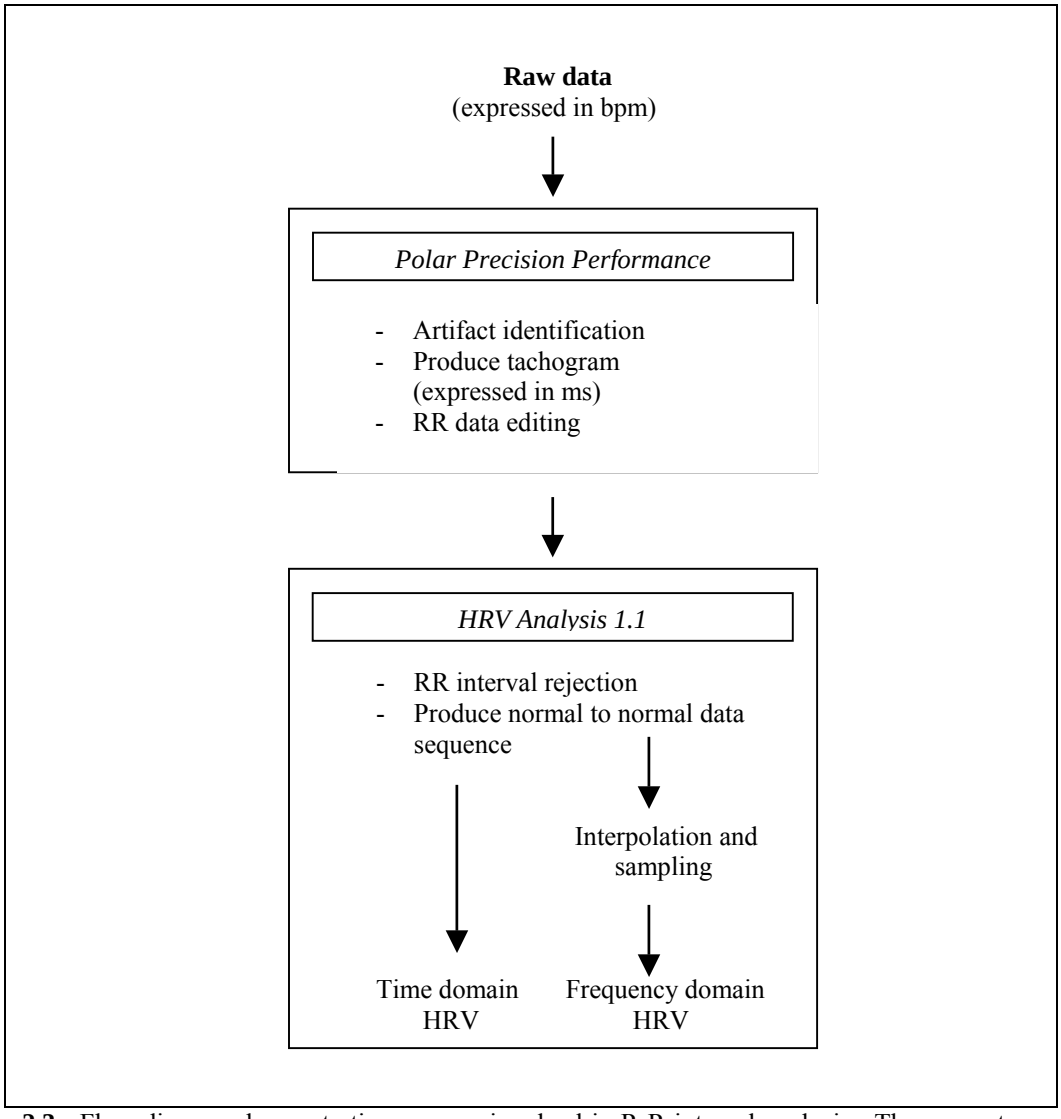
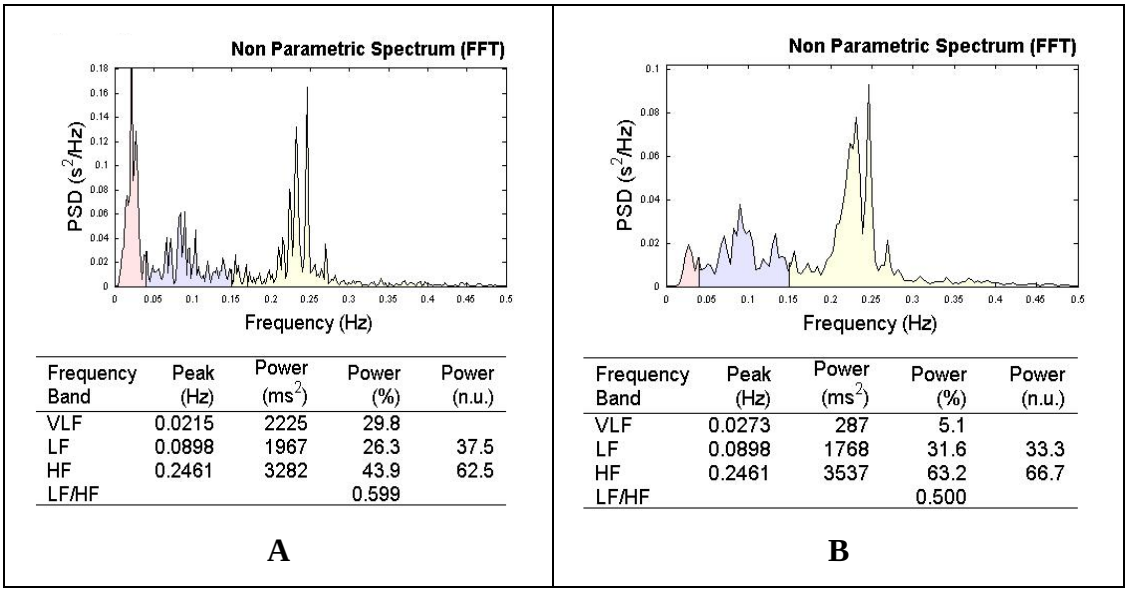


Figure 2.3. Flow diagram demonstrating process involved in R-R interval analysis. The computer program used in each step of analysis is indicated in *italics.*

It was necessary to change certain of the settings in the program before the HRV measures can be calculated. Firstly the ranges for the frequencies were set up at 0 – 0.04 Hz for the VLF band, 0.04 – 0.15 Hz for the LF band, and 0.15 – 0.4 Hz for the HF band. In HRV Analysis 1.1, the R-R series was detrended with the 'smoothness priors' trend and the 'eye' model. λ was used to adjust the soothing according to the researcher's needs. With an increase of λ ($500 < \lambda > 1000$), the lowest frequencies were removed. A decreased λ

(100< λ >400) resulted in the removal of the higher frequencies (17). According to advise from the developers of the software for this specific data set, the regularisation parameter (λ) was set to 500 (26).

R-R interval time series is an irregularly time-sampled series and should be interpolated before the spectrum can be estimated (17). A cubic interpolation rate of 4 Hz was used to minimise the VLF component of HRV, as only the LF and HF bands are important in assessing parasympathetic-sympathetic balance (27). This way the effect of hormonal rhythms (evident in frequencies below 0.3 Hz) was excluded from the analysis. Figure 2.3. demonstrates the effect of the default λ and interpolation rate (1000 and 2 Hz respectively) on the respective frequency bands in comparison to the preferred 500 and 4 Hz setting.



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF	0.0215	2225	29.8	
LF	0.0898	1967	26.3	37.5
HF	0.2461	3282	43.9	62.5
LF/HF			0.599	

A

Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF	0.0273	287	5.1	
LF	0.0898	1768	31.6	33.3
HF	0.2461	3537	63.2	66.7
LF/HF			0.500	

B

Figure 2.3.2. The effect of different settings in HRV Analysis Software on the respective frequency bands for a representative participant. **A** λ=1000, interpolation rate = 2Hz, **B** λ=500, interpolation rate = 4Hz. Note that using the settings in **B**, the VLF component is significantly reduced for the Welch's method (FFT).

Since the data obtained from the respective participants are going to be compared to each other, the same settings were used to analyse all data sets.

2.3.3. Statistical analysis

Statistical analysis for the technique evaluation was performed using BMDP Statistical Software, Inc. and Statistix for Windows, Version 2.0. Both descriptive and inferential statistics were calculated for the four evaluations done in the technique evaluation. Direct and indirect measures were compared with the Wilcoxon statistical test (this test is not

dependant on the distribution of the data). Interpersonal variation (the variation in HRV between subjects) was calculated with the ANOVA statistical test, which tests differences in means. If the variances between the subjects were equal, a normal ANOVA test was applied, if it was unequal, the Welch test was used. The standard deviation of the means calculated for all the respective variables demonstrated the intrapersonal variation (the variation in HRV within the same subject). Finally, the sensitivity of the method to stressors was evaluated by means of the Wilcoxon nonparametric test.

3. Results

3.1 Technique reproducibility (direct vs indirect recordings)

3.1.1 Data summary

The first recording of subject 5 was used to demonstrate technique reproducibility with regard to direct and indirect recordings using Polar heart rate monitors. The analysed data obtained from the direct (interface) and indirect (Polar watch) recordings for a representative subject are set out Table 3.1.1. (The same data is available for all the subjects but is not included in the chapter because it granted identical results).

Table 3.1.1. *Data summary of the indirect and direct measures of a representative subject*

Variable	IND 1	DIR 1	IND 2	DIR 2	IND 3	DIR 3
Time domain						
Mean RR (s)	0.88	0.88	0.89	0.89	0.89	0.89
RR STD (s)	0.04	0.04	0.05	0.05	0.05	0.05
Mean HR (bpm)	68.43	68.46	67.95	67.85	68.00	67.85
HR STD (bpm)	3.62	3.62	4.21	4.24	4.61	4.24
RMSSD (ms)	40.97	41.19	53.17	53.76	54.66	53.76
pNN50 (%)	22.52	22.82	39.44	39.84	39.16	39.84
SDANN (ms)	9.31	8.78	46.15	45.53	10.50	45.53
RR triang. ind.	0.08	0.08	0.11	0.11	0.10	0.11
TINN (ms)	235.00	235.00	325.00	325.00	305.00	325.00
SD1 (ms)	29.10	29.25	37.74	38.16	38.81	38.16
SD2 (ms)	61.65	61.48	87.92	86.97	79.37	86.97
Frequency domain						
LF (ms^2)	198.25	201.33	288.36	299.42	241.54	299.42
HF (ms^2)	452.12	460.25	656.57	664.53	698.54	664.53
TP (ms^2)	684.10	695.69	987.11	1006.51	989.12	1006.51
LF n.u.	30.48	30.43	30.52	31.06	25.69	31.06
HF n.u.	69.52	69.57	69.48	68.94	74.31	68.94
LF/HF	0.44	0.44	0.44	0.45	0.35	0.45

Explanation: Note how the time and frequency domain data for the direct and indirect recordings is almost identical. **Abreviations**: IND, indirect recording; DIR, direct recording; RR triang. ind., RR triangular index.

Table 3.1.1. *Data summary of the indirect and direct measures of subject 5 – continued*

Variable	IND 4	DIR 4	IND 5	DIR 5	IND 6	DIR 6
Time domain						
Mean RR (s)	0.84	0.89	0.71	0.71	0.81	0.81
RR STD (s)	0.05	0.05	0.03	0.03	0.05	0.05
Mean HR (bpm)	71.64	67.76	84.36	84.36	74.39	74.25
HR STD (bpm)	4.48	4.32	4.22	4.22	4.74	4.75
RMSSD (ms)	50.74	53.30	30.01	30.01	48.54	48.47
pNN50 (%)	36.08	38.87	6.44	6.44	34.78	34.51
SDANN (ms)	33.29	13.66	34.42	34.42	30.64	28.87
RR triang. ind.	0.10	0.10	0.06	0.06	0.11	0.11
TINN (ms)	395.00	285.00	190.00	190.00	285.00	285.00
SD1 (ms)	36.01	37.84	21.33	21.33	34.45	34.40
SD2 (ms)	85.44	72.94	64.29	64.29	77.77	76.41
Frequency domain						
LF (ms^2)	275.96	226.46	136.05	136.05	299.37	310.34
HF (ms^2)	639.94	683.31	285.77	285.77	690.04	702.01
TP (ms^2)	951.46	957.25	450.68	450.68	1023.23	1046.12
LF n.u.	30.13	24.89	32.25	32.25	30.26	30.66
HF n.u.	69.87	75.11	67.75	67.75	69.74	69.34
LF/HF	0.43	0.33	0.48	0.48	0.43	0.4

Explanation: Note how the time and frequency domain data for the direct and indirect recordings is almost identical. **Abbreviations**: IND, indirect recording; DIR, direct recording; RR triang. ind., RR triangular index.

3.1.2. Descriptive and inferential statistics

Table 3.1.2. presents the calculated mean, with its standard deviation for each variable. P-values, calculated with the Wilcoxon statistical test, are provided for the difference between direct and indirect values for a specific variable.

Table 3.1.2.a *The mean, standard deviation and p-value for the time domain variables*

Variable	Indirect variable mean (SD)	Direct variable mean (SD)	I – D variable mean (SD)	P-value
Mean RR (s)	0.85 (0.07)	0.84 (0.07)	0.01 (0.02)	0.1250
RR STD (s)	0.04 (0.01)	0.04 (0.01)	- 0.01 (0.01)	0.6250
Mean HR (bpm)	71.76 (6.67)	72.46 (6.36)	- 0.71 (1.56)	0.1250
HR STD (bpm)	4.23 (0.36)	4.31 (0.40)	- 0.08 (0.16)	0.8125
RMSSD (ms)	46.57 (9.54)	46.35 (9.33)	0.40 (1.17)	0.6250
pNN50 (%)	30.38 (13.41)	29.73 (12.99)	0.65 (1.10)	0.1250
SDANN (ms)	29.46 (15.61)	27.38 (14.55)	2.08 (17.83)	0.6250
RR triang. ind.	0.10 (0.02)	0.09 (0.02)	0.01 (0.01)	0.3125
TINN (ms)	274.17 (52.95)	289.17 (71.44)	- 15.00 (47.22)	1.0000
SD1 (ms)	33.19 (6.76)	32.91 (6.61)	0.28 (0.84)	0.6250
SD2 (ms)	74.84 (10.86)	76.07 (10.85)	-1.23 (6.44)	0.4375

p-value calculated with Wilcoxen statistical test, note that there are no significant differences

Table 3.1.2.b *The mean, standard deviation and p-value for frequency domain variables*

Variable	Indirect variable mean (SD)	Direct variable mean (SD)	I – D variable mean (SD)	P-value
LF (ms²)	245.50 (69.73)	239.92 (62.78)	5.58 (34.25)	0.4375
HF (ms²)	576.73 (167.75)	570.49 (166.13)	6.24 (24.81)	0.4375
TP (ms²)	860.46 (237.31)	847.62 (230.39)	12.84 (8.73)	0.6250
LF n.u. (%)	30.06 (2.61)	29.88 (2.20)	0.17 (3.36)	0.6250
HF n.u. (%)	69.94 (2.61)	70.11 (2.20)	-0.17 (3.36)	0.6250
LF/HF	0.43 (0.05)	0.43 (0.04)	0.01 (0.06)	0.6250

p-value calculated with Wilcoxon statistical test, note that there are no significant differences

3.2 Interpersonal variation and intrapersonal variation

3.2.1. Data summary

The individual data for the 6 recordings (on the 6 consecutive days) for each of the nine participants can be found in Table 3.2.1. in the Appendix to this chapter.

DEMONSTRATION OF INTRAPERSONAL VARIATION

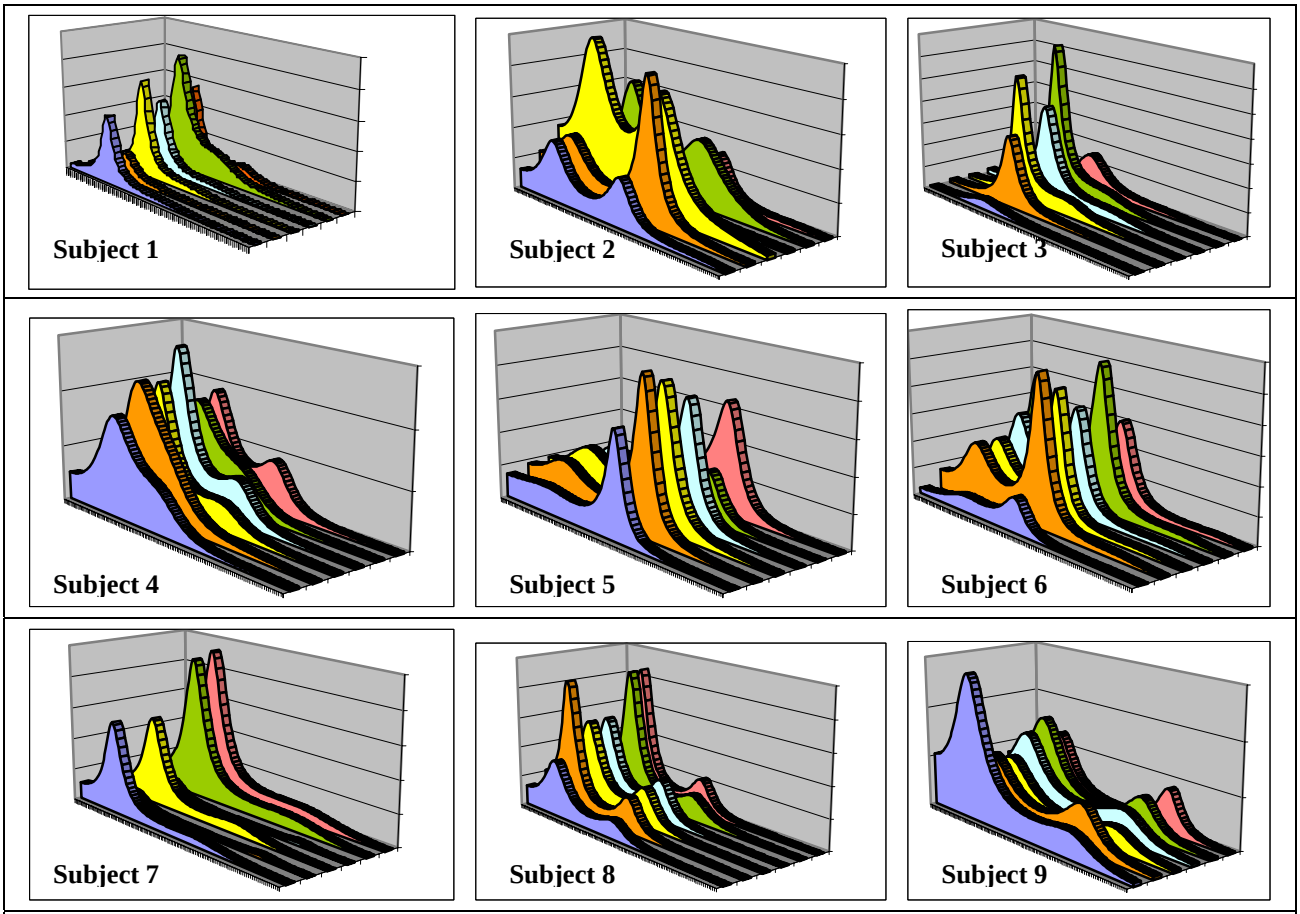


Figure 3.2.1.a Power spectral density graphs of the 6 recordings for each of the nine subjects. The 6 recordings on the 6 consecutive days for a specific subject is presented on the same axes to illustrate the similarity within an individual with regard to the power in the frequency domain. X-axis: Frequency in Hz; Y-axis: Power spectral density (PSD) in s²/Hz. The area under each curve shows the amount of power in the frequency domain.

Key: recording 1 recording 2 recording 3 recording 4 recording 5 recording 6

DEMONSTRATION OF INTERPERSONAL VARIATION

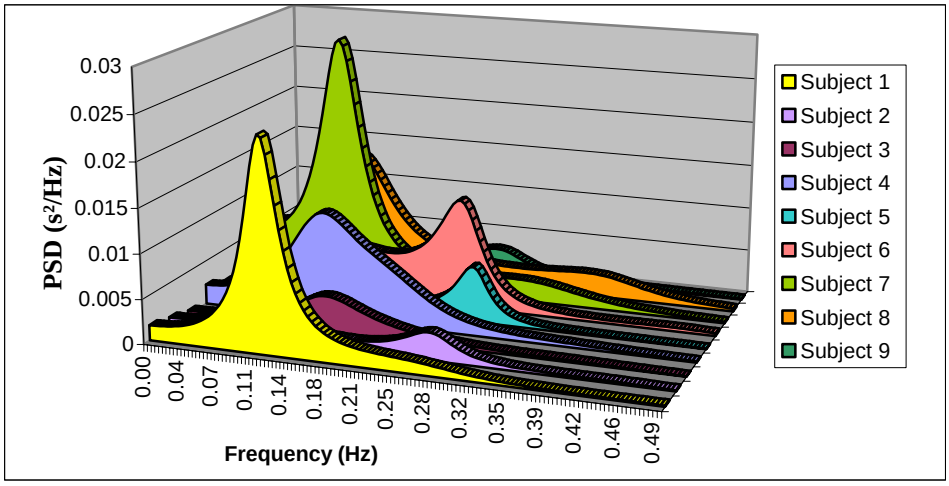


Figure 3.2.1.b Power spectral density (PSD) graph for the first recording of each of the nine subjects. The 9 first recordings are presented on the same axes to illustrate the diversity between individuals with regard to the power in the frequency domain (in comparison to Figure 3.2.1.a).

3.2.2 Descriptive and inferential statistics

For each subject, means and standard deviations were calculated for the 6 recordings (observations) made on the 6 consecutive days (Table 3.2.2.).

Table 3.2.2.a *Time domain means and standard deviations for each subject*

Variable	Subject 1 (6 observ)	Subject 2 (6 observ)	Subject 3 (6 observ)	Subject 4 (6 observ)	Subject 5 (6 observ)
Mean RR (s)	0.86 (0.07)	0.89 (0.07)	0.97 (0.06)	0.87 (0.03)	0.84 (0.07)
RR STD (s)	0.06 (0.02)	0.05 (0.01)	0.07 (0.02)	0.06 (0.00)	0.04 (0.01)
Mean HR (bpm)	70.43 (5.59)	67.74 (4.78)	62.48 (4.10)	70.04 (2.54)	72.46 (6.36)
HR STD (bpm)	5.47 (0.88)	3.97 (0.71)	4.75 (1.01)	5.43 (0.30)	4.31 (0.40)
RMSSD (ms)	54.41 (22.87)	52.81 (16.57)	80.73 (26.88)	52.28 (3.39)	46.35 (9.33)
pNN50 (%)	24.85 (14.46)	26.44 (12.10)	45.24 (17.56)	28.09 (3.04)	29.73 (12.99)
SDANN (ms)	10.62 (4.05)	16.85 (10.66)	21.03 (14.89)	20.67 (6.32)	27.38 (14.55)
RR triang. ind.	0.13 (0.04)	0.10 (0.03)	0.15 (0.04)	0.12 (0.01)	0.09 (0.02)
TINN (ms)	353.33 (81.83)	353.33 (163.02)	398.33 (99.18)	295.83 (12.81)	289.17 (71.44)
SD1 (ms)	38.70 (16.22)	37.54 (11.76)	57.27 (19.06)	37.18 (2.40)	32.91 (6.61)
SD2 (ms)	102.69 (26.53)	82.91 (27.28)	102.98 (28.72)	112.20 (6.83)	76.07 (10.85)
Variable	**Subject 6 (6 observ)**	**Subject 7 (6 observ)**	**Subject 8 (6 observ)**	**Subject 9 (6 observ)**	**p-value**
Mean RR (s)	0.91 (0.06)	0.86 (0.06)	0.84 (0.04)	0.86 (0.05)	0.8886
RR STD (s)	0.10 (0.02)	0.07 (0.03)	0.06 (0.01)	0.06 (0.01)	0.0243*
Mean HR (bpm)	67.70 (3.96)	71.06 (4.43)	72.60 (3.88)	70.48 (4.24)	0.8584
HR STD (bpm)	7.89 (1.18)	6.34 (1.56)	6.35 (0.63)	5.70 (0.70)	0.0184*
RMSSD (ms)	114.16 (27.15)	62.64 (29.51)	59.56 (6.80)	44.67 (8.40)	0.0552
pNN50 (%)	52.83 (11.23)	26.43 (16.02)	32.49 (6.91)	23.02 (7.77)	0.2941
SDANN (ms)	26.87 (12.83)	28.05 (11.47)	22.13 (7.87)	27.48 (12.03)	0.3242
RR triang. ind.	0.18 (0.04)	0.11 (0.04)	0.10 (0.01)	0.12 (0.02)	0.1287
TINN (ms)	510.0 (105.02)	399.17 (180.40)	345.83 (63.52)	309.17 (55.35)	0.0956
SD1 (ms)	81.10 (19.30)	44.65 (20.97)	42.37 (4.84)	31.93 (6.01)	0.0563
SD2 (ms)	161.41 (33.73)	129.90 (42.87)	106.15 (10.29)	109.13 (19.46)	0.0146*

* indicates significant difference ($p \leq 0.05$), standard deviation is indicated in brackets.
Standard deviations indicative of the intrapersonal variation. P-values (calculated with ANOVA) indicative of interpersonal variation. **Abbreviations**: Obsev., observation; RR triang. ind., RR triangular index.

Table 3.2.2.b *Frequency domain means and standard deviations for each subject*

Variable	Subject 1 (6 observ)	Subject 2 (6 observ)	Subject 3 (6 observ)	Subject 4 (6 observ)
LF (ms²)	1295.96 (542.39)	376.84 (202.01)	961.93 (735.74)	768.99 (140.59)
HF (ms²)	637.75 (578.50)	510.42 (295.28)	1559.54 (665.89)	706.84 (129.07)
TP (ms²)	1981.72 (1093.52)	962.99 (532.55)	2553.04 (1268.46)	1522.30 (253.51)
LF n.u.	70.04 (8.64)	42.97 (3.04)	36.25 (12.72)	52.04 (3.75)
HF n.u.	29.96 (8.64)	57.03 (3.04)	63.75 (12.72)	47.96 (3.75)
LF/HF	2.53 (0.80)	0.76 (0.09)	0.62 (0.33)	1.10 (0.18)
Variable	**Subject 5 (6 observ)**	**Subject 6 (6 observ)**	**Subject 7 (6 observ)**	**Subject 8 (6 observ)**
LF (ms²)	239.92 (62.78)	1817.04 (839.80)	1777.50 (1256.14)	661.80 (176.47)
HF (ms²)	570.49 (166.13)	2852.37 (1031.50)	701.38 (528.55)	611.45 (123.61)
TP (ms²)	847.62 (230.39)	4840.75 (1871.99)	2587.51 (1844.06)	1337.29 (264.65)
LF n.u.	29.89 (2.20)	37.84 (4.84)	72.74 (1.87)	51.77 (5.17)
HF n.u.	70.11 (2.20)	62.16 (4.84)	27.26 (1.87)	48.23 (5.17)
LF/HF	0.43 (0.04)	0.62 (0.13)	2.68 (0.25)	1.09 (0.23)
Variable	**Subject 9 (6 observ)**	**p-value**		
LF (ms²)	0.86 (0.05)	0.0009*		
HF (ms²)	0.06 (0.01)	0.0331*		
TP (ms²)	70.48 (4.24)	0.0102*		
LF n.u.	5.70 (0.70)	0.0001*		
HF n.u.	44.67 (8.10)	0.0001*		
LF/HF	23.02 (7.77)	0.0001*		

* indicates significant difference ($p \leq 0.05$), standard deviation is indicated in brackets.
Standard deviations indicative of the intrapersonal variation. P-values (calculated with ANOVA) indicative of interpersonal variation. **Abbreviations**: Obsev., observation; RR triang. ind., RR triangular index.

In Table 3.2.2.a) and b), the standard deviation calculated for the means gives an indication of the amount of variance in HRV parameters within an individual (intrapersonal variation). The p-values calculated for the differences between the respective experimental subjects, is indicative of the amount of variance in HRV parameters between subjects (interpersonal variation).

3.3. Sensitivity and response to stressors

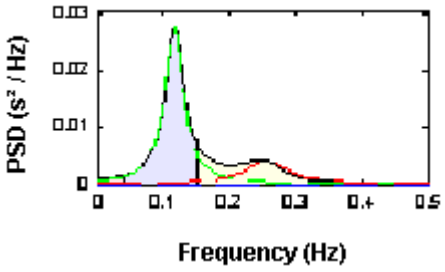
3.3.1. Data summary

During the last two recordings for each subject, the sensitivity of the technique was evaluated by exposing the experimental subjects to either raucous or soothing music. After the results were studied, it became apparent that two distinctive groups could be distinguished on the basis of autonomic balance. The first, the sympathetic dominant group, had a LF/HF ratio > 1 during resting conditions. The other, the parasympathetic dominant group, a LF/HF ratio < 1. The following PSD graphs demonstrate how these two groups differ in their autonomic response to music. The low frequencies (sympathetic activation) are indicated in blue and the high frequencies (vagal activation) in yellow.

SYMPATHETIC DOMINANT GROUP

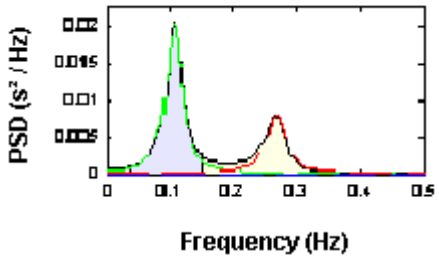
Effect of soothing music on ANS

Subject 1



LF (%)	67.34
HF (%)	32.66
LF/HF	2.06

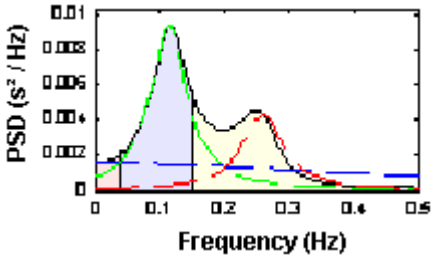
Baseline recording



LF (%)	57.20
HF (%)	42.80
LF/HF	1.34

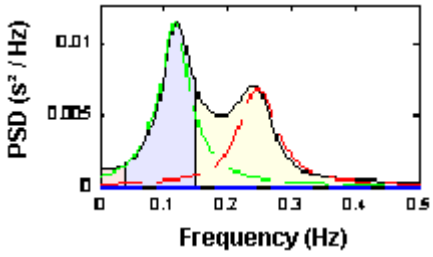
Effect of soothing music

Subject 4



LF (%)	50.08
HF (%)	49.92
LF/HF	1.00

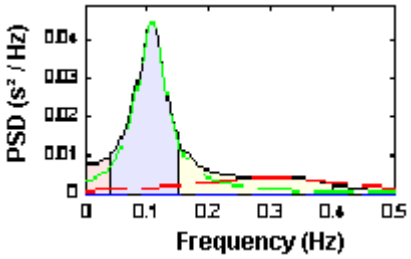
Baseline recording



LF (%)	32.27
HF (%)	67.73
LF/HF	0.48

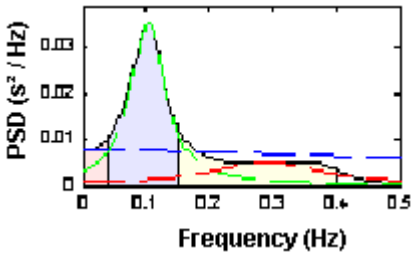
Effect of soothing music

Subject 7



LF (%)	71.50
HF (%)	28.50
LF/HF	2.51

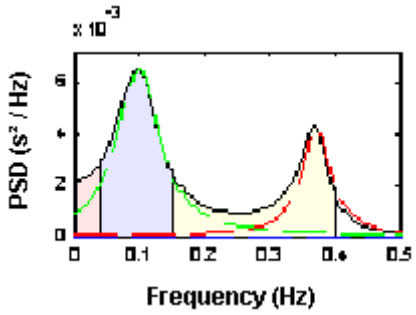
Baseline recording



LF (%)	56.27
HF (%)	43.73
LF/HF	1.29

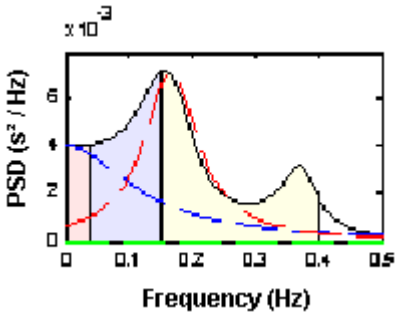
Effect of soothing music

Subject 8



LF (%)	55.33
HF (%)	44.67
LF/HF	1.24

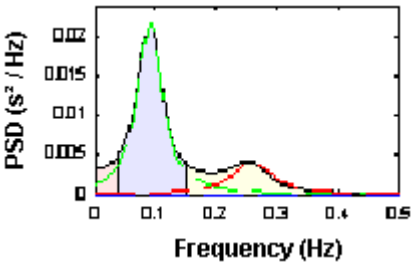
Baseline recording



LF (%)	46.68
HF (%)	53.32
LF/HF	0.88

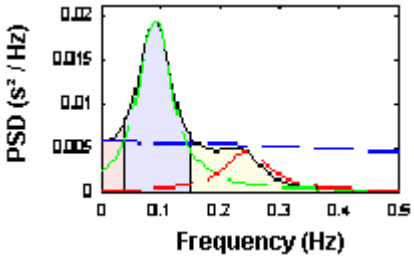
Effect of soothing music

Subject 9



LF (%)	71.49
HF (%)	28.51
LF/HF	2.51

Baseline recording

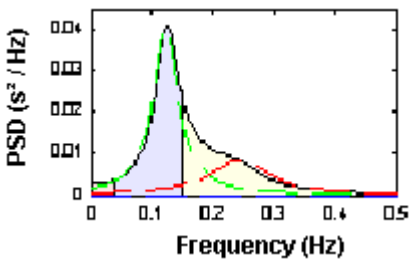


LF (%)	57.81
HF (%)	42.19
LF/HF	1.37

Effect of soothing music

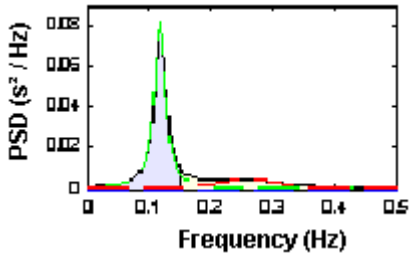
Effect of raucous music on ANS

Subject 1



LF (%)	53.78
HF (%)	46.22
LF/HF	1.16

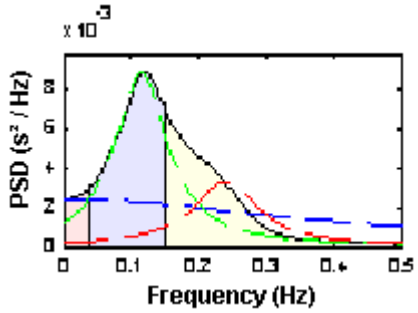
Baseline recording



LF (%)	76.09
HF (%)	23.91
LF/HF	3.18

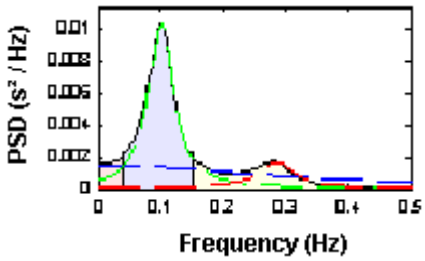
Effect of raucous music

Subject 4



LF (%)	53.54
HF (%)	46.46
LF/HF	1.15

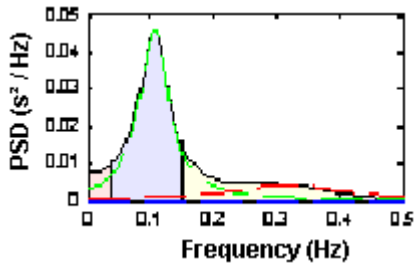
Baseline recording



LF (%)	72.88
HF (%)	27.12
LF/HF	2.69

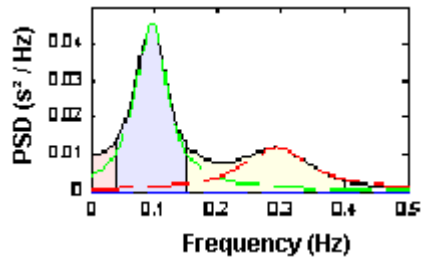
Effect of raucous music

Subject 7



LF (%)	69.98
HF (%)	30.02
LF/HF	2.33

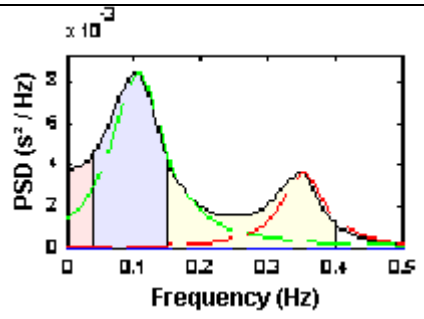
Baseline recording



LF (%)	71.28
HF (%)	28.72
LF/HF	2.48

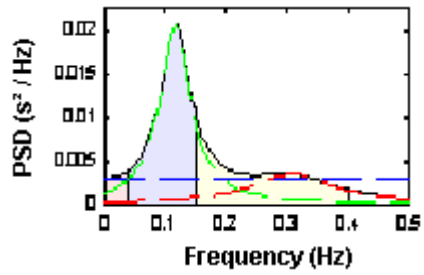
Effect of raucous music

Subject 8



LF (%)	48.79
HF (%)	51.21
LF/HF	0.95

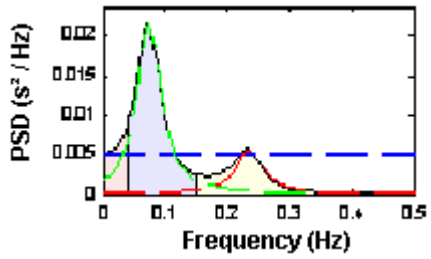
Baseline recording



LF (%)	57.51
HF (%)	42.49
LF/HF	1.35

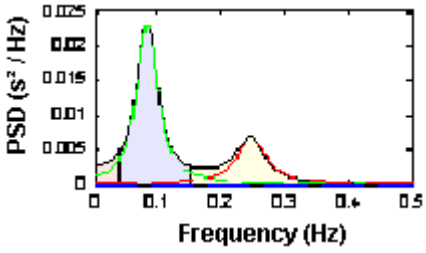
Effect of raucous music

Subject 9



LF (%)	70.85
HF (%)	29.15
LF/HF	2.43

Baseline recording



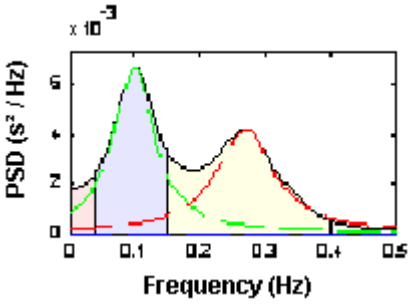
LF (%)	72.79
HF (%)	27.21
LF/HF	2.68

Effect of raucous music

PARASYMPATHETIC DOMINANT GROUP

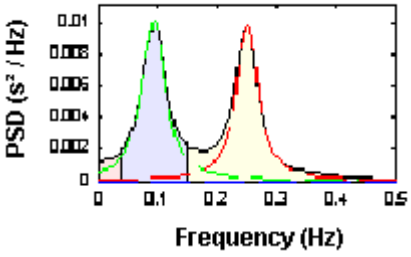
Effect of soothing music on ANS

Subject 2



LF (%)	39.05
HF (%)	60.95
LF/HF	0.64

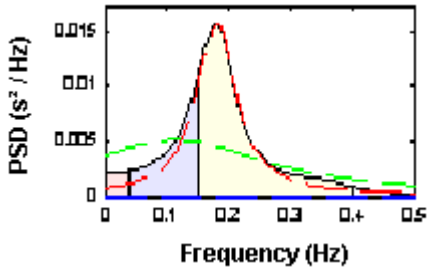
Baseline recording



LF (%)	57.20
HF (%)	42.80
LF/HF	1.34

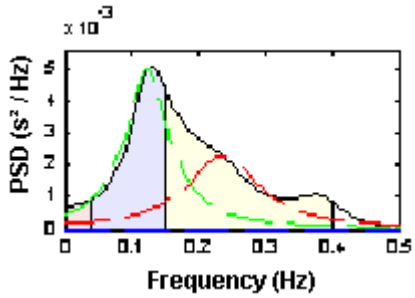
Effect of soothing music

Subject 3



LF (%)	22.95
HF (%)	77.05
LF/HF	0.30

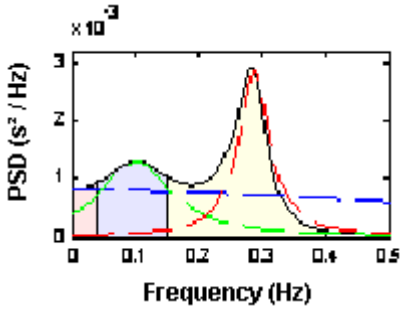
Baseline recording



LF (%)	28.48
HF (%)	71.52
LF/HF	0.40

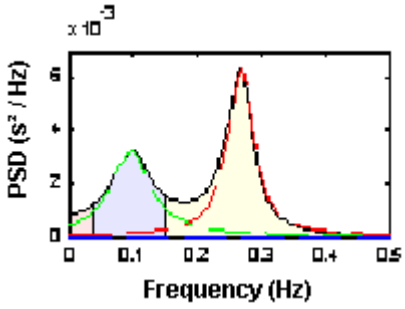
Effect of soothing music

Subject 5



LF (%)	32.25
HF (%)	67.75
LF/HF	0.48

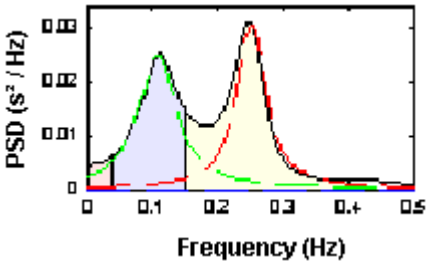
Baseline recording



LF (%)	41.31
HF (%)	58.69
LF/HF	0.70

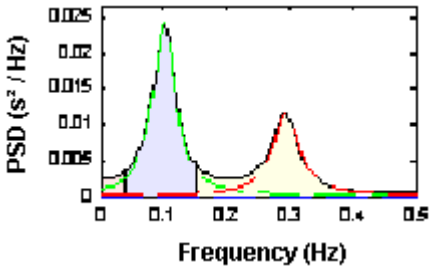
Effect of soothing music

Subject 6



LF (%)	39.38
HF (%)	60.62
LF/HF	0.65

Baseline recording

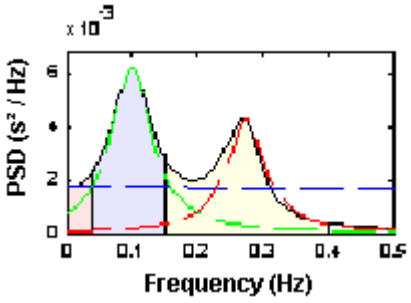


LF (%)	57.70
HF (%)	42.30
LF/HF	1.36

Effect of soothing music

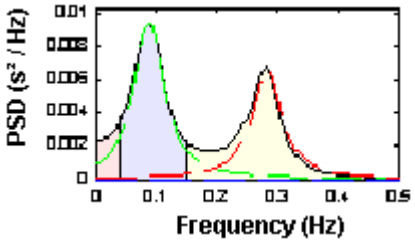
Effect of raucous music on ANS

Subject 2



LF (%)	46.04
HF (%)	53.96
LF/HF	0.85

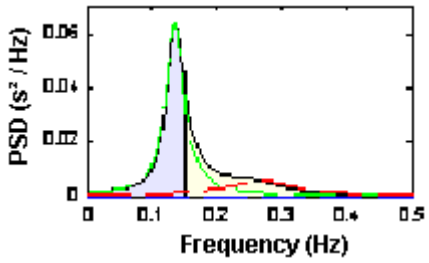
Baseline recording



LF (%)	35.65
HF (%)	64.35
LF/HF	0.55

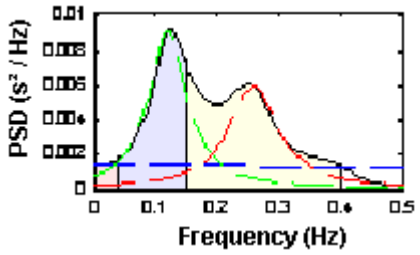
Effect of raucous music

Subject 3



LF (%)	46.81
HF (%)	53.19
LF/HF	0.88

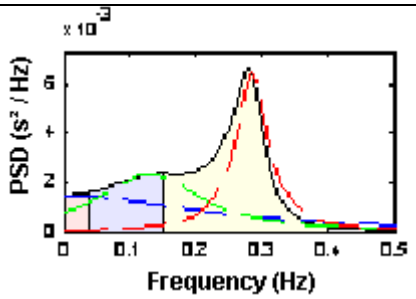
Baseline recording



LF (%)	38.85
HF (%)	61.15
LF/HF	0.64

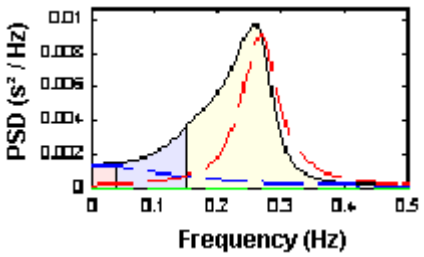
Effect of raucous music

Subject 5



LF (%)	30.26
HF (%)	69.74
LF/HF	0.43

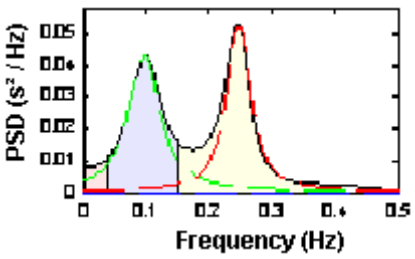
Baseline recording



LF (%)	12.61
HF (%)	87.39
LF/HF	0.14

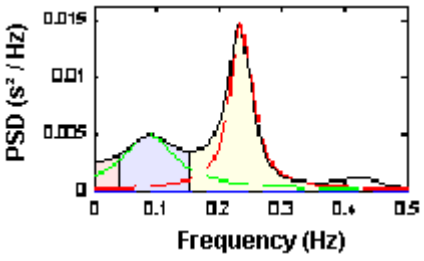
Effect of raucous music

Subject 6



LF (%)	43.71
HF (%)	56.29
LF/HF	0.78

Baseline recording



LF (%)	25.27
HF (%)	74.73
LF/HF	0.34

Effect of raucous music

3.3.2. Descriptive and inferential statistics

Table 3.3.2.a *The means and standard deviation of the effect of different types of music on the autonomic nervous system*

	Baseline		**Soothing music**		**Baseline**		**Raucous music**	
Sympathetic Group		**nu (%)**		**nu (%)**		**nu (%)**		**nu (%)**
Mean HR (bpm)	70.05 (1.98)		66.53 (2.68)		68.79 (5.23)		68.35 (5.71)	
TP (ms²)	2199.90 (1493.20)		1942.00 (606.71)		2603.50 (1490.30)		3247.90 (2231.70)	
LF (ms²)	1406.30 (1105.30)	63.15	927.03 (410.98)	50.04	1515.10 (1013.20)	59.39	1996.90 (1770.00)	70.11
HF (ms²)	704.20 (343.39)	36.85	899.80 (262.56)	50.00	962.27 (559.31)	40.61	877.21 (573.15)	29.89
LF/HF	1.86 (0.71)		1.07 (0.39)		1.61 (0.71)		2.48 (0.68)	
Parasympathetic Group		**nu (%)**		**nu (%)**		**nu (%)**		**nu (%)**
Mean HR (bpm)	70.29 (10.37)		68.76 (8.80)		65.21 (6.65)		65.36 (5.95)	
TP (ms²)	2029.20 (1873.60)		1459.0 (1005.90)		3185.40 (2855.40)		1344.90 (366.12)	
LF (ms²)	687.94 (746.70)	33.41	729.18 (625.72)	46.17	1349.10 (1275.00)	41.71	377.09 (242.37)	28.09
HF (ms²)	1274.90 (1099.90)	66.589	674.50 (326.75)	53.83	1737.60 (1560.00)	58.30	922.98 (238.98)	71.91
LF/HF	0.52 (0.17)		0.95 (0.48)		0.74 (0.21)		0.42 (0.22)	

Table 3.3.2.b *The means, standard deviation and p-values for the change in autonomic activity in response to different types of music*

	Δ (Basal – Soothing music) Means (SD)			**Δ (Basal – Raucous music) Means (SD)**		
Sympathetic Group		**p-value (within)**			**p-value (within)**	**p-value**
Mean HR (bpm)	3.52 (1.82)	p = 0.0591		0.44 (2.95)	p = 0.7874	
TP (ms²)	257.90 (1386.50)	p = 1.0000		- 644.43 (889.90)	p = 0.1775	
LF (ms²)	479.24 (944.11)	p = 0.4185		- 481.79 (837.04)	p = 0.4185	
LF (nu)	13.10 (3.73)	p = 0.0591		- 10.72 (9.73)	p = 0.0591	
HF (ms²)	- 195.59 (408.96)	p = 0.2807		85.06 (477.85)	p = 0.7874	
HF (nu)	- 13.10 (3.73)	p = 0.0591	**p-value**	10.72 (9.73)	p = 0.0591	**p-value**
LF/HF	0.80 (0.36)	p = 0.0591	**(between)**	- 0.87 (0.85)	p = 0.0591	**(between)**
Parasympathetic Group						
Mean HR (bpm)	1.54 (2.54)	p = 0.5839	p = 0.1270	- 0.15 (4.27)	p = 0.8551	p = 0.9025
TP (ms²)	570.22 (1141.20)	p = 0.5839	p = 0.7302	1840.4 (2869.80)	p = 0.5839	p = 0.1508
LF (ms²)	- 41.24 (334.65)	p = 0.8551	p = 0.4841	972.02 (1240.90)	p = 0.1003	p = 0.1508
LF (nu)	- 12.76 (6.48)	p = 0.1003	p = 0.0079*	13.61 (5.23)	p = 0.1003	p = 0.0397*
HF (ms²)	600.37 (849.42)	p = 0.5839	p = 0.2381	814.61 (1572.60)	p = 0.5839	p = 0.5556
HF (nu)	12.76 (6.48)	P = 0.1003	p = 0.0397*	- 13.61 (5.23)	p = 0.1003	p = 0.0079*
LF/HF	- 0.43 (0.32)	p = 0.1003	p = 0.0079*	0.32 (0.08)	p = 0.1003	p = 0.0397*

p-values (within) calculated with Wilxoxon signed rank test, p-values (between) calculated with Wilxoxon rank sum test. *indicates statistical significant difference

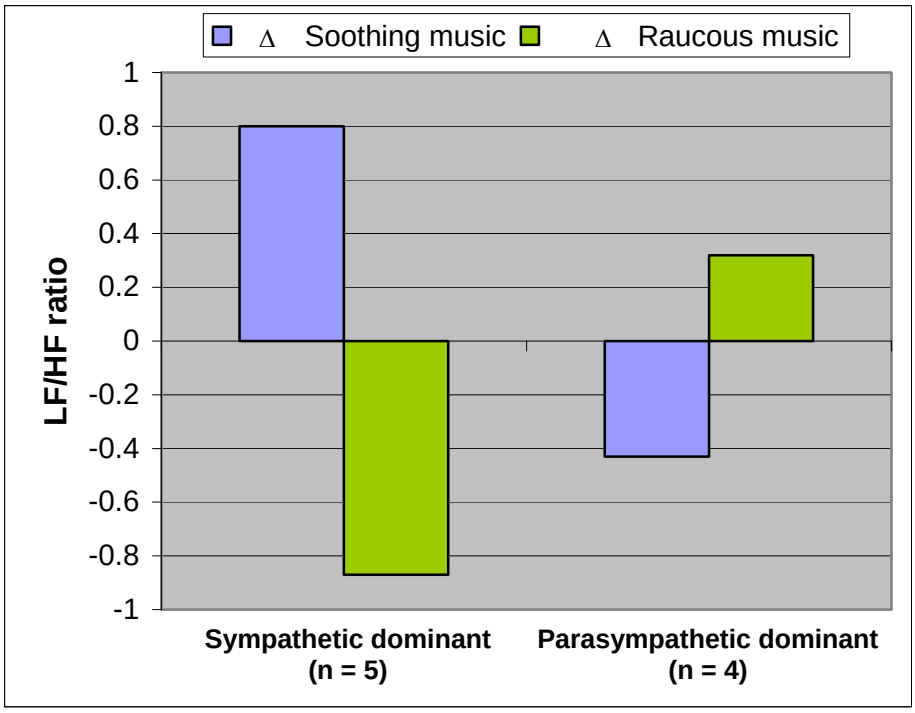


Figure 3.2.2. The mean effect of soothing and raucous music on subjects with different autonomic balances

4. Discussion

The analysis of HRV has attracted an extensive amount of interest in psychology and physiology and has therefore become an important measure in neuropsychology (1). The quantification and interpretation of HRV remains rather complex, though. The inaccurate quantification and interpretation of HRV patterns in psychophysiology (or in any other field) may obscure crucial issues or relationships and may hinder, rather than promote, psychophysiological applications (9).

Although our understanding of HRV is far from complete, it seems to be a marker of both dynamic and cumulative load. As a dynamic marker of load, HRV appears to be sensitive and responsive to acute stress. Under laboratory conditions, mental load – including making complex decisions, and public speech tasks – have been shown to lower HRV (20). As a marker of cumulative wear and tear, HRV has also been shown to decline with the aging process. Although resting heart rate does not change significantly with advancing age, there is a decline in HRV, which has been attributed to diminished efferent vagal tone and reduced beta-adrenergic responsiveness. By contrast, regular physical activity (which slows down the aging process) has been shown to raise HRV, presumable by increasing vagal tone (20).

The aim of this technique evaluation was to become acquainted with the analysis of R-R intervals as a measure of HRV, since this evaluation was to be used in the main fibromyalgia study. Apart from assessing the technique's reproducibility, a primary objective was to see how sensitive the technique would be in the assessment of the autonomic nervous system's response to stressors. Evaluating the effect of music on the sympathetic-parasympathetic balance was an ideal way to reach this goal, since music is a minor stressor. Thus, the method would have to be fairly sensitive to detect changes in autonomic nervous system activity in response to listening to music.

Firstly, technique reproducibility was evaluated. Table 3.1.2.a) and b) (Section 3) contain the means and standard deviations calculated for the mean RR, mean HR, RMSSD, pNN50, SDANN, RR triangular index, TINN, SD1, SD 2, LF, HF, total power and LF/HF ratio. According to the p-values calculated for these time and frequency domain variables, the difference between all the direct and indirect measures was statistically non-significantly ($p > 0.05$ for all of the variables). The similarity of the two measures was plainly seen in the raw data (Table 3.1.1, Section 3) as well. This is an indication that R-R recording using the Polar heart rate monitor (indirect) and interface (direct) has high reproducibility and therefore is a reliable tool in assessing HRV.

In order to determine the intra- and interpersonal variation of the subjects, it was necessary to compare the 6 recordings obtained from each of the nine subjects, firstly within each individual (intrapersonal), and then between the respective subjects (interpersonal). Table 3.2.2.a) (Section 3) summarises the time domain results for each subject. According to the standard deviations calculated for each of the variables, the amount of variability within each subject seemed to be rather similar to the variability in the rest. One participant, subject 4, seemed to have less variability than the rest of the participants, though. The same was observed when reviewing the standard deviations of the frequency domain results. Once again, subject 4 clearly had less variability than the rest of the study group. After completion of the experimental part of the study, this subject revealed that he used anti-depressive drugs (anti-depressants are known to lower HRV). Figure 3.2.1.a (Section 3) visibly illustrates intrapersonal variation in the power spectral components of HRV (frequency domain).

To demonstrate interpersonal variation, p-values were calculated for the differences in the time and frequency domain variables between the respective subjects (Table 3.2.2.a, b). As far as the time domain variables are concerned, statistically significant differences between the subjects were obtained for the standard deviation of the mean RR ($p = 0.0243$), the standard deviation of the mean HR ($p = 0.0184$), and SD2 ($p = 0.0146$). According to the standardisation criteria set out by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, the standard deviation of the mean RR is also referred to as SDANN (1). SDANN and SD2 give insight into the long-term components of HRV (18). Because the long-term variation of HRV is usually associated with the sympathetic modulation, it seems that, according to time domain measures, the subjects differ the most with regard to their sympathetic nervous system modulation of HRV. In the frequency domain, subjects differed significantly from each other for all the power spectral components of HRV (LF: $p = 0.0009$; LFnu: $p = 0.0001$; HF: $p = 0.0331$; HFnu: $p = 0.0001$; total power: $p = 0.0102$; and LF/HF: $p = 0.0001$). These results imply that the subjects had high interpersonal variance in the amount of activity exhibited by the sympathetic (LF and LFnu) and parasympathetic nervous system (HF and HFnu), the total amount of HRV (total power), and autonomic balance (LF/HF ratio). Thus, analysis in the time and frequency domain differed in the regard that frequency domain analysis was able to identify variances between the subjects not detected through time domain analysis. The clear differences in spectral power are illustrated in Figure 3.2.1.b).

During the evaluation of the autonomic nervous system activity in reaction to stressors, it was clear that the effect of music is strong enough to elicit an autonomic response in the subjects (the individual reactions to the different types of music is illustrated in Section 3.3.1.). The deviation from the baseline in response to the music was statistically non-significant for all the HRV parameters evaluated, though (see Table 3.3.2). What is of particular interest is the direction of change in response to the music. It seemed that the two groups of subjects distinguished on the basis of their autonomic balance (LF/HF ratio), the sympathetic ($LF/HF > 1$) and parasympathetic dominant group ($LF/HF < 1$), reacted in opposite ways from one another in response to the music played. This difference in reaction between the two groups is best described by the change in the LF/HF ratio. In response to the soothing music, the sympathetic dominant group's LF/HF ratio decreased ($\Delta = 0.80$, SD 0.36) while that of the parasympathetic dominant group increased ($\Delta = -0.43$, SD 0.32). In response to the raucous music, the sympathetic dominant group showed an increased LF/HF

ratio (Δ = - 0.87, SD 0.85), while the parasympathetic group's ratio decreased (Δ = 0.32, SD 0.08). What this basically implies is that the sympathetic dominant subjects react to the different types of music in the way expected: they seem to be excited by raucous music and calmed by soothing music. The parasympathetic subjects, however, react in a fairly unusual way: they are stimulated by soothing music and calmed by raucous music (see Figure 3.2.2., Section 3). The decreased LF/HF observed in the sympathetic dominant group in response to soothing music, is mainly due to decreased sympathetic activity (Δ LF = 479.24, SD 944.11), with some increased activity in the parasympathetic branch of the autonomic nervous system (Δ HF = - 195.59, SD 408.96). In the parasympathetic dominant group, major decreases in parasympathetic activity (Δ HF = 600.37, SD 849.42) was responsible for the sympathetic dominance (increased LF/HF ratio) during soothing music. As far as the raucous music was concerned, the predominance of the sympathetic branch (increased LF/HF ratio) in the sympathetic dominant group was caused by increases in sympathetic (Δ LF = - 481.79, SD 837.04) and decreases in parasympathetic activity (Δ HF = 85.06, SD 477.85). The parasympathetic group reacted to raucous music with decreases in both sympathetic (Δ LF = 972.02, SD 1240.90) and parasympathetic activity (Δ HF = 814.61, SD 1572.60). In addition to the opposite reaction of the sympathetic and parasympathetic branches observed in the two groups, the sympathetic dominant group also showed a greater reaction to the music played (see Figure 3.2.2.). The difference in the response to music between the two groups was statistically significant for the low and high frequency as well as the LF/HF ratio (soothing music: LFnu $p = 0.0397$, HFnu $p = 0.0079$, LF/HF ratio $p = 0.0397$; raucous music: LFnu $p = 0.0079$, HFnu $p = 0.0397$, LF/HF ratio $p = 0.0079$).

It would be interesting to extend this type of study to a larger sample to determine the reproducibility of these results and to assess the influence of personal preference in music styles on the autonomic response to different types of music. If these results were to be repeated in a larger study, it could hold interesting implications for practises such as music therapy. As soon as normative HRV values are available for the autonomic response to different music styles, music therapy practises could incorporate HRV recordings when examining the effectiveness of their therapies.

5. Conclusions

The technique evaluation of HRV showed that the recording of R-R intervals with the Polar heart monitors could be a reliable method for the evaluation of HRV on the condition that great care is taken not to use simplistic HRV myths in analysing and reporting results. Under carefully controlled laboratory conditions and with standardised protocols, this technique is able to provide reproducible results. The reproducibility of the technique was demonstrated by the similarity between direct and indirect measures as well as relatively low intrapersonal variation within the subjects evaluated.

Out of the results obtained by this study, it became apparent that there is high interpersonal variation between subjects. The high interpersonal variation holds implications for the assessment of the ability of the technique to detect changes in the sympathetic-parasympathetic balance of the autonomic nervous system. When calculating descriptive statistics for heart rate variability measures, high interpersonal variation will result in larger standard deviations for means, which in turn can result in non-significant p-values (even in the midst of clear differences between groups/ changes from baseline to intervention). To eliminate problems such as these, larger study groups are needed.

It is necessary to realise that analysis of HRV in the frequency domain, while providing clues to autonomic function, does not provide simple, unambiguous results. Inaccurate quantification and interpretation of HRV patterns should therefore be avoided by formulating an integrative and interdisciplinary perspective on the origins, quantification, and interpretation of patterns of HRV.

The technique evaluation for this study was presented at the Faculty Day of Health Sciences (UP) and the 31st Annual congress of the Physiology Society of Southern Africa.

C. APPENDIX TO CHAPTER – INDIVIDUAL SUBJECT DATA

Table 3.2.1. *The individual time and frequency domain measures for the 6 recordings (on the 6 consecutive days) for each of the nine subjects*

Subject	Observ	Mean RR (s)	RR STD (s)	Mean HR (bpm)	HR STD (bpm)	RMSSD (ms)	pNN50 (%)	SDANN (ms)	RR tri ind	TINN (ms)
1	1	0.84	0.06	71.63	5.41	47.34	20.77	10.14	0.13	365.00
	2	0.77	0.04	78.29	4.00	27.52	5.97	5.10	0.07	210.00
	3	0.85	0.06	71.43	6.15	49.75	22.28	11.16	0.14	350.00
	4	0.85	0.06	71.56	5.50	49.01	21.86	7.24	0.13	370.00
	5	1.00	0.09	61.06	6.57	96.70	50.29	14.54	0.19	465.00
	6	0.88	0.06	68.61	5.20	56.16	27.94	15.55	0.11	360.00
2	1	0.85	0.04	70.65	3.45	39.79	17.06	7.87	0.07	270.00
	2	0.87	0.05	69.21	3.94	57.70	23.97	11.53	0.09	665.00
	3	0.99	0.07	61.36	5.21	77.74	45.84	36.10	0.15	340.00
	4	0.80	0.03	74.80	3.17	30.07	8.72	9.30	0.07	190.00
	5	0.93	0.05	64.98	3.94	59.15	33.57	14.37	0.11	345.00
	6	0.92	0.05	65.40	4.14	52.43	29.48	21.93	0.11	310.00
3	1	0.86	0.03	69.65	2.93	32.22	9.94	10.28	0.08	225.00
	2	0.93	0.07	64.75	5.05	73.69	49.96	12.32	0.17	380.00
	3	0.98	0.09	61.85	5.51	99.86	50.57	24.03	0.17	415.00
	4	1.03	0.09	59.08	5.35	102.95	56.52	48.68	0.18	475.00
	5	1.03	0.09	59.06	5.45	99.02	55.43	9.07	0.18	510.00
	6	1.00	0.06	60.52	4.21	76.66	49.00	21.80	0.14	385.00
4	1	0.91	0.06	66.85	5.42	56.35	30.82	22.56	0.13	310.00
	2	0.90	0.06	67.13	5.43	56.04	30.20	15.45	0.13	310.00
	3	0.85	0.05	71.47	4.99	49.08	25.30	22.23	0.11	285.00
	4	0.86	0.06	70.58	5.24	52.62	29.02	15.40	0.13	300.00
	5	0.83	0.05	73.28	5.68	48.35	23.34	31.74	0.11	280.00
	6	0.86	0.05	70.92	5.81	51.26	29.89	16.66	0.11	290.00

Table 3.2.1. *The individual time and frequency domain measures for the 6 recordings (on the 6 consecutive days) for each of the nine subjects – continued*

Subject	Observ	Mean RR (s)	RR STD (s)	Mean HR (bpm)	HR STD (bpm)	RMSSD (ms)	pNN50 (%)	SDANN (ms)	RR tri ind	TINN (ms)
5	1	0.88	0.04	68.43	3.62	40.97	22.52	9.31	0.08	235.00
	2	0.89	0.05	67.95	4.21	53.17	39.44	46.15	0.11	325.00
	3	0.89	0.05	68.00	4.61	54.66	39.16	10.50	0.10	305.00
	4	0.84	0.05	71.64	4.48	50.74	36.08	33.29	0.10	395.00
	5	0.71	0.03	84.36	4.22	30.01	6.44	34.42	0.06	190.00
	6	0.81	0.05	74.39	4.74	48.54	34.78	30.64	0.11	285.00
6	1	0.83	0.06	72.72	5.54	64.32	33.13	16.12	0.10	370.00
	2	0.94	0.11	65.12	8.42	130.35	61.62	30.39	0.22	510.00
	3	0.92	0.10	66.92	8.40	116.79	54.77	30.51	0.18	490.00
	4	0.90	0.10	68.15	8.76	117.33	52.36	15.34	0.18	515.00
	5	0.99	0.12	61.99	8.19	144.45	65.18	19.73	0.23	695.00
	6	0.86	0.10	71.31	8.02	111.73	49.93	49.16	0.16	480.00
7	1	0.87	0.07	69.87	7.11	62.17	27.82	23.24	0.13	385.00
	2	0.78	0.03	77.07	4.40	28.14	6.72	26.79	0.07	205.00
	3	0.90	0.07	68.15	6.90	68.53	33.01	47.71	0.11	385.00
	4	0.79	0.03	76.23	4.35	28.77	7.19	13.80	0.07	205.00
	5	0.91	0.09	67.37	7.60	93.57	41.99	33.02	0.16	600.00
	6	0.90	0.10	67.67	7.71	94.66	41.84	23.75	0.14	615.00
8	1	0.89	0.07	67.83	6.82	68.69	35.75	20.22	0.11	465.00
	2	0.87	0.06	69.25	6.01	66.46	39.68	24.35	0.11	340.00
	3	0.78	0.05	78.22	6.25	51.36	20.56	33.75	0.09	330.00
	4	0.81	0.06	75.02	7.17	59.05	31.67	22.12	0.11	345.00
	5	0.82	0.06	74.02	6.50	57.72	29.73	23.09	0.11	320.00
	6	0.85	0.05	71.24	5.37	54.11	37.58	9.27	0.09	275.00
9	1	0.83	0.05	72.73	4.97	38.11	16.49	32.82	0.10	265.00
	2	0.78	0.05	77.09	5.08	30.89	10.45	10.46	0.10	225.00
	3	0.91	0.06	66.35	5.48	49.99	28.92	29.90	0.13	315.00
	4	0.91	0.06	66.65	5.58	49.28	29.77	28.52	0.13	325.00
	5	0.84	0.06	72.13	6.67	52.73	25.89	18.07	0.11	350.00
	6	0.89	0.06	67.93	6.41	47.04	26.57	45.09	0.13	375.00

Table 3.2.1. *The individual time and frequency domain measures for the 6 recordings (on the 6 consecutive days) for each of the nine subject s– continued*

Subject	Observ	SD1 (ms)	SD2 (ms)	LF (ms²)	HF (ms²)	TP (ms²)	LF n.u.	HF n.u.	LF/HF
1	1	33.70	99.20	1209.33	404.59	1613.92	74.93	25.07	2.99
	2	19.59	59.98	450.17	175.82	625.99	71.91	28.09	2.56
	3	35.44	108.84	1616.36	491.61	2107.97	76.68	23.32	3.29
	4	34.89	101.77	1283.37	414.94	1698.31	75.57	24.43	3.09
	5	68.67	143.22	2082.70	1789.65	3872.34	53.78	46.22	1.16
	6	39.91	103.15	1133.81	549.89	1683.70	67.34	32.66	2.06
2	1	28.30	63.89	262.14	295.22	557.37	47.03	52.97	0.89
	2	40.77	73.11	313.02	448.17	761.19	41.12	58.88	0.70
	3	55.28	126.21	745.60	1037.39	1782.98	41.82	58.18	0.72
	4	21.38	48.57	152.50	204.28	356.78	42.74	57.26	0.75
	5	42.03	95.35	396.31	618.54	1014.85	39.05	60.95	0.64
	6	37.46	90.33	391.50	458.90	850.40	46.04	53.96	0.85
3	1	22.88	54.83	205.46	274.10	479.56	42.84	57.16	0.75
	2	52.27	96.52	508.10	1769.84	2277.94	22.31	77.69	0.29
	3	70.83	114.37	2007.02	1849.01	3856.02	52.05	47.95	1.09
	4	73.05	138.61	918.62	2089.42	3008.04	30.54	69.46	0.44
	5	70.20	119.75	1703.57	1935.59	3639.17	46.81	53.19	0.88
	6	54.41	93.80	428.80	1439.30	1868.10	22.95	77.05	0.30
4	1	40.06	113.31	858.76	879.44	1738.20	49.41	50.60	0.98
	2	39.85	115.72	892.43	855.81	1748.24	51.05	48.95	1.04
	3	34.88	107.37	562.90	581.11	1144.01	49.20	50.80	0.97
	4	37.39	100.91	883.26	614.25	1497.51	58.98	41.02	1.44
	5	34.41	119.10	787.79	683.53	1471.32	53.54	46.46	1.15
	6	36.48	116.80	628.83	626.88	1255.70	50.08	49.92	1.00
5	1	29.10	61.65	198.25	452.12	650.36	30.48	69.52	0.44
	2	37.74	87.92	288.36	656.57	944.92	30.52	69.48	0.44
	3	38.81	79.37	241.54	698.54	940.08	25.69	74.31	0.35
	4	36.01	85.44	275.96	639.94	915.89	30.13	69.87	0.43
	5	21.33	64.29	136.05	285.77	421.82	32.25	67.75	0.48
	6	34.45	77.77	299.37	690.04	989.41	30.26	69.74	0.43

Table 3.2.1. *The individual time and frequency domain measures for the 6 recordings (on the 6 consecutive days) for each of the nine subjects – continued*

Subject	Observ	SD1 (ms)	SD2 (ms)	LF (ms²)	HF (ms²)	TP (ms²)	LF n.u.	HF n.u.	LF/HF
6	1	45.67	94.92	436.60	899.03	1335.62	32.69	67.31	0.49
	2	92.58	169.39	1671.28	3466.61	5137.88	32.53	67.47	0.48
	3	83.01	168.87	1748.86	3091.90	4840.76	36.13	63.87	0.57
	4	83.40	171.35	2252.83	3035.02	5287.85	42.60	57.40	0.74
	5	102.62	192.08	3002.02	3865.81	6867.83	43.71	56.29	0.78
	6	79.33	171.86	1790.62	2755.85	4546.48	39.38	60.62	0.65
7	1	44.42	153.77	1877.56	685.35	2562.91	73.26	26.74	2.74
	2	20.10	75.53	324.37	112.49	436.86	74.25	25.75	2.88
	3	48.87	157.57	1808.83	692.06	2500.89	72.33	27.67	2.61
	4	20.55	73.74	366.76	121.45	488.21	75.12	24.88	3.02
	5	66.57	159.02	3291.17	1311.59	4602.77	71.50	28.50	2.51
	6	67.38	159.79	2996.31	1285.34	4281.65	69.98	30.02	2.33
8	1	48.87	119.05	1014.64	702.85	1717.50	59.08	40.92	1.44
	2	47.24	102.89	652.65	797.00	1449.65	45.02	54.98	0.82
	3	36.55	104.23	565.96	487.40	1053.37	53.73	46.27	1.16
	4	42.03	114.08	592.35	624.20	1216.54	48.69	51.31	0.95
	5	41.06	107.33	548.02	575.19	1123.21	48.79	51.21	0.95
	6	38.47	89.30	597.15	482.05	1079.20	55.33	44.67	1.24
9	1	27.19	93.79	679.95	357.82	1037.77	65.52	34.48	1.90
	2	22.06	80.84	427.43	273.70	701.13	60.96	39.04	1.56
	3	35.71	112.76	951.01	588.99	1540.00	61.75	38.25	1.61
	4	35.25	113.81	912.94	602.49	1515.42	60.24	39.76	1.52
	5	37.62	116.77	1380.38	550.61	1930.99	71.49	28.51	2.51
	6	33.73	136.78	1160.80	477.65	1638.45	70.85	29.15	2.43

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