Universidad Zaragoza
1542

# Trabajo Fin de Máster

# INCLUSIÓN DE LA INFORMACIÓN RESPIRATORIA EN EL ANÁLISIS DE LA VARIABILIDAD DEL RITMO CARDIACO PARA LA IDENTIFICACIÓN DE ESTRÉS

# INCLUDING RESPIRATORY INFORMATION IN HEART RATE VARIABILITY ANALYSIS TO IDENTIFY STRESS


Autor/es

Alberto Hernando Sanz

Director/es

Raquel Bailón Luesma

Escuela de Ingeniería y Arquitectura


2015

## Agradecimientos

A mi familia, compañeros y amigos por apoyarme en todo momento y darme ánimos.

A todos los miembros del grupo BSICoS, en especial a Jesús, Eduardo, Pablo y sobre todo a Raquel, por ayudar a que este trabajo salga adelante dedicándole su tiempo, saber y consejo.

# Including respiratory information in heart rate variability analysis to identify stress

## SUMMARY


Stress is defined as the physiological response to a threat. However, when stress response is maintained in time or it is initiated over and over again it leads the subject to a non-healthy situation. The associated social and medical problems of stress are clearly growing and seriously affecting not only adults but also young and children, being considered the health epidemic of $21^{st}$ century.

The main problem with stress is the non-existence of an objective measure. This is the aim of ES3 project, the study of physiological signals, biochemical markers and psychometric questionnaires in order to analyze the organism response to stress. Heart rate variability (HRV) is considered a non-invasive measure of the Autonomic Nervous System regulation of the heart, so it is widely used to characterize stress response. Respiration also changes during mental stress and attentional tasks. Inside the ES3 project, this work focuses in the analysis of respiration and heart rate variability during acute emotional stress.

The first part of the work comprises the recording of database of young healthy volunteers undergoing a protocol aimed at inducing acute emotional stress. Several physiological signals, including the ECG and respiration signals, were recorded.

The second part includes the spectral analysis of HRV in the classical frequency bands, commonly associated to the sympathetic and parasympathetic systems. A time-frequency representation of the modulating signal which carries ANS information is done, and the following frequency bands are defined: low frequency (LF, from 0.04 to 0.15 Hz) and high frequency (HF, from 0.15 to 0.4 Hz). Several indices, reported to be measures of sympathovagal balance, have been extracted in order to study if they are able to discriminate between being stressed or not. Frequency domain HRV indices, computed in classical terms, scarcely show statistical differences during stress.

The third part in this work has focused on the analysis of respiratory information, namely its rate and its stability. Respiration stability in this work is measured as the peakness of respiration spectrum, which is computed as the percentage of the power around the peak with respect to total power. Results show high discriminative power considering respiratory rate information, suggesting that it can discriminate the different stress states. This, however, comes at the cost of losing the excerpts where this rate can not be estimated.

The last part has considered the analysis of HRV taking into account respiratory information. Respiratory frequency is used to define the HF band and to avoid the measurement of power in the LF and HF bands, when respiratory frequency is too low and falls within the LF band. This avoids the overestimation of sympathetic activity and the underestimation of parasympathetic activity that occurs when the respiration rate lies in LF band. This combined HRV and respiratory rate analysis, increases the discrimination power among different stress situations, where a major sympathetic dominance is observed.

# Inclusión de la información respiratoria en el análisis de variabilidad del ritmo cardiaco para la identificación del estrés

## RESUMEN


Se define el estrés como una respuesta fisiológica ante una amenaza. Sin embargo, si la respuesta ante el estrés se mantiene durante mucho tiempo o se inicia muy continuamente desemboca en una situación no saludable para el sujeto. Los problemas sociales y médicos asociados al estrés están creciendo claramente y afectando seriamente tanto a adultos como a niños, siendo considerado el estrés como la epidemia del siglo XXI.

El principal problema del estrés es la no existencia de una medida objetiva del mismo. Éste es el objetivo del proyecto ES3, el estudio de señales fisiológicas, de marcadores bioquímicos y de cuestionarios psicométricos para analizar la respuesta del organismo ante el estrés. La variabilidad del ritmo cardiaco (HRV) es considerada una medida no invasiva de la regulación del Sistema Nervioso Autónomo (ANS) sobre el corazón, por lo que es ampliamente usada para caracterizar la respuesta al estrés. La respiración también varía ante estrés mental y tareas que requieren atención. Dentro del proyecto ES3, este trabajo se centra en el análisis de la respiración y de la variabilidad del ritmo cardiaco ante estrés emocional agudo.

La primera parte de este trabajo comprende la grabación de la base de datos de voluntarios jóvenes y sanos sometidos a un protocolo destinado a originar estrés emocional agudo. Varias señales fisiológicas, incluídas la señal del ECG y la señal respiratoria, han sido grabadas.

La segunda parte del trabajo ha incluído el análisis espectral de la HRV en las bandas de frecuencia clásicas, asociadas comúnmente con los sistemas simpático y parasimpático. Se realiza la representación tiempo-frecuencia de la señal moduladora que contiene la información del ANS y se definen las siguientes bandas frecuenciales: baja frecuencia (LF, de 0.04 a 0.15 Hz) y alta frecuencia (HF, de 0.15 a 0.4 Hz). Varios índices, que se usan como medidas del balance simpato-vagal, han sido extraídos para estudiar su capacidad de discriminar si el sujeto está estresado o no. Los índices del dominio frecuencial de la HRV, calculados según los términos clásicos, apenas muestran diferencias significativas con la presencia de estrés.

La tercera parte del proyecto se ha centrado en el análisis de la información respiratoria, específicamente en su estabilidad y su frecuencia. En este trabajo, la estabilidad respiratoria es medida como la picudez del espectro respiratorio, que se calcula como el porcentaje de potencia alrededor del pico máximo respecto a la potencia del espectro total. Los resultados muestran mayor potencia discriminativa considerando la información de la frecuencia respiratoria, sugiriendo que puede ser un marcador para discriminar la presencia de estrés entre las distintas etapas de la prueba. Esto, sin embargo, se consigue a costa de perder algunas excepciones donde no se puede estimar la frecuencia respiratoria.

La útlima parte considera el análisis de la HRV teniendo en cuenta la información respiratoria. La frecuencia respiratoria se usa para definir la banda de HF y evitar la medida de potencia en ambas bandas cuando la frecuencia respiratoria es tan baja que cae dentro de la banda de LF. Esto evita la sobresestimación de la actividad simpática y la infraestimación de la actividad parasimpática que ocurre cuando la frecuencia respiratoria cae en la banda de baja frecuencia.

La combinación de los análisis de la HRV y la respiración aumenta el poder discriminativo entre las diferentes etapas del test, mostrando una mayor dominancia simpática cuando se está en una situación de estrés.

# Including respiratory information in heart rate variability analysis to identify stress

## AUTHORSHIP AND ORIGINALITY STATEMENT

---

The next page includes the authorship and originality statement (Declaración de autoría y originalidad es español) which reflects that all the work done in this project is done by myself, without being a copy of another project.

TRABAJOS DE FIN DE GRADO / FIN DE MÁSTER

Escuela de Ingeniería y Arquitectura Universidad Zaragoza

# DECLARACIÓN DE AUTORÍA Y ORIGINALIDAD

(Este documento debe acompañar al Trabajo Fin de Grado (TFG)/Trabajo Fin de Máster (TFM) cuando sea depositado para su evaluación).

D./Dª. Alberto Hernando Sanz,

con nº de DNI 76921458-M en aplicación de lo dispuesto en el art. 14 (Derechos de autor) del Acuerdo de 11 de septiembre de 2014, del Consejo de Gobierno, por el que se aprueba el Reglamento de los TFG y TFM de la Universidad de Zaragoza,

Declaro que el presente Trabajo de Fin de (Grado/Máster) Máster de Ingeniería Biomédica, (Título del Trabajo) "Inclusión de la información respiratoria en el análisis de variabilidad del ritmo cardíaco para la identificación de estrés",

es de mi autoría y es original, no habiéndose utilizado fuente sin ser citada debidamente.

Zaragoza, a 18 de noviembre de 2015

Fdo:

# Contents

# List of Figures

# List of Tables

# Chapter 1

# Introduction

## 1.1 Context

This work is developed in the context of these projects, involving Universidad de Zaragoza under fellowship UZ2014-TEC-01; Ministerio de Economía y Competitividad (MINECO), FEDER, under projects FIS-PI12/00514 and TIN2014-53567-R; CIBER in Bioengineering, Biomaterials & Nanomedicine through Instituto de Salud Carlos III, and Grupo Consolidado BSICoS from DGA (Aragón) and European Social Fund (EU). The computation was performed by the ICTS "NANBIOSIS", by the High Performance Computing Unit of the CIBER in Bioengineering, Biomaterials & Nanomedicine (CIBER-BBN) at the University of Zaragoza.

## 1.2 Motivation

Many mental disorders as anxiety, depression, epilepsy, multiple sclerosis or pathological stress have increased significantly over the last few years. Individuals throughout the world are reacting physically and mentally to continuous stressful situations as a result of the modern lifestyle in a constantly changing society. The associated social and medical problems of the stress are clearly growing and seriously affecting not only adults but also young and children.

But how can be correctly defined the stress? It is the physiological response to a threat, either physical or psychological, mainly mediated by the autonomic nervous system (ANS) through its two branches, sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). This response starts in the hypothalamus, which triggers the sympathetic "fight or flight" response to provide the body with the energy to address the perceived danger. Once the threat has passed, the parasympathetic "rest and digest" response restores the body homeostasis. In this way, stress is a necessary survival mechanism and not health-threatening.

However, when stress response is maintained in time or it is initiated over and over again, the body cannot reach its homeostasis. Prolonged stress has been associated with dysfunctions in the immune system[1], psychiatric disorders such as anxiety, depression and Alzheimer [2, 3] and cardiovascular diseases [4, 5]. The World Health Organization has called stress the health epidemic of the $21^{st}$ century.

Despite the high incidence and negative consequences of stress, there is not a reliable tool

for the non-invasive, objective and continuous monitoring of stress level. This is the goal of ES3 project, which includes different physiological signals, biochemical markers and psychometric questionnaires, during physical, emotional and even chronic stress [6]. In this work we will focus on acute emotional stress. The term acute refers to the immediate response of the organism to the stressor, and the term emotional to the individual perception that demands of a personally important situation, task or duty exceeds his or her capabilities and resources to face it.

Heart rate variability (HRV) at rest is widely accepted as a noninvasive measure of the ANS regulation of the heart. Spectral analysis of HRV at rest reveals two main components: i) a high frequency (HF) component in the range from 0.15 to 0.4 Hz, mainly due to respiratory sinus arrhythmia, and ii) a low frequency (LF) component in the range from 0.04 to 0.15 Hz, which reflects both sympathetic and parasympathetic activity. Power in the HF band has been used as a measure of parasympathetic activity. Power in the LF band normalized by power in both the LF and HF bands has been considered a measure of sympathetic dominance. The ratio between the power in the LF and HF bands (LF/HF ratio) is considered a measure of sympathovagal balance [7].

Due to its relation to ANS activity, HRV has been widely used to characterize the stress response. Most of the research on HRV response to stress is focused on the measurement of SNS excitation through the normalized power in the LF band and the sympathovagal ratio. A different approach is considered in [8], where respiratory sinus arrhythmia, as a marker of PNS tone, is proposed to assess stress and vulnerability to stress. In this approach PNS tone is considered to parallel homeostasis and a withdrawal of PNS tone would represent the disruption of homeostasis induced by stress.

Most of the studies suggest higher sympathetic dominance during stress than during resting or relaxing conditions, however changes in specific HRV parameters published in literature are inconsistent even when restricting to an specific emotional/cognitive type of stress. For example, an increase in the LF power has been reported during mental arithmetic [9]. An increase in sympathovagal balance and in normalized LF power during mental task was found in controls but not in patients with a prior myocardial infarction [10]. Mental stressors added during computer work caused a decrease in the HF power and an increase in the LF/HF ratio, but not an increase in the LF power in [11]. In [12, 13] a decrease both in LF and HF powers is reported during mental load added to a normal office task. Lower HF power was also observed during Stroop test and mental arithmetic, while LF power increased during Stroop test and decreased during the arithmetic test [14].

Specific differences in stress stimulus and population are not enough to explain the differences found in the results. Some of the inconsistent results may be due to the methodology applied for the spectral analysis of HRV. Time-frequency analysis could allow to characterize the nearly instantaneous response to acute stress, which may be blurred with time-invariant methods [15]. Moreover, differences in mean heart rate (HR) during stress and relaxing conditions can introduce a bias in HRV spectral parameters, which needs to be compensated for [16]. Finally, it has been shown that changes in respiratory pattern alter the spectral content of HRV [9, 17], and mental stress was reported to alter the breathing pattern, increasing both tidal volumne and respiration rate [13, 18]. Respiratory variability and sigh rate also change during mental stress and attentional tasks [19]. Thus, stress related changes in respiration may alter HRV parameters, obscuring their interpretation in terms of SNS and PNS activations.

In this work we analyze HRV and respiration changes in healthy subjects during acute emo-

tional stress using time-frequency representations. Then, we include information on respiratory frequency in HRV analysis to obtain a more reliable interpretation of HRV parameters for stress assessment.

A preliminary version of this work has been reported to the conference Computing in Cardiology (CinC) which took place in Nize, France in September 2015 [20]. In this paper, respiratory information was analyzed in a subset of the database used in this work.

A complete version of this work has been sent to the IEEE Journal of Biomedical and Health Informatics, in a special issue about mental disorders called "Sensor Informatics for Managing Mental Health". The title of the paper is: "Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment". The paper submitted can be check in the Appendix A.

## 1.3 Objectives

The goal of this TFM is the analysis of HRV and respiratory information during the protocol that induces acute emotional stress over the subjects. Four aims are proposed:

- Recording of a database of young healthy volunteers during a protocol aimed to induce acute emotional stress.
- Analysis of classical HRV indices during protocol and comparison between relaxing and stressful stages.
- Analysis of respiratory parameters during protocol and comparison between relaxing and stressful stages.
- Analysis of alternative HRV indices during protocol, including respiratory rate information to the HRV and comparison between relaxing and stressful stages.

## 1.4 Structure

The following chapters are briefly described below:

- **Chapter 2**: A description of the subjects that make up the database and the stress protocol is done here.
- **Chapter 3**: Description of the methods for:
  - Classical HRV estimation: extraction from the ECG, spectral analysis, bands definition and parameters measured.
  - Respiratory signal processing: with the methods applied to extract parameters related with the stability and the rate of the signal.
  - Alternative HRV estimation: using the respiratory rate information to redefine its bands and controlling the overlapping between them to proceed with the parameters measurements.

- **Chapter 4**: The results of the parameters studied are shown.
- **Chapter 5**: The results of the parameters studied are discussed.
- **Chapter 6**: The conclusions are presented.
- **Chapter 7**: The future expansion of the work is proposed.

# Chapter 2

# Acute emotional stress database

## 2.1 Subjects

A data base of 80 volunteers (40 men and 40 women) with a mean age of $21.84 \pm 3.16$ years was created. These recordings were acquired in the Autonomous University of Barcelona (UAB) [21] and in the University of Zaragoza (UZ), doing the same number of recordings in each University (40 and 40). The recordings in the UZ have been acquired in the context of this TFM.

Inclusion criteria were: to be student of the Autonomous University of Barcelona or of the University of Zaragoza, aged between 18 and 30 years old and non-regular consumer of psychotropic substances, alcohol or tobacco.

Exclusion criteria were: a body mass index higher than 30%, to have been diagnosed with any chronic disease or psychopathology or having more than 70% stress level in visual analog scale.

All participants were informed of the study's targets, protocol details and physiological samples to be collected. Before involvement, participants signed their written consent. The informed consent form is included in Appendix B. The protocol was approved by the Ethics Committee both at the UAB and UZ.

## 2.2 Sessions

Each subject underwent a basal session and a stress session, as Fig. 2.1 shows and as it will be later explained in more detail. These two sessions were completed in days close to each other and at the same hour (10 a.m. or 11.15 a.m., depending of the subject), trying to reproduce the same biorhythms-related stress conditions in both sessions.

Subjects were instructed for the session day to wake up two hours before the session, to have a light breakfast without coffee or tea and not to do any physical exercise, also to avoid alcohol, tobacco or any psychotropic substance consumption 24 hours before each session.

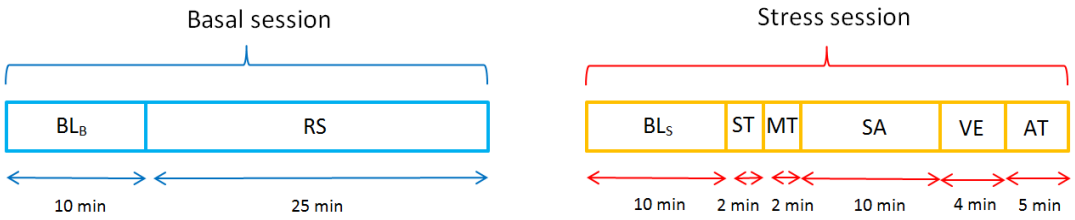



Figure 2.1: Basal and stress sessions with their stages and the time employed in each one.

### 2.2.1 Basal session

The basal session consisted of a 35-minutes-length relaxing audition, divided in two parts as follows:

- Baseline stage ($BL_{\mathrm{B}}$), with 10-minutes-length relaxing audition, that will be repeated in stress session.
- Relax time ($RS$) with 25-minutes-relaxing-audition continuation.

Only the six central minutes of $BL_{\mathrm{B}}$ are considered in this work.

### 2.2.2 Stress session

The stress session tries to induce emotional stress by following a modification of the Trier Social Stress Test [22]. Instead of a speech, the students performed a memory test and an arithmetic task exert while being video recorded, then the video was displayed in front of an audience with a stress anticipation time in between. The stress test of 25 minutes comprises different stages:

- Baseline stage during stress session ($BL_{\mathrm{S}}$): 10-minutes-length relaxing audition.
- Story telling stage ($ST$): 3 stories are told to the subject with a great amount of details. The subject is requested to remember as much details as possible, demanding a great amount of attention.
- Memory task ($MT$): the subject is requested to tell back every remembered detail within 30 seconds for each story.
- Stress anticipation ($SA$): subject is requested to wait for the evaluation of the memory test. The duration of this stage is 10 minutes.
- Video exposition ($VE$): a projection of a video with the subject performance in the memory test is shown. The video showed twice each one of the 3 stories. First, an actor repeats the story in a perfect way, trying to make the subject believe that this is the common case. Subsequently, the subject (recorded during the $MT$ stage) telling back the story is displayed.

- Arithmetic Task ($AT$): the subject has to count down from 1022 in steps of 13. In case of a calculation error, the subject is requested to restart from 1022. Although the subject is not expected to complete the countdown, he is requested to do so within 5 minutes. No subject completed the countdown.

The last five stages are considered stressful. $BL_{\mathrm{S}}$ and $SA$ have longer duration that the other stages. Only the 6 central minutes of them are analyzed in this work. Furthermore, for $BL_{\mathrm{B}}$, only the segment from 2 to 8 minutes (6 central minutes of the first 10 minutes) was analyzed, in order to compare it with the 6 central minutes in $BL_{\mathrm{S}}$.

## 2.3 Biological signals

Different signals are recorded during the entire duration of the sessions, using the software Medicom system (Medicom MTD Ltd, Russia), which can be seen in Fig. 2.2. These are:

Figure 2.2: Medicom MTD Ltd hardware and software.

- Temperature, measured in the cheek and in the little finger.
- Electrocardiogram (EEG), which reflects cardiac activity, using 3 orthogonal non-standard leads.
- Electromiogram (EMG), which reflects muscular activity, using two muscles very sensitive to stress changes, as trapezius and orbital muscles.
- Pulse plethysmography (PPG), which monitors the blood perfusion, measured in the supra-orbital veins and in the medium finger.
- Skin conductance, which controls how good our skin carries electricity, increasing with the stress, and it is measured in two fingers.
- Respiration, measured with a chest band that reflects thorax volume changes.

ECG and EMG were sampled at 1 KHz. The rest of the signals, at 250 Hz.

To complete the information, saliva and blood samples are recollected to study some enzymes related to stress, considered chemical bio-markers [23, 24]. Cortisol and $\alpha$-amylase are measured in the Endocrinology and Radioinmuno analysis services of the Neurosciences Institute of the UAB. An immune-assay is carried out to determine cortisol concentration whereas an enzyme assay analyzed the $\alpha$-amylase enzyme kinetics (Salimetrics, State College, PA, USA). The analysis of prolactin, Co-Pentane and glucose is quantified in Center of Diagnostic Biomedical (CDB) of Barcelona Clinic Hospital. All the samples are processed in the same test in order to avoid any variability inter-test.

Finally, standard psychometric tests and another created by Clinic Hospital Lozano Blesa psychologists and psychiatrists are fill out by the subject in order to obtain a reference of how much stress the test has produced over him/her. These tests can be consulted in the Appendix C.

In this work, only respiratory and ECG information will be analyzed. Complete recordings with quality ECG and respiration signals in both sessions during all the stages and with their corresponding time markers are only available for 48 volunteers (20 men and 28 women, with a mean age of $22.21 \pm 3.34$ years) out of the initial 80 recorded.

# Chapter 3

# Methods

## 3.1 Heart rate variability analysis

### 3.1.1 Heart rate variability signal

The Time Variant Integral Pulse Frequency Modulation (TVIPFM) model is used to represent the control of the heart rate by the Autonomous Nervous System. The explanation of this method is explained in Appendix D.

First, heart beats are detected from Z lead of the recorded ECG signal using an algorithm based on wavelets [25]. Ectopic beats, missed and false detentions are identified [26].Then, an instantaneous heart rate signal $d_{\mathrm{HR}}(n)$, sampled at 4 Hz, is obtained from the beat occurrence time series based on the integral pulse frequency modulation model, which accounts for the presence of ectopic beats [16, 26].

$$d_{\mathrm{HR}}(n) = \frac{1 + \mathfrak{m}(n)}{T(n)} \tag{3.1}$$

where $\mathfrak{m}(n)$ represents the modulating signal which carries the information from ANS and $T(n)$ is the mean heart rate, which is considered to be slow-time-variant by this model.

Then, a time-varying mean HR, $d_{\mathrm{HRM}}(n)$, is obtained by low-pass filtering $d_{\mathrm{HR}}(n)$, with a cut off frequency of 0,03 Hz:

$$d_{\mathrm{HRM}}(n) = \frac{1}{T(n)} \tag{3.2}$$

HRV signal is obtained as:

$$d_{\mathrm{HRV}}(n) = d_{\mathrm{HR}}(n) - d_{\mathrm{HRM}}(n) \tag{3.3}$$

Finally, the modulating signal is estimated as [16]:

$$\mathfrak{m}(n) = \frac{d_{\mathrm{HRV}}(n)}{d_{\mathrm{HRM}}(n)} \tag{3.4}$$

This modulating signal is supposed to carry the information of ANS activity without the influence of HR.

The three last signals are represented in Fig. 3.1, where the correction over the $d_{\mathrm{HRM}}(n)$ terms proposed in $\mathfrak{m}(n)$ can be seen, showing the differences between choose $d_{\mathrm{HRV}}(n)$ or $\mathfrak{m}(n)$ in order to study heart rate variability.

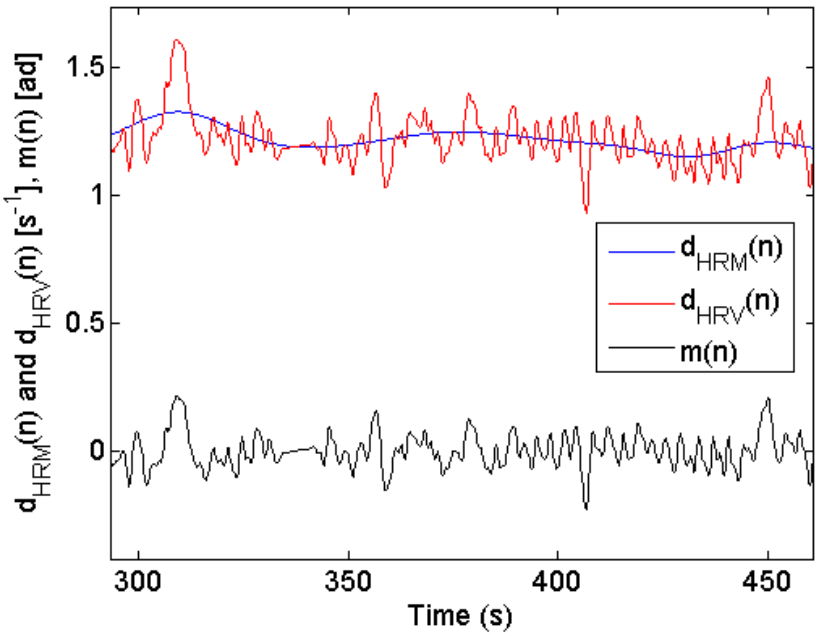



Figure 3.1: Representation of $d_{\mathrm{HRM}}(n), d_{\mathrm{HRV}}(n)$ and $\mathfrak{m}(n)$, where the difference between them can be considered.

### 3.1.2 Time-frequency analysis of HRV

Time-frequency analysis is applied to $\mathfrak{m}(n)$ in order to characterize the rapid response of the ANS to stress. The smoothed pseudo Wigner-Ville distribution (SPWVD) is selected because it provides better resolution than non-parametric linear methods, independent control of time and frequency filtering, and power estimates with lower variance than parametric methods when rapid changes occur. SPWVD of $\mathfrak{m}(n)$ is computed:

$$P_{\mathfrak{M}}(n,m) = 2 \cdot \sum_{l=-L+1}^{L-1} |h(l)|^2 \cdot \left[ \sum_{n'=-N+1}^{N-1} g(n') a_{\mathfrak{M}}(n+n'+l) a_{\mathfrak{M}}{}^{*}(n+n'-l) \right] \cdot e^{-j2l(m/M)\pi}; \quad m = -M+1 \ldots M \quad (3.5)$$

where $n$ and $m$ are time and frequency indices. The analytic signal $a_{\mathfrak{M}}(n)$ is defined as $a_{\mathfrak{M}}(n) = \mathfrak{m}(n) + j \cdot \hat{\mathfrak{m}}(n)$, where $\hat{\mathfrak{m}}(n)$ represents the Hilbert transform of $\mathfrak{m}(n)$. The terms $g(n)$ and $h(l)$ are time and frequency smoothing windows, chosen to be Hamming windows whose lengths are $2 \cdot N + 1 = 203$ and $2 \cdot L + 1 = 1025$ samples respectively [27].

Instantaneous power in classical LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz) bands is computed from $P_{\mathfrak{M}}(n,m)$, yielding $P_{\mathrm{LF}}(n)$ and $P_{\mathrm{HF}}(n)$, respectively. Fig. 3.2 represents these two bands over the time-frequency map.

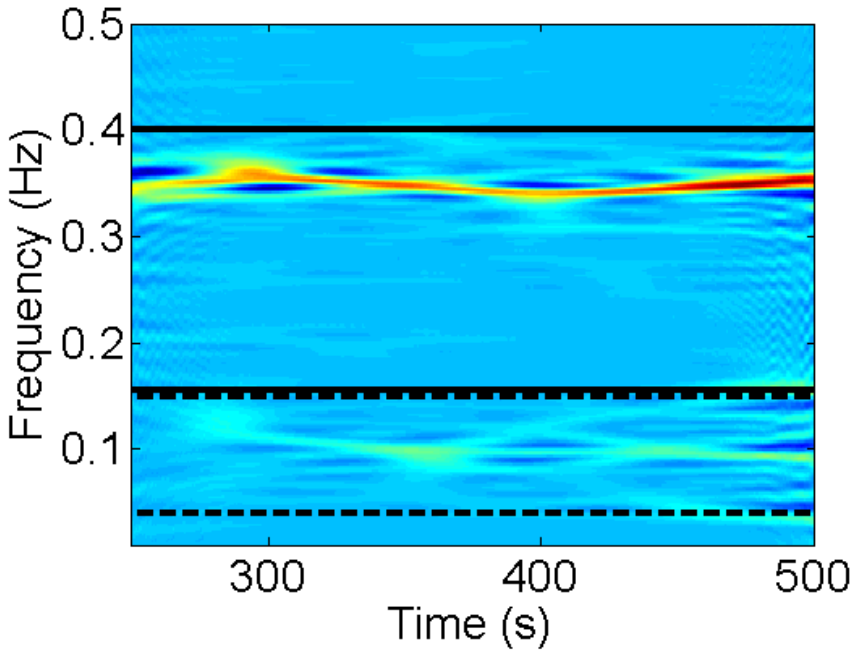



Figure 3.2: $P_{\mathfrak{M}}(n,m)$ for one subject. Solid black lines represent classic HF band. Dashed black lines represent LF band.

Instantaneous power of the SPWVD of $d_{\mathrm{HRM}}(n)$ is also computed and denoted $P_{\mathrm{VLF}}(n)$.

Instantaneous normalized LF power $P_{\mathrm{LF_n}}(n) = P_{\mathrm{LF}}(n)/(P_{\mathrm{LF}}(n) + P_{\mathrm{HF}}(n))$ and LF/HF ratio, $R_{\mathrm{LF/HF}}(n) = P_{\mathrm{LF}}(n)/P_{\mathrm{HF}}(n)$ are also considered.

## 3.2 Respiration analysis

Respiration signal is band-pass filtered (cut-off frequencies of 0.03 and 0.9 Hz) and downsampled to 4 Hz.

Respiratory rate was estimated from this filtered respiratory signal by using an algorithm based on [28]. The method consists in the estimation of the respiratory frequency ($F_{\mathrm{R}}$) from "peaked-conditioned" averaged spectra.

Every 5 seconds, a power spectrum density $S_{\mathrm{k}}(f)$ is estimated by using Welch periodogram from the $k^{th}$ 42 second length running window. Spectra obtained from 12 second-length subintervals overlapped 6 s are averaged. Subsequently, a measure of peakness is obtained from $S_{\mathrm{k}}(f)$ as the percentage of power around the previous estimated respiratory rate $F_{\mathrm{R}}(k-1)$ with respect to the total power within [0.08 Hz, 0.8 Hz] band:

$$P_k = \frac{\int_{F_{\mathrm{R}}(k-1)-\delta}^{F_{\mathrm{R}}(k-1)+\delta} S_{\mathrm{k}}(f)df}{\int_{0.08}^{0.8} S_{\mathrm{k}}(f)df} \cdot 100 \tag{3.6}$$

where $\delta$ value was empirically set as 0.1 Hz. Then, a peaked-conditioned average spectra, $\bar{S_k}(f)$, is obtained by averaging those $S_k(f)$ which are sufficiently peaked:

$$\bar{S_k}(f) = \sum_{l=-L_s}^{L_s} \chi_{k-l} S_{k-l}(f), \tag{3.7}$$

where $L_s$ was set to 2 in order to average a maximum of 5 spectra as in [28], and $\chi_{k-l}$ is a criterion to consider whether the power spectrum $S_{k-l}(f)$ is peaked enough or not:

$$\chi_k = \begin{cases} 1, & P_k \geq 0.65 \\ 0, & otherwise \end{cases}, \tag{3.8}$$

allowing to take part in the average only to those $S_k(f)$ whose $P_k$ is above 65%.

Figure 3.3 displays two spectra as examples, one with $P_{\mathrm{k}} > 65\%$ (peaked enough to take part in the average), and another one with $P_{\mathrm{k}} < 65\%$, (not peaked enough to take part in the average).

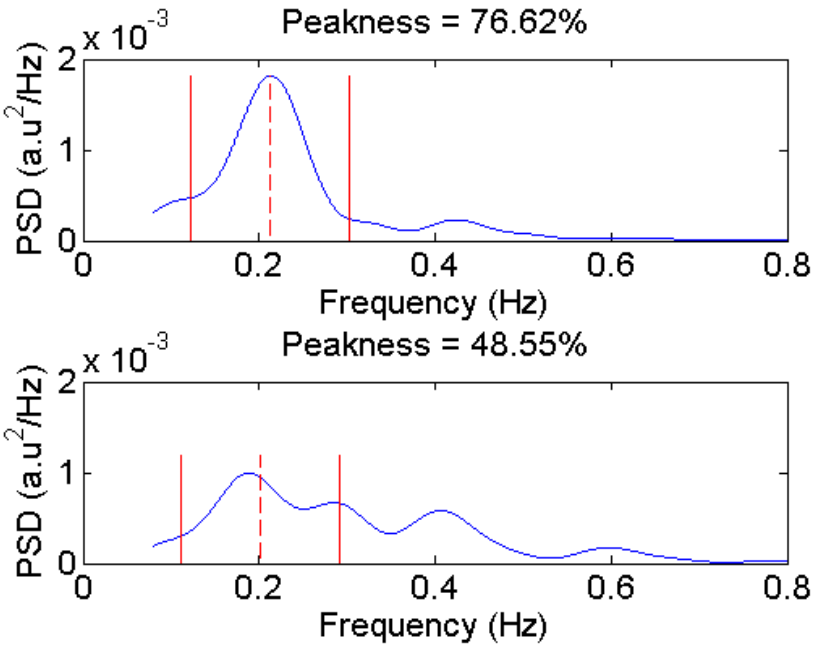



Figure 3.3: Differences between spectra which satisfy the peakness condition and those which do not. Red lines illustrate the limits of the integrating interval of the numerator in $P_{\mathrm{k}}$ and the dashed line marks the previous respiratory rate estimated $F_{\mathrm{R}}(k-1)$ (see eq. 3.6)

Finally, respiratory rate is estimated as the maximum of $\bar{S}_{\mathrm{k}}(f)$ within the entire band:

$$F_{\mathrm{R}}(k) = \arg\max \bar{S}_{\mathrm{k}}(f); \quad f \in [0.08Hz, 0.8Hz] \tag{3.9}$$

Studied parameters were respiratory rate $F_{\mathrm{R}}(k)$, the peakness $P_{\mathrm{k}}$ and the percentage of spectra which take part in the peaked-conditioned average ($N_{\mathrm{k}}$), considering the last two parameters to be related to the respiratory stability.

Respiratory frequency could not be estimated during $MT$ and $AT$ stages since speech modifies respiratory pattern [29] and no spectra would satisfy the peakness criterium.

## 3.3 Heart rate variability analysis including respiratory information

Analysis of respiration revealed changes in respiratory frequency during stress conditions with respect to relax [18, 19]. In order to obtain a more reliable assessment of PNS activity, respiratory frequency estimation is included in HRV analysis redefining the HF band centered at respiratory frequency as in [30]. The method described in Section 3.2 offers an estimation of respiratory rate every five seconds, so a linear interpolation is made in order to obtain a respiratory frequency signal $F_{\mathrm{R}}(n)$ with the same sampling rate than the HRV series (4 Hz).

The VLF and LF bands are the classical bands ([0, 0.04 Hz] and [0.04, 0.15 Hz]), while a new HF band is defined centered in respiratory rate (see Fig 3.4):

$$\Omega_{\mathrm{HF_R}}(n) = [F_{\mathrm{R}}(n) - 0.05Hz, F_{\mathrm{R}}(n) + 0.05Hz] \tag{3.10}$$

Instantaneous power in LF band and in $\Omega_{\mathrm{HF_R}}(n)$ is computed for each subject yielding $P_{\mathrm{LF_R}}(n)$ and $P_{\mathrm{HF_R}}(n)$. In some stages of the test, specially during the basal stage, a low respiratory rate makes $\Omega_{\mathrm{HF_R}}(n)$ overlaps with LF band. In order to avoid the measurement of the same power in both bands, a threshold that delimits the amount of overlapping percentage between $\Omega_{\mathrm{HF_R}}(n)$ and LF band is defined. If at a given time instant $n$, overlapping is higher than experimentally adjusted 50%, $P_{\mathrm{LF_R}}(n)$ and $P_{\mathrm{HF_R}}(n)$ of this subject at that instant are not computed. Figure 3.5 shows an example where respiratory rate (mean respiratory rate is 0.1041 Hz) is within the LF band (inside dashed black lines), so $\Omega_{\mathrm{HF_R}}(n)$ (between solid black lines) overlaps with it. The percentage of overlapping is higher than the fixed threshold (50%) during the whole interval displayed so $P_{\mathrm{LF_R}}(n)$ and $P_{\mathrm{HF_R}}(n)$ are not computed for any time instant within this interval. Normalized LF power and LF/HF ratio are also computed and denoted $P_{\mathrm{LF_{Rn}}}(n)$ and $R_{\mathrm{LF/HF_R}}(n)$.

To sum up, two modifications are considered to the classical analysis in order to estimate better the ANS regulation:

- A new localization of HF band, centered in the respiratory rate: $\Omega_{\mathrm{HF_R}}(n) = [F_{\mathrm{R}}(n) - 0.05Hz, F_{\mathrm{R}}(n) + 0.05Hz]$
- In case that this rate is low and the new band overlaps with LF band, avoid the measurements when the overlapping exceeds the 50%.

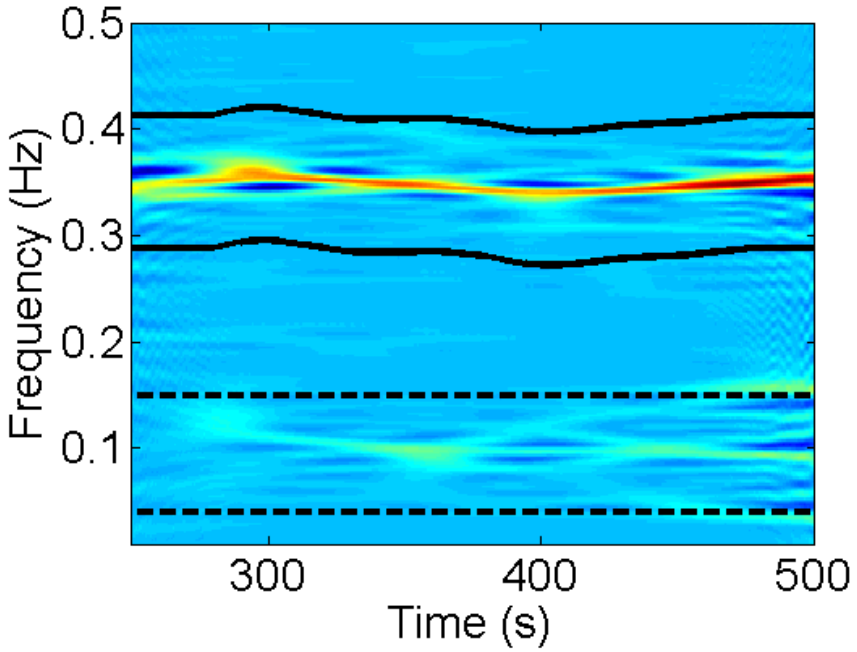



Figure 3.4: $P_{\mathfrak{M}}(n, m)$ for one subject. Solid black lines represent $\Omega_{\mathrm{HF_R}}(n)$. Dashed black lines represent LF band.

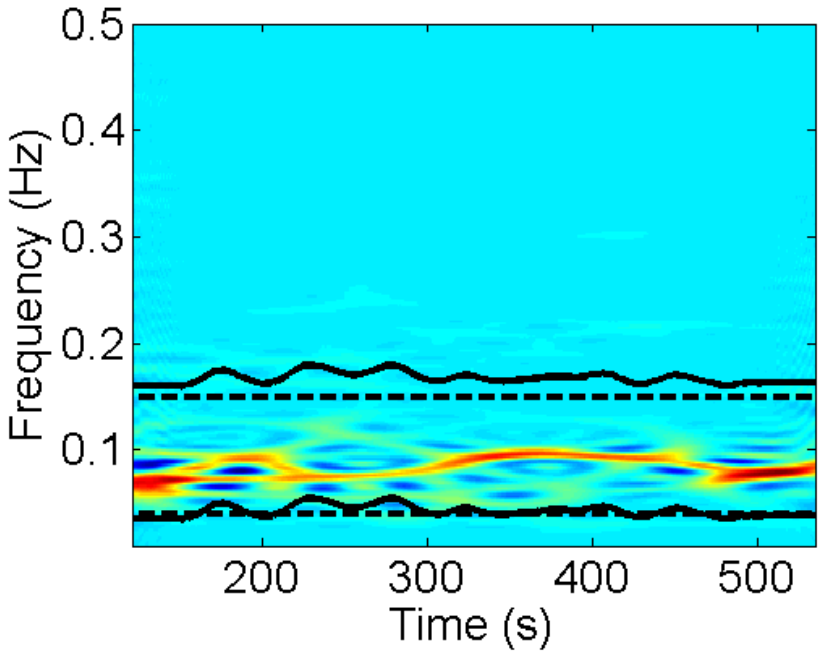



Figure 3.5: $P_{\mathfrak{M}}(n, m)$ for one suject, representing the overlap between the two bands because of a low respiratory rate. Continue black lines represent $\Omega_{\mathrm{HF_R}}(n)$. Dashed black lines represent LF band.

## 3.4 Statistical analysis

Intra-subject mean of each studied HRV index was obtained for each stage of the protocol, yielding the following parameters: $\bar{d}_{\mathrm{HRM}}$ and $\bar{d}_{\mathrm{HRM_R}}$; $\bar{P}_{\mathrm{VLF}}$ and $\bar{P}_{\mathrm{VLF_R}}$; $\bar{P}_{\mathrm{LF}}$ and $\bar{P}_{\mathrm{LF_R}}$; $\bar{P}_{\mathrm{HF}}$ and $\bar{P}_{\mathrm{HF_R}}$; $\bar{P}_{\mathrm{LF_n}}$ and $\bar{P}_{\mathrm{LF_{Rn}}}$; $\bar{R}_{\mathrm{LF/HF}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$. Subindice R denoted HRV analysis including respiratory frequency information parameters. Note that in these last ones, different number of samples take part in the average since those time instants where LF and HF bands overlap more than 50% have been excluded.

In addition, three respiratory parameters are studied too: the intra-subject median of respiratory rate $\bar{F}_{\mathrm{R}}$; of the peakness measure $\bar{P}_{\mathrm{k}}$ and of the percentage of spectra used to compute the peaked-conditioned averaging $\bar{N}_{\mathrm{k}}$.

The non-parametric Friedman statistical test was applied in order to study if there are significant differences between the $BL_{\mathrm{S}}$ stage and each one of the other stages.

# Chapter 4

# Results

Only in 35 volunteers (13 men and 22 women, with an age of $22.49 \pm 3.49$) out of 48, respiratory rate can be estimated in all the stages of the stress session (without talk): $BL_{\mathrm{S}}, ST, SA$ and $VE$. Only in 18 out of these 35 subjects respiratory rate can be estimated in the first basal session ($BL_{\mathrm{B}}$).These stages are going to be analyzed to see if their distribution is similar to the pre-relaxing stage ($BL_{\mathrm{S}}$).

## 4.1 Respiratory parameters

Fig. 4.1 illustrates one example of respiratory rate differences between $BL_{\mathrm{S}}$ and $ST$ stages, showing a higher and less stable respiratory rate during $ST$ than during $BL_{\mathrm{S}}$.

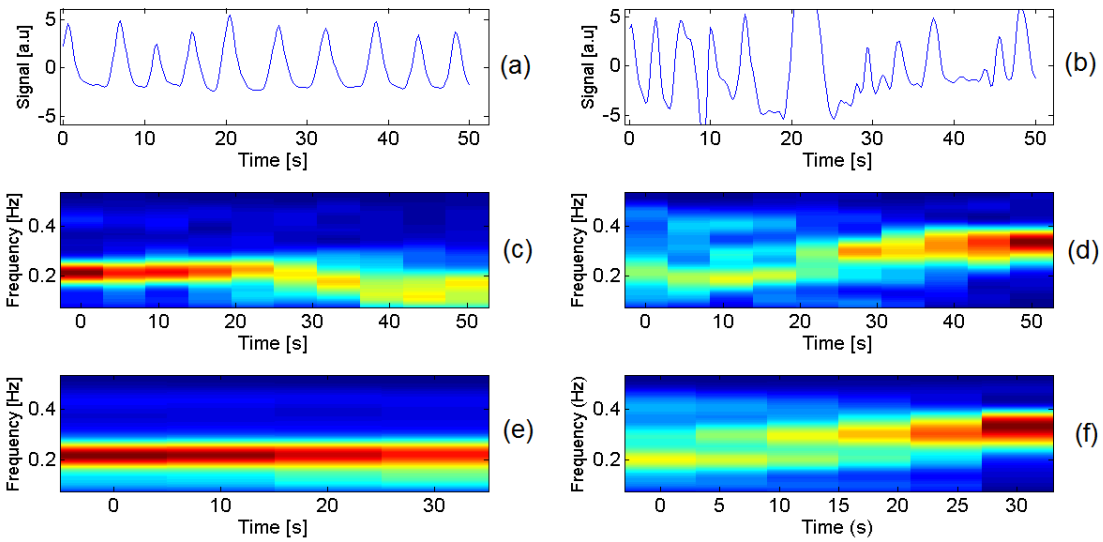



Figure 4.1: a) and b) respiratory signal; c) and d) $S_{\mathrm{k}}(f)$; e) and f) $\bar{S}_{\mathrm{k}}(f)$; in basal stage (left side) and story telling (right side).

Table 4.1 shows the inter-subject median and the median absolute deviation (MAD) of $\bar{F}_{\mathrm{R}}$, $\bar{P}_{\mathrm{k}}$, and $\bar{N}_{\mathrm{k}}$ among the 35 subjects. The respiratory rate is observed to be higher and less stable (lower $\bar{P}_{\mathrm{k}}$ and $\bar{N}_{\mathrm{k}}$) during the stress stages than during the $BL_{\mathrm{S}}$ stage. These differences were statistically significant according to the Friedman test (p-value $< 0.05$).

Table 4.1: Median ± MAD of $\bar{F}_{\mathrm{R}}$, $\bar{P}_{\mathrm{k}}$ and $\bar{N}_{\mathrm{k}}$ from all subjects in the different parts of the test. Significant differences according to the Friedman test are denoted: *, p-value $< 0.05$; †, p-value $< 10^{-3}$; ‡, p-value $< 10^{-4}$.

| Stage | $BL_{\mathrm{S}}$ | $ST$ | $SA$ | $VE$ |
|---|---|---|---|---|
| $\bar{F}_{\mathrm{R}}(Hz)$ | 0.23 ±0.06 | 0.35 ±0.05‡ | 0.29 ±0.04‡ | 0.34 ±0.04‡ |
| $\bar{P}_{\mathrm{k}}$ | 78.43 ±4.96 | 75.15 ±8.75 | 71.87 ±8.06* | 74.19 ±7.39* |
| $\bar{N}_{\mathrm{k}}$ | 87.8 ±12.5 | 54.55 ±10.58‡ | 76.57 ±10.77* | 74.37 ±10.01* |

When comparing both basal stages ($BL_{\mathrm{B}}$ and $BL_{\mathrm{S}}$), results show a slightly lower respiratory rate ($0.211 \pm 0.06$) and percentage of spectra used ($77.87 \pm 5.81$) in $BL_{\mathrm{B}}$. The peakness is very similar in both cases ($80.91 \pm 8.22$). These differences are not statistically significant.

## 4.2 HRV parameters

Fig. 4.2 displays the instantaneous HR signal $d_{\mathrm{HR}}(n)$, the modulating signal $\mathfrak{m}(n)$ and the SPWVD $P_{\mathfrak{M}}(n,m)$ for a subject of the database during one minute of stages $BL_{\mathrm{S}}$ and $ST$. It can be seen a increment in the heart rate signal and a more variable and with more peaks modulating signal $\mathfrak{m}(n)$. The variation of HF band centered at respiratory frequency can be appreciated in the SPWVD, with a low respiratory rate that overlap with LF band in $BL_{\mathrm{S}}$ and just the opposite in $ST$, showing values over the limit of the HF classical band (0.4 Hz).

Table 4.2 shows the inter-subject median and MAD among the 35 subjects when using the classical bands and the proposed respiratory-rate-based HF band $\Omega_{\mathrm{HF_R}}(n)$. With this new localization of HF band, the number of subjects decreases to 24, due to the fact that in 11 subjects the overlap between bands is higher than the 50% in the entire $BL_{\mathrm{S}}$ stage. For this reason, these subjects have been deleted in the study.

Inclusion of respiratory frequency information in HRV analysis do not affect $\bar{d}_{\mathrm{HRM}}$ and $\bar{P}_{\mathrm{VLF}}$ estimation. Parameter $\bar{d}_{\mathrm{HRM}}$ significantly increases during $ST$ with respect to $BL_{\mathrm{S}}$, while no significant differences were found in the other stress stages.

Using the classical bands in HRV analysis, the only parameters with statistical differences are $\bar{P}_{\mathrm{LF}}$ and $\bar{P}_{\mathrm{HF}}$ during $ST$, showing a increase in the power associated to LF band and a decrease in HF band with respect to $BL_{\mathrm{S}}$.

When respiratory frequency information is included in HRV analysis, more significant differences are found. $\bar{P}_{\mathrm{LF_R}}$ increases during $ST$ and $SA$ with respect to $BL_{\mathrm{S}}$, being significant during $ST$. In Fig. 4.3, one time instant of the time-frequency map for $BL_{\mathrm{S}}$ and $ST$ are represented,

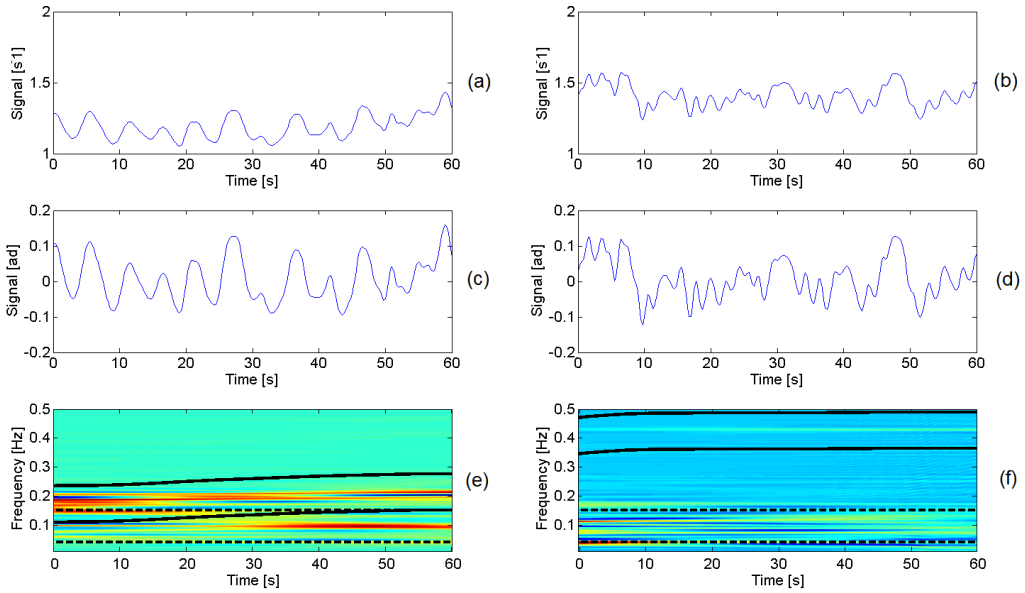



Figure 4.2: a) and b) $d_{\mathrm{HR}}(n)$; c) and d) $\mathfrak{m}(n)$ signals; e) and f) its respective SPVWD in a time-frequency map showing the variations of HF band related to respiration in pre-relaxing stage (left side) and story telling (right side).

Table 4.2: In the left, median ± mad of $\bar{d}_{\mathrm{HRM}}$, $\bar{P}_{\mathrm{VLF}}$, $\bar{P}_{\mathrm{LF}}$, $\bar{P}_{\mathrm{HF}}$, $\bar{P}_{\mathrm{LF_n}}$, $\bar{R}_{\mathrm{LF/HF}}$ from all subjects in the different parts of the test with parameters measured into the classical bands. In the right, median ± mad of $\bar{d}_{\mathrm{HRM_R}}$, $\bar{P}_{\mathrm{VLF_R}}$, $\bar{P}_{\mathrm{LF_R}}$, $\bar{P}_{\mathrm{HF_R}}$, $\bar{P}_{\mathrm{LF_{Rn}}}$, $\bar{R}_{\mathrm{LF/HF_R}}$ from all subjects in the different parts of the test with the HF band redefinition. The measure unit are seconds (s), adimensional (ad) and normalized units (nu). Significant differences according to the Friedman test are denoted: *, p-value $< 0.05$; †, p-value $< 10^{-3}$; ‡, p-value $< 10^{-4}$.

| Classical bands (35 subjects) | | | | | HF band redefinition (24 subjects) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Stage | $BL_{\mathrm{S}}$ | $ST$ | $SA$ | $VE$ | Stage | $BL_{\mathrm{S}}$ | $ST$ | $SA$ | $VE$ |
| $\bar{d}_{\mathrm{HRM}}(s^{-1})$ | 1.24 ±0.16 | 1.4 ±0.19‡ | 1.23 ±0.16 | 1.25 ±0.17 | $\bar{d}_{\mathrm{HRM_R}}(s^{-1})$ | 1.26 ±0.17 | 1.4 ±0.22‡ | 1.25 ±0.16 | 1.28 ±0.17 |
| $\bar{P}_{\mathrm{VLF}}(s^{-2})$ | 0.49 ±0.13 | 0.62 ±0.24* | 0.49 ±0.12 | 0.50 ±0.16 | $\bar{P}_{\mathrm{VLF_R}}(s^{-2})$ | 0.51 ±0.13 | 0.63 ±0.26* | 0.49 ±0.12 | 0.53 ±0.15 |
| $\bar{P}_{\mathrm{LF}}(ad)$ $\cdot 10^3$ | 1.67 ±2.41 | 2.27 ±1.15* | 1.87 ±2.67 | 1.18 ±2.52 | $\bar{P}_{\mathrm{LF_R}}(ad)$ $\cdot 10^3$ | 1.44 ±1.51 | 2.47 ±1.23* | 1.96 ±1.69 | 1.43 ±1.17 |
| $\bar{P}_{\mathrm{HF}}(ad)$ $\cdot 10^3$ | 1.18 ±2.25 | 0.91 ±0.89* | 1.15 ±2.24 | 0.91 ±2.05 | $\bar{P}_{\mathrm{HF_R}}(ad)$ $\cdot 10^3$ | 1.05 ±1.94 | 0.57 ±0.68* | 0.84 ±1.76 | 0.58 ±1.49 |
| $\bar{P}_{\mathrm{LF_n}}(nu)$ | 0.59 ±0.20 | 0.68 ±0.11 | 0.65 ±0.15 | 0.59 ±0.15 | $\bar{P}_{\mathrm{LF_{Rn}}}(nu)$ | 0.58 ±0.21 | 0.85 ±0.14* | 0.69 ±0.16* | 0.67 ±0.18 |
| $\bar{R}_{\mathrm{LF/HF}}(nu)$ | 1.45 ±2.01 | 2.11 ±1.29 | 1.83 ±1.32 | 1.41 ±1.03 | $\bar{R}_{\mathrm{LF/HF_R}}(nu)$ | 1.39 ±2.28 | 5.64 ±4.62* | 2.24 ±2.01* | 2.04 ±2.25 |

showing the spectral power distribution (SPD) in the selected frequencies. This is an example of how $\bar{P}_{\mathrm{LF_R}}$ is higher during stress stages.

$\bar{P}_{\mathrm{HF_R}}$ decreases in all stress stages with respect to $BL_{\mathrm{S}}$, being statistically significant only during $ST$. $\bar{P}_{\mathrm{LF_{Rn}}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$ accounts for variations in the former parameters and displays a significant increase during $ST$ and $SA$ with respect to $BL_{\mathrm{S}}$. These behaviors can be appreciated in Fig. 4.4, where all the parameters studied are compared between the two methods.

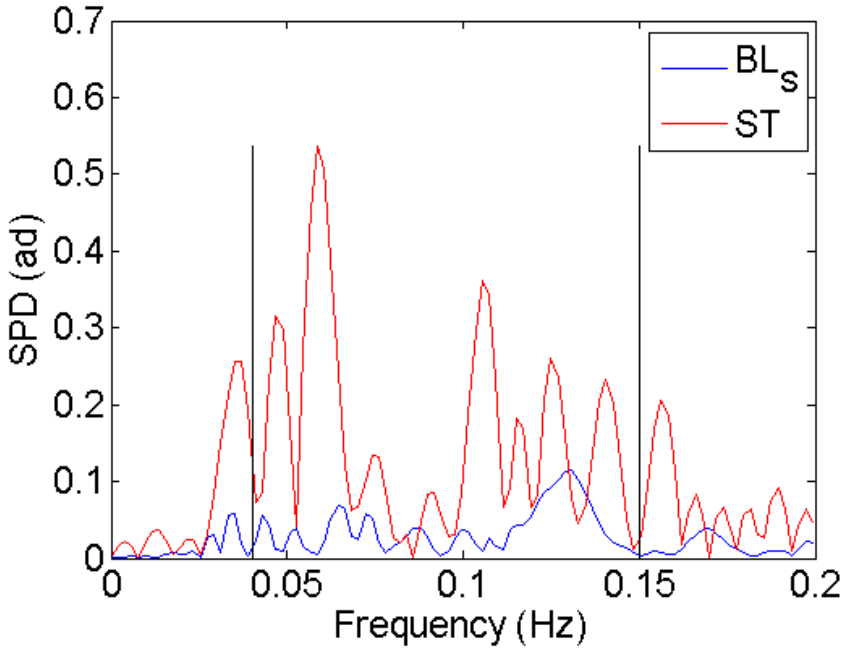



Figure 4.3: One example of how $\bar{P}_{\mathrm{LF_R}}$ (between 0 and 0.15 Hz) is higher in $ST$ than in $BL_{\mathrm{S}}$.

When comparing both basal stages, results in $BL_{\mathrm{B}}$ stage with $\Omega_{\mathrm{HF_R}}(n)$, with only 8 subjects due to the overlapping, are: $\bar{d}_{\mathrm{HRM_R}} = 1.22 \pm 0.17$; $\bar{P}_{\mathrm{VLF_R}} = 0.51 \pm 0.13$; $\bar{P}_{\mathrm{LF_R}} \cdot 10^3 = 1.83 \pm 1.95$; $\bar{P}_{\mathrm{HF_R}} \cdot 10^3 = 1.19 \pm 2.1$, $\bar{P}_{\mathrm{LF_{Rn}}} = 0.61 \pm 0.1$ and $\bar{R}_{\mathrm{LF/HF_R}} = 1.54 \pm 0.54$. An increase in the last four parameters is observed, however, no statistically significant differences are found.

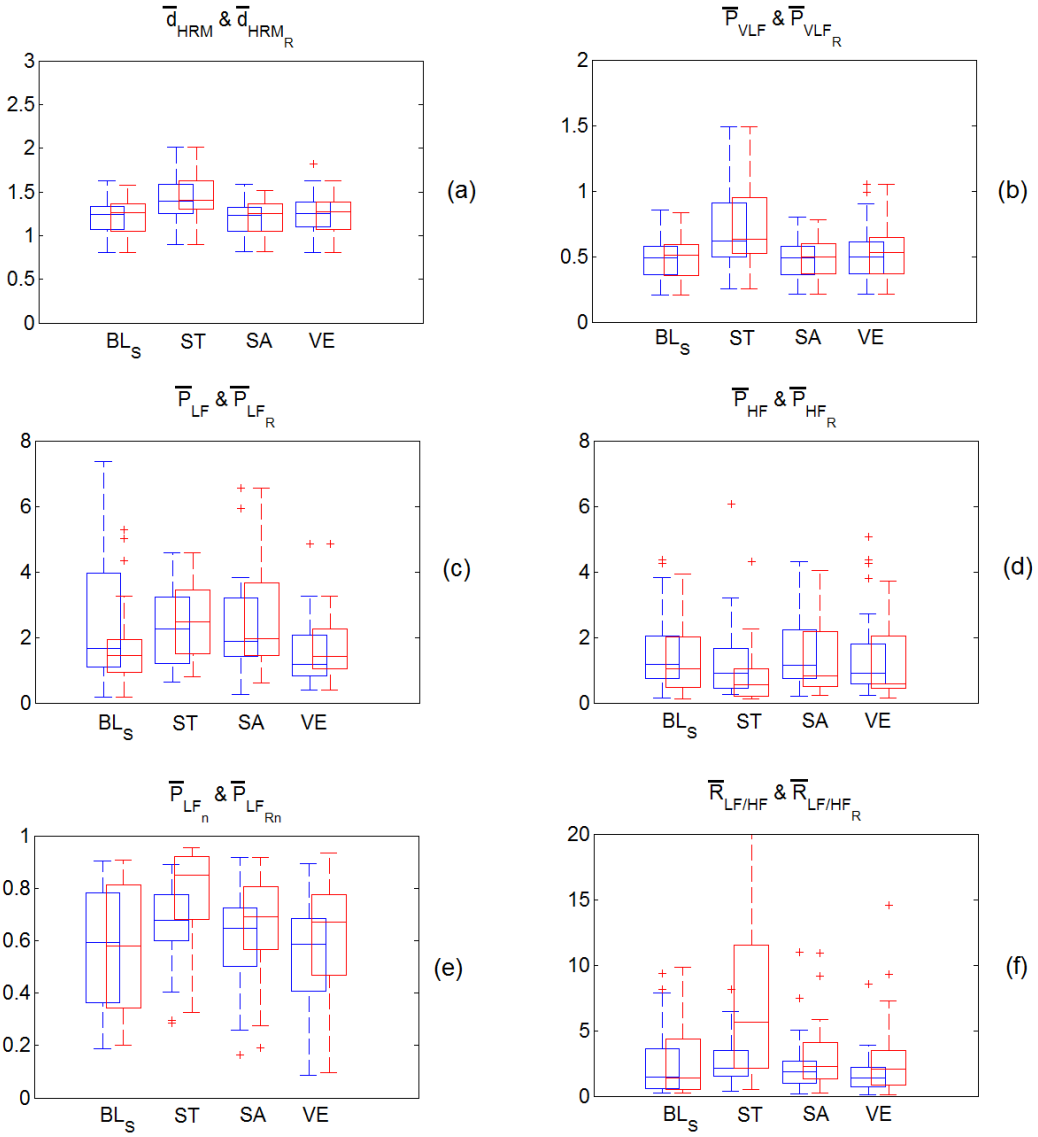



Figure 4.4: Boxplot of the 6 parameters studied in this work: a) $\bar{d}_{\mathrm{HRM}}$ and $\bar{d}_{\mathrm{HRM_R}}$; b) $\bar{P}_{\mathrm{VLF}}$ and $\bar{P}_{\mathrm{VLF_R}}$; c) $\bar{P}_{\mathrm{LF}}$ and $\bar{P}_{\mathrm{LF_R}}$; d) $\bar{P}_{\mathrm{HF}}$ and $\bar{P}_{\mathrm{HF_R}}$; e) $\bar{P}_{\mathrm{LF_n}}$ and $\bar{P}_{\mathrm{LF_{Rn}}}$; f) $\bar{R}_{\mathrm{LF/HF}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$. In blue with classical analysis; in red with the new HF band.

# Chapter 5

# Discussion

In this work respiration and HRV analysis during different stress levels is performed. In addition to the classical HRV frequency bands, a time-varying HF band was considered in order to take into account the respiration effects on HRV. Furthermore, some respiratory-rate-related parameters were also studied.

Respiratory rate was significantly higher (according to the Friedman test) during stressful stages than during relax stage, in agreement with results reported in [9], where the baseline recording presented a lower respiratory rate than in attention or mental arithmetic task.

Spectral peakness and the percentage of spectra accepted to compute the respiratory rate are studied in this work as measures of respiration stability. The more stable respiration is, the more peaked the spectra are and a higher number of them are included in the average. Results obtained for both respiratory stability measures show more stable respiration during relax than during the stress stages of the protocol. The less peaked spectra are found in $VE$, while the fewest spectra used are in $ST$. In [19] respiratory variation measured as the variation of a breath component over a sampling period of 15 minutes is computed as a coefficient that increased with mental task compared to relax situation.

HRV analysis using classical bands showed significant differences with respect to the $BL_{\mathrm{S}}$ stage in only 4 cases: $\bar{d}_{\mathrm{HRM}}$ in $ST$ and $\bar{P}_{\mathrm{VLF}}$, $\bar{P}_{\mathrm{LF}}$ and $\bar{P}_{\mathrm{HF}}$ in $ST$. This observation may be related to the fact that in some cases respiration is in LF band during relax, leading to an overestimation of $\bar{P}_{\mathrm{LF}}$ and an underestimation of $\bar{P}_{\mathrm{HF}}$, confounding classical HRV indices interpretation. In the analyzed dataset, 8 out of the 35 subjects (23%) are breathing with a respiratory rate in the LF band during $BL_{\mathrm{S}}$, and 7 out of the 18 subjects (38.9%) during $BL_{\mathrm{B}}$.

Furthermore, it may also happen that respiratory rate is above classical HF band during stress, as exemplified in Fig. 3.4, leading to an underestimation of $\bar{P}_{\mathrm{HF}}$ and, consequently, an overestimation of $\bar{P}_{\mathrm{LF_n}}$ and $\bar{R}_{\mathrm{LF/HF_n}}$. The percentage of subjects with a respiratory rate higher than 0.35 Hz (so part of $\Omega_{\mathrm{HF_R}}(n)$ is over 0.4 Hz) are: 2.8% in $BL_{\mathrm{S}}$; 48.6% in $ST$; 22.9% in $SA$; 31.4% in $VE$.

When respiratory frequency information is included in HRV analysis, more differences are appreciated between basal and the stress stages. Significant differences with respect to $BL_{\mathrm{S}}$ were observed for the same cases than with classical frequency bands and, in addition, for $\bar{P}_{\mathrm{LF_{Rn}}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$ in both $ST$ and $SA$.

Some works have reported an increase in LF band [9, 10] while others have not found sig-

nificant differences [11]. In our study an increase is observed in $\bar{P}_{\mathrm{LF_R}}$ during $ST$ and $SA$ with respect to $BL_{\mathrm{S}}$, being statistically significant during $SA$.

$\bar{P}_{\mathrm{HF_R}}$ is lower than $\bar{P}_{\mathrm{HF}}$ in all stages since the HF band in $\bar{P}_{\mathrm{HF_R}}$ is narrower than in $\bar{P}_{\mathrm{HF}}$. However, it is appreciated a larger relative reduction during stress stages with respect to $BL_{\mathrm{S}}$ in $\bar{P}_{\mathrm{HF_R}}$ than in $\bar{P}_{\mathrm{HF}}$, supporting the use of respiratory sinus arrhythmia to assess stress, as proposed in [8] and confirmed by [11, 12, 13]. The larger increase of $\bar{P}_{\mathrm{LF_R}}$ in $SA$ than in $ST$, and the larger decrease of $\bar{P}_{\mathrm{HF_R}}$ in $ST$ than in $SA$ may be related to the different types of stressors. For example, during $ST$ there is a large demand of attention while during $SA$ the stress is mainly psychological or emotional.

$\bar{P}_{\mathrm{LF_{Rn}}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$ are significantly higher during $ST$ and $SA$ than during $BL_{\mathrm{S}}$, suggesting a sympathetic dominance. These results are in agreement with those reported in [9, 10, 11, 17]. Note that $\bar{P}_{\mathrm{LF_n}}$ and $\bar{R}_{\mathrm{LF/HF}}$, without the use of respiratory information, do not show significant differences.

In this study , the overestimation of $\bar{P}_{\mathrm{LF}}$ and underestimation of $\bar{P}_{\mathrm{HF}}$ due to a low respiratory rate are avoided by discarding those segments of HRV where respiration is in LF band. However, further studies should consider the separation of PNS and SNS activities in this situation. Another limitation of the study is that the method is only valid for those intervals when respiratory rate can be properly estimated (sufficiently peaked spectra). Future studies should considered the respiration effects over HRV when the subject is speaking.

Despite the fact that the inclusion of respiratory frequency information in HRV analysis allows a more reliable characterization of ANS response to stress in terms of SNS and PNS activities, respiratory frequency presents with stronger statistical differences (lowest p-value) than any HRV index, suggesting its potential for stress assessment.

The complementary information that HRV analysis can add to respiration analysis for stress assessment should be considered in a larger study and should include those cases where respiratory frequency cannot be estimated robustly.

# Chapter 6

# Conclusion

The first part of the work comprises the recording of database of young healthy volunteers undergoing a protocol aimed at inducing acute emotional stress. Several physiological signals, including the ECG and respiration signals, were recorded.

The second part includes the spectral analysis of HRV in the classical frequency bands, commonly associated to the sympathetic and parasympathetic systems. A time-frequency representation of the modulating signal which carries ANS information is done, and the following frequency bands are defined: low frequency (LF, from 0.04 to 0.15 Hz) and high frequency (HF, from 0.15 to 0.4 Hz). Several indices, reported to be measures of sympathovagal balance, have been extracted in order to study if they are able to discriminate between being stressed or not. Frequency domain HRV indices, computed in classical terms, scarcely show statistical differences during stress.

The third part in this work has focused on the analysis of respiratory information, namely its rate and its stability. Respiration stability in this work is measured as the peakness of respiration spectrum, which is computed as the percentage of the power around the peak with respect to total power. Results show high discriminative power considering respiratory rate information, suggesting that it can discriminate the different stress states. This, however, comes at the cost of losing the excerpts where this rate can not be estimated.

The last part has considered the analysis of HRV taking into account respiratory information. Respiratory frequency is used to define the HF band and to avoid the measurement of power in the LF and HF bands, when respiratory frequency is too low and falls within the LF band. This avoids the overestimation of sympathetic activity and the underestimation of parasympathetic activity that occurs when the respiration rate lies in LF band. This combined HRV and respiratory rate analysis, increases the discrimination power among different stress situations, where a major sympathetic dominance is observed.

This work has been presented in the IEEE Journal of Biomedical and Health Informatics, in a special issue about mental disorders called “Sensor Informatics for Managing Mental Health”. The title of the article is: “Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment”.

# Chapter 7

# Future work

One limitation of this study is the number of subject used in the final computation: only 35 out of 80 available subjects were analyzed (43.8%). The percentage of subjects used can be increased if a entire recording is not discarded when only one stage is not correctly recorded. Another possibility to increase the number of subjects is the inclusion of 40 more volunteers recorded in the Polytechnic University of Madrid.

When the modification of the HRV analysis is done including respiratory information, if the overlapping between LF and HF bands exists, the algorithm discarded these measures. In order to improve the analysis, a parametric method can be applied. This method consists in the decomposition of the two bands using the information of the zeros and poles related to each band, computing each one separately, without the overlapping between them. With this approximation the separation of parasympathetic and sympathetic activities can be considered. This may allow not to lose the information of these subjects where the overlapping happens during a whole interval.

Another improvement that should be included in future work is the analysis of that stages where respiratory rate can not be extracted because of talking. In these cases, respiratory rate can not be estimated, so our proposal about redefining the HF band centered in respiratory rate is not useful. Therefore, a modification in the HF band can be done, defining the same in function of the coherence between HRV and respiration.

Finally, these results could be complemented by the processing of the rest of signals extracted in the test. Adding new markers to the respiratory and HRV parameters presented in this work will complete the analysis, creating a more objective and reliable method for the identification of stress.

# References


[1] R. Glaser and J. Kiecolt-Glaser, "Stress-induced immune dysfunction: implications for health," *Nature Reviews Immunology*, vol. 5, pp. 243–251, 2005.

[2] G. M. A. Bao and D. Swaab, "The stress system in depression and neurodegeneration: focus on the human hypothalamus," *Brain Res Rev*, vol. 57(2), pp. 531–553, 2008.

[3] D. Pizzagalli, "Depression, stress, and anhedonia: toward a synthesis and integrated model," *Annu. Rev. Clin. Psychol.*, vol. 10, pp. 393–423, 2014.

[4] M. Kivimäki, P. Leino-Arjas, R. Luukkonen, H. Riihimäi, J. Vahtera, and J. Kirjonen, "Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employees," *British Medical Journal*, vol. 35, p. 857, 2002.

[5] T. Heidt, H. B. Sager, G. Courties, P. Dutta, Y. Iwamoto, A. Zaltsman, C. von zur Muhlen, C. Bode, G. L. Fricchione, J. Denninger, C. P. Lin, C. Vinegoni, P. Libby, F. K. Swirski, R. Weissleder, and M. Nahrendorf, "Chronic variable stress activates hematopoietic stem cells," *Nat Med*, vol. 20, pp. 754–758, 07 2014.

[6] J. Aguiló, P. Ferrer-Salvans, A. García-Rozo, A. Armario, A. Corbí, F. Cambra, R. Bailón, A. González-Marcos, G. Caja, S. Aguiló, R. López-Antón, A. Arza-Valdés, and J. Garzón-Rey, "Proyecto es3: intentando la cuantificación y medida del nivel de estrés," *RevNeurol*, vol. 61, no. 09, pp. 0405–415, 1.

[7] Working group of ESC, "Heart rate variability. Standards of measurement, physiological interpretation, and clinical use," *Eur Heart J*, vol. 17, pp. 354–ÂŰ381, 1996.

[8] S. Porges, "Cardiac vagal tone: a physiological index of stress," *Neuroscience and Biobehavioral Reviews*, vol. 19(2), pp. 225–233, 1995.

[9] L. Bernardi, J. Wdowczyk-Szulc, C. Valenti, S. Castoldi, C. Passino, G. Spadacini, and P. Sleight, "Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability," *Journal of the American College of Cardiology*, vol. 35, no. 6, pp. 1462 – 1469, 2000.

[10] M. Pagani, G. Mazzuero, A. Ferrari, D. Liberati, S. Cerutti, D. Vaitl, L. Tavazzi, and A. Malliani, "Sympathovagal interaction during mental stress. a study using spectral analysis of heart rate variability in healthy control subjects and patients with a prior myocardial infarction," *Circulation*, vol. 83(4), pp. 43–51, 1991.

[11] N. Hjortskov, D. Rissén, A. Blangsted, N. Fallentin, U. Lundberg, and K. Søgaard, "The effect of mental stress on heart rate variability and blood pressure during computer work," *European Journal of Applied Physiology*, vol. 92, no. 1-2, pp. 84–89, 2004.

[12] J. Taelman, S. Vandeput, E. Vlemincx, A. Spaepen, and S. Van Huffel, "Instantaneous changes in heart rate regulation due to mental load in simulated office work," *European Journal of Applied Physiology*, vol. 111, no. 7, pp. 1497–1505, 2011.

[13] D. Widjaja, M. Orini, E. Vlemincx, and S. Van Huffel, "Cardiorespiratory dynamic response to mental stress: A multivariate time-frequency analysis," *Computational and Mathematical Methods in Medicine*, vol. 2013, p. 12, 2013.

[14] Z. Visnovcova, M. Mestanik, M. Javorka, D. Mokra, M. Gala, A. Jurko, A. Calkovska, and I. Tonhajzerova, "Complexity and time asymmetry of heart rate variability are altered in acute mental stress," *Physiological Measurement*, vol. 35, no. 7, p. 1319, 2014.

[15] L. Mainardi, "On the quantification of heart rate variability spectral parameters using time-frequency and time-varying methods," *Phil. Trans. R. Soc. A*, vol. 367, pp. 255–275, 2009.

[16] R. Bailón, G. Laouini, C. Grao, M. Orini, P. Laguna, and O. Meste, "The integral pulse frequency modulation model with time-varying threshold: Application to heart rate variability analysis during exercise stress testing," *Biomedical Engineering, IEEE Transactions on*, vol. 58, pp. 642–652, March 2011.

[17] P. Zhang, W. Tapp, S. Reisman, and B. Natelson, "Respiration response curve analysis of heart rate variability," *Biomedical Engineering, IEEE Transactions on*, vol. 44, pp. 321–325, April 1997.

[18] Y. Masaoka and I. Homma, "R," *IEEE Trans. Biomed. Eng.*, vol. 44, pp. 321–325, 1997.

[19] E. Vlemincx, J. Taelman, S. De Peuter, I. Van Diest, and O. Van Den Bergh, "Sigh rate and respiratory variability during mental load and sustained attention," *Psychophysiology*, vol. 48, no. 1, pp. 117–120, 2011.

[20] A. Hernando, J. Lázaro, A. Arza, J. Garzón, E. Gil, P. Laguna, J. Aguiló, and R. Bailón, "Changes in respiration during emotional stress," *Computing in Cardiology*, 2015.

[21] A. Arza, J. Garzón, A. Hernando, J. Aguiló, and R. Bailón, "Towards an objective measurement of emotional stress: Preliminary analysis based on heart rate variability," pp. 3331–3334, Aug 2015.

[22] J. Hellhammer and M. Schubert, "The physiological response to trier social stress test relates to subjective measures of stress during but not before or after the test," *Psychoneuroendocrinology*, vol. 37(1), pp. 119–124, 2012.

[23] U. M. Nater, B. Ditzen, J. Strahler, and U. Ehlert, "Effects of orthostasis on endocrine responses to psychosocial stress," *International Journal of Psychophysiology*, vol. 90, no. 3, pp. 341 – 346, 2013.

[24] S. A. Urwyler, P. Schuetz, C. Sailer, and M. Christ-Crain, "Copeptin as a stress marker prior and after a written examination: the coexam study," *Stress*, vol. 18, no. 1, pp. 134–137, 2015.

[25] J. P. Martínez, R. Almeida, S. Olmos, A. Rocha, and P. Laguna, "A wavelet-based ecg delineator: evaluation on standard databases," *Biomedical Engineering, IEEE Transactions on*, vol. 51, pp. 570–581, April 2004.

[26] J. Mateo and P. Laguna, "Analysis of heart rate variability in the presence of ectopic beats using the heart timing signal," *IEEE Trans Biomed Eng*, vol. 50(3), pp. 334–343, 2003.

[27] R. Bailón, N. Garatachea, I. de la Iglesia, J. Casajús, and P. Laguna, "Influence of running stride frequency in heart rate variability analysis during treadmill exercise testing," *Biomedical Engineering, IEEE Transactions on*, vol. 60, pp. 1796–1805, July 2013.

[28] J. Lázaro, A. Alcaine, D. Romero, E. Gil, P. Laguna, E. Pueyo, and R. Bailón, "Electrocardiogram derived respiratory rate from qrs slopes and r-wave angle," *Annals of Biomedical Engineering*, vol. 40(10), pp. 2072–2083, 2014.

[29] S. Kumar, M. Al-Absi, J. G. Beck, E. Ertin, and M. S. Scott, "Behavioral monitoring and assessment via mobile sensing technologies," *Behavioral Healthcare and Technology*, pp. 621–624, 2015.

[30] R. Bailón, P. Laguna, L. Mainardi, and L. Srnmo, "Analysis of heart rate variability using time-varying frequency bands based on respiratory frequency," *29th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, pp. 6674–6677, 2007.

# APPENDIXES

# Appendix A

# Sensor Informatics for Managing Mental Health 2015

This work has been presented in the IEEE Journal of Biomedical and Health Informatics, in a special issue about mental disorders called "Sensor Informatics for Managing Mental Health". The title of the article is: "Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment".

# Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment

Alberto Hernando, Jesús Lázaro, Adriana Arza, Jorge Mario Garzón, Eduardo Gil, Pablo Laguna, Jordi Aguiló, Raquel Bailón

***Abstract*—Respiratory rate and heart rate variability (HRV) are studied as stress makers in a database of young healthy volunteers subjected to acute emotional stress, induced by a modification of the Trier Social Stress Test.**

**First, instantaneous frequency domain HRV parameters are computed using time-frequency analysis in the classical bands. Then, respiratory rate is estimated and this information is included in HRV analysis in two ways: i) redefining the high frequency (HF) band to be centered at respiratory frequency; ii) excluding from the analysis those instants where respiratory frequency falls within the low frequency (LF) band.**

**Classical frequency domain HRV indices scarcely show statistical differences during stress. However, when including respiratory frequency information in HRV analysis, the normalized LF power as well as the LF/HF ratio significantly increase during stress (p-value $<$ 0.05 according to Friedman test). This combine HRV and respiratory rate analysis, increasing the discrimination power among different stress situations, where a major sympathetic dominance is observed. LF power increases during stress, only being significantly different in stress anticipation stage, while HF power decreases during stress, only being significantly different during the stress task demanding attention.**

**In addition, respiratory frequency is observed to be higher and less stable during stress than during relax (p-value $<$ 0.05 according to Friedman test).**

**Our results support that joint analysis of respiration and HRV obtain a more reliable characterization of autonomic nervous response in response to stress.**

**Additionaly, considering just respiratory rate information, has shown a even higher discriminative power, suggesting that it can also be an index to discriminate the different stress states. This, however, comes at the cost of losing the excerpts where this rate can not be estimated.**

## I. Introduction

STRESS is the physiological response to a threat, either physical or psychological, mainly mediated by the autonomic nervous system (ANS) through its two branches, sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). This response starts in the hypothalamus, which triggers the sympathetic "fight or flight" response to provide the body with the energy to address the perceived danger. Once the threat has passed, the parasympathetic "rest and digest" response restores the body homeostasis. In this way, stress is a necessary survival mechanism and not health-threatening.

A. Hernando, J. Lázaro, E. Gil, P. Laguna and R. Bailón are with BSICoS Group, Aragón Institute of Engineering Research (I3A), University of Zaragoza, and CIBER-BBN, Zaragoza, Spain.

A. Arza, J. M. Garzón and J. Aguiló are with Microelectronics and Electronic Systems Department. Autonomous University of Barcelona, Bellaterra, Spain.

However, when stress response is maintained in time or it is initiated over and over again, the body cannot reach its homeostasis. Prolonged stress has been associated with dysfunctions in the immune system [1], psychiatric disorders such as anxiety, depression and Alzheimer [2], [3] and cardiovascular diseases [4], [5]. The World Health Organization has called stress the health epidemic of the $21^{st}$ century.

Despite the high incidence and negative consequences of stress, there is not a reliable tool for the noninvasive, objective and continuous monitoring of stress level. This is the goal of ES3 project, which includes different physiological signals, biochemical markers and psychometric questionnaires, during physical, emotional and even chronic stress [6]. In this work we will focus on acute emotional stress.

Heart rate variability (HRV) at rest is widely accepted as a noninvasive measure of the ANS regulation of the heart. Spectral analysis of HRV at rest reveals two main components: i) a high frequency (HF) component in the range from 0.15 to 0.4 Hz, mainly due to respiratory sinus arrhythmia, and ii) a low frequency (LF) component in the range from 0.04 to 0.15 Hz, which reflects both sympathetic and parasympathetic activity. Power in the HF band has been used as a measure of parasympathetic activity. Power in the LF band normalized by power in both the LF and HF bands has been considered a measure of sympathetic dominance. The ratio between the power in the LF and HF bands (LF/HF ratio) is considered a measure of sympathovagal balance [7].

Due to its relation to ANS activity, HRV has been widely used to characterize the stress response. Most of the research on HRV response to stress is focused on the measurement of SNS excitation through the normalized power in the LF band and the sympathovagal ratio. A different approach is considered in [8], where respiratory sinus arrhythmia, as a marker of PNS tone, is proposed to assess stress and vulnerability to stress. In this approach PNS tone is considered to parallel homeostasis and a withdrawal of PNS tone would represent the disruption of homeostasis induced by stress.

Most of the studies suggest higher sympathetic dominance during stress than during resting or relaxing conditions, however changes in specific HRV parameters published in literature are inconsistent even when restricting to an specific emotional/cognitive type of stress. For example, an increase in the LF power has been reported during mental arithmetic [9]. An increase in sympathovagal balance and in normalized LF power during mental task was found in controls but not in patients with a prior myocardial infarction [10]. Mental stressors added during computer work caused a decrease in

the HF power and an increase in the LF/HF ratio, but not an increase in the LF power in [11]. In [12], [13] a decrease both in LF and HF powers is reported during mental load added to a normal office task. Lower HF power was also observed during Stroop test and mental arithmetic, while LF power increased during Stroop test and decreased during the arithmetic test [14].

Specific differences in stress stimulus and population are not enough to explain the differences found in the results. Some of the inconsistent results may be due to the methodology applied for the spectral analysis of HRV. Time-frequency analysis could allow to characterize the nearly instantaneous response to acute stress, which may be blurred with time-invariant methods [15]. Moreover, differences in mean heart rate (HR) during stress and relaxing conditions can introduce a bias in HRV spectral parameters, which needs to be compensated for [16]. Finally, it has been shown that changes in respiratory pattern alter the spectral content of HRV [17], [9], and mental stress was reported to alter the breathing pattern, increasing both tidal volumne and respiration rate [18], [13]. Respiratory variability and sigh rate also change during mental stress and attentional tasks [19]. Thus, stress related changes in respiration may alter HRV parameters, obscuring their interpretation in terms of SNS and PNS activations.

In this work we analyze HRV and respiration changes in healthy subjects during acute emotional stress using time-frequency representations. Then, we include information on respiratory frequency in HRV analysis to obtain a more reliable interpretation of HRV parameters for stress assessment. A preliminary version of this work has been reported [20], where respiratory information was analyzed in a subset.

## II. Materials and methods

### A. Data collection

A data base of 48 volunteers (20 men and 28 women) with an age of $22.21 \pm 3.34$ years is used. These recordings were acquired in the Autonomous University of Barcelona (UAB) [21] and in the University of Zaragoza (UZ). The protocol defined in the following was approved by the Ethics Committee both at the UAB and UZ.

Each subject underwent a basal session and a stress session. These two sessions were completed in days close to each other and at the same hour (10 a.m. or 11.15 a.m., depending of the subject), trying to reproduce the same biorrythms-related stress conditions in both sessions. A chest-band-based respiratory signal and 3 orthogonal non-standard ECG leads were continuously recorded with a sampling rate of 250 Hz and 1000 Hz, respectively, using Medicom system (Medicom MTD Ltd, Russia).

The basal session ($BL_{\text{B}}$) consisted of a 35-minutes-length relaxing audition. The stress session tries to induce emotional stress by following a modification of the Trier Social Stress Test [22]. This session included the following stages:

- Baseline stage during stress session ($BL_{\text{S}}$): 10-minutes-length relaxing audition.
- Story telling stage ($ST$): 3 stories are told to the subject with a great amount of details. The subject is requested to remember as much details as possible, demanding a great amount of attention.
- Memory task ($MT$): the subject is requested to tell back every remembered detail within 30 seconds for each story.
- Stress anticipation ($SA$): subject is requested to wait for the evaluation of the memory test. The duration of this stage is 10 minutes.
- Video exposition ($VE$): a projection of a video with the subject performance in the memory test is shown. The video showed twice each one of the 3 stories. First, an actor repeats the story in a perfect way, trying to make the subject believe that this is the common case. Subsequently, the subject (recorded during the $MT$ stage) telling back the story is displayed.
- Arithmetic Task ($AT$): the subject has to count down from 1022 in steps of 13. In case of a calculation error, the subject is requested to restart from 1022. Although the subject is not expected to complete the countdown, he is requested to do so within 5 minutes. No subject completed the countdown.

The last five stages are considered stressful. $BL_{\text{S}}$ and $SA$ have longer duration that the other stages. Only the 6 central minutes of them are analyzed in this work. Furthermore, for $BL_{\text{B}}$, only the segment from 2 to 8 minutes (6 central minutes of the first 10 minutes) was analyzed, in order to compare it with the 6 central minutes in $BL_{\text{S}}$.

### B. Heart rate variability analysis

First, heart beats are detected from Z lead of the recorded ECG signal using an algorithm based on wavelets [23]. Ectopic beats, missed and false detentions are identified [24].Then, an instantaneous heart rate signal $d_{\text{HR}}(n)$, sampled at 4 Hz, is obtained from the beat occurrence time series based on the integral pulse frequency modulation model, which accounts for the presence of ectopic beats [24], [16].

$$d_{\text{HR}}(n) = \frac{1 + \mathfrak{m}(n)}{T(n)} \tag{1}$$

where $\mathfrak{m}(n)$ represents the modulating signal which carries the information from ANS and $T(n)$ is the mean heart rate, which is considered to be (slow-)time-variant by this model.

Then, a time-varying mean HR, $d_{\text{HRM}}(n)$, is obtained by low-pass filtering $d_{\text{HR}}(n)$, with a cut off frequency of 0,03 Hz:

$$d_{\text{HRM}}(n) = \frac{1}{T(n)} \tag{2}$$

HRV signal is obtained as:

$$d_{\text{HRV}}(n) = d_{\text{HR}}(n) - d_{\text{HRM}}(n) \tag{3}$$

Finally, the modulating signal is estimated as [16]:

$$\mathfrak{m}(n) = \frac{d_{\text{HRV}}(n)}{d_{\text{HRM}}(n)} \tag{4}$$

This modulating signal is supposed to carry the information of ANS activity without the influence of HR.

Time-frequency analysis is applied to $\mathfrak{m}(n)$ in order to characterize the rapid response of the ANS to stress. The

smoothed psedudo Wigner-Ville distribution (SPWVD) of $\mathfrak{m}(n)$ is computed:

$$P_{\mathfrak{M}}(n,m) = 2 \cdot \sum_{l=-L+1}^{L-1} |h(l)|^2 \cdot \left[ \sum_{n'=-N+1}^{N-1} g(n') a_{\mathfrak{M}}(n+n'+l) a_{\mathfrak{M}}{}^*(n+n'-l) \right] \cdot e^{-j2l(m/M)\pi}; m = -M+1 \ldots M \quad (5)$$

where $n$ and $m$ are time and frequency indices. The analytic signal $a_{\mathfrak{M}}(n)$ is defined as $a_{\mathfrak{M}}(n) = \mathfrak{m}(n) + j \cdot \hat{\mathfrak{m}}(n)$, where $\hat{\mathfrak{m}}(n)$ represents the Hilbert transform of $\mathfrak{m}(n)$. The terms $g(n)$ and $h(l)$ are time and frequency smoothing windows, chosen to be Hamming windows whose lengths are $2 \cdot N + 1 = 203$ and $2 \cdot L + 1 = 1025$ samples respectively [25].

Instantaneous power in classical LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz) bands is computed from $P_{\mathfrak{M}}(n,m)$, yielding $P_{\text{LF}}(n)$ and $P_{\text{HF}}(n)$, respectively. Instantaneous power of the SPWVD of $d_{\text{HRM}}(n)$ is also computed and denoted $P_{\text{VLF}}(n)$.

Instantaneous normalized LF power $P_{\text{LF}_\text{n}}(n) = P_{\text{LF}}(n)/(P_{\text{LF}}(n) + P_{\text{HF}}(n))$ and LF/HF ratio, $R_{\text{LF/HF}}(n) = P_{\text{LF}}(n)/P_{\text{HF}}(n)$ are also considered.

### C. Respiratory rate estimation

Respiration signal is band-pass filtered (cut-off frequencies of 0.03 and 0.9 Hz) and downsampled to 4 Hz.

Respiratory rate was estimated from this filtered respiratory signal by using an algorithm based on [26]. The method consists in the estimation of the respiratory frequency ($F_{\text{R}}$) from "peaked-conditioned" averaged spectra.

Every 5 seconds, a power spectrum density $S_{\text{k}}(f)$ is estimated by using Welch periodogram from the $k^{th}$ 42 second length running window. Spectra obtained from 12 second-length subintervals overlapped 6 s are averaged. Subsequently, a measure of peakness is obtained from $S_{\text{k}}(f)$ as the percentage of power around the previous estimated respiratory rate $F_{\text{R}}(k-1)$ with respect to the total power within [0.08 Hz, 0.8 Hz] band:

$$P_k = \frac{\int_{F_{\text{R}}(k-1)-\delta}^{F_{\text{R}}(k-1)+\delta} S_{\text{k}}(f)df}{\int_{0.08}^{0.8} S_{\text{k}}(f)df} \cdot 100 \quad (6)$$

where $\delta$ value was empirically set as 0.1 Hz. Then, a peaked-conditioned average spectra, $\bar{S}_k(f)$, is obtained by averaging those $S_k(f)$ which are sufficiently peaked:

$$\bar{S}_k(f) = \sum_{l=-L_s}^{L_s} \chi_{k-l} S_{k-l}(f), \quad (7)$$

where $L_s$ was set to 2 in order to average a maximum of 5 spectra as in [26], and $\chi_{k-l}$ is a criterion to consider whether the power spectrum $S_{k-l}(f)$ is peaked enough or not:

$$\chi_k = \begin{cases} 1, & P_k \geq 0.65 \\ 0, & otherwise \end{cases}, \quad (8)$$

allowing to take part in the average only to those $S_k(f)$ whose $P_k$ is above 65%.

Figure 1 displays two spectra as examples, one with $P_{\text{k}} > 65\%$ (peaked enough to take part in the average), and another one with $P_{\text{k}} < 65\%$, (not peaked enough to take part in the average). Finally, respiratory rate is estimated as the maximum of $\bar{S}_{\text{k}}(f)$ within the band [0.08Hz, 0.8Hz]:

$$F_{\text{R}}(k) = \arg\max \bar{S}_{\text{k}}(f); \quad f \in [0.08Hz, 0.8Hz] \quad (9)$$

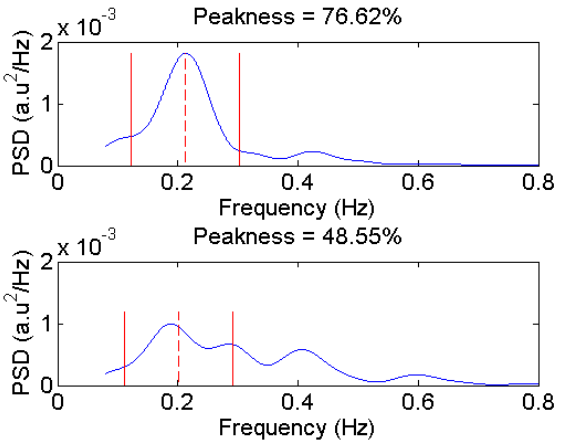



Fig. 1. Differences between spectra which satisfy the peakness condition and those which do not. Red lines illustrate the limits of the integrating interval of the numerator in $P_{\text{k}}$ and the dashed line marks the previous respiratory rate estimated $F_{\text{R}}(k-1)$ (see eq. 6)

Studied parameters were respiratory rate $F_{\text{R}}(k)$, the peakness $P_{\text{k}}$ and the percentage of spectra which take part in the peaked-conditioned average ($N_{\text{k}}$), considering the last two parameters to be related to the respiratory stability.

Respiratory frequency could not be estimated during $MT$ and $AT$ stages since speech modifies respiratory pattern [27] and no spectra would satisfy the peakness criterium.

### D. Heart Rate Variability study including respiratory information

Analysis of respiration revealed changes in respiratory frequency during stress conditions with respect to relax [18], [19]. In order to obtain a more reliable assessment of PNS activity, respiratory frequency estimation is included in HRV analysis redefining the HF band centered at respiratory frequency as in [28]. The method described in Section II-C offers an estimation of respiratory rate every five seconds, so a linear interpolation is made in order to obtain a respiratory frequency signal $F_{\text{R}}(n)$ with the same sampling rate than the HRV series (4 Hz).

The VLF and LF bands are the classical bands used in Section II-B ([0, 0.04 Hz] and [0.04, 0.15 Hz], respectively), while the HF band this time is defined as:

$$\Omega_{\text{HF}_\text{R}}(n) = [F_{\text{R}}(n) - 0.05Hz, F_{\text{R}}(n) + 0.05Hz] \quad (10)$$

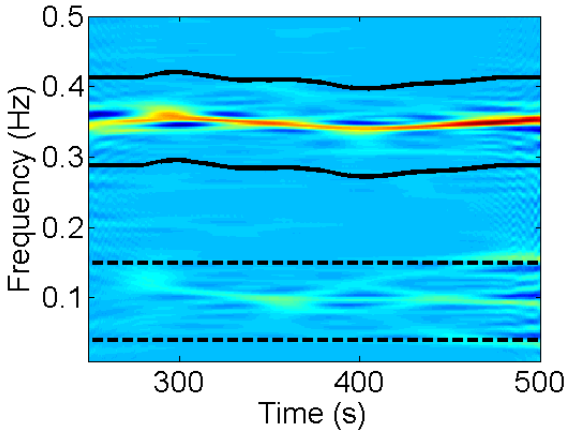



Fig. 2. $P_{\mathfrak{M}}(n,m)$ for one suject. Solid black lines represent $\Omega_{\text{HF}_\text{R}}(n)$. Dashed black lines represent LF band.

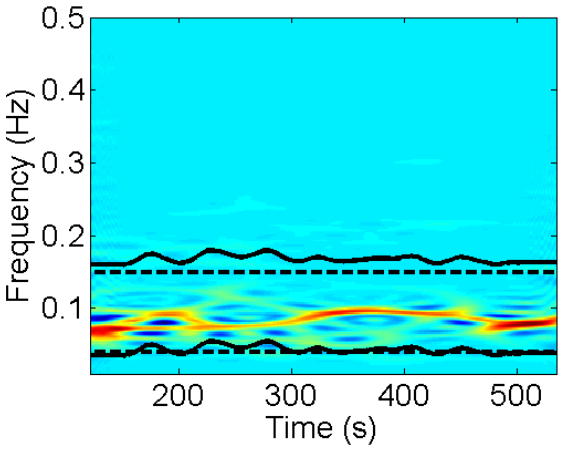



Fig. 3. $P_{\mathfrak{M}}(n,m)$ for one suject, representing the overlap between the two bands because of a low respiratory rate. Continue black lines represent $\Omega_{\text{HF}_\text{R}}(n)$. Dashed black lines represent LF band.

In Fig. 2 the new localization of HF band centered in respiratory rate, can be seen.

Instantaneous power in LF band and in $\Omega_{\text{HF}_\text{R}}(n)$ is computed for each subject yielding $P_{\text{LF}_\text{R}}(n)$ and $P_{\text{HF}_\text{R}}(n)$. In some stages of the test, specially during the basal stage, a low respiratory rate makes $\Omega_{\text{HF}_\text{R}}(n)$ overlaps with LF band. In order to avoid the measurement of the same power in both bands, a threshold that delimits the amount of overlapping percentage between $\Omega_{\text{HF}_\text{R}}(n)$ and LF band is defined. If at a given time instant $n$, overlapping is higher than experimentally adjusted 50%, $P_{\text{LF}_\text{R}}(n)$ and $P_{\text{HF}_\text{R}}(n)$ of this subject at that instant are not computed. Figure 3 shows an example where respiratory rate (mean respiratory rate is 0.1041 Hz) is within the LF band (inside dashed black lines), so $\Omega_{\text{HF}_\text{R}}(n)$ (between solid black lines) overlaps with it. The percentage of overlapping is higher than the fixed threshold (50%) during the whole interval displayed so $P_{\text{LF}_\text{R}}(n)$ and $P_{\text{HF}_\text{R}}(n)$ are not computed for any time instant within this interval. Normalized LF power and LF/HF ratio are also computed and denoted $P_{\text{LF}_\text{Rn}}(n)$ and $R_{\text{LF/HF}_\text{R}}(n)$.

### E. Statistical analysis

Intra-subject mean of each studied HRV index was obtained for each stage of the protocol, yielding the following parameters: $\bar{d}_{\text{HRM}}$ and $\bar{d}_{\text{HRM}_\text{R}}$; $\bar{P}_{\text{VLF}}$ and $\bar{P}_{\text{VLF}_\text{R}}$; $\bar{P}_{\text{LF}}$ and $\bar{P}_{\text{LF}_\text{R}}$; $\bar{P}_{\text{HF}}$ and $\bar{P}_{\text{HF}_\text{R}}$; $\bar{P}_{\text{LF}_\text{n}}$ and $\bar{P}_{\text{LF}_\text{Rn}}$; $\bar{R}_{\text{LF/HF}}$ and $\bar{R}_{\text{LF/HF}_\text{R}}$. Subindice R denoted HRV analysis including respiratory frequency information parameters. Note that in these last ones, different number of samples take part in the average since those time instants where LF and HF bands overlap more than 50% have been excluded.

In addition, three respiratory parameters are studied too: the intra-subject median of respiratory rate $\bar{F}_\text{R}$; of the peakness measure $\bar{P}_\text{k}$ and of the percentage of spectra used to compute the peaked-conditioned averaging $\bar{N}_\text{k}$.

The non-parametric Friedman statistical test was applied in order to study if there are significant differences between the $BL_\text{S}$ stage and each one of the other stages.

## III. Results

Only in 35 volunteers (13 men and 22 women, with an age of $22.49 \pm 3.49$) out of 48, respiratory rate can be estimated in all the stages of the stress session (without talk): $BL_\text{S}, ST, SA$ and $VE$. Only in 18 out of these 35 subjects respiratory rate can be estimated in the first basal session ($BL_\text{B}$).These stages are going to be analyzed to see if their distribution is similar to the pre-relaxing stage ($BL_\text{S}$).

### A. Respiratory parameters

Figure 4 illustrates one example of respiratory rate differences between $BL_\text{S}$ and $ST$ stages, showing a higher and less stable respiratory rate during $ST$ than during $BL_\text{S}$.

Table I shows the inter-subject median and the median absolute deviation (MAD) of $\bar{F}_\text{R}$, $\bar{P}_\text{k}$, and $\bar{N}_\text{k}$ among the 35 subjects. The respiratory rate is observed to be higher and less stable (lower $\bar{P}_\text{k}$ and $\bar{N}_\text{k}$) during the stress stages than during the $BL_\text{S}$ stage. These differences were statistically significant according to the Friedman test (p-value $< 0.05$).

TABLE I
MEDIAN $\pm$ MAD OF $\bar{F}_\text{R}$, $\bar{P}_\text{k}$ AND $\bar{N}_\text{k}$ FROM ALL SUBJECTS IN THE DIFFERENT PARTS OF THE TEST. SIGNIFICANT DIFFERENCES ACCORDING TO THE FRIEDMAN TEST ARE DENOTED: *, P-VALUE $< 0.05$; †, P-VALUE $< 10^{-3}$; ‡, P-VALUE $< 10^{-4}$.

| Stage | $BL_\text{S}$ | $ST$ | $SA$ | $VE$ |
|---|---|---|---|---|
| $\bar{F}_\text{R}(Hz)$ | 0.23 $\pm$0.06 | 0.35 $\pm$0.05‡ | 0.29 $\pm$0.04‡ | 0.34 $\pm$0.04‡ |
| $\bar{P}_\text{k}$ | 78.43 $\pm$4.96 | 75.15 $\pm$8.75 | 71.87 $\pm$8.06* | 74.19 $\pm$7.39* |
| $\bar{N}_\text{k}$ | 87.8 $\pm$12.5 | 54.55 $\pm$10.58‡ | 76.57 $\pm$10.77* | 74.37 $\pm$10.01* |

When comparing both basal stages ($BL_\text{B}$ and $BL_\text{S}$), results show a slightly lower respiratory rate ($0.211 \pm 0.06$) and percentage of spectra used ($77.87 \pm 5.81$) in $BL_\text{B}$. The peakness is very similar in both cases ($80.91 \pm 8.22$). These differences are not statistically significant.

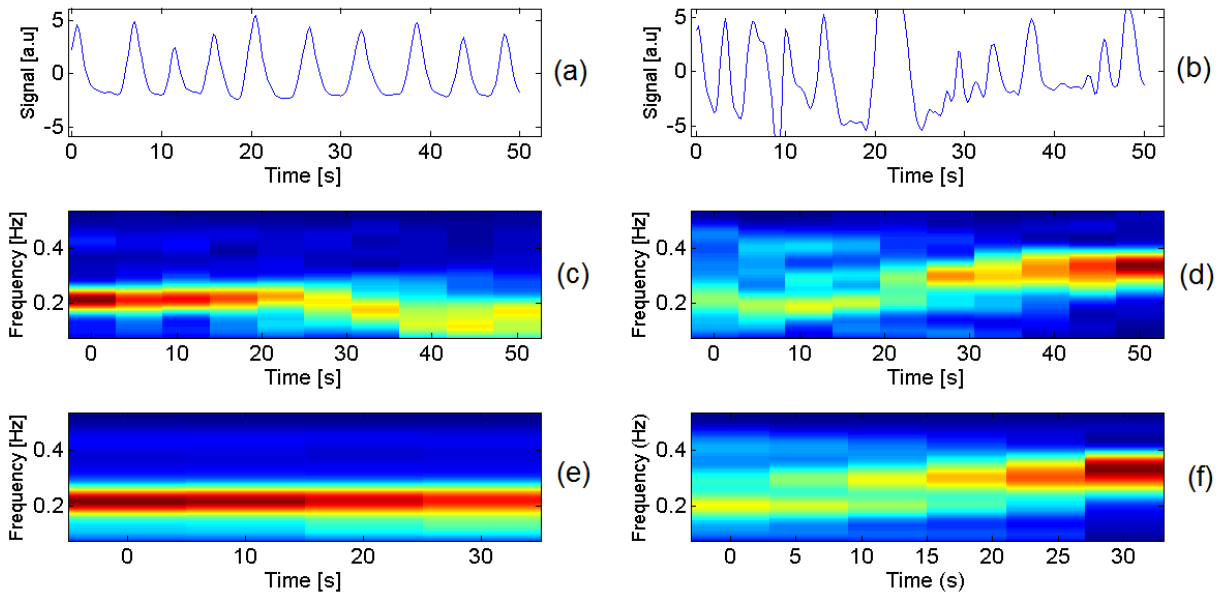



Fig. 4. a) and b) respiratory signal; c) and d) $S_{\mathrm{k}}(f)$; e) and f) $\bar{S}_{\mathrm{k}}(f)$; in basal stage (left side) and story telling (right side).

### B. HRV parameters

Fig. 5 displays the instantaneous HR signal $d_{\mathrm{HR}}(n)$, the modulating signal $\mathfrak{m}(n)$ and the SPWVD $P_{\mathfrak{M}}(n,m)$ for a subject of the database during one minute of stages $BL_{\mathrm{S}}$ and $ST$. The variation of HF band centered at respiratory frequency can be appreciated in the SPWVD, with a low respiratory rate that overlap with LF band in $BL_{\mathrm{S}}$ and just the opposite in $ST$, showing values over the limit of the HF classical band (0.4 Hz).

Table II shows the inter-subject median and MAD among the 35 subjects when using the classical bands and the proposed respiratory-rate-based HF band $\Omega_{\mathrm{HF_R}}(n)$.

Inclusion of respiratory frequency information in HRV analysis do not affect $\bar{d}_{\mathrm{HRM}}$ and $\bar{P}_{\mathrm{VLF}}$ estimation. Parameter $\bar{d}_{\mathrm{HRM}}$ significantly increases during $ST$ with respect to $BL_{\mathrm{S}}$, while no significant differences were found in the other stress stages.

Using the classical bands in HRV analysis, the only parameter with statistical differences is $\bar{P}_{\mathrm{HF}}$ during $ST$, showing a decrease with respect to $BL_{\mathrm{S}}$.

When respiratory frequency information is included in HRV analysis, more significant differences are found. $\bar{P}_{\mathrm{LF_R}}$ increases during $ST$ and $SA$ with respect to $BL_{\mathrm{S}}$, being significant during $SA$. $\bar{P}_{\mathrm{HF_R}}$ decreases in all stress stages with respect to $BL_{\mathrm{S}}$, being statistically significant only during $ST$. $\bar{P}_{\mathrm{LF_{Rn}}}$ and $\bar{R}_{\mathrm{LF/HF_R}}$ accounts for variations in the former parameters and displays a significant increase during $ST$ and $SA$ with respect to $BL_{\mathrm{S}}$. These behaviors can be appreciated in Fig. 6, where normalized power in LF band are compared between the two methods.

When comparing both basal stages, results in $BL_{\mathrm{B}}$ stage with $\Omega_{\mathrm{HF_R}}(n)$, with only 8 subjects due to the overlapping, are: $\bar{d}_{\mathrm{HRM_R}} = 1.22 \pm 0.17$; $\bar{P}_{\mathrm{VLF}} = 0.51 \pm 0.13$; $\bar{P}_{\mathrm{LF_R}} \cdot 10^3 = 2.5 \pm 1.7$; $\bar{P}_{\mathrm{HF_R}} \cdot 10^3 = 1.6 \pm 2.1$, $\bar{P}_{\mathrm{LF_{Rn}}} = 0.61 \pm 0.1$ and $\bar{R}_{\mathrm{LF/HF_R}} = 1.6 \pm 0.54$. A increase in the last four parameters are registered, however, no statistically significant differences are found.

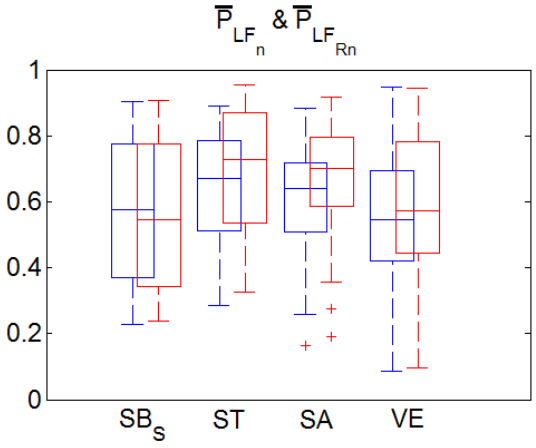



Fig. 6. Boxplot of the normalized power inside LF band in the four stages analyzed. In blue, $P_{\mathrm{LF_n}}$; in red, $P_{\mathrm{LF_{Rn}}}$.

## IV. Discussion

In this paper a respiration and a HRV analysis during different stress levels is performed. In addition to the classical HRV frequency bands, a time-varying HF band was considered in order to take into account the respiration effects on HRV. Furthermore, some respiratory-rate-related parameters were also studied.

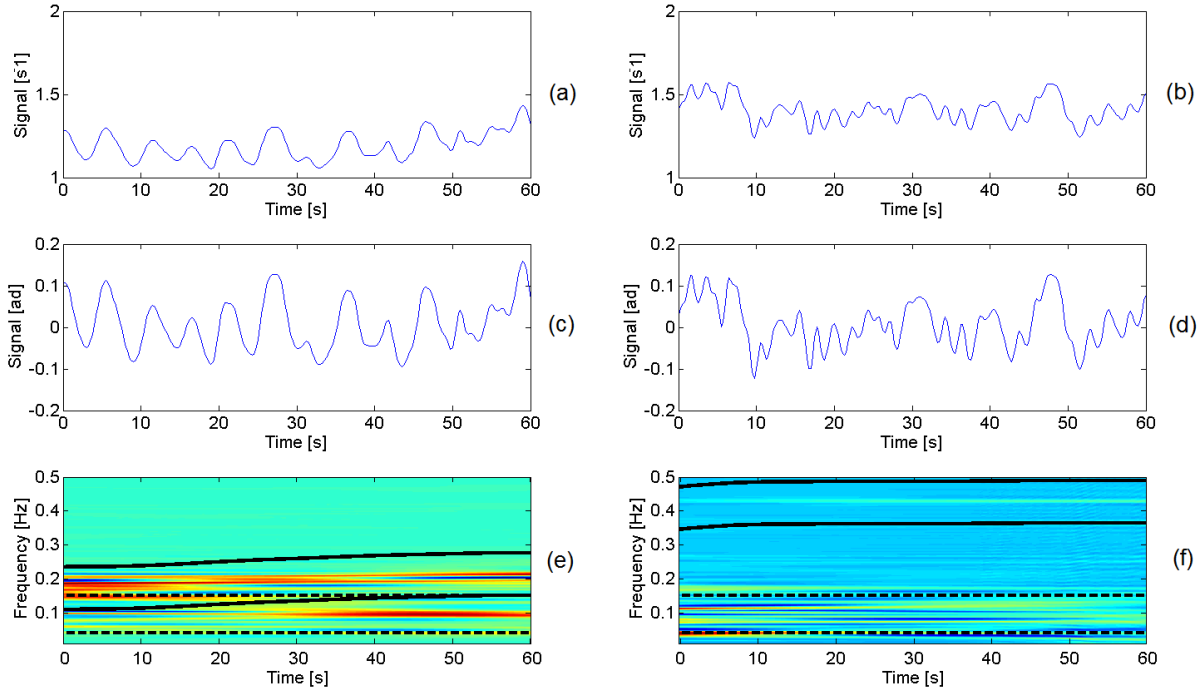



Fig. 5. a) and b) $d_{\text{HR}}(n)$; c) and d) $\mathfrak{m}(n)$ signals; e) and f) its respective SPVWD in a time-frequency map showing the variations of HF band related to respiration in pre-relaxing stage (left side) and story telling (right side).

TABLE II

IN THE LEFT, MEDIAN ± MAD OF $\bar{d}_{\text{HRM}}$, $\bar{P}_{\text{VLF}}$, $\bar{P}_{\text{LF}}$, $\bar{P}_{\text{HF}}$, $\bar{P}_{\text{LF}_\text{N}}$, $\bar{R}_{\text{LF/HF}}$ FROM ALL SUBJECTS IN THE DIFFERENT PARTS OF THE TEST WITH PARAMETERS MEASURED INTO THE CLASSICAL BANDS. IN THE RIGHT, MEDIAN ± MAD OF $\bar{d}_{\text{HRM}_\text{R}}$, $\bar{P}_{\text{VLF}}$, $\bar{P}_{\text{LF}_\text{R}}$, $\bar{P}_{\text{HF}_\text{R}}$, $\bar{P}_{\text{LF}_{\text{RN}}}$, $\bar{R}_{\text{LF/HF}_\text{R}}$ FROM ALL SUBJECTS IN THE DIFFERENT PARTS OF THE TEST WITH THE HF BAND REDEFINITION. THE MEASURE UNIT ARE SECONDS (S), ADIMENSIONAL (AD) AND NORMALIZED UNITS (NU). SIGNIFICANT DIFFERENCES ACCORDING TO THE FRIEDMAN TEST ARE DENOTED: *, P-VALUE $< 0.05$; †, P-VALUE $< 10^{-3}$; ‡, P-VALUE $< 10^{-4}$.

| Classical bands (35 subjects) | | | | | HF band redefinition (27 subjects) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Stage | $BL_\text{S}$ | $ST$ | $SA$ | $VE$ | Stage | $BL_\text{S}$ | $ST$ | $SA$ | $VE$ |
| $\bar{d}_{\text{HRM}}(s^{-1})$ | 1.24 ±0.16 | 1.4 ±0.19‡ | 1.23 ±0.16 | 1.25 ±0.17 | $\bar{d}_{\text{HRM}_\text{R}}(s^{-1})$ | 1.26 ±0.17 | 1.4 ±0.22‡ | 1.25 ±0.16 | 1.28 ±0.17 |
| $\bar{P}_{\text{VLF}}(s^{-2})$ | 0.49 ±0.12 | 0.56 ±0.17* | 0.49 ±0.12 | 0.47 ±0.12 | $\bar{P}_{\text{VLF}_\text{R}}(s^{-2})$ | 0.51 ±0.13 | 0.56 ±0.18* | 0.49 ±0.12 | 0.51 ±0.13 |
| $\bar{P}_{\text{LF}}(ad)$ $\cdot 10^3$ | 1.69 ±2.54 | 1.56 ±1.25 | 2.07 ±2.83 | 1.25 ±5.92 | $\bar{P}_{\text{LF}_\text{R}}(ad)$ $\cdot 10^3$ | 1.48 ±1.44 | 1.95 ±1.28 | 2.17 ±1.7* | 1.41 ±1.24 |
| $\bar{P}_{\text{HF}}(ad)$ $\cdot 10^3$ | 1.26 ±2.25 | 0.73 ±0.89* | 1.2 ±2.9 | 1.02 ±2.13 | $\bar{P}_{\text{HF}_\text{R}}(ad)$ $\cdot 10^3$ | 1.14 ±1.93 | 0.54 ±0.72* | 0.91 ±1.75 | 0.71 ±1.48 |
| $\bar{P}_{\text{LF}_\text{n}}(nu)$ | 0.57 ±0.19 | 0.67 ±0.14 | 0.64 ±0.14 | 0.55 ±0.16 | $\bar{P}_{\text{LF}_{\text{Rn}}}(nu)$ | 0.54 ±0.19 | 0.73 ±0.18* | 0.70 ±0.16* | 0.57 ±0.18 |
| $\bar{R}_{\text{LF/HF}}(nu)$ | 1.35 ±2.19 | 2.05 ±1.53 | 1.79 ±1.03 | 1.2 ±1.46 | $\bar{R}_{\text{LF/HF}_\text{R}}(nu)$ | 1.21 ±1.88 | 2.69 ±4.34* | 2.35 ±1.93* | 1.35 ±2.45 |

Respiratory rate was significantly higher (according to the Friedman test) during stressful stages than during relax stage, in agreement with results reported in [9], where the baseline recording presented a lower respiratory rate than in attention or mental arithmetic task.

Spectral peakness and the percentage of spectra accepted to compute the respiratory rate are studied in this work as measures of respiration stability. The more stable respiration is, the more peaked the spectra are and a higher number of them are included in the average. Results obtained for both respiratory stability measures show more stable respiration during relax than during the stress stages of the protocol. The less peaked spectra are found in $VE$, while the fewest spectra used are in $ST$. In [19] respiratory variation measured as the variation of a breath component over a sampling period of 15 minutes is computed as a coefficient that increased with mental task compared to relax situation.

HRV analysis using classical bands showed significant differences with respect to the $BL_{\text{S}}$ stage in only 3 cases: $\bar{d}_{\text{HRM}}$ in $ST$, $\bar{P}_{\text{VLF}}$ in $SA$, and $\bar{P}_{\text{HF}}$ in $ST$. This observation may be related to the fact that in some cases respiration is in LF band during relax, leading to an overestimation of $\bar{P}_{\text{LF}}$ and and underestimation of $\bar{P}_{\text{HF}}$, confounding classical HRV indices interpretation. In the analyzed dataset, 8 out of the 35 subjects (23%) are breathing with a respiratory rate in the LF band during $BL_{\text{S}}$, and 7 out of the 18 subjects (38.9%) during $BL_{\text{B}}$.

Furthermore, it may also happen that respiratory rate is above classical HF band during stress, as exemplified in Fig. 2, leading to an underestimation of $\bar{P}_{\text{HF}}$ and, consequently, an overestimation of $\bar{P}_{\text{LF}_\text{n}}$ and $\bar{R}_{\text{LF/HF}_\text{n}}$. The percentage of subjects with a respiratory rate higher than $0.35$ Hz (so part of $\Omega_{\text{HF}_\text{R}}(n)$ is over $0.4$ Hz) are: 2.8% in $BL_{\text{S}}$; 48.6% in $ST$; 22.9% in $SA$; 31.4% in $VE$.

When respiratory frequency information is included in HRV analysis, more differences are appreciated between basal and the stress stages. Significant differences with respect to $BL_{\text{S}}$ were observed for the same cases than with classical frequency bands and, in addition, for $\bar{P}_{\text{LF}_\text{Rn}}$ and $\bar{R}_{\text{LF/HF}_\text{R}}$ in both $ST$ and $SA$.

Some works have reported an increase in LF band [9], [10] while others have not found significant differences [11]. In our study an increase is observed in $\bar{P}_{\text{LF}_\text{R}}$ during $ST$ and $SA$ with respect to $BL_{\text{S}}$, being statistically significant during $SA$.

$\bar{P}_{\text{HF}_\text{R}}$ is lower than $\bar{P}_{\text{HF}}$ in all stages since the HF band in $\bar{P}_{\text{HF}_\text{R}}$ is narrower than in $\bar{P}_{\text{HF}}$. However, it is appreciated a larger relative reduction during stress stages with respect to $BL_{\text{S}}$ in $\bar{P}_{\text{HF}_\text{R}}$ than in $\bar{P}_{\text{HF}}$, supporting the use of respiratory sinus arrhythmia to assess stress, as proposed in [8] and confirmed by [11], [12], [13]. The larger increase of $\bar{P}_{\text{LF}_\text{R}}$ in $SA$ than in $ST$, and the larger decrease of $\bar{P}_{\text{HF}_\text{R}}$ in $ST$ than in $SA$ may be related to the different types of stressors. For example, during $ST$ there is a large demand of attention while during $SA$ the stress is mainly psychological or emotional.

$\bar{P}_{\text{LF}_\text{Rn}}$ and $\bar{R}_{\text{LF/HF}_\text{R}}$ are significantly higher during $ST$ and $SA$ than during $BL_{\text{S}}$, suggesting a sympathetic dominance. These results are in agreement with those reported in [9], [10], [11], [17]. Note that $\bar{P}_{\text{LF}_\text{n}}$ and $\bar{R}_{\text{LF/HF}}$, without the use of respiratory information, do not show significant differences.

In this study , the overestimation of $\bar{P}_{\text{LF}}$ and underestimation of $\bar{P}_{\text{HF}}$ due to a low respiratory rate are avoided by discarding those segments of HRV where respiration is in LF band. However, further studies should consider the separation of PNS and SNS activities in this situation. Another limitation of the study is that the method is only valid for those intervals when respiratory rate can be properly estimated (sufficiently peaked spectra). Future studies should considered the respiration effects over HRV when the subject is speaking.

Despite the fact that the inclusion of respiratory frequency information in HRV analysis allows a more reliable characterization of ANS response to stress in terms of SNS and PNS activities, respiratory frequency presents with stronger statistical differences (lowest p-value) than any HRV index, suggesting its potential for stress assessment.

The complementary information that HRV analysis can add to respiration analysis for stress assessment should be considered in a larger study and should include those cases where respiratory frequency cannot be estimated robustly.

## V. Conclusion

Frequency domain HRV indices, computed in classical terms, scarcely show statistical differences during stress. When respiratory rate information is used to guide HRV analysis, it allows to avoid the overestimation of sympathetic activity and the underestimation of parasympathetic activity that occurs when the respiration rate lies in LF band. This combine HRV and respiratory rate analysis, increases the discrimination power among different stress situations, where a major sympathetic dominance is observed. Finally, results showed higher discriminative power considering just respiratory rate information, suggesting that it can also discriminate the different stress states. This, however, comes at the cost of losing the excerpts where this rate can not be estimated.

## Acknowledgment

This work is supported by Universidad de Zaragoza under fellowship UZ2014-TEC-01 and PIFUZ-2011-TRCA-003, by Ministerio de Economía y Competitividad (MINECO), FEDER; under projects FIS-PI12/00514 and TIN2014-53567-R, by CIBER in Bioengineering, Biomaterials & Nanomedicine through Instituto de Salud Carlos III, and by Grupo Consolidado BSICoS from DGA (Aragón) and European Social Fund (EU). The computation was performed by the ICTS 0707NANBIOSIS", by the High Performance Computing Unit of the CIBER in Bioengineering, Biomaterials & Nanomedicine (CIBER-BBN) at the University of Zaragoza.

## References

[1] R. Glaser and J. Kiecolt-Glaser, "Stress-induced immune dysfunction: implications for health", Nature Reviews Immunology, vol. 5, p. 243–251, 2005.

[2] A. Bao, G. Meynen, and D. Swaab, "The stress system in depression and neurodegeneration: focus on the human hypothalamus", Brain Res. Rev. vol. 57(2), p. 531–553, 2008.

[3] D. Pizzagalli, "Depression, stress, and anhedonia: toward a synthesis and integrated model", Annu. Rev. Clin. Psychol., vol. 10, p. 393–423, 2014.

[4] M. Kivim´aaki, P. Leino-Arjas, R. Luukkonen, H. Riihim´aki, J. Vahtera, and J. Kirjonen, "Work stress and risk of cardiovascular mortality: prospective cohort study of industrial employees", British Medical Journal, vol. 35, p. 857, 2002.

[5] T. Heidt, H. Sager, G. Courties, P. Dutta, Y. Iwamoto, A. Zaltsman, C. von zur Muhlen, C. Bode, G. Fricchione, J. Denninger, and et all, "Chronic variable stress activates hematopoietic stem cells", Nature Medicine, vol. 20(7), p. 754–758, 2014.

[6] J. Aguiló, P. Ferrer-Salvans, A. Garc´03a-Rozo, A. Armario, A. Corb´03, F. Cambra, R. Bailón, A. González-Marcos, G. Caja, S. Aguiló, R. López-Antón, A. Arza-Valdés, and J. Garzón-Rey, "Project es3: attempting to quantify and measure the level of stress", Rev Neurol, vol.61(9), p.405–415, 2015.

[7] Working group of ESC, "Heart rate variability. Standards of measurement, physiological interpretation, and clinical use", Eur Heart J, vol. 17, p. 354–381, 1996.

[8] S. Porges, "Cardiac vagal tone: a physiological index of stress", Neuroscience and Biobehavioral Reviews, vol. 19(2), p.225–233, 1995.

[9] L. Bernardi, J. Wdowczyk-Szulc, C. Valenti, S. Castoldi, C. Passino, G. Spadacini, and P. Sleight, "Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability", J Am Col Cardiol., vol. 35(6), p. 1462–1469, 2000.

[10] M. Pagani, G. Mazzuero, A. Ferrari, D. Liberati, S. Cerutti, D. Vaitl, L. Tavazzi, and A. Malliani, "Sympathovagal interaction during mental stress. a study using spectral analysis of heart rate variability in healthy control subjects and patients with a prior myocardial infarction", Circulation, vol. 83(4), p. 43–51, 1991.

[11] N. Hjortskov, D. Rissen, A. Blangsted, N. Fallentin, U. Lundberg, and K. K. Sogaard, "The effect of mental stress on heart rate variability and blood pressure during computer work", J Appl Physiol, vol. 92(1), p.84–89, 2004.

[12] J. Taelman, S. Vandeput, E. Vlemincx, A. Spaepen, and S. Van Huffel, "Instantaneous changes in heart rate regulation due to mental load in simulated office work", Eur J Appl Physiol., vol. 111(7), p. 1497–1505, 2011.

[13] D. Widjaja, M. Orini, E. Vlemincx, and S. Van Huffel, "Cardiorespiratory dynamic response to mental stress: A multivariate time-frequency analysis", Comput Math Methods Med, p. 451–457, 2013.

[14] Z. Visnovcova, M. Mestanik, M. Javorka, D. Mokra, M. Gala, A. Jurko, A. Calkovska, and I. Tonhajzerova, "Complexity and time asymmetry of heart rate variability are altered in acute mental stress", Physiol Meas., vol. 37(7), p. 1319–1334, 2014.

[15] L. Mainardi, "On the quantification of heart rate variability spectral parameters using time-frequency and time-varying methods", Phil. Trans. R. Soc., vol. 367, p. 255–275, 2009.

[16] R. Bailón, G. Laouini, C. Grao, M. Orini, P. Laguna, O. Meste, "The Integral Pulse Frequency Modulation with Time-Varying Threshold: Application to Heart Rate Variability Analysis during Exercise Stress Testing", IEEE Trans. on Biomedical Engineering, vol. 58(3), p.642–652, 2011.

[17] Zhang PZ, Tapp WN, Reisman SS, Natelson BH., "Respiration response curve analysis of heart rate variability", IEEE Trans Biomed Eng, vol. 44(4), p.321–325, 1997.

[18] Y. Masaoka and I. Homma, "R", IEEE Trans. Biomed. Eng., vol. 44, p. 321–325, 1997.

[19] Vlemincx E., Taelman J., De Peuter S., Van Diest I., Van den Bergh O., "Sigh rate and respiratory variability during mental load and sustained attention", Psychophysiology, vol. 48(1), p. 117–120, 2011.

[20] A. Hernando, J. Lázaro, A. Arza, J. M. Garzón, E. Gil, P. Laguna, J. Aguiló, R. Bailón, "Changes in respiration during emotional stress", Computing in Cardiology, 2015.

[21] Arza A., Garzón J. M., Hernando A., Aguiló J., Bailón R., "Towards an Objective Measurement of Emotional Stress: Preliminary Analysis Based on Heart Rate Variability", IEEE Engineering in Medicine and biology Society, 2015.

[22] Hellhammer J., Schubert M., "The physiological response to Trier Social Stress Test relates to subjective measures of stress during but not before or after the test", Psychoneuroendocrinology, vol. 37(1), p. 119–124, 2012.

[23] JP Martłnez, R Almeida, S Olmos, AP Rocha, P Laguna, "A wavelet-based ECG delineator: evaluation on standard databases", Biomedical Engineering, IEEE Transactions, vol. 51(4), p. 570–581, 2004.

[24] J. Mateo and P. Laguna, "Analysis of Heart Rate Variability in the Presence of Ectopic Beats Using the Heart Timing Signal", IEEE Trans Biomed Eng, vol. 50(3), p. 334–343, 2003.

[25] Bailón, R; Garatachea, N; de la Iglesia, I; Casajús, J; Laguna, P., "Influence of Running Stride Frequency in Heart Rate Variability Analysis During Treadmill Exercise Testing", IEEE transactions on Biomedical Engineering, 2013.

[26] Lázaro J., Alcaine A., Romero D., Gil E., Laguna P., Pueyo E. Bailón R., "Electrocardiogram Derived Respiratory Rate from QRS Slopes and R-Wave Angle", Annals of Biomedical Engineering, vol. 40(10), p.2072–2083, 2014.

[27] Kumar S., Al Absi M., Beck J. G., Ertin E., Scott M. S., "Behavioral monitoring and assessment via mobile sensing technologies", Behavioral Healthcare and Technology, p.621–624, 2015.

[28] R. Bailón, P. Laguna, L. Mainardi and L. Srnmo, "Analysis of Heart Rate Variability Using Time-Varying Frequency Bands Based on Respiratory Frequency", IEEE Engineering in Medicine and Biology Society, p.6674–6677, 2007.

# Appendix B

# Written consent

The following pages reflects the written consent that each subject has to fill out and sign in order to make sure that he/she is informed about all study's targets, protocol details and physiological samples to be collected.

## HOJA DE CONSENTIMIENTO INFORMADO

**Título del estudio: Estudio de un sistema de medida del nivel de estrés**

### OBJETIVO DE ESTE DOCUMENTO

El objetivo de este documento es pedir su permiso para incluirle como participante anónimo en este estudio de investigación. El motivo es que es usted estudiante de la Universidad de Zaragoza, sano, y se ha ofrecido como voluntario para participar en este estudio.
Por favor, LEA a continuación la siguiente información para estar seguro/a que comprende perfectamente el objetivo de este estudio y firme en caso de que esté de acuerdo en participar en el mismo.

### OBJETIVO DEL ESTUDIO

Tal y como se le ha informado, este estudio pretende afrontar los problemas médicos y sociales relacionados con el estrés, desarrollando y validando la instrumentación, las técnicas y los protocolos que permitan objetivar la medida del "nivel de estrés" de manera reproducible y de acuerdo con los rankings aceptados por la comunidad médica. El resultado que se persigue es poder disponer de herramientas que faciliten el diagnóstico y seguimiento de pacientes y la comunicación entre profesionales.

### PROCEDIMIENTOS

Si usted acepta participar en este estudio, se contactará con usted en dos ocasiones para:

- Someterte a una entrevista dirigida por un psicólogo y realizada por un entrevistador entrenado para determinar su nivel de estrés de acuerdo con los estándares aceptados sobre el tema.
- Registrar tus datos de presión arterial, temperatura de la piel, electrocardiograma, frecuencia respiratoria, onda de pulso, conductancia cutánea , y electromiografía, así como la señal de voz, siguiendo un protocolo estándar.
- Tomar muestras de sangre y saliva siguiendo un protocolo estándar que se utilizarán para determinar Prolactina, Co-peptina, Glucosa, Cortisol y α-amilasa.
- Realizar un test psicométrico.

Las mediciones se realizarán en el Hospital Clínico Universitario Lozano Blesa en horario de mañana (entre 9.00 y 13.00) y tendrán una duración aproximada de 1 hora.

### RIESGOS E INCONVENIENTES

Las venas y las arterias varían en tamaño de un paciente a otro y de un lado del cuerpo a otro, razón por la cual extraer sangre de algunas personas puede ser más difícil que de otras. Otros riesgos asociados con la extracción de sangre son leves, pero pueden ser: sangrado excesivo, desmayo o sensación de mareo, hematoma (acumulación de sangre debajo de la piel), infección (un riesgo leve cada vez que se presenta ruptura de la piel). Se tomarán precauciones para minimizar estas posibilidades.
El proyecto se realiza con fines de investigación, no asistenciales. Por tanto, no dispondremos en ningún caso de valores de referencia para comprobar que sus muestras se encuentras en límites normales.

### RESPONSABLE

La investigadora responsable del estudio es Raquel Bailón Luesma, Profesora Contratada Doctora de esta Universidad de Zaragoza. Para cualquier consulta Vd. puede contactar con ella a través de correo electrónico rbailon@unizar.es, telefónicamente llamando al 976762876, o personalmente en el despacho D3.20 del Edificio Ada Byron de la Escuela de Ingeniería y Arquitectura.
El investigador responsable del proyecto a nivel nacional es el Dr. Jordi Aguiló Llobet, Catedrático de la Universidad Autónoma de Barcelona. Para cualquier consulta, además de con el investigador que le indicamos más arriba, Vd. puede contactar con él a través de correo electrónico, Jordi.aguilo@uab.cat, telefónicamente llamando al 935 813 557.

### BENEFICIOS

Si, será remunerado. Recibirás 25 euros tras finalizar la segunda sesión.

### GASTOS

Los gastos serán totalmente asumidos por las partes implicadas en el estudio. Como participante en el estudio no tiene ninguna responsabilidad.

**CONFIDENCIALIDAD**

Con la firma de la hoja de consentimiento, da su permiso para la utilización de la información recogida de las muestras tomadas, de los registros efectuados y de las entrevistas realizadas para este estudio de investigación. Su nombre no aparecerá en ningún escrito ni publicación. Las muestras obtenidas en los distintos procedimientos se almacenarán en un lugar protegido y se tomarán las medidas necesarias para mantener su carácter confidencial de manera que nunca se podrá relacionar la muestra con la persona.

Las muestras se destruirán, siguiendo los procedimientos establecidos en cada uno de los laboratorios que se utilicen, una vez que se hayan analizado las muestras y se haya comprobado que no ha habido errores de manipulación.

El acceso a los datos procedentes de las distintas muestras estará restringido a personal investigador. En cualquier caso, y en virtud a lo recogido en la Ley en vigor de Protección de Datos de Carácter Personal Vd. tiene derecho a revocar este consentimiento en cualquier momento y a que los datos procedentes de Vd. sean eliminados de nuestra base de datos y las muestras obtenidas en los distintos procedimientos que pudieran estar almacenadas sean destruidas, mediante un escrito dirigido al investigador principal del proyecto en Zaragoza.

**FIRMA CONSENTIMIENTO**

**Yo ..... (Nombre y apellidos del participante)**

He leído la hoja de información que se me ha entregado.
He podido hacer preguntas sobre el estudio.
He recibido suficiente información sobre el estudio.
He hablado con ..... (Nombre y apellidos del responsable de datos)

Comprendo que mi participación es voluntaria.
Comprendo que puedo retirarme del estudio en cualquier momento y sin necesidad de ninguna explicación-

He recibido una copia de este Consentimiento Informado.

Firma del participante:

Fecha:

---

**Yo ..... (Nombre y apellidos del reponsable de datos)**

He explicado la naturaleza y el propósito del estudio al sujeto mencionado.

Firma del responsable de datos

Fecha:

Firma del investigador responsable

Fecha:

# Appendix C

# Psychometric test

The following pages shows the psychometric test which has to be filled out by the volunteers at the end of each session. Several parts can be found:

- Perceived stress scale reflects feelings and thoughts that the volunteer has had during the last week.
- State trait anxiety shows some sentences that tries to describe the subject.
- The test created by the Clinic Hospital Lozano Blesa psychologists and psychiatrists has two parts too. One related to the symptoms that the subject has when the test has finished (ES3), and another that has to be filled out by the observers according to non-verbal language expressed by him/her during the protocol (EV-ES3).

Focusing in this last test, differences between basal and stress sessions are found. For example, in 26 out of the 35 subjects the difference between both sessions has increased in ES3 or EV-ES3 or in both of them at least 10 points.

## PERCEIVED STRESS SCALE SPANISH VERSION

Las preguntas en esta escala hacen referencia a sus sentimientos y pensamientos **en la última semana**. En cada caso, por favor, indique con una "X" cómo usted se ha sentido o ha pensado en cada situación

| | En la última semana ... | NUNCA | CASI NUNCA | DE VEZ EN CUANDO | A MENUDO | MUY A MENUDO |
|---|---|---|---|---|---|---|
| 1. | ¿Con qué frecuencia ha estado afectado por algo que ha ocurrido inesperadamente? | | | | | |
| 2. | ¿Con qué frecuencia se ha sentido incapaz de controlar las cosas importantes en su vida? | | | | | |
| 3. | ¿Con qué frecuencia se ha sentido nervioso o estresado? | | | | | |
| 4. | ¿Con qué frecuencia ha manejado con éxito los pequeños problemas irritantes de la vida? | | | | | |
| 5. | ¿Con qué frecuencia ha estado seguro sobre su capacidad para manejar sus problemas personales? | | | | | |
| 6. | ¿Con qué frecuencia ha sentido que las cosas le van bien? | | | | | |
| 7. | ¿Con qué frecuencia ha sentido que no podía afrontar todas las cosas que tenía que hacer? | | | | | |
| 8. | ¿Con qué frecuencia se ha sentido al control de todo? | | | | | |
| 9. | ¿Con qué frecuencia ha estado enfadado porque las cosas que le han ocurrido estaban fuera de su control? | | | | | |
| 10. | ¿Con qué frecuencia ha sentido que las dificultades se acumulan tanto que no puede superarlas? | | | | | |

## STATE-TRAIT ANXIETY INVENTORY

Instrucciones: A continuación encontrará unas frases que se utilizan corrientemente para describirse uno a si mismo. Lea cada frase y marque con una "X" aquello que indique mejor cómo se siente usted en este momento. No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando la respuesta que mejor describa su situación presente.

| | ESTADO | NADA | ALGO | BASTANTE | MUCHO |
|---|---|---|---|---|---|
| 11. | Me siento calmado | | | | |
| 12. | Me siento seguro | | | | |
| 13. | Estoy tenso | | | | |
| 14. | Estoy contrariado | | | | |
| 15. | Me siento cómodo (estoy a gusto) | | | | |
| 16. | Me siento alterado | | | | |
| 17. | Estoy preocupado ahora por posibles desgracias futuras | | | | |
| 18. | Me siento descansado | | | | |
| 19. | Me siento angustiado | | | | |
| 20. | Me siento confortable | | | | |
| 21. | Tengo confianza en mí mismo | | | | |
| 22. | Me siento nervioso | | | | |
| 23. | Estoy desasosegado | | | | |
| 24. | Me siento muy «atado» (como oprimido) | | | | |
| 25. | Estoy relajado | | | | |
| 26. | Me siento satisfecho | | | | |
| 27. | Estoy preocupado | | | | |
| 28. | Me siento aturdido y sobreexcitado | | | | |
| 29. | Me siento alegre | | | | |
| 30. | En este momento me siento bien | | | | |

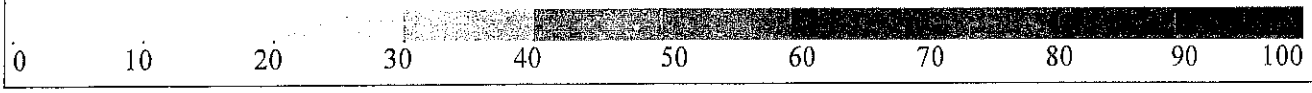
**Instrucciones:** A continuación encontrará unas frases que se utilizan corrientemente para describirse uno a sí mismo. Lea cada frase y señale la puntuación que indique mejor cómo se siente usted en general en la mayoría de las ocasiones. No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando la respuesta que mejor describa cómo se siente usted generalmente.

| | RASGO | CASI NUNCA | A VECES | A MENUDO | CASI SIEMPRE |
|---|---|---|---|---|---|
| 31. | Me siento bien | | | | |
| 32. | Me canso rápidamente | | | | |
| 33. | Siento ganas de llorar | | | | |
| 34. | Me gustaría ser tan feliz como otros | | | | |
| 35. | Pierdo oportunidades por no decidirme pronto | | | | |
| 36. | Me siento descansado | | | | |
| 37. | Soy una persona tranquila, serena y sosegada | | | | |
| 38. | Veo que las dificultades se amontonan y no puedo con ellas | | | | |
| 39. | Me preocupo demasiado por cosas sin importancia | | | | |
| 40. | Soy feliz | | | | |
| 41. | Suelo tomar las cosas demasiado seriamente | | | | |
| 42. | Me falta confianza en mí mismo' | | | | |
| 43. | Me siento seguro | | | | |
| 44. | No suelo afrontar las crisis o dificultades | | | | |
| 45. | Me siento triste (melancólico) | | | | |
| 46. | Estoy satisfecho | | | | |
| 47. | Me rondan y molestan pensamientos sin importancia | | | | |
| 48. | Me afectan tanto los desengaños que no puedo olvidarlos | | | | |
| 49. | Soy una persona estable | | | | |
| 50. | Cuando pienso sobre asuntos y preocupaciones actuales me pongo tenso y agitado | | | | |

## Escala de síntomas de estrés (ES3)

| | Señale la opción que mejor describe cómo se siente en este momento | MUY DE ACUERDO | ALGO DE ACUERDO | NI DE ACUERDO NI EN DESACUERDO | ALGO EN DESACUERDO | MUY EN DESACUERDO |
|---|---|---|---|---|---|---|
| 51. | Siento palpitaciones | | | | | |
| 52. | Siento la boca seca | | | | | |
| 53. | Siento rigidez en el cuello | | | | | |
| 54. | Siento que me falta el aire, suspiro con frecuencia | | | | | |
| 55. | Siento opresión en el pecho | | | | | |
| 56. | Siento escalofríos | | | | | |
| 57. | Siento urgencia para orinar con frecuencia | | | | | |
| 58. | Siento que sudo | | | | | |
| 59. | Siento como si tuviera nervios en el estómago | | | | | |
| 60. | Siento que mi cara se sonroja | | | | | |
| 61. | Me siento mareado | | | | | |
| 62. | Cometo muchos errores | | | | | |
| 63. | No tengo ganas de hablar | | | | | |
| 64. | Me siento enfadado | | | | | |
| 65. | Me siento molesto por todo | | | | | |
| 66. | Siento que me distraigo fácilmente o no me concentro | | | | | |
| 67. | No me siento motivado para hacer cosas | | | | | |
| 68. | Me siento al límite | | | | | |
| 69. | Siento que me impaciento fácilmente | | | | | |
| 70. | Me siento agitado e/o inquieto | | | | | |

En general, ¿Dónde marcaría Ud. su nivel de estrés en este momento?
Siendo 0 = ningún estrés en absoluto y 100 = absolutamente estresado. Por favor, use los cuadrados para indicar los valores intermedios de estrés



## ESCALA VISUAL ANÁLOGA DE ESTRÉS OBSERVADO

| 0 | 50 | 100 |
|---|---|---|

Puntuar en base a los siguientes ítems observados:

1. Nivel de tensión motora observada: ´
    a. Tensión en mandíbula (aprieta dietes)
    b. Tensión de tronco
    c. Tensión de manos (aprieta puños, …)
2. Nivel de malestar emocional (desasosiego) observado:
    a. Expresa angustia
    b. Se le observa angustiad@
    c. Llora
3. Nivel de bloqueo cognitivo
    a. Incapaz de dar ninguna respuesta
    b. Dificultad en contentar a cuestiones sencillas
    c. Expresa impotencia
4. Nivel de inquietud psíquica
    a. Pregunta una y otra vez por la prueba, por lo que va a ocurrir, …
    b. Pone continuas excusas ante errores
    c. Impaciente por acabar

# Appendix D

# Modelo TVIPFM

The Time Variant Integral Pulse Frequency Modulation (TVIPFM) model is used to generate a event-series which represent the time instant of the cardiac beats. This model tries to reproduce the control of the Autonomic Nervous System (ANS) over the heart rate and its block diagram is represented in the Fig. D.1 [16].

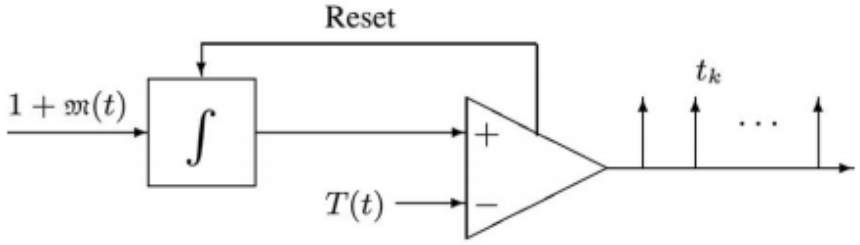



Figure D.1: Blocks diagram of TVIPFM model

In order to relate and derive the ANS influence on the beat occurrence time series $t_k$ , which is the available information, we rely on the TVIPFM model. The TVIPFM model is based on the hypothesis that the ANS influence on the sino-atrial node can be represented by the modulating signal $\mathfrak{m}(t)$, and a beat trigger impulse is generated when the integral of $1 + \mathfrak{m}(t)$ reaches a threshold T(t), which represents the time-varying mean heart period, resetting the integrator. The modulating signal $\mathfrak{m}(t)$ is assumed to be causal, band-limited, and $\mathfrak{m}(t) < 1$.

Assuming that the first beat is in $t = 0$, time instant series $k$ can be generated as:

$$k = \int_0^{t_k} \frac{1 + \mathfrak{m}(\tau)}{T(t)} d\tau \tag{D.1}$$

where $k$ and $t_k$ are the order and the time instant of the k beat. The term inside the integral is called $d_{\mathrm{HR}}(t)$ and it represents the instantaneous heart rate. Heart rate variability is represented by $d_{\mathrm{HRV}}(t)$:

$$d_{\mathrm{HR}}(t) = \frac{1 + \mathfrak{m}(t)}{T(t)} \tag{D.2}$$

$$d_{\mathrm{HRV}}(t) = \frac{\mathfrak{M}(t)}{T(t)} \tag{D.3}$$

Finally, knowing that $\mathfrak{M}(t)$ meets that $\mathfrak{M}(t)<<1$, the estimation of the modulating signal $\hat{\mathfrak{M}}(t)$ can be computed as:

$$\hat{\mathfrak{M}}(t) = \frac{d_{\mathrm{HRV}}(t)}{d_{\mathrm{HR}}(t)} \tag{D.4}$$