UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS

INSTITUTE OF SOUND AND VIBRATION RESEARCH

CARDIOVASCULAR AND RESPIRATORY RESPONSES TO PSYCHOPHYSIOLOGICAL TASKS: METHODOLOGICAL ISSUES FOR ASSESSING AUTONOMIC REGULATION

by

Alessandro Beda

Thesis for the degree of Doctor of Philosophy

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ABSTRACT

Beat-to-beat oscillations in heart-rate and systolic arterial-pressure are known to be modulated by the autonomic nervous system. Changes in the spectra of these signals, and in their interaction, have been used as markers of modifications in autonomic activation between tasks and/or subjects. These cardiovascular indexes have been extensively adopted in psychophysiology to investigate the autonomic reaction to psychological/cognitive challenges. In the current work such challenges were used to study the correlation between birth-weight and autonomic cardiovascular modulation in adult life, in order to investigate the physiological mechanisms underlying the fetal origins of cardiovascular disease.

However, factors other than autonomic modulation may strongly influence the estimation of cardiovascular indexes. In this thesis, two such confounding factors were investigated in detail.

Firstly, the between-task and inter-individual differences in respiratory patterns, especially in tasks involving speech were found to be strongly reflected in cardiovascular indexes. Clear evidence was found that a very significant part of changes in indexes during the psychophysiological experimental protocol considered can be explained by modifications in respiration, without assuming between-tasks or inter-individual differences in autonomic activation elicited by psychological/cognitive processes.

The second factor is the presence of within-task dynamics in the cardiovascular reaction to psychophysiological tasks. The common approach in psychophysiological investigations is to estimate cardiovascular indexes as average values over the whole length of the task. However, the results found show that such an approach may obscure significant within-task changes in the indexes, that might carry useful psychophysiological information. Choosing shorter epochs within the tasks for estimating the indexes has also a notable impact in terms of assessing changes elicited by the tasks.

Since these two factors are intrinsic in the reaction to psychophysiological tasks, they can have a profound impact on the indirect estimates of autonomic reaction through cardiovascular indexes. Controlling them during psychophysiological experiments may be difficult (if not impossible). However, their effects should be minimized, for example by avoiding tasks involving speech and choosing appropriate data epochs for the analysis. These effects must also be considered in the physiological interpretation of cardiovascular indexes, in order to ensure their usefulness in future research, such as in the fetal origins of cardiovascular disease in adult life.

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List of Abbreviations

ANS autonomic nervous system

AP arterial pressure (mmHg)*

AR baroreflex sensitivity estimated using a parametric autoregressive method (ms/mmHg)

BRS baroreflex sensitivity (ms/mmHg)

CI cardiovascular indexes, used in referring to all HRV, SAPV, and BRS indexes

E length of expiration (s)

ECG electrocardiogram (V)

F respiratory air-flow (l/s)

HF high-frequency band: $0.15 - 0.5$Hz

HP heart-period (s)

HR heart-rate (bpm)

HRV heart-rate variability (ms^2 or %)

I length of inspiration (s)

I/E inspiration/expiration length ratio (%)

LF low-frequency band: $0.05 - 0.15$Hz

P_{CO2} partial pressure of CO_2 at the mouth (cmH_2O)

* units reported where appropriate

P_{AIR} air-pressure at the mouth (cmH_2O)

RP respiratory period (s)

RSA respiratory sinus arrhythmia

SAN sinoatrial node

SAP systolic arterial pressure (mmHg)

SAPV systolic arterial pressure variability ($mmHg^2$ or %)

SEQ baroreflex sensitivity estimated using the sequence method (ms/mmHg)

SPC baroreflex sensitivity estimated using an FFT-based spectral method (ms/mmHg)

V respiratory volume (l)

V_T tidal volume (l)

VI variability indexes, used in referring to all HRV and SAPV indexes

Chapter 1

Introduction

1.1 Background

The theory of the fetal origin of cardiovascular diseases was first proposed by D.J.P. Barker [5]. According to his hypotheses, the probability of developing cardiovascular diseases in later life is strongly affected by the nutritional regime and health of mothers during pregnancy. Epidemiological studies have shown that in both men and women, small size at birth is associated with raised death rates from cardiovascular disease in later life. However, the physiological mechanisms linking small size at birth and the development of cardiovascular diseases are not yet clear.

The Medical Research Council Environmental Epidemiology Unit at the University of Southampton has been active in this research field since the theory was first proposed. One of the specific fields of interest has been to test if the link between small size at birth and the development of cardiovascular disease involves abnormal development of the autonomic nervous system. It is well known that the autonomic nervous system is strongly implicated in the control of the cardiovascular system, modulating for example heart-rate and peripheral resistance (resistance of the vessels of the circulatory tree) [18]. Abnormal behaviour of this regulatory system could, in the long term, lead to poor cardiovascular control, even if the abnormality is not evident through acute dysfunction in the short term. Hence, specific research was carried out to investigate the possible correlation between indexes of autonomic activity and size at birth (and other indexes related to fetal growth). To this end the researchers considered the reaction of a cohort of subjects to a set of psychophysiological tests (tests involving cognitive or psychological challenges). Psychophysiological tasks are able to elicit changes

in the autonomic control of the cardiovascular system, through the involvement of limbic and forebrain areas implicated in behavioural processes (e.g. amygdala and medial prefrontal cortex), which have been shown to issue monosynaptic projections to brainstem reflex networks as well as to autonomic source nuclei in the brainstem and spinal cord [18].

Several parameters have been widely used in the literature as indirect indexes of autonomic cardiovascular regulation (for simplicity, they will be referred as "cardiovascular indexes", CI, in the rest of this work). Among others, heart-rate variability in the high-frequencies band (HRV_{HF}, the power of heart-period signal in the band HF: 0.15-0.5Hz) has been used as index of vagal modulation. Heart-rate and systolic arterial-pressure variability in the low-frequencies band (HRV_{LF} and $SAPV_{LF}$, the power of heart-period and systolic arterial-pressure signal in the band LF: 0.05-0.15Hz) have been used as indexes of sympathetic modulation. The spontaneous baroreflex sensitivity in the low-frequency band (BRS_{LF}, the average gain of the frequency response between systolic-arterial pressure and heart-period in the LF band) has been used as index of the strength of the baroreflex mechanism, which modulates the autonomic outflow to the heart in response to changes in blood pressure (detected by baroreceptors at the level of the carotids and the aorta) [73].

1.2 Outline of the research

The current research project was initially motivated by the need for expertise and support in the methodological aspects of the estimation of cardiovascular indexes from the signals previously acquired during these experiments. To this end, a review of the cardiovascular indexes considered in this work, and of their physiological interpretation, was carried out first, and is presented in Chapter 2. Appropriate algorithms for estimation were implemented and indexes were computed for each task of the psychophysiological experimental protocol considered. The results, presented in Chapter 3, show that in women, low birth-weight is associated with smaller values of HRV_{HF} and BRS_{LF}, and higher values of SAP_{LF}, hence strongly supporting the hypothesis of an influence of birth-weight on autonomic modulation of the cardiovascular system.

During the analysis of the data, however, two specific issues emerged which have a significant impact on the results obtained and on psychophysiological investigations in general. The investigation on these two issues resulted in the

core of the research activity reported in this thesis.

The first issue concerns the role of respiration, and especially the respiration associated with speech, in modulating the change in cardiovascular indexes during the tasks considered. This issue emerged mainly from the observations that one of the stress-tasks of the experimental protocol required the subjects to talk (and hence involved a strongly modified respiratory pattern), and that this task produced different outcomes compared to the other stress-tasks (performed silently) in terms of cardiovascular indexes. The potential of changes in respiratory characteristics, such as respiratory rate and tidal volume, to elicit changes in cardiovascular indexes has been repeatedly shown previously [24, 108] and evidence exists that psychophysiological tasks, involving verbalization or not, are in general associated with changes in respiratory pattern when compared to the resting condition. Hence, it was hypothesized that at least part of the changes in cardiovascular indexes can be attributed to changes in respiratory characteristics [28, 85], rather than directly to changes in cental autonomic activation elicited by psychological/cognitive processes. However, as far as the author is aware, very few experimental studies have previously investigated this in detail [11, 104], or explored the potential impact on the use of cardiovascular indexes during speech tasks.

Such a study is of evident importance to understanding to what extent cardiovascular indexes are reliable markers of central autonomic modulation of the cardiovascular system during psychophysiological tasks. As the data available did not include respiration, it was unsuitable for such an investigation. Hence, a separate study was carried out, which included an experimental phase (a cohort of subjects performing a set of psychophysiological tasks, with simultaneous acquisition of the electrocardiogram, arterial blood pressure, and respiration). The analysis of the data involved two steps: investigation of the correlations existing between changes in cardiovascular indexes and characteristics of respiration (presented in Section 4.3), and a study of how much of cardiovascular variability can be explained as solely an effect of respiration, through a model-based investigation of the respiratory modulation of cardiovascular oscillations (presented in Section 4.4).

A second issue that emerged from the analysis of the change in cardiovascular indexes caused by psychophysiological tasks concerns the presence of within-task dynamics of the indexes. In general, for psychophysiological tasks, cardiovascular indexes are computed as averages for the whole duration of the task. Such an

approach may lose important information if the parameters undergo considerable modifications within the tasks. However, this has not been systematically investigated so far. Furthermore, if significant within-task changes in cardiovascular indexes are present, choosing specific epochs within a task to estimate indexes might have a significant impact on the estimated rest-task changes, which in many cases is the focus of the research.

A better understanding of the presence and of the impact of within-task changes in cardiovascular indexes potentially has an important effect in future research in terms of analysis of the data and of planning of experimental protocols. Hence a systematic investigation of the presence of significant within-task changes in cardiovascular indexes was carried out, followed by an analysis of the impact that considering short epochs within the task has on the estimation of rest-task changes. The result of this work are presented in Chapter 5.

The relevance of the issues tackled in this thesis is not limited to the study presented in Chapter 3. The core research activity, reported in Chapter 4 and 5, was aimed at providing results applicable to psychophysiological investigations in general, since modulatory effects of respiration and within-task changes in the indexes can be expected in a wide range of experimental protocols. Nevertheless, specific considerations regarding the implications for the study of the correlation between indexes of autonomic activity and size at birth are reported as well.

1.3 Original contributions

A list of the main original contributions of the author during his PhD research are:

1-Analysis of the impact of respiration on cardiovascular indexes for respiratory patterns associated with verbalization and psychophysiological tasks

The results obtained show that the tasks elicit significant rest-task and inter-individual differences in respiratory pattern, and that such differences are strongly reflected in HRV and $SAPV$ indexes. Thus the assessment of autonomic changes from changes in these indexes, neglecting the influence of respiration, becomes questionable and possibly misleading. For future psychophysiological protocols, respiration should be always monitored, and tasks that limit inter-individual and

between-tasks difference in respiration should be preferred (e.g. avoiding tasks involving speech).

2-Model-based investigation of the respiratory modulation of cardiovascular oscillations during verbalization and psychophysiological tasks

The results confirm and complement those introduced in item 1, showing that simple, linear, and strictly causal models of the respiratory modulation of heart period and systolic arterial pressure can in many cases predict a significant part the inter-individual and between-task differences in HRV and $SAPV$ indexes solely from differences in respiratory patterns, without assuming any change in autonomic activation.

3-Description of within-task dynamics of cardiovascular indexes, and their impact on the indirect assessment of autonomic activation

Systematic investigations regarding a set of psychophysiological tasks (and the initial baseline rest period) revealed the presence of significant within-task changes in HRV, $SAPV$, and BRS indexes. Neglecting these dynamics can have a significant impact on the estimated magnitude of rest-task changes.

4-Development of a method to estimate confidence intervals for the characteristics of a transfer function using a parametric least-squares approach and Monte Carlo simulations

This provides a simple technique for the statistical analysis of physiological systems based on frequency responses estimates obtained by parametric models in individual subjects.

Chapter 2

Literature review on indirect assessment of autonomic activation

This chapter presents a review of the fundamental physiological and methodological aspects related to this project, with the purpose of providing basic background that will help the reader to understand and interpret the results and the implications of the work presented in the following chapters.

In Section 2.1 a brief introduction to the autonomic nervous system, its function and its influence on the cardiovascular system is presented (adapted from [14, 18]). In Section 2.2 basic concepts regarding the physiology and the estimation of indirect indexes of cardiovascular autonomic modulation are introduced. Section 2.3 briefly describes the possible influence that fetal growth can have on the autonomic control of the cardiovascular system. Section 2.4 discusses the use of psychophysiological tests for an in-depth study of the autonomic control of the cardiovascular system. Finally, Section 2.5 summarizes the main concepts presented in the previous sections and sets out why the issues considered in this research project are important, and how they were addressed.

2.1 The autonomic nervous system: an overview

In the original definition of J.Langley (1921), the autonomic nervous system (ANS) is a complex network of peripheral nerves and ganglia, together with associated regulatory system of the brain and spinal cord, which serves to control the smooth muscles and the glands of the viscera. The ANS can be divided into

two peripheral divisions - the sympathetic and parasympathetic branches - and a central division. The brief overview of such divisions presented below is based on [18].

The two peripheral divisions differ in their central origin, peripheral anatomy, neuropharmacology, and functions. Many visceral organs are dually innervated by both branches, and the two divisions are often opposing in their action. Even if the range of regulatory adjustments provided by the two division are quite broad, in general terms, the sympathetic branch can be thought to have mainly a *mobilizing* function (promote end organ increase in activity and metabolism) and the parasympathetic branch a mainly *conservation* function (promote energy intake, reduce energy expenditure, preserve energy reserves). For example, the sympathetic cardiac innervation increases heart rate via the modulation of the activity of the sinoatrial node. In contrast, the parasympathetic innervation slows the beat of the heart. However, the two branches operate by different cellular mechanisms with distinct features and temporal dynamics. As a result, it often occurs that the activity of the two branches is not simply reciprocal, but results in a much more complex interaction that cannot be modeled by simple variations along a reciprocal bipolar continuum extending from maximal sympathetic activation to maximal parasympathetic activation.

The central division is located at the level of the brainstem and the spinal cord, and acts as a complex regulatory system of the activity of the two peripheral divisions. But central regulatory systems extend well above these lower levels of the neuraxis: autonomic regulation has been shown to be affected by cognitive and behavioural processes (e.g. psychological stress, mental workload), through the involvement of limbic and forebrain areas (e.g. amygdala and medial prefrontal cortex). These brain systems not only modulate lower regulatory mechanisms, but issue descending projections that terminate directly on autonomic source nuclei in the brainstem and spinal cord.

2.1.1 *ANS* function: homeostasis VS allostasis

The *ANS* has an historically recognized role in the biological homeostasis of the human body, contributing to maintaining the stability of the internal fluid matrix [14]. This function is implemented by an array of feedback-regulated reflexes (controlled at brainstem level) reacting to perturbations in internal states and generating responses that compensate for these perturbations and restore an ideal set point for internal conditions. On standing up from the sitting position,

for example, gravitational forces result in a pooling of blood in the legs, which could lead to a dangerous drop in blood pressure and circulatory compromise. In healthy individuals, however, baroreceptors located at the carotid level trigger a reflex that involves the activity of both peripheral branches. The results are an increase in heart rate and a sympathetically mediated vasoconstriction, which serve to restore normal blood pressure.

However, the range of regulatory adjustments provided by the ANS is much broader. Through central associative processes, both exteroreceptive and interoreceptive stimuli can come to control the ANS activity in an anticipatory fashion, and can effectively prevent or minimize perturbations prior to their occurrence. A famous example of such a mechanism is the work of Pavlov on conditioning autonomic responses in dogs [91]. Furthermore, although central autonomic regulatory systems do contribute to homeostasis, they may also promote explicit deviations from homeostasis, in accordance with adaptive demands. This is important, as it is not always optimal to maintain homeostatic, steady state conditions. During physical exercise or in the face of a survival threat, for example, there would be considerable adaptive advantage to increasing cardiac output and blood pressure, to enhance blood perfusion of muscles. Hence, the higher (i.e. rostral) levels of the central division of the ANS mediate shifts of the regulatory set point to different levels (allostasis), in order to reach a more 'appropriate' homeostatic point of equilibrium, according to a perceived change in internal and external conditions.

2.2 Indirect indexes of autonomic control of the cardiovascular system

In principle, direct measures of the electrical activity of the autonomic branches allow an exact estimation of the autonomic control of the cardiovascular system. However, this approach presents several major problems [17]. The use of electrodes to detect the activity requires a degree of invasiveness. Such a procedure is not acceptable in humans without anesthesia, which is known to modify the behaviour of the ANS. Hence, in practice, a non-invasive approach is normally necessary.

In the following sections basic concepts will be introduced regarding the physiological basis and the estimation of the most commonly used indirect indexes of the autonomic control of the cardiovascular system: heart-rate variability (Sec-

tion 2.2.1), systolic arterial-pressure variability (Section 2.2.2), and baroreflex sensitivity (Section 2.2.3).

2.2.1 Heart-rate variability (HRV)

Innervations of the sympathetic and parasympathetic (vagal) divisions of the ANS strongly modulate the membrane processes of the sinoatrial node (SAN), which is the natural pacemaker of the heart. The SAN repetitively goes through a depolarizing discharge and then repolarizes to fire again and start the next heart beat. In general terms, vagal activity slows the rate of SAN cells' depolarization, causing a decrease in heart rate, and sympathetic activity speeds the rate of SAN cells' depolarization, causing an increase in heart rate.

Also, the sympathetic system can modulate heart rate indirectly through the release of hormones (catecholamines) in the blood flow, but such a control is very slow to respond (delays of 30s or more, hence causing slow oscillations in heart rate, with a frequency $< 0.03Hz$).

The term heart-rate variability (HRV) is commonly used to define the well known phenomenon of beat-to-beat variations in the length of the heart period [27]. The heart period variations present during resting conditions represent a fine tuning of the beat-to-beat control mechanisms. Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity. The opposite reflex effects are mediated by the stimulation of sympathetic afferent activity. Efferent sympathetic and vagal activities directed at the sinus node are characterized by discharges largely synchronous with each cardiac cycle that can be modulated by central (vasomotor and respiratory centers) and peripheral (oscillation in arterial pressure and respiratory movements) oscillators. These oscillators generate rhythmic fluctuations in efferent neural discharge that manifest as short- and long-term oscillation in the heart period.

The commonly used representation of such fluctuations is the discrete event series, that is the series of the heart period length versus time (indicated at the occurrence of the end of the heart period). Spectral analysis of the discrete event series shows that periodic components of HRV tend to aggregate within several frequency bands [17, 27]:

High frequency band (HF): $0.15 - 0.5Hz$ Sympathetic and vagal nerve activity fluctuates on a breath-by-breath basis, as a consequence of respiratory influences (central and peripheral) on the reflex networks that modulate this

activity. Respiratory-frequency rhythms in autonomic nerves are translated into changes in discharge frequency of the sinoatrial node, a phenomenon known as respiratory sinus arrhythmia (RSA) [19]. Experimental findings in animals and humans support the widely accepted model of the two autonomic branches as low-pass filters, with the parasympathetic nervous system able to modulate heart rate effectively at frequencies up to 0.5Hz, whereas the sympathetic system modulates heart rate with significant gain only below 0.1Hz [17, 102]. Under the widely accepted assumption that respiratory frequency is limited to the HF band (0.15-0.5Hz) [17], the RSA is hence predominately modulated by the vagus. Thus, HRV_{HF} has been proposed as a marker of vagal activation. Evidence exists that HRV_{HF} is proportional to vagal nerve traffic, is abolished by vagal blockade, and is largely unaffected by sympathetic blockade [17, 27, 101, 102]. However, a recent study showed that sympathetic blockade results in a significant increase of respiratory sinus arrhythmia, hence suggesting caution in interpreting HRV_{HF} as a reflection of exclusively vagal modulation [112].

Low frequency band (LF): $0.05 - 0.15HZ$ In accordance with the temporal dynamics of the vagal and sympathetic cardiac innervations as outlined above, both autonomic branches can influence the LF. LF oscillations are originated by a complex interaction of sympathetic and parasympathetic activity, and the interpretation is controversial. Several authors proposed HRV_{LF} as a marker of sympathetic activation, in particular when expressed in normalized units ($HRVn_{LF} = HRV_{LF}/(HRV_{LF} + HRV_{HF})$), based on evidence that it is abolished by sympathetic blockade and increased by stimuli known to increase sympathetic drive [77, 86, 90]. However, others argued that evidence exist that HRV_{LF} is also under considerable parasympathetic influence, in particular that pharmacological blockade of the vagal activity blocks most of the LF oscillation, and that pharmacological blockade of the sympathetic activity may not always appreciably reduce LF fluctuations, and can even enhance them slightly [17, 66]. Detailed discussions of these issues are presented in [17, 27, 90].

Very and Ultra low frequency bands (VLF,ULF): $0 - 0.05Hz$ The physiological origin of these rhythms is still not clear, even if a broad array of stimuli and conditions have been proven to contribute to the VLF (posture, slow variations in breathing patterns, etc.). In view of the limited under-

standing of these rhythms and their minimal application in psychophysiological studies so far, VLF and ULF rhythms will not be considered further.

Estimation of heart-rate variability: HRV_{LF} and HRV_{HF}

Based on the physiological background presented in the previous section, the total power of HRV in the high-frequency band (HRV_{HF}) has been widely used as an index of the vagal control of the cardiovascular system, while the total power of HRV in the low-frequency band (HRV_{LF}) has been more controversially used as an index of sympathetic activation.

In order to estimate HRV_{LF} and HRV_{HF}, the heart-period (HP) is extracted from the electrocardiogram (ECG), or alternatively from the arterial pressure signal (AP), as described in more detail in Appendix A.1. From the HP signal, the total power in specific bands (LF and HF) is commonly computed using either FFT-based non-parametric spectral estimation methods [17, 27] (for example using Welch's algorithm [121]) or parametric autoregressive methods [84] (the use of a model order of 16 has been recommended [21]). The different methods have been reported to produce very similar results [17, 48].

2.2.2 Systolic arterial pressure variability ($SAPV$)

Arterial blood-pressure is controlled closely by the ANS in order to maintain tissue perfusion, through the modulation of the systemic vascular resistance, heart-rate, and cardiac output. Autonomic control of arterial pressure is mainly carried out by the sympathetic nervous system, which supplies all vessels except capillaries. Sympathetic fibres arise from the thoracic and lumbar segments of the spinal cord. These are under the control of the vasomotor center in the medulla, which has distinct vasoconstrictor and vasodilator areas.

When referring to blood-pressure variability, we refer to beat-to-beat variations in the magnitude of arterial pressure, generally the systolic arterial pressure (SAP). In this work only systolic arterial pressure variability ($SAPV$) will be considered. Historically, the study of $SAPV$ and HRV are strictly related [78]. This resulted in a similar spectral approach in the analysis of the oscillations of the SAP signal, with the definition of two frequency bands with different origins, and again based on the assumption that respiratory modulation is limited to the higher frequencies:

High frequency band (HF): $0.15 - 0.5Hz$ The oscillations in this frequency band can be ascribed to the cyclic variation in intrathoracic pressure related to respiration, with breathing mechanically perturbing venous return, cardiac output, and thus blood pressure [126]. Hence, HF oscillations cannot be interpreted as a reflection of autonomic activity. Nevertheless, HF oscillations of blood pressure are detected by baroreceptors at the level of the carotid arteries and the aorta and cause ANS-mediated changes in HR [78].

Low frequency band (LF): $0.05 - 0.15HZ$ The fact that LF oscillations in blood pressure involve primarily the action of the sympathetic nervous system on the vasculature is widely accepted. Nevertheless, the origin of such oscillations is still debated, with the competing hypotheses of a central origin and of the involvement of the baroreflex mechanisms [78, 82]. $SAPV_{LF}$ has been suggested by some investigators as a marker of sympathetic cardiovascular control, based on evidence that this index increases (decreases) as a result of sympathetic excitation (inhibition) [103].

Estimation of blood-pressure variability: $SAPV_{LF}$

As discussed above, the total power of SAP in the LF band ($SAPV_{LF}$) has been widely used as index of the sympathetic control of the cardiovascular system. Despite the debate on which physiological mechanisms generate it, $SAPV_{LF}$ has been advocated as a more reliable index of sympathetic activation than HRV_{LF} [103], since the former is originated by the action of the sympathetic nervous system on the vasculature, while the latter is known to be at least in part under parasympathetic modulation, as outlined above.

For the estimation of $SAPV_{LF}$ the arterial pressure signal (AP) is acquired usually either invasively (catheter connected to pressure transducer) or non-invasively (e.g. plethysmographic devices). The SAP signal is then extracted from the AP signal (the processing applied for this purpose is reported in Appendix A.1). From the SAP signal, $SAPV_{LF}$ is then computed using the same methods as adopted for HRV_{LF} (see Section 2.2.1).

2.2.3 Baroreflex sensitivity (BRS)

The arterial baroreflex seeks to regulate the blood pressure and ultimately to maintain blood flow to the brain and other organs [73]. Baroreceptors sense systemic blood pressure indirectly, by the extent of stretch of receptors in the walls of

the carotid arteries and the aorta. Changes in arterial baroreceptor stimuli result in afferent discharges transmitted to the central nervous system and trigger reflex adjustments that buffer or oppose the changes in blood pressure: a rise in pressure elicits reflex parasympathetic activation and sympathetic inhibition, with subsequent decrease in heart-rate, cardiac contractility, vascular resistance, and venous return. Conversely, a decrease in arterial pressure reduces baroreceptor afferent discharge and triggers reflex increases in heart-rate, cardiac contractility, vascular resistance, and venous return. Thus the baroreflex provides powerful beat-to-beat negative feedback regulation that minimizes short-term fluctuations in arterial blood pressure [73], mediated by the ANS. As introduced above, the actions of the parasympathetic and sympathetic branches of the ANS are partially separable in the frequency domain with a sympathetic activity limited to frequencies below 0.15Hz whilst parasympathetic modulation is capable of acting over a wider range of frequencies (0-0.5Hz) [17]. The impairment of the baroreflex may result in exaggerated blood-pressure fluctuations and in an increased risk of cardiovascular disease. From this pathophysiological link arises the need to estimate how the sensitivity of this reflex changes as a consequence of different stimuli and pathologies.

Baroreflex sensitivity estimation

The most commonly used index of BRS is the change in HP for a unit change in arterial blood pressure (systolic or mean), but different methods have also been used for its estimation. Traditionally, BRS is estimated as the interpolated slope of the $SAP - HP$ curve after the injections of vasoactive drugs or the application of pressure or suction at the neck around the location of the carotid baroreceptors [73]. In more recent times, principally with the growing interest in behavioural and psychophysiological studies, alternative non-invasive methods were developed. In fact the possibility exist that invasive procedures, mechanical stimulation, and interaction with the subjects might elicit changes in autonomic activation (and its indirect indexes) comparable or greater than those triggered by psychological/cognitive processes commonly involved in psychophysiological experimental protocols. The non-invasive methods analyze the spontaneous fluctuations of HP and SAP, each of them using a different approach. The most commonly used are sequence methods [20, 75], FFT-based spectral methods [75, 100], and parametric spectral methods based on autoregressive models [71].

With the sequence methods (which will be referred as SEQ in the rest of this

work) the gradient of a line fitted to changes in HP and the preceding changes in SAP (which are assumed to have caused them) is considered as the index of BRS. Each SAP value is associated with the HP value of the heart beat that caused the systolic peak (or, in other implementations, of the next heart beat [75]). Sequences of consecutive cardiac cycles in which SAP increases (by at least 1 mmHg per beat) whilst HP increases (by at least 1 ms per beat, and up to 5ms have been used in previous works) are analyzed. Similar sequences where SAP and HP decrease together are also used. Only sequences of three beats or longer are considered suitable for BRS estimation. For each suitable sequence, the slope of the regression line is computed and used as the index of BRS [20], if the correlation coefficient is over a given threshold (values of 0.7 or more have been adopted in previous works [75]).

With FFT-based spectral methods (which will be referred to as SPC in the rest of this work), the transfer function between SAP and HP is estimated using a fast Fourier transform approach and BRS is estimated as a weighted mean of the transfer function gain in the specific frequency bands of LF (0.05 - 0.15 Hz) and HF (0.15 - 0.5 Hz) [75, 100]. The weight $\omega(f)$ corresponding to each frequency is usually defined as:

$$\omega(f) = \begin{cases} 1 & \text{if } \gamma(f) \geq k \\ 0 & \text{if } \gamma(f) < k \end{cases} \tag{2.1}$$

where γ is the estimated coherence between SAP and HP at each frequency and $0 < k < 1$ [75]. For k, values near to 0.5 have been widely applied in previous works (e.g. [95, 98]), but it has been suggested that choosing lower values has negligible impact on the results [75]. With this "thresholding", just those frequencies for which SAP and HP are highly coherent are considered in the average.

With the parametric autoregressive methods (which will be referred as AR in the rest of this work), a model identification approach is used to model the reciprocal interaction between SAP and HP [71]. The transfer function and the coherence between SAP and HP are computed from the model coefficients. Then BRS is again estimated as a weighted mean of the transfer function gain in the LF and HF band, following the same approach used for the FFT-based spectral methods. Regarding the order of the model, in previous work the selection was made from a range of values, generally 6 to 16, using objective methods, the Akaike criterion in particular [6, 7, 95]. Adaptive [39] and time-invariant [71]

implementations of the model have been applied, depending on the need to track the within-task changes in the $SAP \rightarrow HP$ transfer function.

All the methods presented proved capable of detecting changes in baroreflex gain, as shown for example in [4, 75]. However, discrepancies exist in the estimated values of BRS [4, 38, 75]. Hence, given that in the literature there is not a clear indication of which should be preferred for psychophysiological experiments, all the three methods were used in the analysis of the data presented in this work, allowing for a comparison of results.

2.2.4 The influence of respiration on indexes of autonomic control

The assumption that there are two separate frequency bands, LF and HF, with distinct characteristics is fundamental for the physiological interpretation of the indexes of autonomic activation described above. Implicit in this is another assumption, namely that the respiratory frequency (or better the spectrum of the respiratory signal) is limited to the HF band. In fact, just in this case the resulting RSA can be assumed to be predominantly mediated by the vagus and its contribution to the spectrum of SAP and HP confidently separated from those of other origins [28].

The physiological mechanisms underlying RSA have been investigated in depth [41], and there is strong evidence that changes in frequency and depth of the respiration cause changes in HRV [24, 108]. In particular it has been shown that RSA decreases with higher respiratory frequencies and increases with increased tidal volumes [53, 70, 108], and that expiratory/inspiratory time ratio is also an independent modulator of HRV_{LF} [110]. Furthermore, several publications have shown that BRS indexes decrease with higher respiratory rates [94]. Hence, if respiratory activity is not properly accounted for, changes in the indirect indexes of autonomic activation could reflect the effect of changes in the characteristics of respiration rather than the result of changes in mean autonomic activation. For these reasons, several authors recommended the use of experimental protocols in which the respiratory pattern of the subjects is controlled, in order to minimize variations in depth of respiration and to limit the frequency range to a narrow band inside the HF [24, 78]. However, the usefulness of such protocols is still debated since the task of controlling the respiration requires cortical effort, which can also change the autonomic control of the cardiovascular system [108]. In sec-

tion 2.4 the possible misleading effect of respiratory patterns during psychological stress tasks is further discussed, with a particular attention to the case of tasks involving verbalization.

2.2.5 Limitation of indirect indexes of autonomic activation

Despite having been studied and used extensively and for a relatively long time, the indirect indexes of autonomic activation are still object of an intense debate. Several authors have presented convincing arguments against the use of HRV, $SAPV$, and BRS as indexes of autonomic cardiovascular regulation [66, 73, 78, 82, 111]. The recently published debate between Parati et al. and Taylor et al. [90], on which this paragraph is based, provides an useful overview of such arguments. The evidence put forth to justify the use of cardiovascular indexes as measures of autonomic outflow is focused on changes in cardiovascular indexes caused by changes in autonomic regulation. In particular, this evidence is based on animal studies investigating changes after surgical modifications of autonomic regulation, human studies of changes induced by manipulation of autonomic cardiovascular control through drug administration or laboratory stimuli, and studies of the effects of diseases affecting the autonomic nervous system (e.g. acute myocardial infarction, hypertension). These studies reported significant correlations between changes in cardiovascular indexes and changes in the modulation of the two autonomic branches, and showed how decreased cardiovascular variability has clinical relevance, for example to predict the risk of death after acute myocardial infarction and to assess the development of diabetic neuropathy [27]. However, results clearly demonstrate that cardiovascular indexes depend on autonomic modulation, it does not automatically make them adequate indexes of the outflow of the two autonomic branches for a wider range of experimental and physiological situations [90]. In fact, as previously pointed out, "changes of breathing frequency and depth, which profoundly alter HRV_{HF}, may not change vagal-cardiac nerve activity at all" [42]. Furthermore, several studies support the idea of a complex interaction of vagal and sympathetic control of low-frequency HR oscillations [42], and evidence exists that low-frequency AP oscillations are originated by a combination of autonomic modulation of central origin and of baroreflex mechanism [65]. Moreover, there is some evidence suggesting that the interaction between SAP and HP might be, at least in the HF band, a reflec-

tion of central common modulators rather than of a baroreflex mechanism [3, 34]. Hence, great caution should be taken in interpreting the cardiovascular indexes as specific markers of specific aspects of autonomic cardiovascular modulation (i.e. vagal and sympathetic modulation, and baroreflex gain), since the underlying physiology is still largely undiscovered [90]. Also, even if all the confounding factors can be controlled or accounted for, it is important to stress that cardiovascular indexes should only be taken as reflections of autonomic cardiovascular modulation, and not of the mean autonomic tone [90].

Despite all the issues presented above, extensive literature exists indicating the usefulness and the clinical relevance of the indirect indexes of autonomic activation [17, 27], especially for those studies in which an invasive approach is not suitable (e.g. psychophysiological tests in humans). It then appears that the use of the variability of the cardiovascular signals is at the moment the only practicable choice for assessing autonomic activation. In fact, despite the evident limitations on the interpretations of the outcomes, the use of cardiovascular indexes is a relatively simple method to obtain non-invasively an insight on autonomic modulation of the cardiovascular system, that can provide useful information regarding the reaction to a broad range of stimuli, and regarding the relationship stimuli$\rightarrow ANS \leftrightarrow$pathologies.

2.3 The possible influence of fetal growth on the development of the ANS

According to the "Barker Hypothesis" on the fetal origins of adult diseases, the probability of developing cardiovascular diseases in later life is significantly increased by reduced growth rates in fetal life and infancy [5]. These growth rates are strongly affected by the nutritional regime and health of mothers during pregnancy. Epidemiological studies show that in both men and women, small size at birth (but also other indexes of fetal growth, such as head circumference or length at birth) is associated with raised death rates from cardiovascular disease in later life. Raised blood pressure increases the risk of coronary heart disease and stroke, and it has been demonstrated that resting blood pressure increases with decreased birth weight [60]. However, the physiological mechanism linking small size at birth and the development of cardiovascular disease is not yet clear. One of the hypotheses proposed in [5] is that abnormal behaviour of the autonomic control of the cardiovascular system in people with small size at birth may

provide this link. Ijzerman et al. showed that low birth weight was significantly associated with shorter pre-ejection period (an indirect index of sympathetic activity) at rest, and during several psychophysiological tasks (reaction time and mental arithmetic) [61]. The research from which the work presented in this thesis developed was primarily focused on further investigation of the hypothesis of an ANS involvement in the fetal origins of adult diseases.

2.4 Cardiovascular and ANS reaction to psychophysiological tasks

2.4.1 Introduction

Psychophysiological tasks stimulate cognitive and behavioural processes, and modulate the activity of the ANS through the involvement of limbic and forebrain areas [13]. Hence, experimental protocols involving psychological stressors have been extensively used to study the physiological effects of cognitive and behavioural processes, investigate the central modulation of ANS function, and to increase the understanding of how psychological stress can evolve to a organic disease. The experimental activities and the results presented in this work belong to this area of research.

According to the literature, stressors are often associated with an increase in sympathetic cardiac control, a decrease in parasympathetic control, or both [13]. Regarding the indirect indexes of autonomic activation, there is a general agreement that HRV_{HF} decreases during stress, supporting the idea of a parasympathetic withdrawal, and several studies report that BRS is reduced as well [43]. More controversial are the findings regarding the index derived from the oscillations in the LF: different studies reported either an increase or a decrease in $SAPV_{LF}$ [43] and HRV_{LF} [50]. Such discrepancies may be due, at least in part, to the different stressors and experimental protocols used by different researchers, and to differences in the parameters considered and the related analysis (e.g. using normalized or absolute values) [13, 50, 89].

2.4.2 Influence of respiration

Respiratory patterns play a fundamental role in modulating the indirect indexes of autonomic activation during psychological stress tasks, which however has been

generally underestimated in past investigations [99]. In has been clearly shown that respiratory rate tends to increase during mental effort tasks [2, 122]. As introduced in Section 2.2.4, such modifications in the respiratory pattern can result in changes of the indexes of autonomic activation unrelated to changes in mean level of sympathetic and vagal activity. This undesired effect can be an important confounding factor when correlating indexes of autonomic activation with other parameters. The hypothesis of a confounding role of respiration is supported by the results presented in [2]. In this work, correlation tests between HRV indexes with mental task-load, showed that HRV_{LF} and HRV_{HF} may be affected by respiratory activity in such a way that it not possible to find a significant correlation with mental task load. Significance was reached only after using respiratory frequency as a correction factor, and for HRV_{LF}, after limiting the analysis to the subjects with frequency in the HF only.

The relevance of respiratory modulation is even bigger for those psychophysiological tasks requiring the subjects to talk. In fact, Bernardi et al. [11] showed that performing the same stress tasks silent or aloud produce a significant difference in HRV_{HF} and HRV_{LF}. Furthermore the same study showed that performing a stress task silently (i.e. reading, mathematical test) results in a statistically significant decrease in HRV_{HF} from resting level, and that the significance disappears when the same task is performed aloud.

2.4.3 Within-task dynamics of cardiovascular indexes

Estimating cardiovascular indexes as average values for the length of each task is a common practice in psychophysiological investigations. Despite this approach being justified when the overall reaction to the task is of interest, it might "average out" valuable information if significant within-task dynamics in the indexes are present [68]. Several studies reported the presence of considerable within-task changes in some cardiovascular indexes (e.g. HR, SAP, and pre-ejection period) [44, 68, 81]. Nevertheless, the evidence available is relatively limited, since a number of cardiovascular indexes, such as indexes of baroreflex sensitivity (BRS) and spectral indexes of HRV and $SAPV$ have never been considered, as far as the author is aware.

From these considerations it appears evident that further investigation is needed on the reaction of the cardiovascular system to psychological stressors, and especially to describe the behaviour of indirect indexes of autonomic activation. The

relative contribution of the modified respiratory patterns and of the within-task changes in autonomic activation on cardiovascular indexes during psychological and cognitive challenges is still an open issue. The experimental activity and the results presented in this work are aimed at contributing to a better understanding in this area of research.

2.5 Objectives of the research project

As described in Section 2.1, the autonomic nervous system (ANS) has among its functions the control of the cardiovascular system, performed in particular through the regulation of heart-rate (HR) and arterial blood-pressure (AP). From HR and AP it is possible to estimate indirect indexes of the activation of the ANS, such as heart-rate variability (HRV) , systolic arterial-pressure variability ($SAPV$), and baroreflex sensitivity (BRS), as outlined in Section 2.2. Nevertheless, the physiological interpretation of these indexes is controversial. However, indirect indexes seems to be the only feasible option currently available to estimate autonomic activation non-invasively in humans.

The present work developed from an epidemiological study carried out at the MRC Epidemiological Resource Center (Southampton General Hospital, UK), aimed at testing the hypothesis that reduced fetal growth causes abnormal behaviour of autonomic control in adult life (see Section 2.3). To this end the reaction to psychophysiological tests in terms of indirect indexes of autonomic activation was considered, and the correlation of such indexes with fetal growth was investigated. The result are reported and discussed in Chapter 3.

During the analysis of the data two issues emerged, that might have a considerable impact in the results found, but, as far as the author is aware, have not been fully investigated in previous work concerning the assessment of autonomic changes through cardiovascular indexes during psychophysiological tests. The investigation of such issues constitutes the core of the research activity presented in this thesis.

The first issue is that the inter-individual and between-tasks differences in respiratory patterns might reflect in indirect indexes of autonomic activation through, especially when considering tasks involving speech, with possibly misleading or confounding effect in the assessment of the autonomic activation. To analyze the impact of such an issue a specific experimental activity was carried out, involving several psychophysiological tasks (some involving speech), and the

reaction in terms of respiratory pattern and cardiovascular indexes, and their correlation was investigated. The results are reported in Chapter 4.

The second issue is the impact of within-task dynamics of cardiovascular indexes on the assessment of autonomic activation. The specific objectives of the work presented in Chapter 5 were to verify for which of the indexes considered in this thesis significant within-task changes are present, and to assess the impact of considering the whole length of tasks for computing cardiovascular indexes, compared to using shorter epochs within the tasks, in terms of estimation of changes in cardiovascular indexes elicited by the tasks.

Chapter 3

Fetal growth and the cardiovascular reaction to psychological stress

3.1 Introduction

In this chapter part of the research work carried out in collaboration with the MRC Epidemiological Resource Center (Southampton General Hospital, UK) is described. This work is part of a wider ongoing research effort to gain a better understanding of the physiological link between reduced fetal growth and the development of cardiovascular disease in adult life (see Section 2.3). The underlying hypothesis is that reduced fetal growth is associated with abnormal behaviour of autonomic control in adult life. This research project was principally aimed at testing the hypothesis on a large sample of data collected previously and then, if appropriate, describing this association.

A group of subjects undertook a protocol of stress-tasks, which are expected to trigger changes of ANS activity modulated by the involvement of limbic and forebrain areas implicated in behavioural processes (as introduced in Section 2.4). Then, cardiovascular indexes were extracted from the arterial blood pressure signal (as described in Section 2.2). Finally, the correlation between cardiovascular indexes and indexes of fetal growth was explored using statistical methods. In the following sections, the details of the experiments, the results, and their interpretation and implications are reported.

The specific contribution of the author in this project included the appropriate implementation of well established signal processing methods and a close

collaboration with the medical counterpart (Dr. Alex Jones, and similar results to those in this chapter are reported in his PhD thesis) in the interpretation of the results. Part of this work has been published [64]. It is important to point out that it was in analyzing the results obtained, that the author identified the main methodological and physiological issues which are addressed in the following chapters. Hence, the main aim of this chapter is to present the background, the motivation, the importance, and the possible practical implications of the research undertaken for this thesis.

3.2 Materials and methods

Experimental protocol

A cohort of 183 adult subjects, 105 men and 78 women with a mean age of 26.3 (SD 0.4), whose fetal growth indexes (birth-weight, birth-length, head circumference, placental weight and gestational age) were available from their birth records (see Table 3.2 for a summary of this data) was considered.

Table 3.1: Summary of fetal growth indexes and demographic data for the whole cohort (data for single subjects are reported in Appendix A.4)

	age (yrs)	bmi (kg/m^2)	weight at birth (g)	length at birth (cm)	head circumf. (cm)	placental weight (g)	gest. age (wks)
mean	26.3	25.3	3430	50.0	34.7	569	39.2
st. dev.	0.4	4.3	532	2.4	1.6	115	1.3

The subjects undertook a protocol of three standard psychological stress tests [118], as summarized in Figure 3.1. The light gray bands in Figure 3.1 represent the stress tests in the following order:

Stroop test: colour-word conflict task in which the subject has to match colors and words presented on a computer screen (as exemplified in Figure 3.2);

Mirror tracing test: a stylus was used to draw the outline of a star that could only be seen in a mirror image. Every time the stylus came off the star a mistake was registered and a beep emitted by the apparatus;

1	2	3	4	5	6	7	8	9	10
Start	REST1	PAUSE1	STRESS1	PAUSE2	STRESS2	PAUSE3	STRESS3	PAUSE4	REST2
15 min	5 min	7-9 min	5 min	7-9 min	5 min	7-9 min	5 min	5 min	5 min
		5 min rest no talk + 4 min instructions	Stroop	5 min rest no talk + 4 min instructions	Mirror Tracing	5 min rest no talk + 4 min instructions	3 min speech preparation + 2 min Speech	5 min rest no talk	

Figure 3.1: Outline of the experimental protocol (as presented in [118])

Speech task: hypothetical confrontational scenario of being unjustly accused of shoplifting by a store detective and having to defend themselves to the store manager and the police.

The dark gray bands represent periods in which the subjects are sitting and are told to rest and not to speak. The white bands represent recovery periods. Before each stress task, the subjects are instructed about the test. The average length of each part is also reported in Figure 3.1.

The design, implementation, and data collection for this protocol was carried out by Dr. A.M.V. Ward et al., and the subjects were recruited from a larger cohort of Australian adults participating in the *Adelaide Family Heart Study* [118].

Data processing

During the experiment, continuous monitoring of finger arterial blood pressure (AP) was performed using a Portapres device (FMS BV, Netherlands), which produced an output sampled at $200Hz$. The recordings of 4 subjects were not considered for the analysis, since large gaps were present in the acquired data, due to problems with the acquisition device. HP and SAP signals, sampled at 4Hz (as recommended [27]), were computed for the rest phase and for each task from the AP signal, using the methods presented in Appendix A.1 (average values for each task were computed as well). Stress-induced increments in HR and SAP indexes (HR_{inc} and SAP_{inc}) were calculated with respect to the rest period preceding all the stress-tasks (block number 2 in Figure 3.1). HRV and $SAPV$ indexes were estimated as the variance of respectively HP and SAP after a zero-phase band-pass filter was applied (pass band $0.05 - 0.15$ for LF

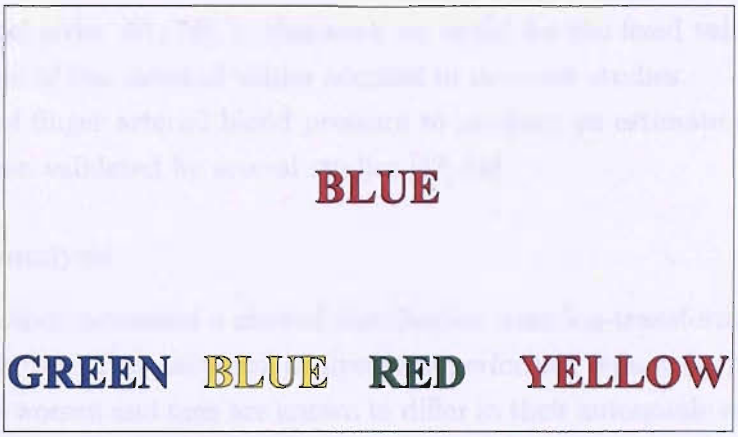


Figure 3.2: Example of a computer based Stroop test. The subject has to associate the colour of the word displayed in the center of the screen with one of the four answers at the bottom (and press the corresponding button). In this case the correct answer is the second from the right, RED, since that is the colour in which the word in center of the screen is displayed. Note that the words and the colors they are displayed in always disagree (e.g. the word RED is never displayed in red).

indexes and $0.15 - 0.5$ for HF indexes), as described in Appendix A.2. The variance was calculated over the full duration of each task. In preliminary tests, this method gave almost identical results to those of HRV estimation methods presented in Section 2.2 (FFT-based and parametric spectral methods), and was chosen due to its computational efficiency (see Appendix A.2). As discussed in Section 2.2.3, given that no clear indication exists regarding the most suitable method to estimate baroreflex sensitivity for psychophysiological tasks, three well established methods were adopted: the sequence method (SEQ), an FFT-based spectral method (SPC_{LF} and SPC_{HF}, computed respectively the LF and HF frequency band), and an autoregressive method (AR_{LF} and AR_{HF}). Details of the sequence method are presented in Section 2.2.3 (in this work, threshold values adopted for changes in HP and SAP were respectively 1ms and 1mmHg, and only sequences with a correlation coefficient larger than 0.7 were considered). For the FFT-based spectral method, the $SAP \rightarrow HP$ transfer function was estimated through the Welch's modified periodogram method [120], using multiple segments of 256 samples (corresponding to 64s), an overlap between adjacent segments of 128 samples, and a Hanning window, providing a spectral resolution of 0.0156 Hz. For the autoregressive method, a time-invariant implementation was adopted [71]. Considering that objective criteria for model order selection tend to underestimate

the true model order [67, 76], in this work we opted for the fixed value of 16, at the higher end of the range of values adopted in previous studies.

The use of finger arterial blood pressure to produce an estimate of HR and HRV has been validated by several studies [47, 80].

Statistical analysis

Parameters which presented a skewed distribution were log-transformed prior to parametric testing. The statistical analysis was performed separately for men and women, since women and men are known to differ in their autonomic cardiovascular control mechanisms [1, 115]. In accordance with log-transformation, geometric means and geometric standard deviations were computed for the parameters. Body mass index [72], phase of menstrual cycle [52], investigator [118], and resting heart-rate [30]) are associated with cardiovascular response to stress (value of these parameters for each subject are reported in Appendix A.4). Therefore, the potential confounding effect of these parameters was adjusted for by using multiple linear regression. Correlations between cardiovascular parameters and indexes of fetal growth were computed using the Pearson's correlation test.

3.3 Results

Figure 3.3 shows the pattern of HR and SAP during the protocol for two representative subjects. Both are higher during the tasks compared to baseline values. The increase starts before the beginning of each task, likely an anticipation effect due to the instructions given to the subjects. Furthermore, it is worth noting that in some situations (e.g. HR during Stroop task for subject 81) the response in terms of HR and SAP appears to change progressively within the task, even if this effect is not consistently present for all subjects (see for example HR during Stroop task for subject 26 in the figure) and tasks, with high inter-individual variability in the pattern of response (as is evident comparing the two subjects shown in Figure 3.3).

In Table 3.2 and Table 3.3 the average values of the different indexes of autonomic activation for the rest phase and the stress-tasks are reported. The direction of change in cardiovascular parameters elicited by the stress-tasks is common to both genders. SAP and HR markedly increased during stress compared to rest. HRV, $SAPV$, and BRS indexes decreased significantly for the Stroop and mirror tasks compared to rest. However, for the speech task, BRS

decreased similarly to the other tasks, while HRV_{HF} tended to decrease without reaching significance, $SAPV_{LF}$ and HRV_{LF} showed a tendency to increase compared to rest, with only $SAPV_{LF}$ reaching statistical significance.

Table 3.4 and Table 3.5 report the results of partial correlation tests between weight at birth and the parameters considered (value of weight at birth and of corrections factors for each subjects are reported in Appendix A.4). Similar results were obtained using other indexes of fetal growth, such as length at birth and head circumference(not reported). There were no significant relationships between weight at birth and the cardiovascular parameters in men. However, in women low weight at birth was associated with greater SAP, SAP_{inc}, $SAPV_{LF}$ and HR_{inc} for both the Stroop and mirror tasks. A positive correlation was found for HRV_{HF} but limited to the Stroop task. It is worth noting that a significant correlation with weight at birth was found also for $SAPV_{LF}$ during the rest phase, and SAP and SAP_{inc} during the Speech task. Considering the different indexes of BRS, those using autoregressive and FFT-based approaches showed a significant correlation with weight at birth for all the tasks, with the exceptions of AR_{LF} for the mirror task (p=0.052) and FFT_{LF} for the speech task (p=0.1). For the sequence method no significant correlations were found. It is also worth noting that, excluding for HR and SAP no significant correlation was found between weight at birth and the rest-task difference in any of the cardiovascular indexes considered.

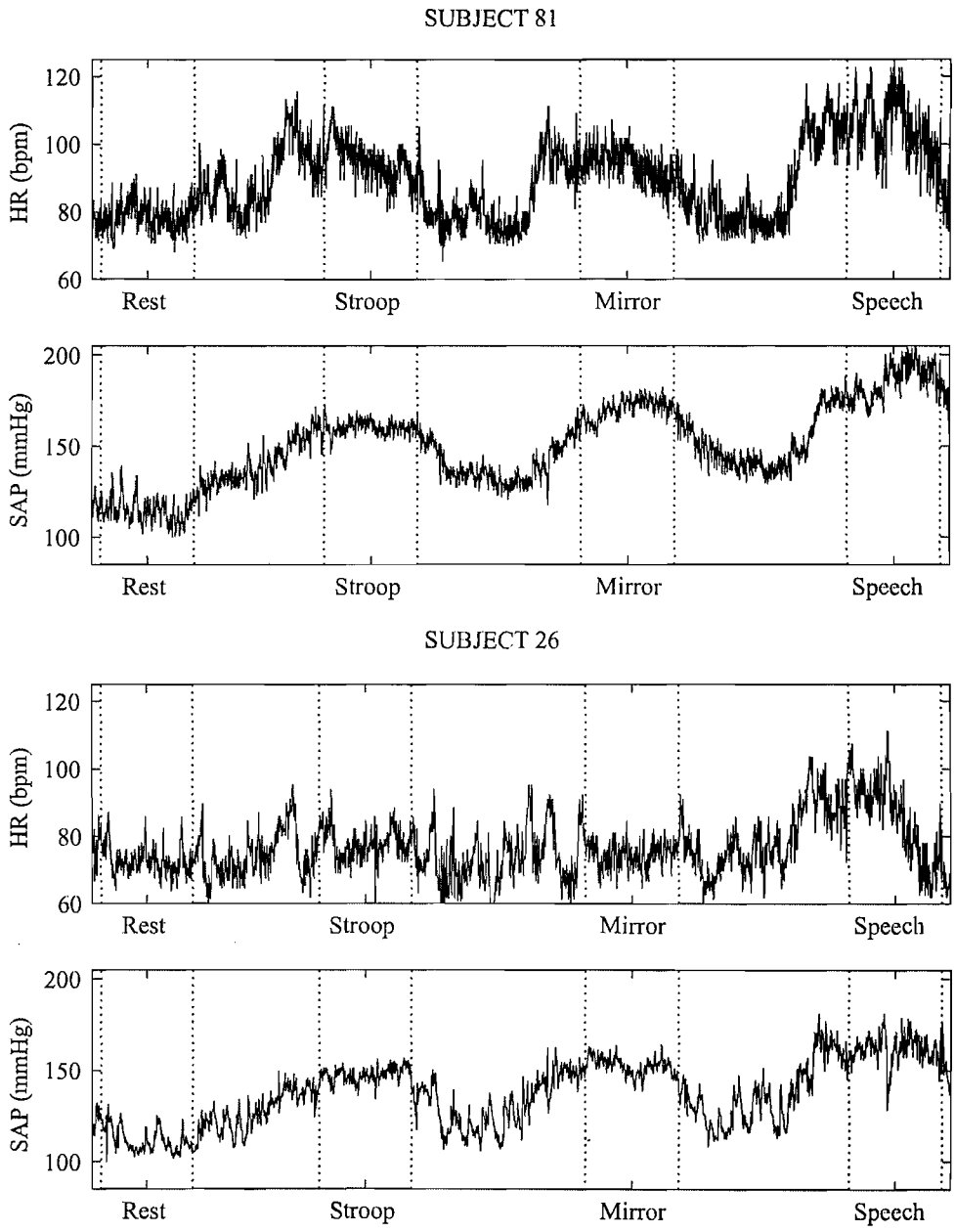


Figure 3.3: Examples of the heart-rate and systolic arterial pressure patterns during the experimental protocol

Table 3.2: Geometric mean (geometric SD) of cardiovascular parameters at rest and during three stress tasks. BRS indexes are reported in Table 3.3.

(a) Men (N = 103)

	Rest	Stroop	Mirror	Speech
$SAP(mmHg)$†	122.4(14.7)	142.8***(17.8)	150.2***(18.0)	163.4***(18.7)
$SAPV_{LF}(mmHg^2)$	13.2(1.7)	9.0***(1.8)	9.6***(1.7)	24.3***(1.7)
$HR(bpm)$	69.8(1.2)	77.0***(1.2)	76.3***(1.2)	83.7***(1.2)
$HRV_{LF}(ms^2)$	1601.9(2.0)	854.5***(2.3)	848.4***(1.9)	1713.9 (1.9)
$HRV_{HF}(ms^2)$	799.4(2.5)	508.2***(2.5)	499.5***(2.3)	693.8 (2.4)

(b) Women (N = 76)

	Rest	Stroop	Mirror	Speech
$SAP(mmHg)$†	122.9(15.2)	139.0***(17.1)	144.9***(18.4)	158.6***(20.4)
$SAPV_{LF}(mmHg^2)$	8.4(1.7)	5.3***(1.8)	6.4** (1.7)	18.4***(1.7)
$HR(bpm)$	73.8(1.1)	81.9***(1.2)	79.7***(1.2)	88.2***(1.2)
$HRV_{LF}(ms^2)$	1105.8(2.1)	557.3***(2.7)	760.4***(2.2)	1289.7* (2.1)
$HRV_{HF}(ms^2)$	932.0(2.9)	475.5***(3.1)	580.4***(3.0)	592.6***(2.4)

SAP, systolic arterial pressure; †, Arithmetic mean (SD); $SAPV_{LF}$, low frequency systolic arterial pressure variability; HR, heart rate; HRV_{LF}, low frequency heart-rate variability; HRV_{HF}, high frequency heart-rate variability. P-values refer to Student's paired t-test comparisons between parameter during a stress task and at rest (after log-transformation, excluding SAP). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 3.3: Geometric mean (geometric SD) of BRS indexes at rest and during three stress tasks.

(a)Men(N=103)

	Rest	Stroop	Mirror	Speech
$AR_{LF}(ms.mmHg^{-1})$	9.4(1.5)	7.8***(1.5)	7.8***(1.5)	7.3***(1.6)
$SPC_{LF}(ms.mmHg^{-1})$	11.9(1.4)	10.1***(1.5)	10.4***(1.5)	8.6***(1.6)
$AR_{HF}(ms.mmHg^{-1})$	10.0(1.7)	7.4***(1.8)	8.6***(1.7)	5.7***(1.8)
$SPC_{HF}(ms.mmHg^{-1})$	14.2(1.7)	12.3***(1.7)	13.6 (1.6)	8.3***(1.7)
$SEQ(ms.mmHg^{-1})$	12.3(1.5)	11.0***(1.5)	11.2* (1.4)	13.1 (1.5)
SEQ: nr. of sequences	8.5(7.1)	5.8 (5.3)	4.9 (4.3)	7.9 (6.8)

(b) Women (N = 76)

	Rest	Stroop	Mirror	Speech
AR_{LF} $(ms.mmHg^{-1})$	10.0(1.7)	7.3***(1.8)	8.1***(1.7)	7.5***(1.8)
$SPC_{LF}(ms.mmHg^{-1})$	11.8(1.5)	10.7* (1.7)	11.4 (1.6)	8.9***(1.7)
$AR_{HF}(ms.mmHg^{-1})$	12.1(1.8)	8.2***(1.9)	9.6***(1.9)	5.9***(1.7)
$SPC_{HF}(ms.mmHg^{-1})$	16.3(1.7)	13.7***(1.8)	14.7* (1.7)	8.8***(1.6)
$SEQ(ms.mmHg^{-1})$	14.1(1.6)	11.8***(1.6)	11.8***(1.5)	13.9 (1.6)
SEQ: nr. of sequences	8.3(6.8)	5.4 (4.7)	5.1 (4.5)	7.7 (6.0)

AR, parametric autoregressive method; SPC, FFT-based spectral method; SEQ, sequence method (also the average (SD) number of suitable sequences for BRS estimation is reported); LF, low-frequencies $(0.05 - 0.15Hz)$; HF, high-frequencies $(0.15 - 0.5Hz)$. P-values refer to Student's paired t-test comparisons between parameter during a stress task and at rest (after log-transformation, excluding SAP). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 3.4: Partial correlation coefficients relating birth weight to cardiovascular parameters (excluding *BRS* indexes) at rest and during three stress tasks.

	(a)Men(N=103)				(b)Women(N=68)			
	Rest	Stroop	Mirror	Speech	Rest	Stroop	Mirror	Speech
SAP	-0.05	0.03	0.05	0.05	-0.16	-0.33**	-0.29*	-0.35**
SAP_{inc}	-	0.1	0.12	0.11	-	-0.32**	-0.29*	-0.34**
$SAPV_{LF}$	-0.03	-0.01	0.08	-0.08	-0.24*	-0.45***	-0.37**	-0.24
HR	0	0.05	0.03	-0.01	0.05	-0.13	-0.08	-0.07
HR_{inc}	-	0.13	0.08	-0.03	-	-0.31*	-0.25*	-0.17
HRV_{LF}	0	-0.09	-0.1	-0.05	-0.01	0	0	0.11
HRV_{HF}	-0.1	-0.13	-0.13	-0.05	0.17	0.27*	0.18	0.22

SAP, systolic arterial pressure; SAP_{inc}, change in systolic arterial pressure between rest and stress; $SAPV_{LF}$, low frequency systolic arterial pressure variability; HR, heart rate; HR_{inc}, change in heart rate between rest and stress; HRV_{LF}, low frequency heart-rate variability; HRV_{HF}, high frequency heart-rate variability; HRV_{ratio}, ratio of low: high frequency heart-rate variability. The effect of resting HR (except HR, SAP and their increments), adult body mass index, investigator and phase of menstrual cycle was removed through partial correlation. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Table 3.5: Partial correlation coefficients relating birth weight to *BRS* indexes at rest and during three stress tasks.

	(a) Men (N = 103)				(b) Women (N = 68)			
	Rest	Stroop	Mirror	Speech	Rest	Stroop	Mirror	Speech
AR_{LF}	0.06	-0.04	-0.13	0.03	0.1	0.39***	0.24	0.28*
FFT_{LF}	0.05	0	-0.14	0	0.12	0.34**	0.25*	0.20
AR_{HF}	-0.12	-0.11	-0.1	0.02	0.27**	0.42***	0.28*	0.29*
FFT_{HF}	-0.12	-0.1	-0.14	0.01	0.2	0.44***	0.26*	0.25*
SEQ	0.05	-0.02	-0.1	0.11	0.08	0.15	0.11	0.21

AR, parametric autoregressive method; FFT FFT-based spectral method; SEQ, sequence method; LF, low-frequencies ($0.05 - 0.15Hz$); HF, high-frequencies ($0.15 - 0.5Hz$). P values refer to Student's paired t-test comparisons between parameter during a stress task and at rest. The effect of resting HR (except HR, SAP and their increments), adult body mass index, investigator and phase of menstrual cycle was removed through partial correlation. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3.4 Discussion

Based on the results presented a number of issues will be addressed:

- supporting evidence was found for the hypothesis of an involvement of the autonomic nervous system in linking size at birth with the cardiovascular response to stress;
- compared to rest values, both HRV_{LF} and $SAPV_{LF}$ decreased significantly during the Stroop and mirror tasks; this is in disagreement with the interpretation of these indexes as markers of sympathetic activation, which is expected to increase during these tasks [56];
- however, for the speech task HRV_{LF} and $SAPV_{LF}$ increased, raising the question if differences in respiratory patterns are at the origin of the discrepancy;

The results presented provide supporting evidence for the hypothesis that the associations between small size at birth and increased HR and SAP responses to psychological stressors may have their origins in autonomic cardiovascular control and baroreflex function, but only in women. In particular, the major findings were that compared with the women of normal birth weight, those with low birth weight showed smaller values for BRS indexes (i.e. a reduced baroreflex sensitivity), bigger values of $SAPV_{LF}$ both at rest and during stress, which may be interpreted as reflecting increased sympathetic activation, and smaller values of HRV_{HF} during the Stroop task, which suggest reduced parasympathetic drive (see Tables 3.4b and 3.5b). These findings were statistically highly significant and in multiple regression analysis were independent of potential confounders (e.g. obesity). The finding that measures of birth size were associated with altered baroreflex sensitivity in women is the first evidence of such a relationship in humans. However, this result is not surprising, considering that previous work already found a significant correlation between birth-weight and hypertension [5], and chronic hypertension has been associated with loss of baroreflex sensitivity [88]. For the findings regarding $SAPV_{LF}$ and HRV_{HF}, it is important to remember that several authors have recommended great care in interpreting these indexes as reliable markers of mean autonomic activation [66, 73, 78, 82]. However, even applying a conservative interpretation of the results, the reported evidence undoubtably shows that fetal growth affects the cardiovascular response

to psychophysiological tasks not only in terms of HR and SAP values, but also in terms of beat-by-beat changes and interaction between HR and SAP. This suggests that for women with low-birth weight there is an impairment of modulation of HR at respiratory frequency, a reduced effectiveness of the modulation of HR in response to changes in SAP, and increased oscillations of SAP in the LF, compared to women with higher birth-weight. Furthermore, even if the physiological interpretation and reliability of cardiovascular indexes is still unclear and debated (see Section 2.2.5), evidence exist that LF oscillations in blood pressure involve primarily the action of the sympathetic nervous system on the vasculature [78], and that HRV_{HF} is principally modulated by the vagus [82]. Hence, the results presented suggest that in women fetal growth significantly affects the response to stressors of both autonomic branches in adult life. In contrast, no significant relationships between size at birth and these cardiovascular parameters were found in the men (see Tables 3.4a and 3.5a).

The presented results agree with previous evidence regarding overall changes in cardiovascular parameters caused by stress-tasks: an increase in HR and SAP [50, 74, 114], and a decrease in HRV and $SAPV$ [43, 74, 105, 107, 114], and decrease in BRS [35, 43, 81, 85]. However, Tulen et. al reported a non-significant change in BRS_{LF} for a Stroop task [114]. This discrepancy with the present results might be explained by the fact that in their study a cohort of only 12 subjects were considered, probably too small to produce statistically significant results.

Table 3.2 shows that compared to rest values, both HRV_{LF} and $SAPV_{LF}$, which are widely used as indirect indexes of sympathetic activation [27, 103] decrease significantly during the Stroop and mirror tasks. This finding is unexpected, since it has been shown that stress-tasks are associated with an increased sympathetic activation [56]. Hence, an increase in both HRV_{LF} and $SAPV_{LF}$ was expected. Nevertheless, a significant decrease in HRV_{LF} in response to stressors (Stroop or mental arithmetic) has been reported in several studies [2, 43, 107]. Other authors reported a decrease in HRV_{LF} without showing significance [22, 56]. For $SAPV_{LF}$, [43, 106] report a decrease for psychophysiological tasks. One possible interpretation is related to evidence that the two indexes are influenced also by parasympathetic cardiac modulation, either directly (HRV_{LF}) or indirectly ($SAPV_{LF}$, through baroreflex feed-forward mechanism) [27, 106, 126]. The expected sympathetic-mediated increase in the indexes for these two tasks might have been canceled by a concomitant vagal withdrawal

(which is indicated by the decrease of HRV_{HF}).

Furthermore, is not immediately evident why the speech task should trigger the opposite response in these parameters than the other two tasks, and it is not clear why the correlation of the parameters with weight at birth is less evident for the speech task compared to the other two. A possible interpretation is related to the strongly modified respiratory pattern during speech compared to the rest phase and the other two tasks. It has been reported that for two stress-tasks (mental stress and reading) HRV_{LF} tends to decrease compared to rest when the task is performed without talking and to increase when the task is performed aloud [11]. A plausible hypothesis is that a similar situation is present in the current data, and that respiratory patterns play a major role in modulating the indexes of autonomic activation (but not necessarily the activation itself) and in confounding their correlation with weight at birth. Further considerations on the topic are presented in Chapter 4.

As described in section 3.2 all the cardiovascular parameters were computed as average values for the length of the tasks. Even if this is the most commonly used approach, it might well not be the most appropriate. Preliminary analysis of the data showed that, at least for part of the cohort, the reaction is not constant throughout a stress-task (e.g. subject 81 in Figure 3.3), in agreement with previous works [44, 68, 81]. Hence, computing an average value for the whole task might mask the presence of dynamics in the response, and ultimately prevent investigators from retrieving potentially useful information related to such dynamics. Chapter 5 reports the results of an investigation into the dynamics in cardiovascular parameters in response to stress, aimed at assessing its impact on the estimation of autonomic activation through cardiovascular indexes and also at proposing modified methods to describe the autonomic cardiovascular control during stress-tasks.

Limitations of the study

In the analysis of the rest-task differences a multiple paired t-tests approach was adopted, which did not take into account the effect of repeated measures. It was decided not to apply a Bonferroni correction [116], given its known limitations [92], leaving to the reader to infer about the impact of repeated measures. Possibly, a repeated measure analysis framework, such as repeated measures ANOVA with post hoc tests [116], could have been a more appropriate approach for this investigation. However, it must be noted that, given the low p-

values reported in Tables 3.2 and 3.3, it is unlikely that any statistical correction would have lead to considerable changes in the interpretation of the results.

Chapter 4

The influence of speech production on indexes of autonomic control of the cardiovascular system

4.1 Introduction

As introduced in Section 2.4, changes in heart-rate variability (HRV), systolic arterial pressure variability ($SAPV$), and baroreflex sensitivity (BRS) indexes have been used in many psychophysiological studies to assess changes in autonomic activation non-invasively. Some of these studies included tasks involving speech, such as role-playing (e.g. the speech task in the protocol analyzed in Section 3, and the protocol used in [25]) or arithmetic calculations (e.g. [50]) performed aloud.

From previous work it is clear that the presence of speech is likely to elicit considerable changes in respiratory patterns compared to rest [55], and that respiration is a powerful modulator of cardiovascular variability, as evident from tests involving spontaneous and paced breathing [17, 23, 54, 93, 108]. Thus the question arises, if HRV, $SAPV$, and BRS indexes during psychophysiological tasks involving speech are driven by respiratory variations, in addition to those arising from any changes in the activity of the autonomic nervous system. If so, associating between-tasks and inter-individual differences in such indexes directly and solely to changes in autonomic activation would be questionable and might confound or mislead the interpretation of the results.

The physiological rationale justifying the use of HRV and $SAPV$ indexes to assess autonomic activation was introduced in Section 2.2. The reliability (or otherwise) of these indexes to assess the autonomic control of circulation has been the object of an intense debate in recent years, and strong arguments have been presented by both sides (an excellent summary is provided in [90]). In particular, one assumption that has been questioned is that respiration is limited to the HF band, which is fundamental for interpreting the power or amplitude of HF oscillations in heart period (HP) as a measure of vagal modulation, and LF oscillations in HP and systolic arterial-pressure (SAP) as indexes of sympathetic regulation [17, 77]. In fact, such an assumption "does not correspond to the enormous inter-individual and intra-individual differences in the respiratory pattern that may be observed in awake subjects under different conditions" [28]. This issue has important implications regarding the estimation and the interpretation of HRV, and $SAPV$ indexes that are relevant to the current work, in particular to tasks involving speech used in the experimental protocol analyzed in Chapter 3. If the spectrum of respiration is not limited to the HF band but also presents considerable power in the LF band, respiratory-related oscillations affect not only the HF band of HP and SAP spectrum, but also the LF band [28]. Therefore, comparing two subjects or experimental settings that present a respiratory frequency (or the most power of the spectrum of respiration) in different bands is likely to result in differences in cardiovascular indexes (HRV_{LF}, HRV_{HF}, $SAPV_{LF}$), without necessarily involving dissimilarities in autonomic activation. Thus, assessing between-tasks changes or inter-individual differences in autonomic activation using HRV and $SAPV$ indexes is highly questionable in situations involving radically differing respiratory patterns, as has already been pointed out in previous works [12, 23].

Potentially, this is particularly relevant for psychophysiological tasks involving speech, if, as expected, the spectrum of respiration during speech presents broadband characteristics. Hence, the rest-task changes in HRV and $SAPV$ indexes may be at least in part a reflection of the rest-task changes in respiration, in addition to direct effects of cognitive/psychological processes elicited by the tasks. Similar reasoning can also be applied for the inter-individual differences in the indexes during a given task involving speech, which may be in part reflections of inter-individual differences in respiration.

For BRS indexes, the effect of different respiratory patterns is more difficult to foresee. These indexes fundamentally provide a quantification of the gain of the

transfer function $SAP \rightarrow HP$, their usefulness being based on the assumption that this is determined only by the baroreflex mechanism. As far as the author is aware, there is no clear indication regarding the impact that respiration can have on the gain of baroreflex. However, it has been suggested that the $SAP \rightarrow HP$ transfer function is also modulated by respiratory related non-baroreflex mechanisms [95], which might vary depending on the respiratory patterns, with a possible confounding effect on BRS indexes.

While previous works [28, 85] have raised the potential problem with respiration and speech for the indirect assessment of autonomic regulation during psychophysiological tasks, very few experimental studies have investigated this in detail [11, 104], or explored the potential impact on the use of HRV, $SAPV$ and BRS indexes during speech tasks. A systematic investigation of this matter is needed, in order to provide a sound basis for a critical interpretation of results such as those presented in Section 3. In particular, such a study would be of critical importance in understanding to what extent changes in cardiovascular indexes during tasks involving speech might be directly attributed to the changes in respiration, and ultimately to investigating to what extent cardiovascular indexes are reliable indexes of average central autonomic activation during psychophysiological tasks involving verbalization.

Since no respiratory signal was acquired for the protocol presented in Chapter 3, a specific experimental protocol was designed and implemented, which involved several psychophysiological tasks with or without speech. The specific objectives of this investigation are:

- to analyze the respiratory patterns during some psychophysiological tasks involving speech, and compare them with rest and tasks performed silently;
- to test for correlations between respiratory parameters, and HRV, $SAPV$, and BRS indexes during the tasks;
- to quantify changes in HRV, $SAPV$, and BRS indexes between tasks in relation to differences in respiratory patterns, and to compare results for a task performed silently and aloud.

The following sections are structured as follows. In Section 4.2, the experimental protocol including the psychophysiological tasks is presented, together with the data acquisition and the methods used to estimate the cardiovascular indexes and respiratory parameters (including respiratory period and tidal

volume). The differences in respiratory parameters and cardiovascular indexes during the tasks, and their correlation, are presented and discussed in Section 4.3 (part of this work has been submitted for publication [8]). In Section 4.4 a linear modeling approach is adopted to investigate the interaction of respiration with heart-period and systolic arterial pressure, in order to quantify the amount of cardiovascular oscillations that can be explained as an effect of respiratory modulation. Based on all the results found, suggestion for future studies are reported in Section 4.5.

4.2 Materials and methods

Experimental protocol

A cohort of 25 young healthy Brazilian students (mean age (SD) 26.5 (4.0), 12 females, 13 males), non-smokers, without known medical conditions or treatments that might affect the cardio-respiratory system, undertook the protocol summarized in Figure 4.1.

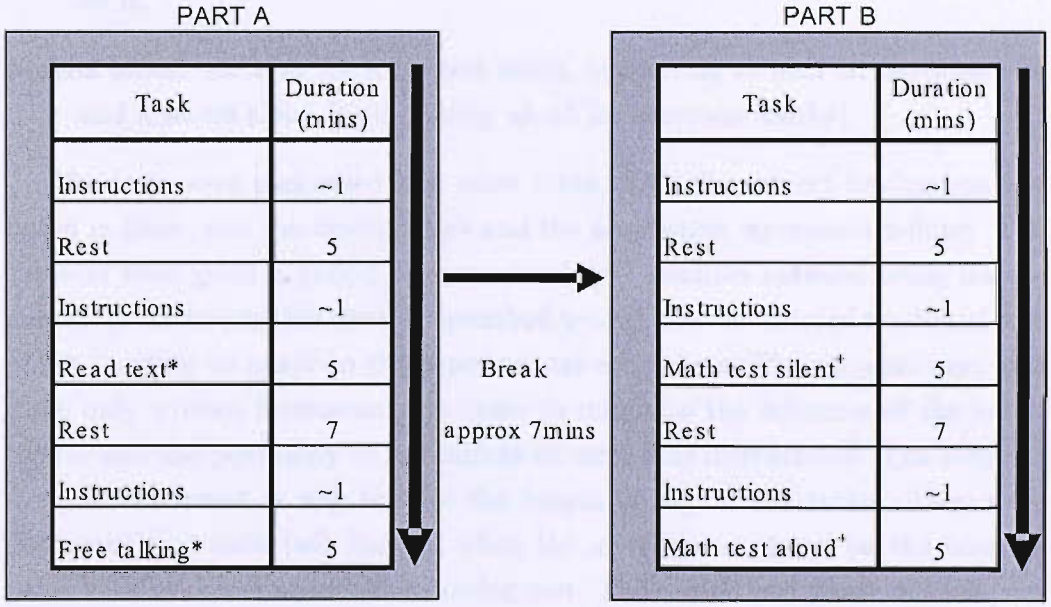


Figure 4.1: Schematic diagram of the experimental protocol. *The order of execution of Read Text\Free Talk was randomized. +The order of execution of Math test silent\Math test aloud was randomized.

The subjects undertook the whole protocol sitting in a comfortable chair, with

their arms on armrests. After instrumentation, the tasks shown in Figure 4.1 were carried out as follows:

Instructions before each task, a panel of instructions was placed on the board in front of the subject, describing the task he/she was going to perform next;

Rest the subject sat comfortably and relaxed, without talking;

Read the subject read a text aloud, as if reading aloud for someone nearby (the text was placed on the board in front him/her);

Talk the subject was instructed to talk about a topic of his/her choice (as if talking to someone sitting nearby), on one of a range of topics, such as daily routine, or a favorite book;

Maths silent the subject preformed a series of subtractions (repeatedly subtracting seven from random numbers of three digits). A sheet with the subtractions was placed on the board, and the subject wrote the answers on it;

Maths aloud same as the math test silent, but having to read all the questions and answers aloud (as if reading aloud for someone nearby).

The tests were performed in a quiet room, with the subject having just the board in front, and the investigators and the acquisition equipment behind. The subjects were given a period of approximately 7 minutes between being instrumented (data acquisition devices described below) and the start of the initial rest phase, in order to adapt to the experimental conditions. The subjects were also given only written instructions, in order to minimize the influence of the investigator and the possibility of incomplete or erroneous instructions. The subjects were not informed at any time of the length of any of the tasks. They were instructed that each task finished when the investigator placed on the board a panel with instructions for the following test. The second rest phase of each part and the break between the two parts were longer than the first rest phase (7 minutes instead of 5). This choice was a compromise between maximizing the recovery time after each stress-inducing tasks, and limiting the overall length of the experimental protocol to about one hour. For the *Read* task, in order to limit reaction related to the topic of the text rather than to the task of reading itself, the selected text to read was of a neutral nature (part of the summary of a book),

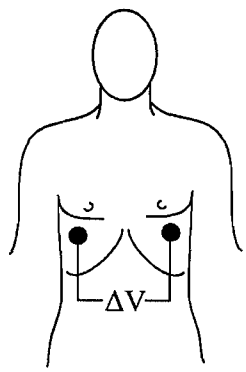


Figure 4.2: Positioning of the electrodes using a non-standard derivation

and its length short enough to allow each subject to read it at least twice during the task.

Data acquisition

During the protocol, the following signals were acquired simultaneously:

electrocardiogram (ECG) One non-standard derivation was acquired (positioning of the electrodes shown in Figure 4.2) using an ECG monitor with analogue output (model SDM-2000, Dixtal, Brazil), in order to extract the heart period series. The reason of the choice of such a derivation, was to minimizing the motion artifacts related to the act of writing during the math tests, and at the same time of producing ECG plots with well defined R peaks, compared to standard derivations.

arterial pressure (AP) Continuous recording of finger arterial pressure was made using a Portapres device (FMS BV, Netherlands), in order to extract systolic arterial pressure series (see Figure 4.3). The device output is an analogue signal generated from the original 200Hz digital signal produced by the device.

air pressure at the mouth (P_{AIR}) P_{AIR} was acquired using a pressure transducer (model 163PC01D48, Honeywell, USA) attached to a face mask that the subject wore throughout the test (see Figure 4.3).

partial pressure of CO_2 (P_{CO_2}) P_{CO_2} was acquired using a capnometer (model CO2SMO DX-7100, Dixtal, Brazil), with the sensor mounted at the end of the mask (see Figure 4.3).

audio A microphone was mounted on the face mask, in order to monitor the performance of the subjects during the speaking tasks. The audio signal was only used in preparing data for analysis, in order to confirm when the subjects were silent or speaking, and to check occasional artifacts such as coughs in the P_{AIR} signal.

time markers Start and end of each task were recorded using a custom made device, which had a button that the operator had to push every time he wanted to mark an event.

All signals were acquired simultaneously (after anti-alias filtering for ECG and P_{AIR}) with a sampling frequency of 1000Hz using a PC with a data acquisition card (model PCI-6024E, National Instruments, USA), except for P_{CO2}, which was acquired using the serial input of the PC (1Hz sampling frequency), given the lack of an analog output in the device. For the audio, a sampling rate of 1000Hz is too low for the speech to be intelligible, but sufficient for the purposes mentioned above.

Data processing

HP and SAP series were extracted from the electrocardiogram and from the continuous blood-pressure signal respectively, as described in Appendix A.1. Mean HR and SAP for each task were computed as well. The time series were then interpolated with a third order polynomial sampled at 4Hz, as recommended [27], and HRV and $SAPV$ indexes were estimated using an FFT-based spectral method. The spectra of HP and SAP were estimated through the Welch's modified periodogram method [120], using multiple segments of 256 samples (corresponding to 64s), an overlap between adjacent segments of 128 samples, and a Hanning window, providing a spectral resolution of 0.0156 Hz. HRV and $SAPV$ indexes were then computed as the total power in the LF band (0.05-0.15 Hz) and the HF band (0.15-0.5Hz), both in absolute units (ms^2 and $mmHg^2$, respectively, and to be denominated as HRV and $SAPV$) and normalized units (%, denominated $HRVn$ and $SAPVn$) [27]. In the remainder of this Section, the term *variability indexes* (VI) will be used in referring to all HRV and $SAPV$ indexes. For *Rest*, only the second half of the task (2.5 min) was considered for the analysis, since preliminary analysis showed that for this task many subjects underwent a progressive decrease in HR and SAP that reached a plateau approximately 2.5 min into the task. Furthermore, baroreflex sensitivity indexes were

computed using three different methods, as described in Section 2.2.3: a sequence method (SEQ), a spectral method (SPC_{LF} and SPC_{HF}, computed respectively in the LF and HF band), and a time-invariant autoregressive method (AR_{LF} and AR_{HF}, computed respectively in the LF and HF band). The details of the implementation of the FFT-based and autoregressive methods are reported in Section 3.2.

Among the different techniques currently used to monitor respiration we opted for the use of a face mask with a set of sensors attached (see Figure 4.3). Such a technique allows direct measures of air-flow (F), respiratory volume, P_{AIR}, and P_{CO2}. Some alternative indirect measures, such as a thoracic belt-plethysmograph (e.g. *Respitrace*, VivoMetrics, USA) are likely to be inaccurate during talking [11].

The main potential drawbacks of this choice are a degree of discomfort related to wearing the device, and the possibility of the device influencing the breathing pattern, the total ventilation, and the expected pressure variations inside the respiratory system. In order to minimize these problems, two different sizes of face mask were available, and each subject used the one he/she considered the most comfortable. Furthermore, different device setups were tested in preliminary work, in order to minimize air-flow resistance as well as dead space. Air-flow resistance is known to influence respiratory patterns, and consequently the amplitude of the respiratory sinus arrhythmia [26]. The presence of a dead space tends to increase the partial pressure of CO_2, and is hence compensated by the subjects with an increase in tidal volume, the latter known to influence respiratory sinus arrhythmia [17, 23]. The final device setup included a P_{AIR} sensor mounted in series with a P_{CO2} sensor (see Figure 4.3). The use of a pneumotachograph was avoided due to the relatively high air-flow resistance offered by the device.

With this configuration, estimates of flow were obtained from P_{AIR}, using a calibration curve, computed in the following way. A series of acquisitions were performed using a syringe of known volume (used to generate oscillatory patterns of air-flow), an accurate pneumotachograph (Fleisch nr.2, Hewlett-Packard, USA), and the acquisition setup (P_{AIR} and P_{CO_2} sensors) mounted in series. Then, from these data a calibration curve to estimate F from P_{AIR} ($F_{EST} = a \cdot |P_{AIR}|^b$) was computed through interpolation using a non-linear least square method [79] (See Figure 4.4). This exponential calibration curve was preferred to other options (e.g. polynomial) since it provided a good tradeoff between limiting the number of parameters and minimizing the mean square error. In fact, with only two parameters, estimates of F through this calibration curve showed a

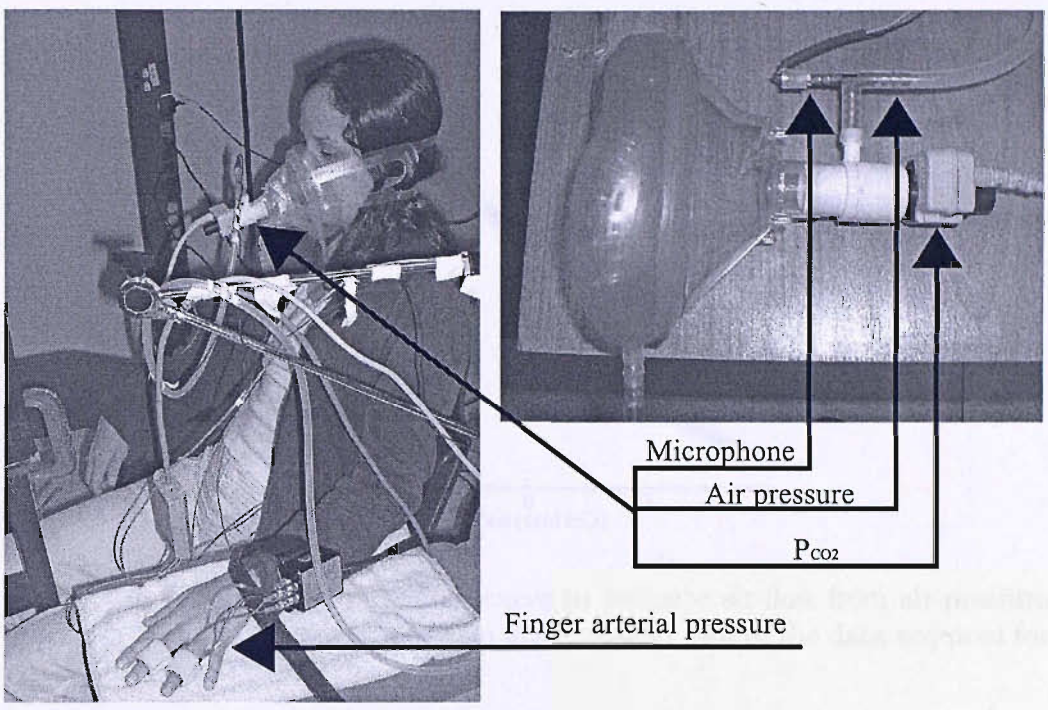


Figure 4.3: Signal acquisition system used for the experiments

median error of 1.2%, with an inter-quartile range of 6%. Furthermore estimates of the syringe volume obtained by integrating the estimated flow showed an error always smaller than 4%, without a bias.

The subjects' respiratory volume signal (V) was estimated from F through numerical integration, and from this the normalized volume in the low-frequency band (Vn_{LF}) was computed: given the spectrum of V, Vn_{LF} is the ratio between the power in the LF band and the sum of the power in the LF and HF bands.

The time t_i of the beginning of each respiratory act (inspiration and expiration) was detected from F using a zero crossing algorithm (see Figure 4.5), in order to estimate respiratory period (RP). Only respiratory cycles longer than 0.1s and with tidal volumes over 100ml were included. Results were checked and corrected by visual inspection. Using the edited t_i series, RP, inspiration/expiration ratio (I/E - the ratio of the duration of the inspiration and expiration periods in each respiratory cycle), and tidal volume (V_T - the difference between minimum and maximum value for V for each cycle), were computed for each respiratory cycle, and median values were computed for each task. The choice of RP and V_T as descriptors of respiration in this work is based on their postulated role as modulators of cardiovascular oscillations [17, 23, 54, 93], while I/E provides com-

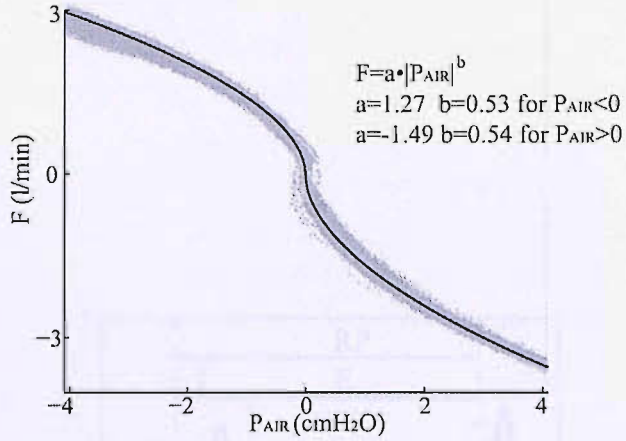


Figure 4.4: Interpolated calibration curve to estimate air-flow from air-pressure at mouth level. In the background (in gray), scatter plot of the data acquired for the calibration.

plementary information regarding the shape of the respiratory waveform and has also been suggested as a possible modulator of respiratory sinus arrhythmia [110].

4.3 Changes in cardiovascular indexes and respiration during the tasks

4.3.1 Introduction

In this section an investigation of the between-task changes and inter-individual differences in cardiovascular indexes and respiratory parameters is reported. In particular, the analysis was aimed at investigating differences in respiratory patterns and cardiovascular indexes between psychophysiological tasks involving speech and baseline rest and the task performed silently (*Maths silent*), and at testing the presence of significant correlations between respiratory and cardiovascular parameters. In Section 4.3.2 the statistical methods applied to this end are presented. The results of the analysis are reported in Section 4.3.3 and discussed in Section 4.3.4.

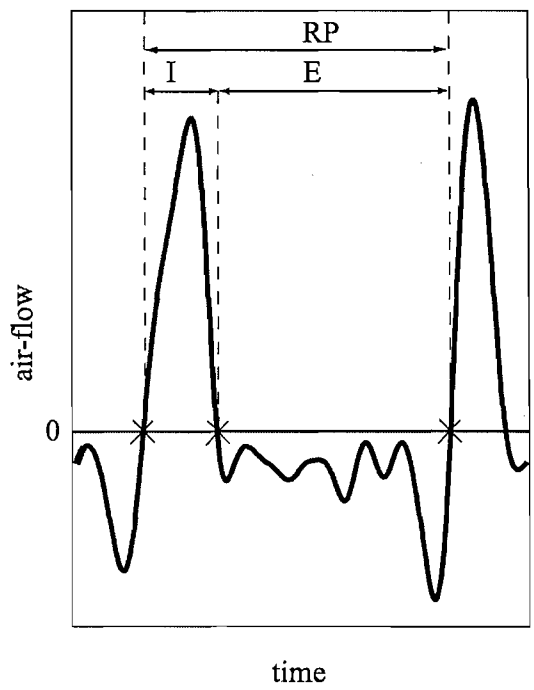


Figure 4.5: Example of computation of the length of inspiration (I), expiration (E), and respiratory period (RP) based on zero crossing detection of the air-flow signal (*Talk* task)

4.3.2 Statistical analysis

The geometric mean was computed for all the parameters in addition to the 5-95% range. The geometric mean was chosen, since the skewness of the distributions called for a log-transform of data. The significance of the change of the parameters for each task compared to rest was estimated using the Wilcoxon signed rank test. The significance of the differences between groups of subjects was estimated using the Wilcoxon rank sum test. The correlation of the cardiovascular indexes with respiratory parameters was estimated using the Spearman rank correlation test. The choice of these non-parametric tests was based on the observed non-normal distributions and on the presence of outliers. Results were considered significant below the p=0.05 level.

4.3.3 Results

Respiration during the tasks

Respiratory patterns changed radically during all the psychophysiological tasks considered, compared to baseline rest, with marked differences between the task performed silently (*Maths silent*) and those involving speech. In Figure 4.6 a typical example for one subject is shown. It is evident that *Maths silent* elicited a decrease in RP (increase in respiratory frequency) and V_T compared to baseline rest, while *Talk* (as well as the other speech tasks, not shown) was associated with an erratic respiratory pattern characterized by high variability in both RP and V_T. The result, in terms of the spectrum of V, is that while for *Maths silent* the power is mainly limited in the HF band, for the tasks involving speech the spectrum is markedly broadband, with considerable power present in both the LF and HF bands (Figure 4.7, top row). The changes in HP and SAP patterns (Figure 4.6) and spectra (Figure 4.7, middle and bottom rows), as expected, reflect these differences in respiratory patterns. It is worth noting that, for a limited number of subjects, even during *Maths silent* a significant amount of power was present in the LF (an example is shown in the next section, Figure 4.17). This is likely to be a reflection of the fact that these subjects, even if asked to remain silent, were sometimes observed to be whispering questions and answers.

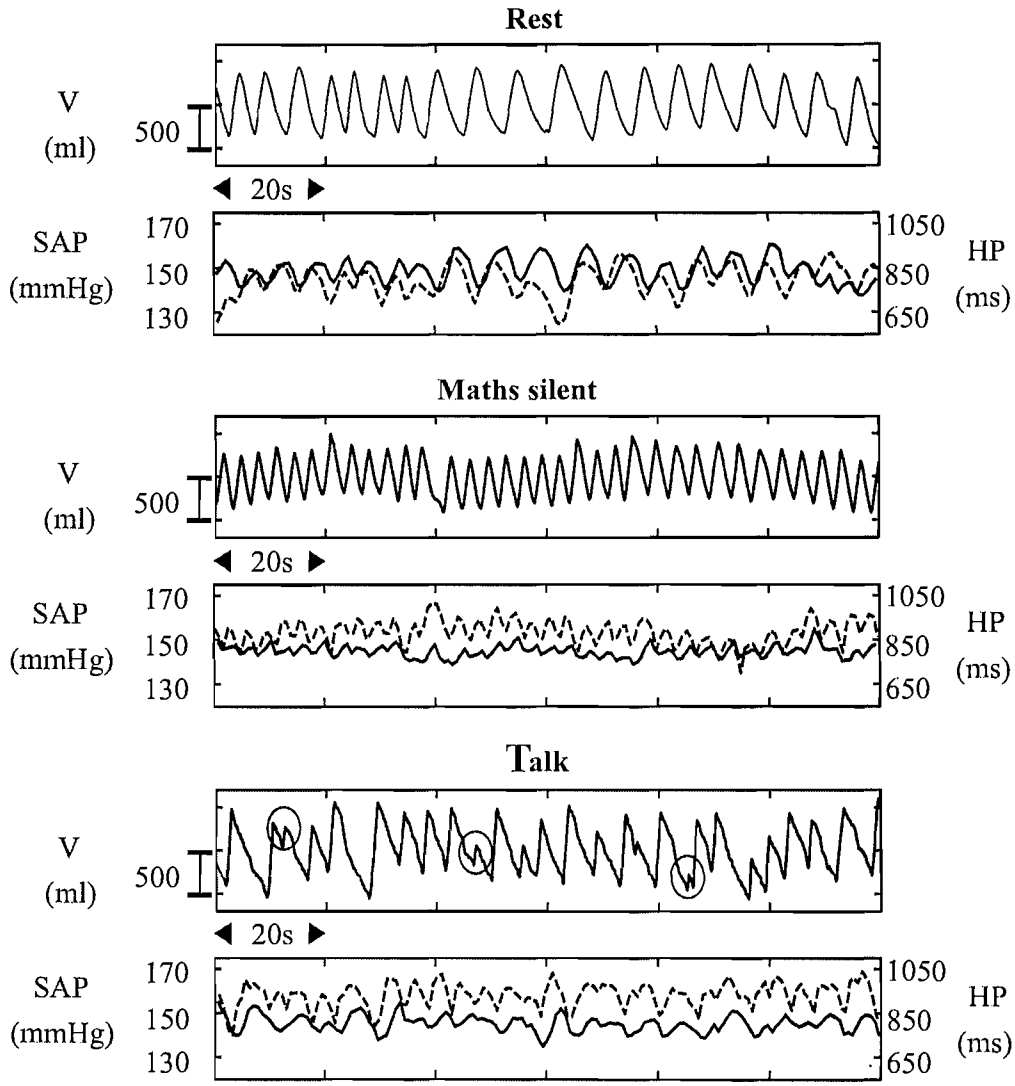


Figure 4.6: Respiratory volume (V), systolic arterial pressure (SAP), and heart-period (HP) patterns for a representative subject during part of three tasks: *Rest*, *Maths silent*, and *Talk* (HP dotted line, SAP solid line). The circles in the plot of V for *Talk*, indicate respiratory oscillations that are too small to be considered complete respiratory cycles.

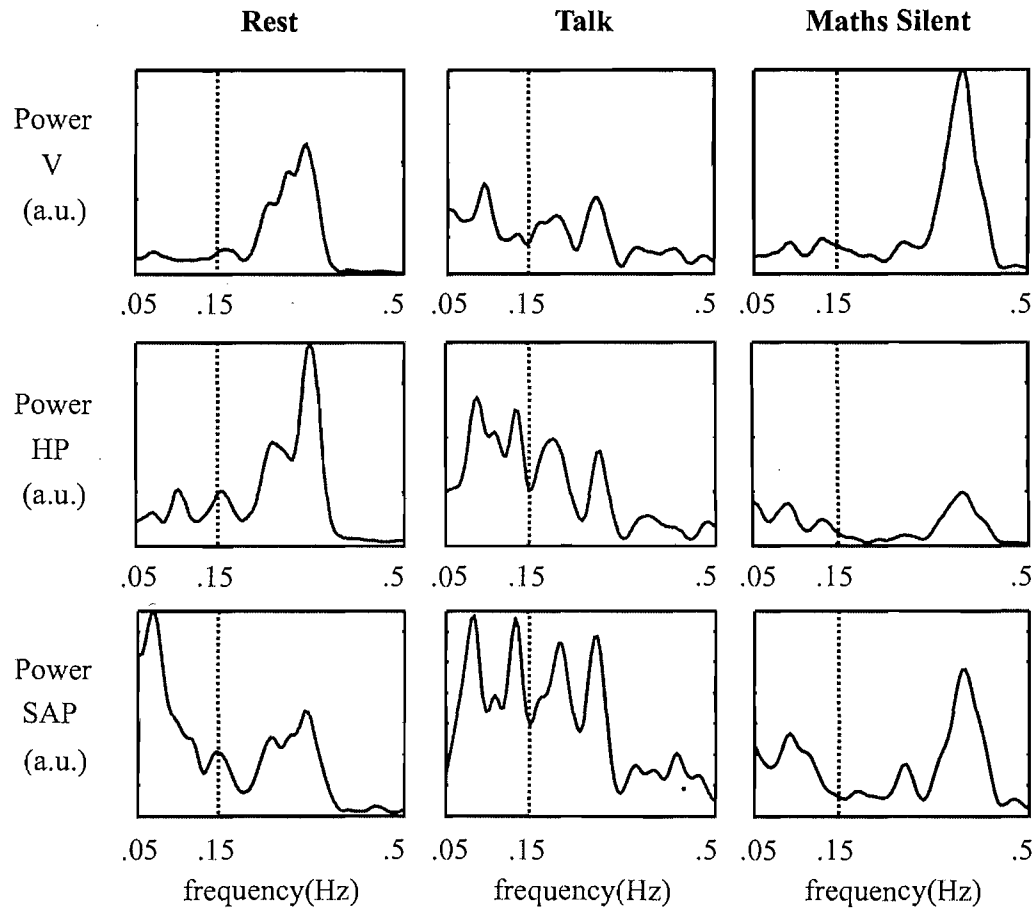


Figure 4.7: Spectrum of V, HP, and SAP (a.u., arbitrary units) for a representative subject during three tasks: *Rest*, *Talk* , and *Maths silent*

Subjects with long RP at rest

A considerable number of subjects showed a low respiratory frequency (long RP) during *Rest*, which may be expected to lead to respiratory-related cardiovascular oscillations in the LF band. This is clearly evident in Figure 4.9, where five subjects out of the 25 are seen to present a median RP of more than 6.7s, and thus a respiratory frequency of less than $0.15Hz$, which is the usually adopted boundary between LF and HF bands. These subjects present much higher HRV_{LF}, as might be expected from the presence of respiratory sinus arrhythmia in the LF. The effect of breathing in the LF range is illustrated further in Figure 4.10, where the spectrum of V, HP, and SAP during *Rest* is shown for three subjects with very different respiratory frequencies. There is a striking similarity between the spectrum of V and the spectra of HP and SAP, with all presenting a clear peak corresponding to the respiratory frequency. However, while for the subject with respiratory frequency in the HF band ($RP = 4.5s$, dotted lines), these peaks are limited to the HF band, for the other two subjects they are located predominantly in the LF band, contributing to HRV_{LF}. Thus, as the respiratory frequency shifts into the LF band, increased HRV_{LF} (as observed in Figure 4.9) is to be expected.

Hence two groups were considered in the further analysis of the data: firstly the six subjects presenting long RP during *Rest* (RP>6s), which include the five subjects with respiratory frequency within the HF band plus a borderline subject with RP=6.1s, and the 19 subjects showing a 'normal' short RP during *Rest*(RP<6s). The subject with 'borderline' respiratory frequency ($RP = 6.1s$) presented a very irregular respiratory pattern, with most of the power of V in the LF band (see Figure 4.8), and was thus included in the first group.

Further evidence of the effect of long RP at rest is reported in Figure 4.11: here the rest-task difference in HRV_{LF} (ΔHRV_{LF}) is plotted on the abscissa, and the RP during the task, on the ordinate. The data for subjects with long RP at *Rest* are plotted as circles, and the remainder as 'X'. Clearly ΔHRV_{LF} differs considerably between the two groups. The group with long RP at rest show a significant decrease in HRV_{LF} between *Rest* and *Read* (top plot, p=0.016), whereas the remaining subjects show the opposite change (p=0.002). When comparing *Rest* to *Maths silent* (Figure 4.11, bottom plot), again the subjects with long RP at rest show a clear decrease in HRV_{LF} (p=0.016), whereas for the others there is little consistent change in LF activity (p>0.05). Similar results were obtained for $SAPV_{LF}$, and considering *Talk* or *Maths aloud* instead of *Read.* It should also

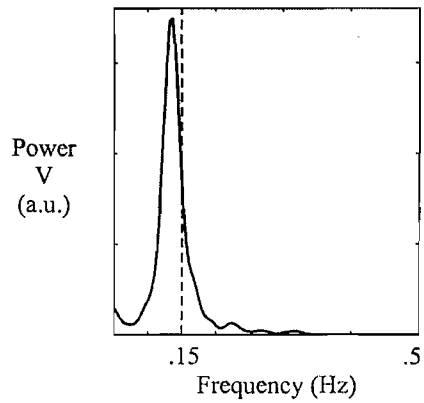


Figure 4.8: Spectrum of V (a.u. arbitrary units) for the subject presenting a median RP at *Rest* of 6.1s. Clearly, most of the power is concentrated in the LF band. For this reason the subject was analyzed together with the group of subject presenting long RP at *Rest*.

be noted that both groups show similar RP during all of the tasks ($p > 0.05$) except of course *Rest*.

The differences in the respiratory parameters and cardiovascular indexes between these two groups during *Rest* are shown in Figures 4.12, 4.13, and 4.14. Here the group average response (together with the 5-95% interval) are given for the group with long RP at rest (grey bar), short RP (black bar), and the entire cohort (25 subjects, white bar). The group of subjects with long RP presents much higher values for V_T and Vn_{LF}($p<0.001$), as well as for HRV_{LF}, $HRVn_{LF}$, $SAPV_{LF}$ and $SAPVn_{LF}$ ($p<0.01$), and lower values for $HRVn_{HF}$ ($p<0.001$). For BRS, there was not a significant difference between the two groups ($p> 0.05$).

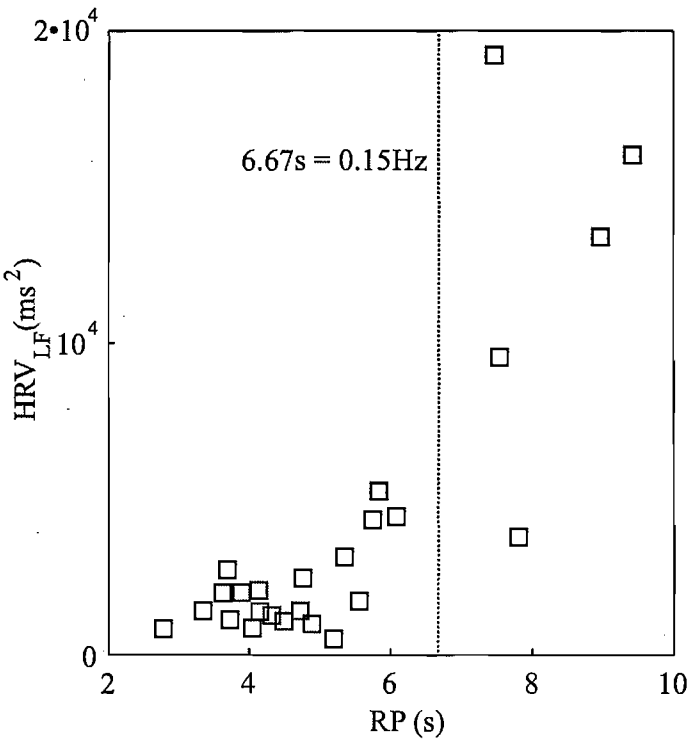


Figure 4.9: Scatter plot of HRV_{LF} vs median RP at *Rest*. The dotted line corresponds to 6.67s (0.15Hz) which is the commonly used boundary between LF and HF band. Five subjects present a respiratory frequency in the LF band. These subjects are characterized by a higher value of HRV_{LF} compared to the rest of the cohort ($p<0.01$).

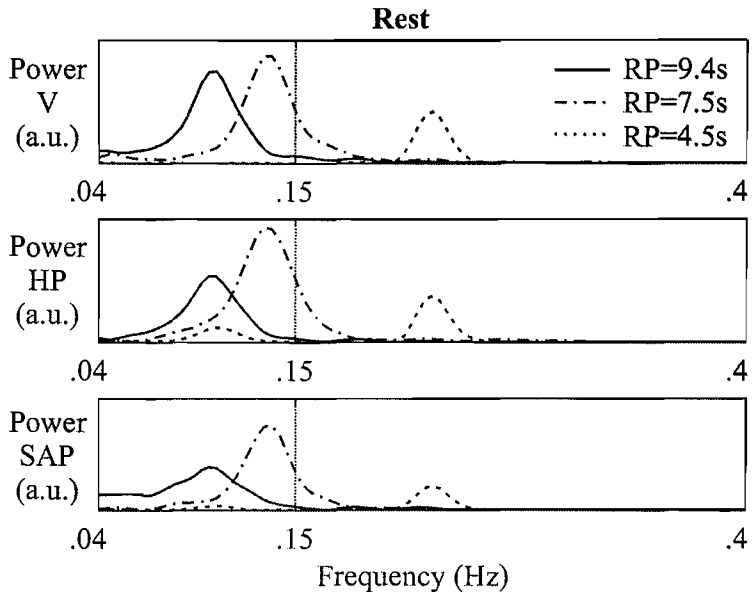


Figure 4.10: Power spectra of respiratory volume (V), heart-period (HP), and systolic arterial pressure (SAP) during *Rest* for three representative subjects with different respiratory frequencies, one within the HF band (RP=4.5s, dotted lines), two within the LF band (RP=9.4s and 7.5s, full and dash-dot lines).

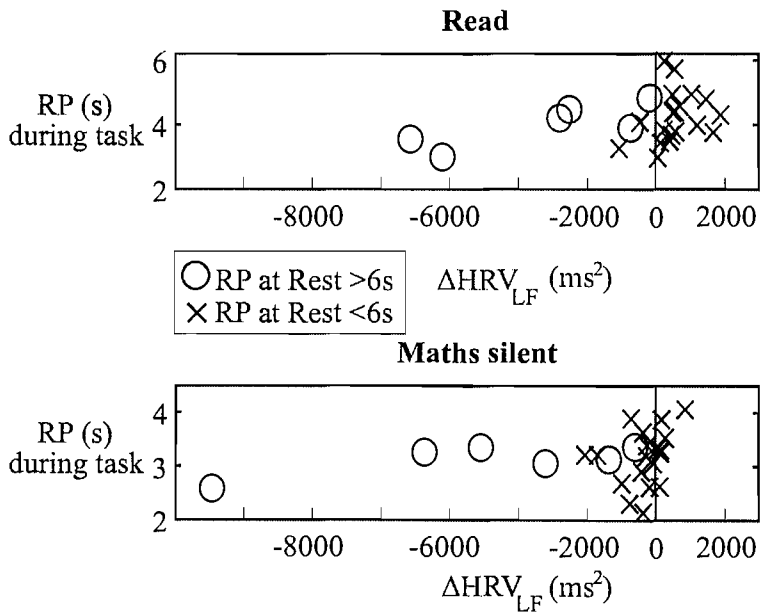


Figure 4.11: Change (Δ) of HRV_{LF} from *Rest* to *Read* (top) and from *Rest* to *Maths silent* (bottom). O: subjects with RP at rest of more than 6s; X: subjects with RP at rest of less than 6s

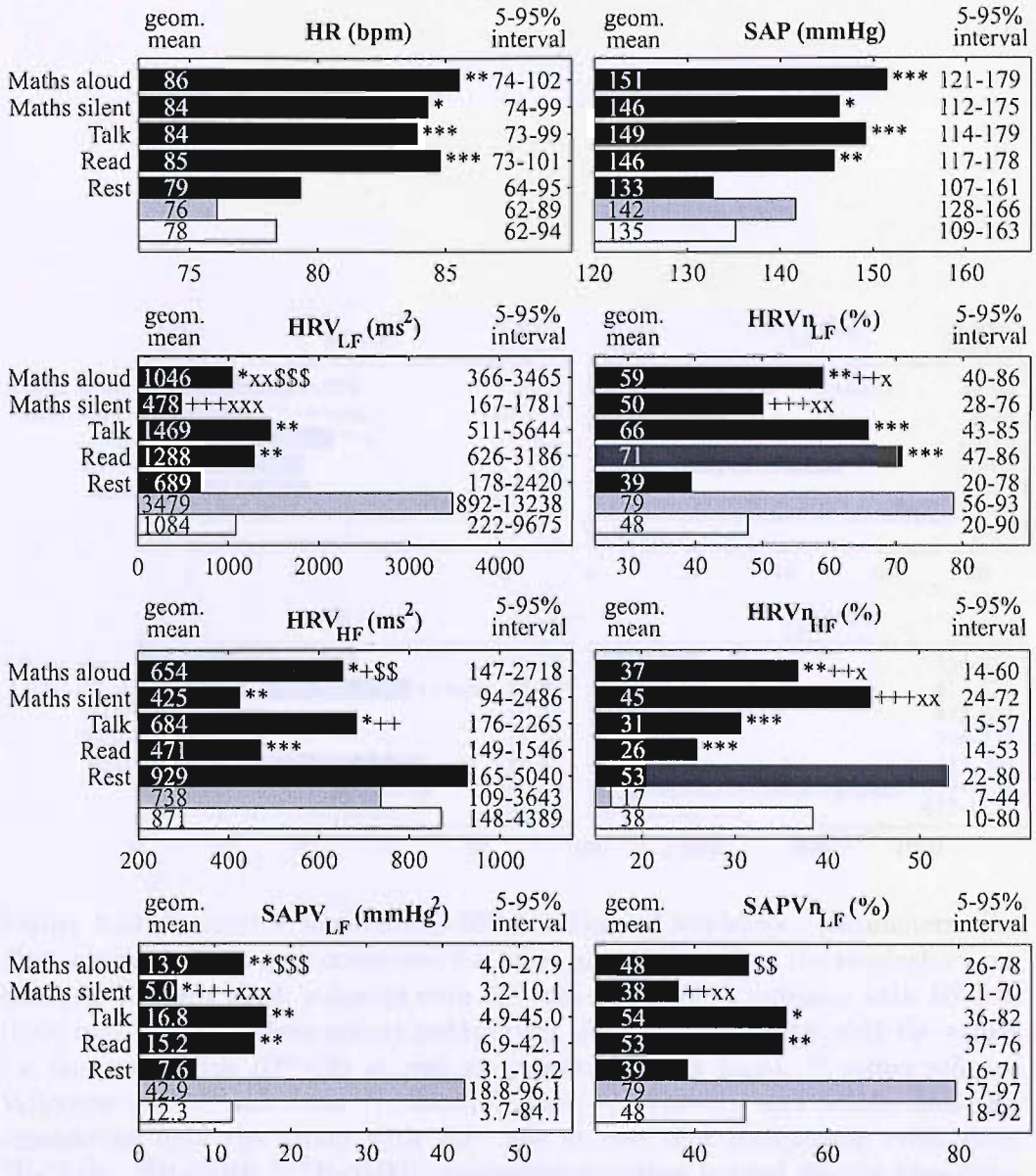


Figure 4.12: Geometric mean and 5-95% intervals of *HP*, *SAP* and *HRV* and *SAPV* indexes. For *Rest*, average values were computed for three groups, based on the respiratory period (*RP*) during *Rest*: subjects with *RP* <6s (black bar), subjects with *RP*>6s (grey bar), and the whole cohort (white bar). For the other tasks, only the values for the group with *RP* <6s at rest are reported (black bars). P values refer to Wilcoxon signed rank tests vs. *Rest*(∗),*Read*(+), *Talk*(x), and *Maths silent*($), considering only the group with *RP* >6s at rest. For comparison with *Rest*, *P<0.05; **P<0.01; ***P<0.001; equivalent notation is used for the remaining statistical tests.

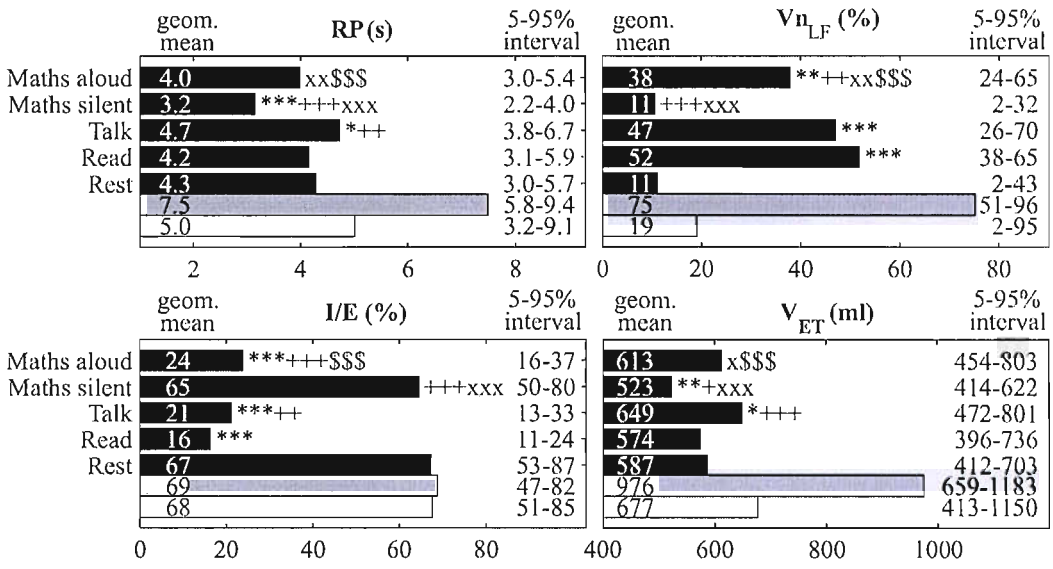


Figure 4.13: Geometric mean and 5-95% intervals of respiratory parameters. For *Rest*, average values were computed for three groups, based on the respiratory period (*RP*) during *Rest*: subjects with *RP* <6s (black bar), subjects with *RP*>6s (grey bar), and the whole cohort (white bar). For the other tasks, only the values for the group with *RP* <6s at rest are reported (black bars). P values refer to Wilcoxon signed rank tests vs. *Rest*(∗),*Read*(+), *Talk*(x), and *Maths silent*($), considering only the group with *RP* >6s at rest. For comparison with *Rest*, *P<0.05; **P<0.01; ***P<0.001; equivalent notation is used for the remaining statistical tests.

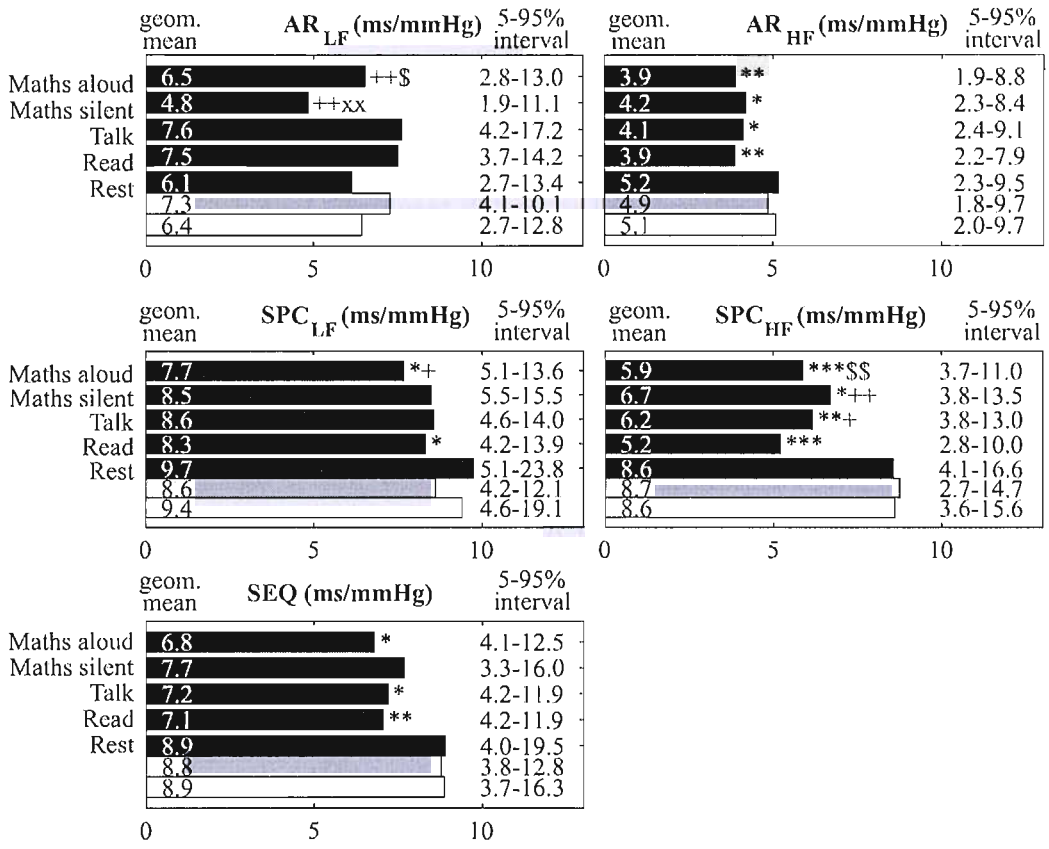


Figure 4.14: Geometric mean and 5-95% intervals of *BRS* indexes (*SPC*, FFT-based spectral method; *AR*, parametric autoregressive method; *SEQ*, sequence method). For *Rest*, average values were computed for three groups, based on the respiratory period (*RP*) during *Rest*: subjects with *RP* <6s (black bar), subjects with *RP*>6s (grey bar), and the whole cohort (white bar). For the other tasks, only the values for the group with *RP* <6s at rest are reported (black bars). P values refer to Wilcoxon signed rank tests vs. *Rest*(∗),*Read*(+), *Talk*(x), and *Maths silent*($), considering only the group with *RP* >6s at rest. For comparison with *Rest*, *P<0.05; **P<0.01; ***P<0.001; equivalent notation is used for the remaining statistical tests.

Responses during the tasks

The average response to all the tasks is shown in Figures 4.12, 4.13, and 4.14. In order to facilitate comparison with *Rest*, only the results for the group with short RP at rest are given (19 subjects, black bars). Results would however be very similar if all subjects were included, as there were no significant differences between the two groups of subjects in any cardiovascular index or respiratory parameters (except for *Rest*, as reported before).

As the figures show, all the tasks considered elicited a significant increase in HR and SAP, without a significant difference between the tasks ($p>0.05$). There is also a consistent decrease in HRV_{HF}, compared to *Rest* (in accordance with the expected vagal withdrawal).

However the results are more diverse for the remaining VI and respiratory parameters, with the response to tasks involving speech differing from *Maths silent*. The latter elicits a decrease in RP (increase in respiratory rate, with concomitant decrease in tidal volume V_T), without significant changes in shape of the respiratory waveform (I/E unchanged), while for the tasks involving speech, there are only modest changes in median RP but dramatic differences in the shape of the waveform, reflected in highly significant decreases in I/E and a shift in power of the respiratory signal to lower frequencies (increase in Vn_{LF}). This is in agreement with the examples shown in Figure 4.6. The differences in Vn_{LF} between the tasks, observed in Figure 4.13, are strikingly similar to those observed in HRV_{LF}, $HRVn_{LF}$, $SAPV_{LF}$ and $SAPVn_{LF}$ (Figure 4.12): compared to *Rest*, there is little change in these indexes for *Maths silent* (with a significant decrease only in $SAPV_{LF}$), but there are significant increases for all three tasks performed aloud.

The results regarding BRS indexes do not provide a clear indication of a difference between tasks performed silent and aloud, and produced inconsistent results regarding the rest-tasks changes (Figure 4.14). SEQ decreased significantly for the tasks involving speech, and significant decrease for all tasks was found for AR_{HF} and SPC_{HF}. For SPC_{LF} the decrease was significant only for *Read* and *Maths aloud*, while for AR_{LF} no significant rest-tasks changes were found ($p>0.12$), with *Maths silent* associated with significantly lower values than the other tasks.

In order to investigate the discrepancies between methods, different values for the order of the autoregressive model were applied, to test the impact of this parameter on the estimated indexes AR_{LF} and AR_{HF}. Model orders of 10 and

30 were used, resulting, as expected from previous evidence [4], in respectively lower and higher estimated values for BRS, but with no appreciable difference in the significance of the estimated rest-tasks changes, compared with the original values for an order of 16. Furthermore, the impulse responses of the transfer function $SAP \rightarrow HP$ estimated to compute the AR and SPC indexes were compared (see Figure 4.15). The transfer functions estimated through the FFT-based approach (used for SPC indexes, see Section 2.2.3) showed evident non causal characteristics, with the response beginning several samples before zero and an evident peak at zero lag. The difference with the transfer functions estimated through the autoregressive model (used for AR indexes, see Section 2.2.3), for which causality without immediate effects (lag=0) is imposed by the model itself, is evident, providing a caveat in interpreting the differences found in the indexes, as will be discussed further on.

Finally, it is important to note that for the subjects with long RP during *Rest*, who have quite different base-line values of VI (Figure 4.12) compared to the remainder of the cohort, the rest-task changes also differ considerably. In fact, for the group with long RP at *Rest*, considerable and significant ($p<0.05$) decreases were found for all respiratory parameters and VI (except I/E and $HRVn_{HF}$), independently of the task. This is clearly in disagreement with the results discussed above, confirming the need for the separate analysis with the two groups adopted in this work.

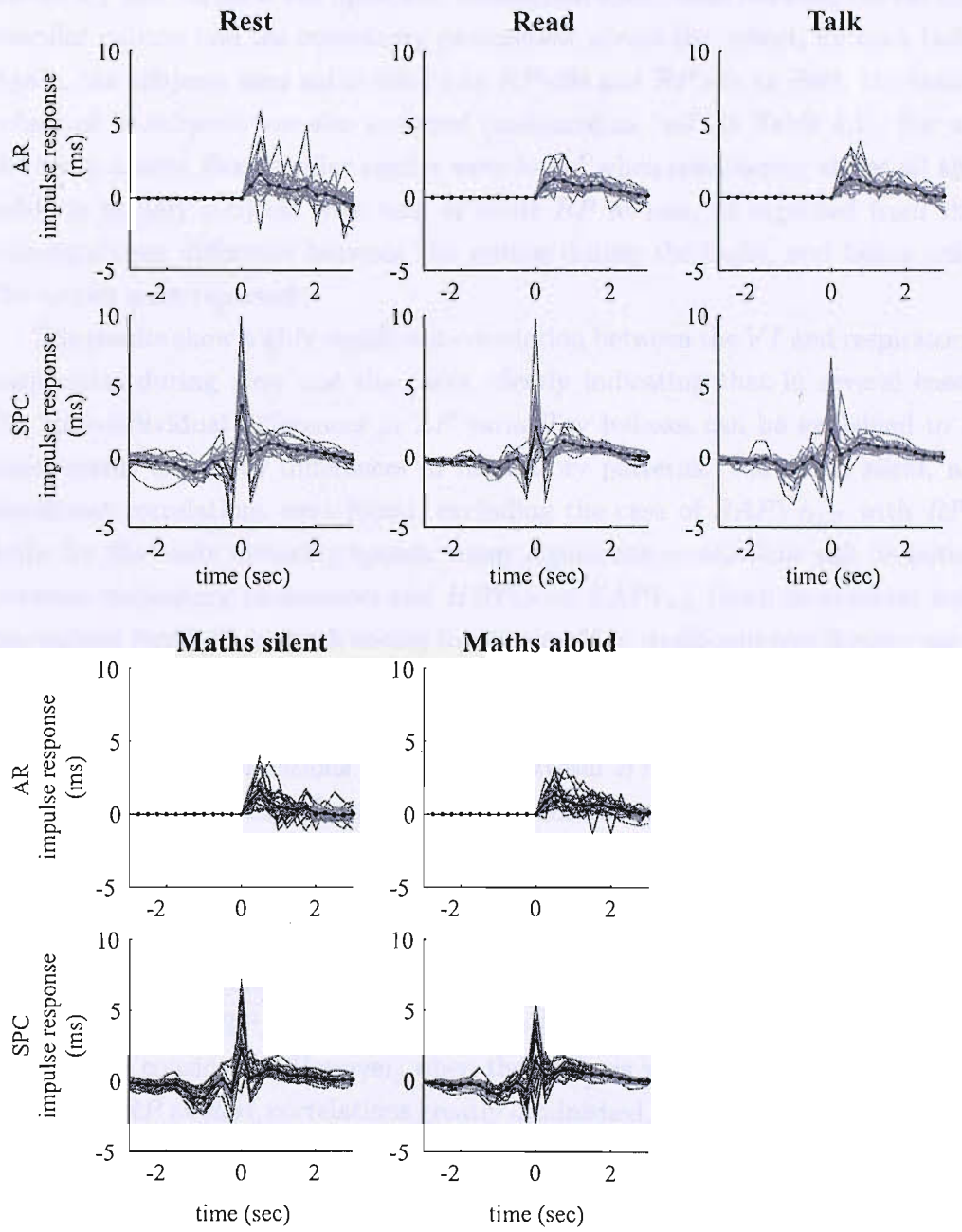


Figure 4.15: Impulse response of $SAP \rightarrow HP$ model, using autoregressive (AR) and FFT-based (SPC) approach. The response of each subject is reported in gray, while the (arithmetic) mean response is in black

Correlations between respiration and VI

Tables 4.1 and 4.2 show the Spearman correlation coefficients between the cardiovascular indexes and the respiratory parameters, across the cohort, for each task. Again, the subjects were subdivided into RP<6s and RP>6s at *Rest*; the entire cohort of 25 subjects was also analyzed (indicated as "*all*" in Table 4.1). For all the tasks except *Rest*, similar results were found when considering either all the subjects or only subjects with long or short RP at rest, as expected from the non-significant difference between the groups during the tasks, and hence only the former were reported.

The results show highly significant correlation between the VI and respiratory parameters during *Rest* and the tasks, clearly indicating that in several cases the inter-individual differences in LF variability indexes can be explained to a considerable extent by differences in respiratory patterns. For *Math silent*, no significant correlations were found, excluding the case of $SAPVn_{LF}$ with RP, while for the tasks involving speech, many significant correlations can be noted between respiratory parameters and HRV_{LF} or $SAPV_{LF}$ (both in absolute and normalized form). It is worth noting that most of the significant correlations were found when Vn_{LF} was considered, while few were found for RP and V_T, and none for I/E (for this reason the results for the latter are not reported).

No significant correlations were found between HRV_{HF} and any of the respiratory parameters in any of the experimental conditions. It should be pointed out that for $HRVn_{HF}$, the Spearman correlation coefficients have the same absolute value as those for $HRVn_{LF}$, since $HRVn_{HF} = 1 - HRVn_{LF}$, and hence they are not reported separately.

During *Rest*, strong and statistically significant correlations between HRV_{LF} or $SAPV_{LF}$ with RP, V_T, and Vn_{LF} were found when all the subjects of the cohort were considered. However, when the analysis was restricted to the group with short RP at *Rest*, correlations greatly diminished, and statistical significance was largely lost. This again suggests that subjects with low RP at rest can have a considerable impact on the estimated cardiovascular-respiratory interaction, as will be discussed further in the next section.

For the BRS indexes, only two statistically significant correlations were found: with RP during *Maths silent* (significant for AR_{LF} and almost for SPC_{LF}, p< 0.065), and with V_T for *Maths aloud* (significant for SPC_{HF} and almost for AR_{HF}, p< 0.06).

Table 4.1: Correlation coefficients between VI and respiratory parameters during the tasks, obtained with Spearman correlations tests. RP<6s: results obtained considering only subjects with RP at rest of less than 6s; all: results obtained considering all the subjects in the cohort. Significance: ***p<0.001; **p<0.01; *p<0.05.

	$SAPV_{LF}$		
	RP	V_T	Vn_{LF}
Rest,all	0.69***	0.67***	0.77***
Rest,$RP > 6$s	0.20	0.31	0.09
Rest,$RP < 6$s	0.33	0.35	0.52*
Read,all	-0.01	0.09	0.42*
Talk,all	0.01	0.33	0.66***
Maths silent,all	0.08	0.19	0.39
Maths aloud,all	0.09	0.37	0.33

	HRV_{LF}		
	RP	V_T	Vn_{LF}
Rest,all	0.60**	0.57**	0.49*
Rest,$RP > 6s$	0.14	0.26	0.20
Rest,$RP < 6s$	0.32	0.33	0.14
Read,all	-0.12	0.16	0.02
Talk,all	0.10	0.41*	0.23
Maths silent,all	0.37	0.02	0.19
Maths aloud,all	0.22	0.14	0.40*

	$SAPVn_{LF}$		
	RP	V_T	Vn_{LF}
Rest,all	0.58**	0.55**	0.81***
Rest,$RP > 6$s	0.88*	0.60	0.94*
Rest,$RP < 6$s	0.10	0.07	0.62**
Read,all	0.35	0.20	0.61**
Talk,all	0.19	0.02	0.69***
Maths silent,all	-0.44*	-0.06	0.13
Maths aloud,all	0.01	0.18	0.38

	$HRVn_{LF}(HRVn_{HF})$		
	RP	V_T	Vn_{LF}
Rest,all	0.43*	0.61**	0.52**
Rest,$RP > 6$s	0.77	-0.08	0.31
Rest,$RP < 6$s	-0.16	0.26	0.04
Read,all	0.07	0.21	0.50*
Talk,all	0.41*	0.28	0.54**
Maths silent,all	-0.10	-0.02	0.13
Maths aloud,all	0.23	0.41*	0.46*

Table 4.2: Correlation coefficients between BRS indexes and respiratory parameters during the tasks, obtained with Spearman correlations tests. RP<6s: results obtained considering only subjects with RP at rest of less than 6s; all: results obtained considering all the subjects in the cohort. Significance: ***p<0.001; **p<0.01; *p<0.05.

	AR_{LF}		
	RP	V_T	Vn_{LF}
Rest,all	0.36	0.19	0.38
Rest,$RP > 6$s	-0.49	-0.09	-0.26
Rest,$RP < 6$s	0.36	0.10	0.35
Read,all	0.03	0.23	0.07
Talk,all	0.26	0.08	-0.24
Maths silent,all	0.45*	-0.13	-0.07
Maths aloud,all	0.17	-0.10	-0.10

	AR_{HF}		
	RP	V_T	Vn_{LF}
Rest,all	0.16	-0.03	0.07
Rest,$RP > 6$s	-0.43	0.14	-0.14
Rest,$RP < 6$s	0.41	0.03	0.19
Read,all	0.12	0.17	-0.07
Talk,all	0.04	-0.12	-0.24
Maths silent,all	0.24	-0.17	-0.07
Maths aloud,all	-0.07	-0.39	-0.09

	SPC_{LF}		
	RP	V_T	Vn_{LF}
Rest,all	-0.05	0	-0.23
Rest,$RP > 6$s	-0.43	0.14	-0.14
Rest,$RP < 6$s	0.04	0.09	-0.22
Read,all	-0.09	0.05	-0.13
Talk,all	0.27	0.11	-0.3
Maths silent,all	0.38	-0.03	0.02
Maths aloud,all	0.13	-0.18	0.11

	SPC_{HF}		
	RP	V_T	Vn_{LF}
Rest,all	0.19	0.01	0.22
Rest,$RP > 6$s	-0.31	-0.03	-0.09
Rest,$RP < 6$s	0.23	-0.11	0.26
Read,all	0.09	0.07	-0.08
Talk,all	0.04	-0.14	-0.11
Maths silent,all	0.08	-0.11	-0.05
Maths aloud,all	-0.08	-0.45*	0.05

	SEQ		
	RP	V_T	Vn_{LF}
Rest,all	0.12	-0.07	0.1
Rest,$RP > 6$s	-0.49	-0.09	-0.26
Rest,$RP < 6$s	0.24	-0.1	0.17
Read,all	-0.03	0.09	-0.15
Talk,all	0.26	0.21	0.02
Maths silent,all	0.10	-0.15	-0.07
Maths aloud,all	0.15	-0.21	0.12

4.3.4 Discussion

Based on the results presented a number of issues will be addressed:

i) the presence of a considerable number of subjects with long RP at rest can potentially bias the interpretation and estimation of average baseline levels of VI, and confound the interpretation of rest-task changes elicited by the speech and non-speech tasks;

ii) in contrast to *Rest*, for tasks involving speech, respiration presents markedly broadband characteristics; for these tasks, interpreting the rest-task changes in HRV_{LF}, HRV_{HF}, and $SAPV_{LF}$ as solely a reflection of changes in autonomic activation elicited by psychological/cognitive processes, ignoring any effect of respiration, appears to be inappropriate and probably misleading;

iii) when speech is present, the inter-individual differences in respiratory parameters are reflected in inter-individual differences in VI; during *Maths silent* no such correlation was observed;

iv) the decrease in HRV_{LF} and $SAPV_{LF}$ observed in *Maths silent*, and the increase noted in tasks involving speech can at least partially be explained by the difference in respiratory patterns between the two types of tasks;

v) BRS changes do not appear to differ significantly between *Maths silent* and tasks performed aloud; however, the discrepancies between the estimates obtained by different methods used strongly limit any inference in this regard, and more generally in the changes in baroreflex elicited by the tasks;

vi) for the tasks involving speech, Vn_{LF} should complement RP and V_T in quantifying the complex respiratory patterns and in investigating the presence of correlations between respiration and VI.

i) VI for subjects with long RP at rest

As shown in Figure 4.9, 6 out of the 25 young, healthy subjects included in this study presented relatively long RP (RP>6s, respiratory frequency <0.17Hz) during *Rest*, and showed the power-spectrum of Vconcentrated in the LF band, resulting in high Vn_{LF} (Figure 6). This is in agreement with some previous reports, where 9 out of 40 subjects [93], and 7 out of 20 [55] were found to have long RP at rest.

For the subjects with long RP it is questionable if autonomic activation can be reliably assessed through VI. A key assumption for using VI as indicators of autonomic activation is that respiration, and consequently respiratory sinus arrhythmia, is confined to the HF band [17]. This assumption has been implicitly accepted in most work on HRV and $SAPV$ to assess autonomic activation in psychophysiological tasks, but clearly the subjects with long RP do not satisfy this. The consequences are evident from considering the examples shown in Figure 4.10. For the subject with the long RP (9.4s, spectra shown as solid lines, and 7.5s, spectra in dash-dot lines), the spectrum of respiratory volume (V) at *Rest* is mostly in the LF band, and the HP and SAP spectra are also mainly limited to the LF band. This is in agreement with previous results on paced breathing at progressively lower frequencies where it was observed that the respiratory peak in the HP spectrum continues to be clearly evident even at low (<0.15Hz) respiratory frequencies (Figure 4.9 in [23]).

Since VI are computed as the total power of HP and SAP spectra in the LF and HF bands, respectively, VI indexes referring to the LF band would be high in subjects with long RP (see Figure 4.9 for the case of HRV_{LF}), and those referring to the HF band very low. Thus in these subjects low HRV_{HF} clearly cannot be taken as an indication of low parasympathetic activation, since the respiratory sinus arrhythmia, whose power is usually assumed to reflect parasympathetic activation [27], is contributing mostly to the LF rather than the HF power of the HP spectrum. Correspondingly, the high values of $SAPV_{LF}$ and HRV_{LF} cannot be taken as indicators of high sympathetic activation, since a considerable contribution to their magnitude comes from respiratory modulation which is known also to be under parasympathetic control [19].

The problems related to the subjects with long RP at *Rest* have also a particular relevance in the assessment of rest-task changes. While at *Rest* an offset in the value of VI is present between subjects with long and short RP (see Figure 4.12), for the other tasks values are similar in all subjects. Thus, if subjects with long RP are not removed from the analysis, this can result, in significant rest-task changes in VI not being detected (e.g. HRV_{LF} during *Read*, see Figure 4.11, top), or being exaggerated (e.g. HRV_{LF} during *Maths* silent, Figure 4.11, bottom). Furthermore, the high correlation between respiration and VI observed in Table 4.1 for the whole cohort during *Rest* probably reflects the shift into the LF band of sinus arrhythmia in those subjects with slower breathing, rather than the frequency dependent modulation of HRV and $SAPV$ by respiration, that may

be the naive interpretation. This is underlined by the observation that correlation is considerably lower and in many cases not significant when only those with 'normal' (<6s) RP are considered, even if it must be acknowledged that this may also be in part due to the smaller size of the group (19 subjects instead of 25 for the whole cohort).

The associations and correlation found cannot be taken as a conclusive proof that respiration is the only origin of the differences in VI between the subjects with long and short RP. There may indeed be difference in autonomic activation, but it would seem unsafe to attribute the differences in VI entirely to the latter effect.

In view of the arguments presented, and of previous evidence [28, 55, 93], we suggest that subjects with long RP at rest should not be included in the analysis of VI for the estimation of rest baseline values. This was carried out in the current work, through separate analysis for subjects with long and short RP at rest (see Figure 4.12).

ii) Influence of speech on respiration and VI

The observed changes in respiration provoked by the tasks generally agree with previous reports, with RP and V_T decreasing for all tasks [2, 55, 122], if all subjects in the cohort are considered (Figure 4.13). Marked differences exist between the responses to *Maths silent* and the tasks involving speech, with the former presenting significantly lower values for RP and V_T. Furthermore, while the values of I/E and Vn_{LF} are similar in *Maths silent* and *Rest*, for the tasks involving speech I/E decreases and Vn_{LF} increases considerably, indicating that the respiratory waveform undergoes radical changes. This is in agreement with results previously reported in the literature [25, 55] and is clearly exemplified in Figure 4.6: while for *Rest* and *Maths silent* the respiratory waveform is approximately periodic and sinusoidal in shape, for the tasks involving speech the pattern is far from sinusoidal (short inspiration and longer expiration) and highly erratic.

The result is that the respiratory pattern during tasks involving speech tends to present markedly broadband characteristics (see the spectra during *Talk* in Figure 4.7 and also Figure 4.7 in [85]), clearly affecting the LF band and increasing Vn_{LF}. Based on current understanding of the interaction between respiration and VI [28], this is expected to lead to increases in HRV_{LF} and $SAPV_{LF}$. This can explain the relatively high values of HRV_{LF} and $SAPV_{LF}$ during task involving speech observed in Figure 4.12, and the lower values for *Maths silent*. In

particular, the striking similarity between Vn_{LF}, HRV_{LF}, and $SAPV_{LF}$ for the tasks can be seen as a direct reflection of the influence of respiration on VI.

However, this argument cannot explain the between-task difference in HRV_{HF}, and in particular why *Maths silent* provides the lowest values in HRV_{HF}, despite presenting more power of the respiratory volume signal in the HF band than the tasks involving speech (Vn_{LF} significantly lower). Vagal withdrawal related to the mental effort is expected, leading to reduced respiratory sinus arrhythmia (in this case limited to the HF band), but, for example, it is not immediately evident why such an effect should be greater for *Maths silent* that for *Maths aloud.* Another characteristic of respiration may provide a partial interpretative caveat of this between-task difference in HRV_{HF}: *Maths silent* has the lowest value of V_T (significantly lower than *Rest*), and such reduced amplitude of respiratory oscillations is expected to lead to a reduced respiratory sinus arrhythmia [24, 54].

In the absence of an independent (and reliable) assessment of autonomic function, it is evidently not possible to quantify the relative contribution of variations in respiration or of modifications in autonomic activation (or their interaction) to the changes of VI, but the results clearly suggest that an interpretation that completely disregards respiratory modulation is at least unsafe.

iii) Correlations between VI and respiration

The correlation between Vn_{LF} and $SAPV_{LF}$, $SAPVn_{LF}$, and $HRVn_{LF}$ during *Read* and *Talk* is striking, indicating that respiratory activity in the LF range is a (highly) significant predictor of inter-individual differences in VI. It should be emphasized that in much previous work, the effects of respiration have been considered to be largely confined to the HF range [19], but the current results indicate that particularly during tasks involving speech, the LF range is also implicated. Thus inter-individual differences in LF variability indexes should not be ascribed purely to differences in autonomic activation, but can be explained by differences in respiration. If this is not taken into account, two subjects with different respiratory patterns during speech might be falsely considered as presenting differences in autonomic activation. This argument can also be extended to the comparison of groups of subjects in whom differences in respiration might be expected.

As pointed out in the results, no significant correlations between HRV_{HF} and respiration descriptors were found for any of the tasks. Thus, different to the LF band, inter-individual variability in HRV_{HF} was not found to be ex-

plained by inter-individual variability in respiration. However, given the high variability and complexity of the respiratory patterns during speech it is possible that the set of respiratory indexes used was not sufficient to reveal cardiorespiratory interactions in the HF band. Furthermore, for normalized HF activity ($HRVn_{HF}$) correlations with Vn_{LF} and RP were found (Table 4.1), but this could be as much due to inter-individual differences in LF as in HF, considering that $HRVn_{HF} = HRV_{HF}/(HRV_{LF} + HRV_{LF}) = 1 - HRVn_{LF}$.

iv) VI for Maths silent vs. aloud

Further striking evidence of the impact of respiration comes from comparing the *Maths* task performed *silently* and *aloud*. When the task is performed silently, HRV_{LF} and $SAPV_{LF}$ decrease compared to *Rest*, while when it is performed aloud, they significantly increase. This is in agreement with previous results [104], and suggests that not only the magnitude but also the direction of rest-task changes may be affected when speech is involved. In the absence of direct assessments of autonomic regulation, the possibility of different levels of autonomic activation during the two realizations of the *Maths* task of course cannot be excluded. However, since no significant differences in HR and SAP were observed (Figure 4.12), it seems unlikely that this is a strong effect. Furthermore, even if this effect is present, it seems very unlikely that opposing changes in VI would be observed when comparing the same task performed silently or aloud. On the other hand, the difference in respiration observed between the tasks provides a very plausible alternative explanation: due to the very large increase in respiratory oscillations in the LF band elicited by *Maths aloud* (increase in Vn_{LF}, see Figure 4.13), an increase in HRV_{LF} and $SAPV_{LF}$ is expected, as discussed in the previous items and in published work [11, 28].

The above may provide an explanation why discrepant results have been reported in previous works on LF cardiovascular oscillations. In tasks involving speech, an increase in HRV_{LF} and $SAPV_{LF}$ was found (e.g. [11, 50, 85]), while when speech was not present a decrease has been reported (e.g. [2, 11, 22, 37, 43, 106, 107]). Clearly the impact speech has on psychophysiological tasks will vary between experimental protocols and subject groups, and respiratory effects may not always be as evident as in the current investigation. However, it is worth noting that the same discrepancy between tasks performed silent and aloud were found for the experiment discussed in Section 3.

The observed decrease in HRV_{LF}, $SAPV_{LF}$, and $SAPVn_{LF}$ during *Maths*

silent compared to *Rest* deserves further comment (Figure 4.12). These results, which agree with previously reported work [106], seem contrary to the expected increase in sympathetic activation during mental effort and stress, which is conventionally associated with increased LF oscillations in HP and SAP [27, 103]. However, it is known that LF cardiovascular oscillations are also influenced by parasympathetic cardiac modulation, either directly (HRV_{LF}) or indirectly ($SAPV_{LF}$, through baroreflex feed-forward mechanism) [27, 106, 126], and it has been proposed that for mild stressors the expected sympathetically mediated increase in LF oscillations may be canceled or even reversed by vagal withdrawal [106], the latter being evidenced in our case by the decrease in HRV_{HF} (Figure 4.12). Our results are in accordance with this argument, reinforcing the critique of the use of LF cardiovascular oscillations as markers of sympathetic activation, at least for the case of mild stressors.

v) BRS indexes

Previous work on tasks involving different forms of mental effort showed a significant decrease in BRS [36, 43, 50, 125]. However the different indexes adopted in this work to estimate BRS changes provided contrasting results (see Figure 4.14).

The discrepancies found between AR and SPC indexes do not appear to be related to the order chosen for the autoregressive model used to compute AR indexes (which can affect the accuracy of the estimate of the transfer function, and ultimately of the AR indexes), since attempts to use different model orders lead to almost identical results in terms of statistical significance of the differences between tasks.

However, the comparison between the impulse responses for the estimated transfer functions (Figure 4.15) showed that the impulse responses estimated using an FFT-based method (from which SPC indexes are computed) clearly present an acausal component (i.e. starting several samples before zero) for all tasks. This is not surprising, and may reflect the known feedback mechanism $HP \rightarrow SAP$ [4, 95], as suggested in a previous work [88]. Furthermore, an evident peak is present at zero, suggesting the presence of important instantaneous interactions between SAP and HP, which might not be related to the baroreflex mechanism, given that the shortest baroreflex latency published from human studies is 0.24s, corresponding to almost one sample at 4Hz [40] (even it must be acknowledged that shorter latencies, even if present, might have not been detected since HP and SAP are not continuous signals with relatively long be-

tween samples intervals). However, the possibility of detecting These effects are obviously not present in the impulse response estimated using the strictly causal autoregressive model. From this point of view, since the aim is to estimate the causal $HP \rightarrow SAP$ modulation, the AR approach would appear more appropriate.

There are also differences between indexes computed using the LF band and those using faster cardiovascular oscillations. In fact, SPC_{HF} and AR_{HF} undergo a significant decrease in BRS for all tasks, in agreement with published results [36, 43, 50, 125]. It has been suggested that coherent fluctuations of HP and SAP at respiratory frequencies are at least in part originated by central oscillators (i.e. brain stem respiratory centers) [3], and by non-baroreflex mechanism [73, 95], implicitly questioning the validity of BRS indexes estimated at respiratory frequencies. For the protocol considered in this work this problem is expected to involve all the BRS indexes computed independently of the frequency band used for estimation, since for the tasks of the protocol involving speech respiratory-related cardiovascular oscillations are present in a broad range of frequencies. However, the relative contribution of these non-baroreflex and central mechanisms have on the estimated $SAP \rightarrow HP$ transfer function for a range of experimental conditions is still unclear [45].

Hence, despite the evidence found that the BRS indexes considered are for many aspects largely uncorrelated with respiration (i.e. no significant differences between subjects with long and short RP at rest, lack of consistent correlations with respiratory parameters for all tasks), the presence of discrepancies between different indexes do not provide a clear picture of the effect of speech-related respiration on BRS estimation, strongly limiting any conclusion on this regard. In fact given the lack of a gold standard for BRS assessment, there is no conclusive evidence on which index, among those considered, should be considered as more reliable [75]. Further investigation is needed, possibly considering a larger cohort [36, 43, 50], in order to exclude the possibility that the lack of significant results found is an effect of insufficient power of the statistical analysis. Furthermore, the use of multivariate models of HP modulation that include also respiration as an input could be considered [95], since, potentially, they are capable of disentangling respiratory-related and respiration-independent coherent oscillations of HP and SAP. These two components, as said above, appear to be related to different physiological mechanisms (central modulation and baroreflex, respectively), that could hence be investigated separately.

vi) Vn_{LF} as a complementary descriptor of respiration

The psychophysiological tasks elicited considerable changes in respiration, which affect not only respiratory frequency, but also the pattern of air-flow. During *Rest* V is approximately sinusoidal and stationary, and then V_T and respiratory frequency (or RP) provide adequate descriptors. However, for the broadband respiratory patterns related to speech, these parameters are not only inadequate, but might also be misleading, since two quite different respiratory patterns may present the same value for RP and V_T. For example *Read* and *Rest* show no significant differences in RP and V_T (Figure 4.13), but radical differences are apparent when visually observing the patterns of V (Figure 4.6), and are quantified by I/E and Vn_{LF} (Figure 4.13). Furthermore, the results of the correlation tests between VI and respiratory parameters clearly show the importance of the additional information provided by Vn_{LF} (see Table 4.1). In fact, for the tasks involving speech, significant correlations for $SAPV_{LF}$ or HRV_{LF} were found with Vn_{LF}, but not with RP or V_T. This suggests that in the study of cardiovascular-respiratory interactions, Vn_{LF} and not only RP (or V_T) should be considered. Vn_{LF} has the advantage over possible alternative means of assessing broad-band respiratory characteristics, in being relatively simple to compute, and derived from uncalibrated signals of respiration (e.g. uncalibrated outputs of plethysmographic belts or thoracic impedance may be adequate).

For the purposes of accounting for the confounding effect of respiration on VI, it has been recommended that RP and V_T should be included as covariates in statistical analysis [17]. The current results suggest that this should be complemented with Vn_{LF} in the case of erratic respiration, as occurs during speech. It should also be pointed out that even in those subjects who did not have their RP in the LF range during *Rest*, there can be a significant LF component in the spectrum of V that can contribute to cardiovascular oscillation in the LF range. This may well be reason why significant correlation between Vn_{LF} and $SAPV_{LF}$ persists for *Rest*, even after removing the subjects with long RP from the sample (Table 4.1). This reinforces the need for Vn_{LF} and not just RP and V_T to be considered in the analysis of respiration, including during the *Rest* phase.

Estimation of RP and V_T during speech

When erratic respiratory patterns are present, as occurs during speech (see Figure 4.6), the estimation of RP (or respiratory frequency) is far from trivial, and

results can be questionable. When the V signal is approximately sinusoidal and approximately stationary (e.g. during baseline rest or during *Maths silent*, see Figure 4.6), estimating RP is relatively simple. It is possible to apply a time domain method (as chosen in the current work), to detect the troughs in the V signal, from which RP can be estimated as the interval between two consecutive troughs. Alternatively, frequency domain methods can be employed [11, 104]: respiratory frequency can then be identified as the one corresponding to the biggest peak in the spectrum of V.

However, applying these two methods to erratic respiratory patterns, such as those associated with speech, is more problematic. For the time domain method, it is it relatively common to find inspiration/expiration cycles that are much smaller in amplitude (V_T) and length (RP) than the remainder, as exemplified in Figure 4.6 for the *Talk* task (indicated by circles). It is then questionable if these should be considered as respiratory cycles. In the current work, cycles with V_T <100ml or RP<0.1s were disregarded, as described in Section 4.2. Evidently, this arbitrary choice for the thresholds could have considerable impact on the estimated average of RP and V_T; however, not selecting cycles biases the estimates to lower values. If a frequency domain method is applied, the broadband nature of respiration during speech (Figure 4.7) makes the estimate of RP from spectral peaks unreliable. In fact, several peaks of comparable size commonly occur (see Figure 4.7), and selecting the biggest as previously employed [11, 104], would be inappropriate in our data involving speech.

Clearly, for tasks involving speech, different methods to estimate RP and V_T could lead to quite different results. The question arises how to operationally define RP (or respiratory frequency) and V_T during speech, or indeed if these indexes are appropriate in the study of VI during erratic respiratory patterns.

Limitations of the study

The face mask setup allowed accurate monitoring of respiratory activity throughout the tasks. It might be argued that the use of a face mask influenced the respiratory patterns, causing long RP and high V_T at rest in some subjects, and interfering with the speech tasks. However, precautions were taken to minimize air-flow resistance and dead space (see Section 4.2), and significant hypo- or hypercapnia were never present (P_{CO2} was always near to 40mmHg). Furthermore a considerable number of subjects with long RP were also found when other methods to monitor respiration, such as belt plethysmography, were ap-

plied [28, 55, 93]. It has also been noted that the presence of a face mask need not modify the respiratory patterns during speech [59].

No direct assessment of autonomic function was carried out in this work (nor is it in most other related works). Without alternative means of reliably evaluating autonomic function, it is not possible to disentangle respiratory effects on VI from those due to changes in sympathetic and parasympathetic activation. However, alternative methods (such as muscle sympathetic response) may not be suitable for the investigation of mild psychological stressors considered here, due to the invasiveness of the procedure.

4.3.5 Conclusions

The conventional use of HRV and $SAPV$ indexes for the assessment of changes in the activation of the two autonomic branches is usually based on the assumption that respiratory modulation of cardiovascular oscillations is confined to the HF band. This is clearly not the case during psychophysiological tasks involving speech, whose erratic respiratory patterns lead to broadband spectra in respiratory volume. The results presented in this work demonstrate clearly the impact this has on the variability of heart-rate and systolic arterial pressure. Thus, for tasks involving speech, inter-individual differences and rest-task changes in HRV_{LF} and $SAPV_{LF}$ can, at least in part, be explained from differences in respiration. Furthermore, a task involving speech can produce an increase in HRV_{LF} and $SAPV_{LF}$ indexes, but a decrease when performed silently - as observed for an arithmetic task performed aloud and silently. Thus the assessment of autonomic changes from changes in HRV and $SAPV$ indexes during these tasks becomes questionable. Hence, in assessing autonomic activation through HRV and $SAPV$ indexes, tasks involving speech (or erratic respiratory patterns in general) should probably be avoided. Respiration should also be monitored for protocols not involving speech, in order to estimate variations in respiratory period and end-tidal volume (which could have a confounding effect on the results) and to remove from the estimation of rest base-line values the subjects with respiration within the LF band (which can potentially bias the results). To this end we recommend that the spectrum of V (and in particular Vn_{LF}, the relative power of the V signal in the LF band) should be calculated to provide physiologically relevant insight into the low-frequency respiratory oscillations commonly encountered during tasks and rest.

4.4 Modeling the influence of respiration on HRV and $SAPV$

4.4.1 Introduction

The results presented in Section 4.3 clearly show how different respiratory patterns are reflected in HRV and $SAPV$ indexes. However, only inter-individual and between-tasks differences in HRV and $SAPV$ indexes and their correlation with respiratory parameters were considered, using average parameters calculated over the duration of each task.

In this section the cardiovascular-respiratory interactions are investigated through mathematical modeling, with the overall objective of assessing to what extent a strictly causal linear model of the respiratory modulation of cardiovascular oscillations applied in individual recordings can explain the results found in Section 4.3. The approach used was the following. The respiratory volume (V) and HP were taken respectively as the input and the output of a parametric control system, and the parameters were identified using a least-squares method. The identified models can then be used to assess if significant differences in the control system exist between different tasks or subjects, and to verify if applying the same model to different experimental conditions allows the prediction of the difference between the cardiovascular indexes associated with these inputs. The same approach was applied to SAP also.

The use of modeling approaches to investigate cardiovascular-respiratory interaction is not new. In several works the $V \rightarrow HP$ and $V \rightarrow SAP$ transfer functions were estimated [9, 10, 102], while others presented more sophisticated models, including the reciprocal interactions between HP and SAP [95], and nonlinear modulations [31]. However, as far as the author is aware, in these studies there is no quantitative assessment of how much of the between-tasks and inter-individual variability in cardiovascular oscillations can be predicted as a direct effect of respiration, in particular for the case of the respiratory patterns involving speech.

For the work presented in this section, the model adopted is a simple linear causal FIR filter relating V to HP and SAP. This approach is similar to that previously adopted for the correction of respiratory influence on cardiovascular oscillations in modeling the baroreflex mechanism [95]. Since no indication was found in the literature about the need of complex models for the purposes of this

work, investigating a linear model was the first choice.

The specific objectives of the work presented in this section are:

- to compare the models identified for different tasks, and to test if the $V \rightarrow HP$ and $V \rightarrow SAP$ control systems change significantly between tasks.
- to estimate how much of inter-individual and between-tasks differences in HRV and $SAPV$ indexes can be explained solely as a direct effect of respiration, by imposing the same model on all the subjects for each task, and on all the tasks for each subject, respectively.

In Section 4.4.2 the modeling methods adopted and the data analysis are described. The results of the analysis are reported in Section 4.4.3 and discussed in Section 4.4.4.

4.4.2 Model identification and statistical analysis

Model identification

For each subject, for each task the $V \rightarrow HP$ and $V \rightarrow SAP$ interaction was modeled with an FIR filter using a least-squares approach (see also Appendix A.3). In the specific case of this work, the output signal used for the identification was either HP or SAP, the input was V (resampled after anti-alias filtering at 4Hz, to be synchronized with the output). The length of the epoch used for estimation was 1200 samples (5 min of each task sampled at 4Hz). Prior to model identification, the signals were high-pass filtered with cutoff frequency of 0.03Hz, based on a third order butterworth filter (described in Appendix A.2) applied in the forward and reverse direction for a zero-phase response. This removed the very low frequencies (and especially the DC component), which were not of interest for this study, given that the lowest frequency considered for HRV and $SAPV$ indexes estimation is 0.05Hz.

The chosen length (order) of the FIR filter to be identified was 40: this choice was based on the assumption that considerable delays between input and outputs are likely to be present, but that these probably do not exceed the length of a complete respiratory cycle. Hence the filter size was chosen in order to obtain an impulse response of comparable length to the longest respiratory cycles found in the data ($\sim 10s = 40$ samples). In preliminary analysis, the identified impulse responses were in agreement with this assumption, converging to values near to zero well within the 40 samples (see Figure 4.16)

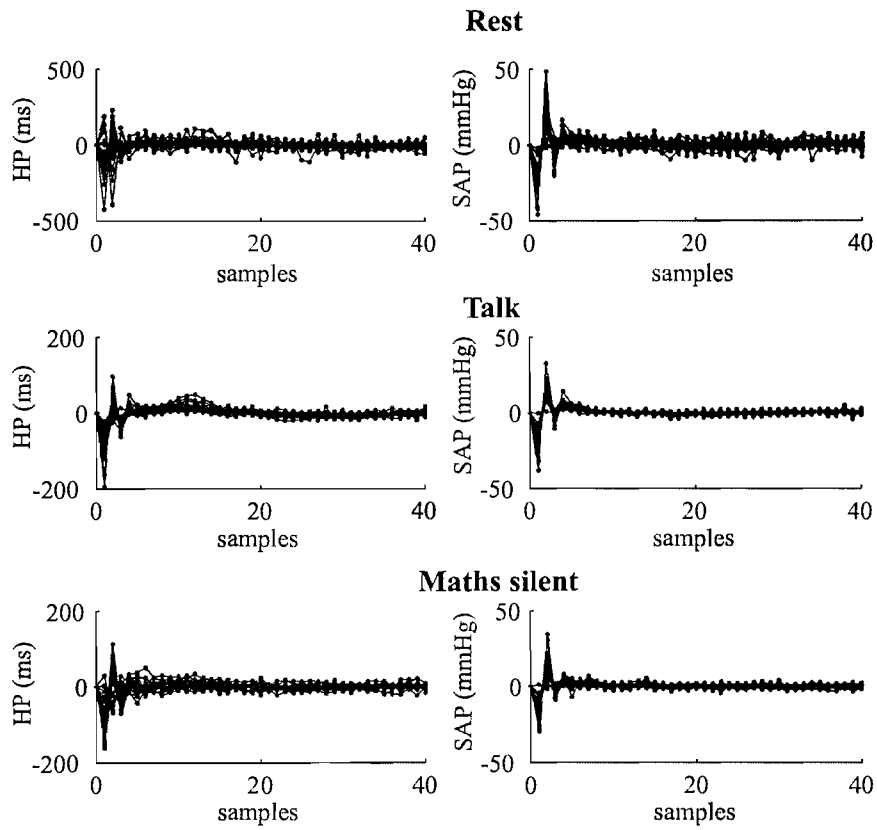


Figure 4.16: Examples of the impulse response of the identified models of the $V \rightarrow HP$ and $V \rightarrow SAP$ interaction for *Rest*, *Talk*, and *Maths silent*. The responses of all 25 subjects are plotted superimposed.

After the system identification, predicted HP and SAP signals were computed by filtering V with the identified FIR filters, HRV and $SAPV$ indexes were then computed from these estimated signals using the methods described in Section 4.2.

Finally, the frequency response (gain and the phase) of each identified FIR filter were computed from the impulse responses, using a frequency resolution of 0.005Hz (corresponding to 90 points in the interval 0.05-0.5Hz, which is the band of interest in this study). For this phase of the work, a model with heart-rate (HR) instead of HP as output was considered, in order to allow direct comparison with previous publications [101, 102]. In addition, the squared coherence between V and either HR and SAP was also estimated for each task, using an FFT approach based on the Welch's modified periodogram method [120], using multiple segments of 256 samples (corresponding to 64s), an overlap between

adjacent segments of 128 samples, and a Hanning window, providing a spectral resolution of 0.0156 Hz.

The standard deviation of gain at each frequency for each model was obtained through a Monte Carlo simulations approach, outlined as follows (details given in Appendix A.3). First, the FIR filter parameters were estimated and the covariance matrix of their estimation error calculated. Then, samples of a multivariate normal distribution are generated in accordance with the parameters. One thousand such sets of samples (realizations) are generated and for each the gain of the frequency response was computed. The result is a sample of 1000 values for the gain at any given frequency, from which the variance can then be computed. This provides a measure of the reliability of the estimated gain at such a frequency. The same approach can then be applied for the estimated phase. A detailed and formal description of the method is presented in Appendix A.3.

This approach, as far as the author is aware, has never been adopted before in this context. It allows for within- and between-subjects comparisons (including hypothesis testing) of the characteristics of the models.

Statistical analysis

In order to assess to what extent the respiratory volume V can predict HP and SAP, and in particular if any prediction is statistically significant (i.e. significantly more than what might be predicted from a random signal) a Monte Carlo method was again employed. Thus, the predicted values obtained using V as the input of the models were compared with those obtained using pseudo-random input signals with similar spectral characteristics. For each subject, 1000 pseudo-random inputs were generated using an autoregressive model of high order (30), whose parameters were identified from the subject's V signal, hence having the same power spectral density as V but random variations in the phase. Then for each task and for each of these pseudo-random input signals, a model was identified as described in the previous section, and the predicted output computed, together with its power (or variance, since the signals have zero mean). Thus, for each subject in each task, 1000 estimates of HP and SAP signals were obtained from the pseudo-random inputs. The normalized mean square error R of these prediction (ratio between mean square error and the power computed from the recorded HP and SAP signals) was compared with the normalized mean square error r_m of the prediction obtained using the model with V as input. The prediction using V was considered significantly better than that using random inputs,

when r_m was smaller than the 5^{th} percentile of R. It is worth noting that this test is equivalent to comparing the power of the predicted signals obtained using V as the input to the models with those obtained using a pseudo-random input. This arises from one of the properties of the linear least-squares model identification adopted here, for which the predicted output and the residuals are uncorrelated.

Differences between tasks in the models identified were investigated through comparisons of the average transfer functions, performed in the following way (a detailed description is provided in Appendix A.3). Considering two tasks A and B and for the $V \rightarrow HR$ transfer function identified for each subject i, lets indicate the estimated gains at a given frequency f as $gA_i(f)$ and $gB_i(f)$, and their variance estimated through Monte Carlo simulations as $sA_i(f)^2$ and $sA_i(f)^2$, respectively. The average paired difference d is then a distribution with mean and standard deviation:

$$\begin{aligned} md(f) &= \frac{1}{K}\sum_{i=1}^{K}\left(gA_i(f) - gB_i(f)\right) \\ sd(f) &= \frac{1}{K}\sqrt{\sum_{i=1}^{K}\left(sA_i(f)^2 + sB_i(f)^2\right)} \end{aligned}$$

where K is the size of the cohort. Under the assumption that d has a normal distribution, the probability of the difference in gain between A and B being zero at a given frequency f is $p = F(0; |md(f)|, sd(f))$, where $F(x; \mu, \sigma)$ is the cumulative density function of the normal distribution with mean μ and standard deviation σ. The hypothesis of a zero difference was rejected if $p < 0.05$. For comparison, the same hypothesis was also tested through a paired t-test.

The percentage of frequencies in the $0.05 - 0.5Hz$ band for which a significant difference in gain was found, was taken as an index of the disagreement between A and B in the identified average transfer functions within that band, and will be denominated *disagreement index* (DI) in the remainder of the analysis. DIs were also computed restricting the comparison to the LF and HF bands.

This statistical testing and the DI estimation were performed for every pairing of tasks in the protocol, and also for the phase of the $V \rightarrow HR$ frequency response. An identical analysis was applied to the $V \rightarrow SAP$ frequency response function also. Furthermore, the DIs between different tasks were computed for each single subject as well, using the same approach (thanks to the estimate of the variance of phase and gain obtained for each subject through Monte Carlo simulations).

In an attempt to quantify how much of the difference in HRV and $SAPV$ indexes between tasks can be explained without assuming any change in the con-

trol system, for each subject the model identified for one task (*Talk*, the rationale of this choice will be discussed further on) was applied to all the other tasks, and the predicted HRV_{LF}, HRV_{HF}, and $SAPV_{LF}$ were computed. Then, the correlation coefficient between each of these "predicted" cardiovascular indexes and the corresponding "true" index (computed either from the HP or the SAP signal) was estimated for each subject across the five tasks, resulting for each cardiovascular index, in 25 (one for each subject) correlation coefficients. These estimates, and the associated p-values, provide an indication of the extent to which between-tasks differences can be explained from variation in respiration (given that for each subject the model adopted was the same for all tasks).

A similar approach was adopted in order to investigate how much of the inter-individual differences in cardiovascular indexes can be explained from respiration. Given a task of the protocol, the same model of $V \rightarrow HR$ modulation was applied to all subjects, and then the correlation, across the cohort, between the predicted and the "true" values of HRV_{LF} for such a task was estimated. Since no objective reason exists to select any specific individual's model to be applied to the whole cohort, the process was repeated for all the 25 subjects, resulting in 25 correlation tests across the cohort (after log-transformation to compensate for the skewness of the distributions). This analysis was performed for each task of the protocol, and extended also to the HRV_{HF} and $SAPV_{LF}$ indexes.

Correlations were tested using the Pearson method, and results were considered significant for $p < 0.05$.

4.4.3 Results

Prediction of HRV and $SAPV$ indexes through modeling

Figure 4.17 and 4.18 show examples of the HP and SAP predicted by the model, as well as recorded signals, together with the corresponding power spectra. The effectiveness of the model in reproducing the main features of the patterns is evident. The example shown was chosen among the group with long respiratory period at rest identified in Section 4.3: it is evident how for this subject the power spectra of SAP ad HP during *Rest* are entirely limited to the LF band, and almost entirely predicted by respiration.

Table 4.3 reports the mean square error of the HP and SAP signal predicted by the models, expressed as percentage of the total power of the HP and SAP respectively. Furthermore, the Monte Carlo simulations showed that this per-

centage is, for each subject during each task, significantly smaller ($p<0.01$) than that predicted by models using pseudo-random signals as input.

Table 4.3: Mean square error of the prediction of the identified models (expressed as percentage of the total power of the signals)

	MSE of prediction of HP		MSE of prediction of SAP	
	mean(%)	range(%)	mean(%)	range(%)
Rest	34	7-74	37	13-63
Read	40	23-63	50	31-72
Talk	38	21-57	40	35-60
Maths silent	62	34-82	55	30-82
Maths aloud	55	35-80	57	27-83

Analysis of the models' transfer functions

From the identified models the frequency responses of the $V \rightarrow HR$ and $V \rightarrow SAP$ transfer functions were computed, of which Figures 4.19 and 4.20 show the average values and their confidence intervals ($\pm$ twice the standard deviation of the average values, calculated from Monte Carlo simulations) for the gain and the phase, together with the average input/output squared coherence.

For the $V \rightarrow HR$ transfer function, the gain shows a maximum within the LF band (approximately at 0.1-0.15Hz), and decreases almost linearly up to 0.4Hz, for all tasks except for *Rest*. The phase also is similar for all tasks, with positive values for frequencies below approximately 0.15Hz, and negative values above, reaching $-\pi/2$ around 0.4Hz. The coherence is high (> 0.5) in the band 0.1-0.35Hz, excluding for *Maths silent* for which the band is narrower (approximately 0.20-0.35Hz).

For the $V \rightarrow SAP$ transfer function, the gain in the $0.15 - 0.5$Hz band is relatively constant for all tasks, with *Maths silent* showing the lowest magnitude, and *Rest* the highest average values. For frequencies below 0.15Hz, all the tasks but *Rest* show a progressive decrease in gain. The phase is similar for all tasks, approximately zero at 0.05Hz and progressively decreasing at higher frequencies, reaching $-\pi$ above 0.35Hz. Low coherence was found only for the lower frequencies (< 0.1Hz approximately) except for *Maths silent*, for which the range of high coherence (>0.5) is limited to the $0.15 - 0.35$Hz band.

It is worth noting that the tasks involving speech presented smaller confidence intervals than tasks performed silently and especially *Rest*. These results are not

unexpected, since in the tasks involving speech a markedly broadband respiration is present (see Section 4.3), and for the reliable identification of the frequency response of a linear system, the presence of power in broad range on frequencies in the input signal is a prerequisite.

In order to quantify the agreement between the average frequency responses for different tasks, the *disagreement indexes* (DI) were computed. DIs for the gain are reported in Table 4.4 (values computed using t-tests are similar, even if slightly smaller, not reported). For the $V \rightarrow HR$ transfer function, the gain for *Read* is very similar to *Talk* (DI= 7% in the 0.05-0.5Hz band). This similarity extents to *Maths aloud*, but limited to the HF band (DI< 30%, while for the LF band DI> 95%). Compared to the three tasks involving speech, *Maths silent* seems to agree with *Maths aloud* (DI= 20% in the 0.05-0.5Hz band), but is different to the other two, especially in the LF band (DI> 80%). *Rest* differs considerably from all the other tasks (DI> 65% in the 0.05-0.5Hz band), except for *Read* and *Talk* in the LF band (DI< 20%). Regarding the phase, only *Rest* differs from the other tasks, in the HF band (DI> 50%, detailed results not shown).

Also for the gain of the $V \rightarrow SAP$ transfer function *Read* and *Talk* show little discrepancy (DI= 15% in the 0.05-0.5Hz band). These two tasks are considerably different from the *Maths* tasks (DI> 60% in the 0.05-0.5Hz band). Also *Rest* differs form the *Maths* tasks (DI> 60% in the 0.05-0.5Hz band), while it shows some similarity with *Read* and *Talk* in the HF band (DI< 20%). Regarding the phase, again only *Rest* differs from the other tasks, excluding *Maths silent*, limited to the LF band (DI> 60%).

It is also worth noting that a low value for the DI can be interpreted as a suggestion but not as a conclusive indication of a negligible difference between two tasks, since it can be originated as well from large dispersion in the data.

The agreement between the transfer functions estimated for each task was investigated also for each single subject, in order to assess to which extent the average results found originate from coherent effects in the whole cohort or from high inter-individual variability. The results, shown in Table 4.5, indicate that, on average, the disagreement is limited for the gain (DI< 50%), and relatively higher for the phase. However, the considerably wide range of the values obtained for DI (in many cases even a 0-100%), suggests that great inter-individual variability exists in the between-tasks changes in the respiratory modulation of cardiovascular oscillations.

Use of the same model for all tasks

For each subject, one model was applied to all tasks and correlation of the resulting predicted values for HRV_{LF}, HRV_{HF}, and $SAPV_{LF}$ the corresponding "true" values was calculated. An example for a representative subject is shown in Figure 4.21. The model identified for *Talk* was adopted for this purpose. This choice was based on the result, shown above, that tasks involving speech result in more reliable estimates of the model (i.e. limited confidence interval), and on preliminary comparisons on some of the subjects with the results obtained applying the model identified for *Read* or *Maths aloud*, which suggested limited differences in the predicted indexes. The results obtained are summarized in Figure 4.22. For a considerable number of subjects (11 for HRV_{LF}, 7 for HRV_{HF}, and 13 for $SAPV_{LF}$) a considerably high correlation coefficient was found (r>0.88), associated with low p-value, (p<0.05, expected since the test was based on five points only). Hence, a considerable percentage of the between-tasks differences in cardiovascular indexes could be predicted by the model: on average over the whole cohort, 62% for HRV_{LF}, 48% for $SAPV_{LF}$, and 27% HRV_{HF} (i.e. values of r^2 of 0.62, 0.48, and 0.27, respectively), with values up to 99% for some subjects.

Use of the same model for all subjects for each task

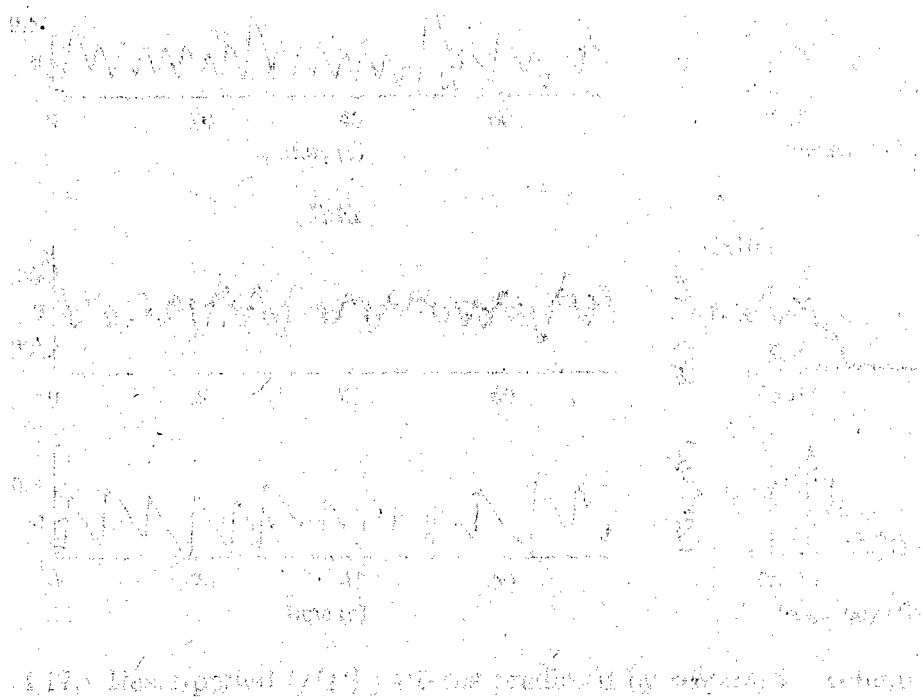
For each task, the model identified for one subject was applied to all the cohort, from which HRV and $SAPV$ indexes were predicted. The correlation between these indexes and those computed from the measured HP and SAP was then computed (after log-transformation, to compensate for the skewness of the distributions), in order to quantify how much of the inter-individual variability in the indexes can be explained by respiration. An example is shown in Figure 4.23. Given the lack of an objective criterion to select a specific model for the purpose, the process was repeated 25 (size of the cohort) times, applying each time the model identified for a different subject as the common model. The results are summarized in Figure 4.24.

For $SAPV_{LF}$, statistically significant correlations ($p< 0.05$) were found, independently of the model adopted, for all tasks, except *Maths silent*, for which the results were however almost significant in all cases ($p< 0.1$). For *Rest*, the results were significant in all cases, and remained so for almost all even when the subjects with long respiratory period were removed from the analysis, i.e. when almost all the power of V was limited to the HF band.

For HRV_{LF}, the correlations were significant for *Talk* and *Rest*, and almost significant ($p< 0.08$) for *Read* and *Maths aloud*, again for all models adopted. However, for *Rest*, when the subjects with long respiratory period were removed, significance was lost (details not shown).

For HRV_{HF} no significant correlation was found (results not shown).

Hence, applying the same model of respiratory modulation of cardiovascular oscillations to all subjects (i.e. assuming no difference in the modulation mechanisms between the subjects) explains a considerable (and statistically significant) part of the inter-individual differences in HRV_{LF} and especially in SAP_{LF} from respiration.



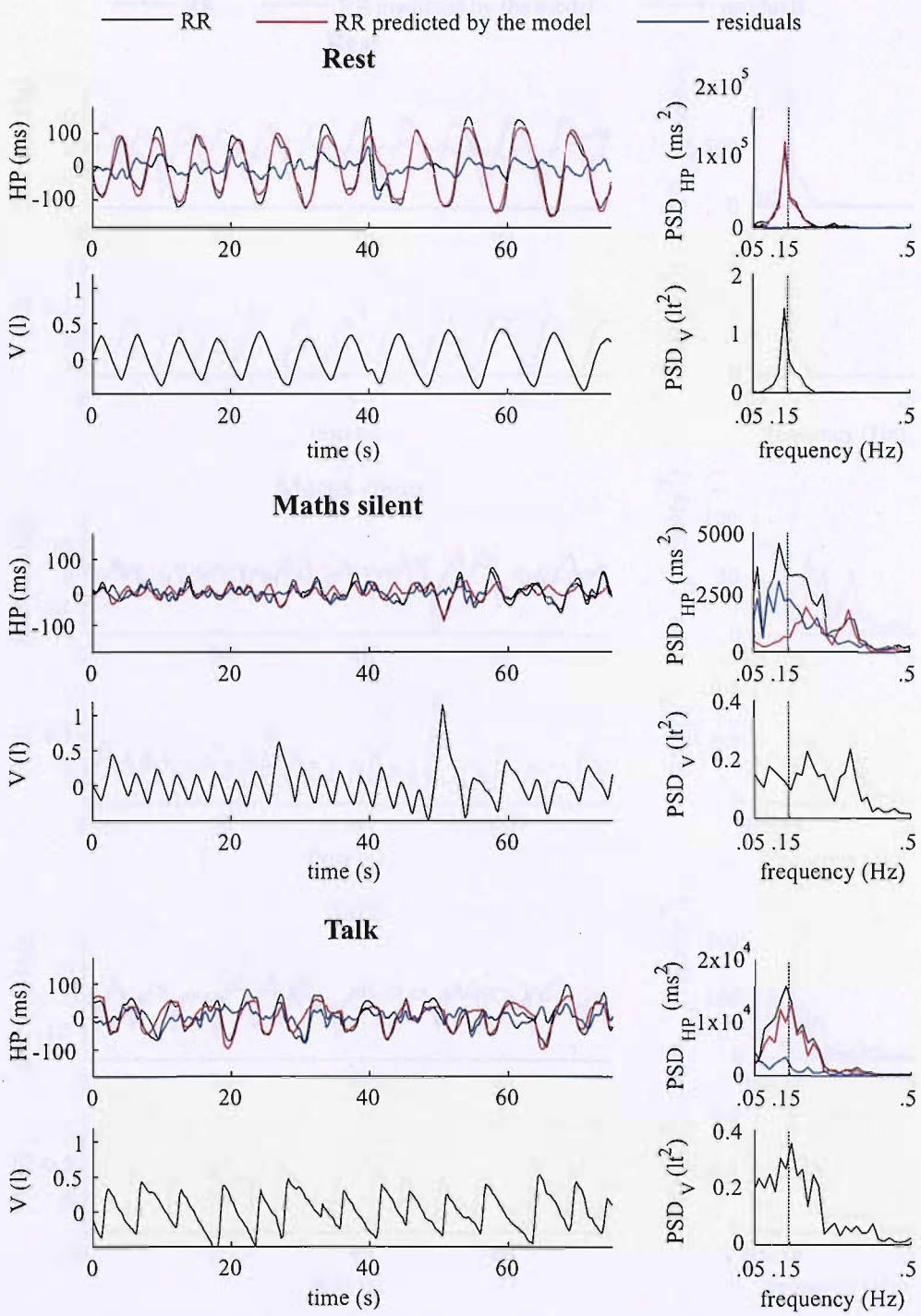


Figure 4.17: Heart-period (HP) patterns predicted by respiratory volume (V), through the model described in Section 4.4.2, for a representative subject during *Rest*, *Maths silent*, and *Talk*. True HP in black, predicted values in red, residuals of the prediction in blue. The corresponding spectra are shown on the right side.

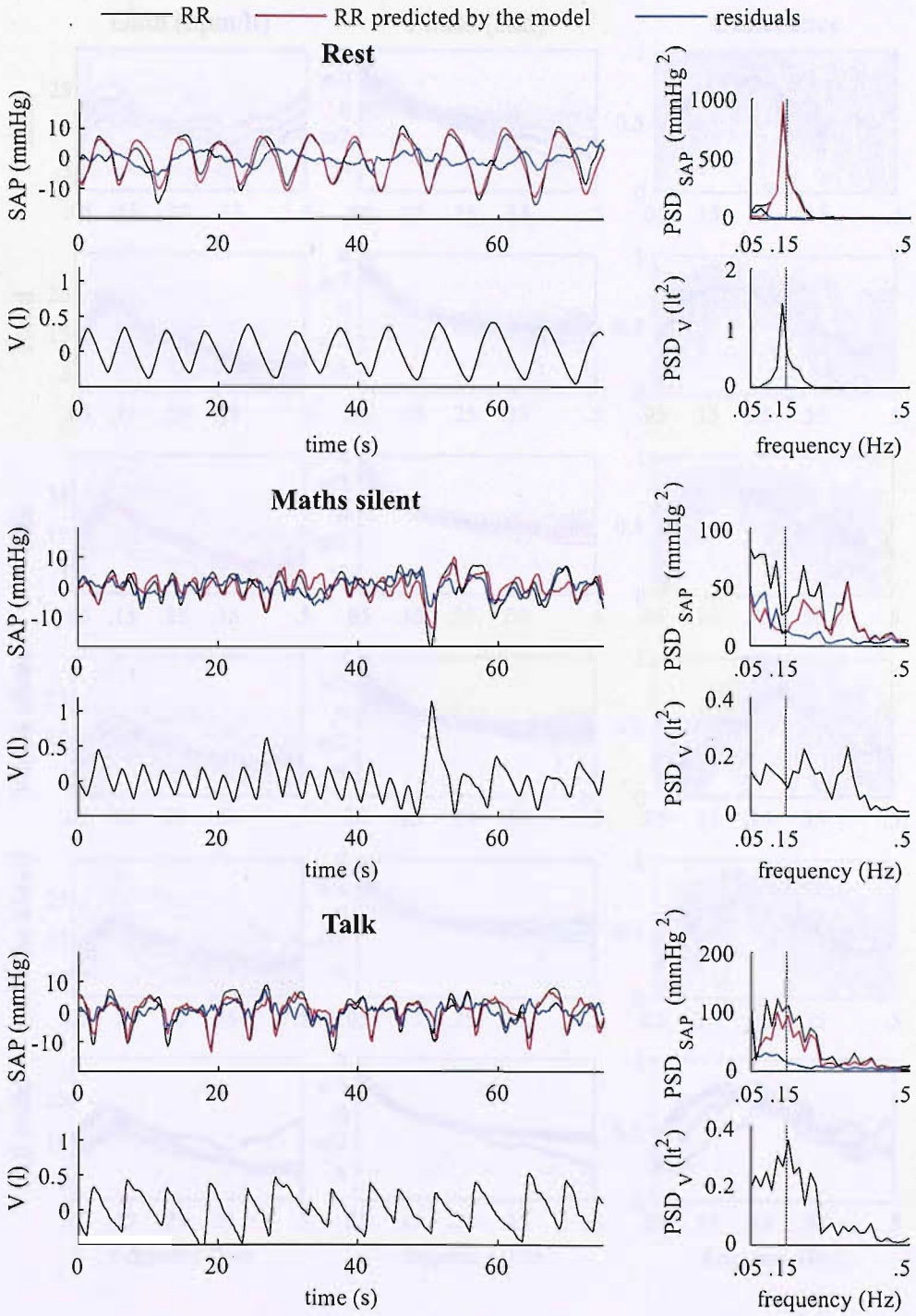


Figure 4.18: Systolic arterial pressure (SAP) patterns predicted by respiratory volume (V), through the model described in Section 4.4.2, for a representative subject (same as Figure 4.17) during *Rest*, *Maths silent*, and *Talk*. True SAP in black, predicted values in red, residuals of the prediction in blue. The corresponding spectra are shown on the right side.

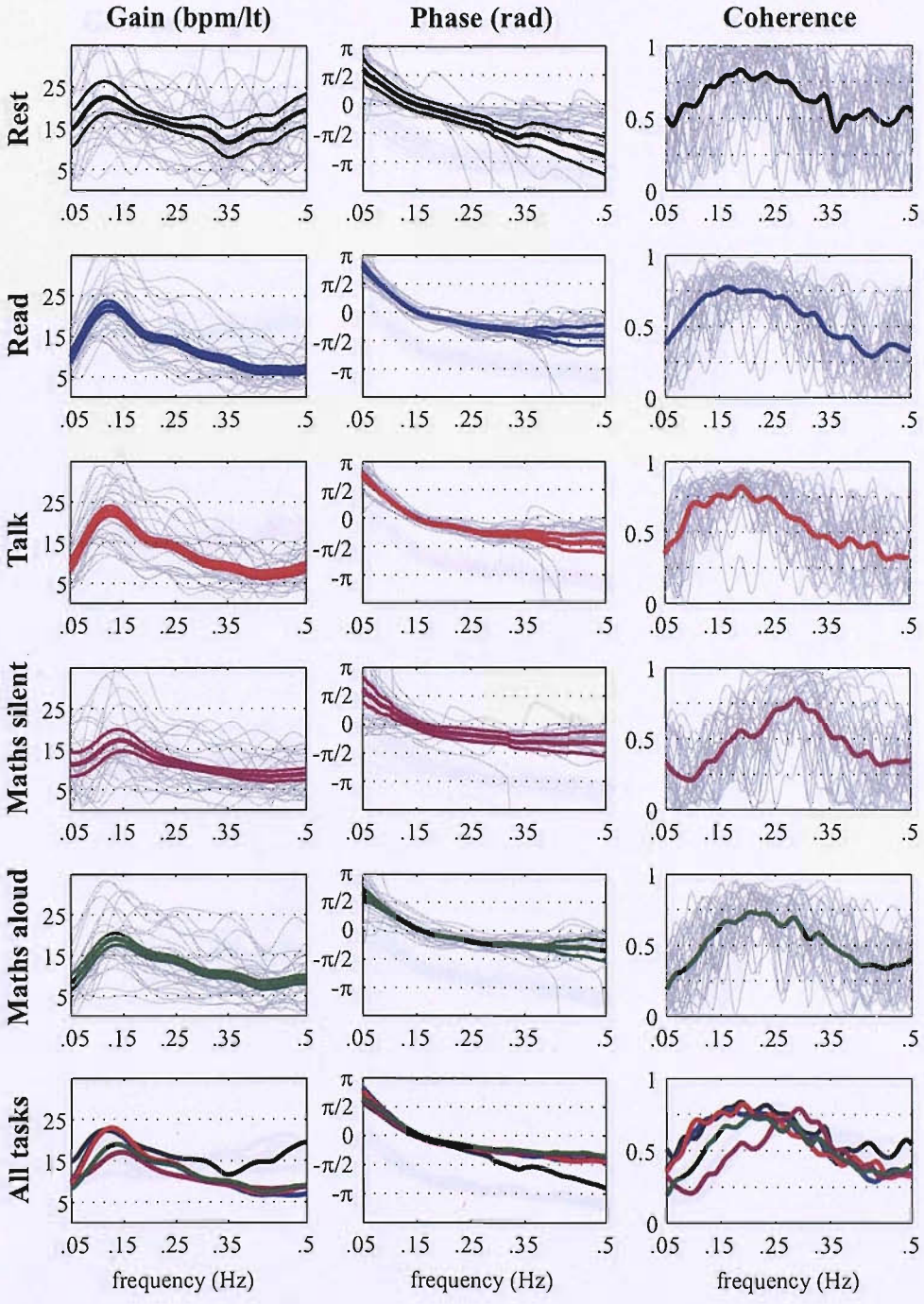


Figure 4.19: Average magnitude (±2∗its standard deviation, computed by Monte Carlo methods) for gain and phase of the $V \rightarrow HR$ transfer function for the whole cohort of subjects for each task. Average squared coherence between V and HR is shown also. In the background (in gray) gain, phase and squared coherence estimated for the single subjects are shown. In the bottom row, the average frequency responses for all tasks are plotted together to allow for comparison.

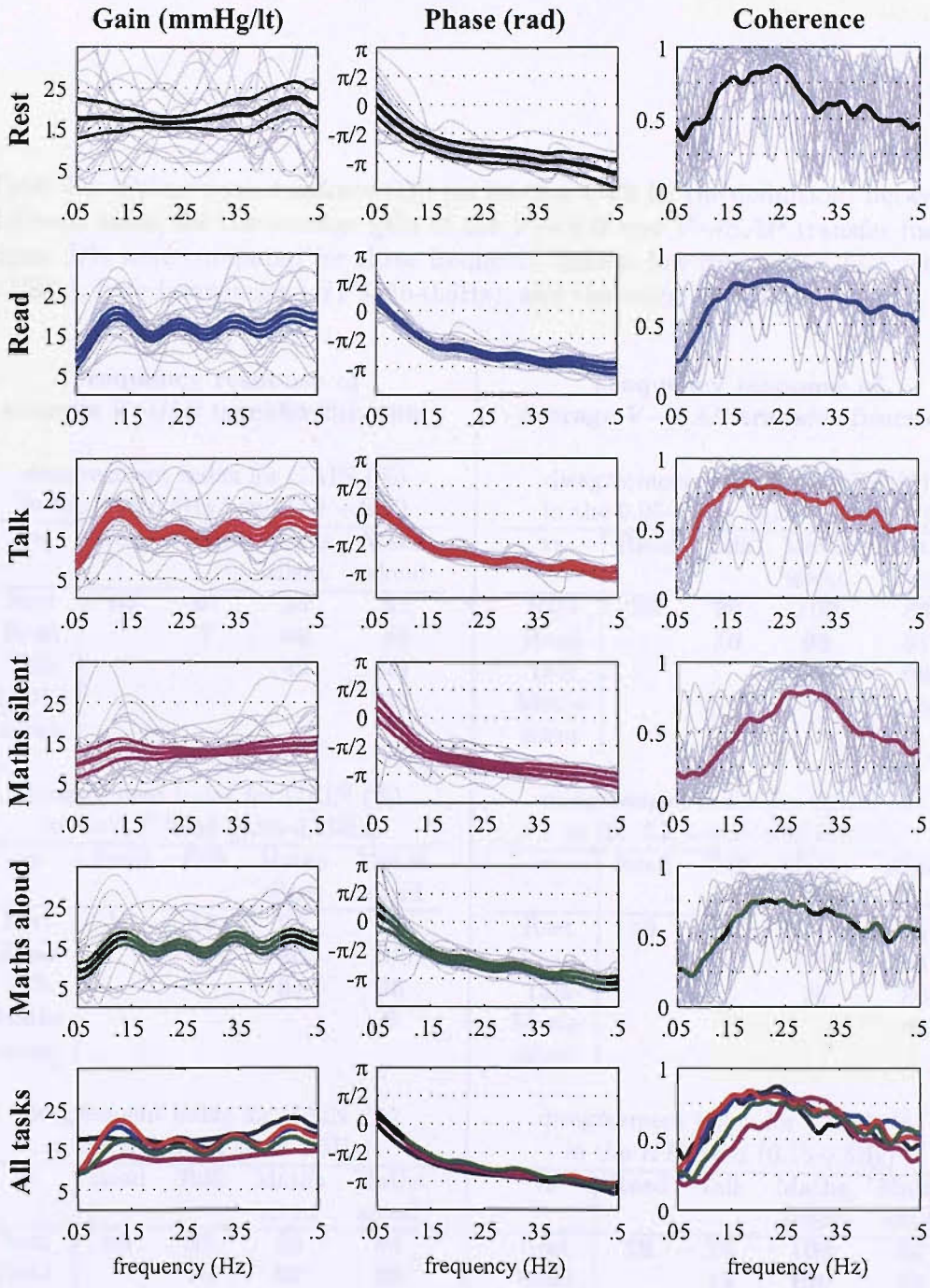


Figure 4.20: Average magnitude (±2∗its standard deviation, computed by Monte Carlo methods) for gain and phase of the $V \rightarrow SAP$ transfer function for the whole cohort of subjects. Average squared coherence between V and SAP is shown also. In the background (in gray) gain, phase and squared coherence estimated for the single subjects are shown. In the bottom row, the average frequency responses for all tasks are plotted together to allow for comparison.

Table 4.4: *Disagreement indexes* (DI, see Section 4.4.2 for the definition) between different tasks, for the average gain of the $V \rightarrow HR$ and $V \rightarrow SAP$ transfer functions. DIs were computed for three frequency bands: low frequencies (LF:0.05-0.15Hz), high frequencies (HF:0.15-0.5Hz), and the union of the two.

Frequency response of average $V \rightarrow HR$ transfer function

disagreement index for GAIN (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**68**	**67**	**94**	**83**
Read		**7**	**66**	**45**
Talk			**49**	**29**
Maths silent				**20**

disagreement index for GAIN (%) in the LF band (0.05-0.15Hz)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**10**	**19**	**95**	**81**
Read		**0**	**86**	**100**
Talk			**81**	**95**
Maths silent				**0**

disagreement index for GAIN (%) in the HF band (0.15-0.5Hz)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**85**	**81**	**93**	**84**
Read		**10**	**60**	**29**
Talk			**40**	**10**
Maths silent				**26**

Frequency response of average $V \rightarrow SAP$ transfer function

disagreement index for GAIN (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**23**	**29**	**100**	**63**
Read		**15**	**95**	**61**
Talk			**95**	**69**
Maths silent				**79**

disagreement index for GAIN (%) in the LF band (0.05-0.15Hz)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**48**	**67**	**100**	**52**
Read		**24**	**76**	**81**
Talk			**76**	**86**
Maths silent				**48**

disagreement index for GAIN (%) in the HF band (0.15-0.5Hz)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**16**	**18**	**100**	**66**
Read		**12**	**100**	**55**
Talk			**100**	**64**
Maths silent				**88**

Table 4.5: Average value (range) of *Disagreement indexes* (DI, see Section 4.4.2 for the definition) between different tasks computed for each subject for the gain and phase of the $V \rightarrow HR$ and $V \rightarrow SAP$ transfer functions, for the 0.05-0.5Hz band ($LF+HF$).

Frequency response of $V \rightarrow HR$ transfer function

disagreement index for GAIN (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**35** (11-76)	**35** (11-79)	**40** (16-77)	**36** (0-82)
Read		**24** (5-55)	**37** (13-72)	**34** (11-71)
Talk			**38** (8-79)	**36** (5-69)
Maths silent				**32** (0-61)

disagreement index for PHASE (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**72** (31-100)	**70** (24-100)	**58** (20-100)	**72** (19-100)
Read		**57** (4-100)	**67** (23-100)	**66** (24-100)
Talk			**69** (31-100)	**57** (16-100)
Maths silent				**66** (17-100)

Frequency response of $V \rightarrow SAP$ transfer function

disagreement index for GAIN (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**34** (3-63)	**33** (2-83)	**35** (7-75)	**38** (9-76)
Read		**34** (4-92)	**48** (18-97)	**43** (8-89)
Talk			**50** (21-98)	**38** (7-75)
Maths silent				**45** (10-96)

disagreement index for PHASE (%) in the 0.05-0.5Hz band (LF+HF)

vs	Read	Talk	Maths silent	Maths aloud
Rest	**34** (0-100)	**26** (0-100)	**41** (0-100)	**38** (0-100)
Read		**45** (4-93)	**68** (20-100)	**54** (7-100)
Talk			**65** (0-100)	**52** (0-100)
Maths silent				**60** (3-100)

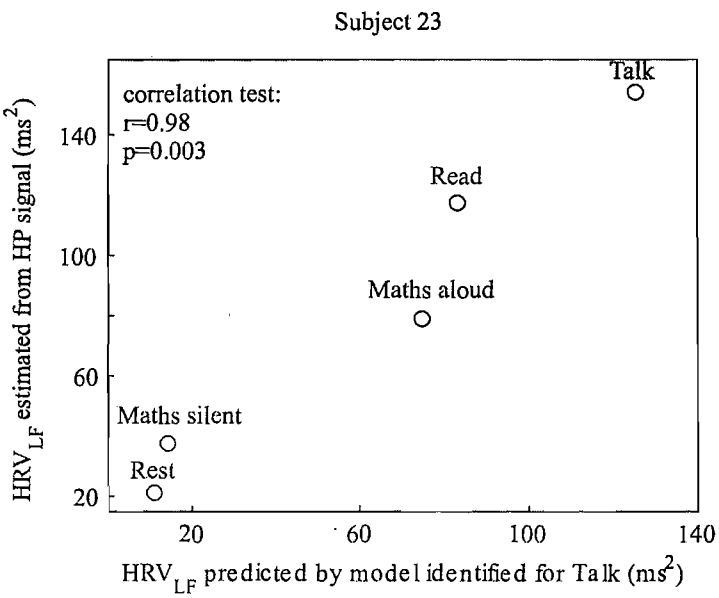


Figure 4.21: Example of within-subject correlation across the tasks between the "true" value of cardiovascular indexes and the corresponding value predicted using the same model for all tasks (the model adopted is the one identified for *Talk*) and V as input.

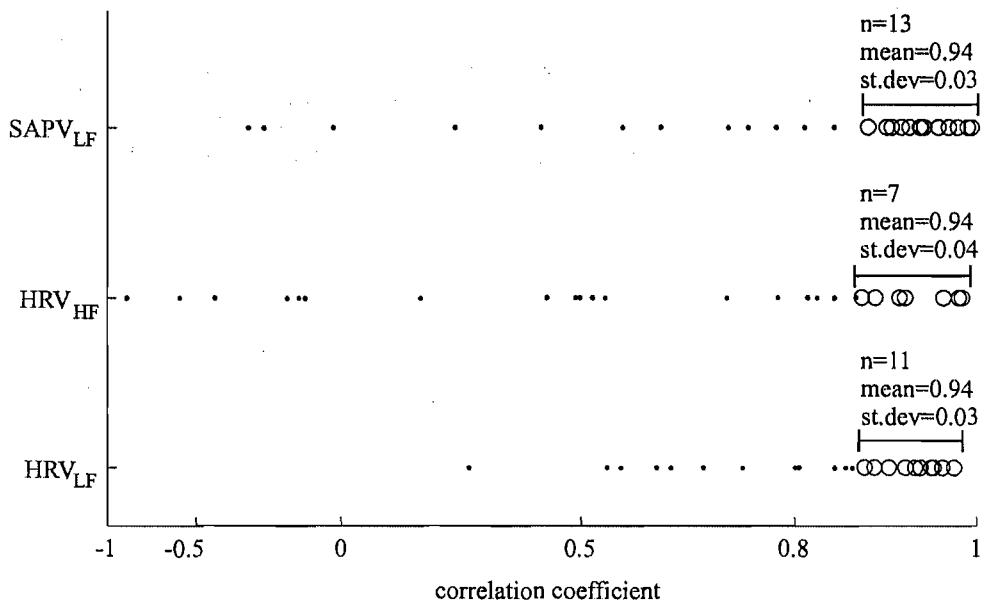


Figure 4.22: Within-subject correlation coefficients across the tasks of the protocol between the "true" value of cardiovascular indexes and the corresponding predicted value using the same model for all tasks (the model adopted is the one identified for *Talk*). Dots represent subjects for which the correlation is not statistically significant (p>0.05). For the other group (represented with circles), the group size, and the mean value and the standard deviation of the correlation coefficient are reported. The scale of the horizontal axis is exponential.

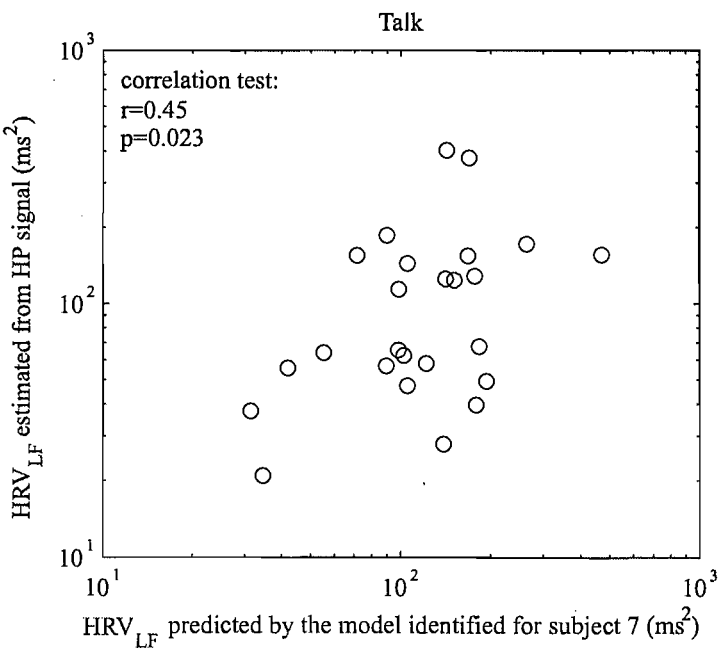


Figure 4.23: Correlation between the "true" value of HRV_{LF} during *Talk* and the corresponding predicted value using the same model for all subjects (model identified for subject nr.7).

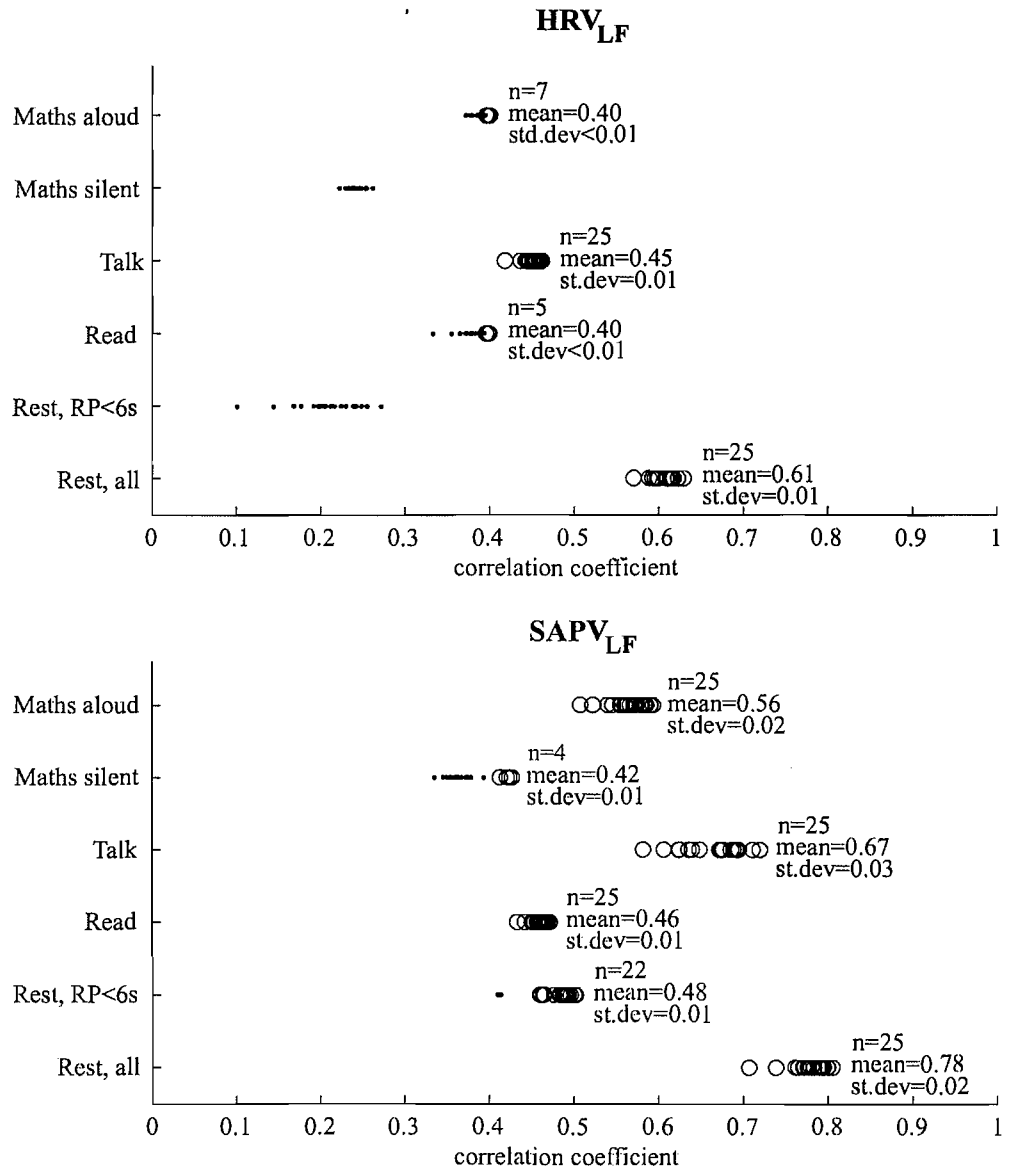


Figure 4.24: Correlation between the "true" value of HRV_{LF} and $SAPV_{LF}$ indexes and the corresponding predicted values using the same model for all subjects. Each marker in the row corresponds to the correlation coefficient obtained using a model estimated from a different subject. For *Rest*, each correlation was computed twice, first considering all the cohort, and then removing the subjects with long respiratory period ($RP>$ 6). Dots represent tests not resulting in a statistically significant correlation (p>0.05). For the remainder (represented by circles) number of cases, mean, and standard deviation are reported.

4.4.4 Discussion

The adopted linear causal model of the respiratory modulation of HP and SAP, allows the prediction of a considerable part of HP and SAP variability, as clearly exemplified in Figure 4.17 and 4.18, and summarized in Table 4.3. As expected, the respiratory volume signal (V) is a statistically significant predictor of the respiratory modulation of HP and SAP, as shown by Monte Carlo simulations. This is in agreement with the known important role of respiration as modulator of cardiovascular variability [19, 54], and indicates that the linear causal model adopted is able to model at least part of such a modulation mechanism. Based on the results presented a number of issues will be addressed in the following discussion:

i) a comparative analysis of the identified models showed that significant differences between tasks exist in the respiratory modulation of cardiovascular oscillations;

ii) however the between-task changes in cardiovascular indexes can be largely explained from differences in respiration even if such differences are neglected;

iii) for many of the tasks considered, inter-individual differences in HRV_{LF} and $SAPV_{LF}$ can be predicted to a considerable extent by differences in respiration, even without taking between-subjects differences in cardiovascular-respiratory interaction into account.

i) Analysis of the frequency response of the models

The results presented in Section 4.3 strongly suggest that the between-tasks changes in cardiovascular indexes are at least in part an effect of modifications in the respiratory pattern. This challenges the idea that during psychophysiological tasks modifications in HRV and $SAPV$ indexes can be attributed mainly to the autonomic modulation of cognitive/psychological processes elicited by the tasks [13], at least for the case of the mild stressors considered in this work. The modeling approach presented in this section attempts to disentangle further the contribution of these two factors. In particular, the comparison of the identified models allows to investigate if significant between-task changes exist in the respiratory modulation of cardiovascular oscillations, known to be under autonomic control [101, 102].

To this end, the models identified for different tasks were compared, through an assessment of the agreement between the frequency responses of the $V \rightarrow HR$ and $V \rightarrow SAP$ transfer functions.

As shown in Table 4.4 and Figures 4.19 and 4.20, considerable differences exist between the tasks. The overall picture that seems to emerge from the results is that the *Maths* tasks elicit a considerable change from *Rest* in the characteristics of the transfer functions, with a generalized decrease in gain (DI> 60% in the 0.05-0.5Hz band), while *Read* and *Talk*, which present strong similarities (DI< 15% in the 0.05-0.5Hz band), show discrepancies with the baseline values that are mainly limited to the HF band for the $V \rightarrow HR$ transfer function (DI> 80% for the gain, DI> 60% for the phase), and to the LF band for the $V \rightarrow SAP$ transfer function (DI> 48% for the gain, DI= 62% for the phase against *Maths aloud*).

Hence, the evidence found suggest that significant rest-task and between-tasks changes in autonomic modulation of the cardiovascular-respiratory interaction are present. However, it must be acknowledged that this result cannot be taken as conclusive. In fact, considering existing evidence that the $V \rightarrow HR$ control system presents non-linear characteristics [57, 58], some discrepancies in the transfer functions (estimated under the assumption of linearity) between tasks presenting different respiratory patterns are expected even without changes in the control system. Hence, in this regard, further investigations involving non-linear models appears needed.

Also, the analysis of the difference between tasks in frequency response performed for each subject (results resumed in Table 4.5), evidenced high inter-individual variability (i.e. discrepancy indexes between two given tasks ranging from 0 to 100%). This is in agreement with the expected high inter-individual variability in the autonomic reaction to psychological/cognitive challenges [13], limiting the possibility to predict the reaction of single subjects from the average results.

As evidenced in the *Results* section, for *Rest* considerably larger error margins (i.e. larger average confidence interval) of the estimates are present compared to the other tasks, especially for the frequencies that are far from the "normal" respiratory frequency for this task (0.2-0.25Hz, equivalent to a respiratory period of 4-5s, see Figure 4.13). A similar effect can be noted, even if to a reduced extent, for *Maths silent*. These results are not unexpected, since the tasks performed silently are associated with a relatively narrow-band spectrum of respiration (see Section 4.3), which is likely to lead to poor estimates of the frequency response

characteristics (i.e. larger confidence intervals) outside this band, compared to tasks involving speech. In Section 4.3 these were shown to elicit a broadband respiration, and hence appear to be a more reliable mean of estimating the $V \rightarrow HR$ and $V \rightarrow SAP$ frequency responses.

The $V \rightarrow HR$ frequency responses found for the tasks involving speech show some agreement with previous results [102], in particular in regard to the presence of a peak in the gain within the LF band and in the positive delay for lower frequencies, both progressively decreasing for increasing frequencies (see Figures 4.19 and 4.25). However, in this work the magnitude of the gain appears higher compared to that found by Saul et al.

Differences in the experimental protocol may help to explain the discrepancies. In particular the posture of the subject (seated vs supine or standing) and possibly the mental effort elicited by the tasks (mild stressors vs controlling the respiration) differ, both of which are expected to influence autonomic control [17, 102]. Also, different methods were used to monitor respiration (direct measure of respiratory air-flow vs two-belt chest-abdomen inductance plethysmography). Furthermore the lower coherence between V and HR found by Saul et al. [102] suggests a depressed respiratory drive or noisier signals, which might also account for the lower gain reported (compare Figures 4.19 and 4.25). Moreover, considering the possibility of the control system being non-linear, as mentioned above, discrepancies between the estimated frequency responses are expected even without dissimilarities in autonomic control, since different respiratory patterns were used to estimate the parameters of the models (speech vs. paced breathing).

In another work from the same authors [101], characteristics of the $V \rightarrow SAP$ frequency response were reported (see Figure 4.26). There is general agreement in the phase, but the gain found in the present work is higher. However, the comparison is limited since no confidence intervals for the average values were reported. Furthermore, differences exist in the protocol that might have had considerable impact in the results: posture of the subjects (sitting vs supine), cohort considered (both sexes with mean age 26.5 vs only males with mean age 21), methods to monitor arterial pressure (plethysmography of finger arterial pressure vs direct measure or radial pressure through catheter) and respiration (direct measure of respiratory air-flow vs two-belt chest-abdomen inductance plethysmography), respiratory patterns used to estimate the parameters of the models (speech vs paced breathing).

Another source of discrepancies with the previous work of Saul et al. could be

the differences in the methods used for the estimation of the frequency response (parametric modeling vs FFT-based approach). However preliminary comparisons of the gain and phase obtained using both methods for some of the subjects of the present cohort suggest that this is probably not a major factor.

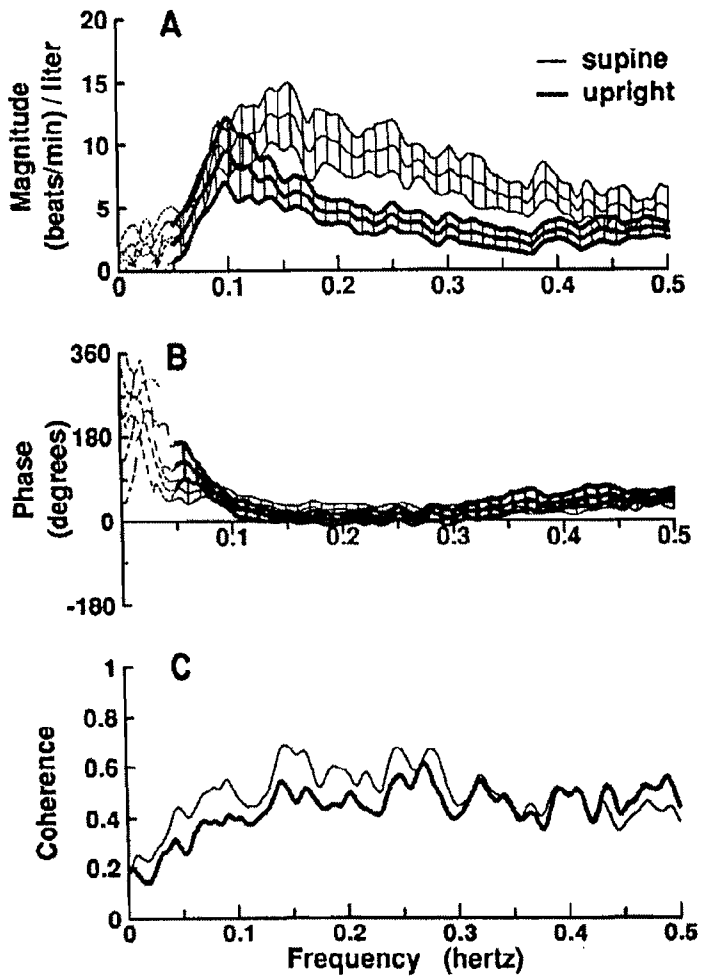


Figure 4.25: Gain, phase, and squared coherence of the $V \rightarrow HR$ transfer function, as reported by Saul et al. [102]. Means ± standard error are shown.

ii) Use of the same model for all tasks

As described in Section 2.2, changes in respiration and changes in autonomic regulation are both expected to contribute to the differences in cardiovascular indexes between tasks. As outlined above, a quantification of the relative contri-

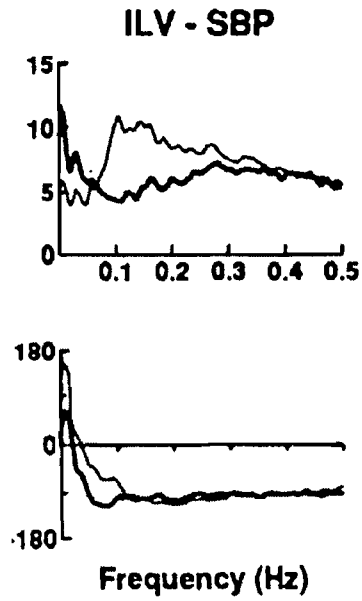


Figure 4.26: Gain and phase of the $V \rightarrow SAP$ transfer function, as reported by Saul et al. [101]. Heavy lines represent data after combined vagal and β-adrenergic blockade, while thin lines represent resting condition, both in supine position.

bution of the former is desirable, to clearly assess the possible confounding effects of respiration in psychophysiological studies.

To this end, for each subject the same model was applied for all tasks, with the objective of estimating how much of between-tasks differences in HRV and $SAPV$ indexes can be explained by changes in the input of the model (i.e. respiration) alone, deliberately neglecting the expected modifications in the model (evidenced in item i). The model chosen was the one identified for *Talk*, since this task provided more reliable estimates of the model (i.e. smaller confidence interval) than tasks performed silently, and with similar values for the predicted indexes compared to applying *Read* and *Maths aloud*.

The results show that for many subjects high correlation ($r>0.88$, with $p<0.05$) exists between the indexes predicted by the model and the "true" values (Figure 4.22). Hence, for many subjects a considerable part of the between-tasks differences in cardiovascular indexes could be predicted by the linear causal model adopted, up to 99% for some. This clearly suggest that even if the respiratory modulation of cardiovascular oscillations undergoes significant between-task changes (as expected from the literature, and confirmed by the results presented in item i), the contribution of such changes to modification of cardiovascular in-

dexes may be considerably smaller than that of changes in respiratory patterns.

Even though this effect was not as strong for all the subjects, the results suggest extreme caution in interpreting average changes in the HRV and $SAPV$ indexes for the whole cohort as a reflection of changes in autonomic activation elicited by cognitive/psychological processes during psychophysiological tasks, if considerable changes in respiration are present. This is in agreement with the conclusions drawn in Section 4.3.

iii) Application of the same model to all subjects for each task

Shifting the focus of the investigation from between-tasks to inter-individual differences, a similar approach to that presented in item *ii* was applied. In this case, for each task, the same model was adopted for all subjects. Again, this approach deliberately neglects part of the physiological reality, namely inter-individual differences in autonomic modulation.

The results show that, for tasks involving speech, a considerable part of the inter-individual differences in HRV_{LF} and $SAPV_{LF}$ can be explained by a causal relationship with respiration (Figure 4.24), independently of the common model adopted. In particular, for *Talk*, about 20% of HRV_{LF} and 45% of $SAPV_{LF}$ between-subjects differences were predicted by the models (the percentages are the square values of the correlation coefficients). For *Read* and *Maths aloud* correlation coefficients are lower, and for HRV_{LF} show only borderline statistical significance ($p< 0.08$). For *Maths silent*, correlation were not significant for HRV_{LF}, and in most of the cases indicating just a trend ($p< 0.1$) for $SAPV_{LF}$. These results are fundamentally in agreement with the evidence reported in Section 4.3, regarding the correlation between respiration and cardiovascular indexes (Table 4.1), and strengthen the conclusion reached that, when tasks involving speech are involved, it is likely that a significant part of inter-individual differences in cardiovascular indexes is linked to the between-subjects variability in respiration. In particular, the present results indicate that such a link can be explained by a causal relationship, since the model adopted is strictly causal, even if the possibility should be considered that coherent cardiovascular and respiratory oscillations can be the effect of a common central modulation [3].

Compared to *Maths silent*, tasks involving speech present higher inter-individual differences in respiratory characteristics (i.e. larger 5-95% intervals in respiratory period, Vn_{LF}, and tidal volume, see Figure 4.13), with *Talk* having the largest variability in respiratory period and Vn_{LF}. The larger inter-individual differ-

ences probably contribute to the observed high correlation coefficients: with high scatter of points, dependence between variables is more likely to be observed. In agreement with the conclusions drawn in Section 4.3, when inter-individual differences in autonomic activation are assessed through cardiovascular indexes, psychophysiological tasks involving speech should probably be avoided, in order to limit the between-subjects differences in respiratory patterns, and consequently the respiratory-related inter-individual differences in HRV_{LF} and $SAPV_{LF}$. However, it must be acknowledged that this does not guarantee "a priori" the absence of a significant confounding effect of respiration, and possibly more radical ways to control respiration (e.g. paced breathing) should be sought in tasks performed silently, as suggested by several authors [23, 49].

For *Rest*, relatively strong correlations were found ($r>0.6$, $p<0.001$). However, when subjects with long respiratory period were removed from the analysis, significance was lost for HRV_{LF}, and the correlation coefficient considerably attenuated for $SAPV_{LF}$. Again, this is in agreement with the results found in Section 4.3, which led to the conclusion that not removing subjects with long respiratory period at rest can lead to a misinterpretation of the correlation found between respiration and cardiovascular indexes. Such a correlation is partly due to an offset in the value of cardiovascular indexes between subjects with long and short RP, which is mainly an effect of the way the indexes are computed (based on a cutoff frequency of 0.15Hz between the LF and HF band), rather than of the dependence of HRV and $SAPV$ on respiration.

Limitations of the study

Clearly the model chosen for this investigation is an oversimplification of the complex interaction between respiration and cardiovascular oscillations. Other approaches might have been used, including for example the presence of feedback loops and non-linearities. However, a simple model appeared to be the most obvious choice for the initial investigation of an issue - the quantification of how much of the cardiovascular oscillations can be predicted solely as an effect of respiration - that, as far as the author is aware, has not been tackled before. Furthermore, the approach adopted proved effective in pursuing the aims of the study - showing that for psychophysiological investigations, differences in respirations can explain to a significant extent between-tasks and inter-individual differences in cardiovascular oscillations.

Moreover, this investigation did not consider the possibility that changes in

autonomic activation might also be directly reflected in changes in respiratory patterns. From this point of view, it is possible that the respiratory modulation of cardiovascular oscillations, rather than confounding the analysis of the results, might actually carry information about changes in autonomic activation. However, if this is the case, investigations on the correlation between autonomic activation and respiration should be carried out. Such investigations are likely to be extremely complex, due to the need to remove or account for the voluntary control of respiration, especially for the case of tasks such as speech or paced breathing, where the respiratory drive is mainly, if not exclusively, voluntary.

Finally, it must be acknowledged that, in many cases, the results found are significant only for a part of the cohort or for specific tasks, rather than being evident for the whole group and for all the tasks in the protocol. Clearly this limits general conclusions regarding the respiratory modulation of cardiovascular oscillations. However the evidence shown provides a sufficiently clear indication that disregarding the modulating effect of respiration is likely to produce misleading and/or confounding results when assessing autonomic activation through cardiovascular indexes.

4.4.5 Conclusions

Modeling of the respiratory modulation of cardiovascular oscillation as a simple linear input/output system provided indications that this modulation undergoes significant changes during psychophysiological tasks, in agreement with previous works showing that cognitive/psychological processes are able to elicit significant modification in autonomic cardiovascular regulation.

However, the results shown confirm and add further evidence in support of the conclusions drawn in Section 4.3, that respiration plays a non-negligible role in determining the between-tasks and inter-individual differences in HRV and $SAPV$ indexes. In particular, for the protocol considered, applying causal models of the cardiovascular-respiratory interaction that deliberately neglect the difference in autonomic regulation, allowed predicting a considerable part of the between-tasks changes (on average over the whole cohort 62% for HRV_{LF}, 48% for $SAPV_{LF}$, and 27% HRV_{HF}, with values up to 99% for some subjects), and up to 45% of the inter-individual differences during a task, solely as an effect of dissimilarities in respiratory patterns. This clearly suggests that even if, as expected, autonomic activation varies significantly between tasks or subjects during psychophysiological tests, its contribution to between-tasks and inter-individual

differences in cardiovascular indexes can be comparable to, or even exceeded by, that of differences in respiratory patterns.

4.5 How to avoid confounding effects of respiration: considerations for future psychophysiological studies

In view of the evidence provided, the investigation of autonomic activation through HRV and $SAPV$ indexes is clearly susceptible to the confounding effects of respiration. Hence, alternatives are desirable. However, cardiovascular variability indexes have the advantage of being based on well-known simply acquirable signals (ECG and AP) and processing [27]. This, as far as the author is aware, does not appear to be the case for other indirect indexes of autonomic activation, for example pre-ejection period [103], which require more complicated acquisition devices and, given their relatively limited diffusion compared to HRV and $SAPV$ indexes, lack comparable standardization and thorough validation.

As far as the author is aware, the only alternative proposed so far in the literature to allow a reliable use of HRV and $SAPV$ indexes is to minimize the differences in respiratory patterns between tasks and subjects, hence removing the issue of respiratory modulation at the origin. In particular, the practical solution that has been suggested is to impose on all subjects the same breathing pattern at rest and during the tasks, with a respiratory frequency strictly within HF band [23, 49]. However, as discussed in Section 4.3.4, this method may be unsuitable for psychophysiological tasks. Controlling respiration is a task requiring mental effort itself, which can potentially modify the autonomic cardiovascular regulation [17], though it has been reported that paced breathing does not result in significant modifications of cardiovascular oscillations [93]. Furthermore, performing a task that requires some degree of mental effort, and at the same time controlling the respiratory pattern is likely to result in a poor outcome in both [99]. Moreover, for tasks involving speech, constraining the respiratory pattern is at least extremely difficult, if not practically impossible.

Hence, the use of HRV and $SAPV$ under paced breathing for the assessment of autonomic activation might be, in principle, appropriate for some psychophysiological tasks, but excludes "a priori" the use of tasks involving speech. The applicability and implications of such an approach to other tasks needs to be fur-

ther investigated. In particular, the modality of pacing requires a deeper analysis, and some standardization is needed.

A radically alternative approach to the investigation of cardiovascular oscillations, previously introduced by Saul et al. [101, 102], and adopted in this work with the use of an original methodology (model identification and Monte Carlo simulations, see Appendix A.3), consists in investigating the transfer functions between respiratory volume and cardiovascular oscillations. Such an approach shifts the object of the study from the signals (heart-period and systolic arterial pressure) to the system originating the signals, and interprets respiration as, citing Eckberg [41], "a precisely controlled experimental tool to tease out and better understand otherwise inaccessible human autonomic neurophysiological mechanisms", rather than as a confounding factor to be removed or accounted for. Saul et al. showed that the gain and the phase of the $V \rightarrow HR$ and $V \rightarrow SAP$ transfer functions undergo significant modifications in response to changes in autonomic regulation (i.e. changes in posture and effect of parasympathetic and β-adrenergic blocking agents), as shown in Figure 4.25 and 4.26 [101, 102]. However, this investigative approach has not been extensively adopted, possibly because of the complexity of the experimental protocol, requiring a complex paced breathing pattern to obtain broadband respiration, which is necessary for a reliable estimation of the transfer functions.

From this point of view the method presented in this study possesses a practical advantage, since the broadband respiratory pattern is obtained simply through making the subject to speak for several minutes. Furthermore, the combined use of least-squares model identification and Monte Carlo simulations allows estimating a confidence interval for each subject for any function of the parameters of the identified model (as explained in Appendix A.3), including gain and phase. This is a more desirable approach than estimating the frequency response only (as for example in the work of Saul et al. [101, 102]), since it allows assessing the significance of the differences between two subjects or within a single subject in different conditions, by means of statistical testing (e.g. the hypothesis test presented in Appendix A.3). Given these advantages this method has the potential to be applied with relative ease in a broad range of investigations, psychophysiological in particular, and to provide information about the reliability of the estimate of any feature (parameter) considered, which is of paramount importance when clinical decisions are to be made.

However, the transfer-function approach requires considerable further inves-

tigation and testing. At present, the evidence regarding the correlation between autonomic activation and features of the transfer function is basically limited to the aforementioned works of Saul et al. [101, 102].

Chapter 5

Dynamics of the cardiovascular response to cognitive/psychological challenges

5.1 Introduction

For studies involving indirect indexes of autonomic activation, estimating the parameters as average values for the length of each task is a common practice. The use of average values is based on the assumption that either during the task there are no significant changes in the physiological response to the stimuli, or that such changes are of limited physiological interest and do not have a significant impact on the conclusions. There are several reasons to question this approach, at least for the specific case of psychological stress tasks. Psychophysiological tests aim at an indirect modification of the autonomic control through the involvement of limbic and forebrain areas implicated in behavioural processes [13]. To Assume that this mechanism results in a step-wise modification of cardiovascular indexes appears an oversimplification, since several studies have reported the presence of within-task changes in some cardiovascular indexes(e.g. HR, SAP, and pre-ejection period) [44, 68, 81]. However the reported results are limited, since a number of relevant cardiovascular indexes, such as indexes of baroreflex sensitivity (BRS) and spectral indexes of HRV and $SAPV$ were not considered. Furthermore, as far as the author is aware, the impact of considering specific short epochs within the tasks in analyzing the response to psychophysiological tasks has not been investigated systematically.

There are a range of benefits that can be derived from a better understanding

of the possible dynamics in the autonomic response to psychological stress. Primarily, it can help researchers in the field to plan more appropriate experimental protocols, in terms of both length and content. For example it may be possible to reduce the length of experimental protocols without compromising the possibility of detecting significant rest-task changes in cardiovascular indexes. Secondly, the dynamics in the response may provide useful additional information about the psychophysiological reaction, which at present is "averaged out" [68]. Finally, it may provide a better basis for discussing the applicability of signal processing methods that have stationarity as a basic assumption.

Preliminary analysis of the data of the experimental protocol described in Chapter 3 suggested that, for the cardiovascular parameters considered (HRV, $SAPV$, and BRS), there exists considerable variations during each task. For example, the average pattern of the sample-by-sample estimate of baroreflex sensitivity in the low frequency (BRS_{LF}) for the Stroop task showed a minimum occurring during the initial part of the task, followed by a progressive return toward baseline values (see Figure 5.1-g).

The work presented in this chapter investigates in more depth the presence of within-task changes in cardiovascular indexes (HRV, $SAPV$, HP, SAP, and BRS) and their impact on the indirect assessment of the autonomic reaction to psychophysiological tasks, through the comparison of estimates of cardiovascular indexes obtained considering short epochs within the same task with estimates obtained considering the whole length of the task.

The chapter is structured in three sections, which reflect different stages of the investigation, aimed at addressing specific issues related to the main objectives. In Section 5.2 a systematic investigation of within-task dynamics for a range of cardiovascular indexes is reported, together with an analysis of the impact that such dynamics have on estimating rest-task changes in the parameters. In Section 5.3, the analysis is focused specifically on BRS indexes, considering also methodological issues related to their estimation. Finally, following on from the results in Section 5.2 that the response to the tasks is characterized by high inter-individual variability, Section 5.5 presents the results of a preliminary study on the use of clustering methods to investigate if different groups of subjects with distinct patterns of response to the tasks are present in the cohort.

5.2 Investigation of within-task changes of cardiovascular indexes

This section reports the results of a systematic investigation of the presence of within-task changes of cardiovascular parameters, for an experimental protocol involving psychophysiological tasks. The aim of this is to critically evaluate the presence of within-task dynamics, and the impact that such dynamics can have on the results. The specific issues investigated are:

- whether significant changes in cardiovascular indexes within different psychophysiological tasks are present, and to verify if their magnitude is comparable to that of rest-task changes;
- if the use of shorter epochs in estimating cardiovascular parameters leads to significantly different results in terms of estimation of rest-task changes compared to analysis of considering the whole length of the tasks.

5.2.1 Material and methods

Experimental protocol

The experimental protocol considered for this work is described in details in Section 3.2. Briefly, a cohort of 179 adult subjects (mean age 26.3), after an initial baseline rest period of 5 minutes, undertook a protocol of three standard psychological stress tests, described in [118], each lasting 5 minutes: a Stroop's word-colour conflict test, a mirror tracing test, and a speech test. The speech test involved two separated sub-tasks: an initial preparation of the speech, followed by an interactive discussion with the investigators. Hence, this task was not considered for this work, since possible within-task changes are likely to be related to the presence of the sub-tasks. Furthermore, in Chapter 4, problems with tasks involving verbalization were identified, related to the confounding effect of respiration.

Data processing

Heart-period (HP) and systolic arterial pressure (SAP) sequences were extracted from the AP signal, and average heart-rate (HR), SAP, heart-rate variability (HRV_{LF} and HRV_{HF}), systolic-arterial pressure variability ($SAPV_{LF}$), and spontaneous baroreflex sensitivity (BRS_{LF} and BRS_{HF}) indexes were estimated over

the entire duration of the tasks, as described in Section 3.2. For the initial investigation of the presence of dynamics in the parameters considered, sample-by-sample estimates of the parameters were computed. For the $SAPV$ and HRV this was achieved by band-pass filtering in specific bands ($LF : 0.05 - 0.15Hz$, and $HF : 0.15 - 0.5Hz$) the SAP and HP signals (after cubic spline interpolation to achieve a constant sampling rate of $4Hz$), and then filtering the square of the resulting signal with a weighted moving average filter (Hanning window of size 80 samples i.e. 20s). A detailed description of this method is reported in Appendix A.2. For BRS, sample-by-sample estimates were computed using a time-adaptive implementation of the autoregressive method [39], using a model order of 16 (motivation of this choice is presented in Section 3.2). For further analysis, in order to investigate dynamics in the response within each task, two epochs were considered: the first $100s$ (which will be referred as $R1$ for the rest task, $S1$ for the Stroop task, and $M1$ for the mirror tracing task), and the last $100s$ of the task (which will be referred as $R3$ for the rest task, $S3$ for the Stroop task, and $M3$ for the mirror tracing task). The average value for each parameter was computed for each epoch: $SAPV$ and HRV indexes were computed as the variance of the band-passed versions of SAP and HP, HR and SAP were computed as mean values for the epoch, and BRS indexes were computed as average values of the sample-by-sample estimates within the epoch. The choice of using $100s$ windows was made after preliminary investigation considering also other two combinations: consecutive windows of $60s$ and of $150s$. This solution appeared to provide the best compromise between avoiding the within-task dynamics to be "averaged out" and limiting the number of intervals to consider. Average values estimated considering the whole length of each task (which will be referred as RT for the rest task, ST for the Stroop task, and MT for the mirror tracing task) were computed as well and used as references for the comparisons.

Statistical analysis

Mean and standard deviation of sample-by-sample estimates were computed for HR and SAP. For all the other parameters, due to the skewness of the distributions, mean and standard deviation were computed using log-transformed data.

For each task, for each of the parameters, the mean differences between the estimates from the shorter epochs ($R3 - R1$, $S3 - S1$, and $M3 - M1$) were computed, together with standard errors. The hypothesis of the difference being

equal to zero was tested.

Then, a similar analysis was applied to the change from rest to task. Mean rest-task differences of the parameters were computed using the different estimates (both those considering only short epochs and those considering the whole tasks), together with standard error. The hypothesis of the rest-task change being equal to zero was tested. Furthermore the hypothesis that estimates of rest-task change that considered short epochs did not differ from estimates that considered the whole task was also tested.

For all the hypothesis testing, paired Student's t-test was applied, and $p = 0.05$ was considered as the threshold to reject the null hypothesis.

5.2.2 Results

Figure 5.1 shows the plots of the sample-by-sample estimates of the parameters for the Stroop task in each subject, together with geometric mean (arithmetic mean for HR and SAP), and 5^{th}-95^{th} percentile. The plots show that the average sample-by-sample estimates undergo within-task changes, shifting from whole task averages (solid horizontal line). However, the magnitude of the dynamics is small compared to the high inter-individual variability of the estimates (gray plots in the background).

Figure 5.2 reports the mean, standard error, and significance of difference between the estimates using the first and the last $100s$ of each task ($R3 - R1$ for the rest period, $S3 - S1$ for the Stroop task, and $M3 - M1$ for the mirror tracing task). Rest-task changes using the whole length of the tasks for the estimate ($ST - RT$ and $MT - RT$) are reported for reference (gray background). Significant differences between estimates using the first and the last $100s$ of the task are present for the rest period (in HR, SAP, HRV_{HF}, and BRS_{HF}), for the Stroop task (in SAP, HRV_{HF}, BRS_{LF}, and BRS_{HF}), and for the mirror-tracing task (in HR, $SAPV_{LF}$, and BRS_{HF}). Furthermore, in several cases the within-task changes found have a magnitude that is quite considerable, compared to the change from rest to the stress-tasks, and with opposite sign. For example, in terms of absolute values, HR undergoes a decrease during the rest period of about 30% of the rest-task increases, SAP during the Stroop task shows a decrease of more than 15% of the rest-task increase, HRV_{HF} shows an increase during the rest period of more than 20% of the rest-task decrease, $SAPV_{LF}$ undergoes an increase during the mirror tracing task of more than 20% of the rest-task decrease, and BRS_{LF} undergoes an increase during the Stroop task of more than 40% of

the rest-task decrease.

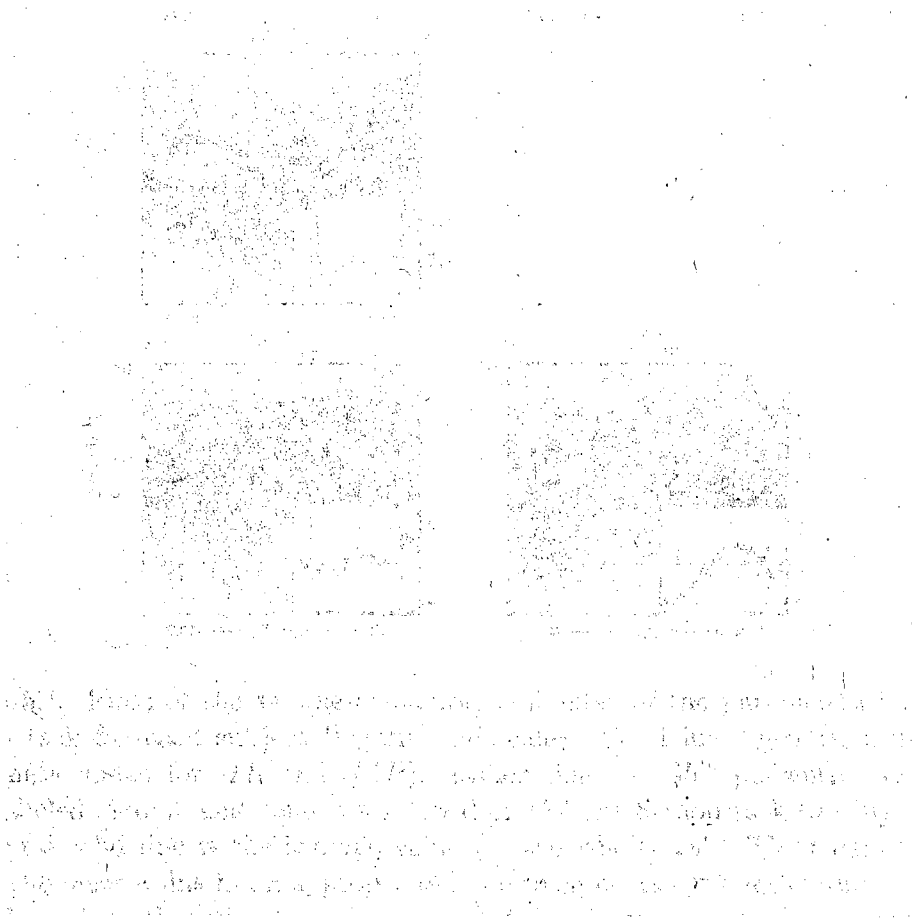
Figure 5.3 reports the mean and standard error of rest-task changes using the last 100s of the rest task ($R3$) to estimate the rest value for each task and either the first or the last 100s of the Stroop and mirror tracing task to estimate the task value (respectively $S1$ or $S3$, and $M1$ or $M3$). Rest-task changes using the whole length of the tasks for the estimate ($ST - RT$ and $MT - RT$) are reported as reference (gray background). All the rest-task change estimates are significantly different from zero ($p < 0.001$). On the right side of each bar plot, for each estimate using short epochs, the mean difference with the corresponding estimate using the whole tasks is reported (Δ, in percent), together with its significance.

The results shown in Figure 5.3 evidence that for all the parameters considered (except HRV_{LF}) the use of the *short-epochs* approach produced considerable and statistically significant differences (p<0.05) compared to the *whole-task* approach in the estimated magnitude of rest-task changes. In particular, for the Stroop task, the use of the $S1$ and $R3$ epochs to estimate baroreflex indexes resulted in a substantial increase in both ΔBRS_{LF} (+27%) and ΔBRS_{HF} (+51%) compared to the *whole-task* approach (see Figure 5.3-g/h). This result appears directly linked to the fact that the use of the whole length of the Stroop task for estimation "averages out" the significant change of the indexes (Figure 5.2-g/h). A similar effect appears to be present for SAP, that shows higher values at the beginning of the Stroop task, progressively decreasing to lower values. This results in higher or lower estimates of the change elicited by the Stroop task if respectively only the beginning ($S1$) or the end ($S3$) of the task is used, compared to the *whole-task* approach.

Also, HR estimates using the *short epochs* approach show an increase between 12% and 26% in the estimated rest-task difference compared to the *whole-task* approach (Figure 5.3-a). This result is likely to be an effect of the tendency of HR to decrease during the rest period, which can be seen in Figure 5.2-a: considering only $R3$ resulted in a smaller estimate of baseline values compared to the *whole-task* approach, and consequently in a larger estimate of the rest-task change.

An unexpected result is that the use of the *short epochs* approach resulted in significantly smaller estimates of the change from rest to the Stroop task for $SAPV_{LF}$ (see Figure 5.3-e). Further investigation showed that this is probably related to the fact that $SAPV_{LF}$ estimated for the central 100s of the Stroop task

(this specific epoch was not considered in this study) is significantly smaller than the estimates using either $S1$ or $S3$ (providing a further evidence for the presence of a complex within-task dynamic for this index). Thus, $SAPV_{LF}$ estimates considering the whole length of the Stroop task are likely to be considerably smaller than those considering only $S1$ or $S3$.



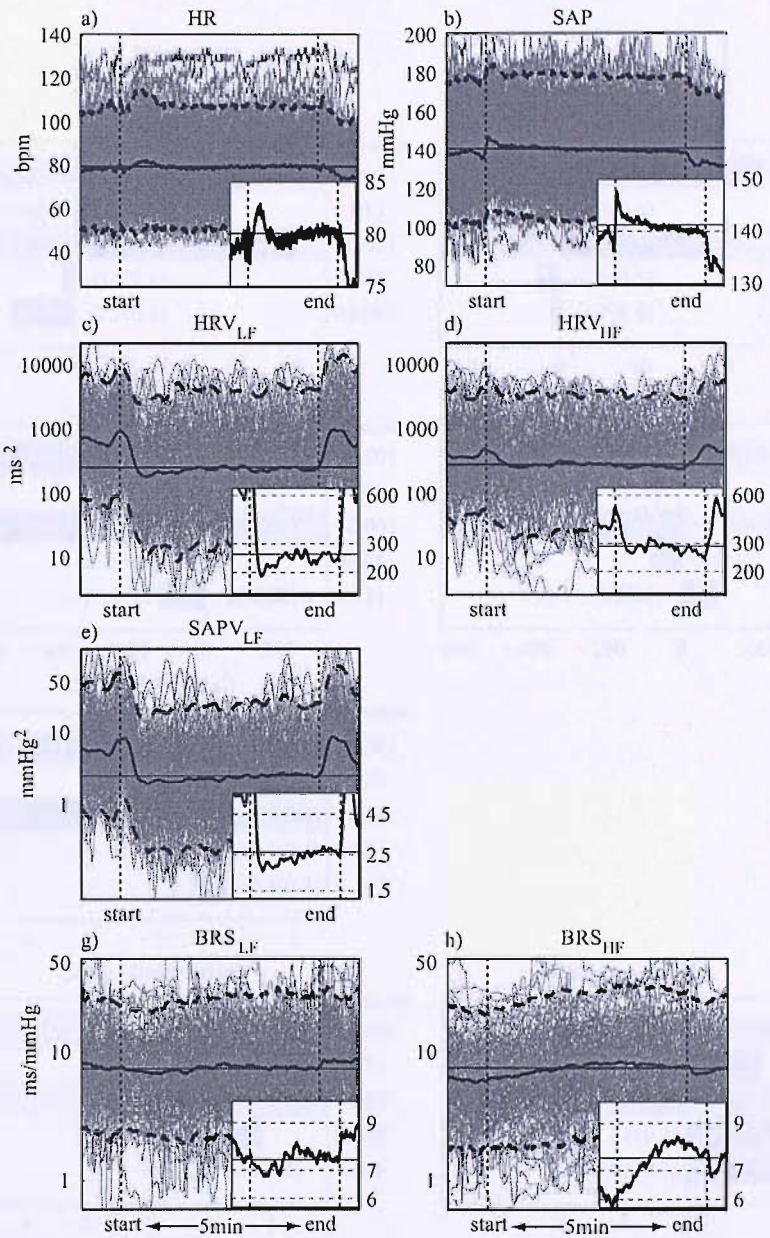


Figure 5.1: Plots of the sample-by-sample estimates of the parameters for the Stroop task for each subject (logarithmic scale). Solid line: geometric mean (arithmetic mean for HR and SAP); dashed line: 5^{th}-95^{th} percentile; dotted lines labeled "start" and "end": start and end of the Stroop task ($5min$). The horizontal solid line is the average value for the whole task. The smaller plot shows the mean value in an appropriate scale to show its dynamics more clearly. The plots show that the average sample-by-sample estimates undergo within-task changes, shifting from whole task averages (solid horizontal line). However, the magnitude of the dynamics is small compared to the high inter-individual variability of the estimates (gray plots in the background).

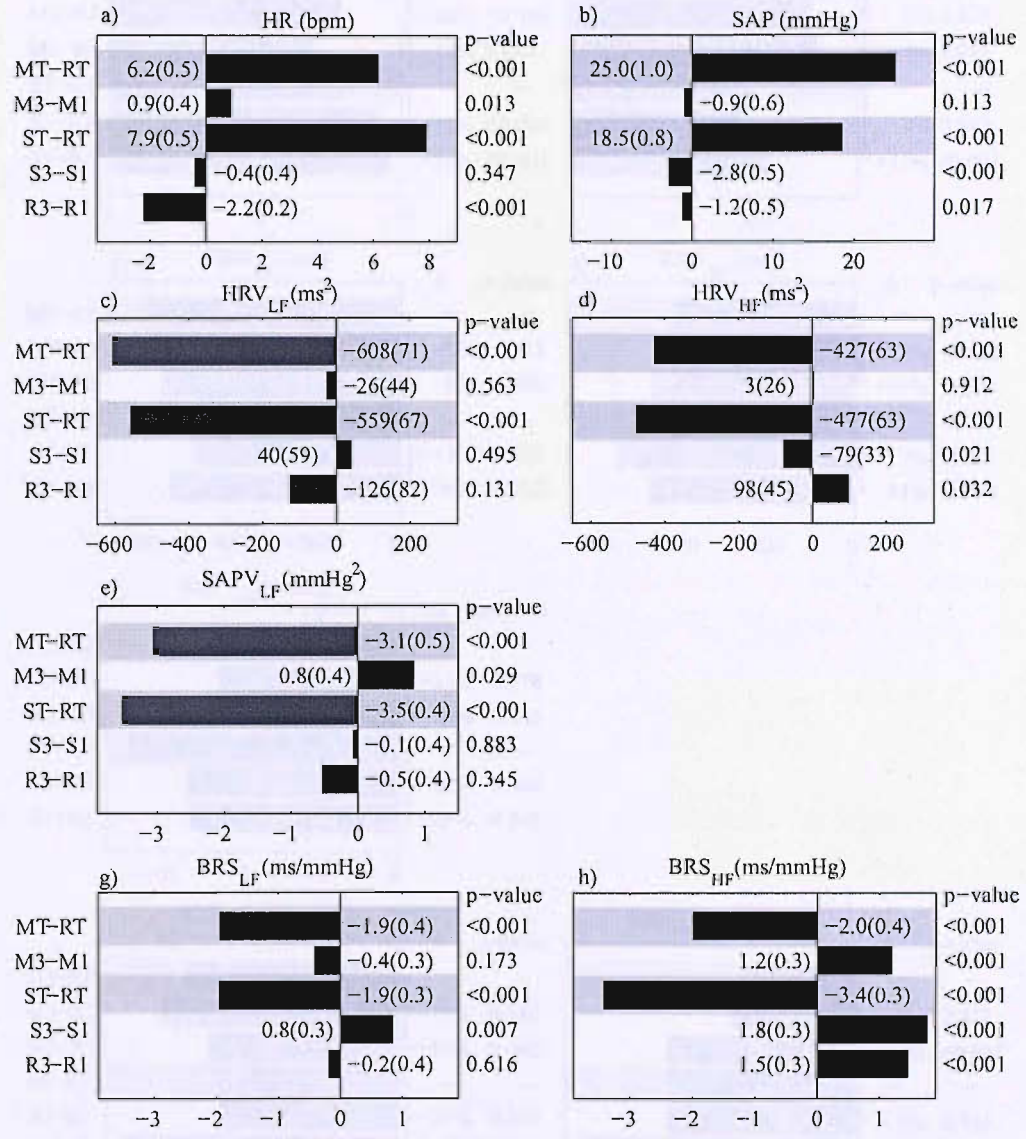


Figure 5.2: Mean (standard error) and significance of change between the first and the last 100s of rest (R3-R1), Stroop (S3-S1), and mirror tracing (M3-M1). Changes estimated using the whole length of the tasks from rest to Stroop (ST-RT), and from rest to mirror tracing (MT-RT) are reported as reference (gray background). For all indexes except HRV_{LF} significant within-task changes where found, in several cases of a considerable magnitude and different sign compared to the average rest-task change (e.g. BRS_{LF}, BRS_{HF}, SAP, and $SAPV_{LF}$).

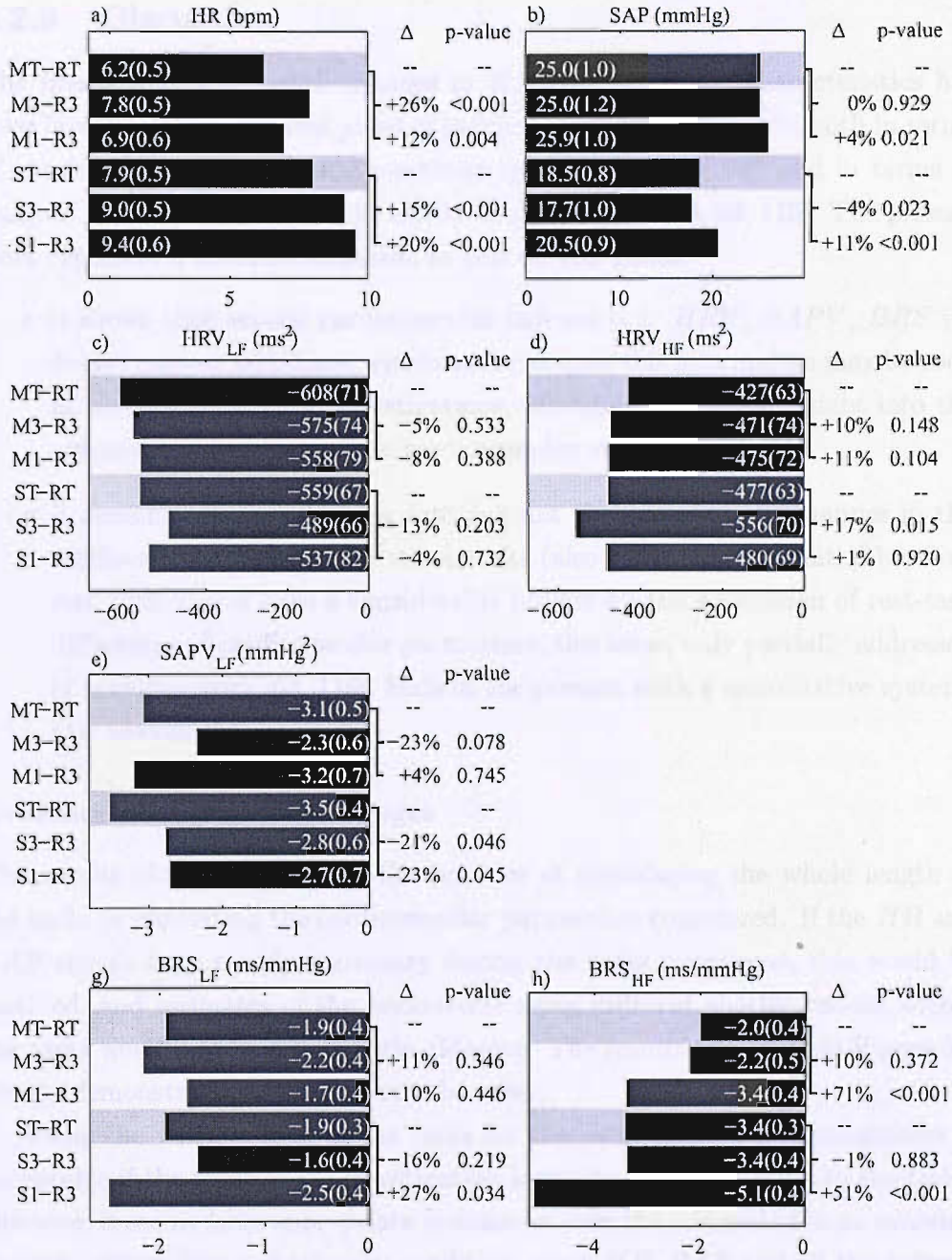


Figure 5.3: Mean (standard error) of rest-task change considering long epochs and short epochs. For the change from rest to Stroop task, the estimate using the whole length of the task is indicated as ST-RT, that using the last 100s of rest and the first 100s of the task as S1-R3, and that using the last 100s of rest and the last 100s of the task as S3-R3. Similar notation is adopted for the change from rest the mirror task (using 'M' instead of 'S'). Δ: mean (in percent) and significance of the difference between the short-epoch estimate and the corresponding long-epoch estimate

5.2.3 Discussion

The presence of within-task changes in HR and AP signal characteristics has been investigated in different areas of psychophysiological research, both in terms of assessing the cardiovascular reactivity to stress [44, 68, 81], and in terms of analysis of the stationarity of the cardiovascular signals [46, 69, 119]. The present work represent a novel contribution to this on two issues:

- it shows that several cardiovascular indexes (i.e. HRV, $SAPV$, BRS indexes) present significant within-task changes; this information may be used in psychophysiological investigations, providing additional insight into the autonomic regulation of the cardiovascular system;
- it demonstrates that taking into account the presence of dynamics in the cardiovascular response to stress tasks (also present in the initial baseline rest period) can have a considerable impact on the estimation of rest-task differences of cardiovascular parameters; this issue, only partially addressed in previous work [68, 119], finds in the present work a quantitative systematic assessment.

Evidence of within-task changes

The results obtained challenge the practice of considering the whole length of the tasks in estimating the cardiovascular parameters considered. If the HR and SAP signals were weakly stationary during the tasks considered, this would be justified, and estimates of the parameters using different shorter epochs within the tasks would not be significantly different. The results presented in Figure 5.2 clearly demonstrate that this is not the case.

Using the whole length of the tasks for the estimation of the parameters is acceptable if the focus of the investigation is the "average" reaction to the tasks. However, it seems more appropriate to consider even the rest period as an evolving process, rather than a stationary condition, since HR, SAP and all the indexes computed in the HF band undergo a significant change during this task. The same applies to the Stroop task (considering the significant within-task change in SAP, BRS_{LF}, HRV_{HF}, and BRS_{HF}), and to the mirror-tracing task (for its significant within-task change in HR and $SAPV_{LF}$).

The presented results agree with previous works on the overall changes in cardiovascular parameters caused by stress-tasks (see Section 3.4 for a detailed discussion of this issue). Regarding the evidence of within-task dynamics, the

results obtained for the Stroop task tend to agree with those previously described [44, 68, 81]: HR and SAP show an initial fast increase, followed by a rapid recovery towards lower values (see Figure 5.1-a/b), even if this dynamic was found to be significant for SAP but not for HR (Figure 5.2-a/b). However, the mirror-tracing task did not replicate these results, presenting a non-significant decrease in SAP and a significant increase in HR (see Figure 5.1-a/b). Several factors might have contributed to the discrepancies between the two tasks. In particular, carryover effects resulting from the fixed task order (mirror-tracing always following the Stroop task), might have led to a reduced cardiovascular reactivity during mirror-tracing as a consequence of previous exposure to a stressful task [68]. Different levels of effort, challenge, or engagement elicited by the two tasks, which are known to modulate the cardiovascular response to stress-tasks [68], could also explain part of the differences. However, an exhaustive investigation of this aspect goes beyond the scope of the present work.

Considering the other cardiovascular indexes analyzed in this work (HRV, $SAPV$, and BRS indexes), as far as the author is aware, no published work investigated their within-task dynamics for psychophysiological tasks, with the exception of the work of Mezzacappa et al. [81]. For that investigation, within-task changes in the square root of the mean squared differences of successive heart periods ($rMSSD$) were considered. $rMSSD$ is an index of high-frequency variations in heart-rate, positively correlated with the degree of vagal cardiac control [17, 27]. The results generally agree with those obtained in this work for HRV_{HF} (as well a marker of vagal activation, see Section 2.2): a within-task decrease for the initial Stroop task (see Figure 5.2-d), and a considerably different within-task dynamic for the following task (suggested in the present work by the non-significant within-task change for the mirror-tracing task, see Figure 5.2-d). However, Mezzacappa et al. used mental arithmetic as the second task instead of mirror-tracing, hence limiting the comparability with the present work.

Impact of within-task changes on estimation of rest-task difference

A considerable number of studies in the psychophysiological field investigated the cardiovascular reaction to stress-tasks through the statistical comparison between rest and task values of cardiovascular parameters computed as averages over the whole tasks (or baseline rest) considered. Kelsey et al. [68] commented that "previous studies of cardiovascular reactivity to stress have typically averaged over factors such as time..., thereby masking possible adaptation effects and obscuring

important information about psychophysiological processes and potential relationships between cardiovascular reactivity and disease". Furthermore Weber et al. [119] suggested that "non-stationarity may result in biased outcomes of significance tests of the effects of task manipulations on the spectral indices of cardiac time series". Hence, taking into account the presence of within-task changes in the indexes, for example considering specific short epochs within the tasks instead of the whole task for parameter estimation, could produce different and additional results compared to averaging over the whole length of the task, both in terms of magnitude and of significance of the estimated rest-task changes.

The present work adds a novel contribution in this direction, quantitatively investigating, for a range of cardiovascular parameters, the difference in estimated rest-task changes between two approaches for parameter estimation: a *short-epochs* approach considering only specific short epochs within the tasks and the rest period, and a *whole-task* approach, considering the whole length of the task and of the rest period. For this investigation, only the last $100s$ of the rest task ($R3$) were used for estimation of baseline values for the *short-epochs* approach. This choice is based on the evidence that HR and SAP decreased consistently from $R1$ to $R3$, indicating an adaptation occurring during the rest period. From this point of view, given that within-epoch stability is a desirable property for baseline assessment [62], the last part of the rest period appears to be a more appropriate epoch to use as a baseline. For estimation of parameters during the tasks for the *short-epochs* approach, two alternatives were considered: the first 100s of the tasks ($S1$ and $M1$), which should include an expected initial peak in cardiac reactivity [68], and the last 100s of the tasks ($S3$ and $M3$), which are likely to include only the period following such a peak.

The results of Figure 5.3 show that for all the parameters considered (except HRV_{LF}) the use of the *short-epochs* approach produced considerable and statistically significant differences compared to the *whole-task* approach in the estimated magnitude of rest-task changes. This evidence clearly challenges the idea that within-task changes can be neglected even if the interest is focused only on the rest-task changes of the parameters, and shows the non-negligible consequences of interpreting the rest-task change as a step change between two steady states, rather then a dynamic transition between two evolving processes.

It could be argued that the increased variance of estimates associated with the use of shorter epochs could result in a reduced capability (higher p-values) in detecting a significant rest-task change when small cohort are considered. How-

ever, the results indicate that this is not true, since when the paired Student's t-test was applied to investigate the presence of a significant difference between rest and task values of the parameters, estimates using the *short-epochs* approach and those using the *whole-task* approach gave similar results in terms of statistical significance.

Concluding, the results suggest that for future psychophysiological investigations a *short-epochs* approach should be preferred to a *whole-task* approach. The *short-epochs* do not lead to an appreciable loss of reliability in detecting significant rest-task changes in the parameters compared to a *whole-task* approach. Moreover, if only the investigation of significant rest-task changes in the parameters is of interest, the use of tasks lasting 5min or more appears unjustified, and shorter tasks can be used (e.g. 100s), since, as discussed above, the results showed that considering only a relative short epoch at the beginning of the stress tasks ($S1$ or $M1$) allowed the detection of rest-task changes with the same statistical significance of using longer epochs.

Limitations of the study

One limitation of this work is that measures of respiration were not acquired during the experimental protocol. Hence, it was not possible to assess if respiration undergoes significant within-task changes, and if these were contributing to the within-task dynamics in cardiovascular indexes. However, even if this fact might complicate the interpretation of the origin of the within-task changes in cardiovascular indexes, it does not affect the validity of the results on the presence of such changes and their impact in the estimation of rest-task differences.

Despite providing evidence regarding the presence and the importance of within-task dynamics, the short epochs approach adopted is not necessarily the only or the most appropriate way to investigate the patterns of cardiovascular reaction. It is possible, for example, that specific features of the reaction (e.g. location of a peak in the pattern, time to reach a certain reduction in a parameter) carry useful information for future psychophysiological studies, rather than the unspecific trends of change considered in this study. This possibility needs to be explored in future research.

5.3 Impact of within-task dynamics on the estimate of rest-task *BRS* changes: a comparison of different methods

In Section 5.2 it was shown that *BRS* indexes undergo significant changes within each task (Figure 5.1-g/h and 5.2-g/h), resulting in significantly different estimates of rest-task changes when specific short epochs within the tasks are considered instead of the whole length of the task. However, in Section 5.2, for the specific case of *BRS* indexes were computed using only one method (parametric approach [39]) of the several that have been proposed in the literature [75]. A gold standard for *BRS* estimation is not available, and it has been shown that the agreement between different methods in estimating *BRS* is limited [75]. Despite the *BRS* reaction to psychophysiological tasks having been repeatedly investigated in the past [36, 43, 50, 81], there is no published study comparing different *BRS* estimates on short epochs within the task rather than the more "traditional" approach of considering the whole task for *BRS* estimation. A better understanding of the advantages and limitations of using shorter epochs for *BRS* estimation might have an important impact on future research, in terms of methods used, interpretation of the results, and chosen length of the experimental tasks.

An issue not considered in Section 5.2 is the applicability of results obtained for the large cohort considered (179 subjects) to studies involving considerably smaller cohorts (e.g. about 30 subjects as in [50, 81]). In particular it could be argued that, when small cohorts are considered, the increased variance of estimates associated with the use of shorter epochs could result in a reduced capability (higher p-values) in detecting a significant rest-task change, even if this is not the case when the whole cohort is considered (as shown in the previous section).

The investigation presented in this section was carried out considering the same methods for *BRS* estimation used in Section 3: a parametric autoregressive method [39], a spectral FFT-based method [75, 100], and a time-domain sequence method [20, 75]. The investigation was focused in assessing and comparing the performance of the methods in estimating *BRS* change elicited by a psychophysiological task (Stroop test). In particular, for each method two different approaches were adopted: a *short-epoch* approach that considered just the initial part of the task for *BRS* estimation, and a *whole-task* approach that

considered the whole length of the task. The specific issues addressed in the investigation are:

- does the *short-epoch* approach result in significantly different estimates of BRS rest-task changes compared to the *whole-task* approach?
- is the *short-epoch* approach different from the *whole-task* approach in its capability of detecting a significant rest-task change in BRS, especially when a small cohort of subjects is considered?
- do any of the methods present specific limitations (or advantages) that indicate it as more or less appropriate for future investigations of BRS in psychophysiological tests?

5.3.1 Materials and Methods

Subjects and recordings

The data used is the same as in Section 3.2.

Estimates of BRS

Three well established BRS estimation approaches were considered: a sequence method (SEQ) [20], a spectral method (SPC) [100], and a parametric autoregressive method (AR) [39]. An overview of the methods is presented in Section 2.2.3, and the details of their implementation for this thesis are described in Section 3.2 (with the latter two methods, the mean gain of the $SAP \rightarrow RR$ frequency response, calculated over the LF, 0.5-0.15Hz, and HF, 0.15-0.5Hz, bands will be used as measures of BRS).

According to the results presented in Section 5.2, considering only a short epoch (100s) at the beginning of the Stroop task showed a larger rest-task difference in BRS compared to considering the whole length of the task. Based on this evidence, in this investigation an even smaller epoch (60s) at the beginning of the task was considered (indicated as $S1$ below). Regarding the initial rest period, the results presented in Section 5.2 showed also significant within-task changes in BRS indexes. Further exploratory analysis using BRS estimates obtained in consecutive $60s$ epochs suggested that the first minute of the rest period presents a significantly lower value compared to the four following minutes, which are characterized by minimal differences between each other (see Figure 5.4 for

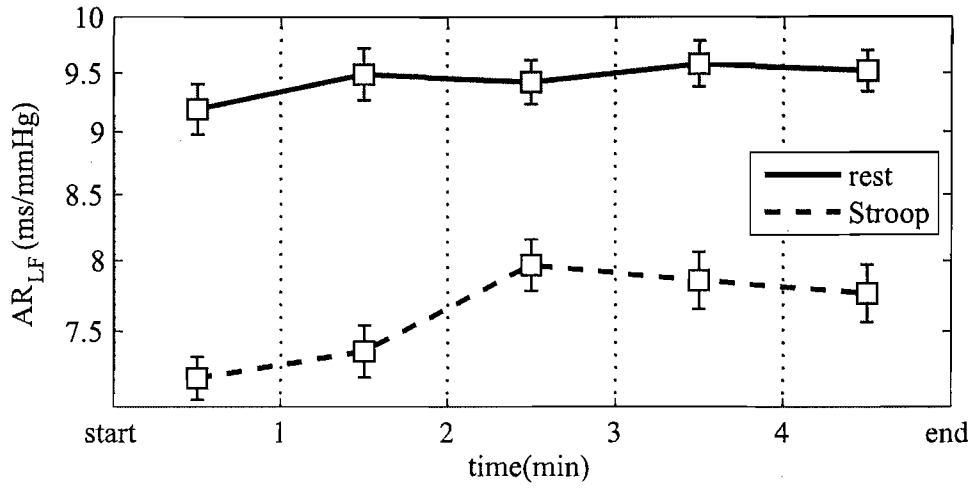


Figure 5.4: AR_{LF} geometric mean ($\pm$95% confidence interval for the mean) over the duration of the rest period and of the Stroop task (Note: y-scale is logarithmic)

an example). Hence, the last four minutes of the rest period ($R4$) were used to compute baseline value of BRS. Furthermore, the more "traditional" approach of considering the whole length of the rest period ($R5$) and of the task ($S5$) were used as a reference for the comparisons.

For the SEQ method the results will be indicated as SEQ^{R5}, SEQ^{R4}, SEQ^{S5}, and SEQ^{S1}, each corresponding to a different epoch considered for the estimation. Similarly for the SPC methods low-frequency (LF : 0.05 – 0.15Hz) estimates will be indicated as SPC_{LF}^{R5}, SPC_{LF}^{R4}, SPC_{LF}^{S5}, and SPC_{LF}^{S1}, and high-frequency (HF : 0.15 – 0.5Hz) estimates will be indicated as SPC_{HF}^{R5}, SPC_{HF}^{R4}, SPC_{HF}^{S5}, and SPC_{HF}^{S1}. For the autoregressive (AR) approach, the results will be indicated respectively as AR_{LF}^{R5}, AR_{LF}^{R4}, AR_{LF}^{S5}, and AR_{LF}^{S1}, for the LF estimates and AR_{HF}^{R5}, AR_{HF}^{R4}, AR_{HF}^{S5}, and AR_{HF}^{S1} for the HF estimates.

Comparison of the methods

The overall aim was to compare the use of specific short epochs ($R4$ and $S1$) to the use of $R5$ and $S5$, for all the BRS estimation methods presented. In particular, it was tested wether the use of $R4$ and $S1$ improves the capability of each method to detect a rest-task change, and allows a significant rest-task change to be detected even in small samples of subjects. In order to do so, two statistical tests were used: a paired t-test, and a resampling test.

The paired t-test was used to investigate the performance of the different methods in detecting a significant rest-task change in BRS. It was carried out between the log-transformed estimates of BRS for rest and stress for each method. The log-transformation was applied due to the skewed distribution of the data.

The resampling test was used to test the ability of the different methods to detect a significant BRS response to stress even in small samples of subjects. The test was structured in the following way. A thousand random subsets of 30 subjects were extracted from the set of 179 recordings. For each subset paired t-tests between the log-transformed estimates of BRS for rest and stress were carried out. The percentage of tests reaching statistical significance for a specific method is an index of the ability of the method to detect the stress-response even in small samples of subjects. In other words, this percentage represents an estimate of the probability that the specific method will detect a significant rest-task difference for a set of 30 subjects. Percentages were compared between different methods and the significance of the differences was estimated using the McNemar test. Similar tests were also carried out using subsets of 10 and 20 subjects.

The agreement between the methods in estimating BRS was investigated using Pearson's correlation, and Bland-Altman plots.

All statistical tests were considered significant for $p < 0.05$.

5.3.2 Results

The use of $S1$ and $R4$ with the SEQ method presented strong limitations. As shown in Figure 5.5, for many subjects these two epochs resulted in few (or no) suitable sequences of rising or falling SAP and HP for estimating BRS (for a definition of suitable sequence see Section 2.2.3). In particular, for $S1$, 39% of the subjects had no suitable sequences. Furthermore, if the assumption is made that the presence of 3 sequences is a minimum requirement for a reliable estimate using SEQ, just 12% of the subjects satisfied this. For $R4$ the problem is similar, even if less dramatic (23% of subjects did not match the requirement). For this reason the use of $R4$ and $S1$ windows with SEQ was not considered in further investigations, and results related to the use of SEQ_{S1} and SEQ_{R4} are not reported. Furthermore, Figure 5.5 shows that even when using $R5$ and $S5$ there is a considerable number of subjects with very few suitable sequences for estimating BRS with SEQ: respectively, 15% and 33% of the subjects present less than 3 sequences suitable for estimation.

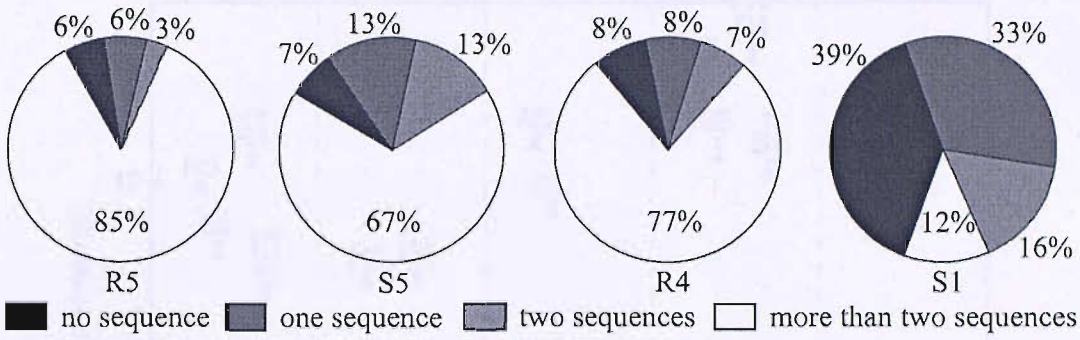


Figure 5.5: Percentage of subject presenting zero, one, two, or more than two suitable sequences for BRS estimation, for different epochs

BRS change between rest and stress

Figure 5.4 shows the presence of dynamics in BRS estimates during both the rest period and the Stroop task (geometrical mean $\pm 95\%$ confidence interval of the mean for estimates of AR_{LF} using consecutive epochs of $60s$). In Figure 5.6, the mean BRS estimates for all the methods are reported (geometrical mean $\pm 95\%$ confidence interval). As expected from the literature, the decrease in BRS caused by stress is evident for all methods, the SPC estimates are higher than the AR estimates [4], and at rest the HF estimates are bigger than the LF ones [75]. For all methods, the mean estimates using $R4$ are always bigger than the ones using $R5$, and the mean estimates using $S1$ are always smaller than the ones using $S5$, without any appreciable difference in the standard errors.

In Figure 5.6 the results of paired t-tests comparing the use of different time windows are reported. For both SPC and AR methods, the difference between using $S1$ and $S5$ is highly significant, both for LF and HF. Furthermore, the difference between using $R4$ or $R5$ is small but significant, except for the case of AR_{LF}.

Figure 5.7 shows that independently of the method used a significant rest-task change is detected. However, the use of the combination $R4 - S1$ results in a significatively bigger estimated rest-task difference (reported in the figure as percentage reduction from rest value) compared to the use of $R5 - S5$. This without an appreciable loss of significance in the estimate of the rest-task decrease ($p< 0.001$ always). AR and SPC produced similar results in terms of estimated difference between rest and stress for LF, but for HF AR estimates are consistently bigger. AR_{HF} using $R4 - S1$ produced the largest results in term

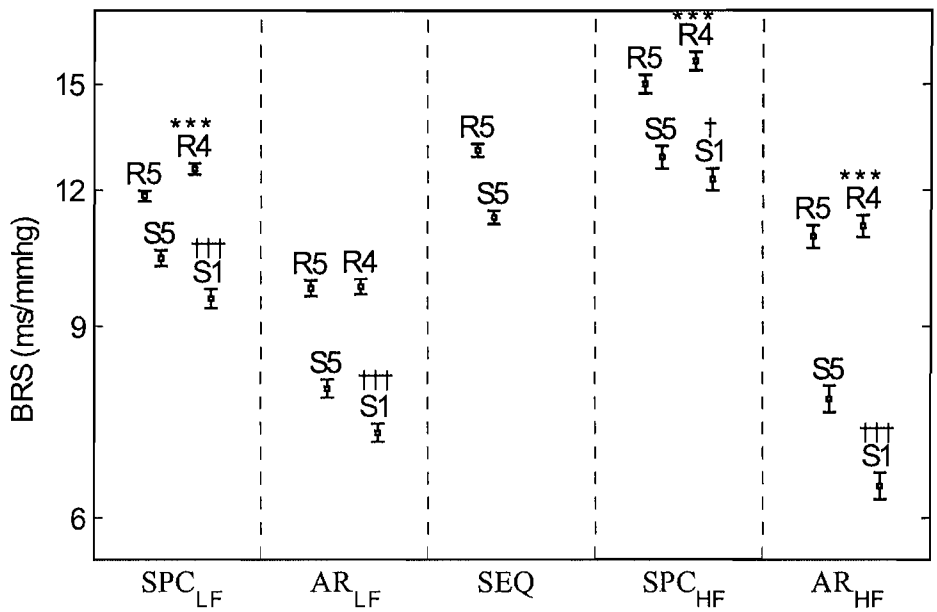


Figure 5.6: geometrical mean (±95% confidence interval) of BRS estimates using different methods and epochs (note: the scale is logarithmic). Difference against $S5$: †††p< 0.001, †p< 0.05; difference against $R5$: ***p< 0.001

of rest-task difference.

Resampling tests

The results of the resampling tests using subsets of 30 subjects are reported in Figure 5.8. The use of the combination $R4 - S1$ consistently enhances the probability of detecting a significant rest-task difference in BRS estimates for small samples of subjects. For AR_{HF}, the detection level is 100% for both $R5-S5$ and $R4-S1$, so no difference exists between the two approaches. For all the other methods, the McNemar test showed a significant ($p < 0.001$) improvement when using the shorter epochs, with an increase between 8% (AR_{LF}) and 39% (SPC_{LF}) in detected significant rest-task changes. Resampling tests using subsets of 20 and 10 subjects (results not reported) showed that the performance of all methods decrease when smaller subsets are used, and that the enhancement due to the use of $R4 - S1$ is more evident for smaller subsets. AR_{HF} produced the best results overall in terms of maximizing the number of significant rest-task changes detected, and SEQ produced the worst.

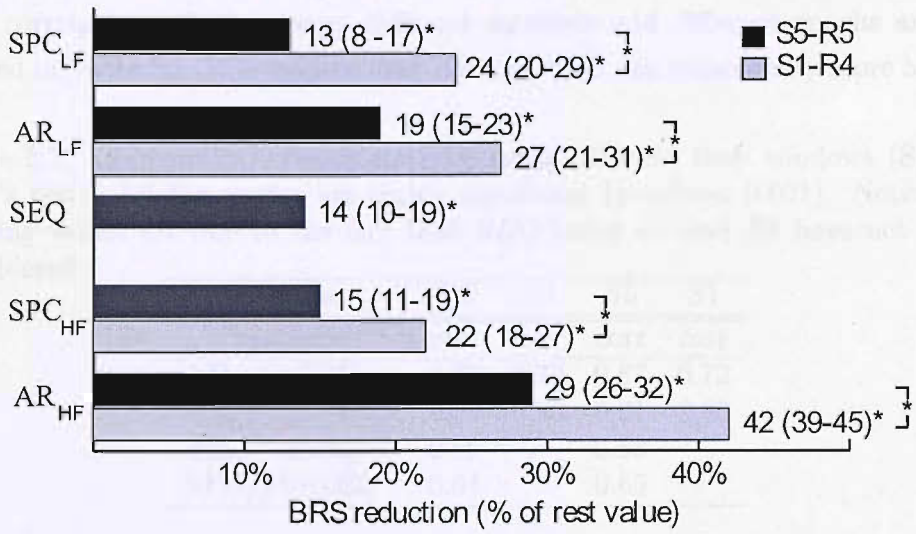


Figure 5.7: Rest-task decrease in BRS (95% confidence interval). Significance of rest-task difference and of difference between $S5$-$R5$ and $S4$-$R1$: *p< 0.001

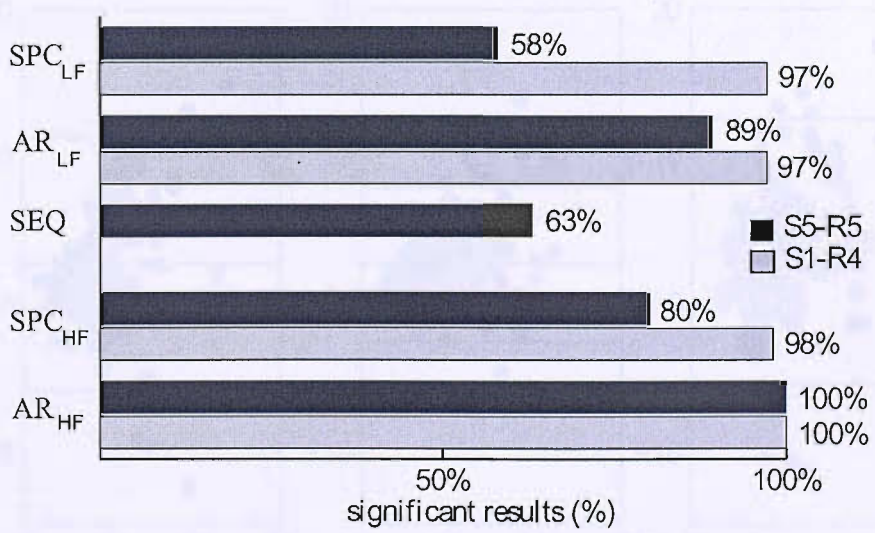


Figure 5.8: Resampling test: probability (in percentage) of finding a significant rest-task difference in BRS if a random subset of 30 subjects is taken from the cohort

Agreement between methods

The correlation values between different methods and different epochs are reported in Table 5.1. It is evident that AR and SPC are consistently more highly

Table 5.1: Correlation between methods using different time windows (Spearman's test). All the results are highly significant (p-value< 0.001). Note: the missing values are due to the fact that SEQ using $S1$ and $R4$ have not been considered

window	$R5$	$R4$	$S5$	$S1$
methods	corr	corr	corr	corr
AR_{LF}VsSPC_{LF}	0.79	0.79	0.87	0.72
AR_{HF}VsSPC_{HF}	0.94	0.96	0.91	0.80
AR_{LF}VsSEQ	0.66		0.63	
SPC_{LF}VsSEQ	0.64		0.65	

correlated than either with SEQ. As expected, using the shorter epoch $S1$ instead of $S5$ reduces the correlation coefficient, while the use of $R4$ instead of $R5$ has a minimal impact. The correlation values for the subsets of estimates during rest are similar to those in the tasks.

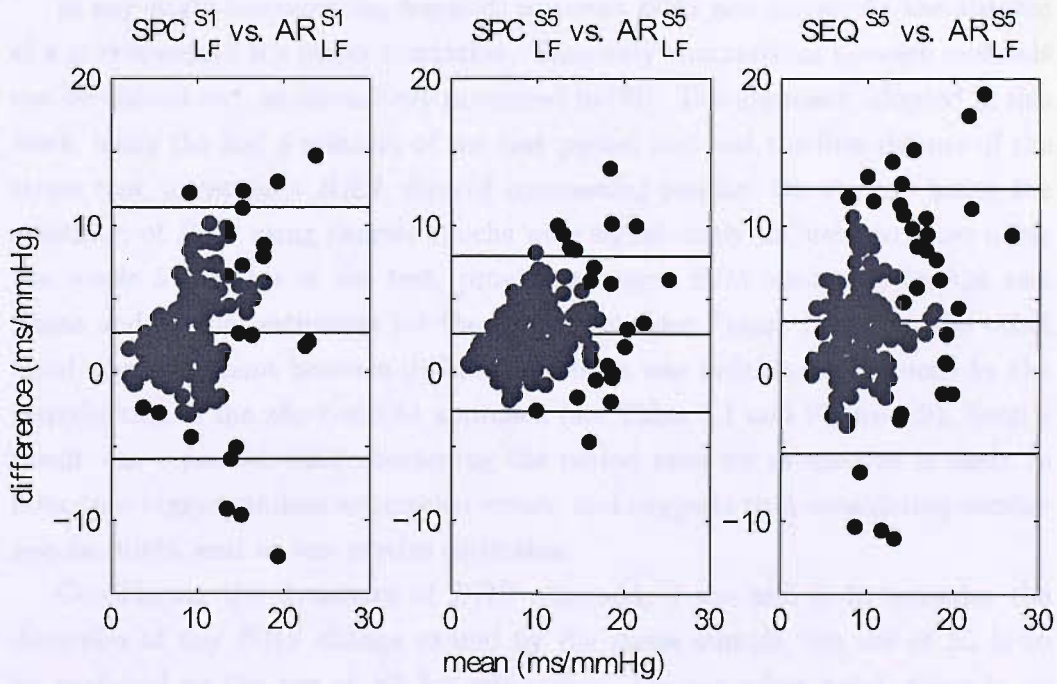


Figure 5.9: Examples of Bland-Altman plots for different combinations of methods and different time windows. Lines represent mean ± standard deviation

Figure 5.9 shows three examples of Bland-Altman plots for different combinations of methods and time windows. In particular, the central plot (SPC_{LF}^{S5} vs AR_{LF}^{S5}) suggests that the absolute difference between AR_{LF} and SPC_{LF} is proportional to the mean value. Comparing the three plots, it seems that the agreement between AR_{LF}^{S1} and SPC_{LF}^{S1} is worse than the agreement between AR_{LF}^{S5} and SPC_{LF}^{S5} (as expected from the reduction in correlation coefficient), but it is comparable (if not better) than the agreement between AR_{LF}^{S5} and SEQ^{S5}. Bland-Altman plots for estimates considering the rest phase or the HF (not shown) show similar characteristics.

5.3.3 Discussion

It can be argued that use of Portapres-derived HP series instead of EGC derived series is not ideal for estimating BRS indexes. However, it has been shown that HRV estimates derived from ECG and AP are practically equivalent for a range of experimental setups including psychological stress-task [80]. Furthermore, other published studies have successfully used Portapres-derived HP series for BRS estimation [43, 81].

In any study investigating methods to assess BRS non invasively, the absence of a gold standard is a major limitation. Thus only comparisons between methods can be carried out, as extensively presented in [75]. The approach adopted in this work, using the last 4 minutes of the rest period and just the first minute of the stress task to estimate BRS, showed contrasting results. On the one hand, the estimates of BRS using shorter epochs were significantly different to those using the whole 5 minutes of the test, providing bigger BRS estimates for the rest phase and smaller estimates for the stress task (see Figure 5.6). On the other hand, the agreement between different methods was indubitably reduced by the introduction of the *short-epochs* approach (see Table 5.1 and Figure 5.9). Such a result was expected, since shortening the period used for estimation is likely to introduce bigger random estimation errors, and suggests that considering shorter epochs might lead to less precise estimates.

Considering the dynamics of BRS observed, if the aim is to optimize the detection of any BRS change caused by the stress stimuli, the use of $S1$ is to be preferred to the use of $S5$ for estimation. On the other hand, there is no evidence supporting the idea that $S1$ is the most informative epoch to study when assessing the BRS response to psychological stress tasks, so that the last 4 minutes of the stress task can be ignored. The results showed that the selection

of the time window used for the estimation of BRS has a strong effect on the estimate, as strong as (or stronger than) the selection of the method used for estimation (SEQ, SPC, or AR). The use of the whole 5 minutes of the stress task for BRS estimation evidently obscures considerable within-task variations of BRS, hence discarding potentially useful information. Furthermore, it seems likely that the last 4 minutes of the stress task contain information about the adaptation of the subject to the stimuli, and deserve further investigation. The use of multiple short epochs for the analysis of BRS dynamic during stress-tasks appears a promising means to investigate this complex issue.

Regarding the difference between the use of $R4$ and $R5$, $R4$ produced significatively even if not very much larger estimates, but it seems evident that this difference had negligible impact on estimated rest-task difference, compared to the impact of considering $S1$ instead of $S5$. However, in future studies using shorter rest periods, the impact might be increased.

Despite an expected increase in random estimation errors given by the use of shorter periods, the t-tests showed that use of $R4 - S1$ allows detecting a difference between rest and stress without any loss of significance compared to $R5 - S5$. The resampling test gave strong evidences that the use of $R4 - S1$ enhance considerably and significantly the ability of the BRS estimation methods to detect a significant difference between rest and stress. Thus the use of $R4 - S1$ provides greater sensitivity to rest-task changes, allowing for smaller sample sizes to be used.

It is worth noting that, among all the methods presented in this section, those using HF for estimation produced the best results in terms of maximizing the rest-task difference and in detecting a significant difference between rest and stress for small samples of subjects. However, "the relative contribution of the arterial baroreflex to this HF component is still not fully accepted" [73]. In particular, Badra et al. suggested that "the close correlation between systolic pressures and R-R intervals at breathing frequencies reflects the influence of respiration on arterial pressure and R-R interval rhythm generators and not baroreflex physiology" [3], in agreement with the results and conclusions of other previous works [29, 34]. This would imply that BRS estimates in the HF band are probably not a reflection of baroreflex modulation. However, further investigation appears required in this matter.

Regarding the SEQ method, the presented results raise strong doubts regarding the usefulness of this method to estimate BRS using short epochs (see

Figure 5.5). It is important to notice that even using the whole 5 minutes of the stress task, there are still a 33% of the subjects with less than 3 sequences suitable for BRS estimation with the SEQ method. This strongly suggests that the SEQ method is probably not suitable for BRS estimation for those tests involving psychological stress and dynamic changes in BRS during the tasks.

5.4 Impact of within-task changes on the investigation of correlations between cardiovascular indexes and weight at birth

Based on the evidence of significant within-task changes in the cardiovascular indexes presented in the previous sections, an analysis of correlations with weight at birth was carried out with the same modality applied in Chapter 3, but considering three consecutive shorter epochs within each stress task (length of 100s) for the estimation of the indexes, and compared with the results obtained considering the whole length of each task.

The results are shown in Table 5.2 (only for the female subjects; for the males no significant correlation was found, in accordance with the results shown in Chapter 3 considering the whole length of the tasks). The correlation found considering the whole task are still present when considering a short epoch in all cases (except for HR_{inc} for the mirror tracing tasks, for which however the results are almost significant, $p < 0.1$). In most of the cases similar correlations were found independently of the short epoch considered. This supports the suggestion reported above that in many cases the use of relatively short tasks is sufficient for studies investigating cardiovascular indexes.

Further analysis was carried out to test if correlation exists between birth weight and the change in cardiovascular indexes between two of the short epochs considered within each task, in order to investigate the impact of fetal growth on the dynamics of the response to psychophysiological tasks. No significant correlation was found. A plausible explanation of this result is that, even if birth-weight is correlated with the overall response to stressors (as shown in Chapter 3), the relative trend of the pattern of the response is influenced more by other factors (e.g. respiration, lifestyle- and health-related).

Table 5.2: Correlation between cardiovascular indexes and weight at birth during different epochs of the tasks, for women only (no significant results found for men). *A*, epoch 0-100s; *B*, epoch 100-200s; *C*, epoch 200-300s; *TOT*, whole length of the task, 0-300s (as used in Chapter 3).

	Rest				Stroop			
	A	B	C	TOT	A	B	C	TOT
SAP	-0.19	-0.16	-0.09	-0.16	-0.35**	-0.32**	-0.30*	-0.33**
SAP_{inc}	-	-	-	-	-0.30*	-0.26*	-0.23	-0.32**
$SAPV_{LF}$	-0.22	-0.04	-0.30*	-0.24**	-0.45***	-0.37**	-0.38**	-0.45***
HR	0.06	0.06	0.08	0.05	-0.16	-0.08	-0.05	-0.13
HR_{inc}	-	-	-	-	-0.28*	-0.20	-0.16	-0.31*
HRV_{LF}	-0.10	0.00	-0.01	-0.01	0.00	-0.07	0.07	0.00
HRV_{HF}	0.10	0.19	0.15	0.17	0.29*	0.25*	0.28*	0.27*
AR_{LF}	0.16	0.06	0.04	0.10	0.45***	0.24*	0.32**	0.39***
SPC_{LF}	0.19	-0.03	0.16	0.12	0.31*	0.16	0.39***	0.34**
AR_{HF}	0.30*	0.25*	0.21	0.27**	0.42***	0.41***	0.37**	0.42***
SPC_{HF}	0.24*	0.22	0.22	0.20	0.48***	0.28*	0.37**	0.44***

	Mirror				Speech			
	A	B	C	TOT	A	B	C	TOT
SAP	-0.30*	-0.33**	-0.33**	-0.29*	-0.32**	-0.37**	-0.31*	-0.35**
SAP_{inc}	-0.24*	-0.26*	-0.25*	-0.29*	-0.27*	-0.31*	-0.24	-0.34**
$SAPV_{LF}$	-0.38**	-0.35**	-0.31*	-0.37**	-0.22	-0.13	-0.24	-0.24
HR	-0.12	-0.02	-0.06	-0.08	-0.11	-0.02	0.02	-0.07
HR_{inc}	-0.23	-0.12	-0.18	-0.25*	-0.18	-0.09	-0.05	-0.17
HRV_{LF}	-0.06	-0.06	0.09	0.00	0.15	0.07	0.03	0.11
HRV_{HF}	0.19	0.08	0.20	0.11	0.26*	0.17	0.16	0.22
AR_{LF}	0.13	0.19	0.25*	0.24	0.30*	0.19	0.16	0.28*
SPC_{LF}	0.19	0.26*	0.44***	0.25*	0.47***	0.11	0.18	0.20
AR_{HF}	0.22	0.23	0.35**	0.28*	0.29*	0.21	0.25*	0.29*
SPC_{HF}	0.31*	0.27*	0.43***	0.26*	0.30*	0.22	0.26*	0.25*

5.5 Clustering methods for characterization of within-task dynamics: an exploratory investigation

In Section 5.2, the presence of average within-task changes in several cardiovascular parameters was shown. However, figure 5.1 clearly demonstrates high inter-individual variability in the pattern of cardiovascular indexes during the stress tasks. One possible explanation of at least part of this variability is that groups of subjects with different patterns of response might be present. If consistent groups are present, it might be hypothesized that these are linked to fetal growth (birth weight), given the initial focus of the current research project. An exploratory analysis of the presence of groups of subjects presenting different patterns of response was performed for HRV indexes. Following the example of previous work in biosignal pattern analysis [33], clustering techniques were chosen as means to identify groups, for the following reasons:

- clustering methods automatically group the data into classes or clusters based on a set of parameters given by the user, so that objects within a cluster have high similarity in comparison to one another, but are very dissimilar to objects in other clusters [51];
- there is no limitation regarding the number, the nature, or the relationship between the parameters of the set to be used for clustering (as long as they are numerical). This property is of great interest for this application, where the large variation of responses among the subjects, which is well known [13], makes it difficult to determine an appropriate set of parameters (extracted from HRV_{LF} and HRV_{HF} sample-by-sample estimates) to investigate the differences in responses between individual subjects. In fact, clustering can be easily applied to a number of different combination of parameters (e.g. mean value of the pattern of response, derivative, difference between two peaks), to determine which is the most effective in evidencing groups with different responses. It is also possible to test if any of the parameters of the set is not significantly different between the clusters, and can hence be removed from the set.
- no a priori assumptions are needed regarding the pattern of responses in each cluster [51];

For pattern processing problems to be tractable, features should be first extracted from the signals [32], which then form the input to the clustering algorithms. The features were extracted from HRV sample-by-sample estimates in the following way for each stress task: the task interval (5 minutes of the task plus one minute preceding and following the task, 7 minutes in total) was divided into 7 overlapping blocks of $90s$ each. HRV_{HF} and HRV_{LF} estimates were normalized using the following equation:

$$P^{norm} = (P - \mu_P^{ST})/\sigma_P^{ST} \tag{5.1}$$

where P is either HRV_{HF} or HRV_{LF} in each of the $90s$ blocks, μ_P^{ST} is the mean value during the stress-task ST (calculated from the set of recorded signals), and σ_p^{ST} is the variance during the stress-task ST. For each subject in each block, the mean and the gradient (computed using linear regression) of normalized sample-by-sample estimates HRV_{HF}^{norm} and HRV_{LF}^{norm} were computed. Clustering methods were then applied to the 14 parameters thus extracted from each of the two signals in order to identify groups of subjects who show a similar pattern of responses.

Among the different methods of clustering [51], two well known and widely used clustering methods were adopted: a partitioning method (k-means) and a hierarchical agglomerative method. K-means is a heuristic algorithm that iteratively relocates the objects to clusters in an attempt to minimize the sum, over all clusters, of the within-cluster sums of objects-centroid distances (where the centroid of a cluster is the object of the cluster presenting the minimum sum of distances from the other objects of the cluster). The agglomerative hierarchical clustering is a bottom-up strategy that starts placing each object in its own cluster and then merges these atomic clusters into larger and larger ones until all the objects are in a single cluster or until certain termination conditions are satisfied. Here, two clusters are merged when their similarity is higher than all the other possible pairings (in our application, the similarity was estimated as the average distance between the elements of the two clusters). For both methods Euclidean distance was used as the measure of the distance between objects. In our preliminary tests, k-means clustering proved to be the best one in creating consistent groups of subjects, and hierarchical clustering the most effective in identifying the subjects who were not part of a consistent group. In the following section, an exploratory analysis using clustering methods is presented.

Exploratory analysis: clustering of the HRV_{HF} response to the Stroop task

For this analysis, the Stroop task of the experimental protocol presented in Section 3.2 was considered, and HRV_{HF} was selected as the index to use for the exploratory analysis. The left column of Figure 5.10 reports the result of clustering the parameters extracted from HRV_{HF} for the Stroop test. After several attempts it was decided to cluster the dataset into 5 groups, since visually this choice appeared to provide a good compromise between consistency within clusters and the number of clusters: a lower number of clusters appeared to fail in effectively grouping subjects with similar pattern of response, and the use of a bigger number of clusters resulted in multiple clusters with similar pattern of response, differing just in minor features.

Considering Figure 5.10, the presence of a trend in clusters 1 and 4 is evident, in accordance with the expected average response [17, 27]: HRV_{HF} decreases at the beginning of the test, maintains a low value during the test and increases again at the end of the test. Clusters 2 and some elements of clusters 3 and 5 show a rather surprising peak during the stress-response. All clusters show considerable dispersion, and some of the responses do not fit well into any of the clusters, and may be considered as "outliers". In order to identify these "outliers" that do not fit well with the general pattern, hierarchical clustering was then applied. The identified "outliers" (objects that, after hierarchical clustering, belong to clusters made of only one or two elements) were then removed from the data-set (see Figure 5.11), and k-means clustering was repeated. The resulting clusters (right column of Figure 5.10) show somewhat greater consistency than before. The elements of Cluster 1 show a response that is fundamentally constant throughout the task, opposed to Clusters 3 and 4, that show respectively a progressive decrease and increase, suggesting the presence of smooth within-task adaptations in the cardiovascular reaction. The other clusters, including those produced from the outliers, evidence considerable within-task fluctuations in HRV_{HF}, suggesting the presence of fast within-task adaptations in the response.

Based on the identified clusters, an investigation was performed to test if significant differences in average birth-weight exists between the subjects belonging to different clusters (using Student's t-test). The result was that the hypothesis of a null difference could not be rejected. This is in agreement with the lack of significant correlations between within-task changes in cardiovascular indexes and birth weight reported in the previous section, suggesting that the impact of

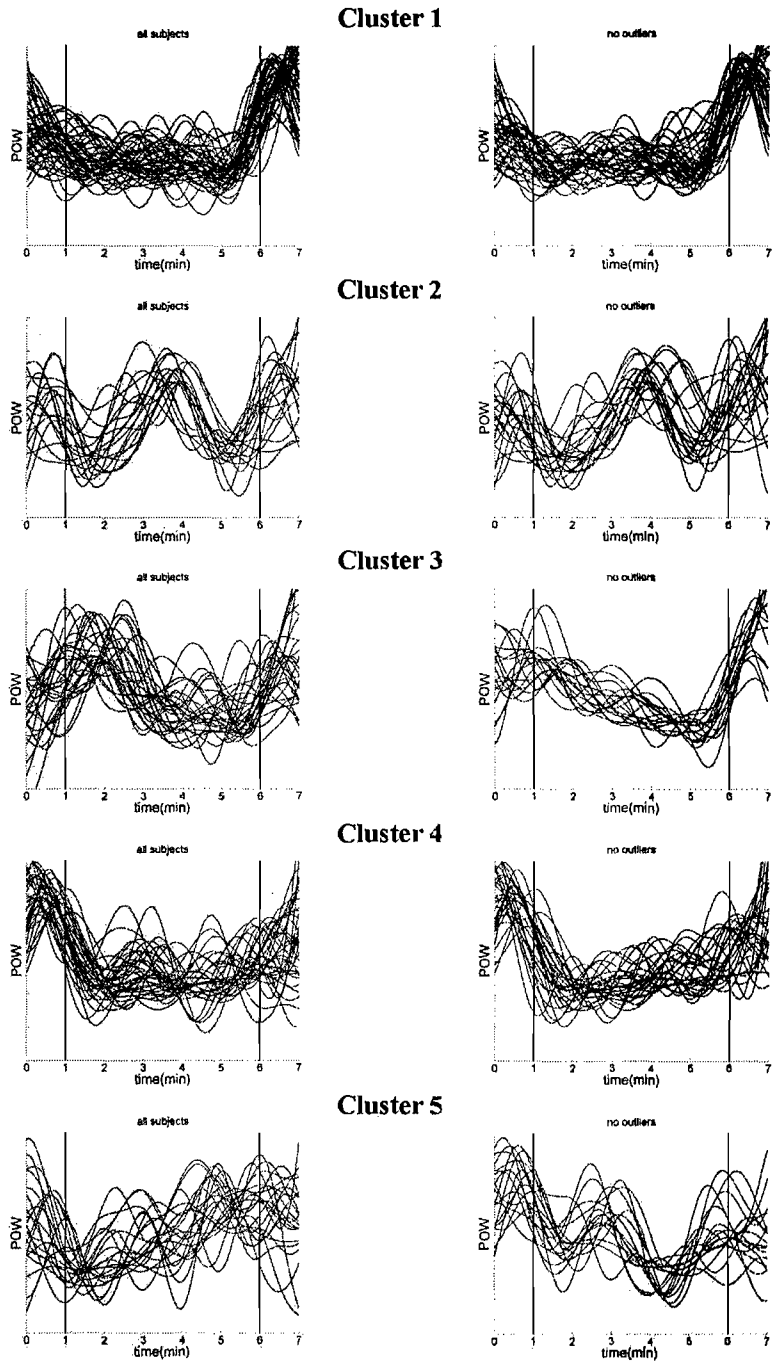


Figure 5.10: Clustering of the responses to the Stroop test, using normalized HRV_{HF} estimates: five clusters using k-means for all subjects (left column) and excluding the outliers (right column). (Power scale is not reported since the magnitudes are normalized to make comparisons easier)

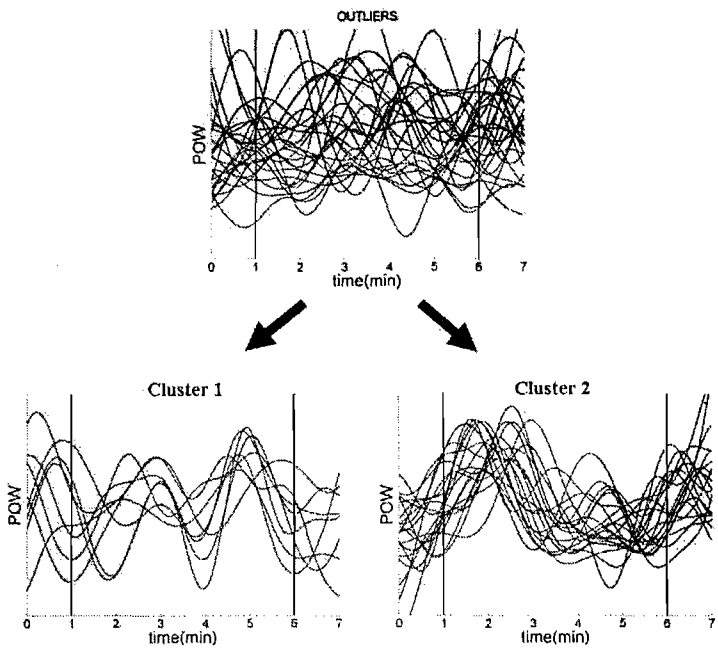


Figure 5.11: "Outliers"of the Stroop test (top). The two clusters (bottom) were obtained applying k-means method. Note that each cluster has a characteristic that differ consistently from the general pattern of response (and from the theoretical expectation of a drop of HRV_{HF} during the stress task with a recovery at the end): Cluster 1 presents a decrease of HRV_{HF} at the end of the task; in Cluster 2 there is an increase of HRV_{HF} at the onset of the stress task.

fetal growth is limited to the magnitude of the cardiovascular response to stress, without appreciable effects on the pattern of such response. From this point of view, it is possible that the choices of normalizing the signals as described in Equation 5.1 and of using average derivatives as parameters for clustering, could have been inappropriate. In fact, such choices were made to produce clusters based on the trend of the pattern during the response, rather than on the absolute magnitude of the response, which was already shown to be significantly correlated with birth-weight (see Chapter 3).

Considering the lack of significant results in this exploratory work (statistical analysis considering also HRV_{LF} indexes and the other tasks produced similar non-significant results, not shown), and doubts that further investigations would bring any significant improvement, this direction of research was suspended.

However, clustering has been proved successful in several aspects, which have the potential to be useful for future applications:

- it is an effective and versatile tool to explore if groups of subjects with common patterns of response are present in large cohorts;
- as it is able to automatically identify consistent patterns within the dataset, it can aid in the difficult task of defining specific indices that quantify individual responses;
- by automatically identifying "outliers" (as defined above), clustering can be helpful in the study of the characteristics of the subjects whose response is incompatible with the expected average response, or in removing these subsets from the cohort.

In particular, in view of the results presented in Section 5.2 that significant average dynamics of several cardiovascular indexes are present during baseline rest and stress tasks, clustering method could be applied to investigate to what extent all the subjects follow such average dynamics. Moreover, clustering methods could be used to test if average dynamics are the result of different groups of subjects presenting distinct patterns of response, rather than the result of an underlying common pattern for all the subjects characterized by high variability.

5.6 Conclusions

The results presented clearly indicate that when studying the cardiovascular reaction to psychophysiological tests the use of shorter epochs is a more appropriate approach than considering the whole tasks for the estimation of cardiovascular parameters. An approach using short epochs takes into account the fact that cardiovascular reaction to stress-tasks undergoes non-negligible within-task change, and allows such dynamics to be investigated. The use of whole-task averages of cardiovascular parameters neglects the presence of within-task dynamics of the parameters, resulting in considerably different estimates of rest-task changes than provided form specific short epochs within the tasks.

Furthermore, the use of a short epoch at the beginning of the task proved to be as effective as the use of whole-tasks averages in detecting statistically significant rest-task changes in cardiovascular parameters. In particular, for BRS indexes this led to significatively bigger estimates of rest-task changes compared to considering the whole period of the task, and provided a more sensitive measure of the response to stress, increasing the chance to detect a significant change when relatively small cohorts (e.g. 30 subjects) are considered. Hence for future studies,

if only the investigation of significant rest-task changes in the parameters is of interest, the use of relatively short tasks (e.g. 100s) could be recommended.

However, the correlation of weight at birth with cardiovascular indexes found considering multiple short epochs within the tasks is similar to that found considering the whole task, and the correlation with within-task changes in the indexes was far from significant. Hence, the overall message that seems to emerge is that in the investigation of the fetal origins of cardiovascular disease in adult life presented in Chapter 3, neglecting the presence of within-task dynamics did not have an important impact on the conclusions reached.

Chapter 6

Summary of the conclusions and final comments

The study of the reaction to psychophysiological tasks is a promising approach to investigate autonomic regulation, in particular how limbic and forebrain areas implicated in behavioural processes modulate the physiological reaction to psychological/cognitive challenges, and how psychological stress and psychological conditions can evolve to a physiological disease [13]. Indirect indexes of autonomic activation have been widely used in such investigations, thought to provide specific indications of the changes in activation of the various components of autonomic regulation [27, 73, 103]. However, factors other than autonomic modulation can strongly influence them and their estimation. This has been underestimated in past psychophysiological investigations, but should be taken into account when interpreting the results.

The work presented in this thesis provides a clear indication that, in the investigation of the autonomic cardiovascular regulation during psychophysiological tasks using cardiovascular indexes, two factors have an important, and possibly misleading, impact on the results:

- the between-tasks and inter-individual differences in respiratory patterns during psychophysiological tests, that can explain to a large extent the rest-task changes and inter-individual variability in cardiovascular indexes, especially when tasks involving speech are considered;
- the within-task dynamics in the cardiovascular reaction to psychophysiological tasks, the presence of which evidence that the cardiovascular reaction to psychophysiological tasks is a complex evolving physiological adaptation

to external stimuli rather than a shift from one steady-state (baseline rest) to another (task).

Since these two factors are intrinsic to the reaction to psychophysiological tasks, the experimental protocols and the methods used for the estimation and the analysis of cardiovascular indexes need to be modified or at least approached critically.

The importance of these two factors has been previously suggested [28, 68]. However, as far as the author is aware, a specific analysis for the case of psychophysiological tasks and for a range of cardiovascular indexes has not been carried out before. The present thesis provides a novel contribution in this direction, showing in particular that the possibility of assessing rest-task and inter-individual differences in autonomic activation through cardiovascular indexes for tasks involving speech is questionable, and that significant within-task changes involve the variability of HR and SAP and the interaction between the two (i.e. baroreflex mechanism), as well as HR and SAP itself.

The evidence presented should help and stimulate researchers in the psychophysiology field to approach more critically the interpretation of cardiovascular indexes in view of the respiratory pattern elicited by the tasks considered, and to adopt the analysis of within-task dynamics as a common practice in the investigation of cardiovascular indexes. With this in mind, the results presented in this thesis provide several guidelines for future investigations:

- respiration should always be monitored during psychophysiological tasks, to assess the dissimilarities in respiratory patterns between task and subjects, and its reflection on inter- and intra-individual differences in cardiovascular indexes;

- subjects presenting respiration in the low frequencies at rest (<0.15Hz) should be removed from the estimation of baseline values of cardiovascular indexes, since these subjects do not match a fundamental requirement for the interpretation of cardiovascular indexes as a reflection of autonomic activation (i.e. respiration limited to the high frequencies, >0.15Hz [17, 28]);

- for similar reasons, tasks involving speech should be avoided whenever possible, since they elicit broadband respiratory patterns resulting in considerable respiratory activity in the low frequencies;

- the use of consecutive short epochs within the tasks, as adopted in this thesis, appears to be a more suitable approach than considering the whole length of the tasks for the assessment of rest-task and between-tasks differences, since it allows detecting and describing the dynamics in the patterns of response within each task, resulting in a more detailed description of the phenomena, providing information that might be "averaged out" otherwise. Such an approach is not without limitations (e.g. time-resolution limited by the length of the epochs, increased number of parameters to analyze) but it constitutes a simple and, according with the results found, reliable approach to investigate the presence of significant changes elicited by psychophysiological tasks without the assumption of the reaction to the stimuli being constant.

- when only the assessment of rest-task changes in cardiovascular indexes is of interest, relatively short tasks (60-100s) can be used without any loss in terms of the capability to detect significant changes, with the evident advantage of shortening the experimental protocols compared to the common practice of considering tasks of 240-300s in duration.

It is advisable that future psychophysiological studies (and among them, those investigating the fetal origins of cardiovascular disease) will follow these guidelines. However, it appears clear from the results of this thesis that the modifications suggested for the experimental protocols and the analysis methods will not be sufficient to produce a reliable estimate of autonomic activation through cardiovascular indexes for every experimental condition. Evidence has been put forward [90] to criticize the use of HRV and $SAPV$ indexes as markers of autonomic activation, in particular about their lack of specificity (i.e. in many cases it is not possible to associate the change in an index to a change in a specific aspect of autonomic regulation). The results found in this thesis support this criticism, at least for the case of mild stressors, in particular for HRV_{LF} and $SAPV_{LF}$, previously advocated as markers of sympathetic activation [17, 103], whose reaction to a mental arithmetic task (expected to elicit a sympathetic reaction) was shown in this thesis to be either an increase or a decrease, depending on the presence or not of speech. This evidence complement and add to results of previous work, especially that of Bernardi et. al [11], in particular in showing the effect of respiration on SAP oscillations, and in evidencing the possible misleading effects for psychophysiological investigations. Furthermore, the presence

of within-task dynamics in cardiovascular indexes raises the question of which is the appropriate way to quantify the autonomic reaction (e.g. which feature of the dynamics, or which epochs to consider for the estimation). It is possible that the answer can vary, depending, among other factors, on the type of cognitive/behavioural mechanisms elicited by the task, and on the indexes considered (given the evidence shown in this thesis that different dynamics are present for different indexes).

The possibility of tackling these and other issues are strongly limited by the absence of gold standards to compare the estimated indexes with (e.g. direct measures of nerve sympathetic and vagal nerve traffic to the heart) since the invasiveness of direct measuring, the sedation required for such a procedure, and the discomfort caused to the subjects can possibly result in a perturbation of cardiovascular autonomic regulation that is large compared to that caused by the mild cognitive/psychological challenges that are under investigation [17, 109]. Hence, great caution in the interpretation of the results appears at present to be the most sensible approach.

However, considerable benefits are likely to come from shifting the focus of the investigations from the characteristics of SAP and HP (i.e. their variability) to the characteristics of the system originating them [96, 123], in particular considering respiration as a fundamental modulator rather than as a confounding factor to be removed or accounted for. The modeling of the respiratory modulation of HP and SAP carried out in this thesis is just one example (together with several available in the literature [71, 95, 102]) of this approach. Nevertheless, much work is needed in this regard to understand to what extent these models are correlated with autonomic activation in their characteristics, and able to disentangle, for psychophysiological tasks, the direct effect of cognitive/psychological processes from others (e.g. changes in respiratory patterns).

Furthermore, currently the research effort on cardiovascular autonomic regulation seems mainly focused on describing and understanding in detail the neurological control itself, and exploiting this knowledge for clinical investigations. Future research into the fundamental problem "why do mammals and other animal species [124] need cardiovascular oscillations?" (e.g. what is the physiological role/benefit of respiratory sinus arrhythmia [124] and of Mayer waves [65]) is desirable. The results can potentially provide a clearer understanding of the implication that modification from normality in cardiovascular oscillations can have on the health, consequently enhancing the prognostic value of their assessment,

through a more organic perspective in which changes in activation of the different components of the autonomic nervous system are the physiological substrate, rather the main focus of the attention.

Concluding, the impact of the studies presented in this thesis on the epidemiologic investigation regarding the fetal origins of cardiovascular disease, that was the starting point of this research project, deserve some comments. The fact that respiratory signals were not acquired for the epidemiologic investigation prevents the interaction between cardiovascular indexes and respiratory patterns from being assessed in the data available. The extent to which inter-individual and between-tasks changes in the indexes were caused by differences in respiratory patterns rather than by changes in activation of the two autonomic branches elicited by cognitive/psychological processes cannot therefore be reliably assessed. However, based on results presented in this thesis and in other works [2], several considerations can be made.

Not taking into account the modulatory effect of respiration on cardiovascular indexes could influence the result of the correlation tests between such indexes and birth weight reported in Chapter 3. However, statistical correction for the influence of respiration (e.g. considering respiratory-rate as a confounding factor in multiple linear regression) would probably have enhanced, rather than weakened, the significant results found. This speculation is based on the absence of any published hypothesis or result regarding a link between respiratory patterns and birth weight. Without such a link respiration is likely to add "noise" to the data, decreasing the correlation observed and leading to fewer statistically significant results. With this in mind, it is interesting to observe that strong correlations between LF cardiovascular oscillations and respiration are to be expected mainly in tasks involving speech (see Chapter 4), and hence the lack of a significant correlation between $SAPV_{LF}$ and birth-weight during the speech task, but not during Stroop and mirror tracing (performed silently), reported in Chapter 3, may well be a direct result of the confounding effect of respiration. On the other hand, if a significant correlation exists between respiration and weight at birth, this would open a new direction of investigation of the link between fetal origins and cardiovascular disease in adult life, and its implication for the respiratory system and its regulation.

Finally, it should be noted that the criticisms, reinforced in this thesis, about the interpretation of $SAPV_{LF}$, strongly suggest that correlation found between weight at birth and $SAPV_{LF}$ during the stress tasks cannot be taken as a reli-

able indication of reduced sympathetic activation during stress in subjects with low weight at birth. This limits the possibilities to infer specifically about the involvement of the sympathetic branch of the ANS in the link between fetal growth and cardiovascular disease in later life. However, even if a more conservative position is taken, that cardiovascular oscillations are just unspecific effects - and not reliable markers - of the cardiovascular modulation of the two autonomic branches, the results regarding SAP_{LF}, HRV_{HF}, and BRS indexes provide new evidence in favor of the hypothesis that fetal growth has a considerable impact on cardiovascular control in adult life, which is not limited to the absolute values of systolic arterial pressure and heart-rate, but involves also complex physiological mechanisms behind the modulation of their beat-by-beat oscillations, and of their interaction.

6.1 Future work

Among the issues that need further investigation pinpointed in the previous chapters, some are of critical importance and are likely to have a considerable impact on the way cardiovascular reaction to psychophysiological tasks is assessed and interpreted in the future. These are discussed below.

Reliable and robust estimation of baseline values for cardiovascular indexes in psychophysiological studies

As shown in Section 4.3, estimation of baseline values of cardiovascular indexes is affected by the presence in the cohort of subjects with long respiratory period (i.e. respiratory frequency within the LF band) at rest. A solution to overcome this problem, as suggested by several authors [23, 49], is to impose a fixed respiratory frequency to all the subjects, through paced respiration.

However, controlling respiration is a task requiring cognitive effort. Hence, it is likely that such a task is not appropriate for estimating baseline values for cardiovascular indexes [17]. However, there is evidence that pacing respiratory frequency itself does not have a significant impact on cardiovascular indexes compared to "normal" rest, at least for those subjects that present a baseline respiratory frequency relatively near to the paced one [93]. Also, it has been shown that using tasks involving minimally demanding cognitive effort as baseline resulted in conditions, named *vanilla* baseline conditions, "equal to or better

than resting baseline conditions using criteria of between- and within-baseline stability, amplitude and significance of responsivity, and generalizability between sessions on separate days" [62]. Since the level of cognitive effort required by pacing respiration is clearly limited, it can be hypothesized that paced breathing might result in *vanilla* baseline conditions as well.

Future work should be aimed at testing this hypothesis. Specific attention should be given to investigating if just controlling respiratory frequency is sufficient to limit respiratory-related inter-individual differences in cardiovascular indexes, since other characteristics of respiration (e.g. end tidal volume and P_{CO2}) might also play an important role in generating between-subject differences.

Alternative approaches to accounting for the influence of respiration on the indirect estimation of autonomic regulation: shifting the focus from signals to systems

As discussed above, the main solution proposed in the literature to limit the confounding effect of respiration on cardiovascular indexes is to limit the between-tasks and inter-individual differences through paced breathing [23, 49]. However, as noted in Section 4.5, this excludes *a priori* the use of tasks involving speech (that inevitably generate between-tasks and inter-individual differences in respiration), and might, when applied simultaneously to a psychophysiological task, result in a poor performance in both [99].

As previously mentioned, a radically alternative approach, that can potentially overcome such limitations, is to shift the object of the study from the characteristics of signals (in this thesis heart-period and systolic arterial pressure) to the characteristics of the system originating the signals [96, 123], including respiration as one of the inputs of such a system. The simple model of the respiratory modulation of HP and SAP adopted in this thesis is an example, but more sophisticated models (e.g. non-linear, or considering multiple inputs) are available in the literature [71, 95, 102].

Future research should investigate if the identified characteristic of the system (e.g. gain of a frequency response) offer a better correlation with changes in autonomic activation than the "traditional" HRV and $SAPV$ indexes. Procedures known to elicit autonomic changes (e.g. drug administration, posture changes, exercise, and acute stressors), should be considered, with the simultaneous monitoring of other indexes reflecting autonomic regulation (e.g. pre-ejection period,

and muscle nerve sympathetic activity). After this needed validation phase, this system-oriented approach might then be applied to psychophysiological tasks.

In this thesis, a method has been presented that, combining least-squares parameter estimation and Monte Carlo simulations, is able to provide for each identification (i.e. subject) confidence intervals of any function of the parameters of the model (e.g. confidence interval for the gain of the frequency response). This will be clearly useful for the aforementioned investigation (but potentially to a range of others also), to have a clear indication of the reliability of the estimates of the indexes considered in each subject (not just across groups), allowing the results to be treated with due caution. Thus it allows to investigate if individual subjects (not just the population averages) undergo significant changes between two experimental situations. Further validation of the method proposed is needed, including the use of simulated data to assess the reliability and the robustness of the confidence interval estimation, and a exhaustive comparison with alternative solutions (e.g. linearization of the problem of estimating confidence intervals [83]).

Psychological/cognitive interpretation of within-task and between-tasks dynamics

Further work appears needed in interpreting the dynamics of cardiovascular indexes observed during psychophysiological tasks. In particular, it would be of great interest to investigate if within-task changes in cardiovascular indexes during such tests parallel within-task changes in the pyschological/cognitive reaction. For example, since the reaction to cognitive stimuli is a combination of emotional and attentional responses [113], an intriguing hypothesis to test is that the evolution in time of cardiovascular reaction reflects the evolution in time of the balance between these two components.

Further research focused on assessing the relevance of within-task changes of cardiovascular indexes, as a means to clarify and to describe the physiological mechanism underlying the reaction to psychophysiological tasks, is desirable. This is aimed at a better understanding of how psychological/cognitive stimuli (e.g. stress) and pathologies (e.g. depression) may cause deteriorations of the physiological health of individuals. Aspects of critical importance will be to investigate how to appropriately describe the within-task dynamics of cardiovascular indexes (i.e. which is the most appropriate set of parameters), and to develop methods to track the psychological reaction during tasks (e.g. monitoring

of perceived challenge, stress, etc. within the tasks).

Also, of great interest would be to investigate the consistency of dynamics in repeated sessions of the same task (or after previous exposure to other cognitive/psychological stimuli) and the dynamics of the cardiovascular indexes after the completion of a task. This would help to gain a better understanding of the importance of carryover effects from previous tasks to the next for the indexes considered, and, in order to minimize their impact, to allow sufficient recovery time between tasks.

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Appendix A

A.1 *DES* extraction and artifact detection

The signal used to compute *HRV* and *SAPV* is the discrete event series (*DES*).

For *HRV* the DES_{HRV} is the series of the heart-period length versus time (indicated at the occurrence of the end of the period). The *DES* can be extracted from the *ECG* or the blood pressure signal in the following way. A series of fiducial points T_i is detected by automatic algorithms: for the *ECG*, the time of occurrence of the *R* wave peaks [87]; for the blood pressure signal, the pulse feet [47, 80]. Hence, each element of DES_{HRV} can be represented as a two components vector, the first component being the length of the heart period and the second component being the time at which the heart period ends:

$$DES_{HRV}(i) = (T_{i+1} - T_i, T_i) \tag{A.1}$$

For *SAPV* the DES_{SAPV} is the series of systolic-arterial pressure peaks amplitude versus time (indicated at the location of the peak). The DES_{SAPV} can be extracted from the blood pressure signal in the following way. A series of fiducial points F_i, which in this case are the systolic peaks in the blood pressure signal, and their time of occurrence T_i are detected by automatic algorithms (such an adaptation of [87], or simpler thresholding-based algorithms). Hence, each element of DES_{HRV} can be represented as a two component vector, the first component being the amplitude of the systolic arterial pressure peak and the second component being the time of occurrence of the peak:

$$DES_{SAPV}(i) = (F_i, T_i) \tag{A.2}$$

Artifacts in the *ECG* or in the blood pressure signal can lead the algorithms to detect some false fiducial points and to fail to detect real fiducial points.

Berntson et al. [15] showed that even a single artifact can contribute substantial spurious broadband power in estimates of heart period variability and that this bias may far exceed typical effect sizes in psychophysiological studies, which are the type of studies considered in this thesis. Hence, the algorithm developed by the same authors [16] was used to automatically detect and remove effects of artifacts from the DES. Furthermore, the DES series is manually edited, in order to check the artifacts detected by the automatic algorithm and to search and remove undetected artifacts.

Finally, the DES series were interpolated with a third order polynomial sampled at $4Hz$, as recommended by [27], in order to apply all those methods for index estimation that require equally spaced sampling, such as FFT-based or autoregressive methods.

A.2 HRV and $SAPV$ estimation using the variance method

As introduced in Section 2.2, the power of the resampled DES_{HRV} and DES_{SAPV} series in specific frequency bands ($LF : 0.05 - 0.15Hz$ and $HF : 0.15 - 0.5Hz$) are used as indexes of autonomic activation. A number of different methods are available for this purpose. For the estimates of HRV and SAP indexes in Chapters 3 and 5, a method based on band-pass filtering followed by estimation of power using a moving window was adopted, and implemented as follows. To band-pass filter the original signal $x(k)$ (sampled at $4Hz$, as described in the previous section) in the band $B = [f_1, f_2]$, two butterworth filters of order $n = 3$ were used, one to low-pass filter with cutoff frequency f_2, the other to high-pass filter with cutoff frequency f_1. Zero-phase filtering was performed for each filter by processing the input data in both the forward and reverse directions. The resulting signal $x_B(k)$ has precisely zero-phase distortion. The frequency response of this band-pass filtering for the LF and HF is shown in Figure A.1. From the band-passed signal $x_B(k)$, the power of $x(k)$ in the band B for the interval $[\overline{k}_1\ \overline{k}_2]$ can be estimated as:

$$POW_B\left[x(k)\right]|_{[\overline{k}_1\ \overline{k}_2]} = \frac{1}{\overline{k}_2 - \overline{k}_1 + 1} \sum_{k=\overline{k}_1}^{\overline{k}_2} \left(x_B(k)\right)^2 \tag{A.3}$$

This method presents the practically useful property that, once $(x_B(k))^2$ is com-

puted, estimation of power for a specific epoch is just a matter of computing an average in the appropriated interval. Furthermore, this can be computed in a computationally efficient way, filtering $(x_B(k))^2$ with a moving average filter (weighted or not). The resulting signal is an estimate of the variation of power over time, and was used for the study of transients or dynamics in the response to different experimental conditions, in Chapter 5 (Figure 5.1).

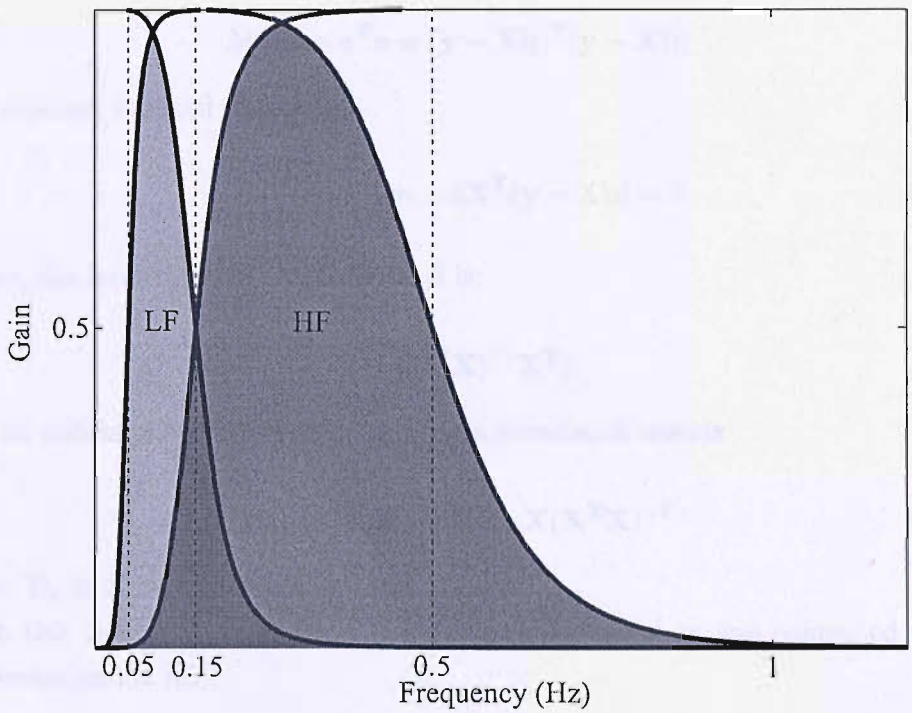

Figure A.1: Frequency response of the band-pass filters in the LF and HF bands described in Appendix A.2

A.3 Estimation of an FIR filter as an ordinary least-squares problem

The ordinary least-squares problem

The ordinary least-squares problem can described in matrix notation as [63]:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{e} \tag{A.4}$$

where $\mathbf{y}$ is an $N \times 1$ vector of the responses, $\mathbf{X}$ is an $N \times p$ matrix of the predictor variables, β is an $p \times 1$ vector of invariant parameters to be estimated, $\mathbf{e}$ is an $N \times 1$ error term with zero mean, N is the number of observations in the sample, and p is the number of parameters to be estimated.

The solution of the least-squares problem is the vector of parameters $\mathbf{b}$ that minimizes the mean square error

$$MSE = \mathbf{e}^{\mathbf{T}}\mathbf{e} = (\mathbf{y} - \mathbf{Xb})^T(\mathbf{y} - \mathbf{Xb}) \tag{A.5}$$

The solution is found imposing

$$\frac{dMSE}{d\mathbf{b}} = 0 \Rightarrow -2\mathbf{X}^{\mathbf{T}}(\mathbf{y} - \mathbf{Xb}) = 0 \tag{A.6}$$

Hence, the least-squares estimator of β is:

$$\mathbf{b} = (\mathbf{X}^{\mathbf{T}}\mathbf{X})^{-1}\mathbf{X}^{\mathbf{T}}\mathbf{y} \tag{A.7}$$

$\mathbf{b}$ is an unbiased estimator of β, and has a covariance matrix

$$\mathbf{C_b} = (\mathbf{X}^{\mathbf{T}}\mathbf{X})^{-1}\mathbf{X}^{\mathbf{T}}\mathbf{\Sigma_e}\mathbf{X}(\mathbf{X}^{\mathbf{T}}\mathbf{X})^{-T} \tag{A.8}$$

where $\mathbf{\Sigma_e}$ is the covariance matrix of the errors.

In this thesis the solution of the least-squares problem was computed using QR factorization [97].

The identification of the FIR filter

The application of the least-squares solution to the problem of identifying the coefficients of an FIR filter with p tabs, given an input and an output signals $x(k)$ and $y(k)$ of length N, is straightforward, taking

$$\mathbf{y} = \begin{bmatrix} y(p+1) & \dots & y(N) \end{bmatrix}^T$$

$$\mathbf{X} = \begin{bmatrix} x(p) & \dots & x(1) \\ \dots & \dots & \dots \\ x(N-1) & \dots & x(N-p) \end{bmatrix}$$

and β as the coefficients of the filter to be estimated.
The time series of the residuals is then computed as:

$$\mathbf{r} = \left[\; r(p+1) \quad \ldots \quad r(N) \; \right]^T = \mathbf{y} - \mathbf{Xb} \tag{A.9}$$

An estimate $\hat{\mathbf{C}}_\mathbf{b}$ of the covariance matrix of the coefficients can be computed from Equation A.8, substituting $\mathbf{\Sigma_e}$ with an estimate $\mathbf{S_e}$ obtained from the residuals, as the biased estimate of the auto-covariance matrix, whose elements s_{ij} are defined as:

$$s_{ij} = \frac{1}{N-p} \sum_{n=p}^{N-|i-j|} r(n)r(n+|i-j|) \quad \forall i,j = 1,\ldots,N-p \tag{A.10}$$

The biased estimate was preferred to the unbiased one because it results in $\mathbf{S_e}$ being positive semidefinite, which is necessary for the Cholesky decomposition introduced below. It is worth noting that, as can be seen from Equation A.6, the least-squares solution imposes:

$$\mathbf{X^T(y - Xb)} = 0 \Rightarrow \mathbf{X^T e} = 0 \Rightarrow \sum_{k=p+1}^{N} e(k)x(k-i) = 0 \quad \forall i = 1,\ldots p \tag{A.11}$$

Hence, the input and the estimated residuals are uncorrelated for lags between 1 and p. A direct consequence is that the total power (i.e. variance) of the output signal is the algebraic sum of the total power of the estimated residuals and the total power of the output predicted by the model (which is a linear combination of past values of the input).

Estimation of confidence intervals for gain and phase of the transfer function through Monte Carlo simulations

The variance of each coefficient of the filter can be estimated from $\mathbf{C_b}$ (the sample variance of the coefficients are the diagonal elements of $\mathbf{C_b}$) or for a linear combination of them. In fact, given a linear combination:

$$\mathbf{d} = \sum_i m_i b_i = \mathbf{mb} \tag{A.12}$$

the sample variance of $\mathbf{d}$ is:

$$s_\mathbf{d}^2 = \mathbf{m C_b m^T} \tag{A.13}$$

However, the most common mean to analyze a filter is through the gain G

and phase Φ of its frequency response H(f), which are non linear functions of the filter coefficients. In fact, while

$$\mathrm{H}(f, \mathbf{b}) = \sum_{k=1}^{p} b_k e^{-2\pi i k \frac{f}{f_s}} \tag{A.14}$$

is a linear function of $\mathbf{b}$ (f_s is the sampling frequency of the input and output signals),

$$\mathrm{G}(f, \mathbf{b}) = |\mathrm{H}(f, \mathbf{b})| = \sqrt{Re[\mathrm{H}(f, \mathbf{b})]^2 + Im[\mathrm{H}(f, \mathbf{b})]^2} \tag{A.15}$$

$$\Phi(f, \mathbf{b}) = \angle \mathrm{H}(f, \mathbf{b}) = -\arctan\left(\frac{Im[\mathrm{H}(f, \mathbf{b})]}{Re[\mathrm{H}(f, \mathbf{b})]}\right) \tag{A.16}$$

are evidently not.

In this case the linear solution A.13 can be applied using a linear approximation around the estimated values of G and Φ (for a detailed presentation of this method see [83]). However, there is no indication that this approximation is justified for a broad range of realistic situations, seriously undermining the reliability of the variance of G and Φ estimated through this method.

An alternative approach is based on Monte Carlo simulations, under the assumption that the identified coefficients of the filter are the sample mean of a multivariate normal distribution with covariance matrix $\mathbf{C_b}$ (which is equivalent to assume that the errors have normal distribution). Using a random number generator it is possible to produce N samples of a multivariate normal distribution with mean $\mathbf{b}$, using a modified Cholesky decomposition approach [117]. For each of these samples the gain and the phase of the transfer function are computed. The final results is a sample of N estimates of the gain and phase, and their histograms can be considered estimates of their sampling distribution. This can then be used to estimate the standard deviation or confidence intervals of gain and phase estimates.

A major limitation of this method is the computational load, since large number of simulations are required for reliable results. However, given the computational power of modern computers, this problem is negligible in many practical cases.

It is worth noting that this method is extremely flexible. In fact, once the N samples of the multivariate normal distribution are generated, they can be used to compute any function of the filters' coefficients, and to estimate their

statistical distribution. This appears highly desirable for practical applications: for example, once a feature of the transfer function is found to have clinical or physiological interest, a confidence interval can be easily estimated using this framework.

Statistical testing of the difference in gain and phase between two identified FIR filters

Given two identified FIR filters ("A" and "B"), a statistical approach can be applied to test the hypothesis H_0 that the difference between the estimated m_A and m_B is zero, where m is either the gain or the phase (or any non-linear function of the filter coefficients). The Monte Carlo simulations approach described above can be used to compute estimates s_A and s_B of the standard deviation of such distributions. Then, the difference is a normal distribution with mean $m_d = m_A - m_B$ and standard deviation $s_d = \sqrt{s_A^2 + s_B^2}$. Assuming that such a distribution is Guassian (if not, a transformation can be applied, e.g. logarithmic), the probability of the difference between m_A and m_B being zero is $p = F(0; |m_d|, s_d)$, where $F(x; \mu, \sigma)$ is the cumulative density function of the normal distribution with mean μ and standard deviation σ. The hypothesis H_0 of a null difference is rejected if $p < \alpha$, where α is the significance level chosen for the test (in this work α=0.05). Given the central limit theorem, the requirement of normality is less stringent, when large number of samples are considered. Alternatively, non-normality can be corrected for by normalizing using transforms.

The generalization of the test to the comparison of the group average between two paired groups $A = \{a1, ..., aK\}$ and $B = \{b1, ..., bK\}$ of identified FIR filters, is straightforward, substituting:

$$m_d = \frac{1}{K} \sum_{i=1}^{K} (m_{ai} - m_{bi}) \tag{A.17}$$

$$s_d = \frac{1}{K} \sqrt{\sum_{i=1}^{K} ({s_{ai}}^2 + {s_{bi}}^2)} \tag{A.18}$$

where K is the size of the groups.

A.4 Epidemiological data of the cohort considered in Chapter 3

In the following table the epidemiological data used for the statistical analysis presented in Chapter 3 is reported, together with baseline values of cardiovascular indexes.

Abbreviations adopted: *subj*, subject code; *bwt*, weight at birth; *bmi*, body-mass index; *inv*, investigator (three different investigators participated in the data acquisition); *mc*, phase of the menstrual cycle (L: luteal, F: follicular).

subj	age yrs	sex	bwt kg	bmi kg/m^2	inv	mc	SAP mmHg	HR bpm	$SAPV_{LF}$ mmHg^2	HRV_{LF} ms^2	HRV_{HF} ms^2	AR_{LF} $\frac{ms}{mmHg}$	AR_{HF} $\frac{ms}{mmHg}$	SPC_{LF} $\frac{ms}{mmHg}$	SPC_{HF} $\frac{ms}{mmHg}$	SEQ $\frac{ms}{mmHg}$
7	26.1	M	3.31	23	3		85.7	69.1	2.9	1640	2799	26.5	31.0	22.6	37.2	31.6
12	27	F	3.07	21.3	1	F	124.2	80.7	8.1	440	469	7.0	8.6	9.0	10.1	8.1
34	26	M	2.75	22.8	1		108.6	66.7	6.1	407	542	7.8	10.8	9.8	11.5	16.8
40	27	F	4.03	22.1	1	F	99.3	58.0	6.2	2447	1627	18.6	17.1	19.8	21.9	5.5
101	26.8	M	3.65	28.1	2		113.4	67.0	5.6	172	42	4.3	3.2	7.1	5.0	21.6
119	26.7	F	3	37.6	2	L	136.7	110.5	8.6	1327	1020	8.7	13.4	14.7	16.9	8.3
139	26.8	F	3.62	32.5	2		111.6	79.6	11.5	2032	2077	14.2	16.4	14.1	22.0	14.9
186	26.6	F	3.49	28.9	2	F	113.7	77.0	3.0	422	573	11.6	10.1	12.5	11.4	11.1
208	26.1	M	4.67	25	2		131.9	71.4	4.1	1175	885	13.7	12.1	22.8	15.5	14.3
228	26.1	M	2.85	27.7	2		132.2	70.1	12.3	524	648	6.2	9.1	8.0	11.3	8.2
231	25.8	M	2.77	23.9	3		119.7	86.3	7.8	680	350	9.1	5.9	9.5	8.9	12.5
250	26.6	F	3.28	27.6	2	L	102.3	79.0	9.2	699	1461	9.0	11.7	9.3	13.6	15.1
263	26.1	M	2.98	26	2		154.1	78.3	2.6	474	374	12.5	7.7	13.4	9.5	10.7
281	26.7	F	3.24	18.7	2	F	121.7	63.1	10.3	1491	1380	14.1	11.8	11.8	12.3	16.3
283	26.1	M	3.55	27.3	2		121.5	62.1	8.7	1582	1406	14.0	16.6	17.3	20.7	18.5
289	26.4	F	2.85	23.4	2	F	119.0	46.6	13.9	2218	3685	13.5	26.2	17.3	35.2	16.8
294	25.8	M	2.9	28.5	1		118.6	68.1	11.8	1254	374	10.8	10.9	15.4	12.6	14.0
324	25.6	M	4.27	26.8	3		124.7	64.2	7.2	2409	2011	13.8	13.4	15.8	22.1	14.6
339	26.4	M	3.69	25.4	2		152.4	78.0	7.0	800	889	15.8	16.2	10.0	19.0	14.6
344	26.4	F	3.05	27.2	2	L	116.5	54.5	21.4	8240	3911	20.1	24.0	22.1	27.0	23.6
346	25.6	M	3.3	27.2	3		108.0	86.0	5.6	650	1014	14.3	14.6	12.3	12.4	15.1
352	25.8	M	3.62	21.4	2		132.8	68.9	2.7	618	1174	8.9	10.4	13.7	15.5	14.5
357	26	F	3.15	18.6	2	F	125.1	71.9	11.0	1398	254	10.3	6.1	13.1	8.4	13.0
361	25.8	M	4.35	29.4	1		103.1	69.5	3.9	696	777	15.9	15.1	11.0	15.7	12.9
365	25.9	M	2.18	32.2	2		92.4	66.7	5.5	1443	3757	15.5	35.0	19.1	45.4	29.5
382	26.2	F	3.34	19.2	2	L	111.7	73.0	4.3	443	225	7.0	9.9	10.2	11.7	10.7
393	25.4	M	2.76	21.6	1		127.4	65.0	3.3	1379	1682	14.1	21.1	19.4	19.6	21.3
404	26	F	3.64	18.1	2	L	155.2	75.4	14.8	1561	304	8.9	7.2	11.9	10.5	11.8
422	25.9	M	4.25	28.8	2		101.3	64.3	11.3	1764	657	10.0	11.5	14.2	13.6	16.4
438	25.9	F	2.84	21.3	2		129.7	53.7	9.1	867	720	6.6	14.4	11.3	19.7	
460	25.5	M	4.21	20.6	3		105.7	67.8	5.3	1602	459	15.6	11.6	19.1	15.5	15.3
468	25.4	M	3.3	24.2	3		138.4	80.2	9.9	1282	1043	18.3	15.1	13.3	19.0	19.2
469	25.8	M	3.11	30.3	2		143.8	78.3	21.0	997	244	4.5	5.6	6.9	9.1	7.8
479	26.1	M	3.9	25.4	2		142.3	89.7	13.7	539	198	4.1	5.6	6.2	10.9	9.7
498	26.1	M	3.11	25.5	2		112.8	89.7	10.8	1580	320	11.3	9.9	11.4	12.9	10.6
503	26.7	F	3.38	20.4	2	L	110.1	74.0	9.4	1356	552	14.3	18.9	15.8	19.4	17.0
516	26.4	F	3.44	30.1	2	L	105.0	58.3	16.3	1136	394	6.3	6.9	8.9	10.6	12.7
519	26.8	M	4.03	25.1	2		135.6	66.2	7.0	1644	1843	12.6	20.7	15.1	22.9	26.5

subj	age yrs	sex	bwt kg	bmi kg/m^2	inv	mc	SAP mmHg	HR bpm	$SAPV_{LF}$ mmHg^2	HRV_{LF} ms^2	HRV_{HF} ms^2	AR_{LF} $\frac{ms}{mmHg}$	AR_{HF} $\frac{ms}{mmHg}$	SPC_{LF} $\frac{ms}{mmHg}$	SPC_{HF} $\frac{ms}{mmHg}$	SEQ $\frac{ms}{mmHg}$
526	25.8	M	2.74	28.8	2		135.5	52.4	6.2	2657	6125	19.4	41.4	22.1	58.7	29.0
528	26.2	F	4	32	2	L	121.6	73.2	7.1	1178	308	11.5	6.0	14.3	8.7	14.2
531	25.9	M	3.15	21.6	2		120.5	79.1	3.4	403	378	8.5	8.8	10.7	10.6	5.2
532	27	F	3.65	29.2	2	L	134.5	72.5	4.7	530	294	8.9	6.6	12.2	8.1	11.1
533	26.7	F	3.59	28.8	2	F	131.7	69.7	7.0	1169	336	13.2	9.7	14.2	11.2	16.8
537	26.2	F	3.75	23.1	2	F			11.5	1136	1085	8.2	19.8	13.1	24.2	10.0
544	26.7	F	3.19	27.8	2	F						6.3	8.3	9.0	10.9	19.0
551	25.6	M	2.96	25.6	1		120.5	59.2	5.2	666	487	8.8	11.7	9.1	12.9	12.3
555	26.7	M	3.5	27.9	2		141.6	72.9	2.9	438	463	9.7	7.5	11.2	7.0	9.6
556	26	F	2.76	23.4	2	L	139.0	82.3	3.5	327	245	6.8	6.7	8.5	10.1	12.3
559	26.8	F	2.9	26.2	2		139.8	81.3	8.9	1029	198	9.1	3.4	8.8	4.9	8.1
561	26.6	M	2.92	22.3	1		104.7	83.1	8.4	867	369	11.9	12.2	11.8	13.6	14.6
562	25.9	F	3.65	28.4	2	L	95.3	83.1	8.8	677	503	6.2	11.5	7.4	11.5	11.3
564	26.4	M	3.2	27.3	2		105.7	71.4	12.3	792	274	6.8	5.4	6.8	8.4	7.2
565	26.2	F	3.17	21.7	2	L	136.6	66.9	12.3	1300	273	8.9	10.7	10.3	13.4	7.6
568	25.7	M	3.57	20.4	2		112.9	76.8	16.1	266	123	3.0	3.6	3.5	4.1	5.3
574	26.4	F	2.8	24.1	2		111.8	75.7	7.7	1042	990	16.0	15.2	9.3	16.1	13.2
583	26.3	F	3.28	21.5	2	F	125.9	82.3	12.4	658	263	6.6	7.0	6.1	7.8	7.1
586	25.7	M	3.79	30.4	2		101.8	63.2	4.8	539	923	7.7	17.6	10.6	24.3	
591	26.1	F	3.64	33.1	2	L	118.1	64.0	5.4	1366	1751	28.8	25.1	20.3	26.6	17.5
594	26.1	F	3.12	20.6	2	L	154.0	69.6	19.3	2564	879	8.1	7.7	9.0	9.2	11.9
598	26.6	M	3.16	21.1	2		140.7	77.9	14.2	340	411	3.0	9.4	5.9	12.3	7.4
601	25.5	M	3.58	20.1	1		115.8	75.2	2.0	435	260	9.0	5.8	13.9	10.5	7.7
602	27	M	3.55	30.9	1		117.8	81.6	16.7	722	175	7.4	4.4	6.7	5.8	8.3
619	26.3	M	3.65	26.8	2		123.8	86.6	13.9	676	555	6.4	9.2	8.7	13.2	12.6
620	25.8	M	3.62	28.2	3		106.3	73.4	14.7	1566	803	8.0	13.4	9.6	14.7	14.5
636	26.7	F	2.72	24.5	1	F	144.7	86.5	23.5	2899	740	9.2	8.4	12.5	13.7	11.4
637	26.7	F	3	22.4	2	L	121.5	62.7	16.9	4008	2634	14.9	21.5	22.4	30.1	20.2
644	25.9	M	4.54	20.2	2		148.3	63.2	26.6	5285	2257	13.6	14.4	16.7	18.5	16.4
655	26.5	F	2.9	26.8	1	F	151.4	71.4	3.2	535	465	9.3	8.7	12.3	11.1	11.6
658	26.2	F	3.27	20.5	2		122.9	57.0	11.0	1812	1493	14.6	18.6	20.7	25.4	23.2
659	26.3	F	3.34	40.9	2	L	126.4	79.7	8.9	530	129	5.7	8.5	8.0	12.8	9.3
667	26.4	F	3.47	27.2	2	F	147.5	66.1	25.5	1934	770	8.1	9.3	13.6	13.7	12.9
672	26	M	3.1	27	1		118.0	72.3	9.5	739	308	6.8	7.9	10.0	9.4	9.8
673	25.8	M	3.92	30.7	2		124.9	78.8	3.6	613	754	14.3	11.5	12.4	13.0	12.0
675	25.9	M	3.48	23.9	2		141.4	73.9	14.0	446	297	6.9	8.2	6.0	11.2	10.1
689	26.6	M	3.63	28.1	1		119.8	88.3	2.5	414	377	6.3	9.2	9.1	13.6	
694	26.6	M	2.32	33.4	2		118.4	62.2	9.9	4527	8077	23.8	39.2	26.2	45.0	36.2
697	25.9	M	1.56	24.2	3		126.5	78.3	4.9	228	341	5.2	10.0	6.1	10.1	10.3
705	26.3	M	3.92	22	2		127.8	70.4	2.7	191	195	6.5	6.9	7.3	8.3	9.6
707	25.6	M	3.94	19.7	2		115.1	63.1	4.4	1677	4232	17.3	22.0	20.3	32.9	21.3
709	25.7	M	3.25	30.1	2		139.8	79.7	9.5	692	448	6.6	8.8	8.5	11.2	9.5
717	26.6	M	3.65	27.6	2		114.9	78.6	12.0	340	579	4.8	9.2	5.8	9.2	7.9
721	26.3	M	3.78	27.1	2		114.6	87.3	3.6	478	495	9.0	10.9	9.3	13.0	15.7
732	26.5	F	2.22	27.2	1	L	102.0	91.6	12.6	321	138	5.0	4.7	6.7	7.2	7.8
742	26	M	3.67	21.5	1		128.2	83.6	9.1	278	555	3.0	8.3	3.3	7.9	8.6
743	25.8	M	3.72	21.5	1		110.9	84.0	7.7	427	407	6.4	10.1	9.6	13.2	7.1
751	26.3	F	3.33	20.2	2	L	145.5	67.6	11.4	3161	1976	15.1	17.4	16.4	26.7	19.0
756	26.8	M	3.88	23.5	2		117.9	58.4	7.7	961	2906	7.2	24.0	8.7	29.2	
759	26.7	F	3.2	22.8	2	L	90.0	70.8	6.9	1109	820	12.5	10.3	13.8	12.1	14.6
763	26.4	F	3.47	22.5	1	F	92.8	90.4	10.7	207	79	3.8	5.6	3.7	6.4	4.7
771	26.1	M	3.5	23	2		122.0	56.1	13.8	4823	3597	14.8	15.4	23.7	28.2	40.9

subj	age yrs	sex	bwt kg	bmi kg/m^2	inv	mc	SAP mmHg	HR bpm	$SAPV_{LF}$ mmHg^2	HRV_{LF} ms^2	HRV_{HF} ms^2	AR_{LF} $\frac{ms}{mmHg}$	AR_{HF} $\frac{ms}{mmHg}$	SPC_{LF} $\frac{ms}{mmHg}$	SPC_{HF} $\frac{ms}{mmHg}$	SEQ $\frac{ms}{mmHg}$
774	26.3	M	3.97	33.3	2		132.0	68.6	2.2	913	1225	15.2	18.9	22.7	24.6	
776	26.3	M	4	23.1	2		155.9	77.4	10.1	1447	820	8.6	10.2	14.0	15.8	14.8
777	26.1	M	3.69	30.1	2		135.1	67.8	3.7	404	611	7.2	10.1	12.4	12.9	14.7
780	26.3	F	3.19	31.8	2	F	119.3	76.9	24.3	2325	373	8.8	6.0	10.8	9.0	10.4
781	25.8	M	3.55	27.5	1		117.3	71.3	6.0	775	598	12.4	15.0	15.7	20.0	13.1
801	26.6	M	3.45	21.6	2		151.8	80.0	19.2	1055	2613	9.2	12.0	9.6	13.2	12.9
803	26.1	F	3.48	21.4	2	L	117.7	58.2	12.5	679	588	6.1	8.9	7.1	16.3	7.5
809	26.6	F	3.52	23.6	2	F	146.8	61.1	7.3	3089	3504	16.7	25.7	21.2	25.6	23.9
814	26.3	M	3.62	22.6	2		147.0	76.1	3.9	471	524	10.8	10.6	11.5	12.4	11.7
822	26.1	M	2.83	21.2	2		111.5	69.1	12.0	806	367	5.4	8.0	8.4	9.7	13.1
825	25.8	M	4.69	24.8	2		101.5	95.5	8.7	304	67	4.3	4.4	8.2	6.5	
826	26.5	F	3.24	22.2	2	F	134.6	65.0	3.5	662	859	8.8	14.9	14.7	15.6	
827	27	F	4.27	37.3	2	F	117.0	78.7	4.8	320	763	9.1	11.2	8.4	13.7	12.2
829	25.5	M	3.76	19.6	3		110.0	67.2	3.5	1130	777	15.0	14.3	22.7	19.8	16.5
834	26.4	M	3.15	34	2		116.5	77.3	12.6	1302	790	9.0	12.4	10.6	16.8	15.3
839	26.6	F	3.33	22.6	2	F	91.6	65.1	2.1	545	1610	32.3	24.2	21.4	25.3	23.4
843	25.5	M	3.62	28.9	3		105.7	58.0	3.3	993	729	14.6	16.7	15.2	20.9	
850	26	M	3.66	25.8	2		105.6	88.9	8.9	406	79	4.7	4.7	7.7	8.6	9.0
853	26.7	F	2.84	25.1	1	F	140.7	116.7	59.1	914	87	3.2	2.7	7.6	5.0	5.3
854	25.9	M	4.15	22.9	1		130.6	65.9	11.2	1909	788	11.6	12.2	15.5	15.7	15.6
855	26.1	M	4.5	31.4	2		128.0	65.9	10.4	1600	1089	9.8	11.1	12.5	16.5	14.0
857	26.6	M	3.5	28.3	2		120.8	70.3	4.8	1183	385	10.3	6.8	14.8	11.7	14.0
858	26.5	F	3.18	21.3	2	F	125.1	72.0	4.8	1395	1094	16.5	13.4	15.5	14.5	14.1
859	26.4	M	3.34	27.6	2		118.1	64.5	7.2	1337	265	10.9	8.9	13.7	14.2	18.1
860	26.7	F	2.11	24.8	2	F	122.0	74.1	8.7	1106	565	8.4	11.2	9.9	8.8	12.7
861	26.1	M	3.8	23.4	2		123.6	80.6	7.0	718	659	10.7	11.1	11.3	17.6	25.4
863	26	M	3.51	22.6	2		105.8	81.0	18.8	1779	326	7.5	6.3	9.6	10.9	4.0
865	25.8	M	3.76	21.6	3		133.3	74.7	3.0	981	717	11.8	11.8	17.2	15.0	12.6
871	26.8	F	3.72	23.7	2	L	135.3	60.8	13.3	539	355	4.6	9.3	6.5	16.5	13.9
878	26.1	F	2.97	20.5	2	L	131.6	70.6	12.1	2039	1146	9.5	14.6	14.2	21.8	9.2
880	26.1	F	3.42	25.9	2	L	119.8	82.0	10.7	1515	2122	11.6	17.0	13.4	21.4	18.1
881	26.1	M	3.42	23.8	1		121.4	70.0	4.4	941	1189	17.1	16.5	16.7	17.9	
884	26.4	F	3.7	25.4	2	L	137.7	73.9	2.9	576	907	11.2	10.1	14.5	11.4	16.6
888	25.5	M	3.58	26.3	1		140.3	74.4	8.7	986	294	11.1	5.9	11.8	6.6	13.7
895	26.3	F	3.9	23.3	2	L	119.7	78.8	8.0	2499	949	12.4	14.3	19.4	15.5	7.2
896	26	M	3.9	24.3	2		119.0	57.6	10.4	339	165	4.1	4.2	5.7	4.4	20.4
906	26.6	F	3.83	21.4	2	F	135.5	80.3	6.1	534	123	8.0	5.6	9.5	6.2	6.1
909	26	M	2.97	19.9	2		125.0	80.7	5.1	944	953	12.4	16.7	12.5	19.6	8.4
913	26.8	M	3.22	31.8	2		122.7	63.8	10.5	2591	2200	8.7	10.9	18.6	17.8	16.5
915	26.8	F	2.79	19	1	L	122.1	76.5	7.6	1119	757	10.0	10.0	13.0	21.8	16.5
919	26.7	M	4.03	26	2		125.3	77.5	1.8	265	491	8.1	8.7	8.7	11.0	14.3
920	26.2	M	4.52	25.7	1		124.5	91.9	11.7	977	398	8.8	6.5	9.6	10.0	9.3
921	25.5	M	3.24	23.9	3		96.3	59.1	9.6	2977	1289	16.3	13.5	27.2	14.2	23.0
940	26.2	M	3.27	24.9	1		108.9	61.5	6.2	2035	1894	21.9	20.6	21.1	19.3	18.8
942	27.7	F	3.8	25.8	2	F	113.2	70.0	12.2	5243	2885	17.7	17.1	20.7	24.9	25.7
948	25.4	M	2.65	24.7	3		119.0	65.9	3.3	983	2224	21.2	21.6	16.9	25.2	21.7
949	26.6	F	3.36	34.5	2	L	132.1	101.7	10.7	149	30	3.1	2.8	3.9	5.2	6.9
954	26.6	F	3.59	22.7	2		121.8	69.2	8.0	2839	2004	17.2	16.5	23.9	21.8	26.7
955	26.2	M	2.9	20.7	2		104.9	77.3	3.2	704	750	20.3	15.1	16.8	16.8	17.3
965	25.5	M	3.64	28.9	3		111.9	82.0	12.8	468	172	5.2	6.3	7.5	10.7	8.1
970	27.2	M	3.7	26.1	1		149.8	90.6	15.5	508	90	4.8	2.3	4.6	3.1	4.7
979	26.1	F	3.17	20.8	2	F	131.7	89.3	2.6	285	67	9.1	5.5	13.0	8.5	12.0

subj	age yrs	sex	bwt kg	bmi kg/m^2	inv	mc	SAP mmHg	HR bpm	$SAPV_{LF}$ mmHg^2	HRV_{LF} ms^2	HRV_{HF} ms^2	AR_{LF} $\frac{ms}{mmHg}$	AR_{HF} $\frac{ms}{mmHg}$	SPC_{LF} $\frac{ms}{mmHg}$	SPC_{HF} $\frac{ms}{mmHg}$	SEQ $\frac{ms}{mmHg}$
988	26.8	F	3.18	26.4	2	L	117.1	80.0	11.9	985	564	8.1	7.4	12.4	13.0	13.8
994	26.4	F	3.07	20.4	2	L	120.3	75.2	13.3	1124	146	8.6	4.4	9.4	5.8	10.6
997	26.3	F	2.95	20.4	2	F	125.6	74.5	4.4	621	326	9.4	10.6	12.7	15.0	7.9
1005	26.8	F	3.1	19.3	1	L	97.2	53.2	10.7	1363	3085	12.2	35.1	19.3	43.6	12.5
1008	26.1	F	2.67	17.4	2	L	123.1	61.9	2.4	1055	2918	13.4	21.8	17.5	22.4	26.2
1011	26	M	4.52	21.2	1		117.4	54.8	6.5	2644	2253	18.1	26.9	22.2	40.2	19.6
1014	26.4	F	2.9	21.7	2	L	120.6	64.2	17.5	3261	1497	16.2	17.7	17.8	21.2	17.8
1017	26.5	F	3.15	40.2	2	L	117.8	72.9	12.7	1811	809	10.2	11.1	13.8	12.7	12.5
1021	26.8	F	4.12	22.5	1	F	128.6	56.3	5.5	1097	972	9.1	19.1	11.1	22.1	19.2
1028	26.7	F	4.41	26.1	2	F	125.6	59.7	14.4	1931	1240	7.9	11.6	13.8	17.9	14.0
1034	26.7	F	3.05	24.4	1	F	112.0	78.3	6.4	722	670	9.1	10.5	11.9	11.0	15.4
1043	26	M	3.57	23.3	2		139.7	57.7	4.6	478	850	9.4	12.9	10.5	13.2	10.2
1044	25.7	M	3.49	25.4	3		127.0	58.9	9.2	1072	1588	9.4	25.7	13.5	31.7	18.6
1052	26	M	3.36	23.3	2		126.7	49.8	9.2	1382	1714	9.1	24.8	14.3	29.9	13.2
1058	26.7	F	2.9	24	2	F	93.0	67.6				12.1	26.2	13.6	31.5	24.1
1061	26	M	3	24.3	2		125.8	80.2	7.8	1099	144	10.0	5.6	10.9	8.7	10.4
1064	26.3	M	4.3	27	2		110.6	68.4	3.1	993	861	14.6	16.2	17.0	19.0	13.8
1068	26.1	M	3.62	27	2		108.1	76.8	6.2	1042	269	11.1	8.2	10.3	10.5	9.9
1071	26.4	M	4.27	28.5	2		123.6	70.7	4.9	2004	2259	19.2	17.9	16.0	17.1	24.8
1077	26.4	M	3.86	21.5	2		122.4	76.7	11.8	794	698	5.1	9.7	6.6	14.3	13.7
1090	26.5	F	3.37	26.8	2	L	96.7	69.3	13.4	1349	459	9.5	10.8	10.5	14.8	13.2
1095	25.8	M	2.66	25.6	1		109.9	77.3	16.6	552	321	3.9	8.5	6.6	12.3	6.4
1099	27.1	F	3.587	27.1	2	L	116.2	73.1	15.2	2809	988	11.4	10.0	15.0	13.4	13.5
1131	26.2	F	2.9	26	2	F	125.4	77.4	16.4	1025	1275	7.5	12.6	11.5	14.5	12.3
1137	26	M	3.54	25.4	2		139.5	74.1	20.4	2311	453	8.6	5.8	9.5	8.3	9.7
1138	26.1	F	2.82	31.4	2	L	133.3	68.3	17.3	1553	874	6.8	10.7	10.0	16.1	13.4
1139	27	F	3.78	30.9	1	F	108.9	76.1	1.9	505	1327	28.2	21.6	12.0	21.5	22.5
1149	26	M	4.46	27.6	2		144.4	85.2	16.6	716	71	5.6	2.1	5.5	3.2	7.6
1150	26.6	F	3.87	21.7	2	L	128.5	66.9	3.4	1211	1791	16.6	21.5	16.5	29.6	
1153	26.2	M	4.71	26.7	3		126.0	71.3	2.9	572	543	9.8	8.9	13.4	10.5	10.6
1158	26.3	M	3.55	24.9	2		106.8	61.1	11.8	1236	1155	10.1	14.6	11.0	17.8	14.3
1169	26.2	M	4.42	25.4	2		146.4	79.4	14.4	2453	381	10.2	4.5	12.1	5.4	12.6
1186	26.5	M	3.38	28.1	1		100.8	74.7	5.5	759	411	13.2	11.1	11.4	16.4	16.1
1189	26.3	F	2.63	18.4	2		119.8	86.4	4.4	234	150	7.5	6.0	8.4	7.5	14.6
1190	25.5	M	2.27	34	1		122.9	59.3	7.1	1391	8872	22.4	41.3	7.5	25.6	33.5
1205	26.1	M	3.36	20.7	2		107.4	83.5	4.1	561	722	14.6	14.4	13.3	16.7	16.5
1207	26.6	M	3.64	24.8	2		134.5	77.9	3.1	818	577	13.0	7.0	14.2	7.3	11.6