Universitat Autònoma de Barcelona

Measurement of Acute Psychological Stress

Adriana Arza Valdés

UNIVERSITAT AUTÒNOMA DE BARCELONA

PhD Thesis

Measurement of Acute Psychological Stress

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by

Adriana Arza Valdés

PhD Advisor: Jordi Aguiló Llobet

Universitat Autònoma de Barcelona,
Departament de Microelectrònica i Sistemes Electrònics
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Measurement of Acute Psychological Stress

Thesis submitted for the degree of Doctor of Philosophy
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Adriana Arza Valdés

Supervised by:
Jordi Aguiló Llobet

Microelectronic and Electronic Systems Department
Autonomous University of Barcelona

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To my mother,

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ABSTRACT

The incidents of anxiety, depression, epilepsy, multiple sclerosis, pathological stress, as well as other different stress related diseases have significantly increased in recent years and it is most probably due to dramatic changes in the daily lifestyle of citizens. The social and medical problems associated to stress are clearly on the rise and seriously affecting mental health and wellbeing not only in adults but also in young adults and children, according to the WHO (World Health Organization). There is currently an increased awareness of stress, specifically with regards to its medical, social and economic importance; and also, because there are several stress-related comorbidities that remain largely understudied. Therefore, stress has become one of the significant focal points of research interest. Despite the efforts made, a scientifically supported, repeatable and usable in daily life measurement method of stress is still not available. An objective and reliable measurement of stress level could be used to compare the level of stress' severity in different individuals or between different sets of professionals. It would also be a crucial tool for the prevention, diagnosis and treatment of several diseases.

Stress can be defined as the disturbance of the homeostatic balance when facing a specific threat to the body (a stressor) that provokes a stress response. The stress response is the body's attempt to deal with the stressor triggering the different reactions that can be broadly summarised as changes in the autonomous nervous system, a hormonal response (adrenal pituitary adrenal axis) and a behavioural change and/or a decrease in cognitive skills. The methods that have been proposed until now for measuring the different aspects of stress considering its multidimensional quality, can be gathered in three groups: psychometric tests, biochemical markers, and physiological markers. Although biochemical samples and psychometric questionnaires are commonly used stress markers, they do not allow continuous or even regular stress assessment.

Therefore, the hypothesis of this PhD thesis is that the stress response level due to a psychological stressor can be determined from a set of physiological parameters, which can be noninvasively and continuously measured. Consequently, the methodology and analysis of results presented are focused on objectively quantifying the physiological component of the stress response level, which can be understood as how close or far an individual is from his/her state of homeostatic balance.

To this end, an experimental study was developed to quantify acute psychological stress in healthy young people using biomarkers from physiological signals, which can be continuously and noninvasively measured. An acute stress response was induced to 40 volunteers by applying a modification of the Trier Social Stress Test (TSST), a widely used and documented protocol for lab-induced stress. Standard psychological tests and biochemical stress markers were used as references for stress levels. A multivariable approach to stress measurement is proposed, which includes as stress biomarkers features derived from finger and face temperature, heart rate variability and pulse photoplethysmogram. The results obtained show that, under the given conditions, stress can be assessed and measured quantitatively and continuously from noninvasive biomarkers, as continuous stress measurement was performed every 1 minute. Based on this approach, a stress monitoring method could emerge in the near future.

Finally, based on the findings from the experimental study and literature review presented in this thesis, a set of guidelines for the development of a stress measurement system were suggested to delineate the specifications for a stress monitoring system in daily life conditions. Also, some key considerations and challenges of this emerging area of study are addressed.

RESUMEN

La incidencia de la ansiedad, depresión, epilepsia, esclerosis múltiple, estrés patológico, así como otras enfermedades relacionadas con el estrés, ha aumentado significativamente en los últimos años, probablemente debido los cambios drásticos en el modo de vida actual. Según la OMS, los problemas tanto sociales como médicos asociados al estrés están claramente en aumento, afectando seriamente la salud mental y el bienestar no sólo de adultos, sino también de jóvenes y niños. Actualmente hay una mayor conciencia respecto al estrés, precisamente por la importancia que tiene desde el punto de vista médico, social y económico; pero también por las grandes lagunas de conocimiento que aún existen en todas las patologías y trastornos relacionados con el estrés. Es por ello que el estrés se ha convertido en una de las líneas de investigación de gran interés. Sin embargo, a pesar de los esfuerzos realizados, no existe aún un método de medición científicamente validado, repetible y que pueda ser utilizado en la vida cotidiana. Una medida objetiva y fiable del nivel de estrés, podría ser utilizada para comparar la severidad del nivel de estrés en diferentes individuos o entre diferentes grupos de profesionales. Sería, además una herramienta crucial para la prevención, diagnóstico y tratamiento del estrés que ayudaría también en el estudio de muchas otras comorbilidades relacionadas.

El estrés puede definirse como la alteración del equilibrio homeostático ante una amenaza específica para el cuerpo (estresor). En este sentido, el estrés no es más que la respuesta de defensa del individuo ante la amenaza percibida. La respuesta del estrés provoca diferentes reacciones fisiológicas en el cuerpo al trata de lidiar con el estresor. Estas reacciones se resumen de manera general como cambios en el sistema nervioso autónomo, una respuesta hormonal (eje suprarrenal adrenal) y un cambio de comportamiento y/o disminución de las capacidades cognitivas. Los métodos que se han propuesto hasta hoy para medir los diferentes aspectos que abarca el estrés

dada su naturaleza multidimensional, se pueden agrupar en tres grupos: cuestionarios psicométricos, marcadores bioquímicos y marcadores fisiológicos. Aunque las muestras bioquímicas y los cuestionarios psicométricos son comúnmente utilizados como marcadores de estrés, los mismos no permiten una evaluación continua, ni siquiera regular del estrés.

La hipótesis de esta tesis doctoral es que el nivel de respuesta del estrés ante un estresor psicológico puede determinarse a partir de un conjunto de parámetros fisiológicos, que pueden ser medidos de forma no invasiva y continuada. Por lo tanto, la metodología y el análisis de resultados presentados en esta tesis se centran en cuantificar objetivamente distintos componentes fisiológicos de la respuesta del estrés, entendiéndose como cuan cerca o lejos está un individuo de su estado de equilibrio homeostático.

Con este fin, se desarrolló un estudio experimental para cuantificar el estrés psicológico y agudo en jóvenes sanos utilizando biomarcadores obtenidos de señales fisiológicas, que puedan medirse de forma continua y no invasiva. Se indujo un estrés agudo en 40 voluntarios aplicándoles una modificación del Test de Estrés Social de Trier (TSST), que es un protocolo ampliamente utilizado y bien documentado para inducir estrés en laboratorio. Se utilizaron test psicológicos estandarizados y marcadores bioquímicos de estrés como la referencia de los niveles de estrés inducido. Se propuso un enfoque multivariable para la medición del estrés, que incluyó, como biomarcadores de estrés, parámetros derivados de la temperatura cutánea periférica (manos) y central (mejilla), la variabilidad de la frecuencia cardíaca y el fotopletismograma de pulso. Los resultados obtenidos muestran que, en las condiciones dadas, el estrés puede ser evaluado cuantitativamente y continuamente a partir de biomarcadores obtenidos de manera no invasiva. Ya que se pudo realizar una medición continua del estrés cada un minuto. Así, basándose en este enfoque, podría surgir un método de monitoreo del estrés en un futuro cercano.

Finalmente, a partir de los resultados del estudio experimental y la revisión bibliográfica, se propone un conjunto de requerimientos y directrices para el desarrollo de un sistema de medida del estrés, delineando las especificaciones del sistema para monitorearlo en la vida cotidiana. Este estudio también aborda algunas consideraciones claves y retos a enfrentar este campo.

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LIST OF ACRONYMS

ANS	……	Autonomic Nervous System
ST	……	Skin Temperature
AT	……	Arithmetic Task
UAB	……	Autonomous University of Barcelona
BS	……	Basal State
BL	……	Baseline Stage
BVP	……	Blood pressure
CPT	……	cold pressor test
ECG	……	Electrocardiography
EDA	……	Electrodermal activity
EEG	……	Electroencephalography
EMG	……	Electromyography
HR	……	Heart Rate
HRV	……	Heart Rate Variability
HF	……	high frequency components
HPA axis	……	Hypothalamic–pituitary–adrenal axis
LF	……	low-frequency components
MT	……	Memory Test
PSS	……	Perceived Stress Scale
PT	……	Psychometric Test
PDT	……	Pulse Decreasing Time
PTT	……	Pulse transit Time
PPG	……	Pulse Wave or Photopletismography
PD	……	Pupil Diameter
RS	……	Relax Stage
Resp	……	Respiration
SC	……	Skin conductance
STAI-s	……	State-Trait Anxiety Inventory of State
STAI-t	……	State-Trait Anxiety Inventory of Trait
STAI	……	State-Trait Anxiety Inventory test
ST	……	Story Telling
SA	……	Stress Anticipation
SRV	……	stress reference variables
SS	……	Stress State
SSS	……	Stress Symptoms Scale
TSST	……	Trier Social Stress Test
VD	……	Video Display
VASS	……	Visual Analogue Scale for Stress
WHO	……	World Health Organization
PCA	……	Principal Component Analysis

1 INTRODUCTION

Stress is the body's response against an extreme situation or a hostile environment that the individuals may perceive as a threat. The General Adaptation Syndrome, as baptized by Seyles in his 1950's article "Stress and the general adaptation syndrome" [1], helps explain the psychological meaning of stress as the body's response to a tension caused by one of the many forms of the struggle for existence regardless of the causal agent.

In fact, the word stress appears first in the Materials Physics in the XV century, defined as "physical exertion that a material object is under". Later it becomes widely used from the year 1855 and onwards. The more general meaning was adopted in Psychology only at the beginning of the XX century [2].

A material subjected to a force, for example stretching, is deformed by the action of this force. The atoms that constitute its structure are separated, increasing the cohesive force of atoms links joins, in turn generating a force or resistance that is opposite to the deformation. This resistance to the action of an external agent was called stress. The deformation ceases when the internally generated resistive force equals the externally applied force and thus a new equilibrium is reached.

It is not surprising that this idea was used later for living beings since it corresponds almost exactly with the intuitive concept that stress has in psychology or psychiatry. This concept is precisely defined as: the external action of an agent moving the homeostatic balance of an individual that triggers a reaction in the individual trying to ensure his/her survival.

In this thesis, we will call a stress factor or a stressor to any insult that alters the body's homeostatic balance, provoking a stress response. A stressor may be an injury, a disease, the

exposure to extreme cold or heat, a death threat or breathlessness due to the lack of oxygen in high altitudes or when staying in enclosed spaces, among others.

Feeling a lack of oxygen, be it true or not, causes the same type of stress to that being experienced when faced with to any other anticipatory emotion. Additionally, anxieties due to a perceived unperformed effort are also stressors. Example, of these are: to having unreachable consumption needs, or demanding oneself to memorize long texts, or expecting to perform a cognitive or mechanical task in period of time so short that would be just unattainable.

In most cases, when the external action ceases the individual gradually returns to its balanced state. However, in some cases, a while after the external action ceases, the subject does not recover the original balanced state, but rather, it maintains a chronic stress state, already pathological. Seyles stated that any agent that causes stress is life-threatening, unless it finds an appropriate adaptive response. The same is true vice versa, any life-threatening agent causes stress and provokes an adaptive response that can succeed or fail this life-threatening agent.

1.1. Motivations

In today's world, stress is understood as a "toxic" factor produced by psychologically traumatic experiences or being under demanding situations over a prolonged period of time. Stress has increased significantly as a result of the lifestyle in a rapidly changing society, which often challenges its citizens. Indeed, many people in the world are physically and mentally subjected to situations that cause a constant high stress level. Because of this and its inherent consequences, the World Health Organization (WHO) has declared stress to be a world epidemic[3].

The incidents of anxiety, depression, epilepsy, multiple sclerosis, pathological stress, as well as other different stress related diseases, have significantly increased in recent years and this is probably due to dramatic changes in the daily life of citizens. The medical, social and economic impacts of stress are currently particularly important in high income countries and it will soon be in most countries of the world. The social and medical problems associated with stress are clearly on the rise and seriously affecting mental health and wellbeing not only in adults but also in young adults and children according to WHO [4].

It has been shown that stress generally has negative consequences with regards to mental health and wellbeing and that chronic stress especially increases the risks of certain pathologies [5]. Moreover, stress can induce small cumulative alterations, with a long-term influence on the development of cardiovascular diseases, hypertension, myocardial infarction, strokes [6], among others, and can even cause sudden death [7]. Behavioural problems, anxiety disorders, depression

or polymorphic co-pathologies, such as drug addiction and changes due to brain atrophy, can also be stress-related [8, 9].

The indirect consequences of stress are diverse. It is known that stressors can promote physiological and behavioural disturbances ranging from immune system dysfunction to psychiatric disorders. For example, scientists at the Max Planck Institute of Psychiatry in Munich have found that stress can cause symptoms similar to those caused by Alzheimer neuropathological changes [10]. Stress indirectly plays an important role in the development of certain diseases such as diabetes [11], Parkinson's [12], multiple sclerosis, epilepsy, etc. Also, stress may influence the progress of traumatic events or the recovery from surgery, among others. Similarly, other problems such as sleep apnea and psychotic episodes may be intensified by stress[13].

In terms of medical impact, pathological stress (stress surpassing the individual's coping limits) along with mental disorders, are now emerging as major factors in global health. It has been estimated that by 2020, neuropsychological disorders will surpass infectious diseases in importance on all world health maps [14]. The different instances of psychiatric and neurological diseases could increase their contribution to the global burden of morbidity from 10.5% to almost 15% by the year 2020 [14]. In comparison, this represents an expected growth rate even higher than the one for cardiovascular diseases. Stress can also be seen as a toxic factor, with an important socio-economic impact. In the US, for example, medical work absences amount to 6 days per year on average, while on the other hand, an average of 25 days accounted for anxiety, stress and related disorders altogether [9]. In addition, it has been estimated in the US that mood disorders will cost more than 50 billion dollars a year in lost productivity. [15].

Even though there is currently an increased awareness of stress, expressly with regards to its medical, social and economic importance, stress remains largely understudied. Up to now, to the best of our knowledge, there is no reliable tool for measuring levels of stress, nor an agreement among professionals and research communities on a single stress assessment method. Therefore, stress has become one of the significant focal points of research interest [16]. Specifically, an objective and reliable measurement of stress level, which could be used to monitor stress conditions in daily life, would be a crucial tool for health professionals to improve the management of mental conditions, as well as the follow-up and prevention of several diseases.

Reliable biomarkers for measuring stress response levels will allow accurate follow-up of patients, the prevention of illnesses, and the detection of a pathological condition in its early stages. Also, they could be used to set the daily dose of a drug in a personalized medicine scenario; to establish threshold levels affecting risky professions; to evaluate more quickly and

economically the effectiveness of medical treatments; to compare the level of stress severity in different individuals or between different sets of professionals, and others. Therefore, it can be argued that the identification of biomarkers of the physiological reaction to stress has become a matter of paramount medical and social need.

1.2. Thesis Framework

The work presented in this PhD dissertation has been developed in the framework of the project “Towards a Body Area Network to Measure Stress Levels” funded by the Spanish Ministry of Science and Innovation and the Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN) from the Carlos III Health Institute of Spain (FIS-PI12/00514 and TIN2014-53567-R).

1.3. Thesis Outline

This thesis comprises eight chapters including this introduction, which are organized as follows:

- Chapter 2 **- Background and Literature Review** presents an explanation on the main concept of stress and its physiological response in the human body. In addition, some of the common methods that have been proposed for measuring stress and the current interest of the market and health industry on stress measuring are discussed. The chapter ends with a brief summary of the stressor stimuli that have been used to elicit stress for purposes of research.

- Chapter 3 - **Hypothesis and Objectives** presents the hypothesis, the main objective and specific objectives of this research of this thesis

- Chapter 4 - **Experimental Measuring of Acute Psychological Stress** introduces an experimental framework designed to select and evaluate a set of biomarkers able to quantitatively assess stress levels using as reference biochemical variables and psychometric tests. This chapter is also dedicated to the design and protocol of an experimental study to induce acute stress to healthy young individuals through a well-defined psychological stressor, the Trier Social Stress Test

- Chapter 5 - **Signal Processing and Feature Extraction** explains **t**he main aspects concerning the signal processing and feature extraction methods used in this research.

- Chapter 6 -**Acute Psychological Stress: Results of the Pilot Study** provides the results of both stress reference variables and stress biomarkers from physiological signal and presents a preliminary approach to a noninvasive and continuous stress measuring method.

- Chapter 7 - **Guidelines for the Development of a Stress Measurement System** presents a set of guidelines to delineate the specifications of a system to monitor stress in daily life conditions. There are also addressed some key considerations and challenges facing this emerging area of study.

- Chapter 8 - **Conclusions and Future** summarizes the conclusions of this thesis and describes future lines of research to expand the results presented in this dissertation.

Additionally, this thesis also includes a section of appendices that presents other contributions related to its main objective. Even though they are not part of the experimental study presented in the core of this thesis, they offer additional elements to the research of suitable methods to measure stress. The appendices are organized as follows:

- Appendix I –**Blood Pressure Variation as Potential Stress Biomarker** summarizes two experimental studies performed to accomplish the estimation of blood pressure variations using a noninvasive procedure, as a condition to become a potential stress biomarker.
- Appendix II-**Patient Empowerment and Stress Reduction** presents a useful application of stress measurement using the experimental framework presented in Chapter 4. This application also provides more insight into the study of the response triggered by another type of stressor.
- Appendix III- **Effects of Limb Movements and Positions** analyses one of the possible factors affecting the resulting values of pulse transit time, namely body motions and changes in limb positions, which represents a step farther in real-life conditions stress monitoring.

2 BACKGROUND AND LITERATURE REVIEW

2.1. Stress Definitions and Physiology

Stress is a commonly used term; however, its precise definition is not unique due to the diversity of stressful stimuli and the variety of bodily reactions in people that should be included in the definition.

One of the first formal definitions, which is also the most commonly accepted in the fields of physiology and biology, is the one given by H. Selye in 1950 [1]: *"Stress is a state of biological activation triggered by the person interacting with external agents that force his or her capacity to adapt".* Another definition also suitable for this research was presented by R. S. Lazarus in 1993 [17]: "*Stress state arises when a specific event threatens the endocrine, physiological or psychological homeostatic stability of the individual".*

A current definition from a psychological point of view, given in 2007 at the Encyclopaedia of Social Psychology [5], is: *"Stress occurs when an individual perceives that the demands of a personally important situation tax or exceed his or her capabilities and resources. The situation can be a major event such as the death of a loved one, an interaction with another person such as a disagreement with a co-worker, or even an internal event such as a realization that one is aging but has not accomplished important life goals. Stress, especially if experienced chronically, can have serious negative physical and psychological consequences."*

Indeed, stress response is a part of our adaptation mechanism. It allows us to face new mental or physical states in front of a stressor. Facing a minor stressor could be doing a cognitive task, running, and such, whereas, a major stressing event could be a major surgery, a death threat, to experience adverse events of war or natural catastrophes.

Stress can be classified, considering the length of time the subject remains under the effects of a stressor or how perdurable its effects are, as follows [18]:

- **Acute Stress** is the immediate response of the organism to a stressor.
- **Chronic Stress** is the resultant state caused by a continuous stressor stimulus over a period of months or due to the inability of the individual to reach a new adaptive state

Conversely, based on the source of arousal, some stressors can be classified as **psychological stressors,** like anticipatory emotions, demanding cognitive situations, among others. On the other hand, there are external physical conditions acting against the organism which can be classified as **physical stressors**, such as in the case of an injury, or illness, or even sudden environmental changes in atmospheric pressure, temperature, oxygen and others, a disease or an injury. However, both types of stress sources generate responses at psychological level and at physical level; though they are not mutually exclusive. For example, the case of an injury is very peculiar since both types of stressors are present. The homeostatic imbalance caused by the injury contributes to the general stress response, but also the feeling of pain, the uncertainty of a new situation and the possible consequences of the injury are contributors to the general stress response.

Psychological stress occurs when an individual perceives that environmental demands tax or exceed his or her adaptive capacity [8]. Examples of psychological stress may include death of a family member, fright of public speaking or appearing ridiculous, pressure to perform during an exam and fear, among others.

The stress response, whether the stressor is either a psychological event or a physical event, triggers a biological response, which prepares the body - the whole system - to face a homeostatic imbalance by achieving a new state adapted to the new situation. These responses, among others, provide the needed energy for an active behaviour, while inhibiting the main anabolic process (digestive tract, growth, reproduction). Under an extreme, prolonged or persistent state of arousal, the body continues secreting extra quantities of biochemicals (hormones, enzymes, etc.), and triggers further processes to maintain the levels of energy, and if arousal persists, the adrenal glands produce anti-inflammatory molecules and depress the immune system. A variety of illnesses, together with exhaustion or even death, may be the outcome of uninterrupted or excessive stress.

The response to acute stress is designed to provide the organism with the alertness, energy, physiological regulation, and immunological activation that are necessary to counterbalance the effects of the stressor in order to survive [18]. When the hypothalamus is stimulated, the vegetative

nervous system is activated and it sends impulses downstream, stimulating the adrenal medulla and the sympathetic system (sympathoadrenal system). In addition, a cholinergic parasympathetic response is also increased.

The primary sensory processing of stimuli is followed by the activation of different areas, including the prefrontal cortex, hippocampal formation, amygdala, septum, and ventral striatum, among others[19], which can be seen as represented in Figure **2.1** . In these areas, signal processing drives to effector areas like the hypothalamic paraventricular nucleus (PVN), which is the key nucleus in the hypothalamic–pituitary–adrenocortical regulation [20].

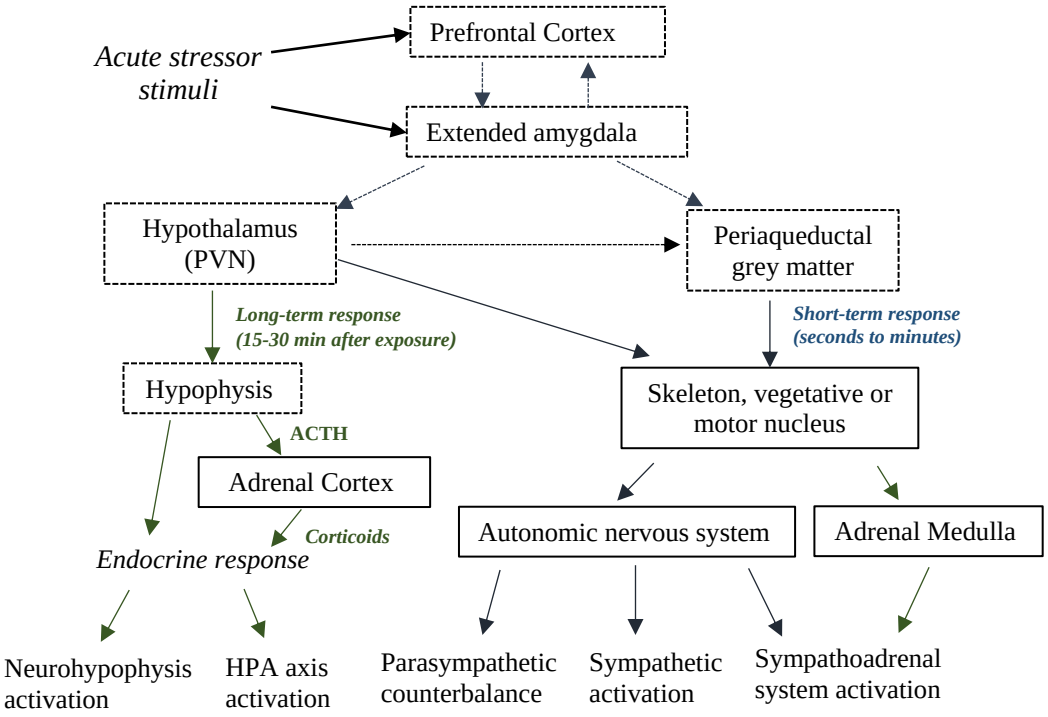

Figure 2.1: Different activation areas involved in processing psychological stimuli.

The sympathoadrenal system is a physiological connection between the sympathetic nervous system and the adrenal medulla. Once activated, norepinephrine and epinephrine (catecholamines) are released directly into the blood, because of that , this system plays a large role in maintaining glucose levels, sodium levels, blood pressure, and various other metabolic pathways that couple with bodily responses to the environment [21].

Sympathetic nervous system activation, together with the release of catecholamines, produces immediate physiological and metabolic changes that prepare the body for action. Examples are increase of the frequency and intensity of the heart contraction, increase of blood pressure, breathing rate, metabolic rate, hyperglycaemia and, lipolysis. Simultaneously, there are modifications in the immunological system[22].

The hypothalamic–pituitary–adrenal axis (HPA axis) activation produces, as a result, the release of glucocorticoids (cortisol in humans) into the bloodstream. Consequently, energy spending is favoured and the inflammatory and immunological response is suppressed. Also, release of mineralcorticoids is produced by HPA axis activation, that provokes retention of sodium ions and water by kidneys, as well as, an increased blood volume and blood pressure.

Physiological changes triggered by the activation of stress mechanisms can be broadly summarized as follows:

1. *Endocrine system:* a) Neurohypophysis activation: vasopressin secretion (copeptin is the pre-hormone) leading to increased blood volume and pressure and increased peripheral blood vessels resistance (vessel-constriction); b) HPA axis activation: cortisol secretion leading to increased catabolism, anabolism inhibition as well as immune system depression, an increased blood volume and blood pressure, and sympathetic nervous system sensitization; c) Sympathoadrenal system activation: release of catecholamines leading to increased blood pressure, breathing rate, metabolic rate, and blood glucose, as well as , change in blood-flow patterns (increased alertness and decreased digestive and kidney activity).
2. *Nervous system:* a) Sympathetic nervous system activation: increased heart rate, stronger heart contractions, increased respiratory frequency and blood pressure, bronchial and pupil dilation, sweating skin, peristalsis inhibition and hyperglycaemia among others reactions related to the "fight or flight" response. [1] b) Parasympathetic counterbalance: rest and digest response, increased anabolism, catabolism inhibition.

A coordinated action of both endocrine and nervous systems is essential for a proper response to stressful situations. The nervous system response is immediate whereas the endocrine system triggers a slower and more complex cascade of reactions. Nevertheless, the stress response is heterogeneous because it arises as a result of the interaction of several variables such as the stressor type, its intensity, the unpredictability in its occurrence, its duration and absence of control. But also, it is related to intrinsic individual traits and thus, it is possible to obtain a broad range of reaction facing the same stressor.

2.2. Measuring Stress: A Review

The earliest measurements of stress focused on the occurrence of life events and the subjective appraisal of stress in one's life and on associated emotions [18]. Accordingly, self-report questionnaires were the most common method of measuring stress in the 1980s. Since then,

several questionnaires have been used to assess the degree of stress an individual perceives by psychiatry, psychology, and the medical community in general. These professionals have been working with those questionnaires, called psychometric tests, as the appropriate tool for psychological stress assessment.

Commonly used psychometric test are the Life Stress Inventory, the Perceived Stress Scale or Visual Analogue Scale for Stress (VASS) and the Social Readjustment Rating Scale. There are several tests to assess post-traumatic stress disorder, anxiety and depression, like the State-Trait Anxiety Inventory test (STAI), and others. VASS reports the self-perceived stress level, while STAI has been initially designed to evaluate anxiety, though further studies show that it is a sensitive predictor of caregiver distress over time and that it can vary with changes in individual's health, life support systems, and other specific characteristics [23,24].

During the past decades, some other researchers have followed a different approach, using physiological variables as stress biomarkers. They have addressed the assessment of stress via its physiological manifestations. Researchers coming from biomedical or biological fields are more keen on using chemical biomarkers [25,26], such as catecholamine, cortisol, alpha-amylase or interleukin-6 [27,28]. Conversely, and more so recently, researchers from engineering fields are mainly using biosignals such as skin temperature (ST) [29], electro-dermal activity (EDA), pulse wave or photopletismography (PPG), electrocardiography (ECG), electromyography (EMG), respiration (Resp), pupil diameter (PD) [30], electroencephalography (EEG), blood pressure (BP), among others [31], as biomarkers for stress assessment.

There are diverse approaches for stress assessment, which measure or assess one or a few more than one of the reactions that arise from the stress response. Moreover, a wide range of psychometric tests and biochemical markers have been established as stress markers [26]. The most frequently used by health professionals are the subject's self-report assessments VASS and STAI, while biomedical and biology professionals use specific biochemical markers such as catecholamine, prolactin, copeptin, glucose, cortisol, alpha-amylase and interleukin-6 [27,28].

Biochemical markers are an invasive and expensive measure and their correct sampling depends on the time elapsed since the stressor has been active, which makes their use difficult for continuous monitoring. Moreover, hormones are not released in a constant way and oscillate throughout the day. Psychometric tests are highly related to individual subjectivity (their own perception of their state) and they lose reliability when they are frequently applied, with the same questions being asked. They can also, be unreliable since they might not be filled in sincerely and may also suffer from recall bias. Thus, there are not very objective measures to assess stress.

Due to all of these reasons, there is an increasing interest in the use of biosignals to evaluate stress levels or emotional states, especially if it can be implemented in a stress monitoring application. The advantage of physiological variables for stress assessment is that they measure the reactions from stress mechanisms and not the subjectively-experienced psychological stress. Consequently, the physiological markers allow to measure the impact of stress on the body since it manifests itself through a physiological response.

One of the first relevant researches, from the engineering approach, was developed in 2005 by the Affective Computing Research Group at the MIT Media Lab [32]. Researchers measured ECG, EMG, Resp and SC in 24 professional drivers in the following three conditions: at rest, driving on a highway and driving in the city, representing low, medium and high stress levels respectively. The results showed that heart rate and skin conductivity metrics provided the highest overall correlations with continuous drivers' stress levels. The stress level was obtained from a questionnaire and a video, and scored from a number of stress indicators in the drivers. This research group has developed several projects in this field [33–35], such as BioMod (2007) and Car PhoneStress, which monitors physiological signals using an integrated mobile and portable device interface with the intention of providing useful information about stress state.

Since then, other research groups have been working in the assessment of stress and emotions. A group from the digital signal processing Lab, at Florida International University, uses physiological signal processing for the detection and recognition of stress state [30,36–39]. Similarly, the Group of Biometrics, Biosignals, and Security at the Madrid Polytechnic University [40–43], has done research focusing on stress detection systems by means of physiological signals. Conversely, there are a number of others studies which are more interested in emotion detection [44–46].

The most important and recent studies about stress detection through physiological signal are summarized in Table **2.1** (see [26,47,48]; for reviews). It can be seen for each study, the stress level or emotion assessed as the target, the references of stress that were used for comparison, the stressor applied to induce a stress response, the biosignals and features employed to measure the stress response, and the employed assessment methods and their results. Most attempts to evaluate stress only distinguish two states: stress or no stress [43]. Though a few of them identify three levels of stress [32,49] i.e. high, medium and low, normally related to the intensity of the stressor.

A variety of methods have been employed to assess the study's results that give reference levels of the induced stress intensity, but without an agreement among professionals on a common and objective assessment method to employ which would allow making comparisons or evaluations of the proposals. Despite its limitations, several studies employ standard psychometric tests or subject's self-report assessments as reference. Other studies employ as

reference the status of the stressor stimulus: active or not, while others use the amount of work load and demand.

Several studies have found relations between stress states and features from physiological signals, mainly with ECG and SC signals [32,43–45,49–54]. The most widely used signal is ECG since its stress marker features, the heart rate (HR) and mainly its variations (HRV), are driven by the autonomic nervous system (ANS) and, more precisely, by its sympathetic and parasympathetic components.

In the last years with the development of image technology and patterns recognitions, some studies have been using facial expressions, body movement, pupil dilation [53] and voice analysis in the assessment of mental states [55]. But they have been only used for states classification and, therefore, they do not provide stress levels measure.

The features employed to assess the stress response are obtained from biosignals using different approaches: from the simple one using the signals themselves directly, to the use of complex signal processing algorithms to extract relevant parameters. The stress assessment methods are diverse too, from simple statistics ones to more complex methods applying sophisticated classification techniques [45], such as support vector machines [43], fuzzy logic, Bayesian networks, classification trees, artificial neural networks [31], and Computer Aided Diagnostic tools [56].

Those studies that are listed in Table **2.1** use different stressors, references of induced stress and methods to assess stress, leading to the conclusion that there is no single model or standard range to classify the levels of stress. Additionally, what is generically called stress corresponds to a variety of situations with common external manifestations. Those all studies employed a similar approach: first, a certain mental state (e.g., stress, certain emotions, mental workload) is induced to the participants while a number of physiological signals are measured. Subsequently, a variety of features are extracted and pattern recognition and machine learning techniques are employed to enable the automatic classification of the emotional states or stress states.

It is obvious that no clear correlation pattern has been found so far between measured physiological parameters and levels of stress. All the studies were carried out at a specific environment and focused on the relation between a set of physiological parameters and stress. The variety of methods employed for the analysis of the data has been also noticed. We should underline that different ranges of magnitude for the definition of the stress level have been proposed but none of this has been standardized yet.

Table 2.1: Summary of stress assessment in literature

Reference	Target	Subjects	Stress References [a]	Stressor [b]	Signals [c]	Features selection [d] (number)	Assessment methods [e]	Results /Accuracy [f]
Healey et al. [32] , 2005	3 stress levels -the rest (low) -highway (medium) -city (high)	24 (drivers)	Questionnaire and video record scored	driving conditions	ECG, EMG (left trapezium), Resp and SC	(13)	SVM	SVM: 97.4% 100% low; 94.7% medium and 97.4% for, high stress
Zhai al. [37] ,2008	2 stress levels -Normal -Stress	32	-	Stroop test	PD, ST(finger), BP and EDA	(11)	Naïve Bayes, DT, SVM	SVM: 90%
De Santos Sierra et al. [43] , 2011	2 stress levels -No-stress -Stress	80, females	-	Hyperventilation , Talk Preparation	EDA and ECG	(4)	GMM, kNN, Fuzzy logic, FiDA, SVM	Fuzzy logic: 99.5%
Wijisman et al. [50], 2013	2 stress levels -rest /stress	30	PSS and VASS	Calculation, memory, logical tasks	ECG, EDA, Resp and EMG (trapezium)	CBA (5)	Generalized Estimating Equations	80%,69.1% (rest /stress)
Seoane et al [49],2014	4 stress states -Neutral -Emotional -Mental -Physical	42	Self-assessment Manikin and profile of mood state	Videos, Math Games, physical load	EDA, ECG, ST (finger), Resp and voice	(195)	LDC	ECG :76.28%
Karthikeyan et al. [51], 2013	2 stress levels -Normal -Stress	40	An Effectiveness report	Mental arithmetic task	ECG, EMG, EDA and ST	- (148)	kNN, PNN–	HRV: 93.75%, ECG: 76.25%, EDA: 70.83%, ST: 75.32%, EMG: 71.25% 90%
Willmann et al. [52] , 2011	2 stress states -Stress -Recovery	109, males	STAI, VASS	video-recorded Stroop colour-word interference test	ECG, EDA, ST (finger EMG (gastrocnemius and trapezius muscles)	-	Comparisons: t tests, R-ANOVA between groups with Low (LA)and high anxiety level (HA)	Physiological activity increased during the stressful situation Recovery for HA was prolonged gastrocnemius muscle tension and prolonged decrease in ST

Reference	Target	Subjects	Stress References [a]	Stressor [b]	Signals [c]	Features selection [d] (number)	Assessment methods [e]	Results /Accuracy [f]
Ren et al. [30], 2013	2 stress levels - relaxation -Stress	42	Self-assessment	Stroop	EDA and PD	- (6)	K⁄, MLP, Naïve Bayes, RF, Jrip	Naïve Bayes and PD: 88.71%
Kurniawan el al.[55] 2013	2 stress levels - relaxation -Stress	203	-	Stroop, TSST, TMCT	Speech, Facial expressions and EDA	-	K-means, GMM, SVM	EDA: 80.72%, Speech: 92.6%, Both: 92.4% (SVM)
Li et al. [54] 2014	5 states - one per stressor	39	A questionnaire	Workload, strange phone calls, audio–videos, threatening letters, exam notification	ECG	Multi-feature fusion (5 to 1)	HMM	96.4%
Mohino et al. [44] 2015	3 emotions -neutral -sadness -disgust	40	Self-assessment and profile of mood state	Videos, Math Games, physical load	ECG, Resp	GA (112 to 10)	neural networks	95.2%
Kukolja et al [45], 2014	5 emotions -sadness, disgust, fear, happiness and neutral	14, females	self-reporting procedure (written questionnaire)	International Affective Picture System database.	ECG, Resp, SC and ST	SFFS, mRMR (288 to 64)	MLP, kNN	SFFS+MLP: 60.3% mRMR+ kNN: 50.33%

[a] Visual analogue scale (VASS), Primary Appraisal Secondary Appraisal (PASA), PSS, STAI

[b] Trier Social Stress Test(TSST), TMCT

[c] Electrocardiogram (ECG), electromyogram (EMG), respiration (Resp), skin conductance (SC), skin temperature (ST) , Electrodermal activity (EDA), Pupil Diameter (PD), Blood pressure (BP)

[d] Correlation based Analysis (CBA) ' Genetic algorithm (GA), Sequential floating forward selection (SFFS), Minimum redundancy – maximum relevance (mRMR),

[e] Linear Discriminant Analysis (LDA), decision tree (DT), Support vector machine (SVM), Fisher Discriminant Analysis(FiDA), k-Nearest Neighbours (k-NN) , Gaussian Mixture Model (GMM), neural networks (ANNs), Hidden Markov Model (HMM), Multilayer perceptron (MLP)

2.2.1. Interest of Market and Health Industry in Stress Measuring

Nowadays, wide variety of devices and mobile app aimed at the detection of stress levels can be found in the market. Among other metrics, they track heart rate, pulse rate or skin's electrical activity to detect mainly emotional states or stress. Some of these devices came from start-ups like *Neumitra Inc* [57] that sells *Neuma* Watch to measure brain activity. Also, *Empatica Inc* [58], offers *Embrace Watch* which monitories seizures, sleep and physical activity. This shows the interest of the potential market in stress measuring.

However, most of the devices that can be found in the market are consumer electronic products not medical devices. They detect some mental load or changes of HR, EDA, but cannot detect a validated stress response level. Galvanic Ltd [59] ,for example, produces a gadget named Pip, sold as emotional stress level measurement device, but it just only detects electrodermal activity levels.

In addition, since 2005 was founded a biometric research platform, called iMotions, which enables researchers to execute multi-sensor research projects on human behaviour. It offers software and hardware products, as well as integrated technologies offering solutions for different research areas. Industries like academic, FMCG's, neuromarketing agencies, market research, gaming and medical iMotions' state of the art research platform to elevate insights with eye tracking, facial expression, EEG, GSR, ECG, EMG and survey technologies. iMotions has offices in Copenhagen, Denmark and Boston, US, and their solutions are utilized in 40 countries around the world

Another good example is *Spire*, a stress-tracking device that clipped to the belt or bra it detects chest motion as inhale and exhale occur. Spire measures breathe in a minute, and it breaks down a person´s breathing patterns into calm, focus, and tense minutes [60].

On the other hand, some patents and patent applications have been registered, showing also the interest of the market in stress measuring. Moreover, there are some patents and patent applications currently requested by major healthcare companies e.g. the multinational Philips N.V., Fujitsu, Spo. Medical Equipment Ltd and Nipro Corporation, among other companies. In Table **2.2** and Table **2.3** patent applications related to the issue in hand. There are patents using electrophysiological signals and movement sensors for stress detection or measurement (see Table **2.2**), while there are other patents for the assessment of emotional or mental states (see Table **2.3**). However, none of them has launched an associated product into the market yet, at least not under the brand or company that applied to the patent, as far as we know.

Table 2.2: Patents for stress assessment

References	Target	Inputs	Notes
Method and System for Monitoring Stress [61] 2016, USA Application Murata Manufacturing co. ltd (Japan)	Stress Monitoring. (Index of stress level)	ECG and PPG	A stress level indication is determined as a function of the beat-to-beat time variation and the relative stroke volume variation. Calibrated for each individual.
Measuring chronic stress [62] 2015 Application USA JouZen Oy (Finland)	Stress o no stress	HRV, Activity levels	A method for measuring stress based on the HR, the HRV, and the activity level of a user. Delimitation of stress or healthy boundaries based on the heart rate. Calibrated for each individual.
Stress measuring device and method [63] 2014 Application USA, WIPO, China, Europe Philips N.V. (Netherlands)	Level of stress of the user (Chronic stress)	Features derived from SC and may be added other signs as ECG, temp, EEG, accelerometers	A stress-measuring device and method for determining a level of stress of a user in particular long-term stress.
Device for determining a stress level of a person and providing feedback on the basis of the stress level as determined [64] 2014 USA patent China and Europe Application, Philips N.V. (Netherlands)	Stress level of a person	motion sensors, body sensor (ECG, EMG, EEG, etc.)	A device for determining a stress related condition of a person and providing feedback about this condition comprises body sensors for detecting stress related body parameters. The body sensors are integrated in a textile structure, so that the tested person does not sense the sensors.
Physiological stress detector device and system. [65] 2009 USA patent Spo Medical Equipment Ltd. (Isrrael)	Normal state or stress physiological on baby or infant	blood saturation and heart pulse rate	A noninvasive device and a system for monitoring and measuring blood saturation and heart pulse rate of a baby or infant is provided. The system activates an alarm when the level falls outside a predetermined range
Continuous monitoring of stress using accelerometer data [66] 2014 Patent USA Fujitsu Limited (Japan)	Stress index of a person	Accelerometer, heart-rate, PPG, and mood state (Stressed, Happy, Alert, Excited, Angry, Unsure, Depressed, Quiet, Relaxed)	In particular embodiments, a method analyse data sets collected from the person when the person is engaged in various activities, and determine a current stress index of the person based on the analysis. Blood pressure is calculated from PTT

Table 2.3: Patents for the assessment of emotional or mental states

References	Target	Inputs	Notes
Wearable mental state monitor computer apparatus, systems, and related methods. [67] 2016 Application USA Vision Service Plan (USA)	Assess metal states: -emotional stress - Fear, -Anger - Sad - Happy	Physiological signals (ECG, Resp, EEG, SC, BP, etc.), Images, voice, questionnaires, environmental factors, etc.	Method of assess the metal state of an individual by using information from one or more of the sensors every 30 minutes, 2 hours or every day. Patent involves using a wearable device to determine one or more environmental factors that are related to the individual's mental state. No further information about the method.
Cognitive biometric systems to monitor emotions and stress [68] 2013, Europe Application Sackett solutions & Innovations LLC(USA)	Emotional state of the subject.	BP, PPG, ECG, EEG, SC, Resp., pupil size, EMG of leg or hand, facial muscles Unusual gestures or motions, changes in bodily movements or sitting postures, Voice changes or expressions, Eyelid fluttering, Pupil movement,	Methods and systems to periodically monitor the emotional state of a subject comprising the steps of: exposing the subject to a plurality of stimuli during a session. Specific diagnostic markers for anxiety disorders, mood disorders, or depression.
Method and device for measuring stress [69] 2007 USA patent 2006 Germany patent 2005 Europe patent Polar Electro Oy (Finland)	Measuring mental load	Heart Rate	The device generates at least two different cognitively loading tasks for the person, the first task determines a reference level and at least one other task determines the loading capacity level. The method generates a value descriptive of the mental load based on the comparison of the pulse parameters. The task requiring memory could be the Stroop task.
Multi-modal sensor based emotion recognition and emotional interface [70] 2015 USA patent Sony Computer Entertainment Inc. (Japan)	Emotional state	Acoustic features, Visual features, Linguistic features, and Physical features (ECG, PPG, movement)	The acoustic, visual, linguistic, and physical features may be analysed with one or more machine learning algorithms and an emotional state of a user may be extracted from analysis of the features

2.3. Stress Elicitation in Research

So far, a variety of stressor stimuli have been employed to elicit stress for research purposes. Frequently used stressor stimuli, can be real situations [33], such as driving, clinical trials [32,45],work stress [71] or written examination [28], but it is also commonly used induced stressors in laboratory e.g mental arithmetic task [51,72,73], public speech task [74,75], Stroop colour word test [73], hyperventilation [43], visualization of film fragments, or cold pressor test (CPT), among others stressors. The Trier Social Stress Test (TSST) was created in 1993 [76] by combining some of the aforementioned stressors. TSST has been one of the most widely used and documented protocols in stress research. Hence, it is currently accepted in experimental psychology and it is, therefore, widely used [25,26,77–79].

In fact, the TSST has been standardized as protocol to induce moderate acute psychological stress in laboratory. It comprises three stages within ten-minute period. The first stage is the anticipatory stress phase, in which the participants are asked to prepare a five-minute speech. During the second stage, participants give the speech in front of an audience while a video recorder is monitoring them. In the final stage, participants execute a mental arithmetic task.

The TSST has demonstrated to exert robust effects on several psychobiological measures in most individuals to whom it has be applied [25], namely :

- Psychological measures: anxiety, negative mood, and perceived stress;
- Autonomic measurements: blood pressure, heart rate, heart rate variability, electrodermal activity, respiration, body temperature, epinephrine, and norepinephrine;
- Endocrine and metabolic measures: adrenocorticotropic- pic hormone (ACTH), plasma and saliva cortisol, prolactin, growth hormone, and glucose;
- Haematological measures: haematocrit, haemoglobin, and plasma volume;
- Immune measures: neutrophils, eosinophils, basophils, lymphocytes, interleukin-6, and tumour necrosis factor alpha (TNF-α);
- Genetic measures: repression/induction profiles of genes in target tissues;
- Psychomotor measures: muscle activity (electromyogram), voice (spectral analyses), limb movements, and dexterity.

A study comparing the TSST to other widely used stressors [80], support its continued use as the prototypical experimental stressor. This study evaluates psycho-physiological stress response patterns to different laboratory stress protocols, giving as a result that perceived stress level values (VASS, Primary Appraisal Secondary Appraisal PASA) are highest in the TSST, followed by

Ergometer, Stroop, and cold pressor test. Additionally, the study shows that the highest HPA axis response (saliva cortisol, saliva alpha-amylase, and heart rate) was found in the TSST, followed by Ergometer, CPT, and Stroop, whilst the highest autonomic response was found in the Ergometer, followed by TSST, Stroop, and cold pressor test [81].

2.4. Summary

Stress, by definition, is a multidimensional concept. The stress response of an individual has different manifestations, which can be broadly summarized as changes in the sympathetic and parasympathetic balance, a hormonal response (adrenal pituitary adrenal axis) and behavioural change and / or decreased cognitive skills. However, individual response is quite diverse depending on the perception or capabilities of the person against the stressor, which can vary depending on factors like his/her genetics, learned skills, expertise, and trainings. Facing the same stressor, the amount of triggered cognitive and physiological reactions and the intensity levels of such reactions varies substantially among persons as well as it varies in the same person facing different stressors. Important terms have been outlined as follows in Table **2.4**.

Table 2.4: Stress terms and its explanations

Terms	Explanations
Homeostasis	The body's internal stability
Stress	Disturbance of homeostatic balance
Stressor	A specific threat to the body, could be psychological or physical
Stress response	The bodies attempt to deal with the stressor
Stress Biomarkers	Mediators which address the specific reactions of a stressor
Stress level	Degree of disturbance of the homeostasis

Several methods have been proposed for measuring stress, but each focuses in a different aspect of the multidimensional concept of stress. These methods can be gathered in three groups:

1. Psychometric tests: focus on the interaction between a person and his environment to study stress. They are no so objective measures to assess stress and cannot be frequently applied.
2. Biochemical markers: allow to measure mainly the endocrine stress response. They need a careful control of the sampling times and most are invasive sampled.
3. Physiological markers: allow to measure the impact of stress on the body. They can be recorded with unobtrusive wearable devices.

There is no agreement among professionals or within the research community neither on a unique stress assessment method nor in a standard stress measurement method. Most probably, this is due to the variety of stimuli that induce stress, the diversity of human reactions to each stressor [48], and the lack of common stress reference among professionals. Hence, up to now, there is not a unique criterion to make an inter-individual or intra-individual evaluation of the stress level response of individuals facing different stressors. Almost all previous studies only made stress assessment (stress/ no stress) using mostly auto-self report, psychometric test, or the condition of being under a specific stress situation.as stress references.

From the analysis of the reviewed literature is appropriate to conclude that, so far, it has not been found a consistent pattern of correlation between measured physiological parameters and stress levels. All studies have been conducted in a specific environment and have focused on proving there is a relationship between a set of physiological parameters and stress using a variety of analytical methods. Despite some stress assessment methods have been proposed, there is neither a standardized metric of stress nor a pattern of references to compare with. Therefore, up to our knowledge, objective, reliable and accepted stress measurement methods do not exist.

On the other hand, several patent applications have been registered, and some gadgets aimed at the detection/ assessment of stress levels are found in the market. This clearly evidences the interest of the health industry and market in a stress detection method and device. However, until now, none of them has implemented a generally used and accepted or medically validated method. Thus, those methods, and new ones, need to be furtherly developed.

Several stress elicitation means have been applied to individuals studied for research purposes. Among them, TSST produces a consistent physiological and psychological response in most of individuals to whom it has been applied [25]. Furthermore, compared with other stressors, the TSST provides a robust and reliable acute stress inducement [80]. For these reasons, TSST is chosen in this research to induce acute psychological stress, as a proven and well-known stress elicitation in research.

3 HYPOTHESIS AND OBJECTIVES

An objective, reliable and repeatable stress measurement, being able to be measured continuously, can be used to compare the level of stress severity in different individuals or between different sets of professionals. It can further be used to establish threshold levels affecting risky or very demanding professions and, also, to prevent several diseases and follow-up on -treatment. This measurement can be useful to set the daily dose of a drug in a personalized medicine scenario and to evaluate quickly and less costly the efficacy of a new medicine; among other uses in numerous applications.

Therefore, identifying biomarkers for their physiological reaction to stress has become a medical need. Measuring the body`s stress response will allow accurate follow-up on patients, prevent illness or detect a pathological condition in its early stages. An objective measurement will also facilitate an easy and precise communication among professionals in order to find more suitable and personalized solutions for the treatment of pathological cases. Furthermore, there is a social need to identify stress induced by physical, emotional and environmental alterations in healthy people that should be met.

Biochemical samples and psychometric questionnaires are commonly used stress markers, but they do not allow continuous or even regular stress assessment due to several factors. The biochemical markers' limitations are their invasiveness and inability to be continuously measured. Additionally, it must be highlighted too that their dynamic response has to be taken into account in order to perform a proper measurement. The relationship, both complex and under-studied, between their body activation and the intensity of the stress perceived also makes them disadvantageous. The flaws of the psychometric questionnaires are their subjective vulnerability and the fact that they are not designed for frequent application.

Taking into account all of the above and aiming a more reliable description of the stress response, it is proposed as a premise of this study the use of a set of biomarkers from physiological signals that can be easily monitored in daily life. This premise was also set with a view to the development of futures methods to monitor stress levels.

Although the ultimate goal is to measure acute stress or chronic stress in daily life, a potential stress measurement should be validated under controlled stress conditions.

Accordingly, **the hypothesis of this thesis** is that the stress response level due to a psychological stressor can be determined from a set of physiological parameters, which can be noninvasively and continuously measured.

The main objective of this thesis is to develop a measurement protocol using the accepted standards to perform an objective and noninvasive stress level measurement.

Due to the promising contributions of blood pressure as a marker of the stress level and facing the fact that it can be not noninvasively and continuously measured, it was included in the thesis work the study of a method for a continued measurement of blood pressure variations using a noninvasive procedure.

The work that constitutes the core of this thesis focuses primarily on the study of acute stress in the healthy and young.

The **specific objectives** are the following:

. Analyse the current forms of stress level assessment: psychometric tests, biochemical samples and physiological signals.
. Establish an appropriate methodology for extracting certain parameters from electrophysiological signals.
. Develop a method for a continued measurement of blood pressure variations using a noninvasive procedure.
. Prove the existence of a statistically significant correlation between selected physiological parameters and the level of emotional and cognitive stress induced, in an individual subjected to a moderate stressor, scientifically accepted as standard.
. Define a set of specifications for the development of a wearable device/system used to measure stress.

4 EXPERIMENTAL MEASURING OF ACUTE PSYCHOLOGICAL STRESS

The literature review leads to the conclusion that there is no agreement within the scientific community in regard to the measurement of stress. Much alike is the lack of consensus on what methodology to apply for obtaining measurements of stress, as well as the use of an objective and a repeatable stress reference scale. Thus, to validate the hypothesis of this thesis, an experimental framework is designed to select and evaluate a set of biomarkers able to quantitatively assess stress levels. In order to use those biomarkers in a future method of stress monitoring, they must be always obtained from continuous, noninvasive measurements of physiological signals. Furthermore, as reference variables of stress have been employed stress markers such as the biochemical variables and psychometric tests previously mentioned in Section **2.2**, which are widely used and accepted in the medical community.

4.1. Methodology of Measurement

The experimental framework that was developed as a part of this thesis work, was designed to be used not only in the pilot study that is the core of this thesis, but also in a group of experimental studies of the ES3 Project [16]. Within the ES3 Project the influence of different stressors on various groups of subjects were evaluated and compared [16], because of that was developed a common experimental framework. A representation of this experimental framework is shown in Figure **4.1**.

The ultimate goal is to measure the level of stress response, which can be understood as how close or far an individual is from his/her state that is considered socially and medically normal. In other words, how close or far an individual is from the state of situation of homeostatic

balance. Therefore, the intended scope of work is to identify the features derived from physiological signals that are best suited to reliably measure stress levels according to the established stress markers, and then to use them for quantitative stress level estimation as a first attempt towards stress monitoring.

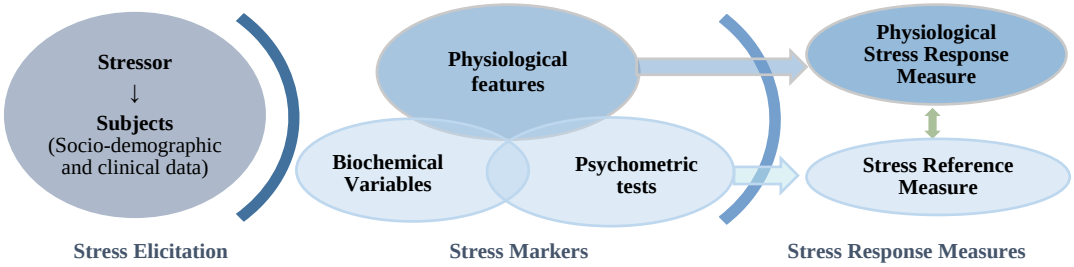

Figure 4.1: Representation of the experimental framework

To avoid hazards and different undesirable effects of the measurement, the physiological signal measurements are carried out using instrumentation, sensors and electrodes with a single brand of equipment (CE certified and calibrated), and following the same procedure for placement of sensors and signal. Furthermore, each of the collected biochemical variables is analysed in the same laboratory. The same psychometric tests are applied in each trial.

The following subsections describe the common design of the developed measurement framework that was applied on the experimental trial presented in this thesis and used in the ES3 Project [16] (Further information on the experimental trial is in Section **4.2**)

4.1.1. A Multimodal Approach to Stress Measuring

The whole stress response of an individual includes different reactions that can be broadly summarized as changes in sympathetic and parasympathetic balances and in hormonal response (HPA- axis), as well as, behavioural changes and decrease in cognitive skills [5]. Therefore, a stress response induces a diversity of body changes affecting different possible stress markers. Considering that conceptually, stress arises when a specific event threatens the homeostatic balance of the individual, in this study the stress level response is determined as a function of the particular variations that the homeostatic imbalance provoke in an individual. This indeed, is an approximation of how much an individual is affected by the specific event. The variations due to the homeostatic imbalance are reflected in a number of physiological reactions that can be measured.

Human bodies are a complex system where stress responses provoke multiple physiological and cognitive reactions. Such stress reactions are dependent on the exposure time to stressors (acute or chronic), but also on certain characteristics of the stressor as they are perceived by the individual, such as its intensity, the ability to control it, and its predictability [19]. Hence, the intra-individual response is quite diverse in terms of different triggered cognitive and physiological reactions facing different stressors. Likewise, the inter-individual response has different levels of intensity of reaction depending on either the particular perception or t capabilities of each person before the same stressor. Thus, a single marker cannot comprehensively evaluate the level of homeostatic imbalance. That is why none of the established stress markers alone, allows a reliable quantitative assessment of the intensity of the stress response.

The above notwithstanding , a foundation of this thesis is that a single stress marker cannot globally assess the individual stress response, as it is also concluded in [48]. In order to be able to compare and assess the stress level response, a method that considers the different stress response reactions all together is required. Therefore, a multivariable approach is proposed in this thesis to assess the individual stress response to a stressor.

4.1.2. Selection of Stress Reference Variables

Stress reference variables are selected from established psychometric tests and biochemical stress markers taking into account the bibliographic review conducted, as well as the criteria of medical doctors and psychologist involved in the ES3 project, as described in [16]. These variables are used to assess the stress levels of the different states induced in the experiment.

4.1.2.1. Psychometric Variables from Psychometric Tests

The selected psychometric variables are well-known psychometric tests, like the following ones: Perceived Stress Scale (PSS), Visual Analog Scale (VASS) and State-Trait Anxiety Inventory test (STAI). Notice that the Stress Symptoms Scale (SSS), a new scale proposed and designed by the Clinic Hospital of Zaragoza's Psychiatric Department was also used.

The PSS measures the degree of overall stress of the individual or the extent to which life situations are appraised as stressful by him/her [82]. The VASS records the stress level that is self-assessed by the individual (his/her own perception). The STAI evaluates anxiety from two different points of view: as a measure of the subject's state (STAI-s) at a given time, and as the trait (STAI-t), or stable tendency of the individual to respond by increasing his/her level of anxiety in stressful situations [83].

4.1.2.2. Biochemical variables from blood and saliva samples

The Biochemical variables selected are the following: Copeptin, prolactin and glucose obtained from blood samples, as well as cortisol and alpha-amylase obtained from saliva samples.

Copeptin is indirectly related to stress, as it is a parameter that reflects the changes in the circulation of vasopressin, a marker of stress[28,84]. Its advantages are that it is quite more stable and non-pulsatile than vasopressin. Copeptin levels are sensitive to situations involving physical stress [84], but can also be sensitive to emotional stress [28]. It has been reported that copeptin levels mirror the individual's stress levels more sensitively than cortisol levels do [85]. Its levels increase immediately as a response to stress and remain high longer than prolactin levels [86].

Prolactin secretion is strongly affected by stress. An increase in its levels as a response to different types of psychological stressors has been reported in humans. The intensity of the response depends on the intensity of the stress stimulus [86]. Comparatively, it has been reported [86] that prolactin secretion depends much more on the intensity of the response than cortisol secretion. Its levels increase immediately as a stress response and it remains 15-20 minutes after.

Glucose metabolic changes in the prefrontal cortex are associated with HPA axis response to a psychosocial stressor [87], probably because they are a reflection of adrenalin release. It has been reported that plasma glucose significantly correlates to anxiety situations [86] because hyperglycemia is one of the consequences of sympathoadrenal activation.

The cortisol is released in stressful situations. It follows a diurnal rhythm, changing over time independently from an experienced stress, which is limitation as a stress marker. (see Figure **4.2**). However, the advantage of its use is that cortisol levels can be determined from saliva collected through noninvasive sampling procedures. There is approximately a 15–20 minutes' delay from the stressor onset until the corresponding rise in salivary cortisol levels happens. This timeframe needs to be considered when using cortisol levels as a stress marker. Cortisol levels peak at the time the individual wakes up and decrease gradually during the day, in such a way that the variations of cortisol levels during the day could be more significant than the variations of cortisol levels due to stress. Therefore, sampling times should be carefully controlled when assessing cortisol levels.

Alpha-amylase (α-amylase) is known as a marker of the activity of the sympathetic nervous system in response to stress. Levels of α-amylase increase in response to physical and/or psychological stress with a maximum peak at 5–10 minutes of exposure to the stressor. Normal values are then quickly re-established. Similarly to cortisol, the secretion of α-amylase has a

circadian physiological pattern: its levels diminish in the first 30 minutes after awakening, which is represented in Figure **4.2** extracted from [88].

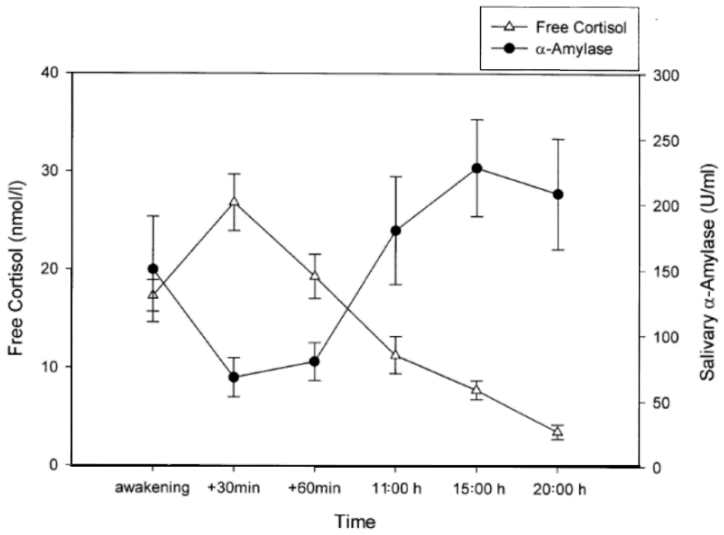

Figure 4.2: Circadian rhythm of free cortisol and saliva α-amylase.

Cortisol and α-amylase were measured in the Endocrinology and Radioimmune analysis service of the UAB Neurosciences Institute. An immunoassay test was carried out to determine cortisol concentration and an enzyme assay analysed the α-amylase enzyme kinetics (Salimetrics, State College, PA, USA). The analysis of prolactin, glucose, and copeptin was quantified at the Centre of Biomedical Diagnostic. All the samples were processed in the same test to avoid any inter-test variability, achieving intra-test variation coefficients lower than 5% in both cases.

4.1.3. Physiological Measures of Stress Response Selection

Stress response triggers changes in various physiological parameters of the body for short or long periods of time that can be measurable, as it was reviewed in Section **2.1**. Those that are mainly reported in literature are summarized in the diagram shown in Figure **4.3**.

Among those physiological reactions to stress, the variables that can be monitored and are suitable to detect the stress response were selected considering the bibliographic review conducted (see Section **2.2**). Also, in view of the possible implementation of a wearable device for monitoring stress, the following signal's selection criteria were used:

- Noninvasive and continuously measured
- Available sensor technology to implement in a wearable device.
- Electrodes or sensors comfortable to wear for long monitoring time.

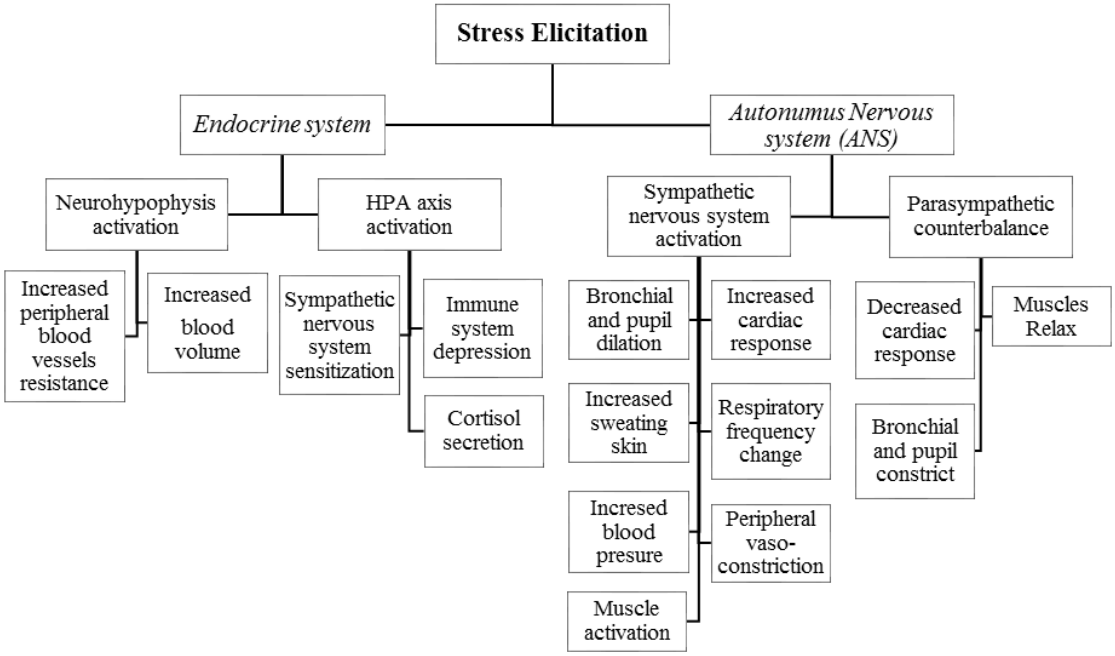

Figure 4.3: Physiological manifestation of stress response.

Changes in blood volume, peripheral blood vessels resistance and cardiac response can be measured by monitoring the pulse wave using a photoplethysmography measurement. Cardiac response and parasympathetic counterbalance are associated to variabilities in heart rate, defined as heart rate variability (HRV), which can be obtained by monitoring the electrocardiography signal. Since the ANS controls the heart activity, measuring cardiac activity seems to be a noninvasive way to evaluate the state of the ANS. Consequently, the above relationship can explain the heart's ability to respond to multiple physiological and environment stimuli.

The increase in blood pressure is another manifestation triggered by both neurohypophysis correlated activation and sympathetic nervous system activation. It is also much related to the increase of cardiac response itself. However, blood pressure cannot be measured noninvasively or continuously. Due to the promising contributions of blood pressure as a stress marker, a research was performed as part of this thesis work to develop a method for a continued measurement of blood pressure variations using a noninvasive procedure (see Appendix **I**). It was concluded that the estimation of PB can be done using features from ECG and PPG but frequently calibrations made from an accurate BP reference measurement per individual (i.e. every 10–15 minutes) are required. However, the occurrence of small variations of BP in short period of times can be detected and followed using certain estimation models. Thus, a continued measurement of BP variations can be done using some parameters derived from noninvasive measurements of ECG and PPG.

Acute stress triggers peripheral vasoconstriction, causing a rapid, short-term drop in skin temperature in homeotherms. This influx of peripheral blood, along with stress-induced thermogenesis, simultaneously increase core temperature [89]. Consequently, skin temperature measurements in the fingers and the face can reflect peripheral vasoconstriction.

One of the effects driven by ANS activation is the need for more oxygen. This increased oxygen demand is reacted with faster and deeper breathing. Much alike respiration, an increase of sweat production is also driven by ANS, innervating the eccrine sweat glands. Both are easily and respectively measured by a pneumographic method (to record expansion and contraction of the thorax) and a skin conductance (SC) sensor (to record electrical properties of skin caused by sweat glands activity). SC is defined as the electrical conductance of the skin in micro Siemens, which is the inverse of skin resistance and is often measured on the palms of the hands due to their high density of eccrine sweat glands.

Muscle activation has been employed on stress detection [32 , 50,52] using EMG signal mainly from the trapezius muscle [90]. Though it is also driven by the sympathetic nervous system activation, muscle activation may be also influenced by other factors like physical activity or environmental factors. The interferences caused by these factors should be taken into account when using features from EMG signals as stress markers.

Pupil dilatation can be tracked by measuring pupil diameter with a camera and image processor techniques, though would be limited if the camera is frontally placed or the camera is added to some gadget like eyeglasses. For these limitations, and because it is a much more expensive sensor to implement, pupil dilatation was not selected for this research. The following are also not considered/selected due to the fact that they cannot be noninvasively measured: bronchial dilation, immune system depression markers, secretion of cortisol or other biochemical samples. The selected physiological signals to measure are those presented in Table **4.1**:

Table 4.1: Selection of physiological signals to measure

Physiological measures	**Physiological manifestation to stress**	**Body Place**
Peripheral skin temperature	Neurohypophysis activation	Finger
Core skin temperature	Neurohypophysis activation	Cheek
Skin conductance	Sympathetic nervous system activation	Hand
Electromyography	HPA axis activation, Sympathetic nervous system activation and parasympathetic counterbalance	3 Orthogonal leads in the thorax
Respiration Rate	Sympathetic nervous system activation	Thorax
Photoplethysmography	Neurohypophysis activation	Index finger
Electromyography	Sympathetic nervous system activation	Trapezius muscle

4.1.4. Measurement Device for Experimental Study

One important condition of the experimental framework design is having a suitable device to acquire the proposed physiological signals. Considering the information previously provided about the common experimental framework measurement and the design of the experimental trial, Medicom's ABP-10 module system(by Medicom MTD Ltd, Russia) [91] was selected based on the following criteria:

- Certification as Medical equipment and CE marking
- Fulfils the technical characteristic of sensors (See Table **4.2**)
- Easy to wear and does not affect subject´s state
- Real time signal visualization
- Access to raw data
- Synchronized measurement of all physiological signals

Furthermore, based on the nature of each signal, the features extracted from it, the timing activation and the variations of each physiological reaction due to the stress response, technical specifications of the sensors were added as requirements for the selection of the measuring device. The required technical specifications of sensors are listed in Table **4.2**. Further information for each signal and feature extraction is detailed in Chapter **5**.

Table 4.2: Technical specifications of sensors

Signal	**Technical specifications**	
Skin temperature	Range	30–50ºc
	Precision	0.01ºc
	Fs	150Hz
Skin conductance	Range	10k–5mohm / 0,2–100us
	BW	0–16 Hz
	Fs	50Hz
Electrocardiography	Derivations	3
	BW	0–150Hz
	Fs	1khz
	Resolution	24 bits
Electromyography	Range	0–2000uv
	BW	20–500Hz
	Fs	1khz
Photoplethysmography	Type	Transmission
	Fs	250Hz
	Resolution	16bit
Respiration	Type	Pneumographic Impedance
	Fs	50hz
	Resolution	20mohm

4.2. Design of Acute Psychological Stress Study in Young Individuals

An experiment was designed to induce acute stress to healthy young individuals through a well-defined psychological stressor. It included a Relax Session and a Stress Session. The following section describes the experimental design and detailed protocol. A procedure document was created with further information about the design and protocol of the experiment and detailed instructions for applications.

4.2.1. Experimental Design

The study is a quasi-experimental pre-post study without control group to examine the effect of a stressor (TSST) on psychological, biochemical and physiological stress markers in a unique group of healthy students. The experiment design included different stress stages for each subject, so that participants could be compared to themselves in basal state and stress state. It included a Relax Session, as a control condition, and a Stress Session, performed in different dates. During both sessions, physiological signals were continuously recorded while standard psychological tests were applied and biochemical markers collected at the end of each session (see Figure **4.4**).

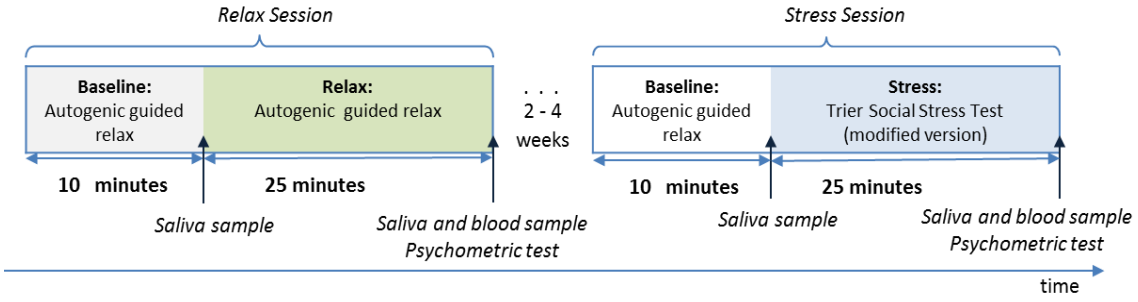

Figure 4.4: Experiment Design.

The Relax Session: included an autogenic, audio-guided relaxation exercise to get a baseline and a longer relaxation to get a basal stress state. The Stress Session, much like the Relax Session, included a baseline stage and also included an induced stress test. At the end of both sessions, blood and saliva samples were collected and a psychometric test applied as stress references. A prior saliva sample was taken 25 minutes before, to obtain the variation of cortisol and alpha-amylase peer session.

A preparatory relaxation period of 10 minutes was conducted at the start of each session to achieve a fairly similar baseline state among participants for both sessions. The state reached after

the Relax Session was considered the basal state while the state reached at the end of the Stress Session was considered the stress state.

The design of each session is given in Section **4.2.3**. Both sessions were carried out in the same room, under controlled temperature and environmental conditions. Before both relax and stress exercises, the autogenic 10-minute relaxation exercise guided by an audio recording was performed to obtain a similar baseline psychological state for all participants.

4.2.2. Participants

Participants were recruited using advertisement on the UAB website, linked the webpage of project ES3 [92]. All interested candidates completed an online form beforehand, to ensure they met the criteria of selection for the study. After individually checking to determine whether they fulfilled the inclusion, participants where included in the study consecutively according they were enrolled in the study. Appointments were made at random.

The UAB Ethics Committee approved the study protocol. All participants were informed about the purpose and characteristics of the study and their rights to withdraw from it at any time during the process. All participants signed their respective consent forms.

The inclusion criteria were: to be a student at the university, between 18 and 30 years old, and a non-regular consumer of psychotropic substances, specifically alcohol and tobacco. The exclusion criteria were: a body mass index higher than 30, any chronic disease or psychopathology, and a stress level higher than 70% on the visual analogue scale.

Participants were instructed to avoid the use of any psychotropic substance, alcohol, or tobacco and to avoid doing any physical exercise 24 hours before each session. They were also told to wake up two hours before the start of the sessions, and have a light breakfast without coffee or tea.

Socio-demographic and lifestyle variables obtained from the questionnaires were: age, sex, BMI, habits (smoking status, coffee intake, consumption of alcohol, consumption of drugs and practice of sport), health condition (chronic disease diagnosed, medication, treatment) and other variables (cohabitation condition, physical activity and job status).

4.2.3. Experimental Protocol

The Relax and the Stress Sessions were performed on different dates, but at the same hour for each participant (see Figure **4.5**). Each session lasted around 40 minutes. The Relax Session had two stages: Baseline stage (BL_R) and Relax stage (RS); while the Stress Session had a

Baseline stage (BL_S) and an acute psychological stress test through: Story Telling (ST), Memory Test (MT), Stress Anticipation (SA), Video Display (VD), and Arithmetic Task (AT).

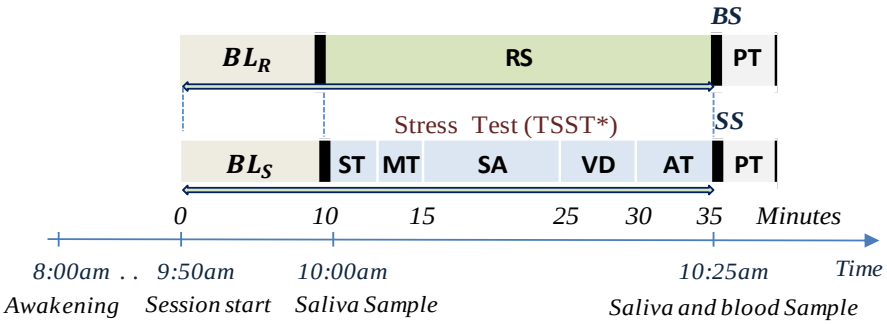

Figure 4.5: Details of the study protocol.

The first saliva sample was taken after the baseline stages (BL_R, BL_S) and the second saliva sample at the very end, after the Relax and the Stress Sessions had ended. A blood sample was taken and psychometric tests were applied at the end of each session. Physiological signals were measured during both sessions. Levels of stress states reached at the end of the Relax and the Stress Sessions were considered basal state (BS) and acute stress state (SS) respectively.

The relaxation was induced by autogenic relaxation guided by an audio recording conforming to Schultz's method [93] at stages BL_R and RS in the Relax Session and BL_S in the Stress Session. Acute psychological stress was generated with a modification of TSST as follows, instead of the speech, the participants performed a memory test while being video recorded. Then the video was shown to him/her along in the company of an audience, after a prior stress anticipation period, much like the one used in [94]. This modification was introduced to compare the results from the Memory Test with the study performed in [94] as part of the ES3 Project [16].

The whole test included five different stages over a 25-minute period. Each stage demanded different conditions, inducing different stress states in the participants. Hence, the TSST as a stressor is divided into these five stages:

1. Story Telling stage: Three different short stories are told to the subjects and they are asked to remember as many details as they can.
2. Memory Test stage: the subjects are requested to repeat each story out loud, telling as many details as they can in a 30-second period per story. During this stage the subjects are notified that they will be video-recorded to assess their performance.
3. Stress Anticipation stage: The participants are asked to go to an adjacent, empty room under the pretext of they should wait until their video is assessed by the group of psychologists. This will increase their anxiety before the next stage.

4. Video Display stage: The participants go back into the previous room, where an unexpected audience of at least three people is waiting for them. The video showing each participant's storytelling is played in front of the audience. Each video is interwoven with another video in which an actor of the same gender is telling most of the details of each story, to increase the subject's arousal.
5. Arithmetic Task stage: Subjects are asked to count backwards from 1022 in decrements of 13 in less than 5 minutes. If they make a mistake, they should start again from 1022.

At the end, the subject is required to complete the psychometric test (PT). Participants were seated the entire time, except at the beginning and at the end of the Stress Anticipation stage, when they walked into the adjacent room.

4.2.4. Reference of Induced Stress

The psychometric and biochemical variables listed in Section **4.1.2** haves been used as reference to compute a stress reference scale applying the multivariable approach (see Section **4.1.1**).

The psychometric tests were self-administered at the end of each session. Biochemical samples were collected by a nurse also at the end of each session. In accordance with the circadian rhythm of cortisol and α-amylase [95], the sessions were scheduled in the morning (9:45h-10:45h), 2 hours after waking up. Two saliva samples were taken in each session; one before the stimulus (relaxation or stress) and the other one at the end of the session [96], (i.e. 25 minutes in-between), to obtain cortisol (Δ cortisol) and alpha-amylase (Δ α-amylase) variation per session.

Along the experiment, the values of stress reference variables (SRV) were rescaled to a 0-100 range of arbitrary units (au) using equation (**4.1**) to avoid biased perception homogenising its ranges.

$$SRV'_p = \frac{SRV_p - SRV_{min}}{(SRV_{max} - SRV_{min})} \cdot 100 \qquad (4.1)$$

Where: SRV_p: Variable SRV value concerning participant p.

SRV'_p: SRV_p rescaled value

SRV_{min} , SRV_{max} : Minimum and maximum values of the variable SRV for all subjects in both sessions.

The rescaling of biochemical variables was performed by splitting the data into two groups by gender, due to different reference ranges between men and women.

Applying the multimodal approach to the stress reference variables, the stress reference level of participant p, in state x (BS or SS), $S_{ref}(p,x)$, can be found in this preliminary approach as a linear combination of n stress reference variables, as expressed by the following equation:

$$S_{ref}(p,x) = \sum_{j=1}^{n} c_j \cdot SRV'_j(p,x) \quad ; x \in \{BS, SS\} \tag{4.2}$$

Where $SRV'_j(p,x)$ is the rescaled value of the *jth* reference variable for the participant p at state x

c_j is the coefficient associated to SRV'_j.

The c_j coefficients were assigned by using a ponderation of the first and second components obtained from the principal component analysis (PCA), concerning the contribution of each variable to the state's differentiation. Further analysis of c_j coefficient values assignation was performed in [97] and it was concluded that variations in the percent values assigned to the coefficients affect slightly the range and absolute values of $S_{ref}(p,x)$ but do not affect the validity of the differentiation between states.

4.3. Discussion

The stress concept establishes that stress arises when a specific event threatens the homeostatic stability of an individual. The ultimate goal of this work is to find an approximate measure of that homeostatic imbalance. We propose to use biomarkers to assess the level of stress response. Also in view of a possible stress monitoring method, the selected biomarkers should be continuously monitored, and, therefore, they must be obtained from physiological signals that are not only continuously but also noninvasively measured.

A common measurement protocol was proposed to study the stress response of distinct homogenous groups of subjects facing different stressors. Hence, it was designed to be used in a group of different experimental studies in order to make a wide stress level assessment. That required to analysing the current forms of stress level assessment: psychometric tests, biochemical and physiological variables.

Up to now, there is no unique criterion to make an inter-individual or intra-individual assessment of the stress level response of individuals when facing different stressors, which would substantially improve study and knowledge of this phenomenon. For that reason, this particular research focuses on a quantitative assessment of the physiological stress response. This

quantitative assessment can be viewed as how much the action of a stressor is affecting the individual since this degree of affection could drive the consequences of the stressor in a medium-term period. Further studies could use the proposal presented hereby and future experimental results could be compared for the advancement of research literature in this field.

Stress, by definition, is a multidimensional phenomenon; so therefore, comprehensive stress response can't be measured via a single marker. The whole stress response of an individual includes a diversity of reactions that can be monitored. However, the responses of individuals are quite diverse depending on the perception or capabilities of each person before the stressor. Consequently, the amount of triggered cognitive and physiological reactions and the intensity level of such reactions can differ from person to person and for the same person against different stressors. Therefore, considering the stress level response as the level of homeostatic imbalance that is reflected in a number of physiological reactions, it can be defined, as it is also concluded in [48],that a single stress marker cannot comprehensively assess the stress response of an individual.

Consequently, a multivariable approach of stress markers is proposed to have a broad measurement of the stress response and to consider extensive aspects triggered by the stressor. This approach can establish the groundworks for applications that aim to monitor stress and improve the research in the field of stress, because it might be a starting point for future inter-individual and intra-individual valid comparisons facing different stressors.

Even though, the selected stress reference variables are well-known stress makers, they cannot be used in view of an implementation of stress monitoring. They, however, are suitable for specific applications or research studies. The VASS scale is the most widely used scale in stress research, but it cannot be used for inter-individual comparisons since it is totally subjective as a self-perceived state and furthermore, its offset could be different for each person. Biochemical variables are related to physiological changes triggered by a stressor. Cortisol is greatly related to the hormonal response to a stressor, but then, again, it cannot be used for inter-individual comparisons without considering its circadian rhythm. Moreover, its levels are also related to other physiological mechanisms. Copeptin is linked to the vasoregulatory mechanism, while prolactin to hypophysis activation. Both are good stress markers; however, they are obtained by using invasive measurements.

Physiological measures of face and finger temperature, ECG, trapeziums muscle EMG, PPG, respiration, and skin conductance were selected to be measured in the experimental trial because they can be used in continuous and noninvasive monitoring. Other physiological measures, such as pupil diameter, were not selected because either they are not easy to implement in a wearable device or its sensors are uncomfortable to wear for long periods of monitoring.

For research purposes in this experimental trial and according to its technical specifications, a device was selected to synchronously measure the selected physiological signals. In a future and real application, a more suitable, comfortable to wear device should be used. It also could be included sensors for real live interactions such as accelerometers for body-motion or ambient temperature, among others.

Using the developed experimental measurement framework, a study was designed to induce acute stress in healthy young individuals through a well-defined psychological stressor. A reliable experimental protocol is proposed, as well as, a well-documented stressor such as the TSST [77] to be applied. The purpose was to induce moderate but significant stress with a validated and widely documented tool to inducing stress.

The design of the experiment took special care in order to maintain both the same time bases for all the electrophysiological variables and the best possible synchronisation between the psychological measures and the stress reference variables collection. The protocols are also designed to ensure that the same schedule is followed between the participants and the reference when taking blood and saliva samples.

The experiment design, as described above, uses a sample of individuals (healthy students) that is subjected to a particular stress stimulus, TSST. However, other physiological responses should be expected using other groups of individuals facing another stressor. A multivariable stress reference level is proposed by merging the above-mentioned stress reference variables (See Section **4.1.2**) into a single scale. As a first approximation, a linear relation between stress level reference and each variable is assumed.

A limitation of the protocol is that the relaxation period is restricted to only 10 minutes, and participants may need longer time to physiologically accommodate to the experimental environment. The study design has two limitations concerning biochemical timing collection: 1) Cortisol and alpha-amylase are measured in the morning hours when intra-individual variability is the highest and 2) alpha-amylase is known to peak at about 10–15 min after stressor onset, whereas it was assessed 25 minutes after the stressor onset.

5 SIGNAL PROCESSING AND FEATURE EXTRACTION

The main aspects concerning the signal processing and feature extraction methods used in this research are listed below. It should be noted, that all signal processing was done with MATLAB R2015.

Most skin conductance recordings from the reported experiment had to be discarded due to a sensor malfunction. Thus, skin conductance values were not considered in any further analyses. Due to the artefact caused by participants' movement, when talking during the Memory Test and Arithmetic Task, respiration and electromyography signals were excluded from this analysis as they were clearly artefacted. Therefore, the set of features considered as possible stress biomarkers was limited to those derived from temperature, ECG and PPG signals. However, cardiorespiratory features from relaxed stages, Story Telling, Stress Anticipation and Video Display are analysed in two work collaborations made within the thesis work. These collaborations are reported in [98–100].

5.1. Temperature

Both face (T_{fa}) and finger (T_{fi}) temperature signals were filtered using a one-dimensional median filter (50 order) and then values from the 20ºC–40ºC range were excluded. Subsequently, the following features related to variations and relationships between face and finger temperatures were computed:

- Temperature Gradient, (ΔT): Temperature variations every one minute, obtained as:

$$\Delta T_j = T_{j+1} - T_j \quad (5.1)$$

Where: j denotes the index of time instant at every minute.

The temperature value T_j was calculated as the mean in one-second time window centered at minute j.

$$T_j = \frac{1}{N_j} \sum_{t_{j-0.5seg}}^{t_{j+0.5seg}} T(t) \quad (5.2)$$

Where: N_j is the number of sample within one-second time window centered at minute j

- Temperature power (T_P) : defined as the average power of temperature via a rectangle approximation of the integral of the Power Spectral Density (PSD) in a time window of one-minute.

  The spectrum estimation method of the periodogram was used, which is a nonparametric estimate of the power spectral density (PSD), defined as:

$$\hat{P}_j(f) = \frac{\Delta t}{N} \left| \sum_{n=0}^{N-1} T_n e^{-j2\pi f_s} \right|^2 \qquad -1/2\Delta t < f \leq 1/2\Delta t \quad (5.3)$$

  Where: Δt: is the sampling interval.

  T_n : the temperature signal sample at f_s.

- Temperature Ratio (T_{ratio}): ratio between values of finger (T_{fi}) and face (T_{fa}) temperatures. $T_{ratio\,j}$ is the mean value of T_{ratio} in a one-minute time window at minute j:

$$T_{ratio\,j} = \frac{1}{N} \sum_{t_j}^{t_{j+1}} \frac{T_{fi}(t)}{T_{fa}(t)} \quad (5.4)$$

  Where: N: is the number sample within a time window of one minute.

5.2. Electrocardiography (ECG)

Three orthogonal ECG leads were sampled at 1 KHz. The ECG signal of lead Y was analysed as in [101] to obtain a time series of *R-peak*. The R-peak series were estimated from the ECG using an implemented algorithm based on the discrete wavelet transform [102]. Ectopic beats, false readings and misdetections were identified and fixed before leading to a new interval series commonly denoted normal-to-normal intervals, or NN intervals. An instantaneous heart rate signal ($d_{HR}(i)$) was obtained from the beat occurrence times using an algorithm based on the integral pulse frequency modulation model, which is taking into account the presence of ectopic beats[103], sampled at 4Hz.

The following parameters were computed, which are explained in detail in [104]:

- Time features: From the fixed NN series, mean heart rate (HR_{mean}), standard deviation of all *NN* intervals (RR_{SDNN}) and root mean square of successive differences of adjacent *NN* intervals (RR_{rMSSD}), which provides a more detailed description of short term variability

$$HR_{mean}(i) = \frac{60}{NN(i)} \tag{5.5}$$

$$RR_{SDNN}(i) = \sqrt{\frac{1}{m-1}\sum_{i=1}^{m}\left(NN(i) - mean\left(NN(i)\right)\right)^2} \tag{5.6}$$

$$RR_{rMSSD}(i) = \sqrt[2]{mean\left(\left(NN(i+1) - NN(i)\right)^2\right)} \tag{5.7}$$

- Frequency features: LF power (*PLF*), ranging from 0.04 to 0.15 Hz, and HF power (*PHF*), ranging from 0.15 to 0.4Hz, were computed in time windows of one minute from the power spectral density (PSD) of the heart rate variability signal (d_{HRV}). The PSD was calculated using the Fourier transform.

  The $d_{HRV}(t)$ signal is obtained subtracting from $d_{HR}(t)$ signal, a time-varying mean HR $d_{HRM}(t)$, which is obtained by low-pass filtering $d_{HR}(t)$ with a cut-off frequency of 0.03 Hz:

$$d_{HRV}(t) = d_{HR}(t) - d_{HRM}(t) \tag{5.8}$$

  The *LF/HF* ratio was computed as well. Power in the very low frequency (*PVLF*) band, ranging from 0 to 0.03Hz, was computed from the PDS of the $d_{HRM}(t)$ signal.

5.3. Photoplethysmography (PPG)

The photoplethysmography signal was recorded from the index finger and it was sampled at 250Hz. A low-pass filter with a cut-off frequency of 10 Hz was applied to the PPG. PPG artefact detector based on Hjorth parameters, as described in [105], was used to suppress artefactual PPG pulses. Those time slots identified as artefacted were not analysed.

Fiducial points in pulse waves were automatically determined from each pulse beat by an implemented algorithm based in [106]. The detected points for the i^{th} pulse in PPG signal can be seen in Figure **5.1** and are listed as follows:

- n_{A_i} → Apex point: The pulse maximum. Each absolute maximum of the PPG signal between two successive QRS complexes detected in the ECG signal is considered to be an apex point of a PPG pulse.
- n_{B_i} → Basal point: The absolute minimum of the PPG signal between 2 successive QRS complexes in the ECG signal.
- n_{M_i} → Middle point: Instant in time when the PPG pulse reaches half of its apex-to-basal amplitude.
- n_{AR_i} → Apex point of the reflected wave: The maximum reflected pulse wave.
- n_{N_i} → Dichrotic notch point: The inflection point between pulse wave and reflected wave.

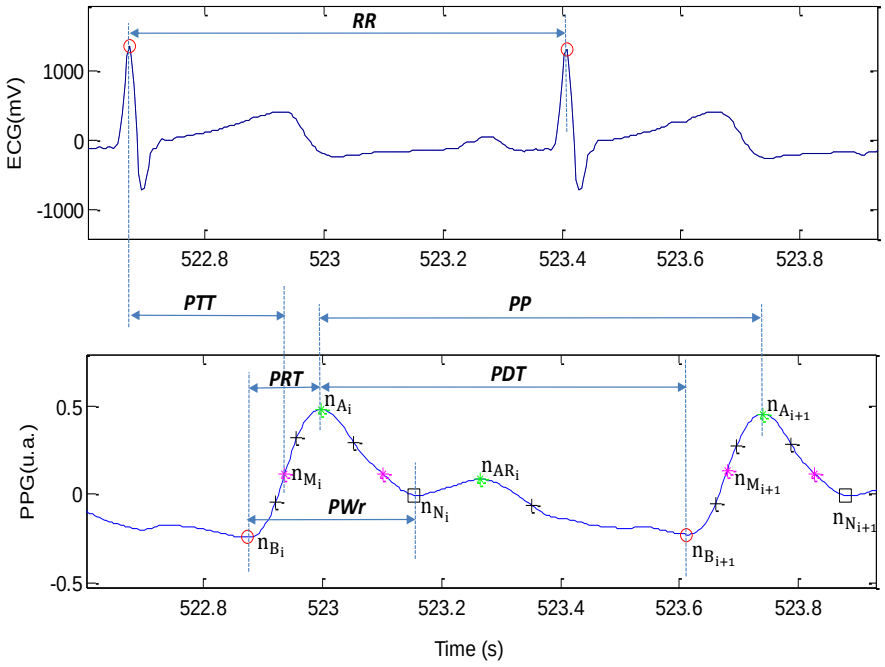

Figure 5.1: Detection points in ECG and PPG signals.

Dichrotic notch point (n_{N_i}) was automatically detected by an algorithm based on the first derivative, as described in [107]. A flow diagram of ECG and PPG signal processing including obtained features is represented in Figure **5.2**.

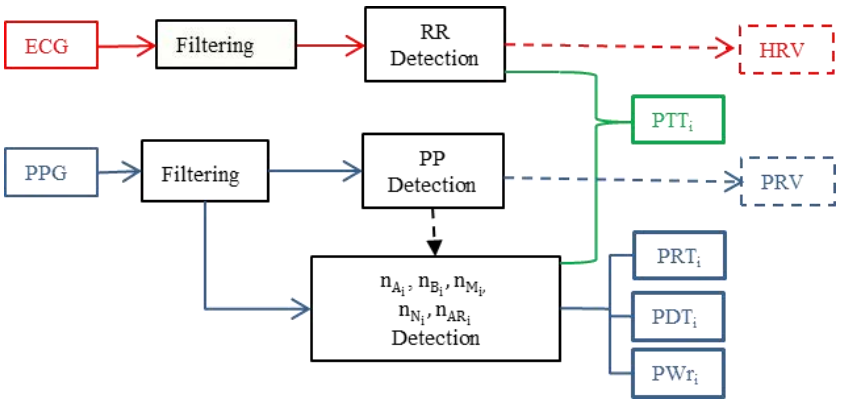

Figure 5.2: Block diagram of ECG and PPG signal processing.

Time-based parameters were calculated (see Figure **5.1**) and listed in Table **5.1**.

Table 5.1: Parameters from the pulse in PPG signal

Acronym	Name	Definition
PP	Pulse Period	The time interval between two consecutive n_{A_i}.
PTT	Pulse Transit Time	The time interval between the maximum of the R wave in the ECG signal and its associated n_{M_i}.
PRT	Pulse Wave Rising Time	The time interval between n_{B_i} and n_{A_i}.
PDT	Pulse Wave Decreasing Time	The time interval between n_{A_i} and $n_{B_{i+1}}$.
PWr	Pulse Width until Reflected Wave	The time interval between n_{B_i} and n_{N_i}.

The fiducial points, n_{M_i}, to obtain PTT are considered to be time-robust because they are located in a very abrupt part of the PPG pulse. The following parameters were computed in non-overlapping time windows of one minute:

- Time Features from *PP(i)* series were similar to the aforementioned time features from the ECG signal (PR_{mean}, PP_{rMSSD}, PP_{SDNN}), see (**5.5**), (**5.6**), (**5.7**).
- Pulse rate variability (PRV) analysis was done with the same procedure used in the previously mentioned analysis of HRV from the ECG signal. Hence, frequency features from *PP(i)* series were obtained as PRV_{PVLF}, PRV_{PLF}, PRV_{PHF}, $PRV_{LF/HF}$.

- Time-based features were studied from PPG-based parameters (*Px*) (see Figure **5.1**). Mean, standard deviation and root mean square of *Px*, in every one-minute time window, were assessed at the j^{th} minute as follows:

$$Px_{mean}(j) = \frac{1}{N}\sum_{i=1}^{N} Px(i) \tag{5.9}$$

$$Px_{STD}(j) = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(Px(i) - mean\left(Px(i)\right)\right)^2} \tag{5.10}$$

$$Px_{rMSSD}(j) = \sqrt[2]{\frac{1}{N}\sum_{i=1}^{N}\left(Px(i)\right)^2} \tag{5.11}$$

Where: *Px:* represents *PTT, PRT, PDT, PWr.*

N: the number of *i* detected beats in a one-minute window.

5.4. Discussion

A set of features was obtained from face and finger temperature, ECG and PPG signals according with previous reported features as stress markers from the reviewed literature. The set of features was obtained also, by looking into other possible features from the analysis of physiological reactions enveloping the stress response.

In addition, a study performed as part of this thesis work related to the measurement of blood pressure variations using a noninvasive procedure (see Appendix **I**), has provided more information about parameters that can mirror some manifestations of the physiological stress response. The results have shown that the occurrence of small variations of BP in short periods of time can be detected using some parameters derived (i.e. *PTT, HR, PW* and pulse amplitude) from noninvasive measurements of ECG and PPG. That is why *PTT, HR* and *PW* were included as stress markers. The *PRT* was also included instead of pulse amplitude, as they are directly linked, to avoid error and artefacts due to displacements in the PPG sensor and body motions.

Variations in face and finger temperature are associated with stress response through vasoregulatory system. In stressful moments, there are both an increase in face temperature due to increased blood volume and a decrease in finger temperature provoked by peripheral vaso-constriction, as reported in [29].

PTT can be easily measured from ECG and PPG. Thus, *PTT* is inversely proportional to pulse wave velocity, which is associated to arterial stiffness and cardiovascular output. Therefore, a decrease in *PTT* is related to an increase in blood vessel resistance and cardiovascular output. Hence, this parameter is also an index of neurohypophysis activation and sympathetic nervous system activation. Additionally, as discussed in Appendix **I**, *PTT* is inversely related to blood pressure (BP)[108], based on the relationship between BP and pulse wave velocity [109,110].

Pulse wave parameters are the length of time between two characteristic points in a pulse beat, because they were selected as time based parameters, avoiding errors of amplitude measurement due to movements in the PPG sensor. *PDT* is related to arterial stiffness, blood volume and diastolic time [111], also highly related to pulse rate, consequently, it is good stress marker.

The *PWr* is a time index of the reflected pulse wave arrival and it is associated with PRT. It is also associated with peripheral central vascular resistance and cardiovascular output, as well as pulse wave velocity [107]. Despite this feature is not commonly used in stress detection, we consider it as stress marker. The *PDT* is an index of diastolic time, also highly related to *PR*. The *PRT* is a pulse wave index and is related to arterial stiffness, blood volume and systolic blood pressure peak [111]. Vasoconstriction is reflected in pulse wave by decreases on its amplitude, which is directly related to PRT.

It is known that the HRV frequency range between 0.04 and 0.15 Hz (low–frequency component, *LF*) is related to both sympathetic and parasympathetic modulation, even though an increase in its power is generally associated to a sympathetic activation. An HRV frequency range between 0.15 and 0.4 Hz (*HF* component) is related to parasympathetic modulation and it is synchronous with the respiratory rate[112]. While *VLF* spectral component takes account of long-term regulatory mechanisms that cannot be resolved in the time window usually considered for the frequency-domain analysis[112].

However, HRV's associations with other components of cardiovascular variability (blood pressure variability, respiration, baroreflex etc.) need to be taken into consideration[113,114]. For example, heart rate variability in synchrony with respiration is expressed within HF component. The respiratory arrhythmia of the heart is the result of the vagal activity and thus it is considered a marker of the parasympathetic activity controlling the heart rate, as is suggested in [100], Thereupon, frequency indices of HRV might be influenced by respiration and, therefore, respiratory information should be taken into account to increase the reliability of HRV as a stress marker. Those associations must be explored and included in futures studies.

On the other hand, *LF/HF ratio* has been used to quantise the sympathic-vagal balance. But there are other studies that suggest that *LF/HF ratio* data cannot quantify cardia sympathic-vagal balance neither in health nor disease [114], since there are other factors that influences the LF/HF ratio, such as respiration rate, diseases and physiological challenges.

In order to validate the hypothesis, of having a continuous measure of stress all along sessions, features from respiration and trapezium EMG signals were not included in the presented analysis because more complex processing of those signals would be necessary to eliminate the influences of speaking and body movements., which is out of the scope of the thesis. This is a limitation of the presented work, because the analysis of features from respiration and trapezium EMG signals could provide more information or redundant data of the stress response, which would be very useful in real life scenarios. Nonetheless, variations of both signals are triggered by a sympatric activation, which is already covered in this work by other features like *PTT, HRV, PRV, PRT* and ΔT_{fi}. In addition, it has been reported that the respiration frequency can be derive from ECG [115,116] and PPG [117,118] signals specifically from PR and PTT [119]. Therefore, indirectly, respiration changes are covered in the variability of these parameters in this work.

In future works, features from respiration and EMG, as well as skin conductance, should be included and analysed both for their interrelationship and their capabilities as stress markers

Even though features from respiration, skin conductance and trapezium EMG signals are not included, the proposed features herein measure different reactions of stress response covering each system activated by a stressor (See Section **2.1**).

6 ACUTE PSYCHOLOGICAL STRESS: RESULTS OF THE PILOT STUDY

This chapter details the results of the experimental study presented in Section **4.2**. The participants', recorded specifically at the end of the Relax and Stress Sessions (basal stated, BS and stress state, SS) were respectively taken as the lowest and highest bands of moderate stress state. This allowed participants to have been involved in all stressing and relaxing activities prior to the reading of their stress states. This was also the time when both the psychometric tests were administrated, and the biochemical samples (a select group of well-known stress markers used for stress level reference) were collected.

A principal analysis was performed between the Relax Stage (RS), as the basal stress level and the Arithmetic Task (AT), as the highest stress level of the study. Since both states are reached during the final moments of each session, there were psychometric, biochemical and biosignal variables to work with.

A. Participants

The study included 40 healthy young participants, who attended the two different sessions. The group includes 23 females and 17 Men subjects. The main socio-demographic and clinical participant data, grouped by gender, is shown in Table **6.1**.

Participants were asked, before the first session, if they felt they were experiencing more stress than usual. Also, the participants stated their stress level on a VASS scale, based on which it was determined whether they met the inclusion criteria or not. Mean and standard deviation for VASS scale values can be found in Table **6.2**.

Those participants who referred themselves as being under higher than usual stress were considered as individuals with a slightly higher baseline stress level. Accordingly, participants

were divided into two groups: 1) those that alluded to being under higher than usual stress, hereby referred to as Baseline-Stressed and 2) those that identified themselves as not being under higher than usual stress, henceforth called Baseline-Unstressed.

Table 6.1: Socio-demographic and clinical participant data

Demographic Data

		Age	IMC	Civil State		Living arrangement			
gender	n	Mean ± SD	Mean ± SD	Married/ couple	Single	Alone	Share flat	Parents	Couple
F	23	21.2 ± 2.1	21.6 ± 2.7	12	11	1	8	13	1
M	17	21.6 ± 3.7	22.8 ± 2.6	5	12	1	7	11	0
F+M	40	21.3 ± 2.8	22.1 ± 2.7	17	23	2	15	22	1

Habits Data

		Sport Practice			Coffee Cups /day		Cigarettes / day			Alcohol		
gender	n	never	seldom	usually	none	less 4	none	less 3	more 3	never	seldom	at times
F	23	4	8	11	15	8	21	1	1	7	16	0
M	17	1	8	8	5	12	12	2	3	0	14	3
F+M	40	5	16	19	20	20	33	3	4	7	30	3

Clinical Data

		Medicaments		Chronic Diseases		Experienced stress higher than usual		
gender	n	yes	not	yes	not	yes	not	reasons
F	23	8 [(1)]	15	5 [(2)]	18	12	11	9 Studies, 4 family, 2 finances
M	17	0	17	0	17	11	6	8 Studies, 5 family, 1 finances
F+M	40	8	32	5	35	23	17	

[(1)]) antihistamine, anticontraceptive; [(2)] 3 Allergy, 2 migraine

Table 6.2: VASS scale results of baseline stress level before the experiment

	Baseline-Stressed		Baseline-Unstressed	
gender	n	Mean ± SD	n	Mean ± SD
F	12	63.33 ± 15.57	11	44.55 ± 17.53
M	11	72.73 ± 9.04	6	41.67 ± 13.29
F+M	23	67.82 ± 13.47	17	43.53 ± 15.79

The finally selected group of students was a socio-demographically, clinically and habit-wise homogeneous data sample. Notwithstanding, further analysis was also performed grouping participants by gender and self-identified baseline stress, taking into account results from previous investigations such as [18,29,120].

6.1. Reference Variables of Stress

Variations in biochemical variables, psychometric test scores and stress reference levels between both sessions were analysed using the Student t-test for paired samples, as this data is normally distributed. The normality of the data was tested using the Kolmogorov-Smirnov test prior to analysis. The results of the psychometric tests are shown in Table **6.3**. As expected, values for the STAI-s, SSS and VASS tests were statistically significantly higher in the Stress Session than in the Relax Session, reflecting the subjects' stress states.

Table 6.3: Inter-subject mean and standard deviation (SD) of Psychometric Test values

Psychometric Test by Groups [range]	n	Relax Session Mean (SD)	Stress Session Mean (SD)	T-test p-value
PSS [0–40]	37	20.5(3.0)	20.0(3.5)	0.625
Women	21	20.4(3.4)	20.2(4.0)	0.958
Men	16	20.5(2.4)	19.6(2.9)	0.426
Baseline-Unstressed	17	19.4(2.7)	19.8(2.3)	0.611
Baseline-Stressed	20	21.2(3.0)	20.2(4.3)	0.383
STAI-s [0–80]	37	15.8(9.4)	26.7(14.1)	1.7e-05*
Women	21	15.5(9.1)	27.1(13.6)	3.8e-04*
Men	16	16.2(10.1)	26.1(15.1)	0.015*
Baseline-Unstressed	17	10.6(3.4)$^{+}$	24.5(15.3)	5.8e-04*
Baseline-Stressed	20	19.6(10.6)$^{+}$	28.6(13.0)	0.01*
STAI-t [0–60]	37	21.1(9.3)	20.0(10.6)	0.384
Women	21	21.8(9.6)	20.0(11.0)	0.085
Men	16	20.1(9.1)	19.9(10.3)	0.659
Baseline-Unstressed	17	16.6(8.1)$^{+}$	16.3(9.5)$^{+}$	0.760
Baseline-Stressed	20	24.4(8.9)$^{+}$	23.2(10.6)$^{+}$	0.415
SSS [0–80]	37	21.6(15.2)	32.9(15.7)	4.2e-05*
Women	21	23.0(16.4)	34.9(15.3)	0.003*
Men	16	19.6(13.8)	30.4(16.3)	0.005*
Baseline-Unstressed	17	15.4(9.4)$^{+}$	28.3(15.7)	0.002*
Baseline-Stressed	20	26.1(17.2)$^{+}$	36.9(15.0)	0.008*
VASS [0–100]	37	27.3(22.5)	52.3(25.1)	4.3e-06*
Women	21	30.7(23.8)	56.2(21.5)	6.2e-04*
Men	16	22.6(20.5)	47.1(29.0)	0.003*
Baseline-Unstressed	17	15.8(14.5)	45.4(24.7)	5.4e-04*
Baseline-Stressed	20	35.8(23.8)	58.2(24.5)	0.003*

T-student test between both sessions, *p-value<0.05 is considered to be significantly different sessions change. $^{+}$ Groups that have significant differences in the same session (p<0.05).

The STAI-s scores reflected an increase of over 50% from the Stress Sessions to the Relax Sessions, while increases in the VASS scores were the highest out of all tests; almost doubling their value. Results of PSS and STAI-t scales were similar in both sessions in that they represent the traits of an individual, thereby indicating coherence in the experiment.

Mean values in almost all psychometric tests for the Baseline-Stressed group were higher than the ones for the Baseline-Unstressed group, approximately 45% higher in the Relax Session and 12% in the Stress Session. Results of STAI-t scale show significant differences between both groups, suggesting that the anxiety level in stressful situations, indeed increases more in the Baseline-Stressed than in the Baseline-Unstressed group. Additionally, in the Relax Session the Baseline-Stressed group did not reach the BS like the Baseline-Unstressed group did according to Total-s, SSS and STAI-s. Conversely, in the Stress Session, the results of the above-mentioned scale did not demonstrate statistically significant differences for either baseline group. This may be an indication that those participants, who had a slightly higher baseline stress level, did not relax as easy as those who had a lower baseline stress level.

Values of biochemical variables in both sessions are given in Table **6.4**. Values of Δcortisol, copeptin and prolactin were significantly different between the sessions. In contrast, glucose and Δα-amylase did not show significant differences, although median values of Δα-amylase show a small increase. This might be because alpha-amylase concentration has a faster dynamic [121] that has a peak at about 10–15 min after stressor onset, whereas the saliva sample was collected 25 minutes after the stressor onset. This limitation of the study design was previously discussed in Section **4.3**.

Table 6.4: Inter-subject mean and standard deviation (SD) of Biochemical variables.

Biochemical variables by Groups	n	Relax Session Mean (SD)	Stress Session Mean (SD)	T-test p-value
Δ α-amylase	38	30.60 (78.0)	50.06 (97.4)	0.287
Δ Cortisol	38	-0.08(0.09)	0.01 (0.15)	0.002*
Women	21	-0.06(0.07)	-001 (0.10)	0.089
Men	17	-0.10(0.11)	0.04 (0.19)	0.012*
Baseline-Unstressed	17	-0.06(0.10)	-0.04 (0.06)	0.343
Baseline-Stressed	21	-0.09(0.09)	0.05 (0.18)	0.003*
Copeptin	38	6.00(3.17)	7.60 (3.74)	0.001*
Women	21	4.56 (1.58) [+]	6.30 (3.35) [+]	0.012*
Men	17	7.86 (3.74) [+]	9.20 (3.66) [+]	0.050*
Baseline-Unstressed	17	5.64(3.52)	6.81 (3.75)	0.080
Baseline-Stressed	21	6.28(2.91)	8.24 (3.70)	0.007*
Prolactin	38	6.34(1.65)	8.78 (4.84)	0.003*
Women	21	6.23(1.65)	9.08 (4.84)	0.016*
Men	17	6.49(1.69)	8.41 (4.96)	0.096
Baseline-Unstressed	17	6.41(1.38)	9.06 (4.76)	0.028*
Baseline-Stressed	21	6.29(1.86)	8.55 (5.00)	0.059
Glucose	38	86.62(22.5)	88.29 (10.7)	0.666

T-student test between both session, *p-value<0.05 is considered to be significantly different. sessions change. [+] Groups that have significant differences in the same session (p<0.05).

Grouping the participants by gender, variations in Δcortisol between sessions were higher in men than in women; while in contrast, prolactin values were higher in women. However, variations of Δcortisol and prolactin values did not demonstrate differences that were statistically significant between gender groups. Conversely, copeptin values did show differences that were statistically significant between gender groups in both sessions; much more so in women than in men. On the other hand, grouping the participants by self-identified stress level, the Baseline-Stressed group had bigger differences in Δcortisol and copeptin. By contrast, the Baseline-Unstressed group reported bigger prolactin differences between sessions.

Table **6.5** shows rescaled values of the reference variables. Mean value variations in the biochemical variables were around 12.0 au, while variations in psychometric tests were higher than 20.0 ua. The VASS scale was the most sensitive, with changes of 27.87 au.

Table 6.5: Inter-subject mean and standard deviation (SD) of rescaled reference variables

Reference variables	n	Relax Session Mean (SD)	Stress Session Mean (SD)	Session changes Mean (SD)	p-value
PSS’	37	60.26(15.67)	57.75(18.46)	-1.56(19.29)	-
STAI-s’	37	30.34(18.13)	51.30(27.06)	20.79(25.46)	1.66e-05*
STAI-t’	37	47.80(22.23)	45.24(25.17)	-1.74(11.98)	-
SSS’	37	32.58(25.36)	51.58(26.15)	20.18(26.31)	4.16e-05*
VASS’	37	30.31(25.04)	58.08(27.88)	27.87(31.35)	4.32e-06*
Δ α-amylase’	37	30.45(16.38)	33.32(23.03)	3.28(25.81)	-
Δ cortisol’	38	26.96(12.44)	39.36(20.15)	12.40(23.85)	0.0027*
Copeptin’	37	33.78(23.18)	46.48(28.49)	12.09(20.83)	0.0011*
Prolactin’	37	12.16(7.65)	23.49(22.36)	10.42(19.98)	0.0030*
Glucose’	35	69.35(22.99)	62.92(23.93)	-6.56(28.15)	-

T-student test between both sessions, *p-value<0.05 is considered to be significantly different.

6.2. Stress Reference Scale

A multivariable approach was applied to stress reference variables to obtain a single scale that encompasses different reactions of the stress response.

Reference variables showing differences that were statistically significant between BS and SS states were used to elaborate the preliminary reference of stress levels, except for SSS scale which is used for the first time in this study. The contribution of each reference variable to this quantitative stress level scale was estimated based on a PCA analysis. As a result, the stress level scale has 50% of psychometric measurement components (25% STAI-s and 25% VASS) and 50% of biochemical measurements components (16% Δcortisol, 16% Copeptin and 18% Prolactin).

By evaluating equation (**4.2**) with the estimated coefficients of the reference variables, the stress level value ($S_{ref}(p,x)$) of participant p at state x could be calculated as follows:

$$S_{ref}(p,x) = 0.25 \cdot VASS'(p,x) + 0.25 \cdot STAIs'(p,x) + 0.16 \cdot \Delta Cortisol'(p,x) + 0.16 \cdot Copeptin'(p,x)' + 0.18 \cdot Prolactin'(p,x)' \quad (6.1)$$

The mean and standard deviation of stress scores values obtained in each session, grouped by participant's gender and self-identified baseline stress, are presented in Table **6.6**. Also, shown in the latter is the result of the comparison using t-student test between mean values from both sessions.

Table 6.6: Inter-subject mean and standard deviation (SD) of stress reference results.

Stress Reference by Groups	n	Relax Session Mean (SD)	Stress Session Mean (SD)	Changes between Sessions Mean (SD)	Changes between Sessions p-value
All	36	27.13(10.69)	45.29(12.93)	17.61(13.65)	4.34e-09*
Women	20	25.83(9.90)	45.50(13.04)	18.18(12.60)	3.46e-06*
Men	16	28.65(11.67)	45.00(13.20)	16.90(15.26)	4.86 e-04*
Baseline-Unstressed	17	21.69(6.44)$^{+}$	40.89(12.31)	19.19(13.32)	2.07e-05*
Baseline-Stressed	19	31.75(11.52)$^{+}$	49.02(12.53)	16.20(14.15)	9.48e-05*

T-student test between both session, * p-value<0.05 is consider significantly different. + Groups that have significant differences in the same session (p<0. 003).

Much like previous results of psychometric tests and biochemical variables, the variations of the stress reference scale among both sessions were statistically significantly different, increasing approximately 66.94% (p-value = 4.34 e-09) from the basal state value. Also, the only differences observed, were those in the Relax Session between Baseline-Unstressed and Baseline-Stressed groups.

Participants who had a slightly higher baseline stress level (Baseline-Stressed group) proved to have higher S_{ref} mean values in both sessions than those who were Baseline-Unstressed. In a similar comparison, the mean changes between sessions for the Baseline-Stressed participants was smaller (16.20au.) than the mean changes for their Baseline-Unstressed counterparts (19.19au.). This also suggests that participants from the Baseline-Stressed group did not relax as much as the ones from the Baseline-Unstressed group. On the other hand, they added to their stress more than Baseline-Unstressed participants.

Figure **6.1** shows S_{ref} values for each participant at both Basal and Stress States, and their differences. The variety of stress levels among participants under the same experimental conditions can be observed, suggesting inter-individuals differences before the same stressor.

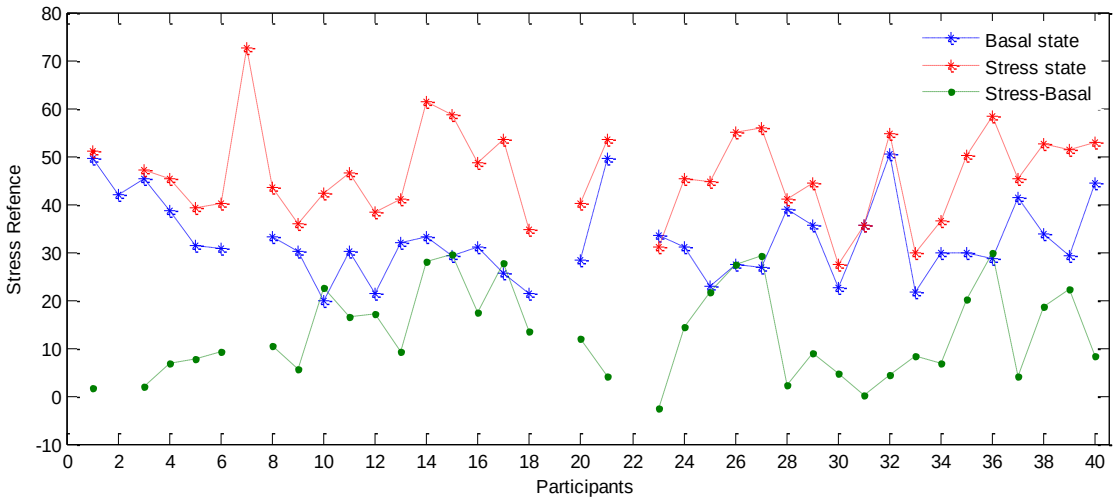

Figure 6.1: Values of S_{ref} at the Basal and the Stress States of each participant.

6.3. Features from Physiological Signals

A set of features related to stress were obtained from ECG, face and finger skin temperatures, and PPG signals as preliminary stress biomarkers. The median and median absolute deviation (MAD) values of the features at the different stages of the experiment are shown in Table **6.7**, which characterize the individuals' states at each stage of the experiment. It can be appreciated how most of the features change from one task to another, and they have significant changes at MT and AT with respect to the other stages. An example of finger and face temperature and respiration signals obtained during both session is show in Figure **6.2**.

A Friedman test (Repeated Measurements Analysis of Variance on Ranks, non-parametric test) was performed for each feature to know whether there was any change in the different stages of the values (a p-value < 0.05 was considered statistically significant, meaning that there are at least two different median stage values). As a result of Friedman test, each of the features has at least one difference per pair compared with their stage values (p-value < 0.05), therefore all of them changed in at least one stage of the experiment.

The statistical differences between the feature values of two different stages were checked using the Wilcoxon Signed Rank Test (non-parametric test), since this data is considered non-normally distributed. Pair-wise comparisons were made between feature values at RS and at each one of the different stages at the Stress Session. Those features whose median at each stage were not significantly different with its median at RS are highlighted in Table **6.7**.

Table 6.7: Inter-subject median and median absolute deviation (MAD) of physiology extracted features

Features	Relax Session		Stress Session					
				Trier Social Stress Test modify				
	Baseline BL_R	Relax RS (BS)	Baseline BL_S	ST	MT	SA	VD	AT (SS)
Temperature								
ΔT_{fi} (°C)	0.15(0.14)	-0.03(0.05)	0.20(0.12)	-0.97(0.41)	-0.86(0.32)	0.06(0.31)	-0.35(0.22)	-0.36(0.24)
$T_{fi_{Ptotal}}$(10^{-3} °C^2.s)	1.229(0.084)	1.255(0.1)	1.226(0.11)	1.16(0.086)	1.049(0.101)	0.926(0.15)	0.94(0.19)	0.775(0.191)
ΔT_{fa} (°C)	0.01(0.02)^	0.01(0.01)	0.05(0.03)	0.02(0.06)	0.08(0.05)	0.01(0.01)	0.13(0.06)	0.07(0.04)
$T_{fa_{Ptotal}}$(10^{-3} °C^2.s)	1.191(0.057)	1.198(0.065)	1.163(0.053)	1.178(0.042)	1.186(0.04)	1.183(0.045)	1.193(0.038)	1.238(0.04)
T_{Ratio}	1.00(0.05)	1.02(0.03)	1.04(0.05)	1.00(0.03)	0.94(0.05)	0.89(0.07)	0.89(0.10)	0.80(0.07)
Heart Rate								
HR_{mean} (bpm)	72.39(6.10)	70.66(6.88)	70.25(7.02)	81.03(8.87)	82.87(8.57)	69.00(7.68)	71.05(9.02)	86.86(11.25)
$RR_{SDNN}(s)$	0.06(0.02)	0.07(0.02)	0.07(0.02)	0.08(0.02)	0.10(0.03)	0.08(0.02)	0.08(0.03)	0.10(0.03)
$RR_{rMSSD}(s)$	0.83(0.08)	0.86(0.09)	0.86(0.09)	0.75(0.08)	0.75(0.08)	0.88(0.10)	0.85(0.10)	0.71(0.08)
HR_{PVLF} (s^{-2})	1.85(0.33)	1.77(0.32)	1.78(0.36)	2.39(0.48)	2.43(0.44)	1.72(0.34)	1.90(0.44)	2.65(0.64)
HR_{PLF} (10^{-3} s^{-2})	1.73(0.88)^	1.28(0.71)	1.17(0.62)	1.39(0.38)	2.15(0.71)	1.24(0.54)	1.12(0.45)	2.41(0.85)
HR_{PHF} (10^{-3} s^{-2})	1.59(0.77)	1.48(0.67)	1.45(0.67)	1.64(0.78)	2.33(0.96)	1.53(0.81)	1.7(0.66)	2.65(1.07)
$HR_{LF/HF}$ (10^{2})	1.24(0.77)^	0.93(0.49)	0.64(0.32)	0.94(0.28)	1.03(0.30)	0.93(0.26)	0.61(0.18)	0.88(0.23)
Pulse Rate								
PR_{mean} (bpm)	72.38(6.30)	71.33(6.96)	70.26(6.87)	80.44(8.50)	83.63(7.33)	68.88(6.93)	71.86(7.92)	85.44(10.12)
$PP_{SDNN}(s)$	0.06(0.02)	0.07(0.02)	0.06(0.02)	0.09(0.02)	0.10(0.03)	0.08(0.02)	0.09(0.03)	0.10(0.02)
$PP_{rMSSD}(s)$	0.83(0.08)	0.86(0.09)	0.86(0.08)	0.76(0.08)	0.74(0.08)	0.88(0.10)	0.85(0.09)	0.72(0.07)
PR_{PVLF} (s^{-2})	1.69(0.26)	1.64(0.35)	1.55(0.32)	2.19(0.44)	2.15(0.39)	1.50(0.32)	1.64(0.47)	2.44(0.62)
PR_{PLF} (10^{-3} s^{-2})	1.67(0.86)^	1.34(0.81)	1.08(0.49)	1.32(0.45)	2.35(0.91)	1.48(0.62)	1.15(0.45)	2.93(1.47)

Table 6.7: Inter-subject median and median absolute deviation (MAD) of physiology extracted features (Cont.)

Features	Relax Session			Stress Session				
				Trier Social Stress Test modify				
	Baseline BL_R	Relax RS (BS)	Baseline BL_S	ST	MT	SA	VD	AT (SS)
Pulse Rate (cont)								
PR_{PHF} *(10^{-3} s^{-2})*	1.93(0.64)	1.45(0.65)	1.69(0.75)	1.78(0.76)	3.08(0.99)	1.73(0.56)	2.33(0.91)	3.49(1.35)
$PR_{LF/HF}$*(10^2)*	1.13(0.74)^	0.82(0.42)	0.62(0.31)	0.77(0.30)	0.80(0.21)	0.68(0.27)	0.49(0.14)	0.90(0.26)
Pulse Wave Rising Time Pulse								
PRT_{mean} *(ms)*	140.43(7.52)	140.46(7.76)	148.74(11.33)	137.74(9.29)	155.34(30.66)	146.97(10.96)	155.57(22.69)	141.74(17.98)
PRT_{STD} *(ms)*	10.93(5.95)	12.65(5.3)	14.93(8.95)	28.82(16.01)	46.36(19.55)	45.94(11.95)	38.29(15.59)	46.89(13)
Pulse Wave Decreasing Time								
PDT_{mean} *(ms)*	696.95(77.43)	700.63(70.39)	703.5(74.43)	596.52(85.71)	577(57.02)	689.87(90.02)	677.71(86.99)	542.42(72.92)
PDT_{STD} *(ms)*	74.11(17.79)	82.95(21.85)	75.13(18.15)	91.68(18.77)	113.42(22.66)	127.92(23.8)	77.74(20.36)	105.74(19.11)
Pulse Width until Reflected Wave								
PWr_{mean}*(ms)*	306.92(12.19)^	305.94(16.8)	315.67(13.27)	317.11(11.71)	327.42(25.25)	305.14(18.32)	316.46(24.03)	311.08(24.09)
PWr_{STD} *(ms)*	18.62(7.29)	17.61(9.5)	20.74(7.93)	35.86(14.18)	53.36(17.4)	59.46(16.52)	42.46(13.59)	52.72(9.7)
Pulse Transit Time								
PTT_{mean}*(ms)*	201.38(13.62)	205.06(15.61)	203.82(13.95)	188.16(15.92)	175.07(13.77)	189.37(11.86)	181.26(17.96)	162.57(18.44)
PTT_{STD} *(ms)*	10.34(4.02)	11.99(5.24)	10.2(2.95)	16.53(7.24)	21.98(10.31)	37.65(8.16)	16.93(7.26)	24.37(7.93)

Inter-subject medians and MAD were obtained in a 3 to5 minute time range depending on the task time. In the case of ST, MT, VD, and AT the whole time interval was taken. For BL_R, BL_S and SA 5-minute range was selected (BL: from minute 3 to 8, SA: from minute 2.5 to 7.5).

^ Marked parameters which have significant difference between both baseline stages

Features that have significant differences between RS values and each stress stages values ($p<0.05$) are highlighted in gray.

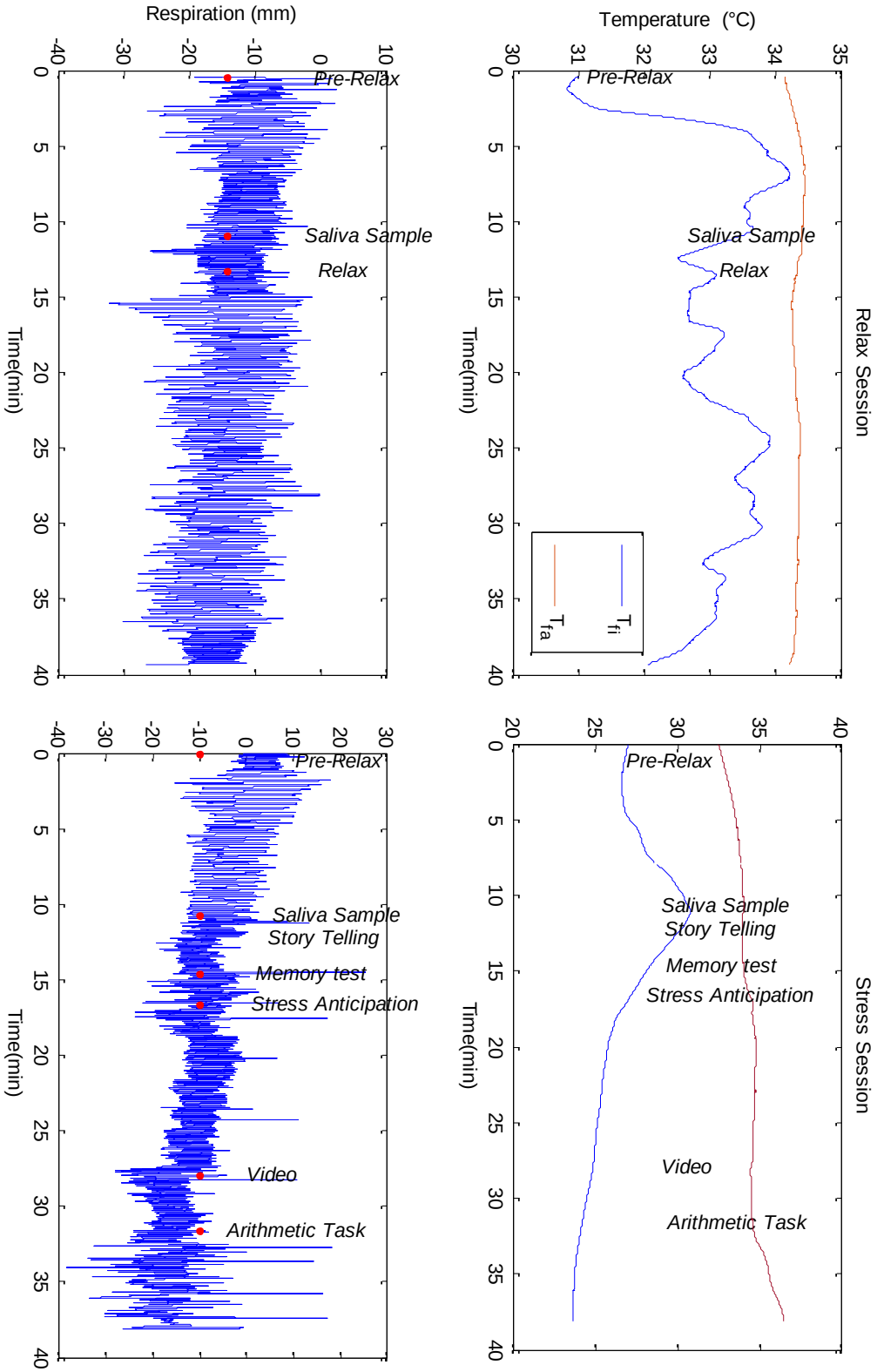

Figure 6.2: Temperature and respiration signals of a participant.

At the start/onset of each session, participants were guided through a relaxation exercise as an effort to obtain a similar baseline state for all participants. Hence, at Baseline stages (BL_R and BL_S) the feature values should not have significant differences between them, and may instead reflect any other difference that is not related to stress. Therefore, features showing statistically different values in both baseline stages were not taken into consideration as stress markers in this experiment. These discarded features from the Baseline stages were $\Delta T_{fa}, HR_{PLF}$, $HR_{LF/HF}$ and PWr_{mean}. Likewise, at stages RS and AT, considered basal stress and highest stress levels respectively, the features should not vary significantly. Accordingly, those features whose medians were not significantly different between stages RS and AT (i.e. $T_{fa_{Ptotal}}$, $HR_{LF/HF}$, $PR_{LF/HF}, PWr_{mean}$, PWr_{mean}), were not taken into consideration as stress markers in this experiment

Notwithstanding the above, those features that were not taken into consideration as stress markers in this experiment do have variations throughout the Stress Session, see Figure **6.3**. Thus, in other experimental or real conditions, their potentials as stress markers could be assessed.

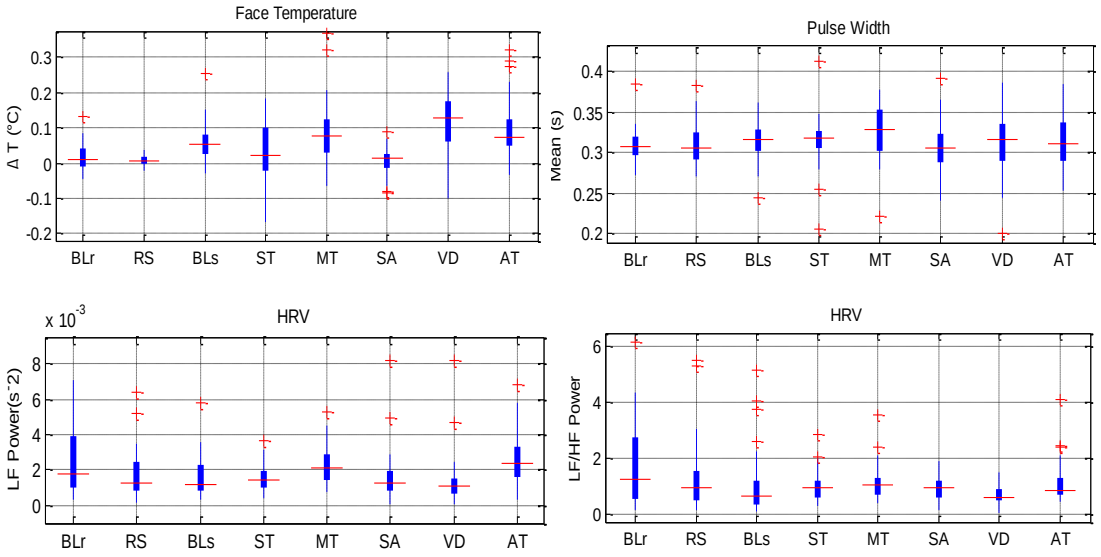

Figure 6.3: The median, 25th and 75th percentiles of ΔT_{fa}, HR_{PLF}, $HR_{LF/HF}$ and PWr_{mean} features during the different session stages

Results for median values of HR_{mean} and PR_{mean}, in the BS were around 70 bpm, while in a demanding stressful task such as ST or MT, they were around 80 bpm. Similarly, for the SS, at the end of AT, or in other words the most stressful stage, they were around 86 bpm. Such results were related not only with the HR feature values but also with the PR time features, as Figure **6.4**. shows.

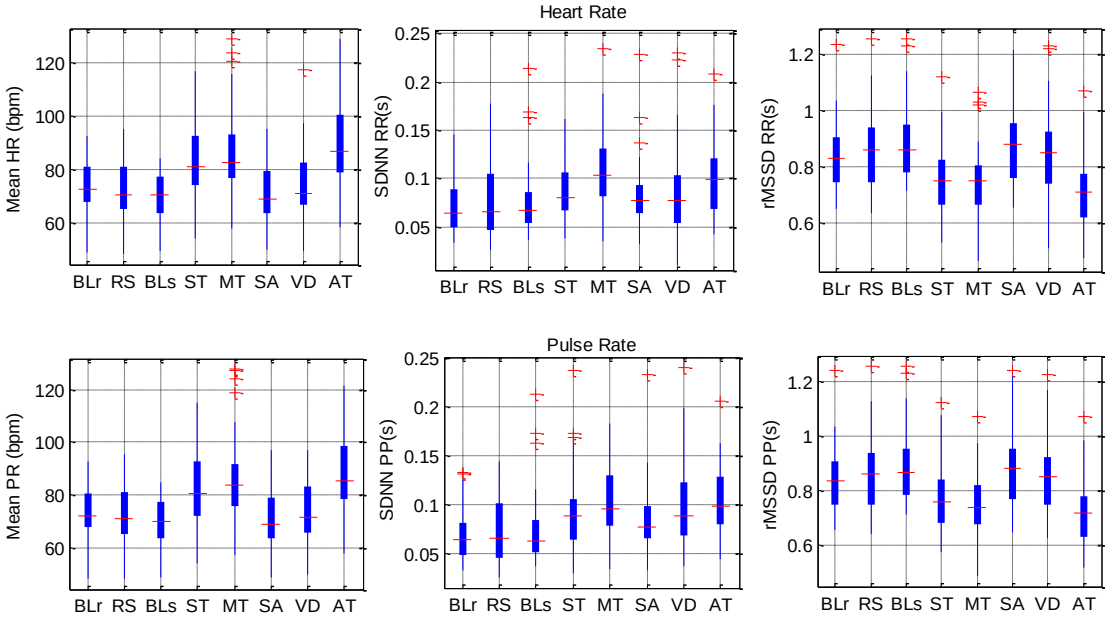

Figure 6.4: Median and 25th, 75th percentiles of HR and PR time features during the different sessions stages.

However, LF and HF power values of PRV and HRV did not have similar values as expected according to previous studies [122,123], this mainly occurs in the Stress Session (see Figure **6.5**). In MT, VD and AT stages, values of PR_{PHF} were around 30% higher than values of HR_{PHF}, while values of PR_{PLF} were around 20% higher than HR_{PLF} values in AS and AT stages.

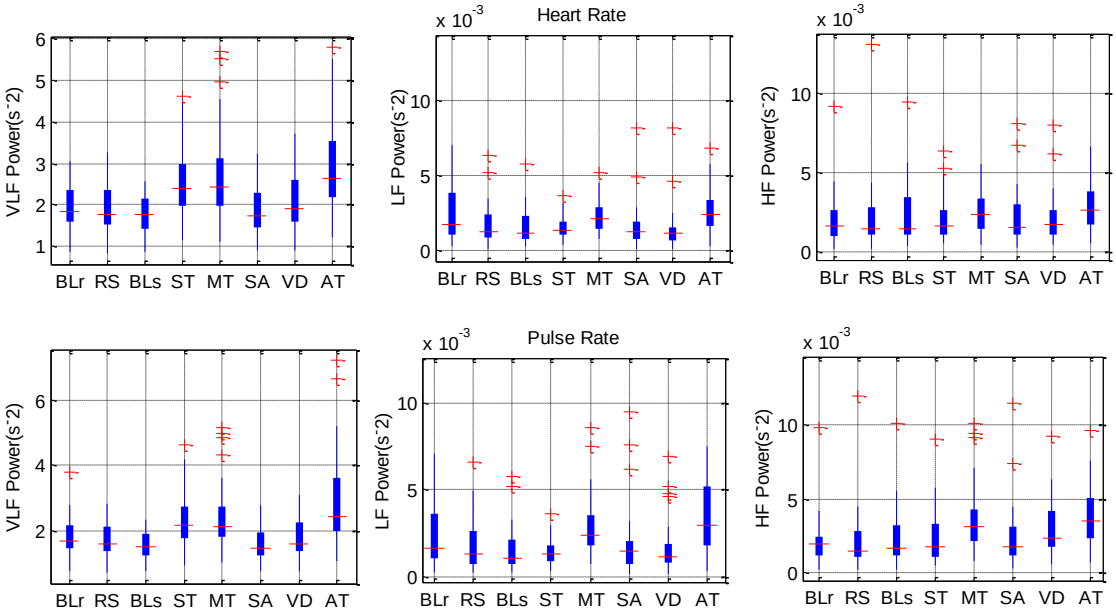

Figure 6.5: The median and 25th and 75th percentiles of HR and PR frequency features during the different sessions stages

These differences were re-examined by visual inspection of the peak-detection algorithm and the time-frequency spectrum of participants. An example of the series obtained in each step comparing the HRV and PRV analysis is provided in Figure **6.6**.

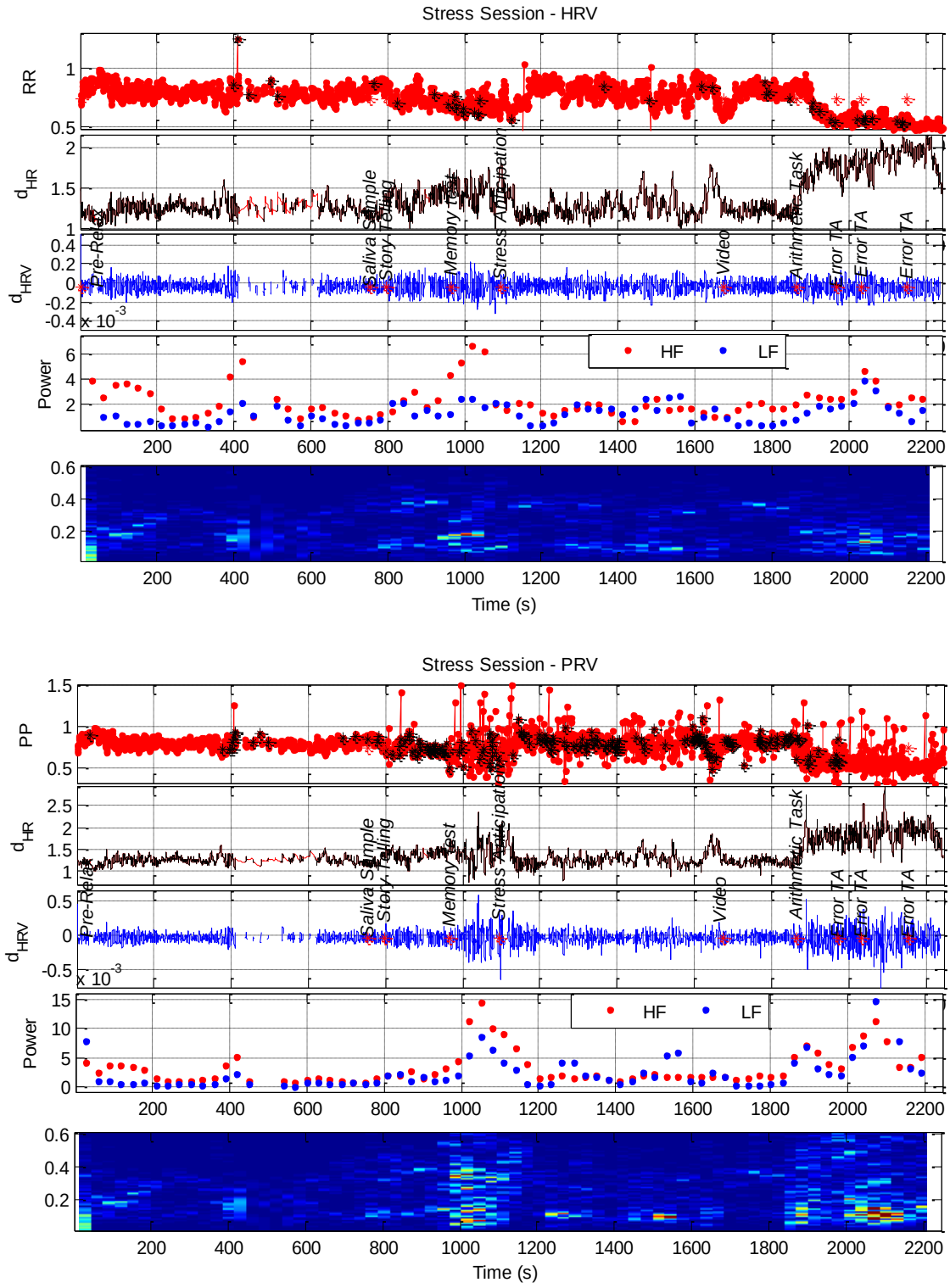

Figure 6.6: HRV and PRV inspection during the Stress Session.

In almost 100% of the cases in which such differences appear, there were also artefacts in PPG signals due to hand-movements and talking. Therefore, differences between PLF and PHF features of PF and HR were caused by using different types of sensors and also different sample frequency for ECG and PPG signals, which were 1kHz and 250 Hz respectively.

An example of HR signal during the Stress Session and a time-frequency spectrum can be found in Figure **6.7**. Said spectrum was obtained from the concatenation of the spectra computed from non-overlapping 30-second windows There are higher variations at MT and AT stages as same as it is shown in the previous Figure **6.6**. Arithmetic Task was the highest stressing moment of the session because of the nature of the task itself and due to the fact that it was done at the end of the 25-minutes stress test period.

However, in the given values in Table **6.7**, there was no significant change in HR_{PHF} for the AT and ST (at both stages participants were talking) with respect to a baseline level contrary to reported in [124]. In this study [124] respiration information was used to ensure that respiratory rate was within the HF range. Herein, it is used standard frequency bands for LF and HF and it may happen that during the relaxed stages respiratory frequency is within the LF band, so the HF band is not measuring respiratory sinus arrhythmia but rather just "noise". During the stressful stages, respiratory frequency increases (e.g see Figure **6.2**) and fall within the HF band, thus increasing the power in the HF band. However, this cannot be interpreted as an increase in parasympathetic activity.

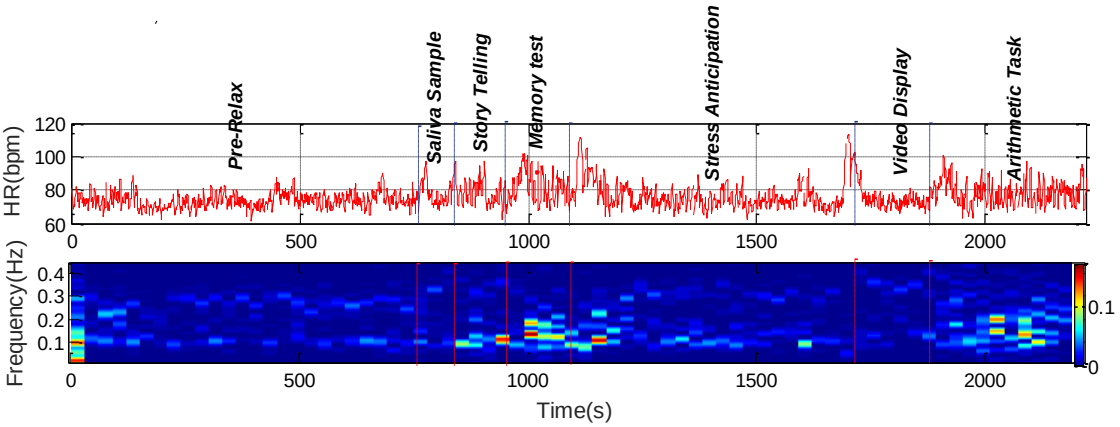

Figure 6.7: Example of HR signal and its time-frequency spectrum during the Stress Session.

6.4. Stress Measurement from Physiological Signals

The following analysis was focused on selecting a set of physiological features and a model able to give similar measures of $S_{ref}(p, x)$ at the two identified states. Furthermore, the selected model was used to estimate the stress level along the different stage sessions.

6.4.1. Stress Biomarkers Selection

The most relevant features from the temperature, ECG and PPG signals that show variations due to the triggered stress response, according to the experimental conditions, were selected as stress markers from the whole set.

So far, for each of participant, two sets of reference variable values were obtained: one at the BS (minimum stress level) and the other one at the SS (showing the stress level reached after the complete TSST). Using these values, linear Pearson correlations between physiological features and stress reference variables were computed. The resulting correlation coefficients can be found in Table **6.8**. As can be noticed a linear dependency between several physiological features and S_{ref} was proven. However, other types of relationships should be studied in future analyses in order to include all possible physiological features suitable to become stress biomarkers.

Table 6.8: Pearson correlation coefficient between physiological features and stress reference variables

	Features	STAI-s	VASS	SSS	Δcortisol	Copeptin	Prolactin	S_{ref}
T_{fi}	ΔT_{fi}	-	-		-	-0.34	-	-
	$T_{fi_{Ptotal}}$	-0.46*	-0.57**	-0.42*	-0.35	-	-0.27	-0.59**
T_{fa}	ΔT^{Fa}	0.34	0.40*	0.29	0.49**	-	0.38	0.46**
	$T_{fa_{Ptotal}}$	0.34	-		0.26	-	-	0.25
	T_{ratio}	-0.51**	-0.60**	-0.45**	-0.39	-	-0.26	-0.62**
ECG	HR_{mean}	0.43*	0.47**	0.37	0.45*	-	0.45*	0.59**
	RR_{SDNN}	-	-	-	-	0.28	-	-
	RR_{rMSSD}	-0.39	-0.43*	-0.36	-0.32	-	-0.41*	-0.51**
	HR_{PVLF}	0.43*	0.47**	0.37	0.50**	-	0.48**	0.59**
PPG	PR_{mean}	0.34	0.36	0.37	0.35	-	0.42*	0.49**
	PP_{SDNN}	-	-	0.35	-	0.28	-	0.32
	PP_{rMSSD}	-0.38	-0.42*	-	-0.29	-	-0.39	-0.49**
	PR_{PVLF}	0.39**	0.39	0.39*	0.42**	-	0.36**	0.51
	PRT_{STD}	-0.42*	-0.46*	-0.41*	-0.29	-	-0.37	-0.53**
	PDT_{mean}	-	-		-	-	-	0.25
	PDT_{STD}	0.29	0.34	0.25	-	-	-	0.41*
	PWr_{STD}	0.28	0.36		-	-	-	0.40*
	PTT_{mean}	-0.47**	-0.51**	-0.43*	-0.41*	-	-0.36	-0.63**
	PTT_{STD}	0.31	0.35	0.26	0.39	-	-	0.44*

* p-value < 0.001. **p-value <0.0001

The PTT_{mean} has the highest Pearson correlation value with a stress reference level (-0.63), and it is also highly sensitive to the different states induced along the experiment. HR time features show a higher correlation with the stress reference scale as well as PR time features.

A subset of features was selected using the following criteria: (1) to have no significant statistical differences between BL_R and BL_S stage values, since equivalent states at the end of both baseline stages were assumed; and (2) to have a Pearson correlation coefficient with the stress reference level higher than 0.4, to ensure that each selected feature and the stress level reference shared at least a 15% of common values (whose coefficient of determination had to be at least 0.15).

Furthermore, in a second step aiming to avoid linear dependencies and redundancies among features in these particular experimental conditions, Pearson correlations between them were computed at each stage. The condition considered for group-related features was to have a correlation coefficient higher than ±0.98 in each one of the stages. One feature was selected per group of related features. Thereby, the set of features given in Table **6.8** was analysed to discern those features with linear dependency throughout this experiment. The following three groups were found: (1) $T_{fi_{Ptotal}}$; T_{Ratio} (2) HR_{mean}; HR_{rMSSD}; HR_{PVLF}; PP_{rMSSD}; PDT_{mean} and (3) PP_{SDNN}; PDT_{STD}. The feature that had the highest correlation value with the stress reference level was selected from each group, namely T_{Ratio}, HR_{mean} and PP_{SDNN}.

Consistently with previously established and already explained premises, the set of features selected only includes features that correlate more than 0.4 with the stress reference, S_{ref} (r>0.4), thus assuring its suitability to be part of the multivariable stress model proposed in this research. The selected features were: T_{Ratio}, HR_{mean}, PRT_{STD}, PTT_{mean}, PTT_{STD} and PWr_{STD}.

6.4.2. Stress Estimation

So far, a stress reference level value was identified for each participant at the BS and the SS state, and also, there were identify those suited features derived from physiological signals usable to estimate the values of the stress reference level, S_{ref}. Therefore, as the physiological signals were continuously measured, the feature changes related to the stress response level were also continuously assessed. Figure **6.8** shows PTT_{mean} and PTT_{STD} values every one minute during the Relax and the Stress Session.

Also in this case, a linear relationship between this subset of features and S_{ref} data was assumed. Feature values of RS and AT stages were associated to the BS and SS states respectively. The coefficients of this linear function were computed using a linear regression

model between S_{ref} values and the values of the selected features at the BS and SS states corresponding to the RS and AT stages. This is an alternative pathway to estimate the stress reference level using physiological signals.

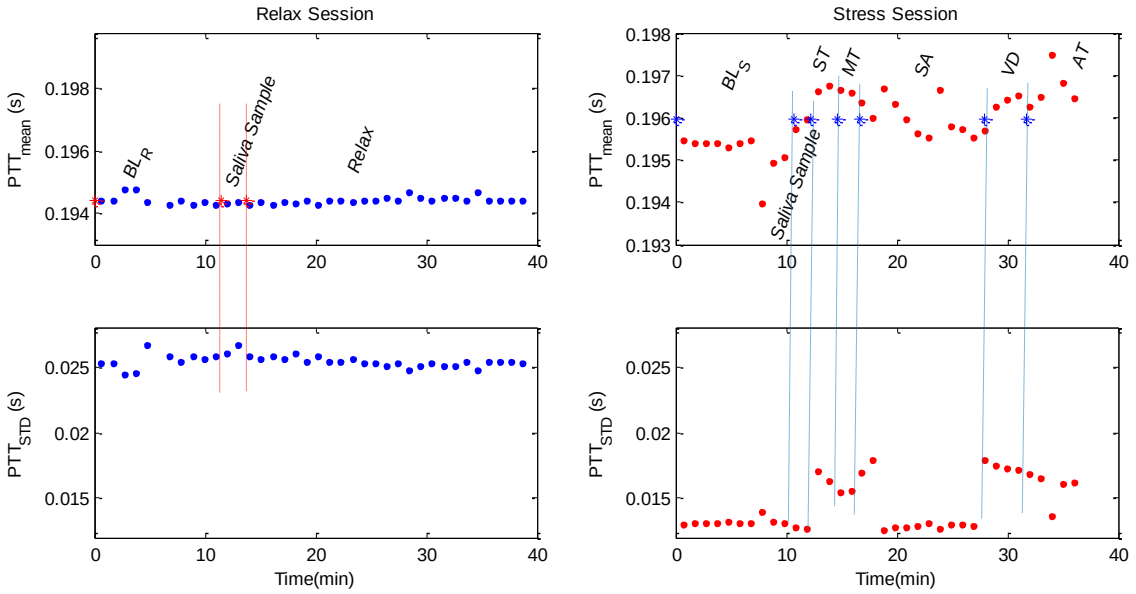

Figure 6.8: An example of PTT_{mean} and PTT_{STD} values per minute during the Relax and the Stress Session.

A preliminary and simple stress estimation through a linear function (S_{est}), see (**6.2**), was performed assuming a linear relation between selected features and the stress level (see Table **6.8**.). The coefficients of the S_{est} function were obtained by linear regression using the data of S_{ref} and the selected features at the BS and SS states. The linear regression RMS error was 9.57 (R-squared 0.67).

$$S_{est}(t) = -40.9 \cdot T_{Ratio}(t) + 0.25 \cdot HR_{mean}(t) + 171.1 \cdot PRT_{STD}(t) - 145.4 \cdot PTT_{mean}(t) - 79.83 \cdot PTT_{STD} - 94.1\, PWr_{STD}(t) + 80.51 \quad (6.2)$$

The obtained values of S_{est} compared to S_{ref} at the BS and SS states for each participant are shown in Figure **6.9**. Two well-defined groups for the basal and the stress states can be observed. The S_{est} values had an increase of 64.13% from the BS to the SS generated states whilst the same result through the S_{ref} values was 66.94%. Accordingly, differences between the BS and the SS were in the range of 18% that, as expected, represents a moderate but significant stress effect. At the BS the values show less dispersion than those at the SS, because a stressor affects individuals differently while the relaxation tends to induce similar basal states in individuals.

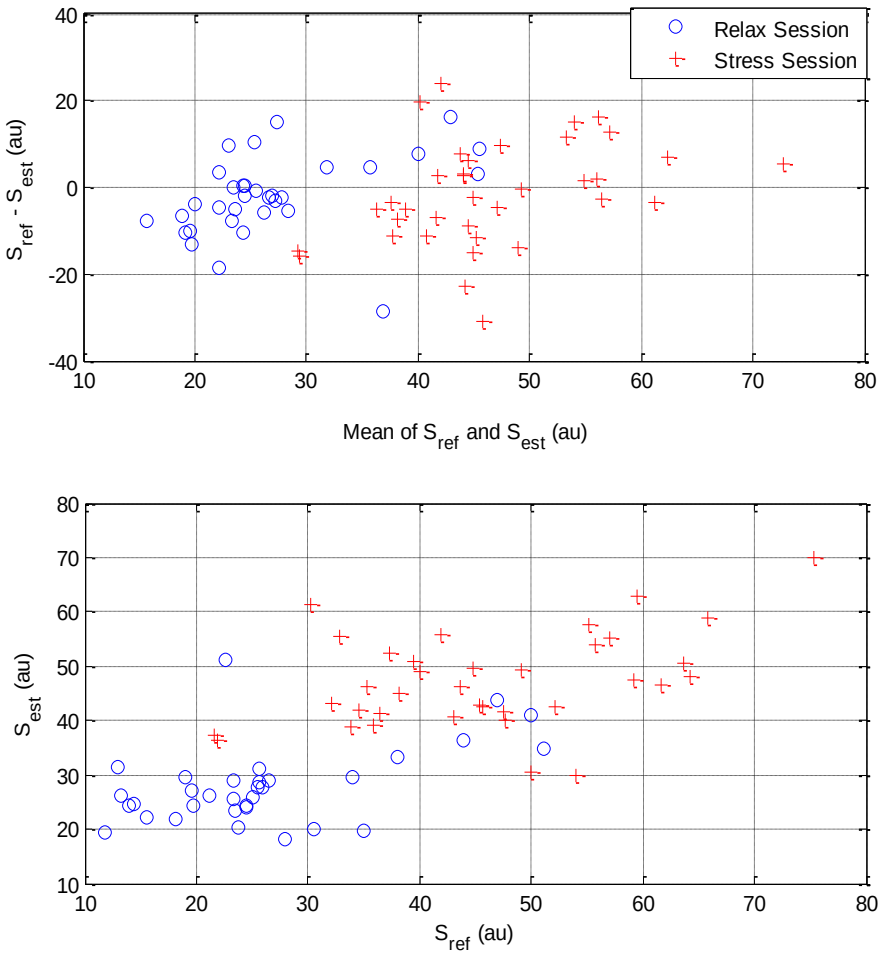

Figure 6.9: The stress level obtained from the reference variables versus the stress level estimated through selected features.

In the Stress Session, participants were subjected to different stimuli at each stage (ST, MT, SA, VD and AT); as a result, the participants' states at each stage had dissimilar feature values. Through the linear equation obtained (**6.2**), S_{est} was calculated for each stage of the experiment, and its inter-participant median are shown in Figure **6.10**.

The inter-participant means of S_{est} are shown in Table **6.9**. The stress level reached in the ST and SA task had similar means although physiological stress markers did not report similar medians. This indicates that similar stress levels could clearly be assigned to different states that may have similar homeostatic imbalance level. Each stage had different demanding or elicited emotional conditions so different emotional/arousal responses were expected. The stages where a relaxation exercise was done showed S_{est} values of around 29.06 au, while S_{est} values rose from 34.58 au. to 47.55 au in the Stress Session.

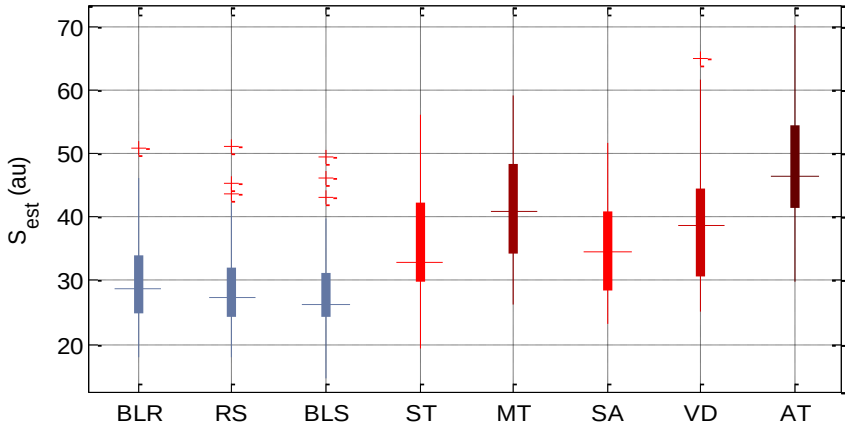

Figure 6.10: The median and 25th, 75th percentiles of the estimated stress throughout both sessions

Table 6.9: Inter-subject mean and standard deviation (SD) of the estimated stress.

	Relax Session		**Stress Session**					
	BL_B	**RS**	**BL_S**	**ST**	**MT**	**SA**	**VD**	**AT**
All	29.03 (8.15)	26.82 (8.47)	25.37 (8.17)	34.00 (8.76)	39.86 (8.72)	34.52 (8.17)	38.53 (9.04)	47.19 (9.31)
Women	29.74 (8.04)	27.53 (8.91)	25.16 (8.63)	34.02 (8.81)	39.86 (7.42)	35.02 (8.66)	38.53 (9.33)	47.71 (8.79)
Men	25.95 (8.18)	24.79 (7.49)	25.47 (7.75)	32.77 (8.94)	39.36 (10.30)	28.53 (6.53)	38.55 (8.89)	46.71 (9.97)
Baseline-Unstressed	28.89 (8.58)	24.79 (7.80)	24.40 (8.42)	32.45 (8.72)	37.94 (7.99)	30.17 (8.24)	36.20 (8.67)	43.57 (7.67)
Baseline-Stressed	29.82 (8.02)	29.25 (8.53)	26.56 (7.77)	36.18 (8.54)	40.87 (9.16)	35.24 (7.95)	40.04 (9.29)	48.86 (10.44)

As physiological signals were continuously recorded, all selected features could be computed over a one-minute window along the sessions. Then using the S_{est} function, a continuous stress estimation was performed every minute for each participant. The S_{est} values from two different participants throughout both sessions can be seen in Figure **6.11**.

Different stress responses from the participants were selected and they are shown in Figure **6.12** to illustrate the diversity of stress responses generated throughout the Stress Session. It can be observed in Figure **6.12** that some participants had higher stress levels than others at the beginning of the session. Some of them greatly reduced their values in BL_S while others remained quite similar.

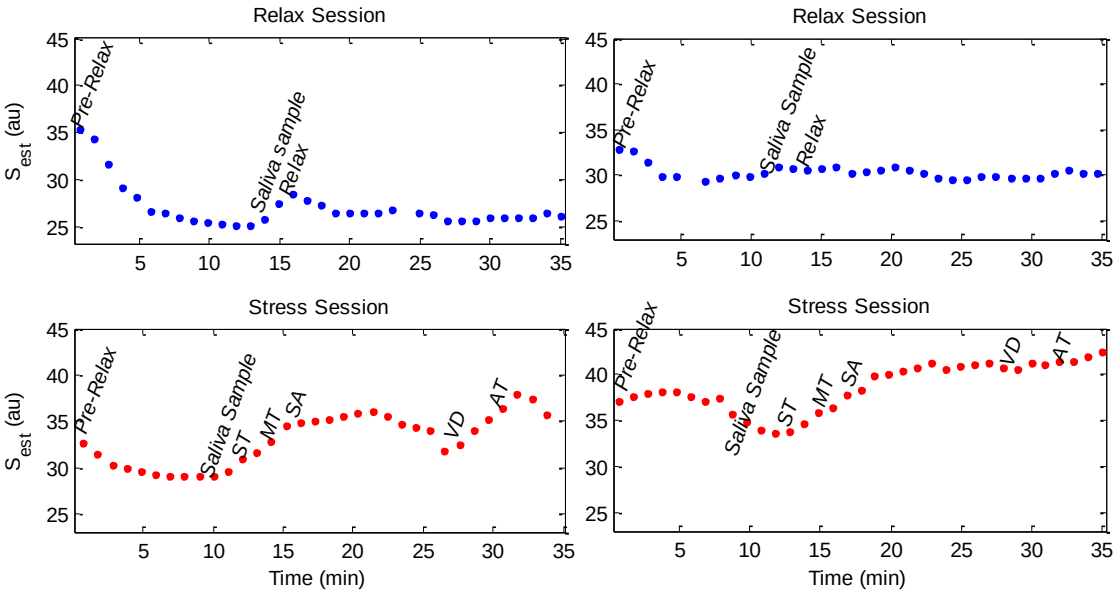

Figure 6.11: Estimated stress from two participants throughout both sessions.

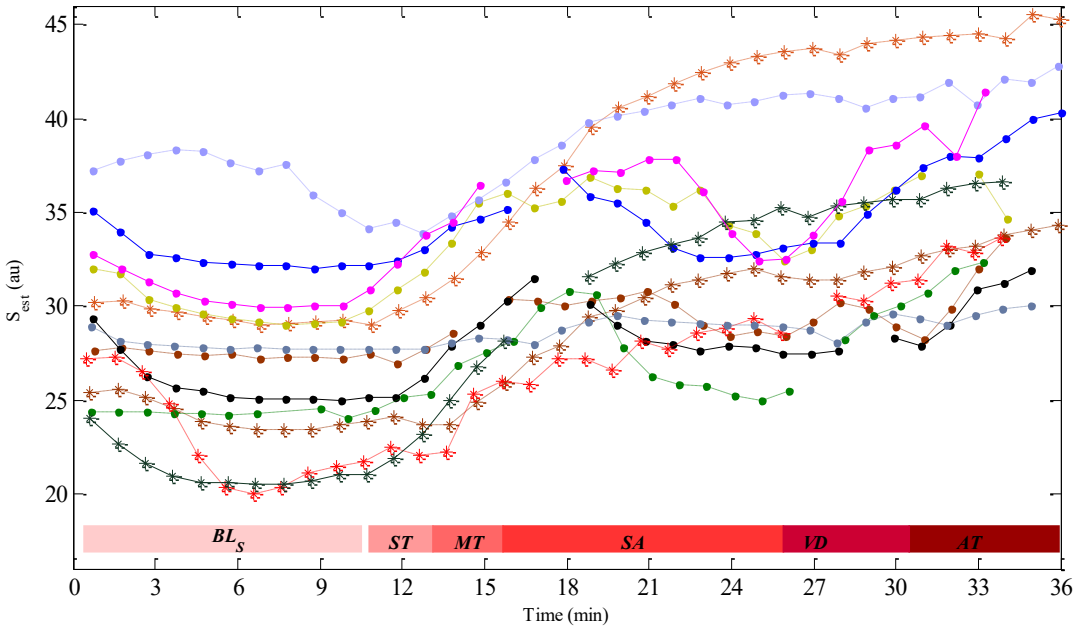

Figure 6.12: Estimated stress throughout the Stress Session

6.4.3. Performance Evaluation

The performance of the method is assessed in terms of stress estimation absolute error (e_A) and relative error (e_R) at the BS and the SS (at the end of both sessions) for each nth participant (see equations (**6.3**) and (**6.4**)). Furthermore, it was analysed the capabilities of this approach to discriminate the different states reached at each stage of the TSST.

$$e_A{}^{SB,SS}(n) = S_{ref}{}^{SB,SS}(n) - S_{est}{}^{SB,SS}(n) \quad (6.3)$$

$$e_R{}^{SB,SS}(n) = \frac{e_A{}^{SB,SS}(n)}{S_{ref}{}^{SB,SS}(n)} \times 100 \quad (6.4)$$

The mean of $e_A(n)$, $e_R(n)$ and its standard deviation were computed for every participant, and the intra-participant mean was calculated ($E_A{}^{SB,SS}$, $E_R{}^{SB,SS}$), which can be seen in Table **6.10.**

Table 6.10: Stress estimation absolute error (E_A), relative error (E_R) at the BS and the SS.

Basal State		**Stress State**	
$\boldsymbol{E_A \pm STD}$	$\boldsymbol{E_R \pm STD}$	$\boldsymbol{E_A \pm STD}$	$\boldsymbol{E_R \pm STD}$
1.92±8.97	19.17±44.00	1.45±12.02	10.12±33.24

Table **6.11** shows the p-values of the paired T- test between estimated stress values for each stage. S_{est} at each of the stages related to the TSST are statistically different to the S_{est} measured at relaxation stages (BL_R, RS and BL_S). These results suggest there were induced different levels of stress. Therefore, this proves the capacity of this quantitative approach to differentiate stress states in a short 25-minute period.

Table 6.11: P-values of the paired T-test among stress measured for each stage

	Relaxed states		**Stressed states**				
Stages	**RS**	**BL_S**	**ST**	**MT**	**SA**	**VD**	**AT**
BL_R	0.125	0.114	0.006	$2.19 \cdot 10^{-5}$	0.012	$8.20 \cdot 10^{-5}$	$5.04 \cdot 10^{-9}$
RS		0.538	$3.17 \cdot 10^{-5}$	$1.51 \cdot 10^{-7}$	$1.82 \cdot 10^{-4}$	$1.98 \cdot 10^{-7}$	$4.17 \cdot 10^{-12}$
BL_S			$6.14 \cdot 10^{-10}$	$2.17 \cdot 10^{-14}$	$1.03 \cdot 10^{-7}$	$5.86 \cdot 10^{-13}$	$1.87 \cdot 10^{-17}$
ST				$4.54 \cdot 10^{-10}$	0.844	$1.57 \cdot 10^{-4}$	$3.50 \cdot 10^{-14}$
MT					$2.22 \cdot 10^{-7}$	0.030	$1.25 \cdot 10^{-8}$
SA						$2.57 \cdot 10^{-7}$	$5.76 \cdot 10^{-17}$
VD							$1.67 \cdot 10^{-14}$

p-values < 0.05 are highlighted in grey

6.5. Discussion

Throughout two sessions (The Relax and the Stress Sessions) a set of stress-related biomarkers was measured, which were compared with established stress markers. At the end of each session, samples of some biochemical variables were extracted and psychometric tests were

administered providing evidence of the two different stress levels of induced states, BS and SS, in agreement with previous studies [25,77].

Until now, the stress response level has not been quantitatively assessed. To our knowledge, there is no objective, reliable, repeatable and easily applicable method to compare either the values of the stress response level of an individual at different stressful moments or to compare the stress state of two different individuals for a fast and reliable follow-up. Current methods cannot define thresholds and have not provided quantitative and reliable estimations of the global stressing effects of an alleged stressor on a population sample and estimations of the impact that a singular decision could have on stress (for more information on reviews [26,47,48]) .

A method to quantify psychologically-induced acute stress is proposed based on the measurement of noninvasive physiological signals. Particularly, a novel preliminary approach is proposed to estimate stress using a multivariable biomarker constituted by features extracted from physiological signals. As far as it is known, this is the first attempt to continuously quantify the stress response from physiological signals.

Each participant's stress response in the basal state and in the acute stress state were assessed using some stress reference variables selected from a set of well-established stress markers. Those variables were then combined in a multivariable scale, which was used as a stress reference level. Finally, suitable features derived from the physiological signals were selected and compared with the stress reference level. Afterwards, those features were used to make a preliminary estimation of the stress level.

The scale of stress level reference shows higher correlations with the physiologic features than with either the psychometric variables or the biochemical variables alone. This suggests that a multivariable approach to the stress level is not only useful but it also can improve the most commonly used methods.

Features extracted from finger and face temperatures as well as electrocardiogram and pulse photoplethysmogram signals were selected to identify the physiological stress response. Most of them show statistically different values between the BS and the SS and a significant linear correlation with the reference variables, as it has been reported in others studies [25].

HR features are highly correlated with the stress reference level as well as PR features, which are quite similar [123]. However, there are differences between PLF and PHF features of PF and HR that mainly occur in the Stress Session, contrary to others studies [122,123]. Those differences are caused by the type of sensors used, which are affected by body movements. Also, the different sample frequency used for ECG and PPG signals might have influenced these results.

Frequency features of HRV are influenced by respiration [112–114]. Thus, respiratory information should be taken into account to increase the reliability of HRV as a marker of stress [100]. This analysis would be an important addition to futures studies.

Other features like, $T_{Ratio,}$ PRT and $PTT_{,}$ are used as stress biomarkers in this study for the first time. In the BL and RS stages, both finger and face temperatures, show low variations following the normal body thermoregulation ($T_{Ratio} \approx 1$). But in stages where a stressor is applied, for instance at ST, MT, VD and AT, the face temperature increases while finger temperature decreases. Similar results are reported in [29]. PTT_{mean} has the highest Pearson correlation value with a stress reference level and it is also highly sensitive to the different states induced throughout the experiment. The lower *PTT* values are reported in stressful tasks, indicating an increase in both blood vessel resistance and blood pressure, while the highest PTT values are observed in relaxing tasks.

Applying the selection criteria explained, only 6 features from the whole set have been retained: T_{Ratio}, HR_{mean} , PRT_{STD}, PTT_{mean}, PTT_{STD} and PWr_{STD}. It is important to note that this selection was tailored to this specific experimental protocol. Therefore, changes in features other than those selected in this experiment could be triggered by either other stressors or in different conditions than the ones used in this experiment, as well as acting in a different population sample or just when a more intensive stress response arise. As mentioned before, the correlation analysis between physiological features reveals that some of them were changing in equal proportions during this experiment. Even if they met the selection criteria, only one feature per related group was used for the estimation performed as their information was redundant. However, all others could be newly included in future studies under different conditions, such as real situations or long term stress monitoring.

The preliminary estimation method gives values of acute physiological stress continuously. Results prove its ability to measure changes from the BS (27.86 au.) to the SS (47.55 au), but also to measure the stress level at different stress states that were produced throughout the sessions It also makes possible to differentiate between four stress levels induced in this experiment (BL_R-RS-BL_S; ST- SA; MT- VD; AT). This preliminary estimation method provides a way to note the tendency of the stress level, which particularly in the ST and MT stages, is to increase. On the other hand, different reactions are observed in the SA and VD stages; since these stages may be related to social phobia or assessment anxiety rather than to stress. In the last stage, the AT also triggers different levels of stress, according to the participant's capabilities to face this task.

There are also some limitations to note regarding this research. Being a preliminary approach, only linear relations between the stress response and the stress reference variables were

analysed. Even though the results obtained strongly support the validity of this approach, other studies including non-linear relations could probably give better results. Likewise, it was assumed that the stress function is linear in the six selected features, but slightly different features and relations that could surely be analysed in future research will expand these results.

Despite the above-mentioned limitations, this lab experiment can be a solid foundation for daily life stress monitoring, by identifying the score function adapted to an individual and, afterwards, by applying this function to monitor daily life stress and track its evolution, as a monitoring of chronic stress.

In Chapter 4 it was discussed that the approach to measure the stress response should be multimodal because, among other reasons, different stressors will trigger different responses. In Appendix **II** ("*Patient Empowerment and Stress Reduction"*) is presented a useful application of stress measurement in patients using the experimental framework previously presented in Section **4.1**, which targeted to evidence the effectiveness of an educational session through markers of psychological stress (i.e. STAI-s, VASS and ECG) measured before and after an educational session. The study was developed under the hypothesis that the referred patients had certain psychological stress generated by a surgery scheduled for the next two to three weeks.

The results allow inferring that the variations of HR_{mean}, RR_{SDNN}, RR_{rMSSD}, HR_{PVLF} were associated to the emotional state in these specific conditions because they were linearly correlated with the stress reference markers (STAI-s and VASS) whose values showed a stress reduction. The HR_{PHF} values (that are associated with parasympathetic activity) decreased after the session in a group of patients that referred being stressed for the upcoming surgery and, contrary to that, increased in the other group that was not stressed by the surgery. Although this was not a result statistically proven, it suggests that a decrease in the parasympathetic activity might be predominant over the sympathetic activity due to fear for and worries about a surgery. Moreover, this result also explains an increase of HR_{mean} that was observed after the Session contrary to what was expected and obtained in the experiment previously presented, a reduction of HR_{mean} in less stressed states.

This application provides more insight into the study of the response triggered by another type of stressor showing that before two different stressors (i.e cognitive demanding task and fear for and worries about the surgery) the homeostatic balance is driven from its normal situation to two different abnormal situations but that both represent a homeostatic imbalance.

7 GUIDELINES FOR THE DEVELOPMENT OF A STRESS MEASUREMENT SYSTEM

A premise of this study was the use of a set of biomarkers from physiological signals able to be easily monitored in daily life setting and with a view to the development of future methods to monitor stress levels. Therefore, obtaining physiological signals noninvasively and in a wearable manner is essential for the practical use of any health monitoring system device.

A set of requirements and guidelines to delineate the specifications of a system to monitor stress in daily life conditions is detailed in this chapter, as a first step towards the implementation of a stress measurement system. Some key considerations and challenges facing this emerging area of study are also addressed.

The main aspects considered when elaborating the set of requirements and guidelines are: (1) the medical and social need to quantitatively and regularly asses stress levels, (2) the multimodal approach required to measure stress as previously discussed (see Section **6.5**), and (3) the state of the art technology concerning possible sensors to be used and (4) the expertise accumulated by the research team and by thesis author throughout the development of this and some other research work in similar fields.

7.1. General Aspects of a Stress Measurement System

7.1.1. Wearable Device and Systems: A Brief Introduction

The accelerated growth of biomedical sensor technologies, low-power circuits and wireless communications, coupled with the need to integrate biomedical sensors for multi-parameter monitoring have led to a new generation of emerging wearable devices that are, in many cases,

integrated in a Body Area Network (BAN). Wearable devices have been used to measure not only human daily physiological signals, but also to measure human physical movements.

Wearable systems generally consist of a set of sensors and/or actuators, a signal processing module, a data transitions module, a core unit and a power management unit. These components are summarised in Figure **7.1**.

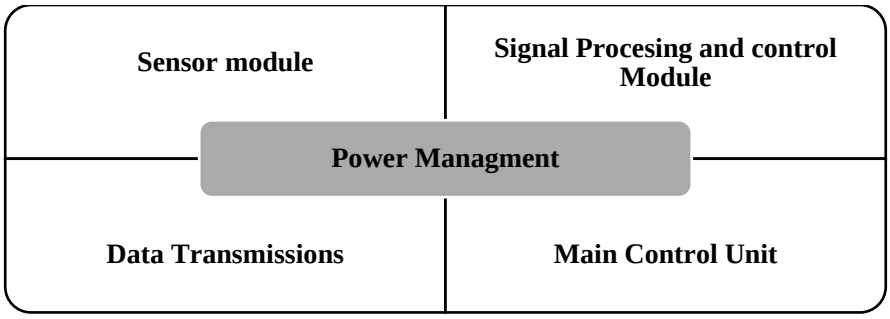

Figure 7.1: Main modules of a wearable system

Wearable systems are characterized by the following:

- Fast and easy-to-use
- Automatic positioning of sensor
- Wireless
- Ambulatory
- Motion artefact resistant
- Comfortable
- Long-term wearable

The sensors of a wearable system or the wearable device itself could be designed as finger rings, ear rings, wristbands, wristwatches, gloves, arm bands, caps, jackets and shoes, among others [125], including in some cases smart textile technology or flexible-stretchable-printable electronics. Wearable devices can currently measure physiological signals, such as skin conductance, electrocardiogram, electroencephalogram, skin temperature, heart rate, respiration rate, body motion, etc. However, certain challenges remain in the field of wearable sensors to monitor human activity and personal healthcare. Some of the major sensor constrains in a wearable device are that the signal-to-noise ratio must be sufficiently high, as humans can be subject to considerable disturbances or noise. Another challenge is the fact that the power supply is limited, as they are always battery dependent. Furthermore, they are generally not comfortable and not wearable in the long-term. Other constrain is that the placing of different transducers close to each other in a contact area can increase signal interferences

In general, wearable systems use different types of existing radio technologies, such as: IEEE 802.11a/b/g/n, Bluetooth IEEE 802.15.1 Bluetooth or low power Bluetooth, IEEE 802.15.3 high speed, IEEE 802.15.4 ZigBee and others. Each one has its own peculiarities, including modulation, data rate, power transmission and power consumption, just to mention a few; which are defined by the operating environment and the main applications. Also, data transitions between the components of a wearable system (if they are not integrated in a one-piece device) could be wired by means of different emerging materials, like smart T- shirt made from a special fabric woven [49,126].

Power Management is a key factor to reach the goal of effectively using long-term wearable devices, not only to prolong their lifespan but also to contribute to the miniaturization of the system since the size of the batteries is a big part of its total dimension. Accordingly, different modes of operation are used, like high-performance mode, low- consumption mode (significantly reducing the operations per second), sleep mode, and real-time streaming operation or recording mode, among others. Additionally, algorithms of optimization for data transmission and computation are included [127,128].

Signal processing can be local or remote. Local processing is advantageous because it reduces the amount of data transmitted wirelessly, reducing power consumption. In turn, this allows for the use of local diagnostic nodes and ensures functionality when low-latency feedback is required or wireless access is not always possible. Therefore, local processing is an energy-efficient form of energy saving at the system level if the ratio of energy spent between the local processing and data transition justifies it.

A variety of studies related to stress detection were presented and discussed in Section **2.2** Some researchers have taken a step forward in stress detection using one-piece wearable devices in real-life environments [129,130], while other studies aiming to develop new wearable devices have presented certain prototypes [44,126]. However, those wearable devices and prototypes only perform a state classification between stress and no stress or arousal and valence.

A selection of commercial solutions for wearable devices, that could be used in a stress measurement system is presented in Appendix **IV**. Among the wearable devices that are currently available in the market, only Empatica's E4 wristband [58] has been classified as a Medical Device class 2a. However, further developments are still needed in order to have a suitable hardware integrated in a wearable device and, above all, to have reliable and accurate methods and algorithms able to measure the homeostatic imbalance induced by the physiological stress response.

7.1.2. Specifications for Application and Use

A multimodal approach using a set of biomarkers was proposed to measure stress levels (see Sections **4.1.1** and **6.5**). Therefore, the design of a stress measurement system (SMSys) should consider not only a one-piece device, but also it may comprise an array or network of sensors placed on different parts of the body. A SMSys should allow for continuous monitoring of stress level measurements for both short and long periods of time.

The main specifications for a SMSys, according to its possible applications and uses, are: to be wearable and non-invasive, to allow for constant monitoring, and to provide information about the environment and the behavior of the subject. In addition, a SMSys should be low-cost and should employ standard off-the-shell (OTS) integrated circuits (ICs).

Other key specifications for a daily life monitoring device are:

- Small size
- Portability
- Long lifespan batteries
- Accurate, easy to use, wireless sensors.

In addition, such a kind of SMSys should fit some more requirements, which are listed below. To meet them rigorously further research and analysis are required. These specifications are:

- The end use should be to measure the level of stress in real time (after a few minutes of previous physiological measurements) for acute stress. Long monitoring time might be needed for chronic stress measurement.
- The method of stress measuring will quantify the physiological response to stress from a set of biomarkers (multivariable or multimodal parameters) and using information from the environment and body motions. These biomarkers will be derived from physiological signals that are synchronously measured.
- The measurement method should also include both the intensity and the response time of activation for each parameter related to the stress source.
- The measurement method should be selective at different types and intensities of stress/stressor and, accordingly, should include an adaptive stress measuring function. For this purpose, the measurement method should use environmental information and possible applied learning techniques; which needs to be studied under the hypothesis that different stressors may induce different homeostatic

imbalances (e.i anxiety, big positive or negative emotions, high demanding conditions, high physical activity).

- The system should include calibrations for each individual based on a questionnaire or biochemical variables to define the subject's threshold values. It should include specific data such as weight, height, age, gender and other sociodemographic variables; as well as the individual's predisposition to stress measured through psychometric tests.
- The measuring system, as it includes more than one sensors (or measuring devices), should be able to control the time-synchronization of the measured signals by accounting their sampling frequencies and time delay due to front-end/HW.
- It may include chronic stress detection using the same method with other thresholds.
- It should include an alarm, which will be triggered when abnormal values are reached. The abnormal values are specific individual thresholds.

7.2. Physiological measurements and Sensor requirements

Herein useful information and guidelines are delineated regarding the physiological signals that were proposed to be included in a SMSys in Section **4.1.3**, such as the relation between physiological signals and the stress response (how they are triggered before a stressor), technical specifications for measuring these signals, and the different types of sensors. Furthermore, certain aspects to consider are detailed in order to obtain convenient features from these sensors. Similarly, sensor constrains are mentioned like, noise, artefacts and misinterpretations that should be avoided for each of them.

Although each physiological signal and each of its extracted features have their own characteristics and specifications; there are some common key elements to analyse when including them in a SMSys, like the following:

- Reacting time related to the stressor: the period of time elapsed since the stressor action to its manifestation in a specific physiological signal or extracted feature; which are:
  - Time-delay from the stressor onset to the activation of feature reaction;
  - Time-period in which the variations of the features remain active;
  - Time-delay from the stressor offset until the feature recovers its normal value
- Relationship between the feature and the stress level: sensitivity to the intensity of the stress response that could be a specific relation/fuction or just an be on/off activation.

- Minimum temporal resolution of a biomarker: minimum time-interval period where a specific feature (biomarker) is extracted from the signal/parameter in order to obtain reliable information of the physiological changes of the stress response.
- Time-delay due to sensor and data transmission (for all measurements synchronization).

Nowadays, different researches are focused on the measurement, analysis, recording and processing of wearable sensors, as well as their integration into wearable multiparametric systems and devices. Some of the latest reports on this topic have been reviewed in [125,131–133] , where the current limitations and challenges of flexible and wearable physical sensing platforms for healthcare and biomedical applications were presented and discussed.

The design of wearable sensors show great challenges, mainly related to power consumption, which in turn is dependent on the sampling frequency, processing and data transmission needs. The contamination of the signals with the intense noise from different external sources or due to the individual's own movement is also a challenge. In addition, sensor monitoring in real time requires large amounts of signal data to be recorded, filtered, analyzed, transmitted and processed. This is why new algorithms and technologies are needed to eliminate artefacts and to recover the actual damaged signals and also to compress the signal data. Other important aspects to consider are security in terms of data protection and the quality of the measurements, which must be reliable and confidential; especially when patients are involved [134].

7.2.1. Skin conductance

Physiology

As already explained in [135], the Skin Conductance (SC), also known as Electrodermal Activity (EDA), refers to the varying electrical properties of the skin in response to sweat secretion by sweat glands. There are three types of sweat glands: eccrine, apocrine and apoeccrine. Eccrine sweat glands are mostly involved in emotional responses as these sweat glands are innervated by sympathetic nerves which accompany psychological processes including emotional arousal.

Different nerve bundles go to the head/face, abdomen, arms/hands, and legs/feet. For example, the nerves that control the sweat glands for the forehead and feet are different from the nerves that control the sweat glands for the fingers; hence the SC response can sometimes vary significantly among different areas of measurement (e.g., fingers, palm, and wrist) [136]. Moreover,

in some cases, depending on which neurological system is activated in the brain, different values of EDA might be measured from the left and right wrists [137].

Features

There are two main components in the SC signal, the general tonic-level that relates to the slower change of the signal and the phasic component that refers to the faster changing elements of the signal, the Skin Conductance Response (SCR). The most common measure of the tonic level is the Skin Conductance Level (SCL) and changes in the SCL are thought to reflect general changes in autonomic arousal.

SCR arises within a predefined response window (1–3 secs to 1–5 secs after stimulus onset) considering that the SCR is elicited by the stimulus when it meets a minimum amplitude criterion (e.g 0.02 to 0.05 μS). In the literature, threshold-amplitudes range between 0.015 μS and 0.3 μS [135]. A threshold-amplitude criterion for counting the number of SC responses has to be established.

The SCR is directly related to a sympathetic activation but its response time is relatively slow (1-2 secs or 1-5 secs). The individual differences in SCL are more reliable associated with psychopathological states [135].

Temporal characteristics of the SCR have been used as biomarkers for emotional arousal and stress stimulus, such as CSR latency (i.e., time from stimulus onset to SCR onset), rise time (time from SCR onset to SCR peak), and half recovery time (time from SCR peak to 50% recovery of SCR amplitude)[137]. A recent study proposed an innovative frequency-domain approach to quantify the sympathetic function using the power spectral density (PSD) of SC comparing the time-domain features of electrodermal activity with the proposed features of PSD during a postural stimulation, Cold pressor, and Stroop test [138]. This study suggests that the PSD analysis of SC is a promising technique for the assessment of sympathetic function.

Measurements and sensors

Typical range values are [0, 100μS], resolution 900 pico Siemens. SC does not usually go above the bottom 25μS in lab studies [137], but a range of [50, 100μS], has been reached in real medical events such as seizures [139]. This measure has the advantage that it is inexpensive to measure.

It has been suggested that motion artefacts can be reduced by detecting a single peak (a SCR) related to a special event [140]. In addition, it has also been proven that change in temperature can drives change of SC [135]. Therefore, both motion activity and temperature should be included in the analysis of SC.

7.2.2. Skin Temperature

Physiology

Acute stress triggers peripheral vasoconstriction [29], due to the activation of the sympathetic nervous system causing a rapid, short-term drop in skin temperature in homeotherms. Also, sympathoadrenal system activation triggers stress-induced thermogenesis (see Section **2.1**), simultaneously changing blood flow patterns.

Podtaev et al. have explained in [141] that the parameters of skin blood flow can be characterized based on the results of high-resolution thermometry and that the spectral range of skin blood flow oscillations has sub-bands that correspond to different factors affecting vascular tone regulation, which are recognized as: myogenic oscillations (0.05–0.14 Hz), neurogenic activity (0.02–0.05 Hz), and endothelium function (0.0095–0.02 Hz) . The frequency of 0.03 Hz is associated with the neurogenic mechanism of vascular tone regulation. The low-amplitude fluctuations of skin temperature (about 0.01 °C) are caused by changes in the tones of small cutaneous vessels and these fluctuations are correlated with these changes in the amplitude.

Consequently, skin temperature (ST) measurements in peripheral limbs and central body can reflect stress-induced reactions, specifically features that mirror short term variations of peripheral ST and the imbalance of the thermoregulation between central and peripheral temperature. As redistribution of blood flow occurs, differences between the right and left lateral parts might be possible

Features

The imbalance of the thermoregulation was analyzed in Section **6.4.1** through the extracted feature T_{ratio} (ratio between values of finger and face temperatures), resulting that T_{ratio} was one of the selected features to estimate the stress levels. Meanwhile, vasoconstriction was assessed using the variation of temperature in one minute as feature and also with the average power of temperature as the integral of PSD in a time window of one-minute.

Measurements and sensors

The most commonly used type of temperature sensors are infrared thermometers and thermistors. A variety of temperature sensors that are used as wearable sensors are available, but only in a few cases, the temperature sensor, classifies as a body temperature variation reference [125]. Temperature sensors still have limitations like: in responsivity to temperature ranging from 30 °C to 50 °C (temperature range of human body), stretchability (most reported thermistors show a strain dependence [132]), and stability. [125]

The optical infrared thermometer has the advantages, when compared with traditional contact sensors. The non-contact measurements, the faster response, and the stable calibration over time are all advantages of this tool.

Results of the experiment presented in Section **6.3** show that ST in the finger could change around 1 ºC in a 1-minute time window and that the ST in the face (placed on the cheek) had very small variation ranging by 0.01–0.08 ºC from the relax state to the stressed state. The sensor used had a resolution of 0.01 ºC and a sampling frequency of 250 Hz.

In accordance with the experimental results and the above information, the use of sensors with resolution equal or below 0.01 ºC and sampling frequency above 50 Hz is recommended.

Body parts of interest are: the forehead, cheeks, neck, armpits, hand palms, thorax, fingertips, and finger bases.

On the other hand, the skin temperature has a strong dependence on environmental temperature fluctuations. Likewise, the skin temperature changes as a result of degraded thermal contact between the skin and the sensor as well as the sweat evaporation from the skin. Therefore, all these constrains need to be addressed for accurately measuring the ST [132].

7.2.3. Respiration

Physiology

A sympathetic activation due to a stressor, provokes an increase in the respiration rate. Additionally, respiration is also known to be related to stress because it is parasympathetic (see Section **2.1**). The normal range of human respiration in rest condition is [0.08, 0.5 Hz], so it can be found in the LF band of the HRV signal. Further information can be found in the Section **7.2.4**

Respiratory sinus arrhythmia (RSA) is a marker of parasympathetic nervous system (PNS) tone [142] and thus, a marker of the disruption of homeostasis induced by stress. It is derived from both the ECG and respiration. RSA describes the variability in RR intervals due to respiration: inspiration shortens RR intervals and expiration grows RR intervals. It is computed by subtracting the shortest RR interval from the longest RR interval within each respiratory cycle.

Features

The respiration rate (F_R) is the most common feature of the respiration signal. Along with this feature, two others are also related to respiratory stability: the Spectral Peakness (P_k) and the Percentage of spectra used to estimate F_R (N_k). All three said features [98,100], using the respiration signal data from the Stress Session of the experiment previously presented as part of their data

set. The respiratory rate was not estimated during the Memory Test and Arithmetic Task stages, since speech modifies the respiratory pattern and no spectra would meet the peakness criteria of the method used. The respiratory rate is observed to be higher and less stable (lower P_k and N_k) during the stress stage than during the baseline stage. Additionally, using the same respiration data other features of the Wavelet Cross-Bispectrum domain were computed from the measure of cardiorespiratory coupling [99], using information of HRV and respiration rate.

Statistical features from F_R are also commonly used, such as the Median (normalized) and variance, as well as frequency features from PSD in different frequency bands (i.e. 0–0.1, 0.1–0.2, 0.2–0.3, and 0.3–0.5 Hz).

Plarre, et al, used in [143] the RSA and the ratio of inhalation duration to exhalation duration (IE Ratio) as a feature for stress classification obtaining good results.

Measurements and sensors

Khan et al. in [132] summarized the type of sensor that could be used in a wearable system, to measure the flow of breath (placed near the nose or mouth) or the expansions and contractions of the chest and abdomen during breathing. An example is the impedance plethysmography that can be embedded or woven into a stretchable insulating matrix formed into a loop around the body. There are also acoustic, piezoelectric and triboelectric sensors of respiration rate that can be integrated in textile technology or formed in path strain sensors. Khan et al. also said that it is critical for the sensor to be as comfortable and conformable as possible as well as to avoid body motions and in some cases heartbeats, as sensing element is in close contact with the body.

Strain sensors are commonly used to measure respiration because they are easily embedded into clothing or daily objects like chairs or beds. At the same time, the inductive/impedance plethysmography has been developed in the forms of clothing and textile belts.

It was shown that the piezoelectric pneumography provided the best robustness to motion artefacts for respiratory rate measurement among inductive plethysmography, impedance plethysmography and, piezoresistive, pneumography [144].

7.2.4. Electrocardiogram

Physiology

The autonomous nervous system (ANS) controls the heart activity, so a sympathetic activation provokes an increase in cardiac response, which can be assessed from an electrocardiogram (ECG) information and, more specifically, from the heart rate signal (HR). The

short-term variations in heart rate (heart rate variability, HRV) have been widely used as a tool for ANS assessment [104,112]. Therefore, HRV can be used to characterize the stress response.

It is important to note that it is necessary to the presence of ectopic beats exclude in HRV analysis, which sometimes are generated by a mass of heart cells not located in the sinus node. It may generate an electrical impulse before the sinus node, thus leading to a new interval series commonly denoted normal-to-normal intervals, or NN intervals.

Respiration modulates the heart rate, which is higher during inspiration than during expiration [142]. Respiration is usually mirrored in the HF band, but in certain conditions, it could be in the LF band, such as the rest and relaxed conditions. It may also exceed the classical range of HF, for example in exercise conditions [145]. To avoid the influence of respiration, in some studies the HF band is centered at the respiratory rate using either a constant or a time-dependent bandwidth [100,146].

Features

The HRV and HR signals are derived from ECG signal. The extracted features can be divided into 2 groups. (1) the time domain features, based on statistical and geometrical measurements of the RR interval series, (2) the frequency domain methods, which are subjected to a PSD estimation from a time-window of NN interval series, usually of 2–5-minute length (as per Section **5.2** and as detailed information in [104]). Recently non-linear features are been used, as reviewed in [47], such as the Entropy that shows information about the HR regulation mechanism, also the Tone and the Complexity features that represents the sympathovagal balance.

The European Society of Cardiology [112] has suggested that a minimum time period of 5 minute is essential in order to measure the heart rate (HR) from ECG signals, and that any measurement lasting less than 5 min is of dubious value.

The following complexities are involved mainly in HRV power spectrum analysis [145]: (1) HR variations with respect to time and (2) inefficient detection algorithm of R peaks. These two problems cause uneven sampling, and cause ectopic beats in the signal that directly reflects the HF and LF bands which are highly sensitive to power spectral analysis of short-term and long-term HRV signal. Another such complexity as the above stated is he influence of respiration rate in heart rate and HRV. This would yield inaccurate estimates of the ANS activity.

The inclusion of respiratory rate information in HRV analysis was proposed in [100] for stress assessment, the same study previously commented in which some respiration features were extracted using data from the experiment presented in previous Chapters. In that study was concluded that frequency domain HRV indices, namely PLF and PHF computed in classical

terms, scarcely show statistical differences during stress. When respiratory rate information is used to guide HRV analysis, it is possible to avoid the overestimation of sympathetic activity and the underestimation of parasympathetic activity that occurs when the respiration rate lies in LF band, as well as the underestimation of parasympathetic activity when respiratory frequency is above 0.35 Hz. This combination of HRV and respiratory rate analysis increases the statistical differences among different stress situations, where a major sympathetic dominance is observed.

The Task Force constituted by the European Society of Cardiology and the North American Society of Pacing and Electrophysiology has standardized the methods of HRV measurement and has also defined the HRV's physiological and pathophysiological correlation [112].

Measurements and sensors

ECG is one of the most commonly used vital signs in health monitoring. It measures the electrical activity of the heart recorded superficially over the skin by two electrodes. These two electrodes can be placed over the skin in positions where the spatio-temporal variations of the cardiac electrical field are reflected enough. This specific electrode-positioning is called lead. [104]. Traditional clinical settings use the standard 12 leads, while in emerging ambulatory ECG monitoring is used the 3-lead configuration. There are also several applications in which single ECG lead configuration is employed, mainly in wearable systems such as [147]

There are many configuration and type of sensor to measure the electrical activity of the heart from the classical wet-electrode to non-contact, dry electrode that can be capacitively-coupled. An evaluation between the type of electrode and the body placement should be done to consider the signal to noise ratio and the suitability in the application.

7.2.5. Pulse Wave

Physiology

The pulse wave is the vascular pressure wave created during heart contraction that is propagated through the walls of the blood vessels to the walls of the arteries. The pulse wave propagates itself by exchanging energy between the flow of blood in the aorta and its walls, given the elastic properties of the walls of the blood vessels. At each bifurcation, a fraction of energy is transmitted to the following arteries while another fraction is reflected backwards. Thus, the reflected wave in the peripheral arteries returns in the opposite direction to the flow.

Therefore, the pulse wave contains not only information of the cardiac response but also of the blood volume and peripheral blood vessels, which are influenced by the parasympathetic

counterbalance, neurohypophysis activation and SNS activation (more detailed explanation is provided Section **2.1**)

Features

More information related to features from pulse wave can be found in Chapter 5, Sections **5.3** and **5.4**. The pulse wave until the reflected wave (PWr) is associated with peripheral central vascular resistance and cardiovascular output, as well as pulse wave velocity [107] The pulse decreasing time (PDT) is an index of diastolic time of blood pressure. The PRT is a pulse wave index and is related to arterial stiffness, blood volume and systolic blood pressure peak [111]. Vasoconstriction is reflected in pulse wave by decreases on its amplitude, which is directly related to PRT. The pulse transit time (PTT) is inversely proportional to the pulse wave velocity, which is associated to arterial stiffness and cardiovascular output.

Measurements and sensors

A photoplethysmography consists of illuminating the tissue and simultaneously measuring the transmitted or the reflected light. The principle is to use a specific wavelength that the oxyhaemoglobin present in blood flow absorbs and then the volume of blood of each cardiac cycle is measured and consequently the pulse wave. In conclusion, plethysmogram represents the pulsatile blood volume changes of peripheral microvasculature that is induced by pressure pulse within each cardiac cycle.

Traditionally, the sensor is placed in direct contact with the skin. Recent research has shown though that sensors can be integrated into daily-life accessories or gadgets like ear-rings, gloves and hats, to achieve unobtrusive measurements. Certain indirect-contact sensors for pulse wave measurement, such as sensors placed over clothing and a digital camera, has been proposed. However, the temporal resolution of the blood volume detected by those methods is restricted by the sample rate of the sensor [125].

Along with the development of new material systems, the fabrication of highly sensitive strain sensors capable of measuring pulse pressure waveforms is making progress, but a more rigorous study has yet to be done on the reliability of these sensors [132].

Signal artefacts caused by motion, electrode placement, or respiratory movement affect the accuracy of measured recordings. These factors increase the difficulty of developing a SMSsys for everyday use. For example, according to Olufsen et al.[148], the cardiovascular responses to postural change from sitting to standing and from standing to varied levels of movement involve interactions between the autonomic nervous system, which regulates heart rate, perspiration and pupil dilation, as well as cerebral autoregulation.

Taking a step further in stress monitoring in real-life conditions, one of the possible factors affecting the resulting values of pulse wave parameters was analysed namely, the effects of limb movements and positions on pulse transit time; which is presented in Appendix **III**.

7.2.6. Electromyogram

Physiology

An Electromyogram measures the electrical activity of the muscles by using electrodes placed over the muscle of interest. A stress response could elevate muscle tone when SNS is activated or to the contrary, muscle tone decreases when PNS is activated. Stress has been found to provoke involuntary reactions on facial and Trapezius muscles [47].

It is explained in [104] that when the muscle activity exceeds the double or triple standard range, the muscle is defined to be activated. Because single spontaneous spikes can easily exceed the SD range, it is useful to define a minimum time (minimum subperiod duration) in which the EMG signal has to constantly stay over the threshold to be accepted as activated (e.g. 50 ms). The same is valid for the offset of the signal, to avoid that single random amplitude gaps trigger the deactivation of muscle activity. An alternative solution of threshold definition would be a percentage amount of the local peak activation found within the analysis period, e.g. 5%. This peak setting produces much more reliable threshold settings and it is independent from the baseline characteristics and variations. Another alternative would be to define a certain microvolt level.

Whatever method is selected, it is absolutely necessary to check the validity of the threshold setting results and onset periods.

Features

Most common features are statistics like standard deviation, Root of Mean Square and minimum and maximum values, as well as, the average number of gaps per minute and the relative time with gaps, the frequency values, among others [47]

Measurements and sensors

The noninvasive electromyogram measurements are performed placing two electrodes over the skin in the muscle fiber orientation and minimizing the electrical cross-talk from other muscles. The common sample frequency for the surface EMG recording is 1kHz since the rage of muscle activation varies from 400–500 Hz [104].

A reliable algorithm of fiducial point detection in the EMG signal has to be used (i.e. baseline and onset/offset time). Additionally, this signal is very noisy due to body motions, electrical interferences and also the noise of electrical activity of the heart contractions when it is recorded on the trunk (e.g. over trapezius muscle).

It is important to remark that the threshold definition varies from person to person and from muscle to muscle. The threshold definition is also related to the area between the two electrodes since the surface EMG measure the gross activity produced by a large number of motor units [104]

The effectiveness of this signal as stress biomarker compared to other biomarkers should be determined in further studies that include them in a SMSys. The challenge is presented by its constraints, regarding the placement of the electrodes on specific body places and the possibility of becoming obtrusive in some situations, as well as the amount of noise that has to be removed.

7.2.7. Type of sensor electrodes

In general, sensor electrodes for ECG / SC / EMG measurements can be classified as:

- Non-contact sensor / contact sensor
- Dry-electrode / wet-electrode

Convectional ones are contact sensors, wet electrode, and usually employ 12 or 15 Ag-AgCl electrodes, with gel in the middle of a pad. Their use often requires cleaning the attachment site and, if necessary, shaving the hair off some parts of the body. Although this type of electrodes provides good signal quality, it is inconvenient and may cause skin irritation, allergic reactions, and inflammation due to toxicological issues of the gels and the adhesive tape in long-term treatments [149]. Furthermore, the gel dehydrates during prolonged use. Thus, wet-electrode systems might be unsuitable for long-term ECG/EMG/SC monitoring.

A possible alternative is a dry-electrode sensor that still has direct contact with the skin. Another possibility is given by capacitively coupled methods [149], though they have some disadvantages when comparing them with wet-electrodes, like poor quality of the signal and greater sensitivity to environmental noise and motion noise.

The main artefact source in a noncontact sensor electrode is the displacement between the electrode and the skin [132]. Its common problems are that they are very noisy, very sensitive to artefactual motion (provoking over saturation in the signal), and to the 50/60 Hz noise

The selection of the type of electrode should consider the signal-to-noise ratio and the wearability of the sensor.

7.3. Summary

Table **2.1** and Table **7.2** summarize the main aspects previously presented with regard to physiological signals and their sensing methods, as well as the parameters that any SMSys should meet. Further studies could optimize the measurement range, resolution and sampling frequency. These studies should also take aim at power saving, better power management, the improvement of signal-to-noise ratio in the design of the sensor-HW and better noise suppression and filtering techniques. In addition, motion movement and body position should be measured not only to remove artefacts or misunderstandings in the parameters/features, but also to include their information in the stress measuring method.

Table 7.1: Summary of parameters from physiological signals for stress measurement method

Physiological signals	Parameters/features
Skin Conductance	- Skin Conductance Level (SCL) / time features - Skin Conductance Response SCR / time features and frequency features
Skin Temperature	- Ratio between values of peripheral and central skin temperatures - Variation of temperature in one-minute average - Power of temperature in one-minute window
Respiration	- Respiration Rate / time features and frequency features - Respiration stability/ Spectral peakness and Percentage of spectra accepted to compute a peaked-conditioned spectra. - The ratio of inhalation duration to exhalation duration*
Pulse wave	- Pulse Period / time features and frequency features - Pulse Wave velocity (PWV) / time features - Pulse Wave Rising Time (PRT) / time features - Pulse Wave Decreasing Time (PDT) / time features - Pulse Width until Reflected Wave (PWr) / time features
Electrocardiogram	- Intervals of *R-peak* (RR, HR=1/RR) / time features - Heart Rate Variability (HRV) / frequency features - Non-linear features (Entropy, Tone and Complexity)*
Electromyography (Trapezius muscle)	- Amplitude / statistical features - Gaps / statistical features - Power spectral features
Electrocardiogram-Respiration	- HRV analysis centered in the respiration rate - Wavelet Cross-Bispectrum* - Respiratory sinus arrhythmia (RSA)
Electrocardiogram-Pulse wave	- Pulse Transit Time (PTT) / time features

*Further research is required to have reliable and accurate measurements

Table 7.2: Summary sensing methods and parameters of physiological signals for a SMSys

Physiological signal	Sensing method	Range and parameter	Fs
Skin Conductance	- Skin electrodes	[0 – 100µS], resolution 900 pS	50hz
Skin Temperature	- Thermistors - Infrared thermometer*	[30 –50 ºC] 0.01ºC resolution	50 Hz
Respiration Rate	- Impedance plethysmography - Piezoresistive* - Peumography	20mohm resolution (Impedance pneumography)	50 Hz
Pulse wave	- Photoplethysmography - High speed digital camera* - Highly sensitive strain sensors*	16–24 bits resolution	0.5 – 1khz
Electrocardiogram	- Skin electrodes	24 bits resolution	1khz
Electromyography (Trapezius and facial muscle)	- Skin electrodes	[0–2000 uv]	Above 1kHz

* Further devolvement is required to have reliable and accurate measurements

8 CONCLUSIONS AND FUTURE RESEARCH

This chapter summarizes the main findings and the conclusions of this thesis. Moreover, the strengths and possible applications of this thesis are described and future research lines are proposed. Finally, a list of the publications produced during this research is provided.

8.1. Conclusions

The methodology and the results presented in this thesis are focused on objectively quantifying the physiological component of the stress response, which can be understood as how close or far an individual is from what is socially and medically considered to be his/her normal state.

From the analysis of the reviewed literature, it is appropriate to conclude that, all previous studies have been conducted in some very specific conditions and have only focused on the detection of stress states by using a variety of analytical methods. Even though previous studies have proposed some stress assessment methods, they fail to provide neither a standardized metric of stress nor a pattern of references to compare it with.

To face the lack of stress measurement procedures, an experimental framework was designed in order to select and evaluate a set of biomarkers able to quantitatively assess stress level. Selected biomarkers were extracted from physiological signals noninvasively measured. On the other hand, some biochemical variables and psychometric tests that are well-known and accepted stress markers were used as stress reference values. They were selected by a multidisciplinary team.

Using the experimental framework, an experimental protocol was designed to induce well-differentiated stress states. It included two sessions, a Relax Session and a Stress Session. In the

Stress Session, a moderate but significant stress was induced through the validated and widely documented tool, the Trier Social Stress Test.

A multivariable approach based on stress markers was proposed to estimate stress levels, in order to have a broad measurement of the stress response, thus considering multiple reactions triggered by the stressor. This approach may establish the groundwork for future applications that aim to monitor stress. It also benefits research in the field of stress allowing intra-individual and inter-individual evaluations to be made using a common index of stress level

A set of features was obtained from face and finger temperature, ECG and PPG signals. Some of these features were previously reported as stress markers from the reviewed literature. Other features were also obtained by looking into the analysis of physiological reactions forming part of the stress response: like T_{ratio}, PTT and PRT, which are used as stress biomarkers for the first time in this study.

A multivariable stress reference scale has been proposed by merging some of the selected stress reference variables

The existence of a statistically significant correlation between the measured set of stress-related biomarkers and the induced-stress level assessed trough the previously mentioned multivariable stress reference scale was proven.

The results obtained from both experimental sessions confirm that a clear stress response is triggered by the proposed protocol

An innovative preliminary approach was proposed to estimate stress using a multivariable biomarker, constituted by features extracted from physiological signals. Results from this approach had showed its ability to continuously measure stress because different stages that occur sequentially in a short period of time can be easily distinguished.

The performed experiment proves that the level of stress response caused by a psychological stressor can be determined from a set of non-invasively and continuously measured physiological parameters, as an alternative to the currently used stress assessment methods.

Therefore, this procedure can be a solid foundation for daily life stress monitoring, by, first, identifying the score function adapted to a particular individual and, then, by applying this function to monitor daily life stress

A set of requirements and guidelines were suggested to outline the specifications for an electronic system to monitor stress in daily life conditions. Some other key considerations and challenges facing this emerging area of study were also addressed. However, further

developments are still needed in order to have a suitable hardware integrated in a wearable device and, above all, to have reliable and accurate methods and algorithms able to measure the homeostatic imbalance induced by the physiological stress response.

8.2. Ongoing work derived from this thesis

The work done in this thesis developed in the framework of the ES3 Project has prompted two other projects “Remote Assessment of Disease and Relapse in Central Nervous System Disorders (RADAR-CNS)- IMI Joint Undertaking Project 115902-1” and the project “Patterns of Autonomic Nervous System Biomarkers in Depression, DEANS. CIBER Intramural project BI07”.

8.3. Future Research

A reliable and continuous stress measurement will be a useful tool for mental health care and wellbeing. Also, it will enable professionals to have easy and precise communication methods in order to find more suitable and personalized solutions for the treatment of pathological cases.

A first approach to measure acute stress was presented in this thesis and future research should validate its potential to be used for stress measurement in daily life under different conditions.

The level of the stress response, as the degree of the homeostatic imbalance, should be further studied. In this regard, new stress biomarkers should be identified.

The results of the experiment presented in this thesis properly motivate further experiments with different stressors and populations in order to validate its extrapolation capabilities and to adapt its use to other conditions. Therefore, a new and more sensitive stress measurement method could emerge as starting point for future inter-individual and intra-individual valid comparisons facing different stressors based on the results presented in this thesis.

Linear dependency between certain physiological features and the stress level were proven as well as it was assumed that the stress function is linear in the six selected features. However, other types of relationships should be studied in future analyses, but slightly different features and relations that could surely be analysed in future research will expand these results.

A patent document concerning a validated stress measurement device has been already submitted. To implement, to industrialize and to commercialize the device drafted in this thesis constitute the challenge to face in the near future.

In addition, there are several issues that are also to be investigated and that can open new lines of research. Here are some directions briefly described for future research that would be a continuation of the work presented in this thesis:

- Deeper analysis of the relationship between the biomarkers' intensity and the stress response level, as well as, their interrelationship before a stressor.
- Extracted features from respiration and electromyography, as well as skin conductance are required.
- Reaction times of the features and their possible dependency with the intensity of the stress response triggered when facing a stressor agent
- Evolution of the features according to certain characteristics of the stressor like the intensity and the time that the stressor remains active.
- Finding proportion of the features, in terms of each feature's impact/weight in the stress measuring method.
- Identifying overlapping features that provide the same information, in order to select best suitable features for a stress measurement system and to possibly implement selective algorithm according to other factors like environmental, resting, sleeping, and physical activity. To use redundant information to obtain more accurate measures of the stress response.
- Characterization of the stress response' dynamics by assessing the stress level curve against time, in terms stress increase, adaptation, decrease or resistance in the case that stress is chronic. Analyzing: the response time; adaptation facing the stressor; time elapsed until return to normal condition or source of stress disappears; and if adaptive response does not succeed, to analyses the resistance (chronic stress)

8.4. Publications

A list follows, with the research papers produced during this research work:

Journal papers

1) Arza A., Garzón JM., Lázaro J., Gil E, Laguna, P , Bailón R., Aguiló J, "Measuring Acute Emotional Stress through Physiological Signals: Towards a Quantitative Assessment of Stress" , *Journal of Biomedical and Health Informatics.* **Under review**
2) Garzón-rey JM, Arza A, Cámara C De, Lobo A, Armario A, Aguiló J. Aproximación a una escala de referencia de estrés emocional agudo. *Revista de neurologia*. 2017;64:1-9

3) Hernando A., Lázaro J., Arza A., Garzón JM., Gil E., Aguiló J., Laguna, P ,Bailón R. “Inclusion of respiratory frequency information in heart rate variability analysis for stress assessment.” *IEEE Journal of Biomedical and Health Informatics*. April 2016:1-1.
4) Aguiló, J, co autors, Arza, A “The ES3 project:Towards a quantitative assessment of stress level.” , *Revista de Neurologia* 2015. http://www.ncbi.nlm.nih.gov/pubmed/26503316
5) Aguiló J, F.-S. P., García-Rozo A, Armario A, C. A. & Cambra FJ, et al. Proyecto ES3: intentando la cuantifi cación y medida del nivel de estrés. *Rev Neurol* (2015).

Conference papers

6) Spyridon Kontaxis ; Jesús Lázaro ; Alberto Hernando ; Adriana Arza ; Jorge Mario Garzón ; Eduardo Gil ; Pablo Laguna. Mental stress detection using cardiorespiratory wavelet cross-bispectrum. in *2016 Computing in Cardiology Conference (CinC)* (IEEE, 2016)
7) Arza, A., Garzón, J. M., Hemando, A., Aguiló, J. & Bailon, R. Towards an objective measurement of emotional stress : Preliminary analysis based on heart rate variability .*. in *Medicine and Biology Society (EMBC), 2015 37th Annual International Conference of the IEEE* (2015)
8) Hernando A., Lázaro J., Arza A., Garzón JM., Gil E., Aguiló J., Bailón R “Changes in respiration during emotional stress”. Computing in Cardiology ,42nd Annual Conference, 6-9 September, 2015.
9) Aguiló, J., Arza, A., & Rey, J. M. G. Marcadores multivariable en el análisis de trastornos mentales. *1er Congreso Iberoamericano de Neurorehabilitación*, México 2015.
10) Arza Valdes, A., Garzon Rey, J. M., Gayoso, M. N. & Aguilo, J. Patient empowerment and stress reduction. in 2014 IEEE *9th IberoAmerican Congress on Sensors* 1–4 (IEEE, 2014). doi:10.1109/IBERSENSOR.2014.6995560
11) Garzon Rey, J. M., Arza, A. & Aguilo, J. Towards an objective measurement of emotional stress. in 2014 *IEEE 9th IberoAmerican Congress on Sensors* 1–4 (IEEE, 2014). doi:10.1109/IBERSENSOR.2014.6995559
12) Arza, A. A., Lázaro, J., Gil, E., Laguna, P., Aguiló, J., & Bailon, R. . Pulse transit time and pulse width as potential measure for estimating beat-to-beat systolic and diastolic blood pressure. in *Computing in Cardiology Conference (CinC*) 887–890 (IEEE, 2013)

APPENDIX

I BLOOD PRESSURE VARIATION AS POTENTIAL STRESS BIOMARKER

Blood pressure variation is a manifestation triggered by neurohypophysis correlated activation and sympathetic nervous system activation and it is also related to the increase of cardiac response. From the literature reviewed (see Chapter 2), it can be concluded that blood pressure measurement could have a great potential as stress marker. However, no wearable continuous blood pressure sensor is available for this purpose yet.

Due to the promising contributions of blood pressure as a marker of the stress level, one of the objectives of this thesis' work was to develop a method for a continued measurement of blood pressure variations using a noninvasive procedure. The study performed to accomplish this objective is summarized in the following subsections.

I.I Blood Pressure Measurement: A Brief Review

Blood pressure (BP) depends directly on the cardiac response and on the arterial and venous system characteristics of each individual. BP can vary abruptly mainly governed by the autonomic nervous system. Its long-term variations are governed primarily by changes in the cardiovascular system or in response to some pathologies. This is one of the most frequently recorded vital signs in medicine.

The most recurrently used methods for BP measuring are based on the external application of pressure on an artery, usually the brachial one, by means of a pneumatic sleeve disposed around the arm (Riva Rocci-1896) that is generally known as blood pressure cuff. BP is also recorded through invasive methods such as the placement of an intra-arterial catheter, but there are other noninvasive methods based on photoplethysmography (PPG) [150–155,156]. Among them, the most widespread method is to measure BP through the pulse wave transit time [157–159], one of the selected stress biomarkers for the stress estimation method (see Section **6.4.1**).

The slope of the pulse transit time (PTT) as a function of systolic pressure provides information about the circulatory system like *PTT* is inversely related to blood pressure (BP)[108], based on the relationship between BP and pulse wave velocity [109,110]. The following table summarizes the different methods commonly used nowadays.

Table I.I: Blood pressure measurement methods.

Category		*Methods*
Invasive		Intra-arterial catheter with a pressure sensor
Noninvasive	*Cuff*	Auscultator, oscillometric, tonometry, ultrasounds, electronic palpation
	Cuffless	Based on parameters from cardiac response measurement (e.g. ECG, PPG)

Generally, BP estimations are done based on the measure of the cardiac response translated into the pulse pressure wave generated in each beat. Also, the estimations may include the characteristics of the arterial system that remain constant under normal conditions (e.g blood viscosity, Artery radius, blood volume and others). Although generally, arterial system is constantly varying depending on the internal regulation of the body and its relation with the environment.

Continuous noninvasive blood pressure measurement has not been feasible so far. However, several studies have proven that blood pressure estimations can be continuously performed using noninvasive procedures, but within short periods of time of just a 10-15 minutes [150–155]. Blood pressure estimation based on PTT is the most general method and it seems promising [152,154] (e.g. in Samsung's Simband, Nihon Koden's patient monitor), but improvement of this technique is still on-going

The study of the PTT was first described by Moens and Korteweg in 1871, and it was extended by Bramwell and Hill in 1923. Moens and Korteweg defined equation (**I.II**), assuming that the artery wall is isotropic and experiences isovolumetric change with pulse pressure.

The velocity of the pulse wave (PWV) can be calculated as the time it takes the wave to travel a distance Δd (see equation (**I.I**)), according to Newton's laws. Since then, the relationship between BP and PTT has been studied but without a proper accuracy of the BP measurement.

$$PTT = {}^{PWV}/_{\Delta d} \qquad \text{(I.I)}$$

$$PWV = \sqrt{\frac{E.h}{2.r.\delta}} \quad \text{(I.II)}$$

Where: PWV- Pulse wave velocity
E - Elasticity of the walls (Young's Modulo)
h - Width of arterial walls
r - Density of blood
δ - Artery radius

The limitations of BP estimation methods are that they require the application of frequent calibration technics and they are not reliable for long monitoring periods [160]. For example, Nihon Koden's patient monitor is based on the PTT method, but every 5-15 minute period a calibration is performed by means of a cuff. The frequency to perform a calibration depends on the monitoring needs, according to the severity of the patient.

Conversely, McCombie et al [161] have proposed a method that allows a complete calibration of PTT to measure BP without the use of an oscillometric blood pressure cuff or an external pressure sensor. This method is based on changes in the transmural pressure acting on the arteries due to changes in hydrostatic pressures that happen when the height of the sensor varies relative to the heart. Those pressure changes are measurable in PTT. The PTT is measured from two PPG sensors placed at a known distance: a main sensor applied to the wrist on the ulnar artery and a sensor on the little finger of the digital artery. BP is estimated by the Moens-Korteweg equation of arterial vessel elasticity (**I.II**) and calibrated by the hydrostatic pressure changes. However, the error obtained with respect to the BP references of the measuring instruments is not given.

Chern-Pin et al [162] made a comparison between the pulse amplitude (PA) and PTT, to estimate the systolic arterial blood pressure BP_{sys}. He found that pulse amplitude was more effective than PTT for estimating BP_{sys} during sleep and this relationship weakened during REM sleep, compared to other sleep states. These results suggest that the PA is potentially more adequate than the time of arrival of the pulse to estimate continuous blood pressure. However, PA is a very artefactual parameter, which may artefacts from body motions, a bad placement of the sensor, a change in blood oxygen saturation, and others.

I.II Blood Pressure Beat-to-beat Estimations

Two studies of blood pressure estimation were done in different experimental conditions. The first study was done in a real-life situation; sampling patients hospitalized in the Intensive Care Unit of the "San Juan de Deu Hospital" form Barcelona. The second study was done

applying a tilt table test to healthy young subjects that provoked significant BP variations due to postural changes.

The general objectives were to develop a method for a continuous measurement of blood pressure variations (*BPV*) using a noninvasive procedure and to estimate beat-to-beat systolic blood pressure (BP_{sys}) and diastolic blood pressure (BP_{dias}) in accordance with the Association for the Advancement of Medical Instrumentation (AAMI). AAMI establishes for BP estimation that the mean estimation error should be less than 5 mmHg and the standard error must be below 8mmHg for BP_{sys} and BP_{dias} [163].

The overall data set used in both studies includes synchronous electrocardiogram signal, pulse photoplethysmography signal and continuous blood pressure signal. Blood pressure is often estimated by means of linear models [109,164,165]. Accordingly, BP was estimated using a linear model that is calibrated for each subject. The calibration was made computing the coefficients of the model by linear regression.

I.II.I. Data processing

A low pass filter with a cut-off frequency of 35 Hz was applied to BP signals. Fiducial points in the BP signals were automatically determined, as it was described in [108], while detected points in ECG and PPG signals were obtained as previously indicated in Sections **5.2** and **5.3** respectively. A representation of detected points are shown in Figure **I.I**.

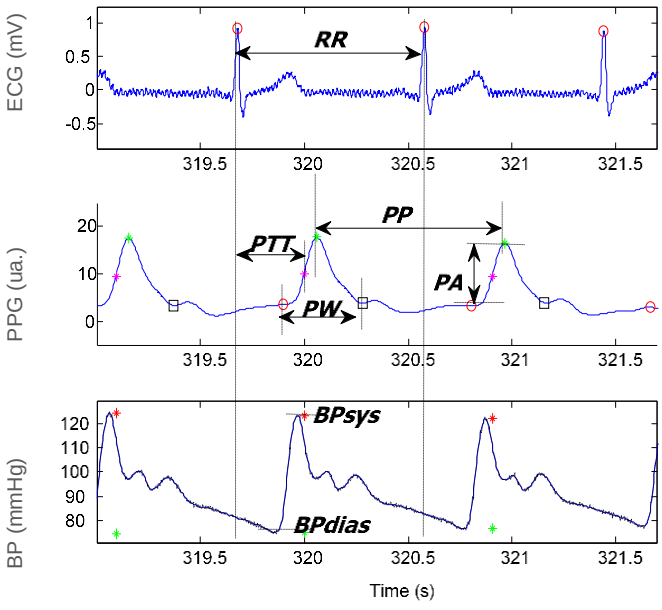

Figure I.I: Computed beat-to-beat parameters for BP estimations.

APPENDIX I

For each beat, n, bounded by ECG R-R interval, the PTT(n), PW(n), PA(n), RR(n) BP_{sys}(n) and BP_{dias}(n) series were calculated as it can be seen in Figure **I.II**.

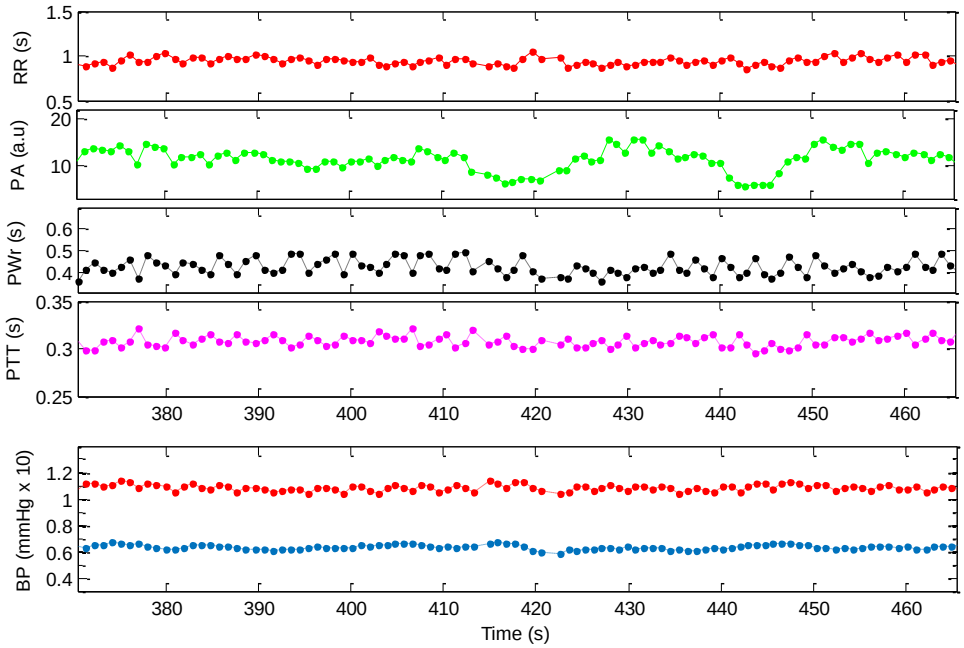

Figure I.II: Example of beat to beat features computed in study 2

Beat-to-beat systolic and diastolic blood pressure were estimated using the following linear models:

$$BP_{S,D}^{a..c}(n) = \beta \times {P_x}^{a..c}(n) \qquad \text{(I.III)}$$

Where: $BP_{S,D}^{a..c}(n)$: represents the series of estimated BP, superscript refers refers to model type model a, b or c and subscript BP_{sys} and BP_{dias}

β: represents the matrix of coefficients that were estimated for each subject by linear regression.

$P_x(n)$: represents matrix of parameters used in each model, e.g.

${P_x}^{a}(n) = [PTT(n)\ PA(n)\ 1]$ or ${P_x}^{b}(n) = [TTP(n)\ RR(n)\ 1]$

Main aspects and results of each study are presented below.

I.II.II. Study 1

The objectives of this study were to compare different noninvasive methods of BP estimation, analyze the validity of a linear relationship between the generally used parameters in the literature (i.e. PTT, PA and HR) and both BP_{sys} and BP_{dias}.

The database was recorded at an Intensive Care Unit (ICU) from 26 young patients (10 Men and 16 Women), aged between 3 month and 16 years. Each patient was recorded for 15 minutes. The PPG, ECG and an invasive BP signal from an ICU patient were recorded with a monitor from Philips (IntelliVue MP70 model). The PPG was recorded from the index finger and, in the case of babies, from the big toe of the foot and with a sampling rate of 250 Hz. The BP was invasively recorded from the femoral or radial artery with an intra-arterial catheter, and with a sampling rate of 250 Hz. The ECG lead V2 was recorded with a sampling rate of 500 Hz.

Linearity was tested using the linear Pearson correlation. Mean values of the correlation coefficients are given in Table **I.II**. Taking into account that the feature series were 15-minute long and their sample frequency was the beat-to-beat occurrence, this correlation results confirm the linear relationship between the TTP, the PA , the HR and the arterial pressure.

Table I.II: Mean correlations between measured BP_{sys} and BP_{dias} and PTT, PA and HR

Parameter	BP_{dias}	BP_{sys}
	C_{Corr}	C_{Corr}
PTT	-0.307	-3.00
PA	-0.333	-0.321
RR	-0.468	-0.358

* p-value < 0.05. **p-value <0.001

Beat-to-beat systolic and diastolic blood pressure were estimated using models a, b and c. In Figure **I.III** shows the chart corresponding to a subject using models a and c. As it can be observed in Table **I.III**. The model $BP^{a}_{S,D}(TTP, AP)$ had the best results for BP_{sys} estimation, while model $BP^{c}_{S,D}(TTP, AP, RR)$ had the best results for BP_{dias}.

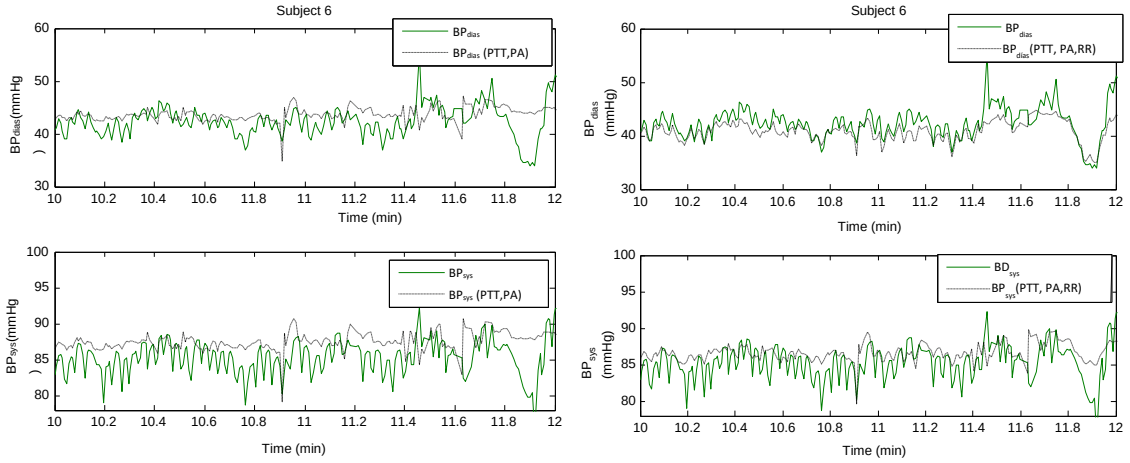

Figure I.III: Example of beat-to-beat blood pressure estimation using models a and c.

Table I.III: Mean absolute error and standard deviation ($E_A \pm SD$) and mean correlations between estimated and measured BP in Study 1.

	BP_{dias}		BP_{sys}	
Model	$\boldsymbol{E_A \pm SD}$ **(mmHg)**	$\boldsymbol{C_{Corr}}$	$\boldsymbol{E_A \pm SD}$ **(mmHg)**	$\boldsymbol{C_{Corr}}$
$BP^a_{S.D}(n) = \beta^a_{S.D} \times [TTP(n)\ PA(n)]$	-0.189 ±5.412	0.358*	-0.315±7.797	**0.293***
$BP^b_{S.D}(n) = \beta^b_{S.D} \times [TTP(n)\ RR(n)]$	-0.833±5.101	0.420*	-0.794±7.878	0.249*
$BP^c_{S.D}(n) = \beta^c_{S.D} \times [TTP(n)\ PA(n)\ RR(n)]$	-0.650±5.108	**0.438***	-0.712±7.909	0.248*

* p-value < 0.05. **p-value <0.001

The results of the BP estimation obtained, although they could be significantly improved, represent a good estimate of blood pressure (error <± 10%) according to AAMI criteria. It was found that RR is more related to the BP_{dias} than PA and TTP. Although, better results were obtained by including them all in the model. On the other hand, PA is more related to the BP_{sys} than PTT and RR, but PA together with the TTP in a linear model presented better results in the BP_{sys} estimation.

I.II.III. Study 2

The objective of this study is to compare PTT and PW as estimators of blood pressure based on the relationship between BP and pulse wave velocity. PW is more sensitive to changes in Systemic Vascular Resistance (SVR) than other indices of pulse wave [166]. The SVR is determined from changes in artery diameter or in blood viscosity. Changes in PW provide valuable evidence with respect to changes in pulse wave velocity too.

The data was collected during a tilt table test from 16 volunteers (10 male and 6 female), aged 28.5 ± 2.5 years, per the following protocol: 4 mins in early supine position, 5 mins tilted head-up in a 70-degree angle, and 4 mins back to later supine position. The table takes 18 seconds to tilt during transitions. The table takes 18 seconds to tilt during transitions (see Figure **I.IV**).

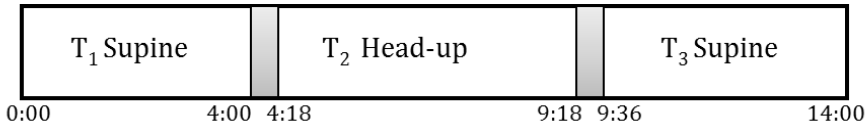

Figure I.IV: Tilt Test Protocol. Table takes 18 seconds to tilt during transitions, marked as filled area.

The PPG signal was recorded from the index finger by Biopac OXYI00C with a sampling rate of 250 Hz. The ECG lead V4 was recorded by Biopac ECG 100C with a sampling rate of 1000 Hz. The BP signal was recorded with a sampling rate of 250 Hz by Finometer system. Further detail on materials and method are in [108].

An illustration of the relationship between PTT, PW, BP_{sys} and BP_{dias} is given in Figure **I.V**, which shows the whole experiment values of these features for each subject.

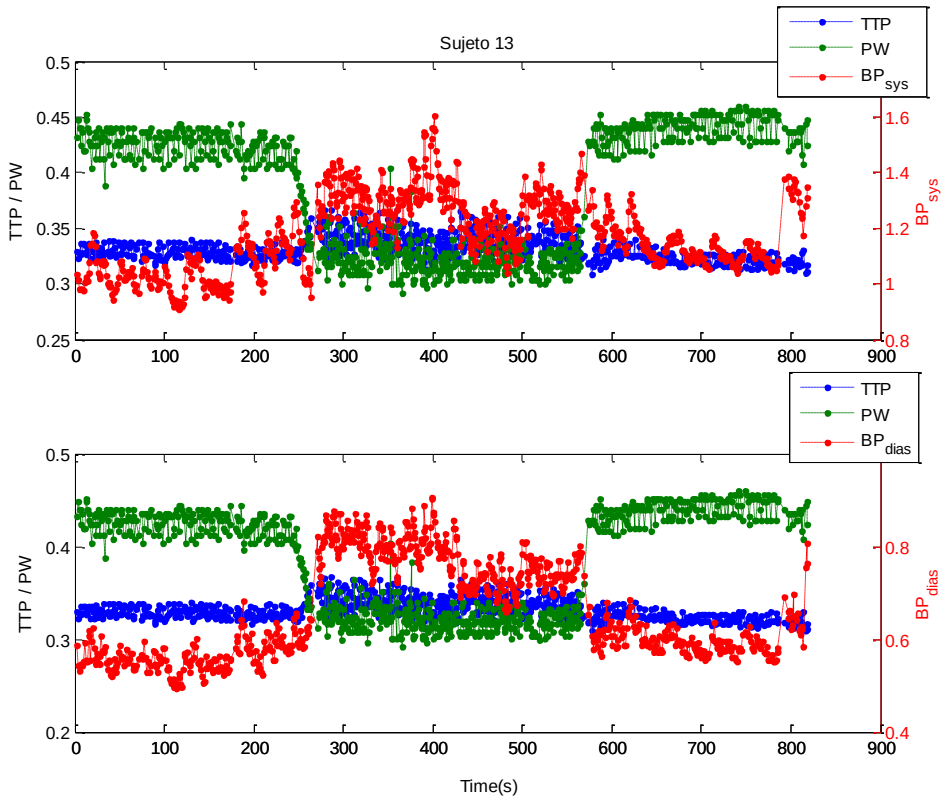

Figure I.V: An example of TTP, PW series related to BP_{sys} and BP_{dias} along the Tilt Table Test.

The calibration coefficients, β (see equation (**I**.)) were estimated for each subject through the linear regression method, taking as reference some points in the BP_{sys} (n) and BP_{dias} (n), respectively. The coefficient selection was studied using different sets of reference points: a range of 25 consecutive beats extracted from TJ Supine, T2 Head-up, T3 Supine and Transition positions, or 5 non-consecutive beats equally spaced extracted from: TJ Supine, T2 Head-up, T3 Supine and Transition positions, or during the whole measurement interval.

Global estimation results are given for the proposed linear models. The calibration coefficients were obtained using 5 equally-spaced beats selected from the whole measurement interval. This set of reference points was the ones that showed better result. The absolute error

mean, its deviations, and the linear Pearson correlation between measured and estimated BP_{sys} and BP_{dias} for models d, e and f are respectively presented in Table **I.IV**.

Table I.IV: Mean absolute error and standard deviation ($E_A \pm SD$) and mean correlations between estimated and measured BP in Study 2.

	$\boldsymbol{BP_{dias}}$		$\boldsymbol{BP_{sys}}$	
Model	$\boldsymbol{E_A \pm SD}$ (mmHg)	$\boldsymbol{C_{Corr}}$	$\boldsymbol{E_A \pm SD}$ (mmHg)	$\boldsymbol{C_{Corr}}$
$BP^a_{S.D}(n) = \beta^d_{S.D} \times [TTP(n)\ 1]$	2.80±6.74	0.240 *	4.40±10.16	0.290**
$BP^b_{S.D}(n) = \beta^e_{S.D} \times [Pw(n)\ 1]$	2.41±5.58	0.403*	3.25±9.47	0.383*
$BP^c_{S.D}(n) = \beta^f_{S.D} \times [TTP(n)\ PW(n)\ 1]$	2.16±5.99	0.504 **	2.72±9.20	0.509**

* p-value < 0.05. **p-value <0.001

The intra-subject mean in the differences of BP at T1 Supine and T2 Head-up positions was 14 mmHg for the measured BP_{dias} and it was 16mmHg for the estimated BP_{dias} (BP^c_D). Variations in PW induced by the tilt of the table are more significant than variations in PTT. The intra-subject mean of PW variation between T1 Supine and T2 Head-up position was 18.7%, while the intra-subject mean of PTT variation between the same positions was 2.8 %.

Figure **I.VI** shows the results from a participant, whose measured and estimated BP_{dias} and BP_{sys} during the complete study and in a time window of 145 seconds are exhibited. It shows that the estimation is able to follow the fine details and it is better in a LF band than in others.

In this study, BP was estimated from PTT and PW using linear models during a tilt-table test. It can be concluded that both parameters can be used to estimate BP, but PW is better than PTT to detect pressure variations due to postural change. However, together they are a promising tool to monitor beat-to-beat systolic and diastolic BP, even in situations with large BP variations (approx. 15 mmHg), such as the tilt table test.

Additionally, the mean estimation error meets AAMI criteria as well as the standard deviation, except for BP_{sys} **,** which was very close to meeting AAMI criteria. The correlation between estimated and recorded series was significant.

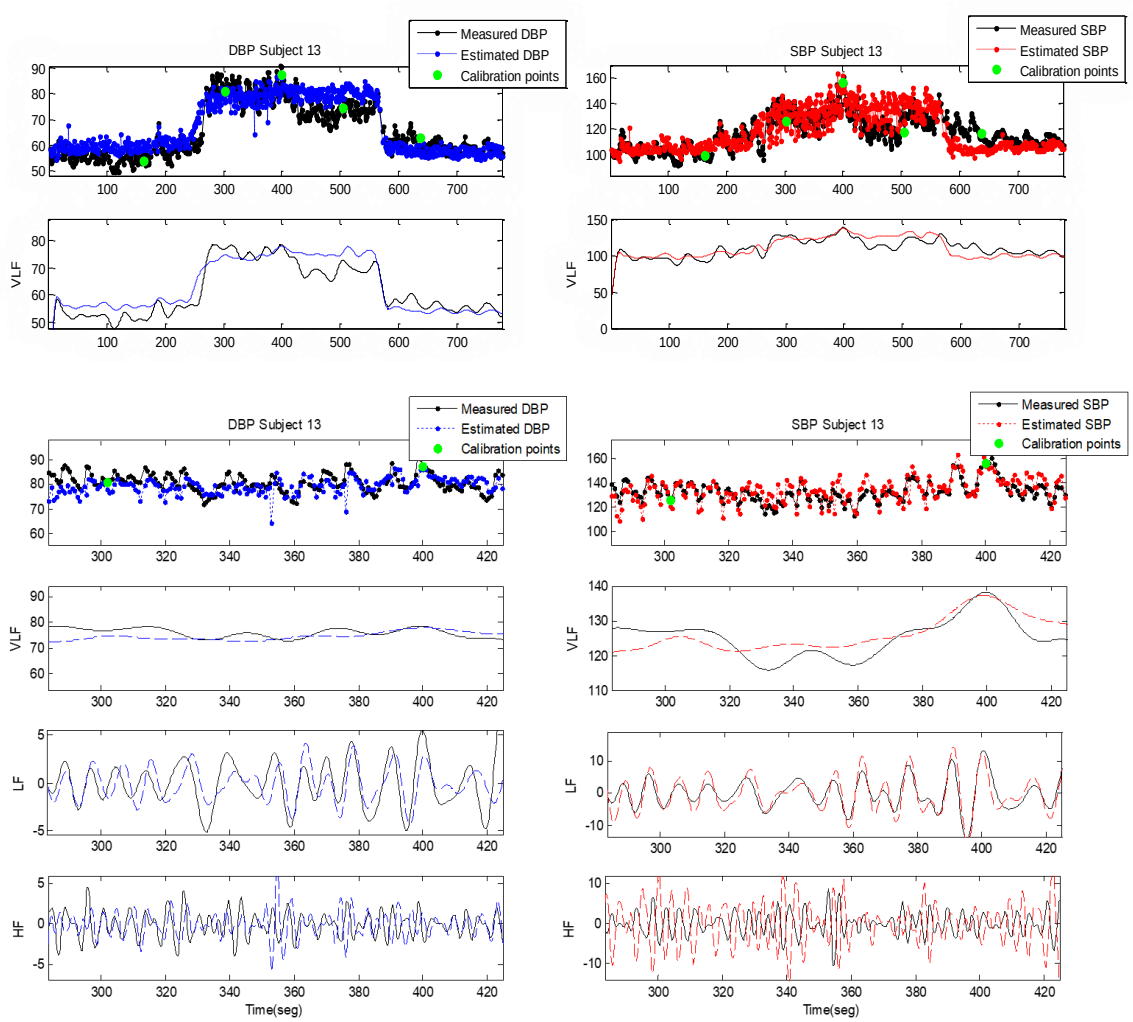

Figure I.VI: An example of measured and estimated BP_{dias} and BP_{sys} and filtered signal in the frequency bands.

I.III Conclusion: Study 1 and Study 2

Two different scenarios were studied: one included pediatric patients hospitalized in an ICU and the other one included healthy young subjects who went through the Table Tilt Test. Different types of BP sensors were used in both studies. An intraarterial BP catheter that provided direct measures of BP was employed in Study 1; while a non-invasive beat-to-beat BP sensor system that measured BP indirectly and on peripherical arteries was used in Study 2. Even though the studies performed had their differences, common conclusions were drawn, as follows:

- There is a linear relationship between the presented features (i.e. PTT, PA, HR and PW) and both BP_{dias} and BP_{sys}.

- BP_{dias} and BP_{sys} estimation can be done by combining the beat-to-beat series of TTP, PA, PW and HR in a linear model. This model must be calibrated for each individual.
- Estimation errors meet the AAMI standard, for short periods of time. When calibrations are made from intraarterial BP signal estimation errors were lower ($E_{BP_{dias}} = -0.650 \pm 5.108$, $E_{BP_{sys}} = -0.315 \pm 7.797$) than calibrating from peripheral noninvasive BP measure, ($E_{BP_{dias}} = 2.16 \pm 5.99$, $E_{BP_{sys}} = 2.72 \pm 9.20$) .
- The accuracy of the BP reference measurement contributed to the quality of the estimations.
- The proposed models are linear, and they characterize the system for specific conditions and they are also able to detect beat-to-beat variation of BP in short periods of time but they are not capable of detecting large changes in BP over prolonged periods of time.

A limitation of both studies was the short periods of time analysed which did not allow to make BP estimations over prolonged periods.

Summarizing: the estimation of PB can be done using features from ECG and PPG but it needs frequent calibration from an accurate BP reference measurement per individual (i.e. every 10-15 minutes). However, the occurrence of small variations of BP in short periods of time can be detected and followed using these estimation models. Thus, continued measurement of BP variations can be done using some features (i.e. PTT, PA, FC and PW) from non-invasive ECG and PPG measurements.

II PATIENT EMPOWERMENT AND STRESS REDUCTION

Stress measurement could be used as a tool to obtain feedback of either assorted types of treatment, diverse protocols for mental diseases or on other situations in which it could improve the patients' mental health and wellness. Nowadays some surgery protocols include Patient Empowerment as a key factor to enhance the mental state of the patient to achieve better surgical results. Since 2011, the "Fast Track Prosthetic Knee Project" at the Clinic Hospital of Barcelona (Spain) includes an educational session as patient empowerment before to a knee arthroplasty surgery [167].

Patient Empowerment is defined as the act of self-regulation. It may be induced through an educational session to promote the patient's individual potential in order to maximize health and wellness. Before a surgery, a large number of patients suffer psychological pre-operative distress (negative psychological stress) and it is proven that those factor are counterproductive for a fast and efficient surgery recovery [168]. It has been proven that the success of surgeries is affected by the patient's state of mental wellness before surgery and during the recovery process [169,167]. Therefore, Patient Empowerment improves the results and satisfaction of patients, who are going to have a total prosthesis in lower limb.

A study on Patient Empowerment was done during the educational sessions regularly performed at the Clinic Hospital of Barcelona. The main objective of this study was to evidence the effectiveness of the Empowerment Session through markers of psychological stress measured before and after an educational session. The study was developed under the hypothesis that the referred patients had certain psychological stress level generated by a surgery scheduled for the next two to three weeks.

A send objective was to provide more insight into the study of the response triggered by another type of stressor using the same framework

II.I Participants, Study Protocols and Methods

This study included 32 volunteers randomly selected that were knee osteoarthritis patients (25 women and 7 men) and with an average age of 72.25 years (range 64-90). The majority (80%-95%) were not active workers and were married or widowed, thus, in general, it was a socio-demographically homogenous group. The evolution of the participants' psychological stress was assessed during the educational session.

Differences in psychological stress before and after the educational session were analysed from psychometric tests (i.e. STAI-s and VASS) and heart rate variability (HRV) features as possible markers of psychological stress.

The presented study is experimental, prospective and observational. Data were collected according to the following protocol: two sets of psychological stress markers were collected from each patient, before the Empowerment Session (Pre-Session) and after the Empowerment Session (Post-Session). The data for each set was collected through a 10-minute electrocardiogram (ECG) recorded while patients were seated and, afterwards, two standard psychometric tests were administrated.

Additionally, patients were asked about whether or not they felt stressed and the ones that did feel stressed were asked whether or not this stress was related to the upcoming surgery. According to their answers, participants were divided into three groups: 1) those who referred not being stressed - due to the surgery (7 patients), hereafter are called Not-Stressed; 2) those that referred being stressed but not related to the surgery, henceforth are called Not Surgery-Related Stressed (6 patients) and 3) those that referred being stressed due to the surgery, henceforth are called Surgery-Related Stressed.

ECG was recorded using the Medicom system, ABP-10 module (Medicom MTD Ltd, Russia), the previous introduced in Section **4.1.4**. Features from heart rate variability (HRV) analysis were obtained as was presented in Section **5.3**.

II.II Results of the Empowerment Session

The mean and standard deviation of the data collected from each psychometric test applied before the session (Pre-session) and after (Post-session), and also the sum of both tests (Total) are shown in Table **II.I**. Each of them showed a decrease after the session.

Grouping patients according to their self-perceived stress before the Empowerment Session, allowed to observed that the means of the Not-Stressed group remained in the same range

before and after the session and that they were lower than the means of the Surgery-Related Stressed group at Pre-Session. The variation of the mean of the Total values at Pre-Session and Post-Session was much bigger in the Surgery-Related Stressed group (around 53.4%) than in the Not-Stressed group (9.3%). The Not Surgery-Related Stressed group reported a variation of 19.2 % in the mean of the Total values at Pre-Session and Post-Session.

Table II.I: Mean and standard deviation (SD) of the psychometric test by stress referring before and after educational session.

	Test	**All**	**Not-stressed**	**Stressed (not Surgery Related)**	**Stressed (Surgery Related)**
Pre-Session	STAI- state (60 au)	18.25 (13.8)	14.20 (12.21)	17.00 (16.7)	19.45 (13.95)
	VASS (100 au)	40.46 (25.04)	24.40 (16.94)	28.20 (21.61)	46.90 (25.32)
	Total^{+} (160 au)	58.71 (36.19)	38.6 (27.5)	45.2 (31.1)	66.36 (38.2)
Post-Session	STAI- state (60 au)	11.37 (11.5)**	10.8 (10.8)	10.4 (10.9)	11.72 (12.31)**
	VASS (100 au)	20.87 (17.24)**	24.20 (16.53)	19.60 (21.23)	20.40 (17.26)**
	Total^{+} (60 au)	32.25 (27.08)**	35.0 (26.2)	36.5 (32.4)*	30.91 (27.5)**

+Sum of both psychometric test values; * p-value<0.001 Wilcoxon signed rank test between Pre and Post session values.

After the Empowerment Session, these three groups had mean values in the same range, as shown in Figure **II.I**, indicating that the emotional state of all patients was quite similar after the Empowerment Session. But, at Pre-Session, the mean of each group had different values. Additionally, the differences between the psychometric test values before and after the educational session were statistically significant only in the Surgery-Related Stressed group. These results suggest the effectiveness of the Empowerment Session, mainly in patients of the Surgery-Related Stressed group.

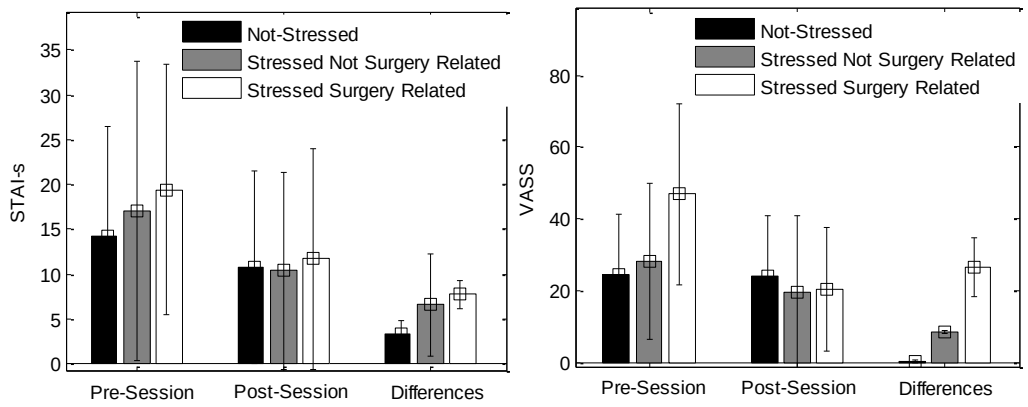

Figure II.I: Mean values of STAI-s and VASS scales by stress referring before The Empowerment Session

Table **II.II** shows the Pre-Session and Post-Session inter-patient means of HRV features before and after the educational session and by group of patients as previously defined. The results showed that there were statistically significant changes in HR_{mean}, RR_{rMSSD}, RR_{rMSSD} and HR_{PVLF} after the Empowerment Session. LF power shows significant differences in the Surgery-Related Stressed group, which could be associated with a decrease of the sympathetic activity after the session.

Table II.II: Median and median absolute deviation (MAD) of the HRV-features by stress referring before and after The Empowerment Session.

	Feature	All	Not-Stressed	Stressed (not Surgery Related)	Stressed (Surgery Related)
Pre-Session	HR_{mean} *(bpm)*	68.93(6.43)	60.62(1.63)	71.52(0.51)	72.63(7.96)
	$RR_{SDNN}(s)$	0.04(0.02)	0.04(0.02)	0.04(0)	0.04(0.02)
	$RR_{rMSSD}(s)$	0.88(0.09)	0.99(0.02)	0.84(0.01)	0.83(0.09)
	HR_{PVLF} *(s^{-2})*	1.71(0.3)	1.34(0.07)	1.84(0.05)	1.90(0.45)
	HR_{PLF} *(10^{-3} s^{-2})*	0.07(0.04)	0.03(0.01)	0.08(0.04)	0.08(0.05)
	HR_{PHF} *(10^{-3} s^{-2})*	0.16(0.09)	0.14(0.02)	0.11(0.05)	0.19(0.12)
	$HR_{LF/HF}$ *(au.)*	0.56(0.34)	0.12(0.12)	0.7(0.24)	0.63(0.26)
Post-Session	HR_{mean} *(bpm)*	74.01(8.02)**	64.22(2.66)	73.93(3.28)	79.37(6.57)**
	$RR_{SDNN}(s)$	0.03(0.01)*	0.04(0.02)*	0.03(0.01)**	0.04(0.02)
	$RR_{rMSSD}(s)$	0.81(0.08)**	0.94(0.04)	0.81(0.04)*	0.77(0.07)**
	HR_{PVLF} *(s^{-2})*	1.99(0.41)**	1.49(0.11)	1.98(0.17)	2.23(0.44)**
	HR_{PLF} *(10^{-3} s^{-2})*	0.07(0.04)	0.04(0.03)	0.09(0.06)	0.07(0.05)
	HR_{PHF} *(10^{-3} s^{-2})*	0.14(0.09)	0.31(0.17)	0.15(0.09)	0.14(0.09)
	$HR_{LF/HF}$ *(au.)*	0.53(0.26)	0.04(0.01)	0.51(0.12)*	0.64(0.27)

*p <0.05, ** p-value<0.001 Wilcoxon signed rank test for median changes between Pre and Post session values;

Results concerning HR_{PHF} and $HR_{LF/HF}$ are interesting to note even the small sample size of the Not-Stressed and Not Surgery-Related Stressed groups in this study.

The HR_{PHF} and $HR_{LF/HF}$ values at Pre-Session and Post-Session, and the difference of both measurements are represented in Figure **II.II**, grouping patients according to their self-perceived stress.

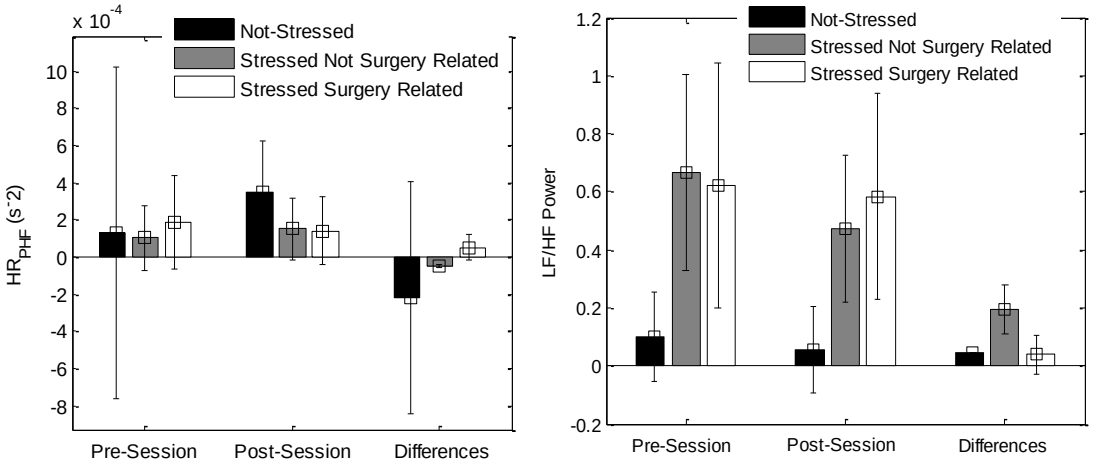

Figure II.II: Mean values of HR_{PHF} and $HR_{LF/HF}$ by stress referring groups before The Empowerment Session.

Another interesting result is the fact that $HR_{LF/HF}$ values are much higher for the Stressed groups than for the Not-Stressed group at both Pre-Session and Post-Session regardless of the cause of their stress (the stressor agent). Also, after the Empowerment Session only the Not Surgery-Related Stressed group decreased significantly $HR_{LF/HF}$ values.

The two results presented above are not conclusive due to the small sample size limitation in this study, but it could be further analysed.

The correlation between each psychometrics scale and each HR-index was computed (see Table II.III). Some HR-features, like LF, Power HF and LF/HF did not have significant linear correlation with the results of the psychometric tests, but they also did not present significant value changes after the session. The ones that really had significant value changes after the Empowerment Session were those related to the psychometric test values.

Table II.III: Pearson correlation and p-value of psychometric tests and HRV-features

	STAI-s	**VASS**	**Total**
HR_{mean}	-0.263 *	-0.260 *	-0.277 *
RR_{SDNN}	-	0.283 *	0.260 *
RR_{rMSSD}	0.282 *	0.289 *	0.305 *
HR_{PVLF}	-0.249 *	-0.251 *	-0.266 *
HR_{PLF}	-	-	-
HR_{PHF}	-	-	-
$HR_{LF/HF}$	-	-	-

*Significant values p <0.05

II.III Conclusion

The psychometric stress markers showed a stress reduction of 16.54% according to the total stress scale value, and a 45.06% of stress reduction according to the initial test values after an Empowerment Session was held. This prove that the patients had a different stress state before and after the Empowerment Session.

The variation of HR_{mean}, RR_{SDNN}, RR_{rMSSD}, HR_{PVLF} between Pre-Session and Post-Session was statistically significant. Moreover, there is significant linear correlation between these HRV-features and psychological stress markers. The results allow inferring that the variation of HR_{mean}, RR_{SDNN}, RR_{rMSSD}, HR_{PVLF} was associated to the emotional state in these specific conditions.

A not statistically proven result suggests that HR_{PHF} has possibilities as a marker to evaluate the effectiveness of an Empowerment Session in patients who refer being stressed by an upcoming surgery.

There are two main limitations in regard to this study. Firstly, the ECG recordings were performed without control on whether the patients were speaking or not, which could affect the results of HR_{PLF}. Secondly, the small sample size of the Not-Stressed and Not Surgery-Related Stressed groups limits the statistical analysis by groups self-assessing their surgery-related stress. However, the higher state of patient's wellness suggests that the effectiveness of an Empowerment Session could be assessed through physiological and psychological markers, as the ones used, in this study.

III EFFECTS OF LIMB MOVEMENTS AND POSITIONS IN PULSE TRANSIT TIME

The principal motivation of this work was to identify changes on features of the pulse wave due to body movements that provoke changes in hydrostatic pressure [150] and that, consequently, provoke changes on the arterial blood pressure wave. Additionally, movement's effect was compared in two PPG sensor placements: the finger or mid-arm.

Accordingly, a short study was carried out to find out the effect of different limb movements and positions (see Figure **III.I**.) on pulse transit time (PTT), which was one of the most promising stress markers found in the main experiment previously presented.

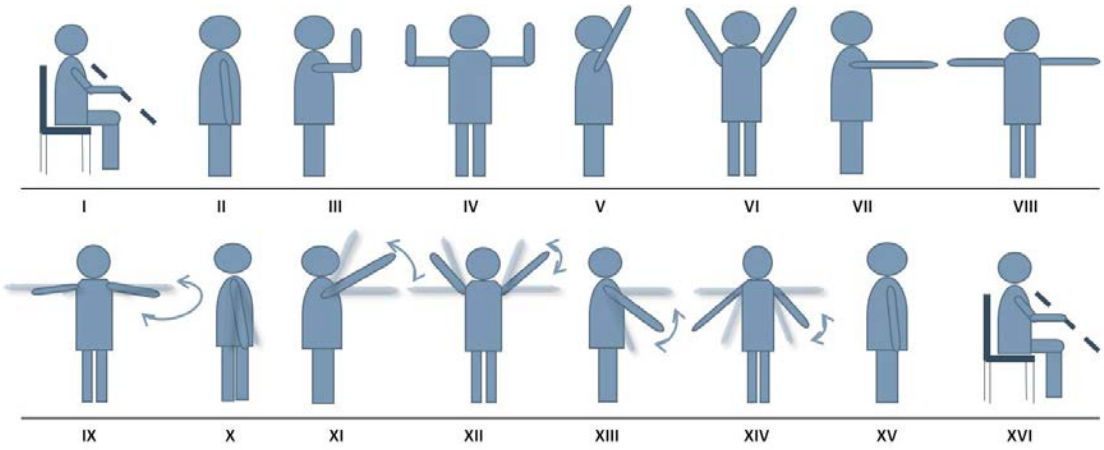

Figure III.I: The different positions and movement tested on the experiment

III.I Participants, Study Protocol and Methods

The study was performed on 11 volunteers that were students of the ESL. The average age of the volunteers was 28 years (range 23-35). None of the subjects had been diagnosed with any chronic disease or psychopathology. They were non-regular consumers of alcohol or tobacco,

with a body mass index lower than 30. Subjects were instructed to take and hold 16 different positions as it is shown in Figure **III.I**. The subjects were instructed to remain at each position for 30 seconds. The first two and last two positions (I-II and XV-XVI) were the same, sitting with the hands resting on a table and standing up respectively. From positions IX to XIV the subjects were asked to move their arms rhythmically as shown in Figure **III.I**.

For each subject two consecutive records were done, Test A and Test B. In Test A, the PPG sensor was placed mid-arm of the left arm as shown in Figure **III.II**. In Test B, the PPG sensor was placed on the index finger of the left hand. Also, a band with the sensors' board was placed around the upper arm or the wrist, in such a way that 3D-acceleration and 3D gyroscope sensors were always as shown in Figure **III.II**.

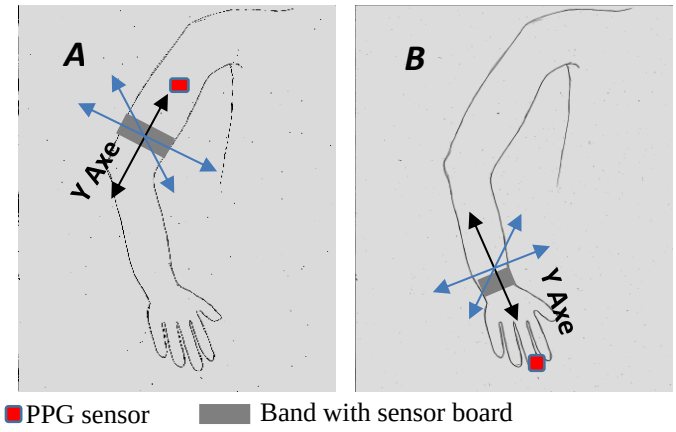

Figure III.II: Body placement of PPG sensor and custom board with 3D accelerometer on the arm in Test A and on the finger in Test B.

The measurements were performed with a custom design board (see Figure **III.III**), which has 3 integrated sensors. The ECG sensor has a proprietary design by SmatCardia [147] while the PPG and the 3D-acceleration and 3D gyroscope are off-the-shelf components. The model LSM330DLC was used to sensor 3D-acceleration and 3D gyroscope. While the PPG sensor was the standard module Nonin's OEM III Module. Two different PPG sensors were used: a reflectance sensor (model 8000R) and a finger clip (Model 8000AA) in Test A and Test B respectively.

The hardware and the core of the firmware were by the Embedded Systems Laboratory (ESL) of the l'Ecole Polytechnique Federale de Lausanne (EPFL) and SmartCardia [147]. The on-board processing was implemented in C, to integrate the ECG and PPG signals, adding 3D-acceleration and 3D gyroscope signals to detect the measures affected by the movement in real time.

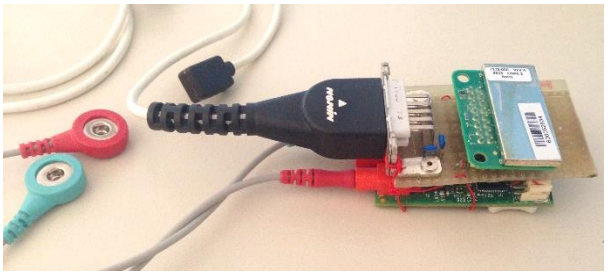

Figure III.III: Custom board with PPG module sensor by Nonin.

The PPG sensor sampling frequency was 75 Hz, and a 3-axis accelerometer data at 50 Hz; while ECG was sampled at 250Hz. The PTT series were obtained as was explained in Section **5.3**. The 3-axis accelerometer and gyroscope data were used to track the arm movement by a developed algorithm. The accelerometer is configured with a range is ±2g, this translates to 0.016g per count.

III.II Results

The obtained median and 25th, 75th percentiles of PTT on each position adopted in Test A and Test B are shown in Figure **III.IV**. The positions were changed every 30 seconds. It can be observed how PPT values changed in both Tests, due to the positions. It can also be appreciated in the figure how PTT_{finger} (Test B) is more sensible to movements than PTT_{arm} (Test A). The PTT values at position VI and XI are the highest, where the position adopted was arms extended out to the side.

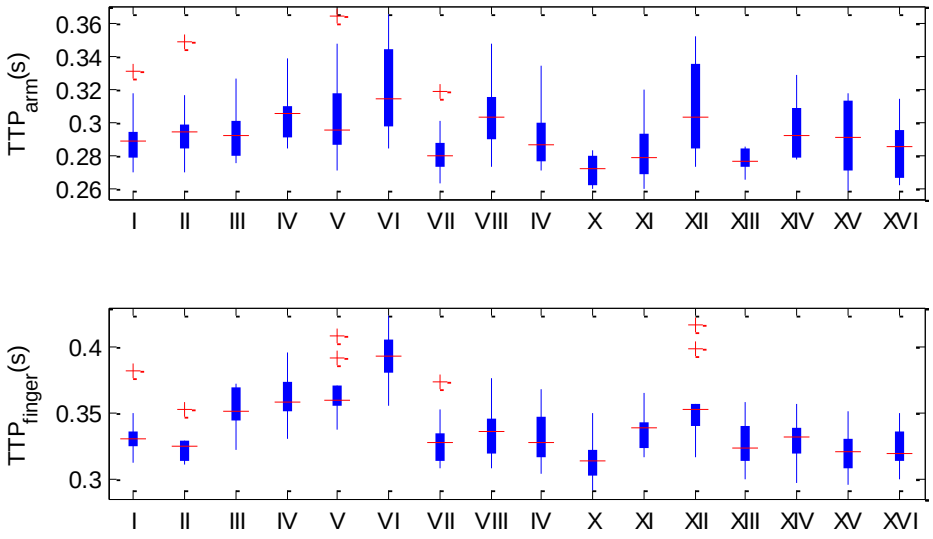

Figure III.IV: The median and 25th, 75th percentiles of PTT on each position in Test A and Test B.

The different heights of the arms respect to the heart (i.e. Up, Levelled, Down, Middle-Up) and the position in which the arms were moving rhythmically are represented in Figure **III.VII** to a better understanding .

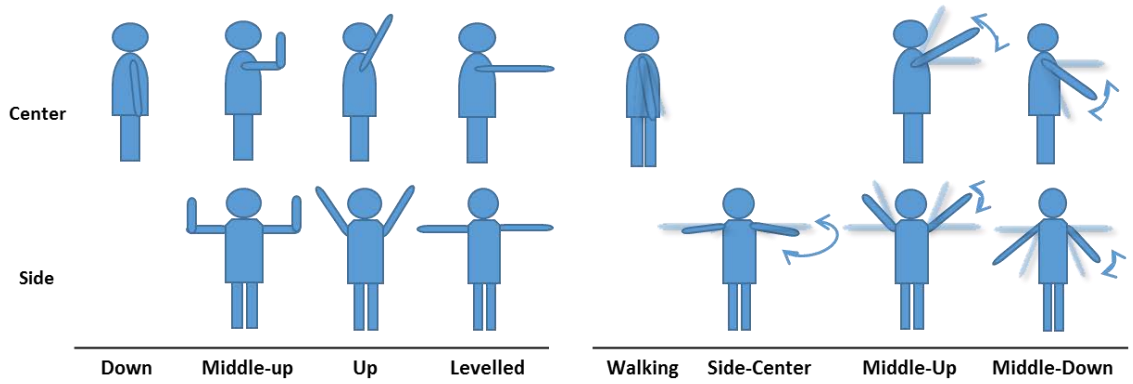

Figure III.V: Representation of the labelled arms' position and movements

Inter-subject mean percent of variation between the first position (P-I) and the positions that did not involve rhythmical movements of the arms are given in in **Test A** and 1-9 % in Test B. The PTT values in Test B (from the finger) were more dependent to movements and positions than in Test A (from the arm), accounting an increase of 50%

Table III.I. The PTT values, in Test B where the positions were centered, changed 10.34% from heart level to arms up, while with the arms extended out to the sides this change was 18.17%. The same results were found in Test A, but just with lower percentage change due to the placement of the sensor on the body which was less affected by the hydrostatic pressure.

An unexpected result was that from Center to Side positions (see Figure **III.VII**) median PTT differences increased around 3-8 % in Test A and 1-9 % in Test B. The PTT values in Test B (from the finger) were more dependent to movements and positions than in Test A (from the arm), accounting an increase of 50%

Table III.I: Inter-subject mean percent of variation respect to position I

		Test B (finger)		**Test A (arm)**	
		Center	**Side**	**Center**	**Side**
Still positions	**Up**	10.34 (V)	18.17 (VI)	4.01	8.44
	Levelled	-1.15 (VII)	0.77 (VIII)	-2.97	4.56
	Down	-4.09 (V)	-	-0.11	-
	Middle-up	5.84 (III)	9.26 (IV)	0.39	4.64

(*) Indicated the position; Up, Levelled, Down, Middle represents de different high of the arms; Center and Side represented the position of the arm as position VII and VIII.

APPENDIX III

A representation of obtained PTT series, 3 axes accelometer and gyroscope signals and hand tracking. during Test B can be found in Figure **III.VI**.

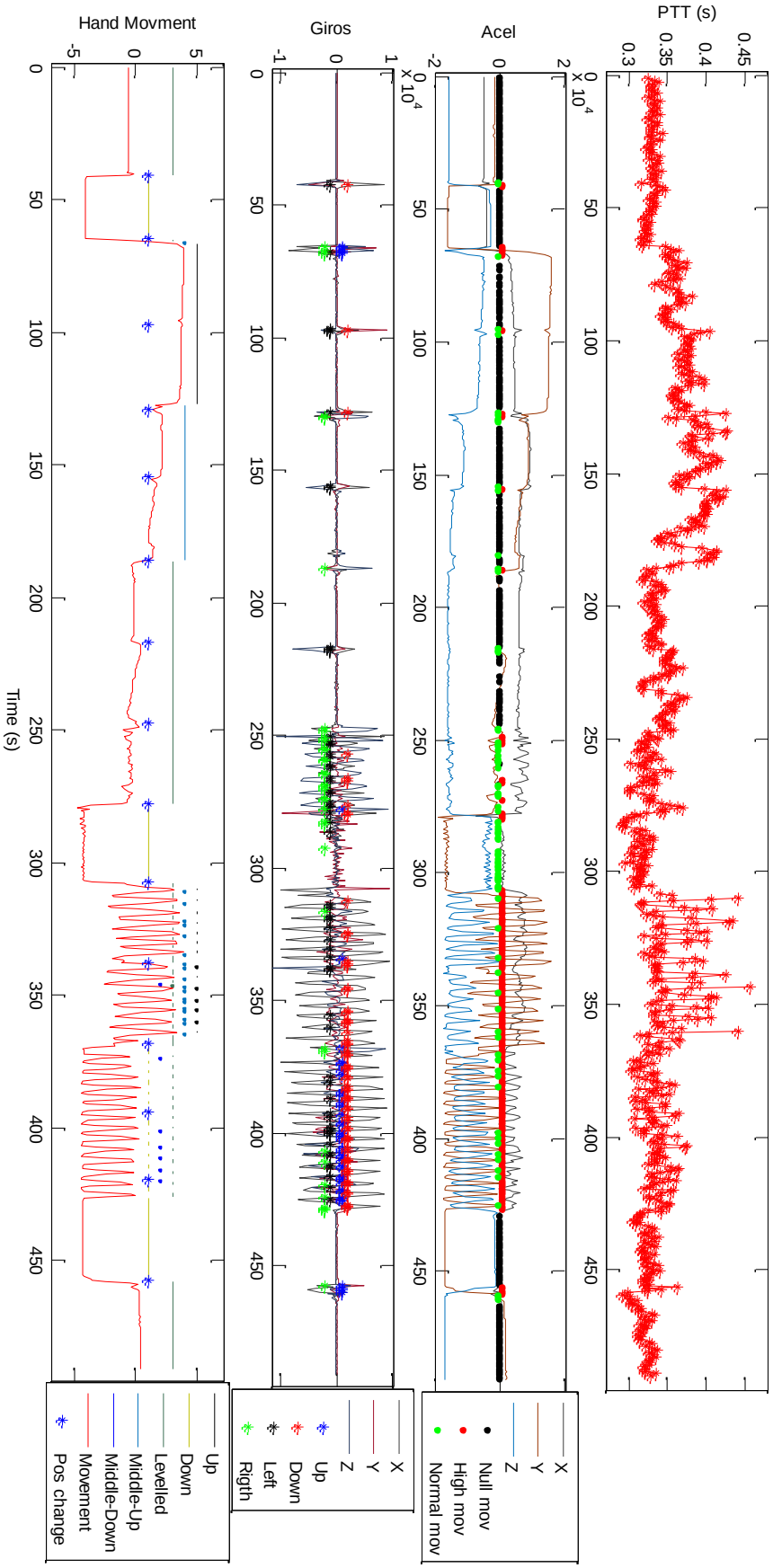

Figure III.VI: An example of obtained PTT series, 3 axes accelometer and gyroscope signals and hand tracking.

APPENDIX III

Obtained PTT series and computed arm movement tracking are also represented in Figure **III.VII** for the positions where the arms were moving rhythmically at different heights (i.e. XI-VV). The PPT variations according to arm movements and changes between the Middle-Up and Middle-Down movement are clearly seen.

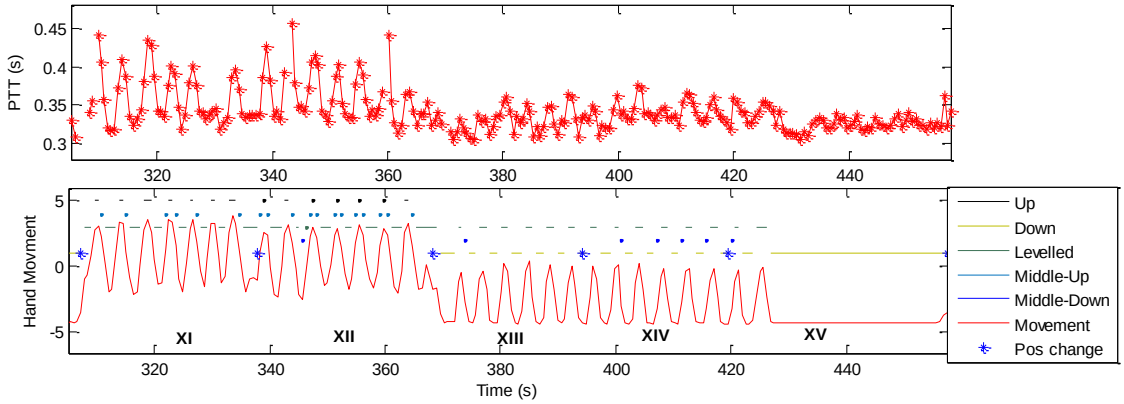

Figure III.VII: A representation of PTT variations for arm movement in Test B from positions XI to XV.

All subjects' PTT values in both Tests are plotted in the graphs shown in Figure **III.VIII** It also shows that PTT measurements from the finger were more dependent to movements and positions than from the mid-arm. However, the measurements of pulse wave were more artefacted in Test A (arm) than in Test B (finger) due to the type of sensor and its body placement. Test A was performed with a reflectance sensor, while Test B was performed with a transmission sensor attached to a finger clip. The first type of sensor was more sensible to small movements. Also, the mid-arm is not as vascularized as the distal phalanx of the finger.

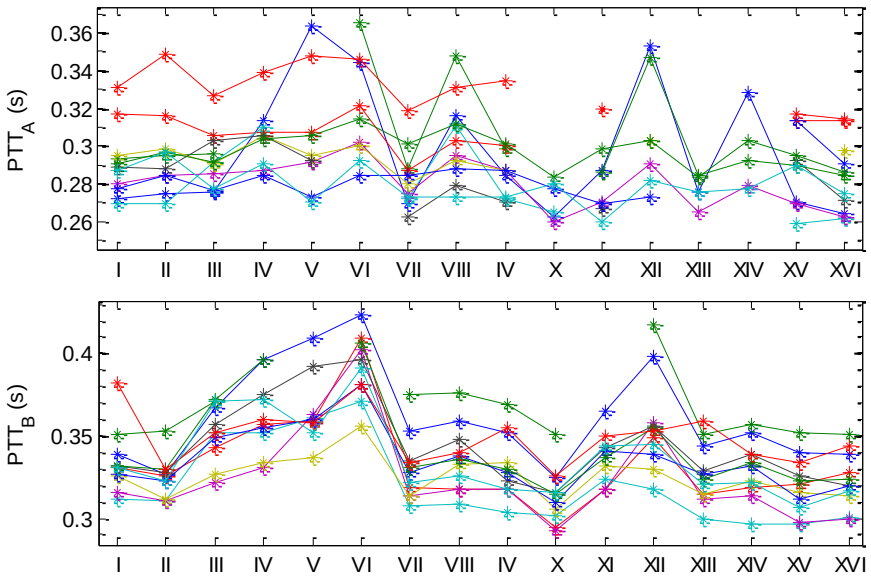

Figure III.VIII: Median PTT values of all subject at each position on Test A and Test B.

III.III Conclusion

From these results, it can be concluded that reliable measurements of cardiovascular system changes (e.g. due to stress) through the pulse wave, and more specifically of PTT, will be obtained in some positions of the arms. A future line of work would be to correct these values in terms of position and movement when PPG is measured on a limb.

The results show that there are not only changes in the PTT at different arm heights but also in having them totally extended or more centered When comparing both measurement configurations, it was found that the arm measurement is less susceptible to changes in position and movements than the finger.

The differences between both used sensor types and placement was also noticed, specific application assessment of its advantages and disadvantages has to be done depending on its uses. The integration of the ECG and PPG signals into a wearable device must be done by adding accelerometers to correct the measures affected by movement.

IV COMMERCIAL WEARABLE DEVICES FOR PHYSIOLOGICAL MONITORING

Angel

Tel Aviv, Israel. (Crowdfunding) http://www.angelsensor.com

-Physiology signals
Heart rate
Skin temperature
Blood oxygen (SpO2)
- Physical activity (such as calories, steps, sleep and activity modes)

Embrace

Empatica Inc. https://support.empatica.com/

-Physiology signals:
Electrothermal activity
Temperature
-Motion sensors : 3-Axis accelerometer
Gyroscop
- Bluetooth Low Energy Smart®

E4 GSR Wristband

Empatica Icn. https://support.empatica.com/

-Physiology signals:
Photoplethysmography
Electrodermal activity
Temperature
- Continuous, real-time data
- Motion sensors : 3-axis accelerometer data
- CE Medical (class 2a).

INYU

SmartCardia http://www.smartcardia.com/inyu/

-Physiology signals:
Electrocardiogram
Breathing Rate,
Skin conductance
- Motion sensors: 3-axis accelerometer data
- Emotional Levels

Qardiocore,

https://www.getqardio.com/

-Physiology signals:
EKG/ECG
Respiratory Rate
Temperature
Galvanic Skin Response
- fs200Hz , resolution 16 bit, band width 0.05-40Hz

-Activity Tracking

Simband

Samsum https://www.simband.io/

-Physiology signals: PPG, ,bio-impedance,GSR, Skin temperature, ECG lead
- 3D accelerometer

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