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A Framework for Objective Mental Health Assessment Using Physiological Data: From Stress Dynamics to Depression

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A Framework for Objective Mental Health Assessment Using Physiological Data: From Stress Dynamics to Depression

Doctoral thesis submitted in the PhD Program of Electronic and Telecommunication Engineering by the Autonomous University of Barcelona

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Un Marco Para La Evaluación Objetiva De La Salud Mental Utilizando Datos Fisiológicos: De La Dinámica Del Estrés A La Depresión

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UNIVERSIDAD AUTÓNOMA DE BARCELONA

TESIS DOCTORAL

Un Marco Para La Evaluación Objetiva De La Salud Mental Utilizando Datos Fisiológicos: De La Dinámica Del Estrés A La Depresión

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que la Memoria de *Tesis UN MARCO PARA LA EVALUACIÓN OBJETIVA DE LA SALUD MENTAL UTILIZANDO DATOS FISIOLÓGICOS: DE LA DINÁMICA DEL ESTRÉS A LA DEPRESIÓN* presentada por Esther García Pagès para optar al título de Doctora en Ingeniería Electrónica y de Telecomunicación por la Universidad Autónoma de Barcelona se ha realizado bajo su dirección en el Grupo de Biomonitorización GBIO (CIBER-BBN) del Departamento de Microelectrónica y Sistemas Electrónicos de la Universidad Autónoma de Barcelona.

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Table of Contents

List of acronyms 1

List of figures 3

List of tables 5

Abstract and Main Conclusions 7

Resumen y Conclusiones Principales 9

I. Introduction 11

1. Mental health 13

1.1. Mental health and stress 14

1.2. Diathesis-stress model 14

1.3. Stress sensitization model 15

2. Pathophysiology of stress 17

2.1. Stress response 17

2.1.1. Central nervous system 18

2.1.2. Hypothalamic–pituitary–adrenal axis 18

2.1.3. Autonomic nervous system 19

2.2. Stress-related illnesses 20

2.3. Dysfunctional emotion regulation 21

3. Major depressive disorder 23

3.1. Disorder course and burden 25

3.2. Treatment methods 26

3.3. Cognitive function 27

3.4. Diagnosis and follow-up 28

4. Biomarkers in mental health assessment 31

4.1. Physiological biomarkers 31

4.1.1. Electrocardiogram 31

4.1.2. Photoplethysmogram 33

4.1.3. Electrodermal activity 34

4.1.4. Respiratory signal 35

4.1.5. Temperature 38

4.2. Physiological stress dynamics 38

5. Objectives and outline of the thesis 41

II. Physiologic stress dynamics for mental health assessment 45

6. Methodology for signals processing 47

6.1. Electrocardiogram 47

6.2. Photoplethysmogram 50

6.3. Pulse arrival time 51

6.4. Electrodermal activity 52

6.5. Respiratory signal 54

6.6. Skin temperature 55
6.7. Final set of features 56

7. Physiological dynamics in stress 57
7.1. Methods 59
7.1.1. Participants 59
7.1.2. Experimental protocol 60
7.1.3. Outcome measures 61
7.1.3.1. Psychometric tests 61
7.1.3.2. Biochemical variables 62
7.1.3.3. Physiological signals 63
7.1.4. Statistical Analysis 64
7.2. Results 64
7.2.1. Participants 64
7.2.2. Outcome measures 65
7.2.2.1. Psychometrics tests 65
7.2.2.2. Biochemical variables 67
7.2.2.3. Physiological signals 67
7.2.3. Stress reference scale 72
7.2.4. Physiological stress model 74
7.3. Discussion 77
7.3.1. Artificial stress environment protocol 77
7.3.2. Reference scale for stress measurement 78
7.3.3. Noninvasive stress measurement 79
7.4. Limitations 82

8. Physiological dynamics in major depressive disorder 82
8.1. Methods 84
8.1.1. Participants 84
8.1.2. Experimental protocol 84
8.1.3. Outcome measures 85
8.1.3.1. Psychometric tests 85
8.1.3.2. Cognitive tests 85
8.1.3.3. Biochemical variables 86
8.1.3.4. Physiological signals 86
8.1.4. Statistical Analysis 87
8.2. Results 88
8.2.1. Participants 88
8.2.2. Psychometric evaluation 90
8.2.3. Static levels of physiological features 92
8.2.4. Dynamics of physiological features 94
8.2.5. MDD model 96
8.3. Discussion 98
8.3.1. Cognitive stress protocol in MDD 99
8.3.2. Predictive outcomes 99
8.3.2.1. Neuropsychological assessment 100
8.3.2.2. Stress dynamics assessment 101
8.3.3. Noninvasive MDD model 104

8.4. Limitations 105

III. Protocol design to assess mental well-being and distress *107*

9. Protocol for physiological dynamics assessment in mental health **109**

9.1. Methods 111

9.1.1. Study design and setting 111

9.1.2. Participants and eligibility criteria 112

9.1.3. Recruitment 113

9.1.4. Study procedure 113

9.1.5. Study variables 115

9.1.6. Physiological variables 117

9.1.7. Statistical analysis plan 118

9.1.8. Sample size 119

9.1.9. Ethical considerations 119

9.2. Results 120

9.3. Discussion 120

IV. Conclusions and future work *123*

10. Summary and final discussion **125**

11. Strengths and limitations **129**

12. Main conclusion **129**

13. Future work **130**

V. Appendix *133*

14. Scientific contributions **135**

14.1. Journal publications 135

14.2. Conference publications 137

15. Acknowledgments **139**

16. References **141**

17. Supplementary information **175**

List of acronyms

ACTH - Adrenocorticotropic Hormone

ANS - Autonomic Nervous System

AT - Arithmetic Task

BD - Bipolar Disorder

BDI - Beck Depression Inventory-II

BP - Blood Pressure

BR - Baseline Relax

BS - Baseline Stress

CBT - Cognitive Behavioral Therapy

CNS - Central Nervous System

CGI - Clinical Global Impression

CRH - Corticotropin-Releasing Hormone

DALYs - Disability-adjusted life years

DSM-V - Diagnostic and Statistical Manual of Mental Disorders, 5th edition

ECG - Electrocardiogram

EDA - Electrodermal Activity

FFT - Fast Fourier Transform

GCs - Glucocorticoids

HC - Healthy controls

HDRS - Hamilton Depression Rating Scale

HPA - Hypothalamic-Pituitary-Adrenal axis

HR - Heart Rate

HRV - Heart Rate Variability

IS - Immune System

MDD - Major Depressive Disorder

mHealth - Mobile Health

MT - Memory Task

NN - Normal-to-Normal Intervals

OCD - Obsessive-Compulsive Disorder

PCA - Principal Components Analysis

PEP - Pre-Ejection Period

PNS - Parasympathetic Nervous System

PPG - Photoplethysmogram

PRV - Pulse Rate Variability

PSM - Physiological Stress Model

PSS - Perceived Stress Scale

RMTs - Remote Measurement Technologies

Resp - Respiration

SA - Stress Anticipation

SA - Sinoatrial node

SCL - Skin conductance level

SCR - Skin conductance response

SCWT - Stroop Color and Word Test

SMNA - Sudomotor Nerves Activity

SNS - Sympathetic Nervous System

SRS - Stress Reference Scale

SSS - Symptomatic Stress Scale

ST - Story Telling

T - Skin Temperature

STAI - State-Trait Anxiety Inventory

SUD - Substance Use Disorders (SUD)

TMT - Trail Making Test

TSST - Trier Social Stress Test

VASS - Visual Analog Stress Scale

VD - Video segments

WHO - World Health Organization

List of figures

Figure 1: Stress-diathesis model interaction with stress sensitization model 16
Figure 2. Role of cortisol in the stress response 18
Figure 3. Autonomic nervous system regulation 20
Figure 4. Diagram of the hypotheses to explain major depressive disorder pathogenesis 24
Figure 5. Electrical conduction system of the heart 32
Figure 6. Recording of a photoplethysmogram 33
Figure 7. Eccrine sweat glands diagram and electrodermal activity signal 35
Figure 8. Respiratory system and modulations of an electrocardiogram signal 37
Figure 9. Inverted-U model for stress reactivity 39
Figure 10. Power spectral density analysis of heart rate variability 49
Figure 11. Photoplethysmography signal processing techniques 50
Figure 12. ECG and PPG signals for pulse arrival time calculation 52
Figure 13. Electrodermal activity signal processing techniques 53
Figure 14. Respiratory signal processing technique 54
Figure 15. Experimental design for physiological dynamics in stress 61
Figure 16. Experimental design for physiological dynamics in depression 85
Figure 17. Comparison of the evolution of the features between depressive and healthy individuals 93
Figure 18. Major depressive disorder model 97
Figure 19. M&M study experimental procedure outline 114

List of tables

Table 1. Summary table of physiological features ____ 56
Table 2. List of extracted features for physiological dynamics in stress ____ 63
Table 3. Sociodemographic data of the healthy students ____ 65
Table 4. Psychometric test results of the healthy students ____ 66
Table 5. Biochemical variable results of the healthy students ____ 67
Table 6. Physiological features of the healthy students ____ 68
Table 7. Eigenvalues and explained variances of PCA of healthy students' data ____ 73
Table 8. Scoring coefficients of healthy students' data ____ 73
Table 9. Stress reference scale results ____ 73
Table 10. Eigenvalues and explained variances of PCA of healthy students' physiological data ____ 75
Table 11. Scoring coefficients of healthy students' physiological data ____ 75
Table 12. Physiological stress model results ____ 76
Table 13. List of extracted features for physiological dynamics in depression ____ 87
Table 14. Demographics and clinical characteristics of the MDD patients and healthy controls ____ 89
Table 15. Psychometric evaluation results of the MDD patients and healthy controls ____ 91
Table 16. Dynamics of physiological features and BDI correlations ____ 95
Table 17. MDD model parameter estimates ____ 96
Table 18. Participants inclusion-exclusion criteria in M&M study ____ 112
Table 19. List of features for physiological dynamics assessment in M&M study ____ 117

Abstract and Main Conclusions

This thesis investigates the dynamics of physiological stress responses and their implications for mental health, with a primary focus on Major Depressive Disorder (MDD). The research encompasses the conception, development, application, and validation of predictive models for the objective and non-invasive evaluation of mental health, applicable for both diagnosis and monitoring.

The first part of this dissertation provides a theoretical framework for investigating the pathophysiology of stress and its role in the development and assessment of mental illnesses such as depression. Stress is an adaptive physiological response activated in situations perceived as threatening. However, when this response is frequently activated, persists for prolonged periods, or does not adequately adjust to the stimulus, it can become a pathological condition linked to various diseases. Thus, the importance of mental health research is emphasized, highlighting the complexities involved in objective measurement. The introduction outlines the challenges in psychiatry, where current assessment methods are largely subjective and prone to bias. Consequently, the state-of-the-art review presented in the introduction delves into the potential of ANS biomarkers, derived from physiological signals, as non-invasive and objective tools for mental health evaluation. Various signals are covered, including electrocardiogram, photoplethysmography, electrodermal activity, respiration, and temperature, discussing their relevance and application.

The primary objective of this thesis is to evaluate stress dynamics using these physiological data and to develop an objective tool to assess the severity of depression. The second part describes the signal processing techniques used to derive ANS biomarkers and the two experimental studies conducted to achieve this objective.

The first study evaluated ANS biomarkers in a healthy population of 120 students. To establish a quantitative reference supported by the usual gold standards, a Stress Reference Scale was first designed by combining scores from self-reported psychometric tests and biochemical stress markers. Subsequently, a regression model based on physiological biomarkers was developed, showing a high correlation with the reference scale, thereby enabling continuous and non-invasive stress assessment. These findings demonstrate the ability of ANS biomarkers to evaluate various types of stress and highlight their potential for depression assessment.

This potential was studied in the second study, involving 40 patients diagnosed with MDD and their respective controls. The role of stress dynamics in measuring depression severity was examined, comparing the ANS function between patients and healthy subjects during a cognitive stress protocol. The analysis included baseline values of the previously studied biomarkers and autonomic reactivity indices during cognitive load. The results indicate that depression is associated with autonomic imbalance, characterized by reduced sympathetic

reactivity and a greater delay in returning to baseline conditions. The multimodal ANS reactivity model demonstrates high diagnostic performance achieving an accuracy of 78%.

The third part focuses on proposing ongoing research to objectively evaluate mental health through a research protocol that automates the evaluation process, accessible remotely and effective for clinical and epidemiological use. A protocol was designed to develop and validate a tool incorporating physiological and cognitive variables to assess stress reactivity. The ongoing multicentric observational study includes young adults aged 18 to 34, classified based on their mental well-being.

Finally, the relevance of this thesis, along with a summary of the main conclusions and future work, are rationalized in the fourth part. The results of this work support the importance of individual variability in stress reactivity and recovery for mental health assessment, proposing objective and noninvasive diagnostic and monitoring strategies. The need to adapt this framework to clinical practice is emphasized, validating the results with larger and more diverse samples, investigating variability among depression subtypes, and conducting prospective studies to better understand dysfunctions in stress dynamics. Additionally, future research is proposed to develop methods for obtaining robust physiological biomarkers, integrating passive data from wearables to improve precision and patient adherence.

Resumen y Conclusiones Principales

Esta tesis investiga la dinámica de la respuesta fisiológica al estrés y sus implicaciones para la salud mental, con un enfoque principal en el Trastorno Depresivo Mayor (MDD). La investigación abarca la concepción, desarrollo, aplicación y validación de modelos predictivos para la evaluación objetiva y no invasiva de la salud mental, aplicables tanto para el diagnóstico como para el seguimiento.

La primera parte de esta memoria proporciona un marco teórico para investigar la fisiopatología del estrés y su papel en el desarrollo y valoración de enfermedades mentales como la depresión. El estrés es una respuesta fisiológica adaptativa que se activa ante situaciones percibidas como amenazantes. Sin embargo, cuando esta respuesta se activa con frecuencia, persiste durante períodos prolongados o no se ajusta adecuadamente al estímulo, puede convertirse en una condición patológica vinculada a diversas enfermedades. Por ello, se subraya la relevancia de la investigación en salud mental y las complejidades de la medición objetiva. La introducción destaca los desafíos en psiquiatría, donde los métodos de evaluación actuales son subjetivos y propensos a sesgos. Por este motivo, la revisión del estado del arte presentada en la introducción profundiza en el potencial de los biomarcadores del ANS, derivados de señales fisiológicas, como herramientas no invasivas y objetivas para la evaluación de la salud mental. Se cubren diversas señales, incluyendo el electrocardiograma, la fotopletismografía, la actividad electrodérmica, la respiración y la temperatura, discutiendo su relevancia y aplicación.

El objetivo principal de la tesis es evaluar la dinámica del estrés utilizando estos datos fisiológicos y desarrollar una herramienta objetiva para evaluar la gravedad de la depresión. En la segunda parte se describen las técnicas de procesamiento de señales utilizadas para derivar los biomarcadores del ANS y los dos estudios experimentales realizados para alcanzar este objetivo.

El primer estudio evaluó los biomarcadores del ANS en una población sana de 120 estudiantes. Con el fin de disponer inicialmente de una referencia cuantitativa soportada por los gold standard habituales, en primer lugar, se diseñó una Escala de Referencia de Estrés combinando puntajes de pruebas psicométricas autorreportadas y marcadores bioquímicos de estrés. Posteriormente, se desarrolló un modelo de regresión basado en los biomarcadores fisiológicos, mostrando una alta correlación con la escala de referencia, permitiendo así una evaluación objetiva y no invasiva del estrés. Estos hallazgos demuestran la capacidad de los biomarcadores del ANS para evaluar varios tipos de estrés y arrojar luz sobre su potencial para la evaluación de la depresión.

Este potencial de valoración se investigó y se desarrolló en el segundo estudio, con 40 pacientes diagnosticados con MDD y sus respectivos controles. Se examinó el rol de la dinámica del estrés en la medición de la gravedad de la depresión, comparando la función del ANS entre

pacientes y sujetos sanos durante un protocolo de estrés cognitivo. El análisis incluyó los valores basales de los biomarcadores anteriormente estudiados, así como los índices de reactividad autonómica durante la carga cognitiva. Los resultados indican que la depresión se asocia con un desequilibrio autonómico, caracterizado por una reactividad simpática reducida y un mayor retraso en el retorno a las condiciones basales. El modelo multimodal de reactividad del ANS demuestra un alto rendimiento diagnóstico logrando una precisión del 78%.

La tercera parte se centra en proponer una investigación continua para evaluar objetivamente la salud mental mediante un protocolo de investigación que automatice el proceso de evaluación, accesible de forma remota y eficaz para uso clínico y epidemiológico. Se diseñó un protocolo para desarrollar y validar una herramienta que incorpora variables fisiológicas y cognitivas para evaluar la dinámica del estrés. El estudio observacional multicéntrico, actualmente en curso, incluye jóvenes de 18 a 34 años, clasificados según su bienestar mental.

Finalmente, la relevancia de esta tesis, así como un resumen de las principales conclusiones y el trabajo futuro se racionalizan en la cuarta parte. Los resultados de este trabajo respaldan la importancia de la variabilidad individual en la reactividad y recuperación del estrés para la evaluación de la salud mental, proponiendo estrategias diagnósticas y de seguimiento objetivas y no invasivas. Se enfatiza la necesidad de adaptar este marco a la práctica clínica, validando los resultados con muestras más amplias y diversas, investigando la variabilidad entre subtipos de depresión y realizando estudios prospectivos para entender mejor las disfunciones en la dinámica del estrés. Además, se proponen futuras investigaciones para desarrollar métodos para obtener biomarcadores fisiológicos robustos, integrando datos pasivos de wearables para mejorar la precisión y adherencia del paciente.

I. Introduction

This introductory part aims to address the context and the state of the art on mental health research and emphasize the relevance of research in this field. It also introduces the stress-diathesis and sensitization models and discusses how they relate to the development of mental disorders.

The body's stress response, the stress-related disorders, and the role that dysfunctional emotion regulation plays in mental health illnesses are all covered in the second section, which explores the pathophysiology of stress.

Major Depressive Disorder (MDD), one of the most prevalent and crippling mental health illnesses, is the subject of the third segment. It covers the nature and severity of the illness, how it affects cognitive function, how it is now diagnosed and screened, and what are the main treatments available.

The applicability of noninvasive biomarkers in mental health is then introduced in the fourth and final segment of this introduction. An overview of physiological signals is given, along with a discussion of the different potential biomarkers and their relationship with health and disease.

Table of contents

1. Mental health ***13***

2. Pathophysiology of stress ***17***

3. Major depressive disorder ***23***

4. Biomarkers in mental health assessment ***31***

5. Objectives and outline of the thesis ***41***

1. Mental health

According to the World Health Organization (WHO), complete mental health is more than just the absence of psychopathological symptoms, mental health is a condition of well-being in which a person recognizes their own potential, is able to handle everyday stressors, can work effectively, and is able to give back to their community [1].

Mental health is essential for socioeconomic, communal, and personal growth. At the beginning of the century, Major Depressive Disorder (MDD), Schizophrenia (SCZ), Bipolar Disorder (BD), Substance Use Disorders (SUD), and Obsessive-Compulsive Disorders (OCD) collectively made up five of the top ten causes of disability globally. Approximately 400 million individuals worldwide experienced mental or neurological illnesses. Still, more than 20 years later, there is a great need for mental health services worldwide, and yet the available resources are insufficient even though the prevalence of these disorders keeps increasing [2].

In 2019, the three disorders that accounted for the largest global burden of disability were MDD, with 280 million cases, alcohol, or SUD, with more than 160 million cases, and SCZ, with 23 million cases [3]. Every year, suicide claims the lives of around 900,000 people. Moreover, mental illnesses frequently impact and are impacted by other illnesses like cancer, heart disease, and infections. For instance, there is proof that depression increases the risk of diabetes and myocardial infarction, two conditions that in turn raise the risk of depression [4]. Stress, alcohol consumption, and low socioeconomic level are only a few of the risk factors that are shared by noncommunicable diseases and mental disorders.

A crucial component of establishing the case for investing in global mental health research is assessing the economic impact of mental disease. There are multiple strategies to calculate the economic cost of mental diseases, but an approximate value was published by [5] using data from 2019. They estimated the attributable mental burden of disability-adjusted life years (DALYs) to quantify the economic burden of mental disorder assigning a monetary value to each DALY. It was projected that the economic impact would be approximately USD 4.7 trillion globally considering healthcare costs and lost productivity.

Mental health research faces several challenges. First, mental disorders are complex and multifaceted, involving a combination of biological, psychological, and environmental factors [6]. This complexity makes it difficult to pinpoint exact causes or develop effective treatments. Second, there is considerable heterogeneity within mental health disorders, meaning that two individuals with the same diagnosis can exhibit very different symptoms [7]. This variability complicates research efforts and needs the development of personalized solutions. Finally, a third challenge is that unlike many physical health conditions, there are no clear biological markers for most mental health disorders [8], making diagnosis, monitoring, and treatment even more complex. These challenges underscore the need for continued efforts in mental health

research, to improve our understanding of these disorders and develop more effective strategies for their prevention and management.

The care of mental diseases requires continuous symptom monitoring, e.g., to track response to therapy or identify early warning signals of relapse. Nowadays, this surveillance depends on self-reported surveys or clinical interviews, which are limited and prone to recall bias because they rely on people's memory of their symptoms [9]. Biomarkers to monitor mental health can not only help in monitoring mental disorders but also shed light on the pathophysiology of these conditions. For instance, studying biological markers, such as changes in brain structure or function, genetic markers, or alterations in the autonomic nervous system (ANS), can help us understand the underlying biological mechanisms of these disorders [10, 11], leading to the development of more effective treatments. Similarly, a relatively new approach is digital phenotyping, which involves using data from smartphones and wearable devices to monitor behaviors and symptoms related to mental health [12, 13]. This approach can provide insights into how these disorders manifest in everyday behaviors, contributing complementary information to clinicians for the personalization of intervention strategies.

1.1. Mental health and stress

In our fast-paced environment, mental stress is common and has a significant impact on both our physical and psychological health. People who frequently encounter stressful or unpleasant situations have a variety of physiological and/or psychological reactions that can either be beneficial to the person or detrimental.

Understanding the nature of this relationship has been a key responsibility of mental health researchers. However, the universe of stressors is made up of a wide variety of events, and in a similar vein, stressors might show up as a variety of distinct mental health effects. Furthermore, a wide range of varied elements such as social context and personal predispositions of individuals have been found to further influence how stressors are experienced and how they affect mental health. Therefore, there is a great deal of intricacy to be comprehended as a result.

1.2. Diathesis-stress model

Researchers from a variety of fields have been examining the psychopathology of socially generated stress, leading to psychological distress, psychiatric disorder symptoms, or other forms of social dysfunction or health issues for more than 20 years. To address these problems, several models of the stress process have been created [14].

A popular contemporary model is the Diathesis-Stress Model, which proposes that stress interacts with individual vulnerabilities to trigger or exacerbate mental health disorders [15]. This model emphasizes the interplay between environmental stressors and genetic, biological, or psychological predispositions.

Diathesis refers to an individual's predisposition or vulnerability to developing a mental disorder. Diathesis can be biological, such as genetic markers [16] or neurobiological abnormalities [17, 18], psychological, including personality traits or cognitive styles, or even socio-cultural, involving family history or socioeconomic status [19, 20]. These vulnerabilities do not cause mental disorders on their own but increase the risk of developing a disorder when paired with stress. People with a high level of diathesis may require only a small amount of stress to trigger a disorder, whereas those with a lower level of diathesis might only develop a disorder after facing significant stress.

Regarding stress, in the context of this model, it represents external pressures or events that challenge an individual's coping abilities. Stress can arise from a wide variety of sources, including traumatic events, significant life changes, interpersonal conflicts, or chronic adversity. The nature, intensity, and duration of stress can vary greatly among individuals.

These two components together are the bases of the diathesis-stress model, which has important implications for treatment and prevention strategies, since interventions can be tailored to be more effective and personalized reducing the impact of stressors, enhancing individuals' coping mechanisms, or addressing the underlying vulnerabilities.

1.3. Stress sensitization model

The stress sensitization model suggests that individuals who have experienced significant stress or trauma are more sensitive to the effects of later stressors [21]. This concept complements the diathesis-stress model as shown in Figure 1. It proposes a mechanism through which the initial vulnerability (diathesis) may be exacerbated or activated by subsequent stress experiences, such as childhood adversity [22], combat experiences, and sexual trauma [23], leading to a heightened stress response or the development of mental health disorders.

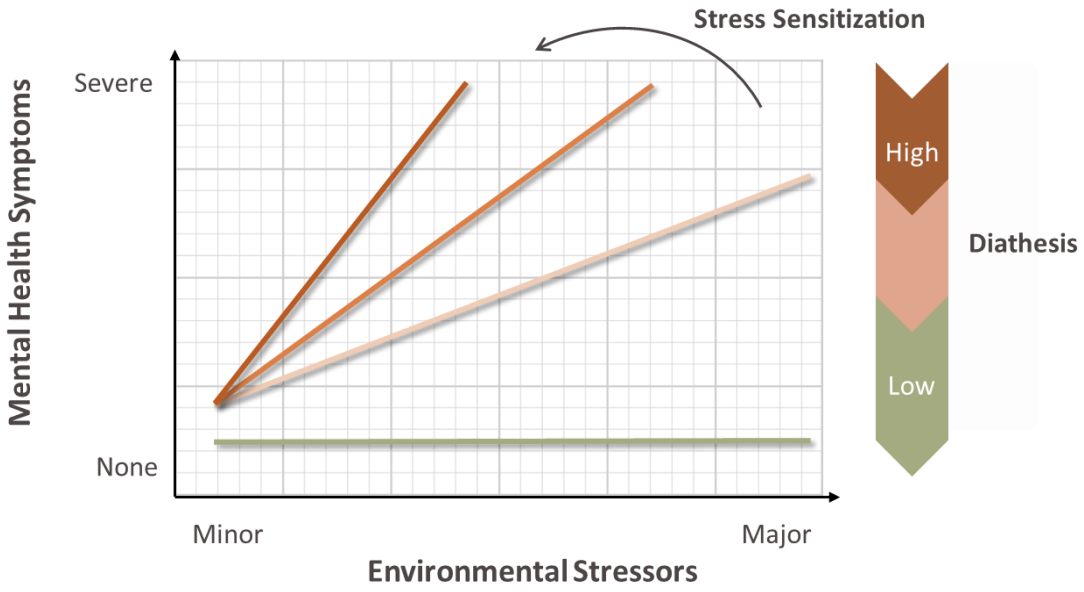
Several moderators and mechanisms within the stress sensitization model can influence an individual's response to stress and their susceptibility to mental health disorders.

Moderators such as genetic predispositions, exemplified by polymorphisms in the serotonin transporter gene (5-HTTLPR), play a crucial role in determining an individual's susceptibility to stress [24]. Additionally, neuroendocrine reactivity can modulate stress responses, while the presence and quality of social support serve as a buffer that may mitigate the effects of stress [25, 26].

Mechanisms underlying stress sensitization include neurobiological changes in critical brain areas like the hippocampus, amygdala, and prefrontal cortex, which are involved in stress regulation and emotion processing [27]. In rodent experimental studies as well as studies of suicide victims, maternal care during early development was found to shape stress reactivity through epigenetic modifications, particularly in the expression of the glucocorticoid receptor

gene in the hippocampus [28]. The findings emphasize that gene-environment interactions, particularly during early development, can lead to long-lasting changes in behavior and physiological responses to stress [29].

Figure 1: Stress-diathesis model interaction with stress sensitization model



Continuous diathesis impact modulated by stress sensitization process. Adapted from [30].

Cognitive and emotional processing mechanisms, such as heightened stress perception and rumination, further contribute to stress sensitization [31]. Emerging evidence also points to inflammatory responses as a significant pathway through which chronic stress may lead to depression and other stress-related conditions [26].

This model highlights the complexity of the stress response and underscores the necessity of multifaceted approaches to mental health treatment and prevention, which consider genetic, neurobiological, cognitive, emotional, and social factors.

2. Pathophysiology of stress

Historically, the concept of stress has evolved from being perceived merely as an external stimulus to a complex interaction between an individual and their environment, leading to a wide range of physical and psychological responses. This evolution can be traced back to 1915 when Walter Cannon introduced the "fight or flight" response, highlighting the body's immediate reaction to threatening situations [32]. The origins of the definition of stress came later from Hans Selye, who explained it as the nonspecific response of the body to any demand [33]. Stress can be understood as a physiological and psychological process that arises when demands from the environment are greater than an individual's perceived capacity for coping. He made a distinction between the acute stress and the reaction to long-term stressors, referring to the latter as the General Adaptation Syndrome. He decomposed this syndrome into three phases explaining the entire stress response: the alarm reaction, the stage of resistance and the stage of exhaustion. When faced with a stressor, the body is initially caught off guard exhibiting a “fight or flight” response. It then tries to resist the change to maintain homeostasis, but eventually resistance gradually decreases and collapses trying to combat the stressor.

2.1. Stress response

The stress response involves intricate biological pathways that bridge the perception of stressors to the body's comprehensive response [34]. At the heart of this response is the hypothalamic-pituitary-adrenal (HPA) axis, a central component of the stress system. Activation of the HPA axis leads to the release of cortisol, a glucocorticoid hormone pivotal in modulating various bodily functions to adapt to stress. Concurrently, the ANS engages, specifically the sympathetic branch, releasing catecholamines like epinephrine (adrenaline) and norepinephrine (noradrenaline), preparing the body for immediate physical action.

However, while acute stress responses can be adaptive and essential for survival, chronic stress exposure can lead to dysregulation of the HPA axis and ANS, contributing to health issues. Prolonged cortisol exposure has been implicated in altering immune function, increasing susceptibility to infections, and contributing to the development of chronic conditions such as cardiovascular disease [35], diabetes [36], and mental health disorders like depression and anxiety [37]. Furthermore, chronic stress has been shown to affect neuroplasticity, particularly within regions of the brain such as the hippocampus, prefrontal cortex, and amygdala, which are crucial for memory, cognition, and emotional regulation [38]. These changes in neuroplasticity have proven to contribute to the development of mental health disorders [18, 39].

2.1.1. Central nervous system

The central nervous system (CNS) plays a pivotal role in the stress response, integrating sensory information and coordinating the body's reaction to stressors. The amygdala, hippocampus, and prefrontal cortex, components of the limbic system, are particularly involved in processing emotional responses to stress. The amygdala triggers the initial stress response, signaling the hypothalamus to initiate the ANS and HPA axis activation. The hippocampus, associated with memory and learning, modulates the stress response based on past experiences of stress. The prefrontal cortex is responsible for executive functions and decision-making processes, including the assessment of the stressor and planning appropriate responses. Dysregulation in these areas due to chronic stress can lead to impaired cognitive functions and emotional dysregulation, exacerbating the stress response [38].

2.1.2. Hypothalamic–pituitary–adrenal axis

The HPA axis represents the basis of the body's response to stress, orchestrating a complex hormonal cascade that regulates energy distribution, immune function, and emotional state. Initiated by the perception of a stressor, the CNS stimulates the hypothalamus to release corticotropin-releasing hormone (CRH). CRH then acts on the anterior pituitary gland, prompting the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. Upon reaching the adrenal cortex, ACTH triggers the synthesis and release of cortisol, a glucocorticoid hormone with widespread effects across the body as shown in Figure 2 [40].

Figure 2. Role of cortisol in the stress response

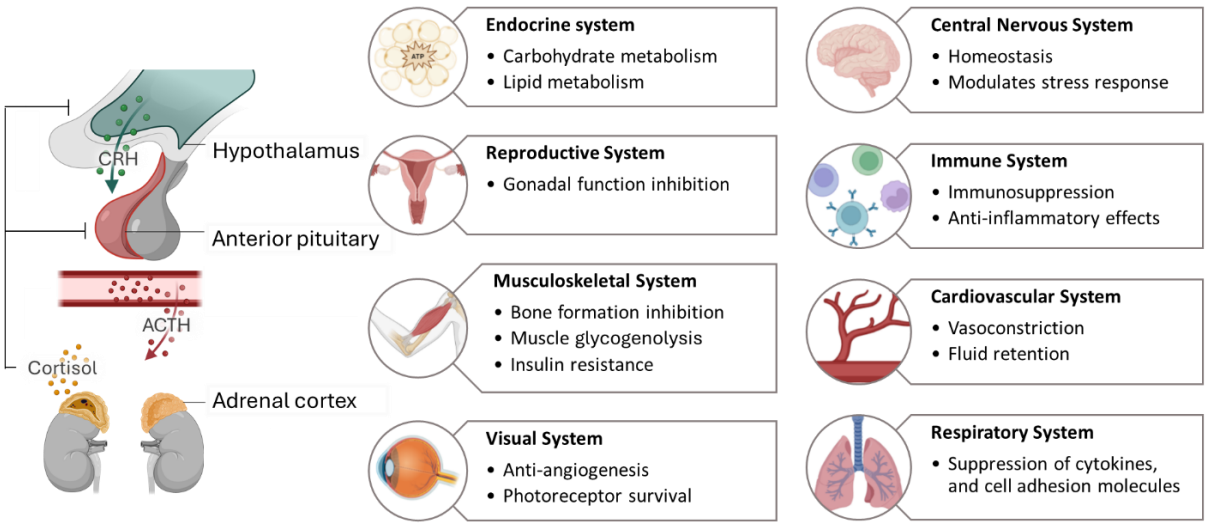


Diagram of the hypothalamic-pituitary-adrenal axis and the effects of cortisol on major organ systems in the human body. CRH: corticotropin-releasing hormone; ACTH: adrenocorticotropic hormone. Adapted from [41].

In normal conditions, cortisol follows a specific circadian pattern, governed by the central pacemaker situated in the suprachiasmatic nucleus, which triggers the HPA axis. Cortisol levels are lowest around midnight, peak in the morning, and gradually decrease to their lowest point throughout the day [42].

During stress conditions, cortisol plays a critical role in mobilizing glucose and lipid reserves for immediate energy, suppressing non-essential functions such as digestion, reproduction and bone formation, and modulating the immune response [43]. Its elevated levels also act on the brain to influence mood, motivation, and fear response, preparing the organism for the fight-or-flight decision. Crucially, cortisol exerts negative feedback on the hypothalamus and pituitary gland, reducing the production of CRH and ACTH to prevent overactivation of the stress response, a mechanism essential for restoring homeostasis once the stressor has been mitigated [40, 44].

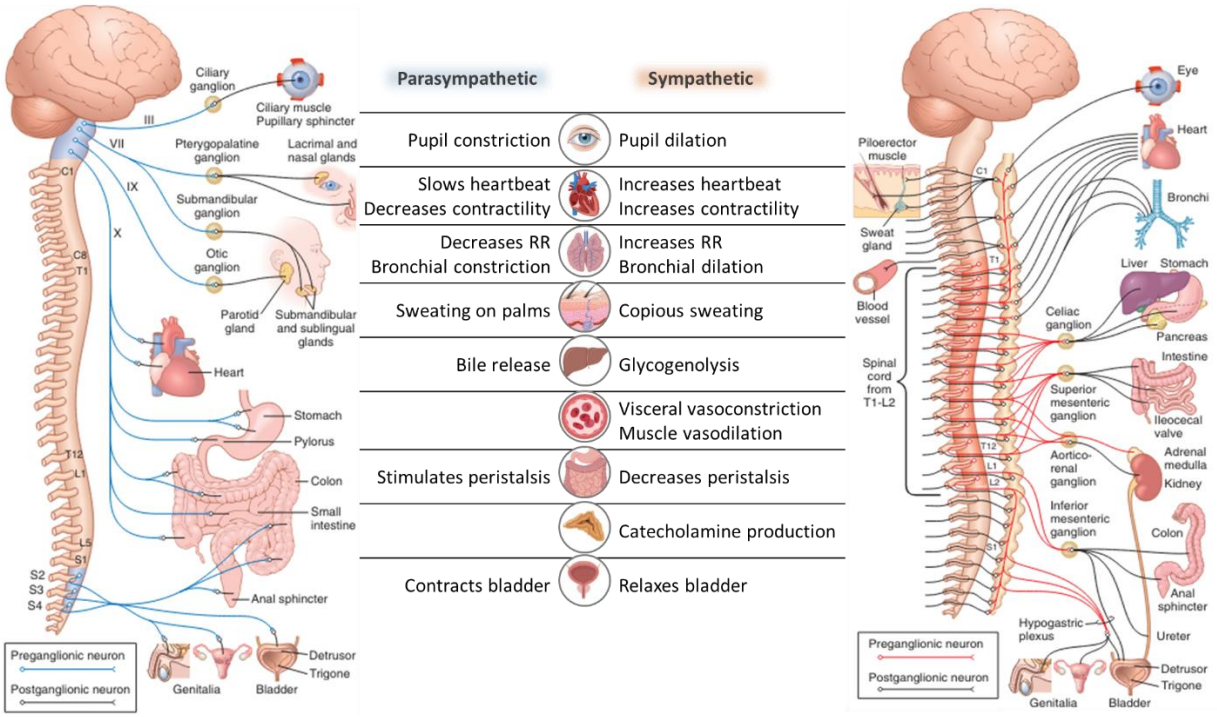
2.1.3. Autonomic nervous system

The ANS is immediately engaged upon stress perception, primarily through the activation of the sympathetic nervous system (SNS), to enact the fight-or-flight response, and inhibition of the parasympathetic nervous system (PNS), responsible for the rest-and-digest response. This rapid mobilization involves the release of catecholamines from the adrenal medulla, which serves to maximize the body's capacity to confront or flee from the perceived threat and away from non-critical functions like digestion.

The main patterns of SNS cardiovascular response to stress include increasing heart rate (HR) and contractility to boost blood circulation to vital organs, vasoconstriction reducing peripheral and visceral blood flow, and vasodilation in muscle tissue. Concurrently, the SNS stimulates dilation of the pupils for better vision, induces piloerection and sweat glands activate to cool the body, collectively known as a “cold sweat”. The respiratory rate increases, and the bronchial tree dilates to supply more oxygen to the bloodstream, enhancing the body's readiness for physical action. The digestive and salivary activities diminish to conserve energy for immediate needs. Additionally, the adrenal medulla increases the secretion of catecholamines as neuromodulators of the peripheral SNS while the kidneys adjust blood pressure (BP) by releasing renin, and the bladder’s relaxation facilitates focus on the stressor at hand. Metabolically, the body shifts towards glycogen breakdown (glycogenolysis) and potassium uptake in muscle cells, ensuring an energy reserve for physical exertion [45].

Conversely, the PNS plays a crucial role in counterbalancing the sympathetic response, promoting relaxation, and recovery processes once the stressor is removed. These activities include sexual arousal, salivation, lacrimation, urination, digestion, and defecation. The parasympathetic system's actions complement those of the SNS, underscoring the importance of a balanced autonomic response to maintain health and well-being.

Figure 3. Autonomic nervous system regulation



Parasympathetic (left) and sympathetic (right) branches of the autonomic nervous system. RR: respiratory rate. The black lines represent postganglionic fibers, and the blue/red lines show preganglionic fibers. Oculomotor nerve (III); facial nerve (VII); glossopharyngeal nerve (IX); vagus nerve (X). Spinal cord segments: cervical (C1-8), thoracic (T1-12), lumbar (L1-5), sacral (S1-4). Adapted from (Hall, 2016).

2.2. Stress-related illnesses

Stress, especially when chronicled, increases the likelihood of developing various diseases, spanning from cardiovascular or autoimmune conditions to mental health disorders. During the stress response, the heightened release of catecholamines along with adrenal glucocorticoids (GCs), triggers a shift towards catabolism. This mobilizes lipid and glucose reserves while also inducing insulin antagonism. Elevated levels of circulating catecholamines and GCs additionally stimulate heightened cardiovascular activity. These effects aim to enhance the availability and dispersion of energy sources [46]. While these responses fulfill the metabolic requirements induced by stress, prolonged exposure to elevated levels of these hormones can lead to insulin resistance [47], hypertension, hyperlipidemia, hypercholesterolemia, abdominal fat accumulation, and an augmented risk of arterial damage, all of which are correlated with an elevated risk of heart disease [48]. Moreover, prolonged stress has also been associated with an elevated risk the metabolic syndrome [49], and shortened telomere length, a marker of cellular aging and a risk factor for cardiovascular events [50].

Regarding the immune system (IS), the inflammatory activity in the body is regulated by various CNS processes, which can prepare the body for potential injury or infection before it occurs. Immune cells are redistributed and trafficked, anticipating a response to pathogens.

Moreover, social stressors such as conflict, evaluation, rejection, or exclusion can also trigger immune responses, especially when perceived as dangerous [51]. IL-1β has been hypothesized to be a key factor in adverse stress effect, as it increases levels of norepinephrine, leading to cascading effects on melatonin and cortisol, which further influence the IS. In chronic stress, high levels of GCs cause resistance to their feedback on the HPA axis, allowing proinflammatory pathways to escape normal inhibition. This creates a vicious cycle where all mediators become imbalanced, affecting the CNS, IS, and HPA axis [41]. These effects could potentially link stress to autoimmune disorders [52] and cancer by amplifying inflammation and inhibiting immunity. Furthermore, excessive stress hormones have the capability to influence tumor and stromal cells within the tumor microenvironment, thereby fostering tumor growth, invasion, and metastasis [53].

Finally, dysregulation of the HPA axis has also been implicated in the pathophysiology of various mental health disorders, including depression, anxiety, SCZ, and post-traumatic stress disorder [54, 55]. Chronic stress is known to affect neurotransmitter systems, including serotonin, dopamine, and norepinephrine, which are critical for mood regulation. Alterations in these neurotransmitter systems can contribute to the development of mood disorders such as depression and anxiety [4]. Elevated glucocorticoid levels as well as pro-inflammatory cytokines due to psychological stressors can also negatively affect neurogenesis and synaptic plasticity, thereby contributing to brain structure alterations, particularly in areas such as the prefrontal cortex and hippocampus, which are involved in executive function and emotional regulation [39, 54, 55].

Social factors also play a significant role in the development of stress-related mental health disorders. Adverse childhood experiences, such as abuse or neglect, have been shown to increase the risk of developing mental health disorders in adulthood by influencing stress response systems and brain development [56]. Additionally, chronic social stressors, such as discrimination, socioeconomic disadvantage, and interpersonal conflicts, can exacerbate stress responses and contribute to the onset and progression of mental health disorders [57]

2.3. Dysfunctional emotion regulation

A critical aspect of the stress response involves difficulties in managing and responding to emotional experiences effectively. Emotion regulation is the ability to manage and modify the occurrence, intensity, duration, and expression of emotional responses [58]. Chronic stress can impair emotion regulation strategies, leading to an over-reliance on maladaptive coping mechanisms. These maladaptive strategies are closely linked to the development of mood and anxiety disorders, as they hinder an individual's ability to cope with stress constructively [59].

Neuroimaging studies indicate that depression is associated with abnormal activation patterns in brain regions involved in emotion regulation, including the hippocampus, prefrontal cortex, particularly the subgenual anterior cingulate cortex, and amygdala [54, 58, 60]. Individuals with

depression exhibit a greater tendency to engage in maladaptive strategies such as rumination, avoidance, and suppression. Rumination perpetuates negative mood states and interferes with effective problem-solving and emotional recovery. Additionally, depressive patients often have impaired cognitive control processes, which include the ability to shift attention, inhibit negative information, and update working memory. These impairments contribute to persistent negative affect and difficulty in adopting more adaptive emotion regulation strategies [58].

This dysfunctional emotion regulation underscores the importance of stress monitoring tools for early intervention and support. Advances in digital health technologies, such as wearable sensors and mobile health (mHealth) applications, offer promising tools for real-time stress monitoring and personalized intervention strategies, potentially mitigating the impact of a dysfunctional stress response on mental health [61].

3. Major depressive disorder

MDD is identified when a person exhibits symptoms such as a low or depressed mood, anhedonia or a diminished interest in enjoyable activities, changes in appetite and/or weight, insomnia or hypersomnia, agitation or psychomotor retardation, lethargy, feelings of worthlessness or guilt, difficulty concentrating, or suicidal thoughts [62]. A common coping mechanism among depressed individuals is self-destructive conduct, that, if left untreated, can often lead to severely crippling effects. MDD lowers the quality of life by seriously impairing functioning and negatively affecting interpersonal connections.

The etiology of MDD is complex, with current models pointing to an intricate interplay among HPA axis dysregulation, neurotransmitter imbalances, inflammatory processes, genetic predispositions, neuroplasticity alterations and environmental stressors [63].

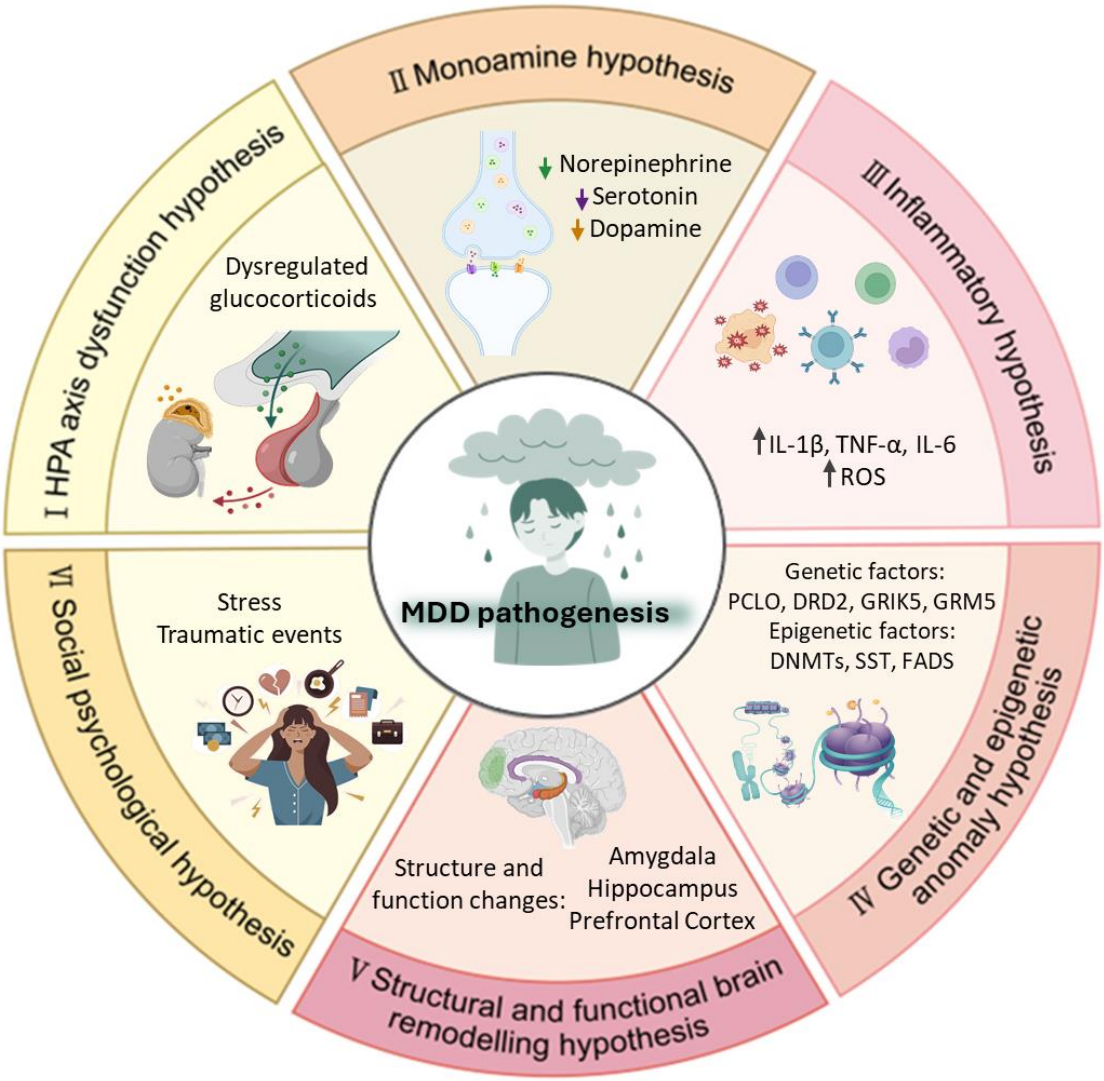
The HPA axis, integral to the body's stress response (explained in section 2.1.2), exhibits notable dysregulation in MDD, leading to unusual cortisol levels. Hypersecretion of cortisol is associated with acute and more severe subtypes of MDD. However, this elevated hormone concentration is notably absent in the atypical subtype of MDD. This dysregulation may contribute to the disorder's pathogenesis through its effects on immune function and neurobiology, further complicating the clinical picture of depression [55].

The monoamine hypothesis suggests that MDD is associated with deficiencies in monoamine neurotransmitters, including serotonin, norepinephrine, and dopamine. These neurotransmitters are critical for regulating mood, arousal, and cognition. For instance, serotonin metabolites have been discovered to be reduced in people who experience suicidal thoughts [64]. For this reason, reduced levels or impaired receptor function of these neurotransmitters are thought to contribute to the development of depressive symptoms.

Genetic studies have consistently demonstrated a heritable component to MDD, suggesting a polygenic inheritance pattern where multiple genetic variations contribute to the disorder's pathophysiology [63, 65]. Variants in genes such as presynaptic vesicle trafficking (PCLO), D2 subtype of the dopamine receptor (DRD2), glutamate ionotropic receptor kainate type subunit 5 (GRIK5), metabotropic glutamate receptor 5 (GRM5), among others, are linked to an increased risk of MDD. Moreover, epigenetic modifications, including changes in DNA methylation and histone modification, also contribute to the disorder by influencing gene expression without altering the DNA sequence, e.g. DNA methyltransferases (DNMTs), transcription levels of somatostatin (SST), fatty acid desaturase (FADS). These genetic factors likely influence the key monoamine neurotransmitter systems, which have long been implicated in mood regulation and represent primary targets for pharmacological intervention [24, 66]. Nevertheless, current hypotheses suggest that depression is mostly linked to intricate brain

circuits and neuroregulatory systems, which in turn lead to secondary disruptions of neurotransmitter systems [67].

Figure 4. Diagram of the hypotheses to explain major depressive disorder pathogenesis



Hypothesis to explain Major Depressive Disorder pathogenesis: (I) Hypothalamic–pituitary–adrenal (HPA) axis dysfunction hypothesis: elevated glucocorticoid levels. (II) Monoamine hypothesis: functional deficiency of serotonin, dopamine and norepinephrine. (III) Inflammatory hypothesis: neuroinflammation induced by reactive oxygen species (ROS) and inflammatory cytokines, interleukins (IL-1β, IL-6), and tumor necrosis factor alpha (TNF-α). (IV) Genetic and epigenetic anomaly hypothesis: certain genes are more susceptible in individuals with MDD, including those involved in presynaptic vesicle trafficking (PCLO), dopamine receptor D2 subtype (DRD2), glutamate ionotropic receptor kainate type subunit 5 (GRIK5), metabotropic glutamate receptor 5 (GRM5), DNA methyltransferases (DNMTs), transcription levels of somatostatin (SST), and fatty acid desaturase (FADS). (V) Structural and functional brain remodeling hypothesis: reduced densities of glial cells in the prefrontal cortex, hippocampus, and amygdala. (VI) Social psychological hypothesis: traumatic or stressful life events are significant risk factors. Adapted from [63].

Beyond neurotransmitter dysregulation, emerging research underscores the importance of neuroplasticity and the role of neurotrophic factors, such as brain-derived neurotrophic factor,

in MDD. Alterations in neurogenesis, particularly within the hippocampus, alongside reduced neurotrophic factor levels, have been associated with the onset and persistence of depressive symptoms, highlighting the critical role of brain structure and function in the disease [68]. Other key areas affected include the prefrontal cortex and amygdala, which are involved in mood regulation, emotional processing, and memory. Patients with MDD often show reduced volumes and altered activity in these regions. Moreover, microglial cells, the primary immune cells in the brain supporting neuronal function, become overactive in depressed individuals and this overactivation contributes to synaptic dysfunction and neuronal damage, exacerbating depression [63, 69].

The role of inflammation in MDD has gained increasing attention, with elevated pro-inflammatory cytokines observed in depressed individuals, suggesting an inflammatory component that may influence brain function and mood regulation [70]. Elevated levels of pro-inflammatory cytokines can disrupt neurotransmitter systems, particularly serotonin and dopamine pathways.

Finally, environmental and psychosocial factors, including stressful life events and adverse childhood experiences, significantly contribute to MDD's risk profile. These external stressors interact with biological vulnerabilities to precipitate the disorder, emphasizing the importance of a multifactorial approach to understanding and treating depression [71].

In sum, the pathophysiology of MDD is characterized by a complex interplay of genetic, neurobiological, and environmental factors. This multifaceted perspective underscores the necessity for integrative treatment approaches and further research aimed at elucidating the underlying mechanisms of MDD, paving the way for innovative therapeutic strategies.

3.1. Disorder course and burden

Merely depression constitutes 4.3% of the worldwide disease burden and is one of the leading causes of disability globally [72], accounting for 11% of all years lived with a disability. Women experience depression about twice as frequently as men do, and the prevalence peaks from adolescence to the third decade of life for both genders, followed by a lower peak from the forties until the sixties [73].

MDD has a pleomorphic course, with wide variations in chronicity and remission. The average length of an episode in population-based samples ranges from 13 to 30 weeks, and 70–90% of MDD patients recover in less than a year. Nonetheless, in outpatient care settings, the percentage decreases to only 25% of remitted patients after six months, and more than 50% of patients continue to have MDD after two years [74].

MDD patients may experience untreated depression episodes for 6 to 12 months. 10-15% percent of those with MDD actually take their own lives, while the remaining two-thirds ponder

suicide [67]. Although it is possible to diagnose MDD based on a single depressive episode of more than two weeks, MDD is recurrent in the majority of cases; recurrence rates are roughly 50% after the first episode, 70% after the second, and 90% after the third. Patients with moderate episodes, no psychotic symptoms, improved adherence to therapy, a robust support network, and well premorbid functioning have a good prognosis with MDD. On the contrary, a bad prognosis can be predicted if there are multiple hospitalizations, an advanced age of onset, a personality issue, and a concomitant psychiatric disease.

The relationship between the onset of MDD and other medical conditions is intricate and potentially bidirectional [63]. MDD has been related globally with a wide range of unfavorable outcomes [73], such as high rates of teen pregnancy, low education, marital disruption, and unstable employment, elevated risk of the onset, persistence, and severity of a wide range of secondary disorders, and an increased risk of early mortality from other pathologies and suicide. People who have MDD are more likely to experience co-occurring anxiety disorders [75], SUD [76], eating disorders [77] or OCD [78]. 10-15% of MDD patients go on to develop BD [79]. Moreover, medical diseases like cancer and cardiometabolic [80], neurological and inflammatory diseases can all be made worse by comorbid depression [4] which has a deleterious combined effect on general health. Depressed symptomatology is linked to lower adherence to treatments [81], worse prognosis and higher mortality rates in a variety of medical conditions [82].

3.2. Treatment methods

Pharmacotherapy and psychotherapy are the two primary initial therapeutic choices for MDD management. Various guidelines agree that medication or a combination of medication and psychotherapy should be used to treat moderate-to-severe depressive episodes [83]. Psychotherapy by itself can be used to treat a minor depressive episode in the beginning [74], but in the case of severe or resistant MDD, with acute suicidality or severe psychosis, Electroconvulsive Therapy has been found to be the most efficacious [84]. Alternative less often used treatments are repetitive transcranial magnetic stimulation and vagal nerve stimulation, deep brain stimulation, and sleep deprivation therapy, mainly applied to individuals with treatment-resistant depression [85].

The main pharmacological MDD treatments target the enhancement of monoaminergic neurotransmission, acting within the synapses, subsequently influencing molecular and cellular changes that lead to a neuroadaptive evolution eventually resulting in therapeutic effect [86]. There are several FDA-approved pharmacotherapies with similar efficacy [87], but their profiles of adverse effects vary. For this reason, Selective Serotonin Reuptake Inhibitors (SSRIs) are the first-line treatment option nowadays instead of the traditional Monoamine Oxidase Inhibitors (MAOIs). Other antidepressant types are Serotonin-Norepinephrine Reuptake Inhibitors and Enhancers, Tricyclic Antidepressants, Serotonin Modulators, Atypical Antidepressants and NMDA antagonists**.** In any case, clinicians need a comprehensive

examination of the patient and there are some guidelines for the management of pharmacotherapy based on the patient's reaction and any side effects [85].

Regarding the psychological approach, there are different methods of psychotherapy with proven contribution to symptoms reduction in depressed patients [88]. One of the most studied is Cognitive Behavioral Therapy (CBT), also known as cognitive therapy. The goal of CBT is to change dysfunctional schemata, or negative thought patterns. It can be used to treat a variety of psychosocial issues that exacerbate symptoms, such as marital disagreement and work stress, and has been shown to reduce the likelihood of a recurrence and strengthen the treatment adherence [89]. A type of CBT is Problem-Solving Therapy that emphasizes teaching adaptive problem-solving attitudes and techniques [90]. In contrast, Behavioral Activation Therapy disregards internal cognitions in favor of a more extreme behaviorist approach. It is based on activity scheduling to re-engage patients in activities that provide positive reinforcement instead of avoidance [91]. Finally, Interpersonal Therapy is another type of psychotherapy where the goal is to improve interpersonal functioning, such as enhancing social skills and assisting with life organization, to reduce symptoms [92].

3.3. Cognitive function

Cognitive impairment is common in MDD, and it is a major predictor of depression severity, health outcomes and societal perspectives [93]. For these reasons, regular cognitive evaluations to understand the extent of cognitive difficulties, can enhance MDD management, helping clinicians predict the impact of MDD on patients' daily lives and tailor interventions accordingly [94].

Cortisol, the stress hormone, has been linked to mood regulation and cognitive processes, and in the context of MDD, elevated cortisol levels have been associated with cognitive dysfunction. This relationship is also rooted in cortisol's effect on brain structures involved in cognitive function, such as the hippocampus, prefrontal cortex, and amygdala [70]. Chronic exposure to high cortisol levels can lead to neurotoxicity and disrupt the balance between neuronal survival and death, leading to cognitive impairments.

Research from different neuroimaging techniques has also pointed to a volume reduction or dysregulated functionality on a dispersed neuronal circuit made up of several prefrontal cortex sectors interacting with temporal lobe components (the hippocampus and amygdala) and subcortical areas (the striatum and thalamus) [95], supporting the neurologic mechanisms implicated in MDD cognitive impairment.

Neurocognitive assessments have gained attention for their potential to uncover cognitive deficits associated with depression, such as problems with memory, attention, and executive function. Tools such as the Trail Making Test, Wisconsin Card Sorting Test and the Color–

Word Stroop task offer insights into the cognitive aspects of depression and have been linked to decreased performance in MDD, with impact sizes varying from 0.32 to 0.97 [96].

Advances in mHealth applications have transformed depression assessment, enabling real-time monitoring of symptoms and behaviors through smartphones and wearable devices [97]. There are several cost-effective and easily accessible neurocognitive solutions for the collection active self-reports, like the THINC-integrated tool (THINC-it), which show promise for routine use in real-life and clinical settings [93].

3.4. Diagnosis and follow-up

The diagnosis of MDD is made following the criteria listed in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V, APA, 2014). It is characterized by the presence of five (or more) of the nine previously mentioned symptoms during at least a 2-week period, representing a change from previous behavior, and one of the symptoms must be depressed mood, or loss of interest or capacity for pleasure. These symptoms are causing clinically substantial suffering limiting the person's ability to perform in social, occupational, or other critical daily life activities. Additionally, clinicians verify that the origin of the symptoms is not a medical condition (such as hypothyroidism), a substance (such as a drug or medicine), or a period of grief (such as following the death of a loved one) shorter than 2 months or characterized by marked functional disability, morbid concerns of worthlessness, suicidal ideation, psychotic symptoms, or psychomotor slowing.

The key differential diagnoses of MDD are with BD and with SCZ [62]. On the one hand, the presence of a history of hypomania or mania, which is defined by a distinct period of elevated mood or irritability, along with at least three of the following symptoms—inflated self-esteem, decreased need for sleep, increased speech, flight of ideas, distractibility, increased activity in goal-directed tasks, and/or involvement in risky behavior—is the only factor that can be used to differentiate BD from MDD [74]. On the other hand, if hallucinations or delusions occur only during major depressive or manic episodes, SCZ can be dismissed.

In clinical practice, there are several specifiers and subtypes to categorize each depressive episode that help providing common ground for a better understanding of the condition, tailoring treatment approaches to individual patients and assessing their prognosis [87]. The specifiers depend on the illness pattern, the severity, the time of onset, the remission status, and the clinical features. This last specifier describes MDD subtypes, being melancholic and atypical the most prevalent [98]. Melancholic subtype is characterized by reduced responsiveness of emotion and mood, a pervasive and unique aspect of low mood that is worse in the morning, anhedonia, guilt, and psychomotor disruption. On the contrary, the symptoms for atypical depression diagnosis are mood reactivity, increased appetite and sleep, leaden paralysis and interpersonal rejection sensitivity that seriously impairs social or professional functioning [62].

Accurately detecting and regularly assessing changes in mental state is the basis and at the same time a major obstacle to the effective treatment of MDD. Nowadays, depression is identified and monitored using interview-based evaluations or self-report questionnaires that ask about past experiences, present circumstances, family history, and the frequency of symptoms that are specific to depression. However, a large portion of this data is dependent on the patient's memory and is subjective, and hence can be influenced by the clinician's opinion. As such, it is vulnerable to biases that could compromise the standard of care [99]. Furthermore, these evaluations are infrequent and programmed since they are usually made only during consultations with clinicians, and hence, only capture a momentary picture of a person's present health regardless of their situation and evolution.

To tackle these issues, recent research has been focusing on Ecological Momentary Assessment studies, which are based on a regular administration of self-reported measures. This approach offers several benefits: it allows for real-time assessments, reducing retrospective bias and increasing accuracy; repeated measurements enable a better understanding of time-dependent processes and dynamic symptom changes; context-specific information is gathered, assessing symptoms in context; interactive assessments offer real-time customizable feedback; and there's higher generalizability due to increased ecological validity and collection of more representative data [100]. The patient's burden, however, may restrict the frequency of questionnaire collection. In fact, when the frequency or duration of a questionnaire is increased, adherence to it decreases [101].

Parallelly, there has been growing interest in precision psychiatry [102], trying to identify biomarkers for prevention and treatment considering the variations in a person's genetics, environment, and lifestyle. However, the translation into clinical practice, in a way that directly benefits patients and clinicians, remains practically nonexistent. In conventional clinical settings, physicians may find it difficult or costly to evaluate potential biomarkers. This is particularly true for endocrinological markers, neuroimaging, and panomics, especially since their cost-effective predictive performance is worse than the traditional questionnaire results [99].

In this context, the surge of remote measurement technologies (RMTs), such as smartphones and wearable devices, offers a promising alternative combining EMA benefits as well as low financial strain being highly useful for researchers as well as less time-consuming and burdensome for patients [103].

This is how the project RADAR-CNS was born [104]. This international research project within the Innovative Medicine Initiative (IMI) framework was a public-private founded project in collaboration with healthcare providers and industry partners. The project aimed to explore the potential of RMTs to monitor and predict health outcomes in patients with CNS disorders, including depression. In this project, our knowledge of CNS and stress biomarkers contributed

to the goal of developing and validating digital biomarkers for continuous, real-time monitoring of patients.

The study successfully collected longitudinal data over 2 years, providing valuable insights into the daily and weekly fluctuations in patients' behaviors and symptoms. This long-term data was crucial for understanding the dynamics of depression and predicting relapses.

The research identified several promising digital biomarkers for depression. These include distinct sleep patterns, such as later sleep onset [105], reduced physical activity [106, 107], and lower HR variability [108–110], which were found to correlate with depression severity.

The project demonstrated the feasibility of collecting continuous, real-time data from patients using RMTs. While the overall dropout rate was around 40%, engagement with the wearable devices was higher than with smartphone apps, suggesting that wearables may be more user-friendly or less intrusive for continuous monitoring. Additionally, the continuous monitoring of these technologies was perceived as beneficial for both patients and clinicians, increasing self-awareness and facilitating better-informed clinical decisions [106]. Still, further research is needed to develop standardized protocols for the use of digital biomarkers in clinical practice.

Although research is still in the early stages, these technologies provide multimodal assessments based on contemporary analysis of behaviors, physiological signals, and subjective experiences. This facilitates a more comprehensive and personalized picture of a patient's health, treatment evolution, and even suggests possible intervention targets [111].

4. Biomarkers in mental health assessment

The emergence of digital technologies has catalyzed an unprecedented exploration into physiological biomarkers that hold promise for advancing our understanding of mental health disorders, straddling the boundaries of biomedical science, engineering and computer sciences.

4.1. Physiological biomarkers

Physiological biomarkers, encompassing measures such as HR, temperature or electrodermal activity, offer a window into the complex functioning of the ANS and its response to various stimuli, stressors, and treatments [112]. The development of these biomarkers is based on the precise, noninvasive, and real-time monitoring of electrical activity generated in our body modulated by the ANS, reflecting the dynamic interplay between cognitive processes and emotional states, and the physiological response.

The development of these physiological biomarkers, however, is not without its challenges. Issues such as data privacy, standardization of measurement protocols, and the interpretation of complex biological signals in the context of individual variability necessitate a collaborative effort across disciplines. Moreover, the translation of these biomarkers from research settings to clinical practice is still under active research and it requires rigorous validation studies to establish their reliability, sensitivity, and specificity in diagnosing mental health disorders and monitoring treatment outcomes.

4.1.1. Electrocardiogram

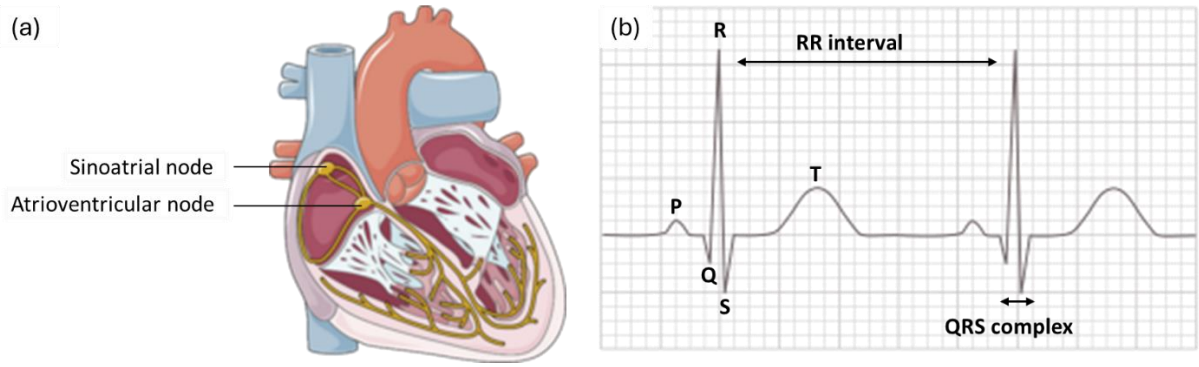
The electrocardiogram (ECG) is a fundamental tool in cardiovascular diagnostics, offering a noninvasive and rich source of data on cardiac electrical activity. Originating from the heart's electrical impulses, the ECG signal offers information about the heart's rhythm and broader aspects of ANS modulating function.

The sinoatrial (SA) node, located in the upper region of the right atrium, is composed of pacemaker cells that spontaneously generate action potentials initiating the cardiac cycle of a typical beat. The ANS has a significant impact on the intrinsic rhythm of SA node activation. Both the sympathetic and parasympathetic nerve systems innervate the heart via the extrinsic intrathoracic ganglia (stellate ganglia, dorsal root ganglia, and sympathetic chain) and the intrinsic cardiac autonomic nervous system. This system is comprised of an extensive epicardial neural network known as ganglionated plexi which coordinates the response to regulate regional contractile, vascular, and physiological functions [113].

In an ECG, each waveform, interval, and segment hold specific physiological significance, simplified in Figure 5, reflecting various phases of the cardiac cycle and hence, illustrating essential information with clinic al implications [114].

The first wave is the P wave, a small upward waveform that represents the electrical activity associated with the atrial depolarization, which is triggered by the SA node and leads to the atria contracting and pushing blood into the ventricles. An abnormal P wave can indicate atrial enlargement or atrial arrhythmia. The QRS complex is the following sharp set of waveforms, consisting of a downward Q wave, a tall R wave, and an S wave dipping below the baseline, that represents the ventricular depolarization. The electrical signal from the atria activates the atrioventricular node which in turn prompts the ventricles to contract and pump blood out to the lungs and the rest of the body. Abnormalities in the QRS complex may suggest ventricular hypertrophy, conduction blockages, or ventricular arrhythmias. Finally, the T wave follows the QRS complex representing the ventricular repolarization, the process by which the ventricles recover electrically and prepare for the next heartbeat. Abnormal T waves can be a sign of myocardial infraction, hypertrophic cardiomyopathy or subarachnoid hemorrhage.

Figure 5. Electrical conduction system of the heart



(a) Cross-section of a healthy heart diagram (left), highlighting the four heart valves, and electrical conduction system including the sinoatrial node and atrioventricular node. (b) Characteristic waves in the ECG.

Beyond traditional cardiac assessment, the ECG has been increasingly utilized to explore the ANS's dynamics through derived measures such as heart rate variability (HRV). The basis for calculating HRV is the R-R interval, which is measured from the peak of one R wave to the next peak, reflecting the HR. When signal artifacts and ectopic beats have been removed, this interval becomes the so-called normal-to-normal (NN) interval. The physiological phenomenon of variation in the time interval between heartbeats is particularly esteemed for its ability to reflect the balance between the sympathetic and parasympathetic branches of the ANS [115].

For this reason, the development of physiological biomarkers from the ECG signal opens new avenues for assessing the ANS and its relationship with medical conditions. Several studies exemplify the expanding interest in leveraging HRV biomarkers for the assessment of cardiovascular events risk [116] or diabetes [117]. However, research has expanded the utility

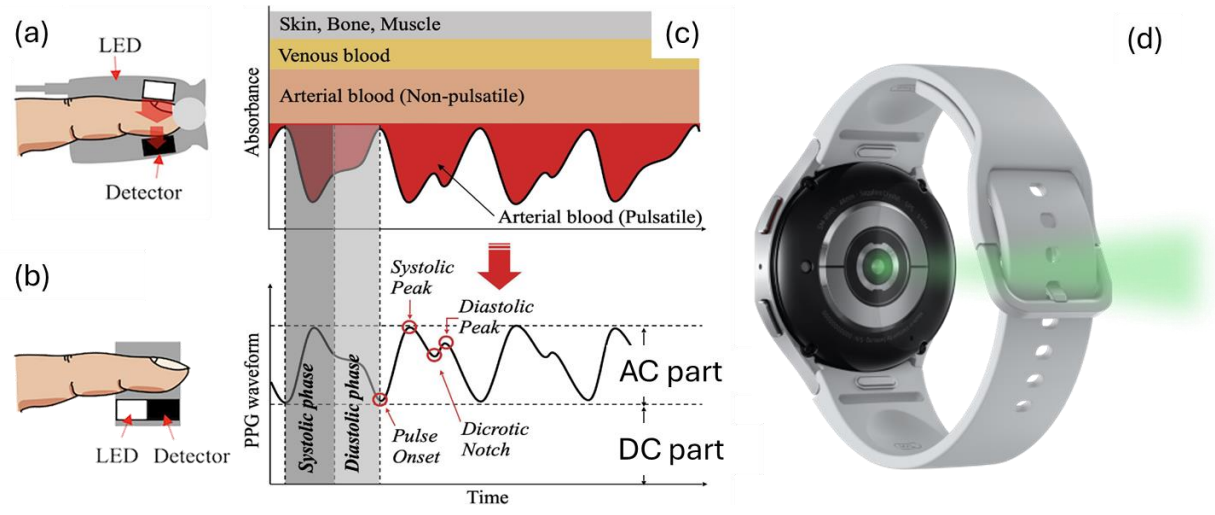
of HRV from assessing systemic physical health issues to encompassing evaluations of psychological states and disorders such as the assessment of the stress or depression severity [118, 119]. These investigations underscore the potential of ECG features, especially HRV, as noninvasive markers heralding a new era in personalized mental health diagnostics and treatment strategies.

4.1.2. Photoplethysmogram

The photoplethysmogram (PPG) is another valuable physiological tool that is becoming increasingly significant in the domain of ANS assessment. This noninvasive method records the blood volume changes within the microvascular bed of tissue using a photoplethysmograph which emits a light signal into the skin and measures the amount of light either transmitted or reflected to the sensor, illustrated in Figure 6. Changes in light absorption during each cardiac cycle reflect the blood pulse wave that corresponds to heartbeats, thus providing data on cardiovascular dynamics [120].

The PPG waveform is composed of a physiological waveform that is pulsatile (referred to as "AC") and is attributed to cardiac synchronous changes in blood volume with each heartbeat. This waveform is superimposed on a baseline that varies slowly (referred to as "DC") and has various lower frequency components that are associated with respiration, SNS activity, and thermoregulation [120].

Figure 6. Recording of a photoplethysmogram



(a) PPG transmission mode. (b) PPG reflectance mode. (c) The characteristic PPG waveform results from light attenuation by tissues. (d) PPG sensor in wearable device. Adapted from [121]

PPG primarily measures the perfusion of blood to the dermis and subcutaneous tissue, offering a more convenient and simpler method for data acquisition compared to ECG, making it highly suitable for wearable technologies applications such as the Empatica, Fitbit or Garmin wristbands [122]. It can be used to derive pulse waveform features as well as pulse rate

variability (PRV) features, a counterpart to HRV, all biomarkers of cardiovascular health that are influenced by the ANS. However, while PPG can serve as an alternative to ECG for extracting heart rate information, it is important to note that this is not universally applicable. Certain pathologies manifest as alterations in ECG morphology that are not detectable via PPG. Therefore, while PPG provides a valuable tool for monitoring cardiovascular health in general, ECG remains indispensable for detecting specific cardiac conditions.

Recent studies have delved into the use of PPG analysis within the field of mental health. Notably, [123] introduced a Python toolbox for photoplethysmography signal analysis, named 'pyPPG.' This tool is specifically designed to advance the research and application of PPG in both clinical and non-clinical settings by providing standardized and validated biomarkers for PPG morphology. Features derived from pulse decomposition analysis, such as the time interval or the amplitude loss between the main wave and reflected waves [124], have been correlated with depression severity. Furthermore, frequency domain analysis of PRV has demonstrated superior predictive capabilities for mental health conditions such as stress, anxiety, and depression compared to time domain features [125].

Nevertheless, while PPG technology has expanded rapidly with integration into wearable devices (Figure 6.D), offering substantial benefits in continuous and remote health monitoring and clinical diagnostics, it also has inherent sources of inaccuracy such as the susceptibility to motion artifacts or to several biases such as skin pigmentation, age, or body mass index (BMI) [126] that need to be considered when studying its reliability.

4.1.3. Electrodermal activity

Electrodermal activity (EDA), also known as galvanic skin response (GSR), is a sensitive biomarker of the ANS's response to both external factors and internal processes like memory and attention. EDA measures changes in the skin's electrical conductance due to the eccrine sweat glands' activity, which is influenced by SNS arousal. The activity of the sudomotor nerves (SMNA) initiates the activation of sweat glands. SMNA oversees various physiological functions, including temperature regulation and sensory perception. It has also been noted that SMNA is associated with emotional states, especially affecting levels of arousal [127].

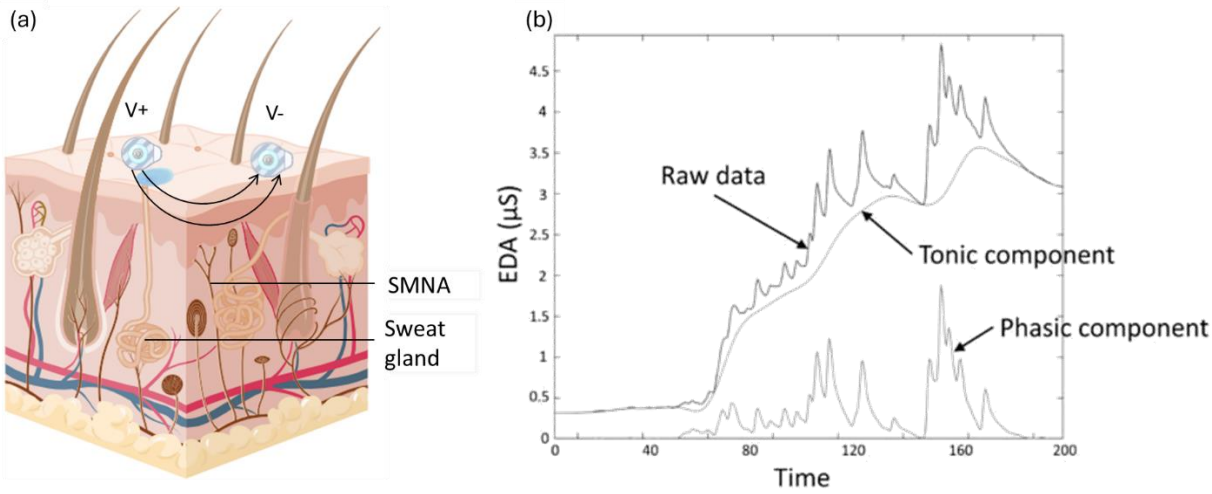
EDA is typically measured using the exosomatic method. This method applies a small external electrical current across the skin and measures the electrical properties, like resistance or conductance. Using two electrodes placed on the skin's surface, commonly on the fingers or palm, where sweat gland density is high, these electrodes apply a small voltage to create a potential difference, and changes in skin conductance are recorded [128].

This method includes the measurement of tonic skin conductance levels as well as the skin conductance response to discrete stimuli (also known as the phasic response). This data can be

used to design ANS biomarkers, particularly useful in psychological research, providing insights into emotional states, stress responses, and various psychiatric conditions [119].

EDA sensors encounter several challenges that must be considered. These sensors measure skin conductance, which can be significantly affected by the specific site of sensor placement due to variations in skin properties across different body areas. For instance, skin thickness, sweat gland density, and peripheral microcirculation differ notably between areas such as the palms, soles, and forehead. Moreover, these sensors are susceptible to artifacts induced by movement when placed on parts of the body that frequently move, such as the fingers or wrists. The sensors can pick up noise from these movements, leading to inaccurate readings.

Figure 7. Eccrine sweat glands diagram and electrodermal activity signal



(a) Eccrine sweat glands diagram and electrical conductivity of the skin. SMNA: sudomotor nerves. (b) Electrodermal activity (EDA) data decomposition into tonic and phasic components from raw signal. Adapted from [129].

Recent advancements in sensor technology have allowed for more precise and less intrusive measurements of EDA designing devices for stress measurement such as the Empatica E4 wristband [130] or the Moodmetric EDA Ring [127]. These improvements have expanded the use of EDA monitoring in both clinical settings and wearable technology, enhancing our understanding of its correlation with psychological phenomena.

4.1.4. Respiratory signal

The respiratory signal is a fundamental physiological activity critical for gas exchange and maintaining homeostasis [131]. Respiration (Resp) involves two primary mechanical processes: inhalation and exhalation. During inhalation, the contraction of the diaphragm and the external intercostal muscles enlarges the thoracic cavity and decreases intrapulmonary pressure, allowing air to flow into the lungs. Conversely, during exhalation, the relaxation of the respiratory muscles and the elastic recoil of the lungs, which increase intrapulmonary pressure

and expel air. Active exhalation, observed during demanding activities, involves the contraction of internal intercostal muscles and abdominal muscles to forcefully reduce thoracic volume and push air out more rapidly.

Measurement of respiratory patterns can be done using piezoelectric chest straps that monitor chest expansion and contraction, through the analysis of changes in thoracic impedance measured with ECG-like electrodes, or more advanced methods like spirometry, which can directly measure the volume and flow of air during breathing.

Breathing is intricately regulated by neural and chemical mechanisms to adapt to the body's varying demands [131]. The respiratory centers in the brainstem, primarily the medulla oblongata and pons, form the neural basis for respiratory rhythm generation and modulation. On the one hand, the medullary respiratory centers comprise the dorsal respiratory group, which primarily controls inspiratory movements by innervating the diaphragm and external intercostal muscles, and the ventral respiratory group, involved primarily in active expiration but also plays a role in increased inspiratory activity during high respiratory demands, such as exercise. On the other hand, the pontine respiratory centers are the pneumotaxic and the apneustic centers. The pneumotaxic is in the upper pons, and this center regulates the switch from inspiration to expiration, helping to control the rate and pattern of breathing. This center is considered antagonist to the apneustic center, located in the lower pons, which provides a constant stimulatory signal to the inspiratory neurons of the medulla, promoting deep, prolonged inspirations unless inhibited by the pneumotaxic center [132].

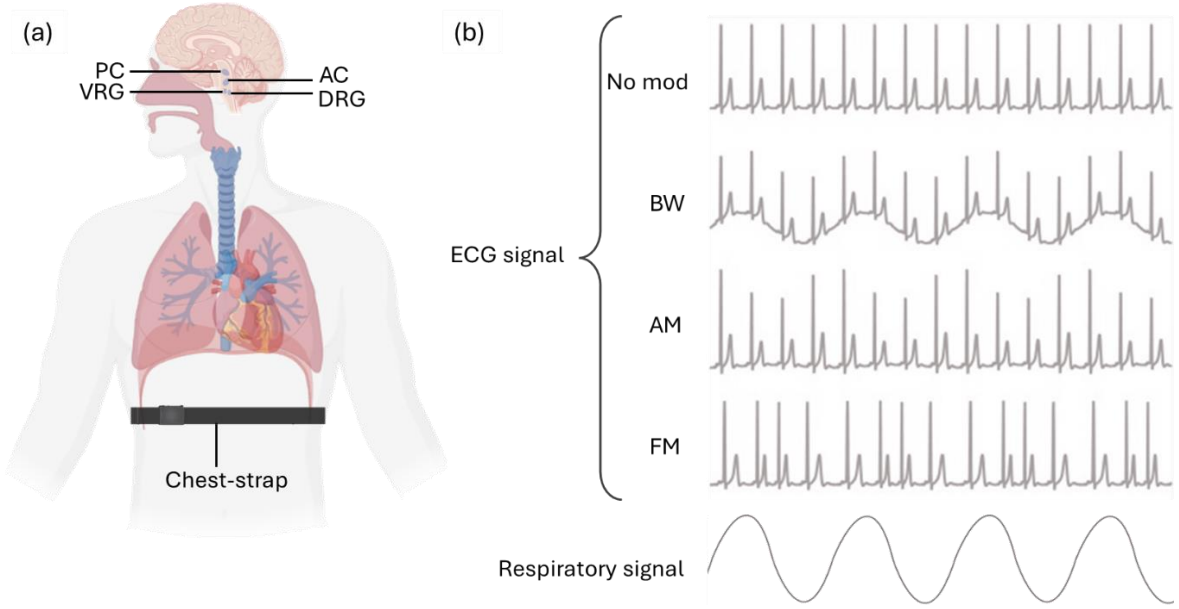
These centers integrate sensory inputs from chemoreceptors and mechanoreceptors and adjust respiratory muscle activity accordingly [133]. Elevated CO_2 levels or decreased blood pH primarily stimulate an increase in ventilation to correct the imbalance, illustrating a predominant chemical control through feedback mechanisms.

Moreover, the ANS modulates the respiratory centers to ensure that respiratory activity is appropriately aligned with the body's changing needs. The SNS generally increases respiratory rates and bronchial dilation during stress or physical activity through the release of catecholamines whereas the PNS predominantly acts to conserve energy by reducing the respiratory rate and constricting the bronchi during restful periods through the vagus nerve.

A noninvasive measure of PNS activity is respiratory sinus arrhythmia (RSA), a naturally occurring variation in HR that occurs during a breathing cycle, and it is considered a key indicator of cardiac vagal tone. This phenomenon is primarily driven by changes in the function of cardiovagal motor neurons, which are partially regulated by input from arterial baroreceptors. During inhalation, these neurons are inhibited by signals from central inspiratory neurons and heightened activity of slowly adapting stretch receptors in the larger airways of the lungs. This reduces their responsiveness to baroreceptor inputs, decreasing vagal tone and consequently increasing HR. Conversely, upon exhalation, the activity of the cardiovagal motor neurons is

restored to normal levels, enhancing vagal tone and reducing HR [131]. Therefore, the frequency modulation due to RSA is characterized by an increase in HR during inhalation and a decrease in HR during exhalation. Additionally, the movement of the thoracic cavity during breathing induces ECG baseline wander, and the expansion, and contraction of the chest cavity, which affects the distance between the heart and the electrodes, modulates the ECG amplitude. Due to these modulations, the respiratory signal can indirectly be derived from the ECG or PPG signals [134–136].

Figure 8. Respiratory system and modulations of an electrocardiogram signal



(a) Diagram of respiratory centers: pneumotaxic (PC) and apneustic (AC) centers, dorsal respiratory group (DRG) and ventral respiratory group (VRG), and a piezoelectric chest strap. (b) Representations respiratory modulations in the ECG signal. Across four respiratory cycles, from top to bottom: no modulation (No mod), baseline wander (BW), amplitude modulation (AM), frequency modulation (FM), derived respiratory signal. Adapted from [137]

Therefore, the respiratory patterns provide crucial insights into the ANS functioning. Apart from the relevance in managing respiratory disorders such as asthma and sleep apnea, where dysregulation of autonomic control can exacerbate symptoms, variations in respiratory patterns are closely associated with different emotional states and can be indicative of physiological arousal or relaxation. The respiratory signal is particularly informative of parasympathetic activity and has been studied for its potential in psychiatric settings to assess stress and anxiety levels [138]. This insight into autonomic functioning is especially relevant in the context of respiratory-based interventions, such as biofeedback, which harness these signals to foster stress reduction and emotional regulation [139].

4.1.5. Temperature

Finally, thermoregulation, primarily controlled by the hypothalamus, reflects the body's ability to maintain its core internal temperature despite external changes. Skin temperature (T) variations can be measured using thermal sensors or more sophisticated imaging technologies such as infrared thermography [140], both useful options for wearable applications.

Peripheral T is influenced by blood flow changes mediated by SNS activity, making it a useful indicator of stress and emotional states. During stress or emotional arousal, sympathetic nerves increase peripheral vasoconstriction, reducing blood flow to the skin and lowering surface temperature. Conversely, relaxation leads to vasodilation, increasing blood flow and surface temperature. For instance, prior research has demonstrated a substantial decrease in temperature in distal areas, such as the fingertips, correlated to the acute stress intensity [141]. On the other hand, temperature rose when sensors were positioned in core areas, such the neck [142].

4.2. Physiological stress dynamics

Exploring the stress dynamics, including reactivity and recovery, offers insight into an individual's inherent capacity to respond to stressors, which varies significantly across different individuals [143] and yields insights into health-related risks that cannot be gleaned from resting measurements [144, 145]. Besides, static physiological measures can be affected by several factors such as age, BMI, or even body position [146].

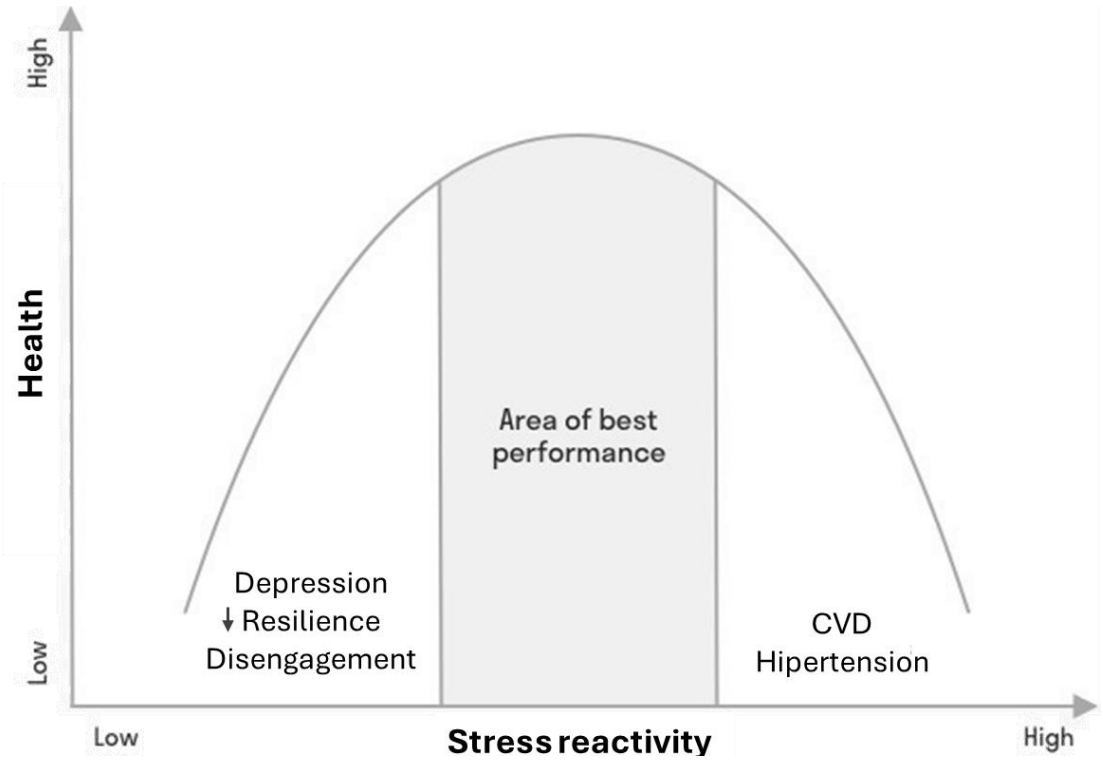
Approximately 0.26 to 0.43 of the variance in stress responses is attributed to genetic factors, with early life environmental factors also playing a significant role [147]. Adverse prenatal and early postnatal environments can have long-term effects on stress reactivity, potentially mediated by epigenetic changes [28]. Research has shown that these reactions are stable over time, suggesting that reactivity may partially explain the endophenotype of increased stress sensitivity [148]. However, laboratory evidence shows that social support [149] and moderate-intensity aerobic exercise [150] reduces self-reported stress and cardiovascular reactivity.

There is no single "gold-standard" indicator of stress reactivity, and subjective measures [151], though useful, have limitations compared to real-time assessments. However, during the 21st century, there has been increasing research on stress reactivity biomarkers.

As mentioned before, stress dynamics are influenced by ANS and HPA axis activity, leading to changes in biomarkers such as BP, HR, and cortisol levels. Apart from these physiological changes, this variability manifests across several domains, including behavioral, subjective experiences, and cognitive functions, and plays a pivotal role in predicting susceptibility to various diseases, independent of other risk factors.

The reactivity process is hypothesized to conform an inverted-U model, describing the relationship between motivation and performance (Y-axis) and physiological arousal (X-axis), such that there is an optimal mid-point in the arousal continuum where performance is best served and both high and low reactivity can be considered maladaptive stress responses [152].

Figure 9. Inverted-U model for stress reactivity



Inverted-U model linking stress reactivity to health outcomes. CVD: cardiovascular disease. Adapted from [152].

For example, high cardiovascular stress reactivity, measured through tests like the Cold Pressor Test, has been linked to the development of hypertension and cardiovascular diseases [153]. On the other hand, there is evidence suggesting that stress reactivity could be risk factors for mental disorders such as depression.

Lower levels of HR reactivity to acute psychological stress are associated with higher levels of behavioral disengagement and lower perseverance [154], type D personality (i.e. tendency towards negative affectivity and social inhibition) [155], less resilience to stressful events [156] and shorter relapse times in SUD [157]. Patients with MDD generally exhibit a blunted physiological response to stress as well. This is characterized by less fluctuation in HR and high frequency HRV during stress tasks [144, 158, 159]. The blunted stress reactivity has been proposed as a potential biomarker for clinical depression, providing a physiological basis for the observed emotional and behavioral symptoms in these patients [144]. The study highlighted the importance of considering covariates such as age, gender, medication, and comorbid anxiety disorders, as these factors can influence HR and HRV measures. Though despite the inclusion

of these covariates, the pattern of reduced stress reactivity in MDD patients remained consistent, reinforcing the robustness of these biomarkers.

To evaluate stress reactivity, laboratory stress tests offer standardized conditions. The Stroop Color and Word Test (SCWT), the Trier Social Stress Test (TSST), and Arithmetic Tasks (AT) appear to be the most popular and promising stressor tests [130, 160].

While stress reactivity assessments in the lab provide valuable insights, real-life assessments are crucial for a more comprehensive understanding. However, a recent study found that while general lab measures did not correlate well with daily stress reactions, affective recovery in the lab showed a significant association with daily-life stress reactivity [161]. This points to the importance of recovery processes in daily stress experiences, rather than just initial reactivity. Moreover, [119] found that individuals with MDD may require more time to recover to a resting state after experiencing stress compared to healthy individuals due to diminished parasympathetic activity.

Despite the potential of using stress dynamics as a diagnostic tool, its application in clinical settings is complicated by the variability in how stress reactivity manifests across different individuals influenced by factors such as sex, age, and preexisting health conditions. Therefore, while the research literature increasingly supports the concept of generalized stress reactivity, the associations between different stress responses are typically moderate, necessitating careful consideration in their application [144].

5. Objectives and outline of the thesis

The overarching aim of the present thesis is to evaluate stress dynamics using noninvasive physiological data and to develop and validate an objective tool for assessing the severity of depression.

To achieve this broad objective, several specific objectives have been set:

Aim 1. To conduct a comprehensive synthesis of current research on mental health, with an emphasis on understanding the pathophysiology of stress and its role in exacerbating mental disorders, particularly MDD.

Aim 2. To analyze current noninvasive, objective tools for assessing stress, focusing on those that utilize biomarkers associated with the ANS.

Aim 3. To construct a stress pattern using clinical and biological variables that encapsulate both psychological and somatic activations in a healthy population.

Aim 4. To characterize the physiological responses to stress and develop a method for the objective and noninvasive measurement of stress using only physiological features.

Aim 5. To examine socio-demographic, lifestyle, psychometric, and cognitive factors that may predispose individuals to MDD.

Aim 6. To develop a multiparametric model to objectively assess the severity of depression and explore the physiological dynamics of stress in individuals diagnosed with MDD.

Aim 7. To design a research protocol that supports ongoing research to objectively evaluate mental health, ensuring reproducibility in large populations suitable for epidemiological and clinical research.

Aim 8. To synthesize the main findings from the research and propose potential directions for future investigations to complement and validate these results.

Accordingly, the succeeding structure has been followed in this thesis to address these goals:

Part I: this part of the thesis introduces the field of mental health and underscores the significance of research in this area. Chapter 1 addresses the first specific aim by providing a comprehensive synthesis of current research on mental health. It focuses on understanding the pathophysiology of stress and its role in exacerbating related disorders, which is further explored in Chapter 2. Chapter 3 covers an overview of MDD, its course, burden, and treatment, and continues to address the first aim by emphasizing socio-demographic, lifestyle,

psychometric, and cognitive factors that predispose individuals to MDD. Finally, Chapter 4 addresses the second specific aim by analyzing current noninvasive, objective tools for assessing mental health, focusing on those that utilize biomarkers associated with the ANS stress response.

Part II: the purpose of this part is to develop an approach for the objective and noninvasive assessment of mental health based on physiological stress dynamics. This section is divided into several stages. Chapter 5 discusses the methodology for signal processing, laying the groundwork for the subsequent analyses. Chapter 6 examines the stress response in a healthy population and addresses the third specific aim by constructing a stress pattern using clinical and biological variables that encapsulate both psychological and somatic activations. The fourth aim is then tackled in Chapter 7, which characterizes physiological responses to stress and develops a tool for the objective and noninvasive measurement of stress using physiological features alone. Chapter 8 addresses the fifth aim by examining socio-demographic, lifestyle, psychometric, and cognitive factors that may predispose individuals to MDD. Finally, the sixth aim is achieved in Chapter 8 by developing a multiparametric model to objectively assess the severity of depression and investigate the physiological dynamics of cognitive stress in individuals diagnosed with MDD.

The following publications are the result of the research detailed in this chapter:

- García Pagès, E., Arza, A., Lazaro, J., Puig, C., Castro, T., Ottaviano, M., Arredondo, M. T., Bernal, M. L., López-Antón, R., Cámara, C. D. la, Gil, E., Laguna, P., Bailón, R., Aguiló, J., & Garzón-Rey, J. M. (2023). Psychosomatic response to acute emotional stress in healthy students. Frontiers in Physiology, 13. https://doi.org/10.3389/fphys.2022.960118
- García Pagès, E., Kontaxis, S., Siddi, S., Posadas-de Miguel, M., De La Cámara, C., Bernal, M.L., Castro, T., R., Laguna, P., Badiella, L., Bailón, R., Haro, J.M., Aguiló J. (in review) Contribution of Physiological Dynamics in Predicting Major Depressive Disorder Severity. Psychophysiology.

Part III: this part of the thesis focuses on promoting ongoing research to objectively evaluate mental health. Chapter 9 addresses the seventh specific aim by designing a research protocol to automate the mental health assessment process, ensuring its reproducibility in large populations suitable for epidemiological and clinical research. This chapter includes the measurement of stress reactivity during cognitive tasks using multiple physiological parameters to validate a remote objective tool for mental health and illness.

Following this chapter's study, the following publication was produced:

- Castro Ribeiro, T., García Pagès, E., Ballester, L., Vilagut, G., García Mieres, H., Suárez Aragonès, V., Amigo, F., Bailón, R., Mortier, P., Pérez Sola, V., Serrano-Blanco, A.,

Alonso, J., & Aguiló, J. (2024). Design of a Remote Multiparametric Tool to Assess Mental Well-Being and Distress in Young People (mHealth Methods in Mental Health Research Project): Protocol for an Observational Study. JMIR research protocols, 13, e51298. https://doi.org/10.2196/51298

Part IV: the eighth specific aim is addressed in this part by synthesizing the main findings from the research and proposing potential directions for future investigations. Chapter 10 provides a summary and final discussion of the research, while Chapter 11 critically analyzes the strengths and limitations of the study. Chapter 12 draws the main conclusions, highlighting the contributions to the field, and Chapter 13 proposes future research directions to complement and validate the results obtained in this thesis.

Part V: the appendices are included in this last part. Chapter 14 lists the scientific contributions, including journal and conference publications resulting from the research conducted during the thesis, and Chapter 15 provides a comprehensive list of references used throughout the thesis.

II. Physiologic stress dynamics for mental health assessment

This section delineates the comprehensive methodologies employed to investigate the physiologic dynamics for the stress measurement and its application to assess depression severity. Central to this thesis are the signal processing techniques utilized to analyze physiological data. Moreover, each study applies a stress environment protocol and assesses key biochemical variables and psychometric tests used as gold standard underpinning the evaluation of these data sets.

Table of contents

6. Methodology for signals processing _____ 47

7. Physiological dynamics in stress _____ 57

8. Physiological dynamics in major depressive disorder_____ 82

6. Methodology for signals processing

The specific signal processing methods are detailed in this section. It reports the types of physiological signals analyzed, including their acquisition, preprocessing, and the analytical techniques applied to extract meaningful features for ANS assessment.

The final set of signals used in the present thesis, selected due to their relation to the stress response, was the following: electrocardiography (ECG), photoplethysmography (PPG), electrodermal activity (EDA), respiration (Resp) and skin temperature (ST).

All the signals were recorded using the Medicom 83 system, ABP-10 module (Medicom MTD Ltd, Russia), and processed using MATLAB software using 1-minute windows for feature extraction.

6.1. Electrocardiogram

The ECG signal was measured at a sampling frequency of 1000 Hz, using three orthogonal leads on the chest: right-left axis (X), from the fifth intercostal space along the middle axillary line on the right and symmetrically on the left; head-to-feet axis (Y), down from left costal margin to the left medial infraclavicular region; and front-back axis (Z), from the fourth intercostal space on the left from breastbone to near the spine at the same level.

The basis for the HRV analysis is the time difference between successive heartbeats represented by the R-R interval. HRV reflects the heart's ability to adapt to changing circumstances, thereby allowing for the evaluation of the ANS's state. To that end, heartbeat detection was performed using the wavelet transform-based algorithm reported by [162]. However, not all heartbeats are suitable for HRV analysis. Missed beats or false detections, often due to low-amplitude QRS complexes or artifacts, result in R-R interval series with erroneous values. Abnormal changes in R-R intervals can also occur due to electrical impulses originating from regions of the heart other than the SA node, known as ectopic beats, and do not reflect ANS modulation on the SA node's depolarization pattern. Therefore, R-R intervals adjacent to ectopic or mis-detected beats cannot be used for HRV analysis. We used the algorithm described in [163] to identify and remove such beats by imposing a threshold for the maximum deviation permissible for changes in heart rate, and to suggest an interpolated beat correction. Segments of up to three interpolated beats have been accepted and are assumed to be suitable for HRV analysis. The resulting intervals are termed normal-to-normal (N-N) intervals. Out of the 320 files analyzed in the present work, only 9 files were discarded due to poor signal quality, which prevented accurate beat detection.

There are two main methods classically used in literature for HRV analysis: time-domain and frequency-domain analysis. Regarding the time-domain analysis, the mean heart rate (mHR)

was extracted as well as two types of HRV indices. The standard deviation of the NN intervals (SDNN) from direct measurements of the NN intervals, and the root mean square of successive variations between NN intervals (RMSSD), from the variations in NN intervals, both expressed in milliseconds. SDNN represents both SNS and PNS activity whereas RMSSD represents vagally mediated short-term fluctuations in HRV since the difference operation's effect highlights the high-frequency component of the NN interval series.

These series of beat intervals can be graphically represented by the interval tachogram. Another representation, referred to as the inverse interval tachogram, is based on the inverse of the R-R intervals, and provides information about instantaneous rate rather than time. These tachograms signals are indexed based on beat occurrences. However, to create a temporal reference, these signals are transformed into interval function or inverse interval function, respectively. Unlike beat occurrences, these transformed signals are sampled according to time occurrences and are therefore non-uniformly distributed. Consequently, it is necessary to convert these signals into uniformly sampled time-domain signals. This conversion allows for the frequency-domain analysis in hertz and enables more sophisticated variability analyses, particularly when correlating heart rate with other physiological time-domain signals such as respiration.

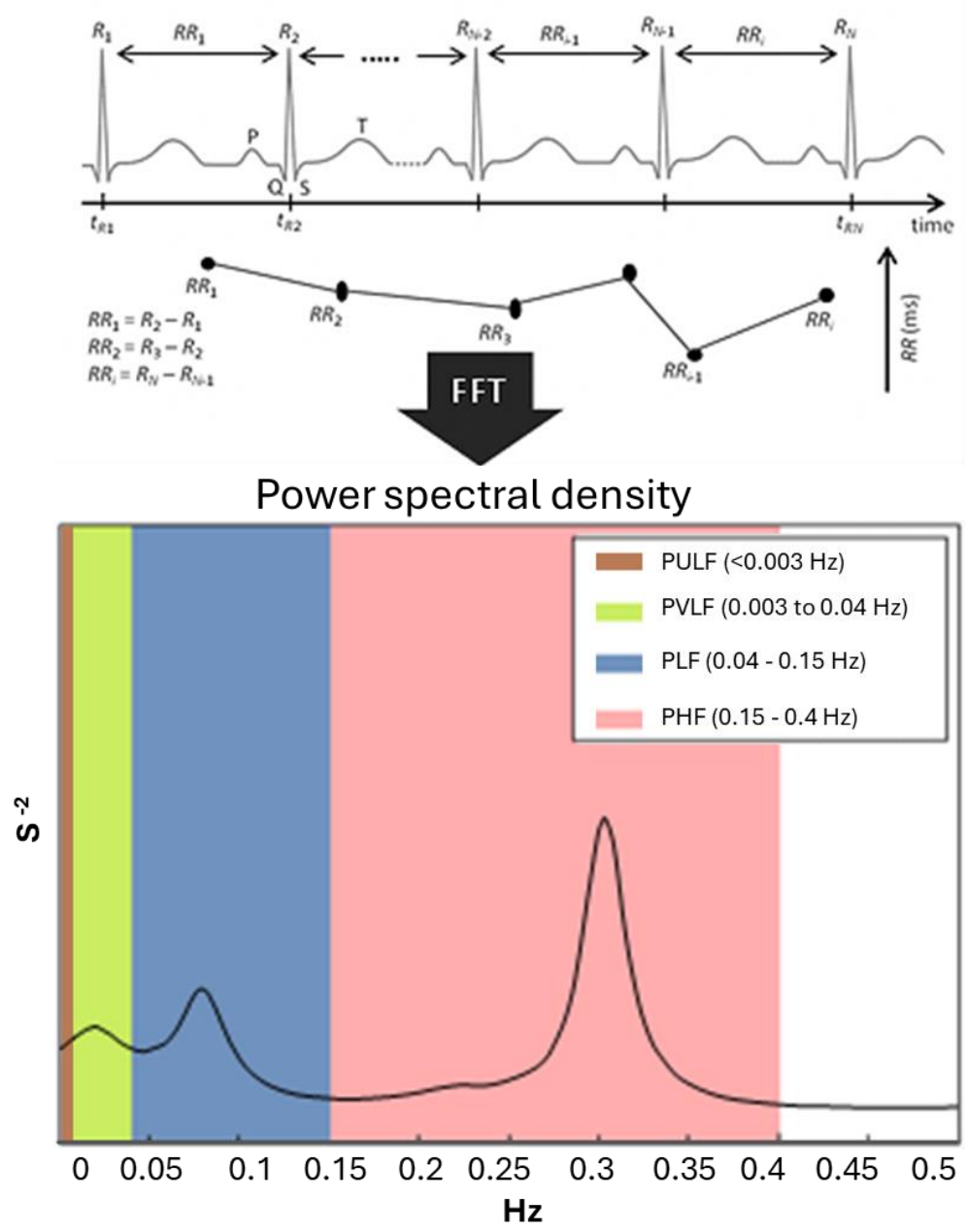
Regarding the frequency-domain analysis, HRV indices are derived from the power spectral density (PSD) of the equidistant inverse interval function signal (Figure 1Figure 10). Two primary spectral components were identified from short-term recordings: the power in the low-frequency (PLF) band, which spans from 0.04 to 0.15 Hz, and the power in the high-frequency (PHF) band, which spans from 0.15 to 0.4 Hz. The PLF represents a mixture of sympathetic and parasympathetic activity, often associated with baroreflex and vasomotor control, whereas the PHF correlates with the RSA and vagal activity, serving as a marker for PNS regulation of the heart, provided the respiratory rate falls within this frequency band. Finally, the ratio between the two indices, the LF/HF ratio, was calculated as a measure of the balance between sympathetic and parasympathetic activity, meaning that an elevated LF/HF ratio may indicate a shift towards sympathetic dominance.

HRV signals, as well as the PRV counterpart, are intrinsically limited by their sampling frequency, which corresponds to the mHR. According to the Nyquist-Shannon sampling theorem, components with frequencies higher than half of the mHR should not be considered to avoid a type of distortion called aliasing. For this reason, the power in the high-frequency (HF) band may overestimate or underestimate parasympathetic activity. To address this, the upper limit of the HF band was adjusted to half the mHR. Using this extended HF band (exHF), it is also possible to estimate the LF/exHF ratio [164].

Additional frequency-domain features are the power in the very low frequency band (PVLF), which spans from 0.0033 to 0.04 Hz, and the power in the ultra-low frequency band (PULF), which spans from frequencies equal or lower than 0.003 Hz. However, the necessary duration

for recording these frequency-band measurements, which ranges from a minimum of 5 minutes to 24 hours, is not suitable for the purposes of our study [165].

Figure 10. Power spectral density analysis of heart rate variability



Schematic diagram for the power spectral density analysis of the heart rate variability obtained by applying the Fast Fourier Transformation (FFT). Classification in four regions: Ultra-Low (PULF), Very-Low (PVLF), Low (PLF) and High (PHF) frequency powers. Adapted from FFT_qCO by Patricia Pineda Vidal / CC-BY-SA-4.0.

Finally, HRV analysis can be influenced by various physiological and physical factors. Therefore, in the studies, individuals were matched by age and gender, and substance use was an exclusion criterion.

6.2. Photoplethysmogram

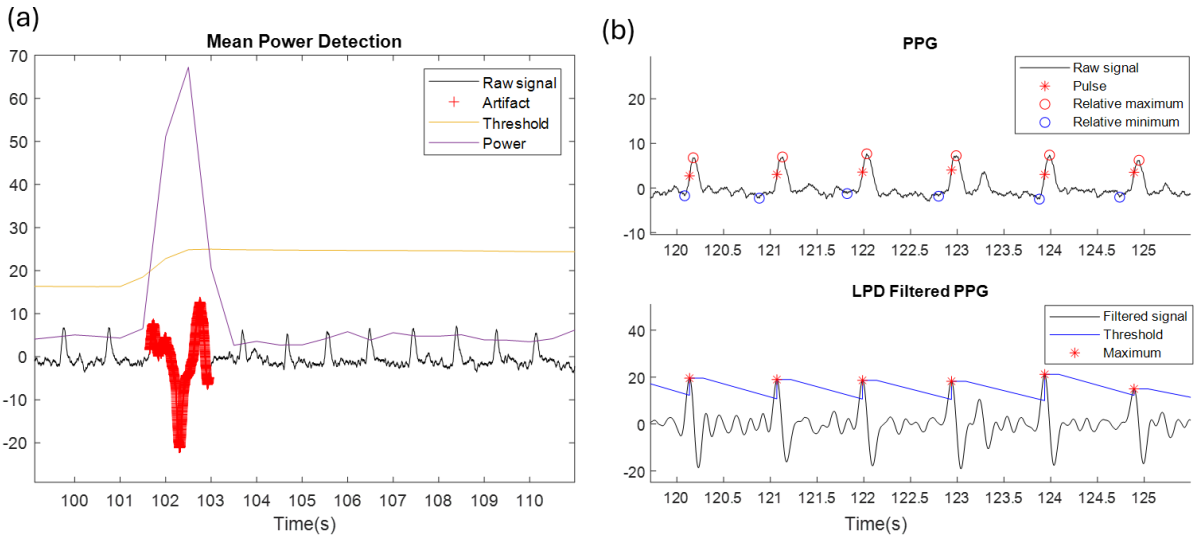
The PPG signal was measured at a sampling frequency of 250 Hz, with a sensor positioned on the middle finger of the non-dominant hand.

The conditioning of the PPG signal to filter out noise and baseline wander was carried out with a low-pass FIR filter with a cutoff frequency of 35 Hz and order 50, and a high-pass FIR filter with a cutoff frequency of 0.3 Hz and order 5000.

The artifacts in the PPG signal were identified as abrupt changes in amplitude that are short in duration. The power of the signal was estimated using a moving 1-second window approach in steps of 0.5 seconds. Specifically, the power at each point in the signal was determined by calculating the variance of the signal within a 1-second window centered at that point. To obtain a smooth estimate of the signal's power, linear interpolation was used between the power values calculated at each step. This resulted in an average power value for each point in the signal. An artifact was then defined as a point in the signal where the associated average power exceeded the mean power plus three standard deviations of the average power from the preceding minute (illustrated in Figure 11.a). For the first minute of data, the mean and standard deviation are calculated from all available data points up to that time.

In addition to detecting short-duration artifacts, the algorithm also identified long-duration changes in signal amplitude, which indicated low-quality signal segments. The detection of this type of artifact or low signal quality segments was carried out based on Hjorth parameters in windows of two seconds displaced every half-second using the algorithm described in [166].

Figure 11. Photoplethysmography signal processing techniques



(a) Detection of short-duration artifacts. (b) Pulse detection at the maximum up-slope point of the low-pass differentiator filtered (LPD) signal.

The detection of heartbeats or pulses in the artifact-free signal was carried out using the method described in [167]. This pulse detection algorithm involves two main phases: linear filtering and adaptive threshold-based detection.

In the first phase, a linear phase band-pass FIR filter is applied, designed using a least-squares technique for the 7.7 Hz to 8 Hz frequency band, which is where the pulses are expected to occur in the PPG signal.

The fiducial point for pulse localization consists in the maximum up-slope point of the low-pass differentiator filtered signal (Figure 11.b). Therefore, for the second phase, starting from the first local maximum identified in the filtered signal, the algorithm looks for points where the filtered signal surpasses a certain threshold. This threshold is initially set to the maximum value of the first 150 ms of the signal. After this period, the threshold decreases at a rate of 0.2. It is assumed that the pulse will occur approximately 300 ms after the filtered signal crosses the threshold. The precise timing of the pulse is determined by identifying the point where the pulse wave reaches a mean value within this 300 ms window.

Once pulses are detected, the algorithm verifies the presence of ectopic or non-normal beats and proposes corrections using the same method employed for ECG analysis, reported in [163]. Segments with up to three interpolated or corrected beats were considered normal. Of the 320 files analyzed in this study, only 12 files were discarded due to poor signal quality that hindered accurate beat detection.

Finally, the same time- and frequency-domain features from HRV analysis were extracted for PRV analysis.

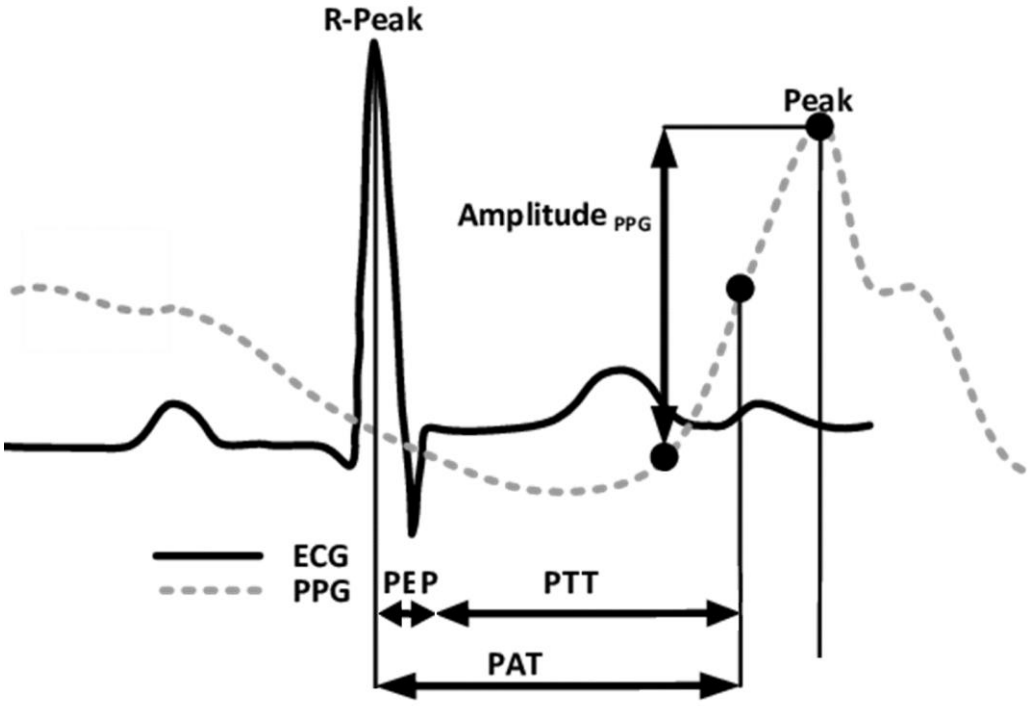
6.3. Pulse arrival time

Pulse Arrival Time (PAT) is a cardiovascular metric used to estimate the time it takes for a pressure wave generated by the heartbeat to travel from the heart to a peripheral site. In essence, PAT encompasses both the electrical and mechanical components of cardiac activity: the depolarization and initiation of ventricular contraction and the subsequent propagation of the pulse wave through the arterial tree. Therefore, this measurement was calculated from the R peak detected in the ECG signal to the pulse detected in the PPG signal, ensuring that the timing of the detected pulse was always greater than the detected beat.

PAT is closely related to the Pulse Transit Time (PTT), but it incorporates an additional component, the pre-ejection period (PEP), illustrated in Figure 12. However, since pinpointing the precise timing of aortic valve opening can involve invasive procedures, PAT is regarded as a promising method for continuous, noninvasive monitoring of BP and cardiovascular health [168].

The mean PAT (mPAT) and standard deviation of PAT (stdPAT) data were provided for each time window. To extract these features, it is essential to accurately detect the heartbeat in both ECG and PPG signals, and synchronization between these signals is of utmost importance. Of the 320 files analyzed in this study, 18 were discarded due to poor ECG or PPG signal quality.

Figure 12. ECG and PPG signals for pulse arrival time calculation



Pulse Arrival Time (PAT) is determined by measuring the time interval between the ECG R-peak and the maximum derivative of the PPG pulse. Pulse Transit Time (PTT) and pre-ejection period (PEP) are also illustrated.

6.4. Electrodermal activity

The EDA signal was measured at a sampling frequency of 250 Hz between the second phalanx of the ring finger and the index finger of the non-dominant hand. A mild direct current is supplied to the skin's outer layer beneath the electrodes and the electrical response of the skin is measured.

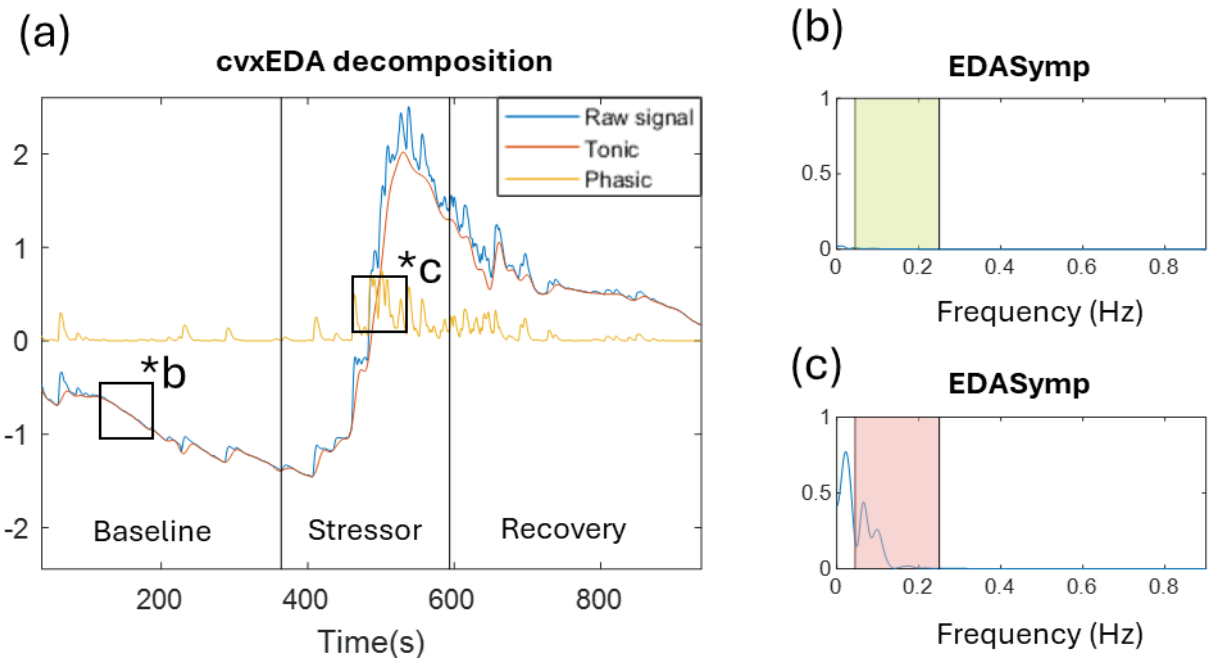
EDA is comprised of two distinct parts: the phasic component, or skin conductance response (SCR), which arises when SMNA is activated and captures immediate and short-duration responses; and the tonic component, or skin conductance level (SCL), which is associated with the overall level of sympathetic arousal, reflecting sustained or cumulative responses over time. The SCR appears as peaks of varying magnitude and duration in response to stimuli, whereas the SCL represents the baseline conductance and can differ between individuals due to various physiological states. The EDA signal is a fast-changing SCR superimposed on a gradual SCL.

To analyze these components, first the EDA signal was visually inspected to delete motion artifacts and linearly interpolated. The 1-minute windows with interpolated segments larger than 25% were discarded and the signal was then resampled at 4 Hz.

For the EDA processing and decomposition, we applied the convex optimization model (cvxEDA) reported in [169], illustrated in Figure 13.a. This model proposes a time-invariant linear system for the skin conductance signal, composed of the sum of vectors representing the tonic and phasic components, along with an independent Gaussian noise term with zero mean and standard deviation, accounting for measurement errors and model inaccuracies.

The information in the SCL signal is usually found below 0.05 Hz frequency, while SCR activity typically ranges between 0.05 to 1.5 Hz, with an average sudomotor fiber activation observed at 0.62 Hz [127]. After applying the Fourier transform to the decomposed EDA signal, the following metrics were calculated: the mean and standard deviation of the tonic and phasic components (mTonic, stdTonic, mPhasic, and stdPhasic), the maximum value and area under the curve of the phasic component (maxPhasic and aucPhasic)

Figure 13. Electrodermal activity signal processing techniques



(a) Application of the cvxEDA decomposition procedure to the EDA signal. Blue: Z-score normalized raw EDA signal. Orange: estimated tonic component. Yellow: estimated phasic component. (b-c) Power spectrum analysis of the EDA signal obtained by applying the Fast Fourier Transformation (FFT), colored boxes indicating the 0.045-0.25Hz band corresponding to the EDASymp feature.

Additionally, the original signal underwent filtering with a band-pass FIR filter of order 500 and cutoff frequencies of 0.01 Hz and 0.9 Hz for power spectral density analysis to compute the sympathetic component of the EDA (EDASymp, illustrated in Figure 13.b-c). This feature is defined as the power within the 0.045–0.25 Hz frequency band, as reported by [170].

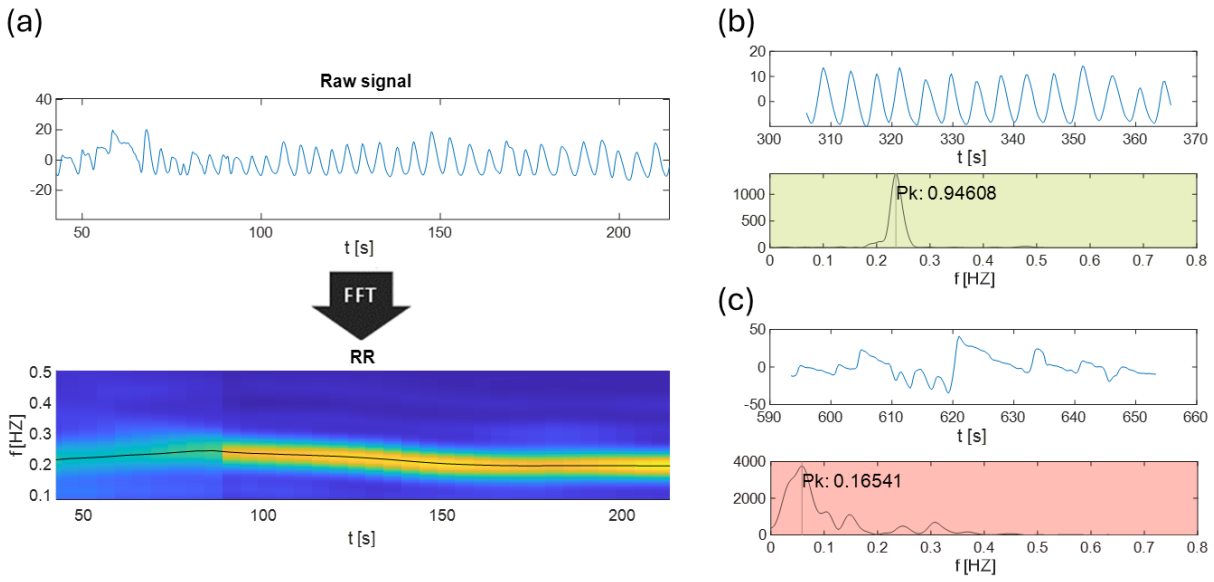
It is important to note that for the EDA analysis, the two sessions for 40 subjects from the first study (out of a total sample of 120) were excluded due to equipment configuration issues. In the second study, 32 files were discarded due to poor signal quality. Consequently, out of the 320 files analyzed in total, the results are based on the remaining 208 files.

6.5. Respiratory signal

The respiratory signal was measured at a sampling frequency of 250 Hz with a chest band.

Following the preprocessing proposed in [171], the respiratory signal was filtered using a 500th-order low-pass FIR filter with a cutoff frequency of 0.9 Hz. Subsequently, a 500th-order high-pass FIR filter with a cutoff frequency of 0.03 Hz was applied, and the signal was resampled at 4 Hz.

Figure 14. Respiratory signal processing technique



(a) Raw respiratory signal and spectrogram by Fast Fourier Transformation (FFT). (b-c) Power spectrum analysis of the 1 min segment respiratory signal, vertical line indicating respiratory rate and its peakness (Pk), green indicating accepted segment (>65%), red indicating rejected segment (<65%).

To find the respiratory rate, the method reported in [171] was used. The respiratory rate (RR) was determined by identifying the frequency corresponding to the maximum peak (Pk) of the power density spectrum, which was calculated using a Fast Fourier Transform (FFT). If the Pk exceeded 65%, the RR was accepted as the respiratory rate, as illustrated in Figure 14.

The specific interest in quantifying ANS activity in respiration, particularly mediated by the parasympathetic branch, we analyzed respiratory frequency during periods of the experimental sessions when the subjects were not speaking. Of the 320 files analyzed, only 9 were discarded due to poor signal quality that interfered with accurate RR detection.

6.6. Skin temperature

The T signal was measured in the little finger of the non-dominant hand at a sampling frequency of 250 Hz.

A visual inspection of the recorded signals indicated the absence of significant artifacts, thus no preprocessing was needed. The mean value (mT) was calculated for each session period in one-minute windows.

To determine the rate of temperature change, successive differences of the mean temperature value every 10 seconds were used as the gradient. For one-minute windows, the mean gradient (mGrad) and median gradient (medGrad) values were associated with this window.

Of the 320 files analyzed, only 5 were discarded due to poor signal quality.

6.7. Final set of features

Table 1 summarizes the final set of features extracted from all physiological signals.

Table 1. Summary table of physiological features

Signal	Technical specifications	Feature	Units	Description	References
ECG or/and PPG	ECG: Range: 1-5mV BW: 0-150Hz SF: 1kHz PPG: Range: 850-950nm (infrared) BW: 0-20Hz SF: 250Hz	mHR	bpm	Mean heart rate	[119, 162, 163, 166, 167, 172]
		SDNN	s	Standard deviation of NN intervals	[119, 162, 163, 165–167, 172, 173]
		RMSSD	s	Root mean square of differences between adjacent NN intervals	[119, 162, 163, 165–167, 172, 173]
		PHF	s^{-2}	HRV high frequency band power (0.15 - 0.4 Hz)	[119, 162, 163, 165–167, 172–174]
		PHFex	s^{-2}	HRV power in extended high frequency band power (0.15 - mHR/2 Hz)	[162, 163, 166, 167, 175]
		PLF	s^{-2}	HRV low frequency band power (0.04 - 0.15 Hz)	[119, 162, 163, 165–167, 172–174]
		PVLF	s^{-2}	HRV very low frequency band power (0.003 - 0.04 Hz)	[119, 162, 163, 165–167]
		PULF	s^{-2}	HRV ultra low frequency band power (<0.003 Hz)	[162, 163, 165–167]
		LF_HF	dl	Ratio of low-frequency to high-frequency power	[119, 162, 163, 165–167, 172–174]
		mPAT	ms	Mean pulse arrival time	[168, 176–178]
		stdPAT	ms	Standard deviation of the pulse arrival time	[179]
EDA	Range: 0.01-100µS BW: 0-16Hz SF: 250Hz	mTonic	µS	Average of the tonic component, i.e. SCL	[169, 179, 180]
		stdTonic	µS	Standard deviation of the tonic component	[179, 180]
		mPhasic	µS	Average of the phasic component, i.e. SCR	[169, 179, 180]
		stdPhasic	µS	Standard deviation of the phasic component	[179, 180]
		maxPhasic	µS	Maximum peak value of the curve of the phasic component	[179, 180]
		aucPhasic	µS·s	Area under the curve value of the phasic component normalized by the length of the session, i.e. SCRs	[179, 180]
		EDASymp	μS^2	Power spectral density of EDA signal (0.045 - 0.25 Hz)	[170, 179, 180]
T	Range: 0-50ºC Fs: 250Hz Precision: 0.01ºC	mT	°C	Mean temperature	[141, 142, 179]
		mGrad	°C	Mean temperature gradient	[179]
		medGrad	°C	Median temperature gradient	[179]
Resp	Range: 0-10 Ω BW: 0-2Hz SF: 250Hz	RR	Hz	Respiratory rate	[171, 179, 181, 182]
		Pk	%	RR peak percentage in the power spectrum	[171, 182]

BW: bandwidth. SF: sampling frequency. bpm: beats per minute. dl: dimensionless. SCL: skin conductance level. SCR: skin conductance response.

7. Physiological dynamics in stress

Stress was declared a global epidemic by the WHO in the 21st century. In addition, the prevalence of stress has been linked to negative effects on both psychic and physical health [183]. However, the level and/or duration required for its transformation into a pathogenic agent is unknown. Chronic stress arises when the stress response is prolonged, and a rearrangement of the homeostatic equilibrium may take place. These new equilibrium conditions can affect the subject's well-being and even negatively affect their health. Again, depending on the stressor, the time and the subject himself, the equilibrium may never be reached, and the subject's health may be severely affected. In this case, we talk about post-traumatic stress disorder. On the opposite side, acute stress arises when the stress response remains for minutes or a few hours affecting the homeostatic equilibrium.

Among the strategies reported in the literature for stress measurement, three different approaches can be highlighted [184]: the assessment of the stress stimulus, the measurement of psychological perception of the stress stimulus and the measurement of the physiological changes under a stress stimulus.

The first strategy is based on the information gathering of events that occur in an individual's lifetime, assessing the effect they produce and understanding that the accumulation of stressful events generates a higher affectation level. This strategy assumes that any event similarly affects all individuals. The Holmes and Rahe Stress Scale [185] is a well-known instrument based on this strategy, where a list of possible events is associated with a score.

The second approach focuses on the perception that an observer has of a subject exposed to a stimulus. This approach usually uses questionnaires. Clearly, this method is subjective and comparing subjects from different observers is difficult. Another alternative within this strategy is the self-perception of the subject, on behalf of the own psychosocial environment. For example, the Derogatis Stress profile [186] has 77 questions rating the stress level based on personality traits and the emotional response.

The third approach is based on the measurement of the physiological response of the subject to a stress stimulus, namely caused by the ANS and HPA axis activation. This response can be measured using anatomical, endocrine, hematological, electrical, thermal, immune and genetic markers. Although the physiological approach seems to be the most objective, it can be invasive, not very specific and may leave the subjective perception of the stress aside.

In any case, to characterize the stress response, a stress stimulus is needed. It could be based on physical elements, social interaction, emotional reactions or cognitive tasks. For example, sinking the hand or foot in cold or hot water are types of stimuli known as the Cold Pressor Test and the Warm Water Test. Similarly, Project ES3 [187] has studied the response to stress caused

by high ambient temperatures with high humidity simulated in a climatic chamber [188]. Exposure to videos of corneal transplant surgeries has made it possible to characterize cortisol and alpha-amylase as stress biomarkers [189].

The Trier Social Stress Test (TSST) is a widely accepted protocol for robustly inducing moderate acute stress in human populations using different stimuli. TSST has made it possible to study the relationship between psychological and physiological responses in recent decades [190]. Perhaps its usefulness lies in the simulation of a combination of a memory task, a social interaction event followed by an arithmetic task.

An increase in salivary, plasma and serum cortisol levels has been reported on TSST application, as well as in the androgen precursor dehydroepiandrosterone (DHEA) and its metabolite DHEA -sulphate (DHEA-S). Higher levels of adrenaline [191] and salivary α-amylase [192] have also been reported following the TSST. Furthermore, the IS is sensitive to stress and has been linked to the HPA axis activation. Pro-inflammatory cytokines are able to activate the HPA axis and alter glucocorticoid secretion, as well as GCs can affect cytokine production. Pro-inflammatory cytokine interleukin-6 (IL-6) levels can increase by 50% in response to TSST and remain elevated 20 minutes after stress exposure. Elevated levels of IL-6 following the TSST are associated with a higher HR during the arithmetic task and a lower cortisol response [193].

Within the chain of secretions caused by the activation of the HPA axis are arginine vasopressin (AVP) and prolactin. AVP has a small molecular size and a short average lifetime that makes it difficult to measure. However, the concentration of Copeptin derived from the same AVP precursor has been proposed as a more stable surrogate, although its physiological interpretation is unknown [194]. On the other hand, it is well established that prolactin secretion and plasma glucose levels are affected by stress. An elevation of their levels in response to different types of psychological stimuli in humans has been described and the intensity of the response depends on the intensity of the stressful stimulus [195].

Additionally, for a noninvasive measure of the ANS response to stress, HRV derived from the ECG signal is commonly used [115]. The psychophysiological factors that influence the stress level can be reflected in the HR and its variability (HRV). These are both mediated by the ANS in its sympathetic and parasympathetic branches, which is reflected in the low-frequency and high-frequency components, depending on whether one or the other branch was predominant, respectively. A meta-analysis shows that there is a reduction in the HRV, an increase in the LF band parallel to a reduction in the HF band, and/or an increase in the LF/HF quotient, thereby reflecting an increase in sympathetic stimulation and parasympathetic inhibition during a stressful event [196].

Photoplethysmography is an uncomplicated and inexpensive optical measurement alternatively used for HR monitoring purposes among others, more and more present in wearables nowadays.

This signal can be analyzed in order to extract similar information to the HRV, that is, the PRV. Moreover, the PPG signal together with the ECG signal provides useful information about the time lapse between the two, the Pulse Arrival Time, which is inversely proportional to the pulse wave velocity, which is in turn associated with arterial stiffness and cardiovascular output. Therefore, a decrease in PAT is related to an increase in blood vessel resistance and cardiovascular output, as well as being inversely related to BP [197].

There are also reports of an increase in the respiratory rate, RR, and its variability, RRV [181, 198], and even an alteration affecting the synchronization between them [199] as a response to the action of a stressor. Increased EDA has also been observed during TSST [200]. Finally, in other studies a relationship has been established between stress and BP, the electrical characteristics of the skin, and T [142, 193].

To sum up, the stress response is mainly generated through the hypothalamic centers and the hypophysis, which activate the ANS and the HPA axis simultaneously but with different timings, with the former being faster than the latter. Consequently, to establish the methodology for an objective, noninvasive, and continuous measurement of stress, as intended in this thesis, it is necessary to explore the physiological changes induced by both pathways and integrate these changes into a global result. To that end, two steps were needed.

The first step is to generate a Stress Reference Scale (SRS) as a linear combination of psychometric tests and biochemical variables to better differentiate between stressed and relaxed states. However, since the SRS has limited temporal resolution and is invasive, the second step is to identify a set of physiological features to generate a model aimed at the proposed target: establishing a methodology that allows for the objective, noninvasive, and continuous measurement of stress, which could lead to more practical and extensive applications.

For this purpose, an experimental study was performed on healthy university students, designed to guarantee a synchronous and concurrent measure of psychometric tests, biochemical variables and physiological features related to acute stress using a modification of the TSST to induce levels of stress based on different stimuli.

7.1. Methods

7.1.1. Participants

Students from the University of Zaragoza, the Technical University of Madrid and the Autonomous University of Barcelona, Spain were recruited consecutively using advertisements on websites. Through the web portal of the project, a participation survey was carried out among interested students. In the survey, the objective of the project and the conditions of participation were explained in a general way, as well as the inclusion and exclusion criteria. The conditions

for inclusion in the study were to be a healthy student aged between 19 and 30 years old and a BMI lower than 30, while any participant with psychopathology, regular consumption of psychotropic substances, or when the baseline stress level was greater than 70% on the visual analogue scale, was excluded. Moreover, they could not consume tobacco, alcohol, or stimulants of the nervous system (caffeine, theine, taurine, etc.) for at least 8 h prior to the study. Those interested who meet the inclusion criteria were selected, up to a maximum of 60 participants per site and they were contacted to indicate the date and place of the first session. All participants signed an informed consent form approved by the ethics committee of each institution.

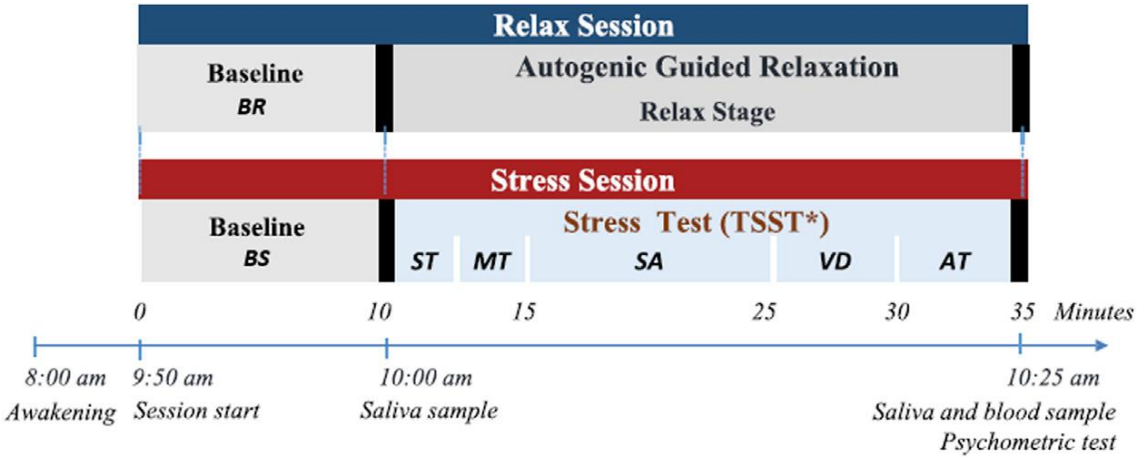
7.1.2. Experimental protocol

The proposed experimental design is an observational and transversal study, in which everyone is compared with himself or herself in a relaxed condition and in an acute stress condition in two measurement sessions. Each session took place on different days, one or 2 weeks from each other, at the same time of day normally 2 h after waking up. Both sessions started with a relaxation period for 10 min with the help of an autogenous relaxing audio to prevent the arriving state from interfering with the study, named baseline relax (BR) and baseline stress (BS). At the end of each session, a nurse took a blood sample. In addition, two samples of saliva were extracted per session at the end and at the beginning, which for the stress stage, it was 25 min before the end of the session, which corresponds to the end of the baseline stage.

During the first session, i.e. relax session, the relevance of the study and the protocol were explained to the participant and the informed consent was signed. Then, sensors were placed, and clinical and socio-demographic data were documented. Afterwards, the subject was left alone in the room, with dimmed lights, in a comfortable position and with an audio for autogenous relaxation for 25 min following Schultz's method [201]. After the BR stage, the first saliva sample was taken. The next 25 min were designated the Relax (R) stage, and, after that, saliva and blood samples were collected, and the psychometric tests administered. During this second session, i.e. stress session, after the 10 min BS stage, the first saliva sample was collected and the stress protocol began, which included three short stories, a memory test, a stress anticipation period, the video presentation, and an arithmetic task. The memory test was carried out with an interviewer and the subject alone in a room and consists of two stages. Within the first stage, referred to as Story Telling (ST), the subject was asked to listen carefully to three stories, which he/she will have to reproduce with the greatest number of details during the second stage, the Memory Task (MT), with a 30 s time limit for each story. The MT stage was video recorded. Under the pretext that the video will be evaluated by experts to assess memory performance, the subject remains alone in the room without knowing how long he/she will be there. This stage aims to increase the state of anxiety before the next stage, and it was labelled as Stress Anticipation (SA). After 10 min, the interviewer, and an audience of at least three people entered the room to make the public presentation of the video segments (VD). The recorded videos of the subject were interspersed with videos from the control segments, which

are equivalent video segments where a dummy participant responds to the same stories correctly. This unknown subject was matched in age and gender for each participant. Finally, during the arithmetic task (AT) the subject was asked to count down from 1022 to 0 in steps of 13. If he/she made an error, he/she had to start over from the beginning (i.e., 1022). The subject had 5 min to complete the task, even though the subject was not expected to finish the task. After that, the second saliva sample and blood draw took place along with the psychometric tests. Figure 15 summarizes the process followed during the two sessions.

Figure 15. Experimental design for physiological dynamics in stress



Experimental design sessions. BR: Baseline relax. BS: Baseline stress. ST: Story Telling. MT: Memory test. SA: Stress anticipation. VD: Public video display. AT: Arithmetic Task.

7.1.3. Outcome measures

7.1.3.1.Psychometric tests

The psychometric tests that were self-administered are the Perceived Stress Scale, the Visual Analog Scale, and the State- Trait Anxiety Inventory. All tests are validated, supported, well documented by the medical world, and have a contrasted and accepted Spanish language version. The Symptomatic Stress Scale, designed at the Clinical Hospital of Zaragoza in the Project ES3 framework, was also administered.

The two sessions took place on different days to prevent the learning effect or memorization of the questionnaires, which could invalidate the psychometric test results.

The State-Trait Anxiety Inventory (STAI) [202], and consists of 40 questions related to the subject's introspection about his feelings of discomfort, worry, tension and stress [189]. The scale aims to measure two components: a trait (STAIt) that would quantify the relatively stable individual differences in responding to situations perceived as threatening or the tendency to perceive situations as more threatening. The second component refers to the state (STAIs) in a transitional period characterized by a feeling of tension, apprehension, and increased activity

of the ANS [203]. Each of the components is measured through a subset of 20 questions out of 40 on the scale. The STAIt and STAIs have total ranges from 0 to 60, with a higher score corresponding to higher detected anxiety. The Cronbach's alpha of the scale, both in its trait and state component, is greater than 0.9 [204].

The Visual Analog Stress Scale (VASS) is a simple method to detect changes in mood and subjective perceptions [205]. It consists of a single question: In general, where would you say your stress level is at this moment? Being 0 = no stress at all and 100 = absolutely stressed. The use of the VASS has shown high internal consistency in the measurement of transient and subjective psychological states (Cronbach's alpha range: 0.84–0.96) [206].

The Perceived Stress Scale (PSS) was proposed in the late 20th century by Sheldon Cohen and his colleagues with a set of 14 items that were later reduced to 10. This scale assesses the extent to which life situations are assessed as stressful based on feelings and thoughts over the past month. Intrinsically, it considers the influence of how well the subject can handle a stressful situation given his or her coping resources, which ultimately offers a characterization of a person's trait [207]. A higher score corresponds to a higher level of perceived stress. The short European Spanish version of 10 items demonstrated adequate reliability with Cronbach's alpha 0.82 [208].

The Symptomatic Stress Scale (SSS) is a psychometric test designed on behalf of the ES3 Project [187] by the psychiatry research group of the Hospital Clínico de Zaragoza. It tries to evaluate the effect of the stressful stimulus on the subject from the somatic and psycho-cognitive symptoms that the subject perceives of himself (Appendix 4). The scale is of the Likert type and consists of 20 questions.

7.1.3.2.Biochemical variables

The biochemical variables considered in the present study are the concentrations of hormones, enzymes or molecules, analyzed in blood or saliva samples that offer information about the HPA axis and the ANS.

The biochemical variables measured in saliva were cortisol and α-amylase, whereas the biochemical variables measured in blood were prolactin, copeptin, and glucose. It was proposed to hold the morning sessions because of the circadian rhythm of cortisol and α-amylase, specifically the sessions were scheduled in the morning (09:45–10:45 h), 2 h after waking up. Moreover, it helps to have control of the foods consumed by the subjects and in the awakening, two factors that could invalidate the biochemical variables. All subjects were asked to avoid coffee and tea before the session and refrain from exercising or drinking alcohol in the last 24 h. The saliva collection was performed in Salivette tubes following the manufacturer's recommendations (Sarstedt AG & Co., Nümbrecht, Germany). Chewing the cotton swab provided with the tube and avoiding swallowing saliva for 1 min to obtain an adequate amount

of sample. Subsequently, the sample was preserved on ice until it was centrifuged in the laboratory (15 min at 1315 g, 4°C) to separate the saliva from the cotton swab. Then it was aliquoted in duplicate in Eppendorf tubes with the subject identifying data, type of session and date. The tubes were kept frozen at −20°C until processing. The concentration of cortisol and α-amylase in saliva were quantified through commercial kits. A commercial immunoassay technique (Salimetrics, State College, PA, United States) was used for cortisol and a kinetic enzyme assay was used for α-amylase (Salimetrics, State College, PA, United States), capable of measuring the activity of the enzyme in international units/ml of saliva (U/ml).

The extraction of blood was carried out in two tubes: the first with EDTA anticoagulant and the second with gel separator. Both were preserved on ice until centrifuged in the laboratory at 3000 rpm for 10 min. The plasma and serum were then aliquoted separately in tubes with the identifying data of the subject, type of session and date. The tubes were kept frozen at −20 °C until processing at the Biomedical Diagnostic Center of the Hospital Clinic de Barcelona by molecular absorption and immunoassay spectrometry techniques to determine the concentration of prolactin, copeptin and glucose.

7.1.3.3.Physiological signals

Details concerning feature extraction are described in section 6. Table 2 summarizes the final list of features used in this study.

Table 2. List of extracted features for physiological dynamics in stress

Physiological signal	Extracted feature	Definition
Electrocardiography or/and Photoplethysmography	mHR (bpm)	Mean heart rate
	SDNN (s)	Standard deviation of NN intervals
	RMSSD (s)	Root mean square of differences between adjacent NN intervals
	PHF (s^{-2})	HRV high frequency band power (0.15 - 0.4 Hz)
	PHFex (s^{-2})	HRV power in the extended HF band power (0.15 – mHR/2 Hz)
	mPAT (ms)	Mean pulse arrival time
	stdPAT (ms)	Standard deviation of the pulse arrival time
Electrodermal Activity	mTonic (µS)	Average value of the tonic component, i.e. SCL
	stdTonic (µS)	Standard deviation of the tonic component
	mPhasic (µS)	Average value of the phasic component
	stdPhasic (µS)	Standard deviation of the phasic component
	maxPhasic (µS)	Maximum peak value of the curve of the phasic component
	aucPhasic (µS·s)	Area under the curve value of the phasic component normalized by the length of the session
	EDASymp (μS^2)	Power spectral density of EDA signal (0.045 − 0.25 Hz)
Skin Temperature	mT (°C)	Mean temperature
	mGrad (°C)	Mean temperature gradient
	medGrad (°C)	Median temperature gradient

7.1.4. Statistical Analysis

The results of the psychometric tests and biochemical variables were analyzed using mixed models with the subject as a random factor. All analyses were run in SAS 9.4 software. To fulfil the assumption of normality required by the model, the Box-Cox transform with $\lambda = 0$ was used when it was necessary. Principal components analysis (PCA) was performed to find the so-called SRS weights, and the most relevant features extracted from the physiological signals. Afterwards, we used linear regression with these physiological features to generate the Acute Stress Model. Before performing the PCA analysis, the features to be included in the model were re-scaled to a range of 0 - 100 to avoid unfair weighting given the differences in the order of magnitude of the values of each feature.

7.2. Results

7.2.1. Participants

The study included 120 healthy young students, recruited from the University of Zaragoza, the Technical University of Madrid and the Autonomous University of Barcelona, Spain. After individual checking for criteria fulfilment, 40 candidates per site were able to participate in the two different sessions.

The population studied corresponds to a group of 60 men and 60 women. Their average age is 22 ± 3 years and in normal ranges according to the WHO based on their BMI [209]. 10% of the sample subjects suffered from asthma, respiratory allergies, migraine or intestinal reflux, but they were treated, and it did not represent an inconvenience to carry out the measurement sessions. Most of the subjects had extracurricular activities, mainly sports, language courses or some type of artistic activities.

The perceived stress measured prior to the inclusion of the study did not exceed 47 units on average where from a scale from 0 to 100 it would be considered from no to mild stress. The self-perception of stress in subjects who were not drug consumers was higher (VAS = 46.21) than in those who consumed at least once a day (VAS = 15.3) but lower than in those who consumed less than once a day on average (VAS = 52.08).

The consumption habits were mainly non-toxic in the measured sample. In other words, non-use of tobacco or drugs and occasional consumption of liquor prevailed. The consumption or not of coffee was evenly distributed between men and women. Regarding the health status of the subjects based on Table 3, it can be defined as good or healthy. Given that most practice sports regularly or occasionally, they do not have diagnosed degenerative diseases and the consumption of pharmaceutical drugs was limited to the use of oral contraceptives. Finally, the majority of the subjects had a partner or emotional relationship, lived in family homes or shared a flat which suggested that there was no anomaly in the capacity for social interaction. In

conclusion, the 120 subjects who participated in the measurement sessions met the proposed inclusion/exclusion criteria.

Table 3. Sociodemographic data of the healthy students

Variable	Categories	N=120
Age (m, std)		21,992 (2,865)
BMI (m, std)		22,145 (2,760)
Gender (n,%)	Female	60 (50)
	Male	60 (50)
Smoker (n,%)	No	100 (83)
	Yes	20 (16)
Coffee (n,%)	No	59 (49)
	Yes	61 (50)
Alcohol (n,%)	None	22 (18)
	Occasional	88 (73)
	Moderate	10 (8)
Drugs (n,%)	No	104 (86)
	Occasional	13 (10)
	Habitual	3 (2)
Chronic diseases (n,%)	No	108 (90)
	Yes	12 (10)
Pharmaceutical drugs (n,%)	No	94 (78)
	Yes	26 (21)
Relationship (n,%)	No	33 (27)
	Yes	87 (72)
Origin (n,%)	Rural	22 (18)
	Urban	98 (81)
Cohabitation (n,%)	Alone	6 (5)
	Family	76 (63)
	Shared flat	38 (31)
Sport (n,%)	No	21 (17)
	Occasional	40 (33)
	Habitual	59 (49)

Data are mean (m), standard deviation (std), number of subjects (n) and percentage (%).

7.2.2. Outcome measures

7.2.2.1.Psychometrics tests

The psychometric scores were calculated following the guidelines available in the CIBER-SAM bank of instruments and methodologies in mental health. Table 4 summarizes the results obtained from the psychometric test administered.

The PSS scores were calculated as the sum of all the items with the reverse scores for questions 4, 5, 7, and 8. The results for each of the sessions and by gender were not significant. The studied sample had approximately three more units in the total score (2.76 ± 3, p-value < 0.0001) than the normative data of the PSS reported [208]. This indicates that the studied population was more likely to perceive a situation as stressful or was more sensitive to it. There was no statistical evidence that this feature changed from one session to the next or that it was influenced by gender.

For the STAI, scores were calculated as the sum of all the items with the reverse scores for questions 1, 2, 5, 8, 10, 11, 15, 16, 19, 20, 21, 23, 26, 27, 30, 33, 34, 36 and 39. In the case of the STAIt, there were no statistically significant changes observed, which is consistent with the result of the PSS. On the other hand, there was an increase in the scores for STAIs in the stress session compared to the relaxation session, regardless of gender. This result supports that the stressful stimulus applied in the stress session influenced the state of the subjects. Women had a significantly lower trait (p-value = 0.0009) than the score reported in [204], but an equivalent score to men.

The scores of the VASS and the SSS had statistically significant increases that were consistent with the State Anxiety Inventory. Again, this result provides evidence of the stressful effect of the stress session and the correct application of the stimulus. Likewise, there were no differences related to sex.

Table 4. Psychometric test results of the healthy students

	Total		Female		Male	
	RS	SS	RS	SS	RS	SS
PSS	20.68±(2.91)	20.00±(3.25)	20.65±(3.16)	20.41±(3.28)	20.70±(2.67)	19.59±(3.21)
STAIt	19.93±(9.53)	19.52±(9.53)	20.83±(9.80)	19.71±(9.84)	19.02±(9.24)	19.33±(9.29)
STAIs	15.99±(8.41)*	23.45±(11.37)*	16.03±(8.75)*	25.05±(11.10)*	15.95±(8.13)*	21.83±(11.52)*
VAS	32.68±(21.36)*	51.02±(21.18)*	34.85±(23.58)*	52.56±(19.48)*	30.50±(18.84)*	49.45±(22.85)*
SSS	21.21±(13.39)*	28.84±(15.05)*	21.92±(14.41)*	30.73±(14.64)*	20.50±(12.37)*	26.91±(15.36)*

*Data are Mean and Standard deviation. RS: Relax State. SS: Stress State. PSS: Perceived Stress Scale. STAIs: State Anxiety Inventory. STAIt: Trait Anxiety Inventory. SSS: Symptomatic Stress Scale. VAS: Visual Analog Scale. * Statistically significant differences between RS and SS (p<0.05).*

The Symptomatic Stress Scale (SSS) was validated with a sample of 71 individuals by JM Garzón and published in his PhD thesis [210]. The score is the sum of all the items with no reverse scoring. Internal validation of the SSS scale was carried out as an instrument for measuring acute emotional stress through a descriptive and inferential statistical analysis following the guidelines and procedures described in [211]. Significant differences in the means of the relaxation and stress sessions were observed through a t-test for repeated measures with a p<0.0001 in all state tests and no difference in the trait tests as expected. Cronbach's Alpha Coefficient was determined for each of the two sessions from the descriptive statistics for each of the questions on the scale. No negative correlations or Cronbach's Alpha Coefficient less

than 0.8 were found, that is, the scale has internal consistency or reliability in both measurement states for the entire set of questions.

7.2.2.2.Biochemical variables

The differences between the second sample and first sample of cortisol and α-amylase concentrations are shown in Table 5. Concerning cortisol, there was a lower decrease in concentration in the stress session compared to the relax session, that is, in both cases, between the two measurements of each session, the circadian cycle is observed, a decrease in cortisol and an increase in α-amylase. However, the lower decrease in cortisol in the stress session shows a greater release of cortisol opposite to the variation due to the circadian cycle. And the same occurs with α-amylase, there is a greater increase in the stress session than in the relaxation session. Both results showed greater activity in the HPA axis and in the sympathetic system during stress. Moreover, men had a higher concentration of cortisol than women while no differences were found in α-amylase concentrations.

Table 5. Biochemical variable results of the healthy students

	Total		Female		Male	
	RS	SS	RS	SS	RS	SS
ΔCortisol ♂	-0.06±(0.09)*	-0.02±(0.11)*	-0.04±(0.07)*	-0.02±(0.08)*	-0.08±(0.11)*	-0.01±(0.13)*
Δα-Amylase	9.22±(56.48)*	49.71±(77.76)*	10.50±(50.09)*	46.00±(78.31)*	7.92±(62.73)*	53.48±(77.69)*
Copeptin ♂	6.28±(3.64)*	7.28±(3.97)*	4.74±(2.71)*	5.67±(3.46)*	7.75±(3.81)*	8.88±(3.82)*
Glucose	87.14±(14.77)	88.22±(9.80)	85.36±(14.95)	87.03±(8.28)	88.87±(14.51)	89.39±(11.05)
Prolactin ♂	7.95±(3.62)*	9.16±(4.54)*	8.92±(4.27)*	10.45±(4.93)*	7.02±(2.58)*	7.92±(3.77)*

*Data are Mean and Standard deviation. RS: Relax State. SS: Stress State. * Statistically significant differences ($p<0.05$). ♂ Statistically significant differences between gender.*

As expected, there was a statistically higher value for prolactin and copeptin in the stress session with respect to the relaxation session. However, glucose levels were not significantly altered by the stressful stimulus applied.

Likewise, it is noteworthy that the values of all the variables were within the normal range of clinical use. It was also observed that the values were affected by sex, finding a higher concentration of copeptin in men than in women. On the contrary, the prolactin concentration in women was higher than in men. In fact, the concentration of prolactin in men in the stress session is equivalent to that of women in the relaxation session.

7.2.2.3.Physiological signals

The results on the physiological features extracted during each protocol stage are presented on Table 6.

Table 6. Physiological features of the healthy students

Electrocardiography				
		Mean	Std	No Diff
mHR [BPM]	BR	73,6	11,55	BS SA VD
	R	72,85	11,22	BS VD
	BS	72,99	11,85	BR R VD
	ST	82,81	15,84	MT
	MT	83,74	16,41	ST
	SA	73,85	12,92	BR VD
	VD	73,22	12,84	BR R BS SA
	AT	89,74	17,71	
SDNN [s]	BR	0,13	1,2	R BS ST AT
	R	0,11	0,87	BR BS ST
	BS	0,07	0,08	BR R ST AT
	ST	0,22	1,01	BR R BS SA VD AT
	MT	0,35	1,62	
	SA	0,31	6,38	ST VD AT
	VD	0,59	3,85	ST SA AT
	AT	0,18	0,8	BR BS ST SA VD
RMS [s]	BR	0,11	1,71	R BS ST
	R	0,07	1,24	BR BS ST
	BS	0,03	0,11	BR R ST
	ST	0,25	1,42	BR R BS SA AT
	MT	0,38	2,26	VD
	SA	0,36	9,05	ST AT
	VD	0,77	5,45	MT
	AT	0,19	1,16	ST SA
PLF [s^{-2}]	BR	0,51	1,22	BS ST
	R	0,5	0,85	R ST SA
	BS	0,83	4,13	BR R BS SA
	ST	1,22	2,43	AT
	MT	0,5	0,61	BR BS ST
	SA	0,68	2,5	
	VD	0,86	2,24	MT
	AT	0,24	0,27	R BS ST SA VD
PHF [s^{-2}]	BR	0,23	0,25	BR ST SA VD
	R	0,28	0,42	BR ST SA VD
	BS	0,57	3,34	BR R BS SA VD
	ST	0,75	2,02	
	MT	0,26	0,35	BR R BS ST VD
	SA	0,34	0,82	BR R BS ST SA
	VD	0,48	0,94	
	AT	0,27	0,29	R BS ST SA VD
PHFex [s^{-2}]	BR	0,26	0,38	BR ST VD
	R	0,31	0,46	BR ST SA VD
	BS	0,62	3,41	BR R BS SA VD
	ST	0,87	2,26	
	MT	0,3	0,41	BR BS ST VD
	SA	0,38	0,9	BR R BS ST SA
	VD	0,58	1,18	
	AT	380,56	489,64	ST MT SA AT
LF_HF [DL]	BR	299,32	423,79	ST MT SA
	R	297,94	391,1	VD
	BS	284,94	253,19	BR R MT SA AT
	ST			

	MT	284,73	382,89	BR R H SA AT
	SA	295,95	352,11	BR R ST MT AT
	VD	209,97	310,99	BS
	AT	311,26	492,72	BR ST MT SA
Photoplethysmography				
mHR [BPM]	BR	73,68	11,36	BS SA VD
	R	73,08	12,93	BS SA VD
	BS	73,37	11,74	BR R SA VD
	ST	83,12	16,72	MT
	MT	85,4	17,27	H
	SA	72,04	12,43	BR R BS VD
	VD	72,78	12,55	BR R BS SA
	AT	88,68	19,17	
SDNN [s]	BR	0,02	0,01	R BS H
	R	0,02	0,01	BR BS H
	BS	0,02	0,01	BR R H
	ST	0,02	0,01	BR R BS VD AT
	MT	0,02	0,02	
	SA	0,08	1,3	
	VD	0,02	0,02	ST AT
	AT	0,02	0,01	ST VD
RMS [s]	BR	1,23	0,19	BS SA VD
	R	1,22	0,22	BS SA VD
	BS	1,23	0,2	BR R SA VD
	ST	1,39	0,28	MT
	MT	1,43	0,29	H
	SA	1,21	0,21	BR R BS VD
	VD	1,22	0,21	BR R BS SA
	AT	1,48	0,32	
Electrodermal Activity				
mTonic [µS]	BR	-0,18	0,68	R
	R	-0,26	0,69	BR
	BS	-1,09	0,57	
	ST	0,10	0,55	SA MT VD
	MT	0,12	0,62	SA ST VD
	SA	-0,03	0,52	ST MT
	VD	0,18	0,58	ST MT
	AT	0,40	0,79	
stdTonic [µS]	BR	0,11	0,12	BS
	R	0,15	0,19	ST
	BS	0,10	0,16	BR
	ST	0,16	0,15	S MT R AT VD
	MT	0,20	0,22	SA ST AT VD
	SA	0,19	0,16	ST MT VD
	VD	0,18	0,19	SA ST MT AT
	AT	0,22	0,23	ST MT VD
EDASymp [μS^2]	BR	0,38	1,17	BS R
	R	0,90	3,05	BR BS
	BS	0,44	4,66	BR R
	ST	5,96	50,54	MT AT
	MT	1,73	4,01	ST AT
	SA	1,08	3,63	
	VD	1,65	3,80	
	AT	1,85	4,94	ST MT
mPhasic [µS]	BR	0,07	0,14	BS
	R	0,10	0,19	BS

	BS	0,07	0,17	BR R
	ST	0,38	0,45	SA MT AT VD
	MT	0,39	0,39	ST AT VD
	SA	0,22	0,25	ST VD
	VD	0,28	0,30	SA ST MT AT
	AT	0,35	0,35	ST MT VD
stdPhasic [µS]	BR	0,07	0,12	BS
	R	0,10	0,16	
	BS	0,06	0,14	BR
	ST	0,17	0,28	MT AT VD
	MT	0,21	0,23	ST AT VD
	SA	0,17	0,17	
	VD	0,19	0,20	ST MT AT
	AT	0,19	0,21	ST MT VD
maxPhasic [µS]	BR	0,29	0,48	BS R
	R	0,42	0,66	BR BS
	BS	0,25	0,51	BR R
	ST	0,82	1,03	MT AT
	MT	0,93	0,88	ST AT
	SA	0,70	0,68	
	VD	0,80	0,76	
	AT	0,88	0,86	ST MT
aucPhasic [µS·s]	BR	4,10	8,05	BS MT R AT SA VD
	R	5,99	11,31	SA BR BS MT AT VD
	BS	4,08	9,96	SA BR MT R AT VD
	ST	21,84	25,49	
	MT	22,67	23,15	SA BR BS R AR VD
	SA	12,88	14,15	BR BS MT R AT VD

	VD	16,59	17,49	SA BR BS MT R AT
	AT	20,36	20,73	SA BR BS MT R VD
Skin Temperature				
MeanT [°C]	BR	33,71	3,33	
	R	34,7	2,83	H
	BS	34,31	3,28	H
	H	34,03	2,63	R BS
	MT	32,5	2,74	SA
	SA	32,3	3,49	MT
	VD	31,68	3,69	
	AT	30,79	3,52	
meanGrad [°C]	BR	0,03	0,07	BS
	R	0	0,04	SA
	BS	0,03	0,07	BR
	ST	-0,07	0,07	MT
	MT	-0,07	0,07	H
	SA	0	0,08	R
	VD	-0,04	0,06	AT
	AT	-0,04	0,06	VD
medGrad [°C]	BR	0,03	0,07	BS
	R	0	0,04	SA
	BS	0,03	0,07	BR
	ST	-0,07	0,07	MT
	MT	-0,07	0,07	H
	SA	0	0,09	R
	VD	-0,04	0,07	AT
	AT	-0,04	0,06	VD
Pulse Arrival Time				

mPAT [ms]	BR	281,69	69,46	R BS ST MT SA
	R	277,41	65,41	BR BS ST MT SA
	BS	270,06	66,77	BR R ST MT SA VD AT
	ST	259,48	77,5	BR R BS MT SA VD AT
	MT	277,9	90,85	BR R BS ST SA VD AT
	SA	268,59	69,31	BR R BS ST MT VD AT
	VD	256,59	68,02	BS ST MT SA AT
	AT	258,3	88,29	BS ST MT SA VD

stdPAT [ms]	BR	15,12	29,98	R BS ST SA VD
	R	13,69	29,43	BR BS ST SA VD
	BS	14,64	39,88	BR R ST SA VD
	ST	19,88	52,31	BR R BS MT SA AT
	MT	30,11	49,36	ST AT
	SA	18,41	56,6	BR R BS H
	VD	12,76	40,03	BR R BS
	AT	22,66	42,88	ST MT

EDA values multiplied by 100. DL: Dimensionless. BR: Basal relax. R: Relax. BS: Basal stress. ST: Storytelling. MT: Memory test. SA: Stress Anticipation. VD: Public video display. AT: Arithmetic Task. No Diff: No significant difference with the stage listed (p-value < 0.05).

For the HRV, regarding the temporal indices, none of them differed between the basal moments but they were significantly different compared to the AT stage, which is the moment of the highest stress level. However, it is surprising that the moments of relaxation, VD and SA did not differ. The frequency indices in both the classic and the extended bands differed between the two moments BR and BS, failing to meet one of the selection criteria. The only frequency indices that met this criterion were the indices in the HF band, although this was only calculated when the subject was quiet. In conclusion, the HRV features that were considered adequate for estimating the stress level were: the mean value (mHR), the standard deviation (SDNN) and the mean square value of the HR (RMS) and in those cases where the subjects were not speaking, the indices on the HF band. Similar results were found in the PRV analysis.

When combining both signals, no significant differences were found between the two baselines BR and BS for both mPAT and stdPAT. In the stages where the stressful stimulus was applied, a decrease in pulse transit time and greater variability were observed.

All the features from the EDA except the skin conductance level met the selection criteria of being the same at the baseline moments of both sessions and showed a significant difference between the moments of relaxation and the moments in which the stressful stimulus was applied. It is important to highlight that, for the EDA analysis, 40 subjects of the total sample of 120 were discarded due to configuration problems with the measurement equipment, therefore the results presented correspond to only 80 subjects.

Finally, for the T features, only the temperature gradient in the finger (mGrad, medGrad) met the selection criteria since there were no differences at the two baseline moments (BS and RB). A temperature decrease was observed during moments of stress, which was more intense in stages ST and MT than VD and AT.

7.2.3. Stress reference scale

Following the methodology suggested in [210], a SRS was computed as a weighted average of the psychometric test scores and biochemical variables. The weights for each element were assigned in accordance with a PCA being proportional to the scoring coefficient and the variances of the component. To define the SRS, the components with an eigenvalue higher than 0.9 that together explained at least 75% of the total variance were selected.

As shown in Table 7, the first five components fulfilled the selection criteria of an eigenvalue higher than 0.9 and accumulated explained variance at least 75% of the total variance. The resulting scoring coefficients for the five components are shown in Table 8 and the highest values are highlighted. The first and the fourth component had the highest scores for the psychometric tests. Components 2 and 3 can be associated with the HPA activation since the cortisol, copeptin and prolactin scores are the most relevant among these components. Finally,

the last component, the fifth, has the peak value for the α-amylase that gives this component a relation to the SNS.

Table 7. Eigenvalues and explained variances of PCA of healthy students' data

Comp	Eigenvalue	Dif	% VAR	VAR Acum
1	2.78	1.56	31%	31%
2	1.22	0.12	14%	44%
3	1.10	0.12	12%	57%
4	0.99	0.04	11%	68%
5	0.95	0.16	11%	78%
6	0.78	0.20	9%	87%
7	0.58	0.22	6%	93%
8	0.36	0.11	4%	97%
9	0.24		3%	100%

Comp: component. % VAR: Percentage of explained variance. VAR Acum: Percentage of accumulated variance.

Table 8. Scoring coefficients of healthy students' data

Component	1	2	3	4	5
STAIs	90	10	-1	0	4
SSS	87	2	13	1	-4
VAS	79	12	-13	-3	23
STAIt	69	-6	5	31	-20
PSS	9	0	-11	93	5
Cortisol	5	77	29	20	0
α-Amylase	3	3	3	4	97
Copeptin	3	1	92	-11	3
Prolactin	7	75	-27	-21	5
% VAR	40%	17%	16%	14%	13%

VAR Prop: Percentage of total variance explained by component for computing component scores.

The resulting SRS computed for a population of 120 young people was:

SRS = 0.14*PSS + 0.11*SSS + 0.11*STAIs + 0.10*VAS + 0.08*STAIt + 0.16*Copeptin + 0.13*αAmylase + 0.09*Cortisol + 0.08*Prolactin

Table 9. Stress reference scale results

SRS		N	Mean	Std	Min	Q1	Median	Q3	Max
Session*	RS	114	39,40	6,70	27,28	35,09	38,01	42,70	62,34
	SS	113	45,40	7,78	29,81	40,26	45,34	51,01	71,31
Gender	F	112	42,89	7,89	28,59	37,56	41,08	47,30	71,31
	M	115	41,90	7,80	27,28	36,40	40,43	47,94	60,13

**p<0.05. RS: relax state; SS: stress state; F: female; M: male*

There were no significant differences between male and female SRS scores, although, as expected, the SRS was significantly higher in the Stress Session compared to the relax session.

7.2.4. Physiological stress model

To generate the Physiological Stress Model (PSM), first of all, feature values that were significantly different at the baseline stages of both sessions were discarded considering that their values were reflecting conditions other than the stress. Moreover, features affected by speech such as respiratory rate were also excluded to avoid intrinsic noise.

Unlike the SRS, where values can be associated only with a state of stress (SS) or a state of relaxation (RS), the physiological features selected are, in a way, "continuous". Although, due to their method of calculation, they are associated with different moments of the session, and, therefore, offer information on the different intensities of stress reached at this time.

To select the relevant features, we associated the values of the SRS for the state RS to the features calculated at the R stage of the first session measurement, and the values of the SRS in the SS state to the last moment of the second measurement session, that is, to the AT stage.

Two sequential selections were done in order to simplify further analysis. Firstly, from each set of features showing statistically significant linear dependencies, only one was selected. From this set of features, only those having a significant correlation with the SRS score were selected in a second step. Only 18 out of the initial 80 physiological features were finally selected: from the ECG the HRV_SDNN, HRV_RMS, HRV_PHFex, HRV_PHF and HRV_mHR; from the PPG the PRV_SDNN, PRV_RMS and PRV_mHR, from the PAT the mPAT and stdPAT; from the EDA the stdTonic, stdPhasic, maxPhasic, mPhasic, aucPhasic and EDASymp; and from the T the mGrad and medGrad. It was considered that this number of features could be easily handled to give results in real-time without requiring large computing capabilities.

Table 10 shows the eigenvalues and explained variances for the components resulting from the PCA analysis on the set of features. It is observed that the first five components are the only ones that have an eigenvalue greater than one and with them, 87% of the variance of the data is explained, which is sufficient for the type of analysis that was carried out.

For the five components selected, Table 11 shows the coefficients or associated weights of each feature. It is observed that each feature loads in a single component with quite a difference compared to the others. This fact can be understood as a great collinearity between the features that make up each group and therefore an evident redundancy in the information they offer. To select the features included for each of the components, only those that had a coefficient greater than 60 were selected.

Table 10. Eigenvalues and explained variances of PCA of healthy students' physiological data

Comp	Eigenvalue	Dif	% VAR	VAR Acum
1	5.79	1.27	32	32
2	4.52	2.35	25	57
3	2.17	0.27	12	69
4	1.90	0.54	11	80
5	1.36	0.72	8	87
6	0.63	0.17	4	91
7	0.46	0.03	3	93
8	0.43	0.11	2	96
9	0.31	0.13	2	98
10	0.19	0.06	1	99
11	0.12	0.08	1	99
12	0.04	0.01	0	100

Comp: component. % VAR: Percentage of explained variance. VAR Acum: Percentage of accumulated explained variance

Table 11. Scoring coefficients of healthy students' physiological data

Feature	C1	C2	C3	C4	C5
HRV_mHR	21	-7	91*	-4	15
HRV_SDNN	11	77*	-40	0	6
HRV_RMS	1	79*	-34	-1	3
HRV_PHF	3	94*	18	0	0
HRV_PHFex	5	94*	21	-1	2
stdTonic	79*	3	1	-5	5
mPhasic	98*	5	9	-5	1
stdPhasic	98*	2	7	-3	1
maxPhasic	98*	2	8	-4	1
aucPhasic	98*	5	9	-5	1
EDASymp	94*	7	10	-7	3
PRV_mHR	19	-6	93	-3	14
PRV_SDNN	9	65*	-56	4	7
PRV_RMS	0	65*	-57	1	0
mPAT	-2	-10	7	1	90*
stdPAT	10	21	14	-4	86*
meanGrad	-10	0	-3	99*	-1
medGrad	-9	1	-4	99*	-2

*Percentage of total variance explained by component for computing component scores. * values>60.*

The first component was associated with all the features extracted from the EDA, which in turn is associated with the state of the sympathetic system. The phasic component from the EDA signal revealed to provide greater information among the EDA parameters' dimension. We selected the standard deviation of the phasic component because it showed better correlation with the SRS.

The group of features associated with the second component was composed of the HRV and PRV indices related to the power in the HF band. The features HRV_PHFex and HRV_PHF had the highest coefficients associated with this component, but HRV_PHFex was selected since its weight was slightly higher.

The mean values of HR estimated from the ECG and PPG were in the third component group. The differences in weight between the two features were very small and although the weight corresponding to the one coming from the PPG (PRV_mHR) was strictly greater, in this case, other reasons were considered. On the one hand, taking the HRV_mHR feature (from the ECG) would avoid having to deal with the greater number of artefacts that PPG undoubtedly generates and, in turn, would eliminate the need to take the PPG signal into consideration since the previous group already took the ECG. On the other hand, using the PPG represents greater comfort for the subject, since the signal can be extracted from a watch or bracelet and adhered electrodes would not be necessary. However, this option makes no sense if we keep the choice of HRV_PHFex in the previous group (which already requires having the ECG). On the contrary, selecting PPG in both groups would definitely mean greater comfort for the subject at the cost of losing precision in the second component.

The mean and median of the hand temperature gradient were the features that form the fourth group. The coefficient associated with the fourth component was identical for both features. mGrad was selected since calculating the mean is computationally less expensive than the median.

Finally, the last group, associated with the fifth component, corresponded to the pulse transit time features. Among these, we selected the mean value (mPAT) for having the highest coefficient associated with component five. From the selected features and using a linear regression model, the SRS was estimated, which resulted in the following equation with a determination coefficient R2 of 0.97:

$$PSM = 0.5 \cdot mGrad + 0.4 \cdot PRV\_mHR + 0.2 \cdot HRV\_PHFex + 0.06 \cdot mPAT + 0.01 \cdot stdPhasic$$

Table 12 shows the descriptive statistics of the physiological stress model. A net difference between the stress states SS and relaxation states RS is preserved.

Table 12. Physiological stress model results

SRS		N	Mean	Std	Min	Q1	Median	Q3	Max
Session*	RS	114	40.48	3.62	26.84	38.45	40.7	42.54	52.98
	SS	113	44.64	4.74	30.71	41.87	44.55	48.03	52.83
Gender	F	112	41.35	4.01	30.71	38.69	41.18	43.56	52.83
	M	115	40.87	4.14	26.84	38.81	40.83	43.18	52.98

*RS: relax stage; SS: stress stage; F: female; M: male. *p<0.05*

Similar to the SRS results, there were no significant differences between male and female PSM scores, although, as expected, they were significantly higher ($p<0.05$) in the Stress Session compared to the relax session.

7.3. Discussion

Stress is a phenomenon that we can perceive, but hardly quantified. The aim of this study was to establish a quantitative, continuous, objective and noninvasive measure of the level of acute emotional stress in healthy young subjects. From a practical point of view, this tool will help clinicians improve a more accurate diagnosis and during the follow-up of therapeutic interventions while facilitating a common ground among professionals.

7.3.1. Artificial stress environment protocol

First of all, from the literature, we have seen that there have coexisted three distinct approaches to stress assessment. The first one relies on the subject's lifetime stressful experiences, which generate a higher affectation level, the second one evaluates the psychological perception of a stressful stimulus and the third one measures some variables of the physiological response of the subject [184]. Gathering information from these three approaches, we have designed a protocol that includes two different sessions. The relax session in which a state of relaxation is achieved with music. The stress session begins with a brief relaxation time to reach similar conditions to the previous session. The stress state is then achieved using the TSST as a moderate stressor. At the end of both sessions, various psychometric tests and biochemical markers are administered as accepted gold standards for stress assessment in humans and animal models respectively. The psychometric tests evaluate the psychological affectation due to the sequence of events while the biochemical variables evaluate the response of the endocrine system. Additionally, several physiological variables, reported in the literature as stress markers, are also measured synchronously throughout the sessions to assess ANS activation.

The TSST is an experimental protocol that has made it possible to study the relationship between psychological and physiological responses in recent decades [190]. Its usefulness lies in sequentially including various common types of stressors: a MT, a social interaction event and an AT, all without previous warning.

The reasoning behind the two separate sessions in the design of the present study considered the effect that could resemble the commonly known as "white coat syndrome", in which participants are altered due to the clinical or experimental setting, although they do not exhibit it in other settings. On the one hand, participants knew what to expect during the second session regarding the sensor placement and the experimental setting and on the other hand, the features that were significantly different in both basal states (BR and BS) were discarded considering their variations were not directly related to stress. Furthermore, this protocol allowed all subjects to be their own control reducing possible biases.

7.3.2. Reference scale for stress measurement

Once the experimental setting was designed, our initial scope was to find a comprehensive scale for stress measurement that could be later used as reference for more practical use cases offering synthesized information on both the response corresponding to the «cogno» or of the psyche as well as of the somatic response to a stressful stimulus.

To that end, there are two sets of highly correlated systems that activate simultaneously when faced with a stressful stimulus, generating a single response. The first is associated with changes in the psyche or «cogno», i.e. changes in thinking, the generation of emotions, the perception of reality, behavior and social interaction, among others. The second set is associated with somatic changes from the system or organ level to the molecular level. This in turn is activated by two separate although interrelated and cooperative pathways: the HPA axis and the ANS.

From the cognitive point of view, our study results clearly showed the stress reactivity caused by the artificial stressor; the questionnaires evaluating the imminent stressful situation, i.e. STAIs, VASS and SSS, showed at least a 10% increase in the stress session, whereas the trait questionnaires, i.e. STAIt and PSS, remained the same. These results are in accordance with the reported literature [190, 212].

Regarding the HPA axis, results show a lower decrease in cortisol concentration during the stress session compared to the relax session. Previously documented patterns of higher anticipatory salivary cortisol responses during the TSST in men compared to women have also been found [213]. Cortisol release also increases the glucose availability for a fight or flight response although our results do not show significant differences.

The synthesis and release of cortisol in humans are subject to a circadian rhythm, with high levels in the early morning that decrease as the day goes by. Additionally, the secretion of cortisol is stimulated as a result of the activation of the HPA axis triggered by a stressful stimulus. Even though the morning cortisol may be difficult to compare during the cortisol awakening response, stress reactivity in healthy subjects has been proven to show greater response compared to the evening results [214].

Outside the adrenal gland, cortisol is mainly bound to corticosteroid-binding globulin. The remaining part, called free cortisol, represents the biologically active part of the hormone. The cortisol found in saliva corresponds to the free cortisol that diffuses passively into the salivary glands and its concentration is associated with the levels of free cortisol in plasma [215]. Although salivary cortisol can be altered as a consequence of a variation in the CBG concentration [216], we analyzed it since it still has numerous advantages over its measurement in plasma, as it does not require any equipment or specialized personnel for its collection.

The activation of the HPA axis also gives rise to arginine vasopressin (AVP) and prolactin hormones secretion. AVP has a small molecular size and a short average lifetime that makes it difficult to measure. Therefore, we determined the concentration of a co-secreted glycopeptide, known as copeptin, as a surrogate biomarker, although physiological interpretation is unknown [194, 217]. Both prolactin and copeptin blood concentrations were elevated after the stressor.

Regarding the ANS, the sympathetic activation of the salivary glands increases the secretion of α-amylase while that of the parasympathetic increases the volume of secreted saliva [218]. Furthermore, the concentration of α-amylase also changes depending on the circadian rhythm of the subject inversely to cortisol [219].

The greater increase in the stress session compared to the relax shows the effect of the TSST, which has been associated with increased plasma catecholamines due to the presence of stressful stimuli. Therefore, there is a presumption that α-amylase in saliva could be considered a noninvasive substitute for the measurement of catecholamines and even cortisol [219].

To elaborate the SRS, all the above variables that respond to the activity of the HPA axis and the SNS that significantly changed as a result of the TSST were considered in the principal component analysis and weighted according to their relevance. Each coefficient of each variable is proportional to the scores in the component and explained variances of the component. The inclusion of the trait psychometric test (i.e. PSS and STAIt), allowed the Scale to have the information of predisposition (trait) of the individual to respond to a stressful stimulus.

The SRS scale shows that 54% of the score corresponds to Psychometric tests, rather than 60% proposed in [210]. The difference can be explained due to the inclusion of the Symptomatic Stress Scale (SSS) test and α-amylase since the increase up to 120 participants showed significant concentration differences between Relax and Stress states, unlike the subset of 40 participants used in [175]. The SRS was validated for the type of population and stimulus of the present study and is independent of gender.

Once validated, the proposed SRS represented a quantitative assessment of stress on a scale from 0 to 100 in which SRS = 0 means the total absence of stress (no response to stimuli) and SRS = 100 means the highest level of distress.

7.3.3. Noninvasive stress measurement

Finally, our protocol included the synchronous monitoring of several noninvasive stress biomarkers. We extracted several stress-related physiological features found in the literature to study their evolution under different stress intensities and to find the most relevant ones for a continuous acute stress measurement.

In [220] an approach to estimate stress level every 1 minute from physiological signals was evaluated on a subset of the database. Results supported the feasibility of quantifying the stress level using a multiparametric measure of the physiological stress response through biomarkers derived from the processing of ECG, PPG and T signals. Moreover, the relationship between the features extracted from the signals and the SRS showed higher correlations than with either the psychometric variables or the biochemical variables alone. This suggested the feasibility of the SRS used to assess the stress levels.

In the present study, the conditioning and treatment of physiological signals was performed on ECG, PPG and ST, and EDA was also added. From them, more than 80 features widely reported in the literature or that are associated with physiological processes related to stress were extracted.

The HRV features that were considered adequate for estimating the stress level were the mHR, the SDNN, RMS and HF band, in line with previous analysis in the data subset [221] where they significantly changed between highest stress stages (MT and AT) and all other stages of the session.

Regarding PRV, in [175] the complementary or additional information of PRV with respect to HRV was investigated for stress assessment. While significant differences were found between PRV and HRV at baseline, no such differences were observed during stress stages, suggesting that pulse arrival time variability is higher during relax than during stress. Changes in the morphology of the PPG signal induced by stress were studied using a Gaussian mixture modelling technique, revealing significant differences in features related to the position and width of the systolic and reflected waves during stress stages with respect to baseline [222].

For the joint analysis of the ECG and PPG signals, we extracted the PAT features which reduced during the stressor. These are reported to be a proxy for vasoconstriction and hence higher BP.

The PAT is the time difference between the peak of the R-wave in the ECG signal (corresponding to left ventricular depolarization) and a fiducial point in the PPG waveform (as measured by a pulse oximeter attached to the fingertip). However, it is often erroneously described in literature as pulse transit time (PTT) [168]. The PAT includes not only PTT, but also the time delay between the electrical depolarization of the heart's left ventricle and the opening of the aortic valve, known as pre-ejection period (PEP). PEP can vary depending on contractility, SNS activity, preload and afterload. In our study, we did not separate the PEP from the analysis, although the literature suggests that the time elapsed between the R-peak and the R-wave gives the best systolic BP prediction [197].

For the T features, the experiments reported in the literature do not converge to the same conclusion. In this study, the mean temperature decreases rapidly during the first stages of the

TSST, remains at its lowest values until the end of the whole task and it has been reported that more than an hour is needed post-stress for temperature to return to baseline [223].

Finally, EDA features measure a person's sweat level in glands since the skin is usually an insulator but its conductance changes when there is sweat as a consequence of sympathetic activation. Skin conductance has been found to have a linearly varying property with respect to emotional arousal, and apart from being used to classify different states such as anger and fear, it is also to detect the stress level while performing a task [224]. The EDA can be divided in two independent components; the first in which rapid, immediate responses are observed and of short duration, known as phasic component, and the second, the tonic component, is associated with the level of arousal of the SNS, that is, a sustained or accumulated response. Our results show this arousal in both components of the time-domain analysis and in the frequency-domain analysis, supporting the sympathetic arousal.

From the PCA analysis, the selected final set of 5 features explaining 87% of the variance of the data were:

$$PSM = 0.5 \cdot mGrad + 0.4 \cdot PRV\_mHR + 0.2 \cdot HRV\_PHFex + 0.06 \cdot mPAT + 0.01 \cdot stdPhasic$$

This model takes into account all the before mentioned features emphasizing changes in the increase of the mHR and the temperature decrease. Contrary to what the literature suggests, the power in the extended high frequency band of the HRV analysis increases with stress, which is usually related to parasympathetic activity, as reviewed by [196]. This could be explained because the power of the extended band of the HF goes from 0.15 Hz to half the HR. Therefore, if the HR increases during the stress stage, the band is extended and consequently the power can increase. Moreover, speech should be taken into account to increase the reliability of HRV as a marker of stress as frequency features of HRV are influenced by respiration [171]. During the relax state, respiratory frequency is within the LF band, so the HF band is not measuring respiratory sinus arrhythmia but just "noise". On the contrary, during the stress tasks, respiratory frequency increases and falls within the HF band, thus increasing the power in the HF band. However, this can be misinterpreted as an increase in parasympathetic activity. An approach to better estimate the sympathovagal balance after separating respiratory influences from the HR was developed in [225]. Finally, a minor contribution goes to the mean pulse transit time and the standard deviation of the phasic component of the EDA.

Our previous publication on a subset of the database [220] where we designed a similar model for the noninvasive measurement of stress, the set of extracted features was more limited and the rescaling of the magnitudes to balance weightings given the differences in the order of magnitude of the values of each feature was not carried out. However, both models are able to quantitatively measure the stress changes over time and show a good correlation with the reference scale designed showing that the methodology used is reliable and can be adapted to the conditions needed for a specific end-user application. Moreover, after all the analysis, both

models include the mHR and mPAT. Therefore, these features show robustness and should be considered for future stress assessment methods.

The purpose of this analysis is to achieve a continuous, noninvasive measurement that allows quantitatively assessing different levels of stress in a repeatable manner and from an approach that groups the different aspects of the psychosomatic changes induced due to a stressor. In fact, the Physiological Stress Model can be calculated throughout the entire session. The highest values of the model can be observed in MT and AT and the lowest in BR, R BS, as expected. It is important to highlight that due to the type of re-scaling that was carried out to "uniform" the ranges of the features, this model will never reach a minimum of 0 or a maximum of 100, which is adequate for the type of moderate stress in healthy students being studied.

7.4. Limitations

The main limitation of the current study is the specific target subject of study, narrowed to healthy young students and a perfect acute stress experimental setting although not applicable in a wider population and other types of stress. Further studies including clinical and sociodemographic data such as age, gender, BMI or fitness may enhance the model's robustness.

The ultrashort term analysis for the HRV is a trade-off between usability and reliability and should also be more carefully studied. Moreover, respiration and speech inclusion into the analysis could greatly improve the reliability of the measurement.

Finally, only linear relations between the stress response and the stress reference features were analyzed in this approach, although including non-linear relations could contribute to a more accurate measure of the stress reactivity nature.

8. Physiological dynamics in major depressive disorder

According to a systematic analysis for the Global Burden of Disease in 2019 [226] there has been a significant increase in the prevalence of mental disorders, paralleling the surge in mHealth technologies (Naslund et al., 2015). The emergence of smartphones and wearable devices, which use embedded sensors to unobtrusively measure human behavior and physiological responses, has opened a world of opportunities for advancement in mental health diagnosis and patient follow-up.

MDD is one of the most prevalent mental health disorders. It affects over 7% of the adult population in Europe, and it is about 50% more common among women than men [227]. Since patients with MDD are at increased risk of suicide, close monitoring, and follow-up by mental health workers become necessary to ensure safety and adherence to mental health treatment.

Undoubtedly, the use of mHealth technologies to monitor these patients could facilitate non-intrusive yet thorough tracking and enable timely intervention if needed. However, valid and reliable objective methods to monitor patients at risk are still lacking [104]. At present, clinical interviews are the most reliable method for determining the etiological diagnosis of MDD relapses. A considerable proportion of this data is subjective, that is, it is predicated on the patient's self-report and is subject to the clinician's interpretation. As a result, it is susceptible to a multitude of biases that have the potential to compromise the quality of healthcare provision. To mitigate these concerns, it might be advantageous to utilize physiological data and examine its association with the severity of depression.

While extensive research on depression has been conducted, our understanding of the etiology and pathophysiology remains limited, leading to the stress-diathesis model becoming a prevalent explanation for the onset of MDD [228, 229]. This model posits that mental disorders develop due to a combination of genetic vulnerability (diathesis) and environmental stressors. In this context, individuals might have different stress reactivity patterns that lead to a predisposition to developing a disorder when exposed to significant stressors.

Stress reactivity refers to the physiological response that an individual has to stressors. Reactivity to stressors yields insights into health-related risks that cannot be gleaned from resting measurements [144, 145]. This can include digital biomarkers such as changes in HR, BP, rapid breathing and other bodily functions that can be objectively monitored using wearables. For instance, in the NESDA cohort [230], while there were no observable differences in HRV between depressed and non-depressed individuals during resting states, hyporeactivity was prominent when a stressor was introduced. Similar results were found in [231] where high frequency-HRV (HF-HRV) reactivity, but not resting HF-HRV at baseline, was predictive of higher scores on suicidal ideation. Another multi-site study, the EUDOR-A project, found that a hyporeactive EDA response pattern was associated with a history of suicide attempts as well as with an increased risk of such behavior during a 12-month follow-up period after being tested [232]. A systematic review on this topic also found that electrodermal hypoactivity seemed to be a reliable feature of depression and a valid marker of suicidal risk [233]. Finally, decreased autonomic reactivity assessed by dynamic changes in the photoplethysmographic waveform was also associated with higher depression severity in a published work from our group with the same participants of the present study [124]. Most studies typically explored just one digital measure, with only a handful attempting to integrate measures such as EDA with HRV [234]. Nevertheless, their sensitivity and specificity were not sufficient to support the use of these markers alone as suicide risk indicators in clinical practice.

Considering these limitations, our research aimed to enhance the understanding of MDD physiological response and to design a multiparametric model that could lead to remote monitoring of depressed patients. To the best of our knowledge, this is the first study designed to evaluate stress reactivity through multiple physiological signals during a mild cognitive stressor, using two validated neuropsychological tests to assess cognitive executive functions

as well, and psychometric tests as the gold standard for mental health evaluation. It has been established that diminished executive functioning is evident during the initial occurrence of depression [235], and this impairment endures even after remission [236] and exacerbates recurrent episodes [94]. This approach allowed us to objectively measure multiple and synchronous physiological signals, providing a more accurate representation of an individual's stress response profile while assessing their executive functioning.

8.1. Methods

8.1.1. Participants

Forty MDD patients and forty healthy controls (HC) were recruited consecutively at the Hospital Clínico Lozano Blesa (Zaragoza, Spain) and the Acute Unit at the Sant Joan de Déu Numancia (Barcelona, Spain) from October 2016 until December 2019. Following the Declaration of Helsinki, all participants voluntarily signed an informed consent form validated by the ethics committee of each institution. The Clinical Research Ethics Committee of the Hospital Clínico Lozano Blesa approved the protocol the 8th of June 2016, identification number PI16-0156, while the Medicine Research Ethics Committee of the Sant Joan de Déu Research Foundation approved it the 13th of December 2016, PIC-148-16.

The selection criteria for the patient group were based on the DSM-V [237] for non-psychotic MDD and to fall within the age range of 18 to 65 years. Depression severity was assessed with a score higher than 19 on the Hamilton Depression Rating Scale (HDRS) [238]. Participants with other comorbidities such as cardiovascular, HPA axis related disorders or neurological disorders were excluded. The use of benzodiazepines was also reported.

For the HC group, the inclusion criteria were to be a healthy individual without comorbidities such as cardiovascular, endocrinological or neurological disorders, and whose age, sex, and BMI ($\pm$ 3 kg/m^2) matched those of the patient group. Any participant with psychopathology or regular consumption of psychotropic substances was excluded. Moreover, all participants were asked not to consume tobacco, alcohol, or stimulants of the nervous system (caffeine, taurine, etc.) for at least 8 hours before participation in the study.

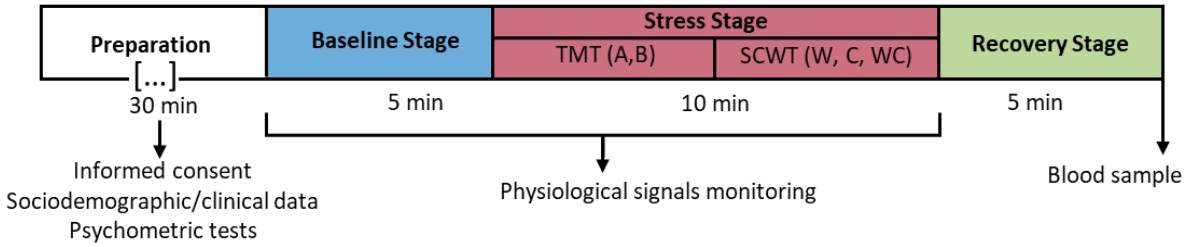
8.1.2. Experimental protocol

The experimental protocol (summarized in Figure 16) had a duration of about 1 hour and it consisted of a preparation period followed by physiological signals monitoring divided into three stages.

During the preparation period, a licensed psychologist asked for the sociodemographic and clinical data and conducted a psychological evaluation. Afterwards, the individuals were asked to fill in the self-reported psychometric tests.

Once all the questionnaires were completed, a technician placed the medical sensors according to the Medicom system (ABP10 module of Medicom MTD, Ltd, Russia) manual. For the first stage, the baseline stage, participants were requested to stay quiet in a seated position. During the second stage, the stress stage, stress was induced in participants using two cognitive tests: (a) the Trail Making Test and, (b) the Stroop Color and Word Test, consisting of a non-verbal and verbal stressor, respectively [239]. Afterward, in the recovery stage, individuals were requested to relax and remain silent for 5 minutes. Finally, following the conclusion of the three session stages, blood samples were taken for biochemical analysis, aiming to explore potential physiological changes linked to depression.

Figure 16. Experimental design for physiological dynamics in depression



Scheme of experimental procedure. TMT: Trail Making Test; SCWT: Stroop Color and Word Test.

8.1.3. Outcome measures

8.1.3.1. Psychometric tests

For the determination of mental health status and the level of depression, a psychologist administered the Hamilton Rating Scale for Depression (HRSD) [238, 240, 241], the Beck Depression Inventory (BDI) [242–244] and the Clinical Global Impression (CGI) [245] through an interview. Additional psychometric tests to determine the level of stress were self-administered. The State-Trait Anxiety Inventory (STAI) [202, 204] for trait and state anxiety, the Perceived Stress Scale (PSS) [207, 208] to assess stressful life situations, and the Visual Analogue Scale for stress (VASS) [246].

All the psychometric tests used are validated, well documented by the medical world, and have a contrasted and accepted Spanish language version. Psychometric tests required, on average, 15 to 20 min to be completed, and participants did not need any special education or training to complete them.

8.1.3.2. Cognitive tests

For the Stress Stage, we used 2 tests that require cognitive control and the engagement of executive functions, such as attention, cognitive flexibility, and processing speed. Therefore,

they can be stressful for individuals, especially under time pressure or when high performance is demanded.

- Trail Making Test (TMT). The TMT is a neuropsychological test of visual attention, processing speed, and task switching. It consists of two parts in which the individual is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy. In the first part (TMTa), the individuals are required to connect, by drawing a line, consecutive numbers, while in the second one (TMTb), they are required to connect numbers and letters in an alternating progressive sequence. The TMT was scored based on the time it takes to complete the test [247].

- Stroop Color and Word Test (SCWT). This psychological test measures a person's selective attention capacity and skills, as well as their processing speed ability. The individuals are required to read, as fast as possible in a pre-established period of 45 secs, from three different lists, the names of: (a) color-words printed in black ink (STROOP_W), (b) different color patches (STROOP_C), (c) ink color of color-words printed in an inconsistent color ink (STROOP_WC, incongruent condition). To determine the pure interference score (STROOP_I), the difference between the STROOP_WC and the estimated STROOP_WC (STROOP_WC') were calculated. The higher the resulting score, the less susceptible the subject is to interference [248].

8.1.3.3.Biochemical variables

The biochemical variables considered in the present study offer information about the HPA axis and the ANS. The biochemical variables measured in blood samples were prolactin, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and glucose. The samples were kept frozen at -20°C until processing at the Biomedical Diagnostic Center of the Hospital Clínic of Barcelona.

8.1.3.4.Physiological signals

Details concerning feature extraction are described in section 6. The final list of features considered in this study is summarized in Table 13.

Finally, for all the above-mentioned features, the average for each stage was used as static feature and the intra-individual difference of a static feature between the different stages is used as a dynamic index of autonomic reactivity ($\Delta(F)$). The reactivity and recovery indices were calculated by subtracting the value of the feature at the baseline (B) and recovery (R) stages from the value at the stress (S) stage and denoted as $\Delta(F)_B^S$ and $\Delta(F)_R^S$ respectively, following the strategy previously used in [124] with the same participants.

Table 13. List of extracted features for physiological dynamics in depression

Physiological signal	Extracted feature	Definition
Electrocardiography or/and Photoplethysmography	mHR (bpm)	Mean heart rate
	SDNN (s)	Standard deviation of NN intervals
	RMSSD (s)	Root mean square of differences between adjacent NN intervals
	PHF (s^{-2})	HRV high frequency band power (0.15 - 0.4 Hz)
	PLF (s^{-2})	HRV low frequency band power (0.04 – 0.15 Hz)
	LF_HF (DL)	Ratio of low-frequency to high-frequency power
	mPAT (ms)	Mean pulse arrival time
	stdPAT (ms)	Standard deviation of the pulse arrival time
Electrodermal Activity	mTonic (µS)	Average value of the tonic component, i.e. SCL
	stdTonic (µS)	Standard deviation of the tonic component
	mPhasic (µS)	Average value of the phasic component
	stdPhasic (µS)	Standard deviation of the phasic component
	aucPhasic (µS·s)	Area under the curve value of the phasic component normalized by the length of the session, i.e. SCRs
	EDASymp (μS^2)	Power spectral density of EDA signal (0.045 − 0.25 Hz)
Skin Temperature	mGrad (°C)	Mean temperature gradient
Respiration	RR (Hz)	Respiratory rate
	Pk (%)	RR peak percentage in the power spectrum

8.1.4. Statistical Analysis

All analyses were run in SAS 9.4 software (SAS Institute, Cary, NC, United States). To compare the distribution of categorical variables across groups, several statistical tests were applied, each chosen based on specific criteria and assumptions. The McNemar Test was used when comparing paired binary variables while when analyzing categorical variables with more than two groups, the Bowker test was employed. The Chi-squared test was used to compare proportions between groups. However, Fisher's exact test was preferred when dealing with small sample sizes. The choice between these tests is determined by the criterion that less than 25% of cells should have expected frequencies below 5, following Cochran's guidelines. The selection of the most suitable test was made on a case-by-case basis to ensure accurate analysis.

For the comparison of quantitative variables, the paired t-test was chosen when comparing two normally distributed groups, and the Wilcoxon Signed-Rank Test was used in the cases where the assumptions for the Paired t-test were not met. This non-parametric test does not rely on normal distribution assumptions and is robust when dealing with skewed or non-normally distributed data. The appropriateness of these tests was confirmed through the Shapiro-Wilk test. Finally, mixed effect models were used to assess confounding factors that may interfere, considering the subject as a random factor and visit, group, and their interaction as fixed effects.

Regarding the physiological features, the multivariate maximum likelihood method was used to handle missing data. To fulfill the assumption of normality required by the model generation methods, the square root or a log transformation was applied when necessary. With the resulting complete dataset, cognitive and physiological features were analyzed with linear models considering BDI and confounding factors as explanatory variables. Finally, to assess the relationship between MDD severity with respect to the outcome features and confounding factors, we used a linear model with the BDI score as the response variable. Interactions between variables were also explored. The performance of the resulting model was assessed using logistic regression with the MDD condition in this case as the response variable. The significance level was set at 0.05. Post-hoc comparisons were corrected for multiplicity using Tukey's correction.

8.2. Results

8.2.1. Participants

The study included 80 recruited participants, 40 candidates per site were eligible to participate. Table 14 displays the baseline characteristics of the sample. The population studied corresponds to a group of 33 men and 47 women with an average age of 45 ± 13 years. Nearly half of the population with depression had some form of non-psychiatric chronic disease, while only 20% of the HC group did. Since the disease was managed, it did not represent an inconvenience to carry out the measurement sessions.

Despite our efforts to control patient-control matching by BMI (± 3 kg/m^2), patients consistently exhibited a higher BMI. This difference was statistically significant, albeit with a mild effect size (r=0.25). The age and gender matching between patients and controls was found to be accurate, ensuring that age-related factors did not confuse our results.

Interestingly, we did not observe any differences in biochemical parameters between the two groups, with one exception: IL-6 levels were elevated in patients. This remained true even after we removed outliers and confirmed that the number of individuals with allergies was similar in both groups.

Most patients were taking anxiolytics (75%) and taking antidepressants (82%). Of the 40 patients, 26 combined both types of medication whereas only three patients did not take any medication.

In our study, we found no differences in having children, coffee consumption, or living environment between the two groups. We found a significant difference in the marital status between the HC and the MDD group. Among the HC, married individuals were prevalent. However, in the MDD group, there was a significant increase in the number of single or divorced individuals.

Table 14. Demographics and clinical characteristics of the MDD patients and healthy controls

Variable	HC	MDD
BMI (m, std)**	24.78 (4.64)	27.15 (5.17)
Age (m, std)	44.33 (12.27)	45.40 (13.35)
Glucose (m, std)	85.53 (18.37)	85.37 (14.93)
Prolactin (m, std)	10.89 (5.78)	9.28 (5.65)
IL6 (m, std)**	1.79 (1.35)	2.82 (1.91)
TNFα (m, std)	3.99 (4.40)	3.36 (4.26)
Marital status (n,%)		
Married	26 (65)	15 (37.50)
Separated	3 (7.50)	8 (20)
Single	10 (25)	14 (35)
Widower	1 (2.50)	3 (7.50)
Gender (n,%)		
Female	24 (60)	23 (57.50)
Male	16 (40)	17 (42.50)
Children (n,%)		
No	16 (40)	19 (47.50)
Yes	24 (60)	21 (52.50)
Education (n,%) **		
Elementary education	2 (5)	14 (35)
High school	5 (12.50)	17 (42.50)
University	33 (82.50)	9 (22.50)
Occupation (n,%)**		
Employed	33 (82.50)	11 (27.50)
Retired	2 (5)	6 (15)
Student	3 (7.50)	4 (10)
Unemployed	2 (5)	19 (47.50)
Environment (n,%)		
Rural	5 (12.50)	10 (25)
Urban	35 (87.50)	30 (75)
Tobacco (n,%)*		
Non-smoker	34 (85)	21 (52.50)
Smoker	6 (15)	19 (47.50)
Coffee (n,%)		
No	10 (25)	9 (22.50)
Yes	30 (75)	31 (77.50)
Alcohol (n,%)*		
No	10 (25)	24 (60)
Occasional	22 (55)	13 (32.50)
Moderate	5 (12.50)	3 (7.50)
High	3 (7.50)	
Sports (n,%)*		
No	7 (17.50)	19 (47.50)
Occasional	7 (17.50)	11 (27.50)
Usual	26 (65)	10 (25)
Chronic disease (n,%)*		
No	32 (80)	21 (52.50)
Yes	8 (20)	19 (47.50)
Medicines (n,%)**		
No	26 (65)	5 (12.50)
Yes	14 (35)	35 (87.50)
Anxiolytic (n,%)**		
No	40 (100)	10 (25)
Yes		30 (75)
Antidepressive (n,%)**		
No	40 (100)	7 (17.50)
Yes		33 (82.50)

**p<0.05. **p<0.005*

Educational level emerged as a significant differentiator between the groups. A substantial 82.5% of the controls had completed university education, while only 22% of the patients had achieved this level. Most patients had either completed secondary education or discontinued education after primary school. Occupation also showed clear differences between the groups. The majority of controls were employed, while most patients were either unemployed or retired.

Finally, in terms of lifestyle habits, we found significant differences in smoking and alcohol consumption. Nearly half of the patients were regular smokers compared to only 15% of the controls. On the contrary, 62% of the controls consumed alcohol occasionally or moderately, while 60% of the patients did not consume alcohol at all, although this might be due to contraindications with medication. Regarding physical activity, most controls regularly engaged in sports activities, whereas only 25% of patients did so and 47% led sedentary lifestyles.

8.2.2. Psychometric evaluation

In terms of mental health outcomes shown in Table 15, both the BDI and the HDRS results show that the MDD group had moderate to severe symptoms of depression whereas the HC group exhibited minimal or no symptomatology, as expected. All psychometric questionnaires pointed towards a higher severity of mental distress in the MDD group; both state and trait anxiety were around 3 times higher, and patients showed about twice the stress level compared to the HC.

This psychological distress was further corroborated by the cognitive test performance. Patients with MDD completed fewer words in the SCWT; 28 less on average for the STROOP_W part, 21 for the STROOP_C part, and a lower but still significant difference in the STROOP_WC part (14 words less). Results also showed lower resistance to interference among the MDD group, although the difference was not statistically significant ($p = 0.26$). Additionally, concerning the TMT, patients took almost double the time to complete both subtasks.

To mitigate the potential distortion of depression assessment due to the influence of confounding variables associated with cognitive scores, we analyzed the most demanding subtasks for each test (STROOP_WC and the TMTb) controlling for age, gender, education level, and medication.

Results showed that age had a significant effect on STROOP_WC scores when controlling for BDI score ($F = 6.03$, $p = 0.02$), although age did not have a significant effect on TMTb scores. The interaction between age and depression was not significant in either test.

Table 15. Psychometric evaluation results of the MDD patients and healthy controls

Variable		HC	MDD
CGI (n,%)**	Healthy	40 (100)	
	Mild		9.00 (22.50)
	Moderate		21.00 (52.50)
	Severe		9.00 (22.50)
	Extreme		1.00 (2.50)
HDRS (m, std)**		2.13 (2.59)	22.20 (6.51)
BDI (m, std)**		3.75 (3.71)	27.48 (12.25)
PSS (m, std)**		11.15 (4.50)	26.93 (7.05)
STAIs (m, std)**		9.45 (7.28)	30.25 (10.19)
STAIt (m, std)**		12.70 (7.91)	37.33 (10.45)
VASS (m, std)**		23.06 (19.46)	44.83 (27.55)
TMTa (m, std)**		24.88 (9.41)	43.60 (22.37)
TMTb (m, std)**		58.70 (28.48)	100.73 (46.20)
STROOP_W (m, std)**		111.65 (19.32)	83.60 (20.75)
STROOP_C (m, std)**		77.10 (13.82)	56.10 (11.95)
STROOP_WC (m, std)**		49.33 (13.10)	35.13 (12.74)
STROOP_WC' (m, std)**		45.36 (7.42)	33.26 (7.11)
STROOP_I (m, std)		3.96 (8.34)	1.86 (9.82)

**p<0.05. **p<0.005. CGI: Clinical Global Impression; HRSD: Hamilton Rating Scale for Depression; BDI: Beck Depression Inventory; PSS: Perceived Stress Scale; STAI: State-Trait Anxiety Inventory for state and trait anxiety; VASS: Visual Analogue Scale for stress. TMT: Trail Making Test parts a and b; STROOP: Stroop Color and Word Test subscores, word (W), color (C), word-color (WC), estimated word-color (WC') and interference (I).*

Regarding the gender effect, neither the gender itself nor the interaction with depression were independent predictors of the cognitive scores. This was also the case when analyzing the consumption of antidepressants and anxiolytics, as well as the anxiety trait measured by the STAIt score. However, the antidepressant effect on the TMTb score was close to the significance level ($F = 2.92$, $p = 0.09$).

Finally, education had an independent significant effect on STROOP_WC scores ($F = 4.18$, $p = 0.02$), whereas the interaction between education and BDI was not significant. In other words, the effect of depression severity on performance was consistent across different education levels. On the contrary, no significant effects were found for TMTb scores when controlling for depression. However, Tukey's post-hoc test showed significant differences between the mean scores in both tests showing poorer test performances at lower educational levels. For the STROOP_WC, fewer words were completed comparing Elementary Education vs University (-20.60 words) while for the TMTb scores, it took more time to complete the task (47.53 seconds).

8.2.3. Static levels of physiological features

Physiological data analysis showed several distinct feature reactivity patterns between MDD patients and HC. Results on the physiological features extracted during each protocol stage are presented in Figure 17 and the extended table in the supplementary material (Appendix 11).

From the ECG and PPG signals, results showed that the mHR increased during the stress stage and decreased during subsequent recovery in healthy individuals. However, this change was not as pronounced in patients, who also exhibited a higher HR during the entire session. HRV features showed similar patterns in the two groups even though all features both from time and frequency analysis showed reduced variability in patients. Particularly, the PHF feature extracted from the ECG signal showed significantly better sensibility to separate both groups compared to the PPG extracted feature, although they show a high correlation as expected.

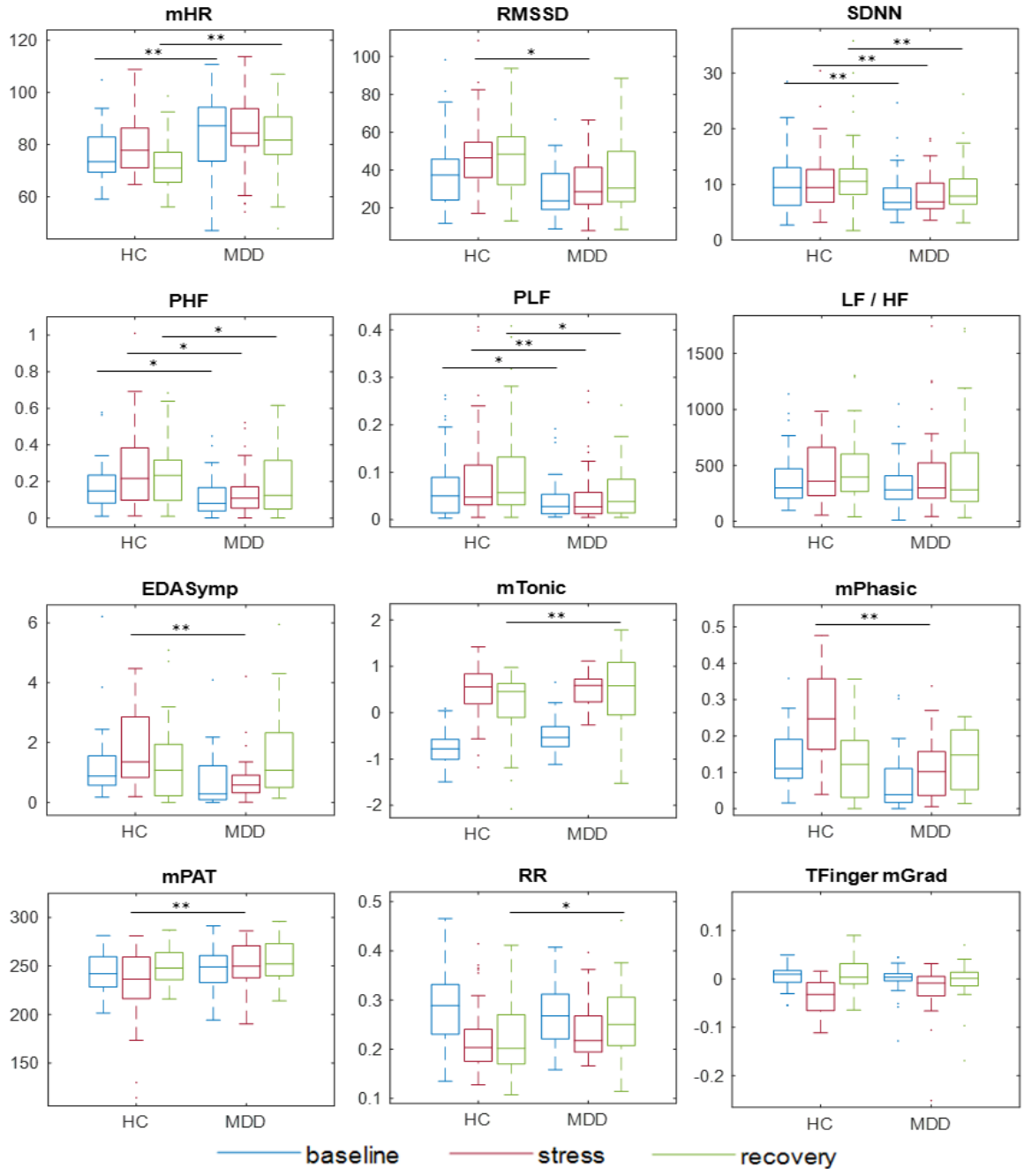
Contrary to the mHR pattern, the mPAT decreased with stress and increased during the recovery stage in HC, while it remained constant in patients, indicating poor cardiovascular control. Similarly, HC exhibited a greater decreasing gradient of the finger temperature during the stress stage compared to the baseline and recovery stages, while the patient group maintained a more uniform temperature. No differences were found for the face temperature.

EDA showed interesting trends as well. In healthy individuals, the EDA tonic and phasic components increased under stress conditions and decreased during subsequent recovery. However, in MDD patients, this stress state was maintained throughout the session or even continued to increase. Regarding the frequential analysis, the EDASymp feature showed similar results of sympathetic dysregulation.

Finally, the respiratory signal exhibited similar results in all participants except for the respiratory frequency during the cognitive tests, where patients showed significantly higher values, 15.5 breaths per minute compared to 13.2 for the HC group.

We examined the interactions between various physiological parameters concerning the intake of antidepressants using mixed models. Our analysis encompassed all parameters, however, our findings indicated no significant differences attributable to antidepressant consumption. Furthermore, our analysis extended to consider potential confounding factors such as tobacco use, coffee and alcohol consumption, and gender. However, none of these factors significantly impacted the physiological parameters under study.

Figure 17. Comparison of the evolution of the features between depressive and healthy individuals



**p<0.05. **p<0.005. mHR: mean heart rate; RMSSD: root mean square of successive differences between normal to normal intervals; SDNN: standard deviation of normal to normal intervals; PHF: HRV high frequency band power; PLF: HRV low frequency band power; LF/HF: PLF and PHF ratio; EDASymp: EDA band power; mTonic: mean value of the EDA tonic component; mPhasic: mean value of the EDA phasic component; mPAT: mean pulse arrival time; RR: respiratory rate; TFinger mGrad: mean finger temperature gradient.*

As expected, there were significant differences between the mHR of participants who do not practice sports compared to those who regularly participate in sports.

Finally, we found that with increasing age, the SDNN is reduced in the baseline stage with a significant negative correlation (r = -0.54) indicating less adaptability to stressors, while mPAT increased with age, albeit with a weaker correlation (r = 0.22).

8.2.4. Dynamics of physiological features

To assess stress response, we employed a strategy of using the difference between the stress and the baseline stages for the reactivity feature ($\Delta(F)_B^S$), and between the stress and the recovery stages for the recovery feature ($\Delta(F)_R^S$). This approach allowed us to focus on the personal disposition that underlies individual differences in responses to stressors, a potential vulnerability factor. Table 16 shows these dynamic features and their correlation with the BDI score.

Regarding the dynamic features related to the cardiovascular system, the $\Delta(mHR)$ showed a significant negative correlation with BDI scores (r = -0.40) both for the reactivity and recovery. This suggests that as BDI scores increase, indicating higher levels of depression, there is a decrease in HR dynamics. The HC group had higher $\Delta(mHR)$compared to the MDD group. HRV features showed similar dynamics in both groups as expected from the similar patterns found in Appendix 4. Moreover, mHR dynamics were significantly negatively correlated with age, both reactivity and recovery values (r = -0.29 and r = -0.25 respectively), indicating a lower response to the cognitive stressor with age, although this association disappeared when controlling for BDI score. Lastly, the dynamics of the mPAT showed a significant positive correlation with BDI scores, indicating that higher depression levels are associated with lower vascular response to a stressor. Regarding the PAT, the recovery feature had lower interdependence with depression than the reactivity feature but was still significant. The HC group had a larger recovery, $\Delta(mPAT)_R^S$ (-20.89 ms) compared to the MDD group (-3.61 ms). In this case, the mPAT dynamics were positively correlated with age (r = 0.31 for reactivity and r = 0.29 for recovery), although they lost this association when controlling for depression.

Concerning the electrical characteristics of the skin, recovery features also showed to be more relevant than the reactivity features. Most recovery features showed significant negative correlations with depression, including $\Delta(EDASymp)_R^S$ (r = -0.28), $\Delta(medTonic)_R^S$ (r = -0.25), $\Delta(medPhasic)_R^S$ (r = -0.29), $\Delta(stdPhasic)_R^S$ (r = -0.27) and $\Delta(aucPhasic)_R^S$ (r = -0.26). These results reveal the divergent pattern in EDA seen in Figure 17; while the HC group returned to a neutral condition during the recovery stage, resulting in a positive recovery value, the MDD group remained equal or even showed higher activation, and hence, negative values. In relation to the reactivity features, only

the $\Delta(medPhasic)_B^S$ (r = -0.27) and the $\Delta(aucPhasic)_B^S$ (r = -0.24) exhibited significant differences between both groups although with a slightly weaker correlation to the BDI.

Table 16. Dynamics of physiological features and BDI correlations

Feature	Reactivity ($\boldsymbol{\Delta(F)_B^S}$)				Recovery ($\boldsymbol{\Delta(F)_R^S}$)			
	HC	MDD	BDI		HC	MDD	BDI	
Electrocardiography			r	p			r	p
mHR (m, std)	4.83 (7.21)	0.45 (3.83)**	-0.40	<0.01	8.49 (7.81)	3.08 (3.68)**	-0.40	<0.01
RMSSD (m, std)	0.06 (0.32)	0.03 (0.14)	0.00	0.98	-0.01 (0.37)	-0.10 (0.20)	0.01	0.96
SDNN (m, std)	0.23 (0.31)	0.13 (0.24)	-0.06	0.61	-0.05 (0.30)	-0.12 (0.30)	-0.05	0.67
PHF (m, std)	0.11 (0.24)	0.05 (0.14)	-0.17	0.13	-0.01 (0.26)	-0.03 (0.20)	-0.09	0.42
PLF (m, std)	0.23 (0.41)	0.10 (0.20)	-0.20	0.07	0.01 (0.45)	-0.12 (0.31)	-0.12	0.31
LF_HF (m, std)	0.15 (0.55)	0.13 (0.52)	-0.04	0.72	0.01 (0.68)	0.02 (0.79)	0.00	0.97
Photoplethysmography								
mHR (m, std)	4.28 (6.40)	0.46 (3.74)**	-0.35	<0.01	7.60 (6.85)	3.51 (4.46)**	-0.40	<0.01
RMSSD (m, std)	0.11 (0.29)	0.18 (0.25)	0.22	0.05	0.04 (0.31)	0.03 (0.32)	0.03	0.78
SDNN (m, std)	0.14 (0.26)	0.16 (0.24)	0.13	0.25	-0.08 (0.28)	-0.09 (0.28)	0.10	0.37
PHF (m, std)	0.07 (0.18)	0.06 (0.17)	-0.17	0.14	0.00 (0.20)	0.00 (0.19)	-0.06	0.58
PLF (m, std)	0.10 (0.35)	0.10 (0.32)	-0.10	0.37	-0.09 (0.45)	-0.07 (0.41)	0.06	0.57
LF_HF (m, std)	0.03 (0.55)	0.00 (0.45)	-0.10	0.39	-0.13 (0.69)	-0.05 (0.75)	0.12	0.31
Pulse Arrival Time								
mPAT (m, std)	-14.88 (29.57)	3.88 (9.72)**	0.41	<0.01	-20.89 (31.54)	-3.6 (12.06)**	0.32	<0.01
stdPAT (m, std)	0.28 (0.47)	0.24 (0.39)	-0.06	0.60	0.28 (0.41)	0.22 (0.40)	-0.11	0.35
Electrodermal Activity								
EDASymp (m, std)	0.51 (0.84)	0.26 (1.07)	-0.11	0.34	0.57 (1.09)	-0.37 (1.02)**	-0.28	0.01
mTonic (m, std)	1.05 (0.88)	1.01 (0.93)	0.01	0.92	0.36 (1.27)	-0.18 (1.19)*	-0.25	0.02
stdTonic (m, std)	0.09 (0.54)	0.36 (0.56)*	0.31	0.01	0.10 (0.45)	-0.06 (0.42)	0.02	0.89
mPhasic (m, std)	0.14 (0.11)	0.05 (0.09)*	-0.27	0.02	0.18 (0.20)	-0.01 (0.17)**	-0.29	0.01
stdPhasic (m, std)	0.14 (0.27)	0.11 (0.39)	-0.07	0.53	0.13 (0.35)	-0.17 (0.37)**	-0.27	0.02
aucPhasic (m, std)	7.77 (5.91)	4.33 (4.02)**	-0.24	0.03	6.87 (7.84)	0.42 (5.37)**	-0.26	0.02
Respiration								
RR (m, std)	-0.06 (0.09)	-0.03 (0.06)	0.13	0.24	0.00 (0.09)	-0.02 (0.08)	-0.15	0.20
Pk (m, std)	-0.03 (0.04)	-0.02 (0.03)	0.06	0.61	-0.03 (0.05)	-0.02 (0.04)	-0.12	0.32
Skin Temperature								
TFace_mGrad (m, std)	-0.01 (0.02)	0.00 (0.01)	0.06	0.58	0.00 (0.02)	0.00 (0.01)	-0.05	0.67
TFinger_mGrad (m, std)	-0.04 (0.04)	-0.02 (0.04)*	0.30558	<0.01	-0.04 (0.05)	-0.02 (0.03)**	0.22	0.05

** p<0.05. * p<0.005. For a description of the abbreviated features, please refer to Table 13.*

Finally, while the dynamics of the respiratory features showed no significant relationship with depression, the ST on the finger, showed significant positive correlations with the BDI scores, $\Delta(TFinger\_medGrad)_B^S$ (r = 0.31), $\Delta(TFinger\_medGrad)_R^S$ (r = 0.22). The HC group had lower means for both reactivity and recovery, $\Delta(TFinger\_medGrad)$ (-

0.04ºC) compared to the MDD group (-0.02ºC), indicating that a smaller response to the stressor and lower thermoregulation adaptability are related to depression.

8.2.5. MDD model

To obtain the MDD model (MDDm), different sequential selections were made in the feature selection process to simplify further analyses. Firstly, from all the dynamic features showing statistically significant linear dependencies, only one was selected. From this set of features, only those having a significant correlation with the BDI score were selected in a second step.

For the generalized linear modeling, cognitive and physiological variables were included in the final set of features. The model was adjusted by age, gender, and BMI, and interactions were also considered. A backward stepwise approach was employed to select the predictor variables, utilizing random cross-validation with 5 partitions. It should be noted that depression severity scores were transformed using the square root (log transformation was avoided due to zeros in the BDI scores) to increase the linear relationship with the autonomic reactivity indices.

The final regression models for MDD considering the gender were the following:

Women → $\sqrt{BDI}$ = 6.59 + 0.05 · BMI - 0.12 · STROOP_WC' - 0.03 · $\Delta(mHR)_R^S$

Men → $\sqrt{BDI}$ = 12.95 - 0.18 · BMI - 0.12 · STROOP_WC' - 1.04 · $\Delta(mHR)_R^S$

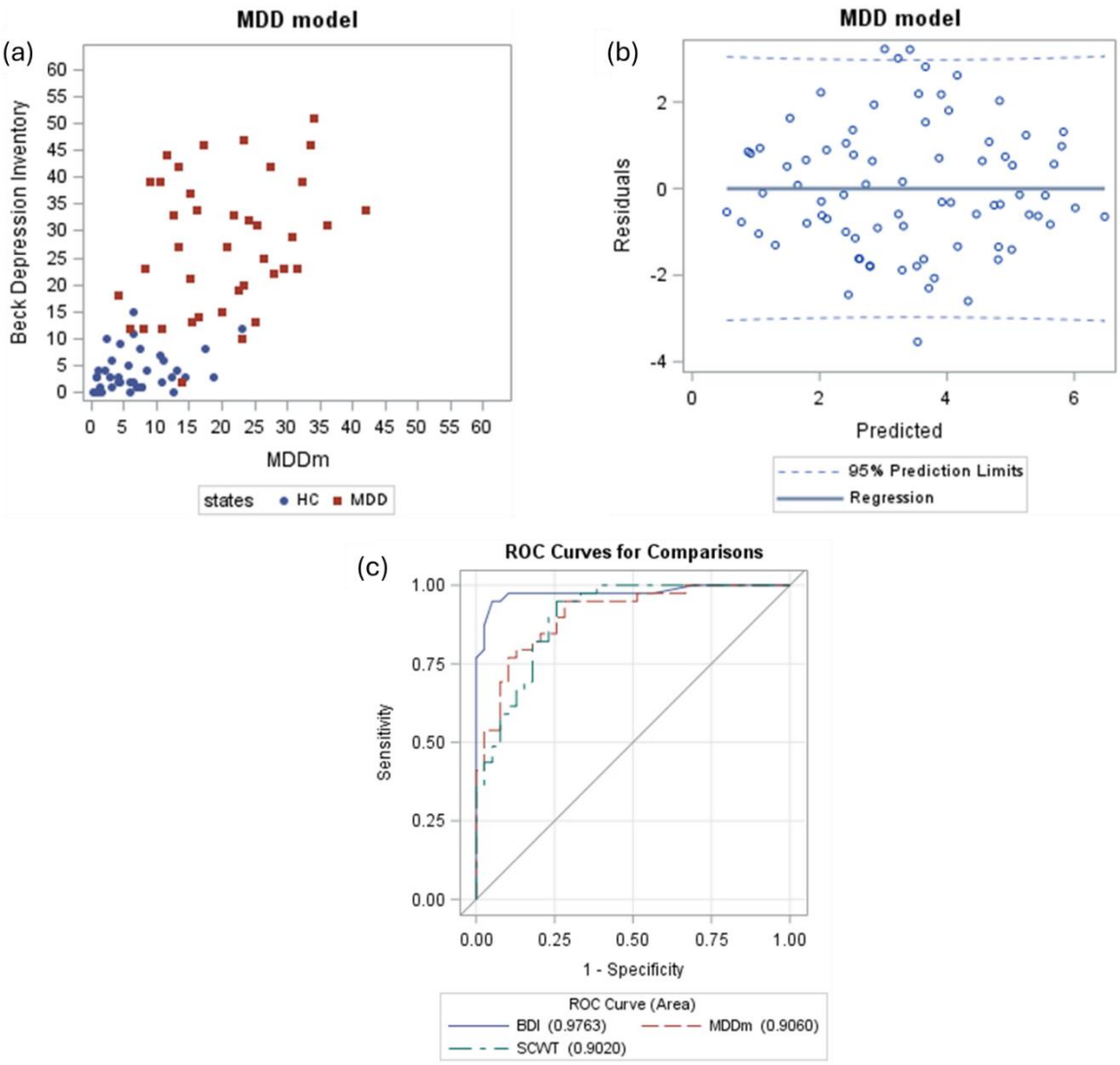
Table 17. MDD model parameter estimates

Parameter	Est	Std Est	SE	t Value	Pr > |t|	Cross Validation Estimates				
						1	2	3	4	5
Intercept	12.95	0.00	2.02	6.42	<.0001	13.31	13.12	11.20	13.27	14.08
Gender female	-6.36	-1.51	2.14	-2.98	<.01	-6.42	-5.82	-5.06	-7.12	-7.75
Gender male	0.00	0.00	.	.	.	0.00	0.00	0.00	0.00	0.00
BMI	-0.18	-0.43	0.06	-2.72	<.01	-0.18	-0.18	-0.12	-0.20	-0.22
$\boldsymbol{\Delta(mHR)_R^S}$	-1.04	-0.50	0.34	-3.06	<.01	-1.09	-1.12	-0.75	-1.05	-1.26
BMI*female	0.23	1.46	0.08	2.84	<.01	0.23	0.21	0.19	0.26	0.28
BMI*male	0.00	0.00	.	.	.	0.00	0.00	0.00	0.00	0.00
STROOP_WC'	-0.12	-0.54	0.02	-5.25	<.0001	-0.12	-0.12	-0.11	-0.11	-0.12
$\boldsymbol{\Delta(mHR)_R^S}$*female	1.01	0.39	0.40	2.53	.01	0.91	1.04	0.72	1.37	1.24
$\boldsymbol{\Delta(mHR)_R^S}$*male	0.00	0.00	.	.	.	0.00	0.00	0.00	0.00	0.00

Est: estimate; Std Est: standardized estimate; SE: standard error; $\Delta(mHR)_R^S$: recovery dynamic feature of the mean heart rate; STROOP_WC': estimated stroop word-color cognitive subtask.

At first glance, the MDDm seems to score higher depression severity in men than women, as can be seen by the intercepts in our models (12.95 and 6.59 respectively). However, upon closer inspection, both $\Delta(mHR)_R^S$ and BMI exert a multiplying factor that reduces the final score to a larger extent in men compared to women.

Figure 18. Major depressive disorder model



(a) MDD model performance. (b) MDDm residuals plot. (c) Accuracy assessment of the BDI, MDDm and SCWT models.

The coefficient of determination of the model was $r^2 = 0.45$, meaning that 45% of the variance of the depression severity score is explained by the predictors in the model. According to the standardized estimates of the derived model, gender is the most relevant factor in predicting BDI score. The second factor observed is the BMI. Within the demographic under study, it appears that men with higher depression tended to lose

weight whereas it had the opposite effect on women. The cognitive component (STROOP_WC'), which is observed to be equivalent across both genders, significantly contributes to the model, independent of sociodemographic variables such as gender and BMI. Lastly, an elevated reactivity of mHR extracted from the ECG notably diminishes the model's score, with a more pronounced effect observed in men as compared to women. Even though age was included in the analysis, it was discarded during the selection process.

Figure 18a shows a scatter plot of the predicted MDDm values vs the actual BDI scores while Figure 18b displays a graph with the residuals of the regression model, which are the differences between the actual values and the predicted values. The graph provides a visual representation of how well the regression model fits the data, without any specific trend that may influence the results of the prediction. Finally, Figure 18c represents a graph with the ROC curves comparison of the BDI, the final MDD model, and the model without the physiological data, i.e. $\Delta(mHR)_R^S$ to analyze its added value (marked as SCWT, green line on Figure 18c). The graph is a visual representation of the performance of these methods in detecting depression. While both proposed models showed a high area under the curve, 0.91 for the MDD model with the mHR dynamics, and 0.90 for the SCWT model without physiological data, correlation analysis indicated that the MDDm correlated better than the SCWT model with the BDI score (r = 0.71 and r = 0.61 respectively).

The same MDD model, replacing the $\Delta(mHR)_R^S$ from the PPG instead of the ECG, gave similar results, with an area under the curve of 0.91. Finally, according to Youden's J statistic, we found an accuracy of 78%, a sensitivity of 97% and a specificity of 59%.

8.3. Discussion

While MDD is an increasingly prevalent problem worldwide, the lack of objective tools for diagnosing and monitoring this condition hinders early detection, treatment research, and the assessment of disease progression. This study aimed to design and validate a multiparametric model to assess depression severity, using the analysis of several physiological signals to evaluate differences in autonomic reactivity and recovery to a mild cognitive stressor and including relevant clinical data. From a practical point of view, this tool was designed to help clinicians find a more accurate diagnosis and follow-up therapeutic interventions while facilitating a common ground among professionals, thereby significantly improving patient care and outcomes.

8.3.1. Cognitive stress protocol in MDD

Currently, the stress-diathesis theory constitutes the most empirically robust etiological model of various mental health disorders, including depression [228, 229]. The significant individual differences in the risk for disease following stress exposure highlight the moderating influence of inherent psychophysiological vulnerabilities.

The protocol employed in this study holds significant relevance due to its ability to assess stress dynamics in MDD patients and compare it to paired HC. Monitoring individuals during a baseline state provides a baseline measure of physiological signals, but at the same time, this measure can be altered by many confounding factors such as menstrual cycle phase, age, diet, physical activity, and sleep [249, 250]. However, the introduction of neuropsychological tests to induce stress followed by a recovery period allows for a dynamic assessment of an individual's physiological response to stressors and it offers insights into the specific vulnerability trait rather than their physical health transient condition [143]. While there are discrepancies on whether reactivity or recovery features are better indicative of mental health disorders, our results are similar to the results in [124, 251] which showed that changes in autonomic reactivity from the baseline to the stress stage have lower predictive power compared to changes from stress to recovery. These results suggest that the recovery may amplify differences in ANS regulation between individuals with MDD and HC. Focusing on the recovery period, we can obtain measurements that are less likely to be influenced by transient factors such as anxiety or nervousness related to being in a clinical setting.

Certainly, when executing protocols that involve monitoring physiological signals in response to cognitive stressors in future investigations, a multitude of factors warrant careful consideration. Empirical evidence suggests that determinants such as age, gender, BMI, educational attainment, medication regimen, and presence of comorbid conditions can modulate the outcomes, thereby potentially confounding the primary objective of the study [252, 253].

In summary, by providing an objective measure of stress reactivity and recovery, this protocol could contribute to a more standardized and objective assessment of MDD, ensuring that all participants experience the same conditions, thereby reducing potential confounding variables.

8.3.2. Predictive outcomes

The design of our study was developed to capture an objective and comprehensive evaluation of cognitive executive function and physiological stress reactivity and

recovery. For this reason, the study was divided into two main predictive outcome groups: the neuropsychological assessment and the stress dynamics assessment.

8.3.2.1.Neuropsychological assessment

Cognitive impairment has been reliably associated with MDD with effect sizes ranging from d = 0.32–0.97 [96], not only in the acute phase of illness, but some reports indicate that this impairment might be long-lasting despite symptom reduction and recovery [254].

In the present study, the neuropsychological assessment was evaluated using two cognitive tests, mainly analyzing two specific components of executive function. First, we examined the ability to inhibit habitual behaviors (prepotent or automatic responses), using the SCWT, and second, the TMT to assess the attention to switch between task goals.

Results from Table 15 show that patients with MDD completed fewer words in all the SCWT subscores and lasted longer to complete the TMT showing a deficit in processing speed and both inhibiting and switching components of executive function. These results are also in line with a systematic review [252] that summarized evidence of worse overall performance on the SCWT and TMT among people with MDD, supporting a deficit in both cognitive effort and processing speed and suggesting that depression is not only characterized by psychomotor slowing but also involves a specific deficit in executive function. However, the results of the analysis were inconclusive due to sample heterogeneity regarding concomitant variables such as the subtype of depression, the age, the number of lifetime episodes of depression, and current medication. Therefore, we incorporated several gathered concomitant variables into our analysis, including the educational level, age, gender, and current medication status of the participants.

Regarding the educational level, our results suggest that education had a significant effect on the STROOP_WC score, both on its own and when controlling for depression severity (BDI score) and the interaction between BDI and education. However, it is important to note that the effect of BDI on test performance did not significantly differ across levels of education. The TMT scores have been associated with education level as well as age and sex [255]. However, they do not report information about mental health. In our study, the analysis between education level, gender, and age did not show a significant relation with the TMT when controlling for depression, although age had a significant effect on STROOP_WC scores indicating that the age of the individual significantly predicted worse inhibition of automatic responses.

Finally, concerning antidepressive intake, the cognitive deficits observed in depressive patients are not solely attributed to the side effects of medication. This was supported by

numerous studies that have identified significant executive function deficits in individuals with depression who are not on medication [256, 257]. Our study results also reinforce these findings since the effect of antidepressants on the cognitive test scores was not significant when controlled by depression severity, although the antidepressant effect on the ability to switch between task goals (TMT score) was closer to the significance level. However, it should be noted that we only had information on the number of antidepressant and anxiolytic medications, not the dosage.

In conclusion, these findings provide further evidence of the cognitive executive deficits associated with MDD. It also highlights the influence of factors such as education level and age on these cognitive abilities. However, more research is needed to fully understand the complex interplay between these variables.

8.3.2.2.Stress dynamics assessment

Regarding the physiological assessment, another key area of the stress response, two systems are particularly significant within the physiological domain: the activity of the SNS, and the production of cortisol from the adrenal cortex, which is driven by the activity of the HPA axis [258]. Several meta-analyses conclude that cortisol activity in MDD is characterized by blunted stress reactivity [37, 259] but in this study, we investigated the stress reactivity mediated by sympathetic activation, leveraging the noninvasiveness of monitoring the ECG, PPG, EDA, Resp and T signals.

Our findings revealed several distinct reactivity patterns between MDD patients and HC. From the ECG and PPG signals, it was observed that the mHR increased during the stress stage and decreased during recovery in healthy individuals and that these dynamics were negatively correlated with age. However, this change was not as pronounced in the individuals with MDD, as seen in Figure 17, who also exhibited a higher mHR during the entire session. Several studies found similar trends [109, 158, 159, 251, 260] but the negative correlation between depressive symptoms and HR reactivity remained significant even after accounting for potential confounding factors such as gender, age, occupational status, physical activity, smoking, task performance, and the use of antidepressant and antihypertensive medication. For these reasons, they discarded excessive reactivity as a mediator between depression and cardiovascular disease outcomes. However, on the other hand, impaired mHR recovery has been associated with an increased risk of cardiovascular disease [48, 261] and it is also connected to several processes that are commonly present in depression, including anxiety, worry, and persistent negative thinking [262, 263].

Ultra-short-term classical HRV features (i.e. HRV analysis windows < 5min) showed similar patterns in the two groups, although overall reduced variability was observed in

patients. Even though current literature is far from uniform regarding the validity of ultra-short-term recordings of HRV [264, 265], the observed trend is in line with the review in [144] were most studies also found lower mHR and PHF reactivity in individuals with depression compared to HC. On the other hand, the importance of considering respiratory information into HRV spectral analysis when assessing stress response has been highlighted [225, 266]. Indices based on linear and nonlinear cardiorespiratory interactions published in previous studies utilizing the same sample have shown to provide better discrimination potential MDD biomarkers than classical HRV features [267, 268]. Discussion of the analysis and interpretation of HRV in the presence of confounding factors such as respiration is beyond the scope of this study but the interested reader is referred to other publications elaborating on the subject [269]. Lastly, it is worth mentioning that the PPG features show slightly lower performance than their ECG counterparts when comparing groups, although they have a high correlation, making them appropriate for wearable applications. However, it must be pointed out that the PPG waveform may vary due to age, posture, ambient temperature, relaxation, and acclimatization [120, 270], so future analysis on wearable signal processing and mental health prediction using this signal should consider these variables.

Contrary to the mHR pattern, the mPAT decreased with stress and increased during the recovery stage in controls, while it remained constant in patients, reinforcing low cardiovascular reactivity. This blunted response to stress could be indicative of allostatic overload or motivational dysregulations [251, 260, 271], or it could also be that greater aortic stiffness is the origin of the hyporeactivity. According to [272], greater aortic stiffness measured as the carotid to femoral pulse wave velocity was associated with MDD and the presence of depressive symptoms in men aged 60 years or younger, and to a lesser extent in women of the same age. However, this association disappeared in men and women older than 60 years, probably overshadowed in later life, where other factors, such as cardiovascular disease and atherosclerosis, could have a bigger impact on the development of depression than aortic stiffness. In any case, both hypotheses may not be mutually exclusive, and a combination of factors likely contributed to the observed results in MDD patients and support the contention that blunted reactivity is an indicator of risk for poor psychological and physical health. Given the cross-sectional design of the current study, drawing robust conclusions on this matter is challenging.

Blunted peripheral temperature reactivity was also a hallmark of MDD. HC exhibited a greater decreasing gradient of the finger temperature during the stress compared to the baseline and recovery stages, while the patient group maintained a more uniform temperature. Limited literature evaluates the temperature dynamics in response to a stressor and its relation to MDD. We only found one study that supports that depression reactivity was negatively correlated with mHR and T [273]. [274] evaluated T in MDD patients, using a recall of unpleasant stressful experiences of a medium intensity as a

stressor, and they also found no between-stage differences, although they could not relate the lack of reactivity to depression due to the lack of HC group comparison. Lastly, T and sleep time-related features were the most significant features of a machine-learning model using real-life wearable data [275].

In contrast to the previous dynamic patterns, the EDA in MDD patients increased during the stress stage in coherence with the healthy participants, though to a lesser extent, but this activation was maintained throughout the session or even continued to increase during the recovery stage. EDA has been found to vary linearly with emotional arousal and can be used not only to classify different emotional states such as anger and fear but also to detect stress levels while performing a task [276]. Several systematic reviews [233, 234] discuss the literature up to date related to EDA hyporeactivity and its relevance as a biomarker for depression, recurring episodes [232], and suicidal behavior [277] although few explore the recovery dynamics. Our findings show that EDA recovery features were negatively associated with depression indicating that even though patients' reaction is weaker, they might require more time to return to their baseline levels. In [278, 279] recovery features were also predictive of depression even though their focus on the features was more as input data for a support vector machine model.

Finally, the respiratory signal was the least informative data in predicting depression. Features exhibited similar results in all participants except for the respiratory frequency during the cognitive tests, where patients showed significantly higher values. However, results should be analyzed carefully since the SCWT uses speech which can alter respiration patterns. In [280] MDD patients who had suicidal ideation were observed to have a faster breathing rate and a reduced amplitude of respiratory sinus arrhythmia compared to those patients without suicidal ideation. In any case, the measurements were taken in a baseline situation without a stressor agent, and in a subsequent study with an expanded database [281], they found higher model accuracies with cardiac features instead of respiratory features.

The study also examined several confounding factors under study such as antidepressant intake, tobacco use, coffee, and alcohol consumption. Despite these considerations, the results demonstrated no significant impact of these variables on the physiological features under investigation. Notably, BMI and gender were identified as influencing factors for HR and EDA features, corroborating previous literature [282–284]. Given their reported association with depression, these factors were integrated into our model. It is recommended that future studies with larger sample sizes account for these potential confounders.

Overall, these findings indicate that a greater physiological flexibility to cope with the cognitive stressor is related to better mental health. Therefore, stress dynamic response

provides valuable insights into the physiological correlates of depression and highlights potential markers for its objective assessment.

8.3.3. Noninvasive MDD model

To conclude the discussion, the main aim of our study was to find a comprehensive model for depression severity measurement that could be later used as an objective reference for clinicians offering synthesized information on both the response corresponding to the cognition as well as of the somatic response to a stressful stimulus.

Our protocol included the synchronous physiological monitoring of reactivity and recovery to extract several noninvasive stress biomarkers that are altered in depression. However, after an exhaustive literature analysis and results comparison (please refer to sections 5.4 and 6.1), the recovery features were selected for modeling purposes. In particular, the stepwise backward selection process selected HR as the most relevant for the task at hand, and accordingly, it is the most prevalent in literature [144, 158, 159, 251, 260, 285]. It is pertinent to highlight that the EDA features and their interplay with gender were on the verge of attaining significance for their incorporation into the final model. Nonetheless, within the confines of our reduced sample size, they did not contribute substantially to the enhancement of the model's predictive precision.

The resulting model (Table 17) showed that BMI appears to have a protective influence in men, while it intensifies the severity of depression in women. Similar results were published in a systematic review [286], where the pooled odds ratio showed a protective effect of being overweight in men, whereas it showed an increased likelihood of depression in women. These findings could also indicate different coping mechanisms for depressed men and women, the former being appetite loss while the latter would be overeating or inactivity as a result of depression leading to weight gain. Despite our intention to assemble a group with comparable BMI, we observed a modest relation with depression that could be even more noticeable in a sample selected at random. Therefore, adding these demographic or medical history variables as context enhances the personalization of the model to determine a more accurate mental state of the individual [287, 288].

The cognitive flexibility component (STROOP_WC'), which is observed to be equivalent across both genders, significantly contributes to the model, independent of sociodemographic variables such as gender and BMI.

Lastly, a large mHR recovery ($\Delta(mHR)_R^S$), because of a good recuperation after an elevated reactivity, notably diminishes the model's score (i.e. less depression severity), with a more pronounced effect observed in men as compared to women. Similarly, [158]

also found that men tended to be more reactive than women. Furthermore, this feature, extracted from the PPG signal, showed similar model performance and given its computational simplicity, it could be easily measured using wearable technology.

There are several updated literature reviews on the state of the art of multimodal physiological models for the detection and classification of depression severity [119, 281]. The main physiological signals used are the ECG (or PPG), EDA, and electroencephalography (EEG). While several EEG models achieve high accuracies above 90%, they involve a small number of individuals [281]. Moreover, this technology requires special equipment preparation, and it is more invasive than the others, making them less promising and/or practical for a broader application.

[281] developed a model to classify MDD using a multi-modal approach combining ECG, PPG, and Resp data achieving 96.6% accuracy. In machine learning models, the goal is prediction, and it's assumed that there is sufficient data to fit models with high complexity and many parameters [289, 290]. However, most of the published models assessing depression severity use machine learning algorithms without appropriate sample sizes which may lead to a lack of reproducibility of their results in other populations. In our approach using statistical models, the goal was to understand the causes of the observed changes and provide measures of variable importance, which can be useful in understanding the relationships between variables.

Ultimately, the scientific achievement of this research contributes to the objective assessment of MDD, and its potential for replication in a mHealth environment thanks to wearable technology and smartphones reinforces its relevance in the field [275, 291, 292].

8.4. Limitations

The main limitation of the current study is the small sample size. Moreover, validation in independent datasets or analysis of depression subtypes were not performed. However, to mitigate this point, 5-fold cross-validation was used to reduce possible overfitting.

Another limitation of our study is the use of two artificial cognitive stressors during the experimental setting which may challenge the replication of the results or comparison with other studies. Moreover, the differences between these two tests were not explored as they were out of the scope of this study. In any case, the results should be cautiously interpreted, and they may vary in a real-life setting with other cognitive stressors (e.g., work deadlines, performance evaluations, university exams, etc.).

Additionally, to assess the evolution of the stress dynamics during the cognitive tests, ultra-short-term analysis for the physiological features was a trade-off between usability

and reliability and should also be more carefully studied. Moreover, linear and nonlinear cardiorespiratory interactions inclusion into the analysis could greatly improve the reliability of the HRV measurement.

Finally, only linear relations between the predictor variables and the MDD reference (i.e. BDI scores) were analyzed in this approach, although including non-linear relations could contribute to a more accurate measure of the stress reactivity and MDD severity.

III. Protocol design to assess mental well-being and distress

This part focuses on a protocol for developing and validating a remote tool that incorporates both physiological and cognitive variables to objectively assess mental well-being and distress. The research targets young individuals, categorizing them into three groups: those with high mental well-being, those experiencing mild to moderate psychological distress, and those diagnosed with depression or anxiety. The goal is to create a comprehensive, remotely accessible mental health assessment tool that is effective for clinical and epidemiological use.

Table of contents

9. Protocol for physiological dynamics assessment in mental health _____ 109

9. Protocol for physiological dynamics assessment in mental health

Mental health conditions are one of the leading causes of disability worldwide and are estimated to reduce life expectancy by 10 years [293]. For instance, depressive disorders were considered the eighth cause of disability in Spain in 2000, rising to fifth in 2019 [294]. Depression, anxiety, and stress-related disorders impose a major economic impact and burden on the public health system. To date, the prevalence of depression in the Spanish population is close to 5%, and the annual cost is estimated at €6145 (US $6648) million [295].

Young people and university students are populations of particular interest. Approximately 75% of mental health conditions have an early onset before the age of 24 years, and several risk factors (including genetic, early life adversity, family, community, and environmental factors) are involved in the development and course of these conditions [296]. Moreover, the recent COVID-19 pandemic has aggravated this situation [297]. Several systematic reviews and meta-analyses have indicated a high prevalence of mental health conditions among young people, with a pooled prevalence for depression of 31% to 33.6%, anxiety of 28% to 39%, sleep problems of 40%, and suicidal ideation of 12.3% [298–300], in line with the results of longitudinal studies suggesting a possible worsening of mental health in this population in recent years [301]. There is a need for early identification and prevention of mental health conditions, which includes the design and implementation of mental health promotion activities that lead to an increase in emotional well-being [302].

In recent decades, interest in mental health research has been steadily increasing, recognizing it a crucial aspect of overall health, rather than simply the absence of related conditions [1]. According to the [303], mental health is characterized by individuals' ability to effectively manage typical stressful situations, develop their potential and skills, and contribute productively to both themselves and the community. This comprises an adequate stress response and recovery as well as maintaining cognitive abilities such as attentional level and proper time of response. Stress reactivity is this capacity to respond to a stressor. It is a disposition that underlies individual differences in response to stressors and is assumed to be a vulnerability factor for the development of mental health conditions [145]. In this context, monitoring physiological response during a stress-inducing task could yield different reactivity patterns, offering valuable insights to differentiate between mental well-being and distress.

In clinical practice, self-reported questionnaires are commonly used to assess the severity of mental health symptoms, quality of life, and mental well-being. Nevertheless, several studies have reported limitations of these tests related to memory biases and distortions

in retrospective recall [119, 304]. To expand such assessments, including physiological biomarkers information will improve the characterization of endophenotypes (research domain criteria) [305]. Owing to technological advances, small sensors can measure physiological data for behavioral health, interventions, and outcomes (digital phenotyping) [306, 307]. Given the significance of stress reactions as complex phenomena encompassing psychological, cognitive, and physiological reactions involving the ANS and the neuroendocrine system, which, in turn, can affect other bodily systems, exploring these dynamics could enhance our comprehension of mental distress. Hence, physiological data monitoring including a stress-eliciting task may have an important role in early detection and intervention in mental health care. HRV, pulse arrival time, breathing parameters, EDA, and T are physiological variables broadly used to study the stress response and gather information about ANS functioning [142, 223, 308, 309].

To progress in this field, the use of wearables in mental health research shows promise, offering increased accuracy in data collection and reduced participant burden. Wearable devices allow researchers to passively monitor individuals in real time and gather data outside of traditional laboratory settings, that is, along with everyday life situations, providing a more holistic understanding of mental health status [310]. Currently, many studies on stress detection are conducted in controlled environments because accuracy decreases when conducted in real-time environments [130]. In addition, different instruments to measure perceived stress are used, which hinders the comparability of results [311], or a small number of signals are usually collected [12, 312]. However, previous studies have shown optimistic results for further advancement in the field for the objective assessment of mental health status and stress. A study analyzing data from 510 participants wearing a Fitbit device during a 2-year follow-up [109] showed a correlation between decreased resting HR variation during the day and the severity of depression, whereas the mHR at night was higher in participants with more severe depressive symptoms. In line with these results, a decreased autonomic reactivity measured through dynamic changes in photoplethysmography waveform morphology was associated with a higher degree of depression in the study by [124]. Sano et al [12] conducted an observational study among university students using wearable sensors that collected EDA and ST, and using psychometric questionnaires as reference, they found an accuracy of 78% and 87% to classify into high or low stress groups and high or low mental health groups, respectively. Similarly, Sano et al [313] found an accuracy of 90% in classifying stress and mental health groups. From a literature review [130], it was observed that HR and EDA are the most regularly used sensory signals, offering the most promising results and high accuracy for detecting stress.

Effective prevention interventions require strategies to identify early risk groups according to risk factors through the development of predictive models. In addition, from

a mental health promotion perspective, effectively assessing mental well-being would help identify the right time to intervene, evaluate the efficacy of the therapy applied, empower the citizens, offer stress-reducing programs, and prevent negative consequences. Here, we present the development and evaluation of a novel multiparametric tool to improve mental health assessments and to facilitate the evaluation of risk and protective factors as well as the effectiveness of promotion and prevention interventions.

This study aims to design and validate a remote multiparametric tool, including several physiological and cognitive variables, to objectively assess mental well-being as well as mental distress (i.e., symptoms of depression and anxiety) among young people for epidemiologic and clinical studies. The specific objectives of this study are (1) to develop an assessment tool for mental well-being and distress based on the most relevant physiological and cognitive variables; (2) to validate the assessment tool using self-reported measures and evaluate the tool reliability and accuracy; and (3) to develop and establish a protocol to automate the measurement process, ensuring that it can be reproduced in large populations.

9.1. Methods

9.1.1. Study design and setting

This is a multicenter observational study of the mHealth Methods in Mental Health Research (M&M) project, currently ongoing, being conducted by the Autonomous University of Barcelona (UAB) and Parc de Salut Mar (PSMAR).

Three different mental health states will be studied: (1) high mental well-being, (2) presenting mild to moderate psychological distress, and (3) depressive or anxiety disorder (diagnosed by a mental health professional). For the high mental well-being and the mild to moderate psychological distress groups, a web-based mental health questionnaire is being distributed among UAB students for screening and analyzed to determine the participant's eligibility. Participants who meet the selection criteria are consecutively included. For the mental health condition group, the patients are being referred from the Institute of Neuropsychiatry and Addictions-PSMAR, the Hospital Sant Joan de Déu, and the Psychology and Speech Therapy Service of the UAB. The assessments are planned at 2 time points and are being conducted at the site of recruitment (UAB, Institute of Neuropsychiatry and Addictions-PSMAR, or Hospital Sant Joan de Déu). The second assessment takes place after 1 month of the first assessment.

9.1.2. Participants and eligibility criteria

The 3 above-mentioned participant groups are being recruited according to the inclusion and exclusion criteria described in Table 18. To ensure a homogeneous sample in terms of age, participants aged between 18 and 34 years are being recruited in all 3 groups.

Table 18. Participants inclusion-exclusion criteria in M&M study

Groups	Inclusion criteria	Exclusion criteria	Site of recruitment
High mental well-being	No history of emotional distress for at least a year PHQ-4 score: <3 and SWEMWBS score: ≥30	Cognitive impairment or damage, including presence or history of head trauma, dementia, or intellectual disability (IQ ≤80) History of SCZ or other psychotic spectrum disorders Problems understanding Spanish or Catalan	University campus
Mild to moderate psychological distress	Recent history of mental health issues PHQ-4 score: 3-8 or SWEMWBS score: 20-29	Cognitive impairment or damage, including presence or history of head trauma, dementia, or intellectual disability (IQ ≤80) History of SCZ or other psychotic spectrum disorders Problems understanding Spanish or Catalan	University campus
Mental health condition	Diagnosed with current depression or anxiety by a mental health professional	Cognitive impairment or damage, including presence or history of head trauma, dementia, or intellectual disability (IQ ≤80) History of SCZ or other psychotic spectrum disorders Problems understanding Spanish or Catalan The symptomatology has an organic origin or is owing to the physiological effects of a substance Presence of acute suicidal ideation Being medicated	Mental health services

PHQ-4: Patient Health Questionnaire-4. SWEMWBS: short version of Warwick-Edinburgh Mental Well-being Scale.

For the high mental well-being and mild to moderate psychological distress groups, inclusion criteria are assessed for eligibility through a web-based questionnaire that contains questions about mental health history (eg, “Have you ever experienced any mental health issue?”). The Patient Health Questionnaire (PHQ; PHQ-4) [314] is used to screen for anxiety and depression symptoms, and the short version of Warwick-Edinburgh Mental Well-being Scale (SWEMWBS) [315, 316] is used to evaluate mental well-being. The cutoff points to be considered as high mental well-being are based on data from a representative sample of young adults of Catalonia from the Catalonia Health

Survey conducted in 2016 [317], in which a median score of 30 points in SWEMWBS was found. Individuals with SWEMWBS well-being score ≥30 and PHQ-4 <3 points are classified in the high well-being group. Individuals with SWEMWBS score between 20 and 29 points or a PHQ-4 score between 3 and 8 are classified into the mild to moderate psychological distress group.

9.1.3. Recruitment

The primary recruitment pathway for nonpatients is the dissemination of the study through institutional mail or social media and the placement of posters in public areas of UAB. The information includes a link or QR code to answer a web-based questionnaire. To facilitate the recruitment of the mild to moderate psychological distress group, the Psychology and Speech Therapy Service of the UAB is collaborating by inviting students who attended the service to participate in this study. In both cases, once the responsible researcher confirms the eligibility criteria, the participant is contacted to schedule the first assessment.

For the mental health condition group, the patients who meet the criteria are identified at the consultation with the psychologist or psychiatrist, who briefly informs them about the study and suggests participation. The research assistant contacts the interested patients by phone and makes an appointment for the first assessment. Written informed consent is provided by all participants before starting the first assessment interview.

9.1.4. Study procedure

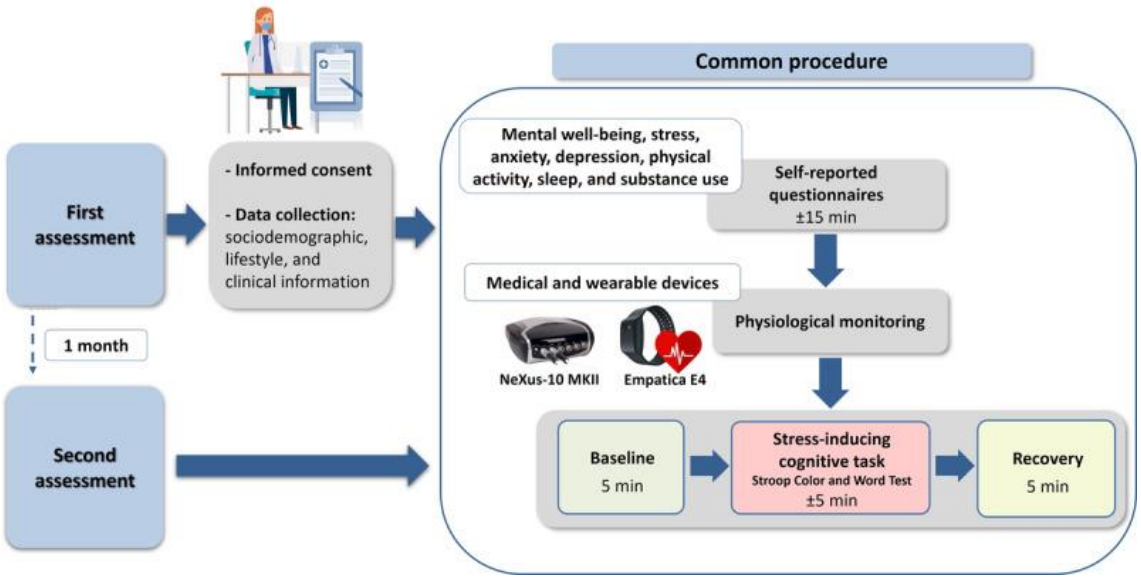
All participants who agree to participate are asked to abstain from tobacco, alcohol, caffeine, or any other beverage or stimulating substance for 2 hours before the study. Figure 19 shows the complete schematic of the experimental procedure.

At the first assessment, the participants are fully informed about the study procedure and are requested to sign the informed consent form. This visit includes an ad hoc interview conducted by a qualified examiner to collect individuals' sociodemographic, modifiable lifestyle factors, health-related variables, and clinical data through the management software Qualtrics (Silver Lake). Subsequently, a psychological assessment is carried out. The participants respond to 7 self-reported questionnaires using the same software. These questionnaires aim to estimate the current mental well-being, stress perception, symptoms of anxiety and depression, physical activity, sleep quality, and substance use. All these measures are described in the Study Variables section.

The physiological assessment consists of recording different stress-related physiological signals using (1) the medical-graded device NeXus-10 MKII (Mind Media BV) and (2)

the wearable E4 Empatica wristband (Empatica Inc). PPG, EDA, and T will be the physiological signals recorded simultaneously by both devices. The ECG and respiration can only be measured using a medical-graded device. This device is used to obtain a more accurate measure for preliminary analysis and, thereafter, validate the predictive model with the wearable device.

Figure 19. M&M study experimental procedure outline



The study experimental procedure outline for the first and second (conducted after 1 month) assessments includes recruitment, obtaining informed consent, and data collection. A research assistant performs the same procedure for both assessments in this observational study (mHealth Methods in Mental Health Research: M&M project).

The wristband is placed on the nondominant wrist and the PPG (middle finger), EDA (middle phalanges of the second and fourth digits), and T (fingertip of the fifth finger) sensors are placed on the nondominant hand to avoid excessive movement artifacts. An adjustable elastic band is placed over the abdomen to measure the respiration signal. For lead 1 of the ECG signal, electrodes are positioned below the right collarbone and below the left rib cage, whereas for lead 2, electrodes are positioned on the fifth intercostal space along the midaxillary line on the left side and symmetrically on the right side. The reference electrode is placed on the left collarbone.

This part of the procedure lasts approximately 15 minutes and is divided into three different stages: (1) baseline (green block in Figure 19): participant in a resting state, sitting comfortably with eyes open; (2) cognitive task (red block in Figure 19): corresponds to the stress-inducing stage, when the individual is submitted to a cognitive task, the Stroop Test [248]; and (3) recovery (yellow block in Figure 19): when the individual's physiological responses are expected to return to the baseline levels. All

physiological signals and variables of interest will be detailed in the Physiological Variables section.

A second assessment is then scheduled 1 month apart and includes the same psychological and physiological assessments. This follow-up session is intended to allow test-retest reliability and account for random errors that could occur in a single session.

9.1.5. Study variables

The following outcome measures are used:

- Depression: It is evaluated using the PHQ-9 [318, 319]. It is a Likert-type scale used to screen the severity of depressive symptoms according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. All 9 items are rated from 0 (not at all) to 3 (nearly every day). Total scores can range from 0 to 27, with higher scores indicating more severe depression. Furthermore, 5, 10, 15, and 20 represent the cutoff points for mild, moderate, moderately severe, and severe depression, respectively [320].
- Anxiety: The Generalized Anxiety Disorder-7 [321, 322] is an instrument for screening the presence of symptoms of anxiety as listed in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. It is a 1-dimensional scale with scores for all 7 items ranging from 0 (not at all) to 3 (nearly every day). The total score was categorized into 4 severity groups according to the original authors: minimal (0-4), mild (5-9), moderate (10-14), and severe (>15).
- Mental well-being: The SWEMWBS [315, 316] is an instrument used to assess mental well-being. This unidimensional scale comprises 7 items ranging from 1 (none of the time) to 5 (all of the time). The higher the total score, the greater the perception of well-being.

Covariates: Sociodemographic variables include age, gender, nationality, marital status, living status, educational level, and occupation.

Current physical and mental health conditions, previous mental health treatments, medication, and suicidal thoughts and behaviors are evaluated through items in the ad hoc interview. Perceived stress is evaluated using the 10-item PSS [208, 323]. It is a 5-point Likert scale with questions about the frequency of feelings and thoughts during the last month, with each item ranging from 0 (never) to 4 (very often). Higher scores indicate higher levels of perceived stress. To assess substance use problems, the CAGE-Adapted to Include Drugs [324] scale is used. It is an adaptation of the original CAGE questionnaire [325] for conjointly screening for alcohol and drug problems based on

lifetime. The scale contains 4 yes or no questions, and a higher score indicates substance use problems.

Modifiable lifestyle variables are assessed using an ad hoc interview, including coffee consumption, cigarette smoking, alcohol and drug consumption, and BMI. Physical activity is measured using the short form of the International Physical Activity Questionnaire [326]. This questionnaire comprises 7 open-ended questions about individuals' 7-day recall of physical activity. According to the total energy expenditure in metabolic equivalent of task (i.e., 1 metabolic equivalent of task is the energy cost of sitting quietly) in minutes per week, the physical activity level is determined as low or inactive, moderate, or high.

Sleep is evaluated using the Medical Outcomes Study Sleep Scale [327]. This questionnaire contains 12 items about a 4-week recall, divided into 8 subscales (sleep adequacy, optimal sleep, quantity of sleep, awakening shortness of breath or with headache, snoring, sleep disturbance, somnolence, and global index of sleep interference). In general, higher scores indicate greater sleep problems. The quantity of sleep score is the patient-reported number of hours of sleep per night, and optimal sleep is scored as 1 (7 or 8 h of sleep per night) or 0 (any different response).

The comfort level is evaluated by asking participants if they are currently experiencing higher stress than usual and identifying its potential causes.

The Spanish version of the standard SCWT is applied [248] as a cognitive stress-inducing task. This test is extensively used to assess cognitive inhibition and processing speed. Furthermore, it has also been shown to be a reliable method to induce mental stress in experimental settings [328]. The individuals are required to read the 3-color cards as fast as possible in a fixed time of 45 seconds each. The stimuli presented on the first 2 cards are congruent, that is, read names of colors or name different colors. In contrast, the last card represents the incongruent stimuli, that is, name the color of the ink instead of reading the color.

Three direct scores are derived by tallying the correct responses for each condition: (1) W (word) represents the number of colors read on the first card (where colors are written in black ink), (2) C (color) represents the number of elements identified on the card of colors (where name colors are represented with strings of XXXXs), and (3) CW (color word) represents the number of items correctly identified on the third card (where colors are printed in an ink that does not correspond to the color name, requiring participants to say the colors of the ink). Two other scores will be calculated from these: (1) predicted CW (PCW): $(W \times C / W + C)$ and (2) interference: $(CW - PCW)$. A higher score indicates a greater ability to inhibit interference.

Direct scores are then converted to T scores, with a preset mean of 50 and SD of 10, so that they can be more easily compared in similar age ranges (in this study, young adults aged between 18 and 44 years). The limits considered normal are between 35 and 65 T points in any of the scores [248].

9.1.6. Physiological variables

Details concerning feature extraction are described in section 6. The final list of features considered in this study is summarized in the following in Table 19. A literature review was conducted to select the most relevant variables for assessing stress response and mental health.

Table 19. List of features for physiological dynamics assessment in M&M study

Physiological signal	Extracted feature	Definition
Electrocardiography or/and Photoplethysmography	mHR (bpm)	Mean heart rate
	SDNN (s)	Standard deviation of NN intervals
	RMSSD (s)	Root mean square of differences between adjacent NN intervals
	PHF (s^{-2})	HRV high frequency band power (0.15 - 0.4 Hz)
	PHFex (s^{-2})	HRV power in extended high frequency band power (0.15 - mHR/2 Hz)
	PLF (s^{-2})	HRV low frequency band power (0.04 - 0.15 Hz)
	PVLF (s^{-2})	HRV very low frequency band power (0.003 - 0.04 Hz)
	LF_HF (DL)	Ratio of low-frequency to high-frequency power
	mPAT (ms)	Mean pulse arrival time
	stdPAT (ms)	Standard deviation of the pulse arrival time
Electrodermal Activity	mTonic (µS)	Average value of the tonic component, i.e. SCL
	mPhasic (µS)	Average value of the phasic component
	aucPhasic (µS·s)	Area under the curve value of the phasic component normalized by the length of the session, i.e. SCRs
	EDASymp (μS^2)	Power spectral density of EDA signal (0.045 - 0.25 Hz)
Skin Temperature	mT (°C)	Mean temperature
	mGrad (°C)	Mean temperature gradient
Respiration	RR (Hz)	Respiratory rate
	Pk (%)	RR peak percentage in the power spectrum

A summary of the findings can be found in Appendix 12 [168, 169, 171, 329–333].

The raw signals recorded with the Empatica E4 wearable device (ie, EDA, PPG, and ST) will be also analyzed in MATLAB (The MathWorks Inc) using a similar procedure, given the different format files. Furthermore, the stress reactivity, that is, the difference between the stress-inducing cognitive task stage and the baseline stage (stress−baseline), and the stress recovery, that is, the difference between the stress and the posterior recovery stage

(stress−recovery), will also be computed for each variable to determine the most relevant set of variables to be considered to design the final model.

9.1.7. Statistical analysis plan

An initial descriptive analysis will be conducted for all study variables, for the overall sample and stratified by study group. The quantitative variables will be summarized, assuming normal distribution (Shapiro-Wilk normality test), using the mean and SD. The qualitative variables will be summarized using the relative and absolute frequencies. Physiological variables that present a skewed distribution will be logarithmically transformed.

A parametric test (Pearson correlation) or nonparametric test (Spearman correlation) will be, accordingly, applied as a descriptive measure of the association between quantitative variables.

To develop a useful tool for assessing mental distress and well-being, the initial step of the statistical analysis plan will involve variables reduction using two methods: (1) a correlation analysis to prioritize the most relevant variables for the prediction of the primary outcome measures and (2) a principal component analysis to find the directions of maximum variance in the data and reduce collinearity. Subsequently, the generated components that account for at least 85% (51/60) of the sample's variability will be included. From these components, 2 separate analyses will be conducted. First, a generalized linear model will be fitted to predict the values of each primary outcome measure. Second, a model will be developed to differentiate the 3 groups identified in the study, each representing a different level of mental health. The models will be fitted using standardized variables and performing k-fold cross-validation to quantify the model's performance using R2 for the regression model and area under the curve for classification models. Known-group validity will be assessed by comparing the mean scores of the tool among the groups at baseline: diagnosed with current depression or anxiety, symptoms of mental distress, and high mental well-being. The predefined hypothesis that higher model scores are predicted for individuals with higher well-being will be evaluated using the Jonckheere-Terpstra test, and Cohen effect sizes will be computed for each category as compared with the lowest category (mental health condition), considering small (0.2), moderate (0.5), and large (0.8) effect sizes [334].

The model test-retest reliability will be assessed with a 2-way random effect intraclass correlation coefficient (ICC), taking repeated evaluations of the same unchanged individuals, to assess the extent to which measures remain stable. A change will be considered a clinically relevant change in the outcome scores, i.e., ≥4 points for the

Generalized Anxiety Disorder-7, ≥5 points for PHQ-9, or ≥3 points for SWEMWBS) [335–337].

The significance level will be set at α=.05. Statistical analysis will be performed using SAS (version 9.4; SAS Institute).

9.1.8. Sample size

As a proof-of-concept study, we plan to include 20 individuals per group (a total of 60 individuals×2 evaluations). For the assessment of known-groups validity of the developed tool, with this sample size of 20 individuals per group, and a type I error rate α=.05 on a 1-sided t test, we will have power of 0.80 to detect a difference between 2 groups corresponding to an effect size of 0.8. For a moderate effect size of 0.5, the power will decrease to 0.47 [306].

Concerning the assessment of test-retest reliability of the tool, assuming a 15% (9/60) loss in follow-up or nonstable participants from baseline participants, an ICC of 0.6 under the null hypothesis, and a type I error rate of α=.05, a sample size of 51 participants with 2 observations per participant achieves a power of 0.90 to detect a hypothetical ICC value of 0.8 under the alternative hypothesis [306].

9.1.9. Ethical considerations

This study protocol was approved by the research ethics committees of both institutions (2021/10163 for PSMAR and 5912 for UAB). This study is in line with the principles established by national and international regulations, including the Declaration of Helsinki and the Code of Ethics. Ethics approval has been obtained in Barcelona from the independent PSMAR Clinical Research Ethics Committee and the Research Ethics Committee of the UAB. Informed consent is requested from all participants before their inclusion in the study. Participants are explained that they can withdraw from the study at any time without giving a reason and that they can request to delete all the data collected from them.

All personal data will be handled following Regulation (European Union) 2016/679 of the European Parliament and the Council on the protection of natural persons concerning the processing of personal data and on the free movement of such data and the National Organic Law 3/2018, of December 5, on Personal Data Protection and the Guarantee of Digital Rights. Physiological and psychometric data will be pseudoanonymized to guarantee privacy in data analysis and will be stored in a research database following the General Data Protection Regulation of the European Union.

Participants receive a 10€ (US $11) gift card for enrolling in the study, and participants receive another gift card of the same value if they complete the second assessment.

9.2. Results

The project was granted in February 2022 and the approval from ethical committees was obtained between April and May 2022. Participant recruitment started in October 2022 and is expected to continue through June 2024. Different recruitment strategies are implemented, including advertising campaigns and invitation letters at the university and recruitment of patients by psychologists.

As of July 2023, a total of 41 participants completed the first and second assessments. The sample corresponds mainly to the group with mild to moderate psychological distress (n=20, 49%), followed by the group with high mental well-being (n=13, 32%) and, finally, those diagnosed with an anxiety disorder (n=8, 20%). At this point, preprocessing and quality checks of the data are ongoing, and the statistical analysis will subsequently begin. The first results are expected to be published in September 2024.

9.3. Discussion

There is a growing interest in remotely assessing mental health through changes in the ANS functioning and its association with mental health and well-being [338]. There is significant evidence to support the notion that changes in the ANS can be inferred from changes in physiological variables, such as HRV, EDA response, and peripheral temperature [220, 329, 339]. However, there is a challenge in designing a robust predictive model that allows this assessment to be easy and objective to be systematically used in epidemiology and clinical settings. To fill this gap, it is important to carefully measure electrophysiological signals considering the updated standards of measurement [340], following a structure of 3-time point experimental design: considering a basal condition, during a stress-inducing task to investigate the stress reactivity, and a recovery stage. This study is applying a similar methodology used in previous research that reached good reliability in its predictive models [308, 341]. However, in this novel approach, individuals with a clinically diagnosed mental health condition are being enrolled, which may allow for a better distinction between different profiles of mental health through the stress reactivity pattern and cognitive performance.

The SCWT is a well-known neuropsychological test reported as a reliable moderate mental stressor, provoking significant physiological changes such as reduced HRV and increased EDA and BP [330, 342, 343]. Furthermore, it is a useful tool for evaluating cognitive processes and has the advantage of not generating a significant learning effect [344]. Cognitive inhibition is compromised in multiple mental health conditions; for

example, individuals with anxiety disorders tend to have longer reaction times and higher error rates on the SCWT, particularly in the incongruent condition, which suggests difficulties with selective attention and response inhibition [345]. Thus, although the impaired performance of the task may be indicative of an underlying neurological disorder, a good result performance may add another layer of confidence in evaluating mental well-being.

There is a lot of research committed to investigating biomarkers and stress reactivity in patients diagnosed with depression and anxiety disorders [119, 346]. The evidence supports differences in physiological behavior in patients with depression and anxiety compared with healthy individuals. Considering this, the decision to include 3 groups with different mental health states will facilitate the detection of patterns that could discriminate them more accurately. The age range selected is justified by the typical early age of onset of mental health conditions and the need for assertive responses to prevent a poor prognosis. Moreover, the recruitment is planned to be carried out in part at a university, considering that university students have been reported as a susceptible population with a higher risk to manifest mental health symptoms [347], which enables us to recruit the mild to moderate psychological distress group effortlessly. To minimize interindividual variation in physiological measures and the influence of external factors, we propose 2 different sessions with the same participants, which will also increase the statistical power [340].

mHealth, which includes physical devices, sensors, software, and other technologies, has been proposed to improve clinical care as it enables data collection, symptom monitoring, and provision of interventions [348]. In this sense, it could be a valuable resource to reach both regular patients and those who do not receive adequate care [303]. Specifically, the use of wearables provides a new and unobtrusive way to monitor physiological biomarkers and gather continuous information about individuals' daily lives and clinical symptoms for both clinical and research purposes. These devices with multiple embedded sensors can be useful in following up patients and remotely assessing their mental well-being through robust models that combine a set of relevant biomarkers [312].

Nevertheless, to ensure that autonomous nervous system biomarkers are effectively used for objectively measuring mental well-being in health services, it is necessary to undertake substantial work in identifying the most useful biomarkers and comprehending the possible obstacles and enablers of widespread adoption. To advance in this direction, this study intends to carry out a preliminary model validation by enrolling a preselected sample with different states of mental well-being in a laboratory-controlled condition; subsequently, we plan to explore its application in larger populations and in a real-life context.

To summarize, our study aims to design a comprehensive multiparametric model combining physiological and cognitive variables to assess the mental well-being among young people. The self-reported questionnaires currently used in clinical settings will be used as a reference to select the best model fit. This novel approach proposes shifting the paradigm to assessing mental well-being rather than measuring the severity of symptoms or a mental health condition. We believe that this change may allow health professionals to properly recommend prevention strategies and increase the possibility of intervening before the diagnosis of a mental health condition. To effectively develop a model that can be easily calculated by a wearable device, we first take a measurement of a standard medical device to ensure the best quality of physiological signal and then establish the final predictive tool.

IV. Conclusions and future work

This final section of the thesis summarizes the main findings and compiles them into a general conclusion. It begins with a recap of the results from each publication mapping them back to my original thesis aims. Then, strengths and limitations are discussed, and finally, the directions for future research are examined.

Table of contents

10. Summary and final discussion ____ ***125***

11. Strengths and limitations ____ ***129***

12. Main conclusion ____ ***129***

13. Future work ____ ***130***

10. Summary and final discussion

The main aim of the present thesis was to provide a comprehensive analysis of stress dynamics and its implications for mental health assessment, particularly for the development and validation of a noninvasive tool for assessing the severity of MDD. Eight specific aims were established and addressed them through this manuscript and the three different published studies: two observational cohort studies — one to evaluate stress in a healthy population and another to assess MDD severity in patients — and a protocol.

To establish a theoretical framework, the first aim was to synthesize current research on mental health, particularly the pathophysiology of stress. Stress is highly prevalent in our fast-paced society and has a significant impact on mental health. The immediate stress response is coordinated by the hypothalamus through the activation of the ANS, regulating physiological adaptations such as increased heart and respiratory rates. Simultaneously, the HPA axis triggers cortisol release, essential for mobilizing glucose reserves for immediate energy, modulating the immune response, and suppressing non-essential functions.

While this response to stress is necessary for survival and homeostasis regulation, continuous exposure to stress can lead to physiological adaptations that alter the neuroendocrinological systems involved in the stress response. Moreover, chronic stress disrupts neuroplasticity, particularly in the hippocampus, prefrontal cortex, and amygdala. This structural brain changes, combined with the dysregulation of the stress response, negatively impact cognitive, emotional, and behavioral processes, ultimately leading to the development of mental disorders such as MDD.

MDD is one of the most prevalent mental disorders. The etiology is described as combination of genetic predisposition, neuroendocrinological imbalance, and environmental stressors, namely influencing neurotransmitters like serotonin, and disruptions in neuroplasticity and the HPA axis which are critical for mood regulation and cognitive function. While screening tools commonly used in clinical practice, such as questionnaires, are valuable, they can sometimes be time-consuming and prone to biases. Given that the combined effect of dysregulated stress response and neurobiological changes in the brain can disrupt ANS function, the second aim of this thesis was to analyze current objective tools for assessing stress dynamics, with a focus on those utilizing noninvasive biomarkers related to the ANS. These noninvasive screening tools based on ANS biomarkers could complement existing psychometric scales, enhancing the accuracy of depression diagnosis and follow-up.

Along the experimental work done in this thesis, it has been demonstrated that PPG's role in measuring blood volume changes noninvasively makes it a great substitute for ECG to assess autonomic nervous system functioning, and it is highly suitable for continuous monitoring through wearable devices at least in movement-free conditions. Its application in mental health to gauge stress and emotional states via the analysis of PRV represents a significant leap in integrating physiological data with psychological assessments. Nonetheless, PPG signals face limitations such as susceptibility to motion artifacts, variability in pressure applied by the sensor, and variability due to skin tone and sensor placement. These limitations must be considered, especially in clinical or critical care settings where accuracy is paramount.

Despite EDA sensors not being as commonly used as PPG sensors, they have provided highly specific measurements of the SNS's response. However, these sensors measure skin conductance, which can be significantly affected by the specific site of sensor placement or movement artifacts. Therefore, there is a need for meticulous placement, calibration, and noise filtering to ensure accurate readings.

Lastly, even though respiration as well as thermoregulation provide noninvasive biomarkers that correlate with stress and emotional states, their use in clinical and research settings is limited.

The emphasis on individual variability in stress reactivity and recovery introduced a more nuanced perspective in stress research. Recognizing that stress responses are not uniform allows for more targeted and effective interventions tailored to individual physiological and psychological profiles.

These sections were instrumental in laying the groundwork for understanding how physiological stress responses can be quantified and used as noninvasive mental health biomarkers. However, prior to the development of the screening tools, we needed to develop a robust stress pattern using clinical and biological variables that encapsulated both psychological and somatic activations, which was the third aim of this thesis.

The endocrine biomarkers from the 120 young healthy subjects indicated a less pronounced reduction in cortisol levels during stress sessions compared to relaxation sessions. This extended cortisol awakening response underscored the significant role of cortisol in stress responses. Moreover, the use of salivary cortisol and salivary α-amylase as biomarkers provided a practical and noninvasive alternative for stress measurement, effectively substituting more invasive measures such as plasma catecholamines.

The integration of endocrine biomarkers, which contributed 46% to the total score according to our PCA analysis, alongside trait and state psychometric scores, which

contributed 54%, was used to offer a balanced perspective encompassing both the HPA axis response and perceived stress levels. This integration led to the development of the SRS, providing a comprehensive pattern of the stress response.

Following the development of the SRS, the dynamics of the physiological stress response were characterized. The SRS served as a reference for creating an objective, noninvasive, and continuous tool for stress assessment based solely on ANS biomarkers, thereby achieving the fourth objective of this thesis. This approach utilized physiological features to enable a more precise and continuous evaluation of stress levels.

Physiological signal processing was performed on 240 files with ECG, PPG, EDA, Resp and T data, to extract over 80 ANS features widely reported in the literature or associated with physiological processes related to stress. Linearly dependent features were excluded and only those having a significant correlation with the SRS score were selected. Principal component analysis facilitated the selection and prioritization of a final set of five most relevant features for estimating stress levels that accounted for 87% of the data variance. The features incorporated into the model included the peripheral temperature gradient, mHR, power in the high-frequency band of HRV, mean pulse arrival time, and the standard deviation of the phasic component of EDA. Consequently, the developed tool offers a quantitative, continuous, and noninvasive measure of stress in healthy students, underscoring the significance of ANS biomarkers for mental health assessment.

Further exploring mental health assessment through ANS biomarkers, the physiological stress dynamics in individuals with MDD were investigated. However, given the multifaceted etiology of MDD, the socio-demographic, lifestyle, psychometric, and cognitive factors that may predispose individuals to MDD were also examined as part of the fifth aim of this thesis.

The observational cohort study highlighted significant differences between the 40 control and the 40 MDD subjects, such as higher BMI, differences in marital status, and lower levels of employment and education in the MDD group. Psychometric assessments revealed significantly higher levels of mental distress in the MDD group, with state and trait anxiety being approximately three times higher, and stress levels about twice as high compared to the control group. Moreover, patients with MDD showed a pronounced decline in cognitive performance, particularly in processing speed and executive function tasks. Further analysis revealed that educational level significantly influenced cognitive scores, although the impact of depression severity on cognitive performance remained consistent, irrespective of the educational background of the subjects. Age also significantly affected cognitive scores, predicting poorer inhibition of automatic responses.

Building on these findings, the sixth aim was to develop a multiparametric model that included the relevant risk factors as well as ANS biomarkers to objectively assess the severity of depression. To that end, the physiological dynamics of cognitive stress in individuals diagnosed with MDD were explored.

There were differences in stress static and dynamic features using a cognitive stress protocol to evaluate how individuals with MDD reacted to stress, compared to HC. The results revealed that recovery biomarkers were more indicative of ANS regulation differences, suggesting that how individuals recover from stress could be more critical in understanding MDD.

Higher depression levels were associated with reduced variability in HR during both stress reactivity and recovery phases, whereas the control group exhibited more pronounced HR dynamics, suggesting better physiological adaptability to stress. Similar results were found in temperature and pulse arrival time dynamics, indicating that higher levels of depression are associated with a blunted thermoregulatory and cardiovascular response to stress. Recovery metrics in EDA were also more relevant than reactivity metrics, showing that individuals with MDD may not return to baseline levels as efficiently after stress, indicating prolonged physiological arousal or delayed recovery. Finally, the study developed a regression model incorporating cognitive and physiological variables, adjusted for age, gender, and BMI, to predict depression severity. The model indicated gender-specific differences in the impact of BMI and HR recovery on depression severity, with significant implications for personalized medical approaches.

Continuing with ongoing research to objectively evaluate mental health, a research protocol that supported ongoing research to objectively evaluate mental health was designed, ensuring reproducibility in large populations suitable for epidemiological and clinical research. This seventh goal aimed to incorporate the measurement of the stress dynamics using multiple ANS biomarkers from a wearable device to validate a remote multiparametric tool. The published protocol is still ongoing, enrolling university students and mental health patients. The ultimate goal is to provide a robust, remote, and easily accessible method to monitor and evaluate mental health, particularly in settings lacking extensive medical resources.

Finally, to summarize the main findings, including the strengths and limitations of the present thesis, and discuss potential directions for future investigations, the last aim is included in the upcoming sections.

11. Strengths and limitations

This thesis highlights the potential for noninvasive and low cost (both economically and computationally) tools for mental health assessment. These tools could provide an objective assessment using remote technologies without any special training and they could be administered repeatedly reflecting improvement or worsening of mental health in response to treatment, particularly in stress and depression. Furthermore, these tools can provide common ground for researchers and healthcare providers in combination with traditional methods.

Although many studies have employed machine learning approaches to predict depression, such as k-nearest neighbor, support vector machine models or decision trees, this thesis presents bivariate and multivariate associations using linear mixed models and a posterior cross-validation to enhance the validity of the results. Machine learning techniques are powerful, especially in handling high-dimensional data and small sample sizes [130]. However, they are often criticized for their lack of interpretability and the risk of overfitting the data. The multivariable approach used in this thesis based on a set of physiological features provides a clear and interpretable evidence base for future feature selection and ensures that our findings are robust and clinically relevant.

The main limitation of this research is the small sample sizes utilized. While the exploratory nature of these studies provides initial insights, validation in independent datasets or larger populations is essential to ensure the generalizability and systematic application of the findings. Moreover, health conditions, sleep patterns, and habits related to alcohol or smoking can significantly impact an individual's physiology. The studies analyzed in this thesis were specifically designed to exclude these cases to avoid biases. Therefore, it is crucial to closely monitor these factors as they may influence the tool's accuracy.

Additionally, to evaluate the evolution of stress dynamics during cognitive tests, the ultra-short-term analysis of physiological features served as a compromise between usability and reliability. Therefore, this approach warrants more thorough investigation, and the inclusion of more advanced signal processing techniques could enhance the features robustness.

12. Main conclusion

This thesis presents a comprehensive analysis of physiological stress dynamics and their implications for mental health assessment, with a particular focus on MDD. It establishes

a methodological framework for developing noninvasive predictive models based on ANS biomarkers, extracted using signal processing techniques. These methodologies encompass a replicable protocol for mental health assessment and two multiparametric models for measuring acute stress and the severity of MDD.

The results achieved provide a better understanding of mental health through the developed multimodal approach, paving the way for enhanced diagnostic and therapeutic strategies. It underscores the utility of ANS biomarkers and suggests new avenues for research and clinical practice.

13. Future work

Adapting the framework outlined in this work for clinical application represents the ultimate goal for the future. However, several areas require further exploration.

Improperly worn devices and unrestricted movement of subjects are the primary challenges in real-time monitoring. In controlled environments, subject movements and stressors are limited, allowing researchers to ensure devices are worn correctly and obtain precise results. However, in real-world settings, movements are unrestricted and unmonitored, and subjects often engage in multiple activities simultaneously. Moreover, many wearables do not provide access to the raw signal but only to derived parameters, which may not be reliable under unfavorable conditions. This complexity can hinder the accuracy and effectiveness of stress detection systems.

Therefore, a primary focus should be on developing methods to derive robust physiological biomarkers, including artifact detection and signal processing algorithms capable of managing diverse and unpredictable data. This entails exploring acceptable rates of missing data and methods to ensure data reliability. Additionally, integrating a wider range of passive data from wearables or smartphones, such as sleep, GPS, or speech in natural settings, could significantly improve outcome accuracy and even patient adherence.

The reliance on laboratory-based stress tests, such as the SCWT and TSST, while providing controlled conditions, may not fully capture the complexity and variability of stress responses in real-world environments. Real-life stressors are often more diverse and unpredictable, potentially influencing stress dynamics differently. For this reason, future studies on stress research should fully encapsulate a real-world stress dynamics design, and incorporating real-time data collection methods like Ecological Momentary Assessment can provide more accurate reflections of daily stress processes.

Future research on depression should aim to refine and validate these findings for broader application, adhering to rigorous reporting standards to ensure reproducibility and transparency. Studies should involve larger and more diverse samples, and devise strategies to increase sample sizes and engage participants effectively, thus minimizing data gaps. Furthermore, it is important to investigate the variability among depression subtypes and conduct prospective studies to determine whether stress dynamics dysfunction is a consequence or correlate of depression, or a marker of vulnerability to future depression.

Certainly, the rapid advancement of artificial intelligence and digital tools for clinical practice, especially remote monitoring technologies, will enhance precision psychiatry and the interaction between patients and therapists in the context of mental health. Consequently, research should also examine how digital health tools can be effectively integrated into clinical practice, overcoming challenges such as interoperability, increased clinician workload, and the disparity in digital access among patients.

V. Appendix

Table of contents

14. Scientific contributions _____ *135*

15. Acknowledgments _____ *139*

16. References _____ *141*

17. Supplementary information _____ *175*

14. Scientific contributions

14.1. Journal publications

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17. Supplementary information

Appendix 1. State-trait anxiety inventory (STAI)

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

	ESTADO	Nada	Algo	Bastante	Mucho
1	Me siento calmado				
2	Me siento seguro				
3	Estoy tenso				
4	Estoy contrariado				
5	Me siento cómodo (estoy a gusto)				
6	Me siento alterado				
7	Estoy preocupado ahora por posibles desgracias futuras				
8	Me siento descansado				
9	Me siento angustiado				
10	Me siento confortable				
11	Tengo confianza en mí mismo				
12	Me siento nervioso				
13	Estoy desasosegado				
14	Me siento muy «atado» (como oprimido)				
15	Estoy relajado				
16	Me siento satisfecho				
17	Estoy preocupado				
18	Me siento aturdido y sobreexcitado				
19	Me siento alegre				
20	En este momento me siento bien				

Instrucciones: A continuación, encontrará unas frases que se utilizan corrientemente para describirse uno a sí mismo. Lea cada frase y señale la puntuación de 0 a 3 que indique mejor cómo se siente usted en general en la mayoría de las ocasiones. No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando la respuesta que mejor describa **cómo se siente usted generalmente.**

	RASGO	Casi nunca	A veces	A menudo	Casi siempre
21	Me siento bien				
22	Me canso rápidamente				
23	Siento ganas de llorar				
24	Me gustaría ser tan feliz como otros				
25	Pierdo oportunidades por no decidirme pronto				
26	Me siento descansado				
27	Soy una persona tranquila, serena y sosegada				
28	Veo que las dificultades se amontonan y no puedo con ellas				
29	Me preocupo demasiado por cosas sin importancia				
30	Soy feliz				
31	Suelo tomar las cosas demasiado seriamente				
32	Me falta confianza en mí mismo				
33	Me siento seguro				
34	No suelo afrontar las crisis o dificultades				
35	Me siento triste (melancólico)				
36	Estoy satisfecho				
37	Me rondan y molestan pensamientos sin importancia				
38	Me afectan tanto los desengaños que no puedo olvidarlos				
39	Soy una persona estable				
40	Cuando pienso sobre asuntos y preocupaciones actuales me pongo tenso y agitado				

Appendix 2. Perceived stress scale (PSS)

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

En el último mes/semana …		Nunca	Casi nunca	De vez en cuando	A menudo	Muy a menudo
1	¿Con qué frecuencia ha estado afectado por algo que ha ocurrido inesperadamente?					
2	¿Con qué frecuencia se ha sentido incapaz de controlar las cosas importantes en su vida?					
3	¿Con qué frecuencia se ha sentido nervioso o estresado?					
4	¿Con qué frecuencia ha manejado con éxito los pequeños problemas irritantes de la vida?					
5	¿Con qué frecuencia ha estado seguro sobre su capacidad para manejar sus problemas personales?					
6	¿Con qué frecuencia ha sentido que las cosas le van bien?					
7	¿Con qué frecuencia ha sentido que no podía afrontar todas las cosas que tenía que hacer?					
8	¿Con qué frecuencia se ha sentido al control de todo?					
9	¿Con qué frecuencia ha estado enfadado porque las cosas que le han ocurrido estaban fuera de su control?					
10	¿Con qué frecuencia ha sentido que las dificultades se acumulan tanto que no puede superarlas?					

Appendix 3. Visual analogue stress scale (VASS)

Nivel de confort:

¿Considera que actualmente está sometido a un nivel de estrés más elevado de lo habitual?

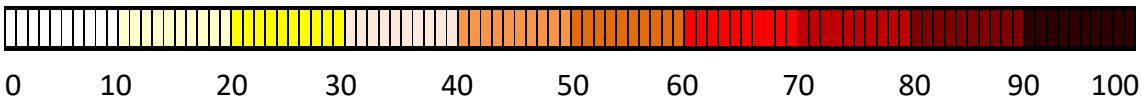
(1. No, 2. Sí) _____

En caso afirmativo, indique la razón o razones principales que considera que causan dicho estrés (p.e., causas familiares, estudios, relacionadas con el puesto de trabajo, situación personal, etc.): _____

En general, ¿Dónde marcaría usted su nivel de estrés en este momento?

Siendo 0 = ningún estrés en absoluto y 100 = absolutamente estresado.

Por favor, use las rayas para indicar los valores intermedios de estrés:



Appendix 4. Stress symptoms scale (SSS)

Señale la opción que mejor describe cómo se siente en este momento.

		Muy de acuerdo	Algo de acuerdo	Ni de acuerdo ni en desacuerdo	Algo en desacuerdo	Muy en desacuerdo
1	Siento palpitaciones					
2	Siento la boca seca					
3	Siento rigidez en el cuello					
4	Siento que me falta el aire, suspiro con frecuencia					
5	Siento opresión en el pecho					
6	Siento escalofríos					
7	Siento urgencia para orinar con frecuencia					
8	Siento que sudo					
9	Siento como si tuviera nervios en el estómago					
10	Siento que mi cara se sonroja					
11	Me siento mareado					
12	Cometo muchos errores					
13	No tengo ganas de hablar					
14	Me siento enfadado					
15	Me siento molesto por todo					
16	Siento que me distraigo fácilmente o no me concentro					
17	No me siento motivado para hacer cosas					
18	Me siento al límite					
19	Siento que me impaciento fácilmente					
20	Me siento agitado e/o inquieto					

Textos de prueba de memoria:

Historieta 1

Ana Pérez de la provincia de Málaga que estaba empleada como mujer de la limpieza en una casa comercial denunció en la comisaria de la estación que le habían robado en la calle Mayor la noche antes y le habían quitado 200 pesetas. Ella tenía cuatro niños pequeños y este dinero lo necesitaba para ellos ya que hacía dos días que no habían comido. Los policías conmovidos por la historia de la mujer hicieron una recolecta para ella.

Historieta 2

Muchos de los niños de una escuela del norte de Francia murieron o quedaron gravemente heridos y otros seriamente lesionados al hacer explosión una bomba en la escuela del pueblo. Los niños salieron despedidos por encima de un barranco hasta una colina a una considerable distancia de la escuela. Solo dos niños quedaron ilesos.

Historieta 3

Juan Gracia de 63 años de edad alcalde de Berga provincia de Barcelona cuando planificaba su campaña electoral empezó a notar dolores de espalda. Estuvo ingresado tres días en el Hospital Clínico para que le hicieran exploraciones médicas. Se le diagnosticó una enfermedad vírica inofensiva. Luego, con su mujer Carmen y sus dos hijos Antonio y Tomas continuaron la campaña.

Prueba aritmética:

En este punto, los jueces piden al participante que reste, en serie, el número 13 de 1022 con la mayor rapidez y precisión posible. Cada vez que el participante comete un error, uno de los jueces interviene, pedirle al participante que comience los cálculos nuevamente desde el número inicial (1022) - "Detener. Por favor, empieza de nuevo".

Appendix 6. Clinical Global Impression (CGI)

Gravedad de la enfermedad (CGI-SI)

Basándose en su experiencia clínica, ¿cuál es la gravedad de la enfermedad en el momento actual?

0. No evaluado
1. Normal, no enfermo
2. Dudosamente enfermo
3. Levemente enfermo
4. Moderadamente enfermo
5. Marcadamente enfermo
6. Gravemente enfermo
7. Entre los pacientes más extremadamente enfermos

Mejoría global (CGI-GI)

Comparado con el estado inicial, ¿cómo se encuentra el paciente en estos momentos? (Puntúe la mejoría total independientemente de que a su juicio se deba o no por completo al tratamiento)

0. No evaluado
1. Mucho mejor
2. Moderadamente mejor
3. Levemente mejor
4. Sin cambios
5. Levemente peor
6. Moderadamente peor
7. Mucho peor

Appendix 7. Hamilton depression rating scale (HDRS)

1- ESTADO DE ÁNIMO DEPRIMIDO_____[ ]

0 *Ausente*
1 *Ligero*: actitud melancólica; el paciente no verbaliza necesariamente el descenso del ánimo
2 *Moderado*: llanto ocasional, apatía, pesimismo, desmotivación....
3 *Intenso*: llanto frecuente (o ganas); introversión; rumiaciones depresivas; pérdida del gusto por las cosas
4 *Extremo*: llanto frecuente (o ganas); frecuente tendencia al aislamiento; contenidos depresivos exclusivos en el pensamiento o la comunicación verbal; pérdida de la capacidad de reacción a estímulos placenteros

2- SENTIMIENTOS DE CULPA_____[ ]

0 *Ausente*
1 *Ligero*: autorreproches, teme haber decepcionado a la gente
2 *Moderado*: ideas de culpabilidad; sentimiento de ser una mala persona, de no merecer atención
3 *Intenso*: la enfermedad actual es un castigo; meditación sobre errores, malas acciones o pecados del pasado; merece lo que padece
4 *Extremo*: ideas delirantes de culpa con o sin alucinaciones acusatorias

3- SUICIDIO_____[ ]

0 *Ausente*
1 *Ligero*: la vida no vale la pena vivirla
2 *Moderado*: desearía estar muerto o piensa en la posibilidad de morirse
3 *Intenso*: ideas o amenazas suicidas
4 *Extremo*: serio intento de suicidio

4- INSOMNIO INICIAL (si toma hipnóticos y no puede evaluar, puntúe 1)_____[ ]

0 *Ausente*
1 *Ocasional*: tarda en dormir entre media y una hora (<3 noches/semana)
2 *Frecuente*: tarda en dormir más de una hora (3 ó más noches /semana)

5- INSOMNIO MEDIO (si toma hipnóticos y no puede evaluar, puntúe 1)_____[ ]

0 *Ausente*
1 *Ocasional*: está inquieto durante la noche; si se despierta tarda casi una hora en dormirse de nuevo (<3 noches/semana)
2 *Frecuente*: está despierto durante la noche, con dificultades para volver a conciliar el sueño; cualquier ocasión de levantarse de la cama (excepto para evacuar), o necesidad de fumar o leer tras despertarse debe puntuar 2 (3 ó más noches seguidas por semana)

6- INSOMNIO TARDÍO (si toma hipnóticos y no puede evaluar, puntúe 1)_____[ ]

0 *Ausente*
1 *Ocasional*: se despierta antes de lo habitual (<2 horas antes; <3 días por semana)
2 *Frecuente*: se despierta dos o más horas antes de lo habitual 3 ó más días por semana)

7- TRABAJO Y ACTIVIDADES_____[ ]

0 *Ausente*
1 *Ligero*: ideas o sentimientos de incapacidad o desinterés. Distíngalo de la fatiga o pérdida de energía que se puntúan en otra parte.
2 *Moderado*: falta de impulso para desarrollar las actividades habituales, las aficiones o el trabajo (si el paciente no lo manifiesta directamente, puede deducirse por su desatención, indecisión o vacilación ante el trabajo y otras actividades).
3 *Intenso*: evidente descenso del tiempo dedicado a sus actividades; descenso de su eficacia y/o productividad. En el hospital se puntúa 3 si el paciente no se compromete al menos durante tres horas/día a actividades (Trabajo hospitalario o distracciones) ajenas a las propias de la sala. Notable desatención del aseo personal.
4 *Extremo*: dejó de trabajar por la presente enfermedad. No se asea o precisa de gran estímulo para ello. En el hospital se puntúa 4 si el paciente no se compromete en otras actividades más que a las pequeñas tareas de la sala o si precisa de gran estímulo para que las realice.

8- INHIBICIÓN_____ [ ]

0 *Ausente*
1 *Ligera*: ligera inhibición durante la entrevista; sentimientos ligeramente embotados; facies inexpresiva.
2 *Moderada*: evidente inhibición durante la entrevista (voz monótona, tarda en contestar las preguntas).
3 *Intensa*: entrevista difícil y prolongada; lentitud de movimientos al caminar.
4 *Extrema*: estupor depresivo completo; entrevista imposible.

9- AGITACIÓN_____ [ ]

0 *Ausente*
1 *Ligera*: mueve los pies; juega con las manos o con los cabellos
2 *Moderada*: se mueve durante la entrevista, se agarra a la silla; se retuerce las manos; se muerde los labios; se tira de los cabellos; mueve ampliamente los brazos, se muerde las uñas, las manos...
3 *Intensa*: no puede estarse quieto durante la entrevista; se levanta de la silla.
4 *Extrema*: la entrevista se desarrolla "corriendo", con el paciente de un lado para otro, o quitándose la ropa, o arrancándose los cabellos; el paciente parece desconcertado y "desatado".

10- ANSIEDAD PSÍQUICA_____ [ ]

0 *Ausente*
1 *Ligera*: tensión subjetiva e irritabilidad.
2 *Moderada*: tensión objetiva, evidente; preocupación por trivialidades.
3 *Intensa*: actitud aprensiva evidente en la cara y el lenguaje.
4 *Extrema*: crisis de ansiedad observadas, la ansiedad forma la mayor parte del contenido de su comunicación espontánea, verbal o no verbal.

11- ANSIEDAD SOMÁTICA_____ [ ]

0 *Ausente*
1 *Ligera*: un solo síntoma o síntoma dudoso o varios síntomas de un mismo sistema.
2 *Moderada*: varios síntomas de distintos sistemas.
3 *Intensa*: múltiples síntomas de varios sistemas simultáneamente.
4 *Extrema*: numerosos síntomas persistentes e incapacitantes la mayor parte de las veces.

12- SÍNTOMAS SOMÁTICOS GASTROINTESTINALES_____ [ ]

0 *Ausentes*:
1 *Ligeros*: pérdida de apetito, pero come sin necesidad de estímulo; sensación de pesadez en el abdomen.
2 *Intensos*: pérdida de apetito, no come aunque se le estimule, o precisa de gran estímulo para comer; precisa o solicita laxantes o medicación para sus síntomas gastrointestinales.

13- SÍNTOMAS SOMÁTICOS GENERALES_____ [ ]

0 *Ausentes*:
1 *Ligeros*: fatigabilidad, pérdida de energía, pesadez en extremidades, espalda, cabeza; algias en el dorso, cabeza, músculos.
2 *Intensos*: fatigabilidad y pérdida de energía la mayor parte del tiempo; cualquier síntoma somático bien definido o expresado espontáneamente.

14- SÍNTOMAS GENITALES (preguntar siempre)_____ [ ]

0 *Ausentes*: o información inadecuada o sin información (emplear lo menos posible estas dos últimas).
1 *Ligeros*: descenso de la líbido; actividad sexual alterada (inconstante, poco intensa).
2 *Intensos*: pérdida completa de apetito sexual; impotencia o frigidez funcionales.

15- HIPOCONDRÍA_____ [ ]

0 *Ausente*:
1 *Ligera*: preocupado de sí mismo (corporalmente).
2 *Moderada*: preocupado por su salud.
3 *Intensa*: se lamenta constantemente. Solicita ayuda, etc.
4 *Extrema*: ideas hipocondríacas delirantes.

16- PÉRDIDA DE INTROSPECCIÓN_____ [ ]

0 *Ausente*: se da cuenta de que está enfermo, deprimido.
1 *Ligera*: reconoce su enfermedad, pero la atribuye a la mala alimentación, al clima, al exceso de trabajo, a una infección viral, a la necesidad de descanso, etc.
2 *Moderada*: niega estar enfermo o el origen nervioso de su enfermedad.

17- PÉRDIDA DE PESO_____ [ ]

0 *Ausente*:
1 *Ligera*: probable pérdida de peso asociada a la enfermedad actual; pérdida superior a 500 gr/semana ó 2,5 kg/año (sin dieta).
2 *Intensa*: pérdida de peso definida según el enfermo; pérdida superior a 1 kg/semana ó 4,5 kg/año (sin dieta).

PUNTUACIÓN TOTAL_____ [ ]

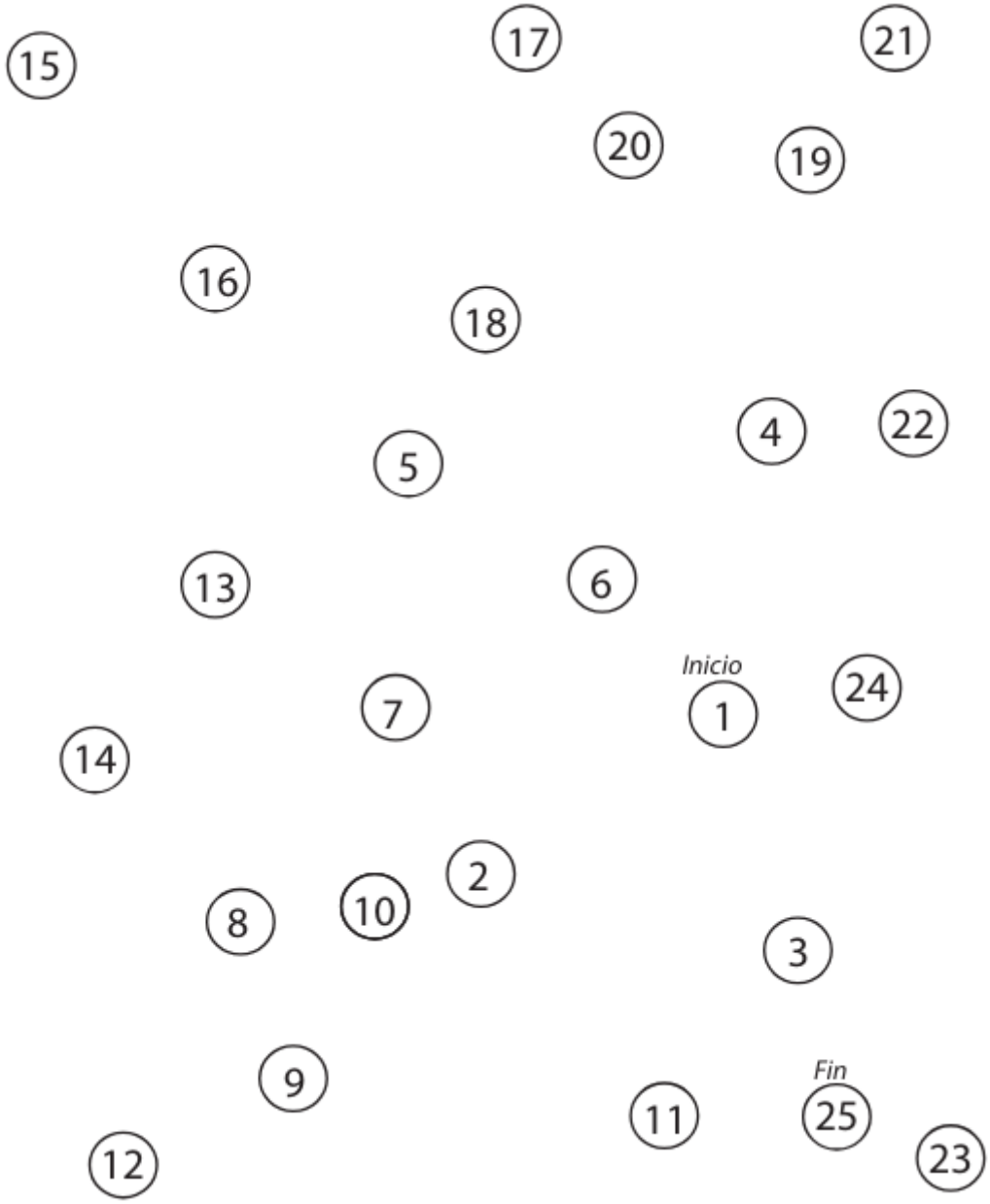
Appendix 8. Patient health questionnaire (PHQ9)

Durante las últimas 2 semanas, ¿cuan qué frecuencia le han molestado los siguientes problemas?		Nunca	Varios días	Más de la mitad de los días	Casi todos los días
1	Tener poco interés o placer en hacer las cosas	0	1	2	3
2	Sentirse desanimado/a, deprimido/a, o sin esperanza	0	1	2	3
3	Con problemas en dormirse o en mantenerse dormido/a, o en dormir demasiado	0	1	2	3
4	Sentirse cansado/a o tener poca energía	0	1	2	3
5	Tener poco apetito o comer en exceso	0	1	2	3
6	Sentir falta de amor propio – o que sea un fracaso o que decepcionara a si mismo/a su familia	0	1	2	3
7	Tener dificultad para concentrarse en cosas tales como leer el periódico o mirar la televisión	0	1	2	3
8	Se mueve o habla tan lentamente que otra gente se podria dar cuenta – o de lo contrario, esta tan agitado/a o inquieto/a que se mueve mucho más de lo acostumbrado	0	1	2	3
9	Se le han ocurrido pensamientos de que sería mejor estar muerto/a o de que haría daño de alguna manera	0	1	2	3
	add columns:		+	+	
	TOTAL:				

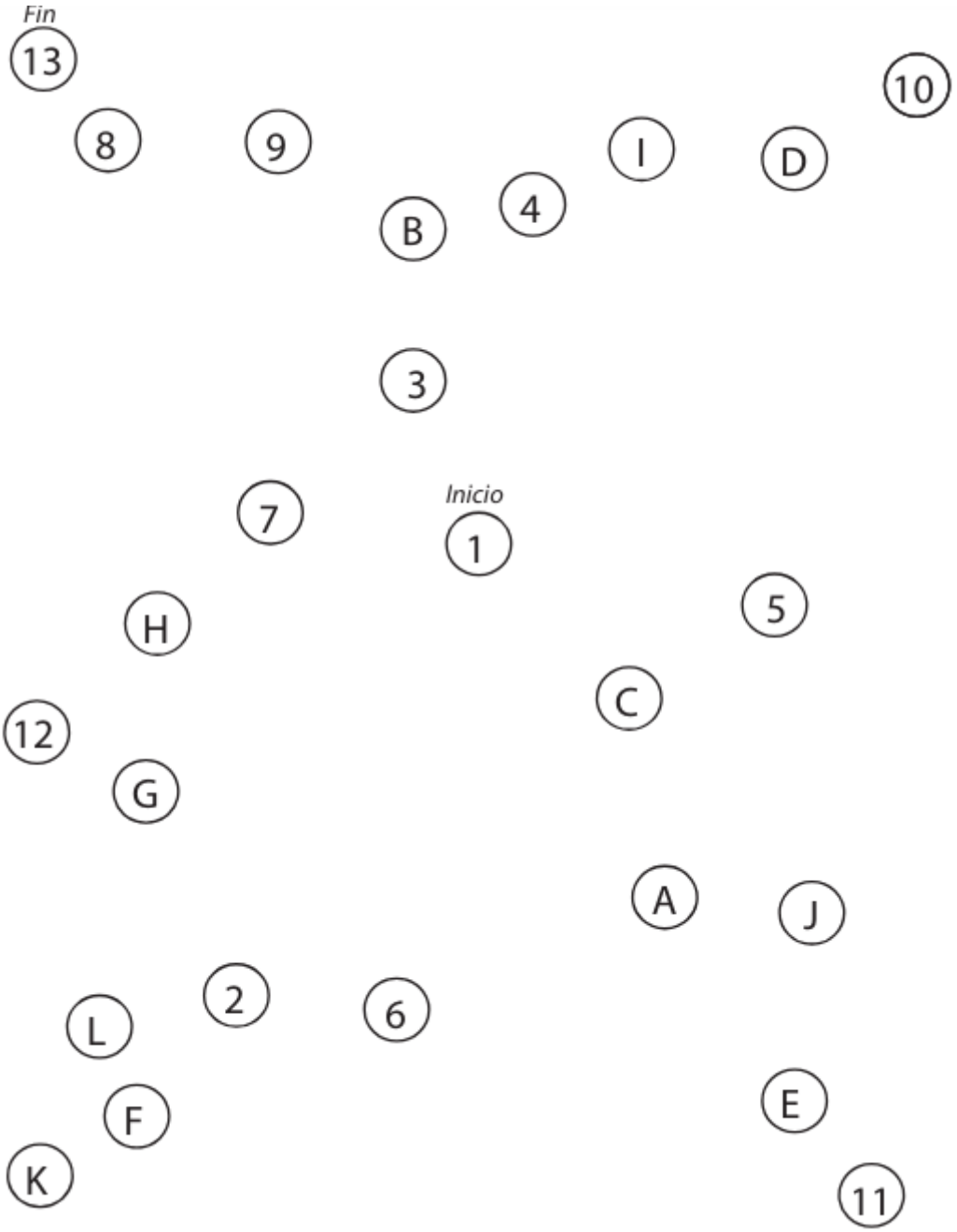
10	Si usted se identificó con cualquier problema en este cuestionario, ¿cuan difícil se le ha hecho cumplir con su trabajo, atender su casa, o relacionarse con otras personas debido a estos problemas?	Nada en absoluto Algo difícil Muy difícil Extremadamente difícil	_____ _____ _____ _____

Appendix 9. Trail making test

Parte A



Parte B



Appendix 10. Stroop Color and Word Test (SCWT)

ROJO	**AZUL**	**VERDE**	**ROJO**	**AZUL**
VERDE	**VERDE**	**ROJO**	**AZUL**	**VERDE**
AZUL	**ROJO**	**AZUL**	**VERDE**	**ROJO**
VERDE	**AZUL**	**ROJO**	**ROJO**	**AZUL**
ROJO	**ROJO**	**VERDE**	**AZUL**	**VERDE**
AZUL	**VERDE**	**AZUL**	**VERDE**	**ROJO**
ROJO	**AZUL**	**VERDE**	**AZUL**	**VERDE**
AZUL	**VERDE**	**ROJO**	**VERDE**	**ROJO**
VERDE	**ROJO**	**AZUL**	**ROJO**	**AZUL**
AZUL	**VERDE**	**VERDE**	**AZUL**	**VERDE**
VERDE	**ROJO**	**AZUL**	**ROJO**	**ROJO**
ROJO	**AZUL**	**ROJO**	**VERDE**	**AZUL**
VERDE	**ROJO**	**AZUL**	**ROJO**	**VERDE**
AZUL	**AZUL**	**ROJO**	**VERDE**	**ROJO**
ROJO	**VERDE**	**VERDE**	**AZUL**	**AZUL**
AZUL	**AZUL**	**ROJO**	**VERDE**	**ROJO**
ROJO	**VERDE**	**AZUL**	**ROJO**	**VERDE**
VERDE	**ROJO**	**VERDE**	**AZUL**	**AZUL**
ROJO	**AZUL**	**ROJO**	**VERDE**	**ROJO**
VERDE	**ROJO**	**VERDE**	**AZUL**	**VERDE**

XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX
XXXX	XXXX	XXXX	XXXX	XXXX

ROJO	AZUL	VERDE	ROJO	AZUL
VERDE	VERDE	ROJO	AZUL	VERDE
AZUL	ROJO	AZUL	VERDE	ROJO
VERDE	AZUL	ROJO	ROJO	AZUL
ROJO	ROJO	VERDE	AZUL	VERDE
AZUL	VERDE	AZUL	VERDE	ROJO
ROJO	AZUL	VERDE	AZUL	VERDE
AZUL	VERDE	ROJO	VERDE	ROJO
VERDE	ROJO	AZUL	ROJO	AZUL
AZUL	VERDE	VERDE	AZUL	VERDE
VERDE	ROJO	AZUL	ROJO	ROJO
ROJO	AZUL	ROJO	VERDE	AZUL
VERDE	ROJO	AZUL	ROJO	VERDE
AZUL	AZUL	ROJO	VERDE	ROJO
ROJO	VERDE	VERDE	AZUL	AZUL
AZUL	AZUL	ROJO	VERDE	ROJO
ROJO	VERDE	AZUL	ROJO	VERDE
VERDE	ROJO	VERDE	AZUL	AZUL
ROJO	AZUL	ROJO	VERDE	ROJO
VERDE	ROJO	VERDE	AZUL	VERDE

Appendix 11. Physiological features of the MDD patients and healthy controls

		Baseline	Stress	Recovery
Electrocardiography				
mHR	HC	75,73 (9,88)**	80,56 (12,08)	72,07 (10,53)**
	MDD	83,63 (14,66)	84,08 (14,54)	80,99 (12,84)
L_RMSSD	HC	2,21 (0,51)	2,27 (0,49)*	2,28 (0,61)
	MDD	2,02 (0,44)	2,05 (0,43)	2,15 (0,44)
L_SDNN	HC	3,56 (0,46)**	3,79 (0,41)**	3,83 (0,43)**
	MDD	3,22 (0,51)	3,35 (0,48)	3,48 (0,54)
L_PHF	HC	-1,85 (0,35)*	-1,74 (0,42)*	-1,72 (0,45)*
	MDD	-1,98 (0,26)	-1,93 (0,32)	-1,90 (0,32)
L_PLF	HC	-1,39 (0,45)*	-1,17 (0,57)**	-1,17 (0,55)*
	MDD	-1,63 (0,44)	-1,53 (0,46)	-1,41 (0,56)
L_LF_HF	HC	5,73 (0,65)	5,88 (0,70)	5,87 (0,80)
	MDD	5,59 (0,82)	5,72 (0,80)	5,70 (0,90)
Photoplethysmography				
mHR	HC	75,69 (9,89)**	79,97 (11,29)	72,36 (10,69)**
	MDD	83,40 (14,41)	83,86 (14,16)	80,35 (12,65)
L_RMSSD	HC	2,28 (0,42)*	2,39 (0,31)*	2,34 (0,47)
	MDD	2,05 (0,43)	2,23 (0,37)	2,20 (0,41)
L_SDNN	HC	3,56 (0,44)**	3,70 (0,38)**	3,78 (0,46)**
	MDD	3,23 (0,54)	3,40 (0,45)	3,48 (0,55)
L_PHF	HC	-1,83 (0,32)	-1,75 (0,35)	-1,75 (0,39)
	MDD	-1,91 (0,27)	-1,86 (0,31)	-1,85 (0,32)
L_PLF	HC	-1,38 (0,48)*	-1,28 (0,50)*	-1,19 (0,59)*
	MDD	-1,61 (0,42)	-1,51 (0,50)	-1,44 (0,58)
L_LF_HF	HC	5,56 (0,64)	5,59 (0,66)	5,72 (0,75)
	MDD	5,45 (0,59)	5,45 (0,55)	5,50 (0,87)
Pulse Arrival Time				
mPAT	HC	240,85 (21,28)	225,97 (36,26)**	246,86 (21,94)
	MDD	245,58 (24,13)	249,46 (24,08)	253,07 (21,70)
L_stdPAT	HC	1,87 (0,38)	2,16 (0,36)	1,88 (0,32)
	MDD	1,83 (0,28)	2,08 (0,41)	1,86 (0,28)
Electrodermal Activity				
L_EDASymp	HC	-0,08 (1,08)	0,43 (0,80)**	-0,14 (1,18)
	MDD	-0,51 (1,58)	-0,25 (0,95)	0,12 (1,26)
mTonic	HC	-0,75 (0,55)	0,29 (0,81)	-0,07 (0,91)**
	MDD	-0,56 (0,41)	0,45 (0,85)	0,62 (0,92)
L_stdTonic	HC	-1,17 (0,41)**	-1,08 (0,35)	-1,18 (0,36)
	MDD	-1,53 (0,50)	-1,17 (0,33)	-1,11 (0,30)
mPhasic	HC	0,14 (0,11)	0,28 (0,15)**	0,11 (0,14)
	MDD	0,10 (0,11)	0,16 (0,14)	0,16 (0,15)

L_stdPhasic	HC	-1,37 (0,36)	-1,23 (0,32)*	-1,36 (0,39)
	MDD	-1,58 (0,54)	-1,47 (0,42)	-1,30 (0,42)
aucPhasic	HC	11,05 (5,79)*	18,82 (8,45)**	11,96 (5,60)
	MDD	8,02 (4,89)	12,35 (5,39)	11,93 (4,39)
Respiration				
RR	HC	0,29 (0,07)	0,23 (0,07)	0,22 (0,08)*
	MDD	0,27 (0,06)	0,24 (0,06)	0,26 (0,07)
Pk	HC	0,72 (0,04)	0,69 (0,02)	0,72 (0,05)
	MDD	0,72 (0,03)	0,70 (0,02)	0,72 (0,04)
Skin Temperature				
TFace_mGrad	HC	0,00 (0,01)	0,00 (0,02)	0,00 (0,01)
	MDD	0,00 (0,01)	0,00 (0,01)	0,00 (0,00)
TFinger_mGrad	HC	0,01 (0,03)	-0,03 (0,04)	0,01 (0,04)
	MDD	0,00 (0,03)	-0,02 (0,05)	0,00 (0,04)

**p<0.05. **p<0.005. Data are mean and standard deviation. L: log transformation. For a description of the abbreviated features, please refer to Table 1. Summary table of physiological featuresTable 1.*

Summary of the literature review of the physiological variables assessed in the mHealth Methods in Mental Health Research (M&M) Project

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The physiological variables included in the mHealth Methods in Mental Health Research Project are shown below:

a) **Heart rate variability (HRV),** which reflects the fluctuation in time intervals between adjacent heartbeats, is one of the most commonly used for this purpose [349]. It can be assessed by electrocardiogram (ECG) or photoplethysmography (PPG). A reduced heart rate variability has been associated with poor ANS functioning, negative cardiovascular outcomes and cognitive impairment. On the contrary, greater HRV has been linked with better performance, health and adaptability [329].
b) **Pulse arrival time (PAT)** has been extensively studied as a surrogate of blood pressure (BP), as it could estimate BP noninvasively [350]. Many studies induced mental stress to investigate changes in this parameter, such as in [331] which observed a reduction in PAT during the Stroop word color test, and [308] which reported the same behavior during the Trier Social Stress Test (TSST).
c) **Respiratory parameters (Resp)** are also broadly studied in experimental research and used in interventions, such as biofeedback, addressed to reduce psychological symptoms present in anxiety and other mental disorders [332, 333]. The respiratory rate (RR) is expected to increase during stress exposure; however, it is easily affected by speech [351]. The spectral peak (Pk) is studied as an indicator of respiration stability, therefore, the higher the peak, the more stable the respiration [352].
d) **Electrodermal activity (EDA)** is another common biosignal selected to the assess stress level in mental health disorders, more precisely it reflects changes in the electrical properties of the skin induced by the sweat glands, which are under the direct control of the sympathetic branch of the ANS [309]. This signal can be decomposed into a phasic, i.e., the fast-changing component in response to external stimuli, and a tonic component, i.e., the slow-varying baseline EDA which is an indicator of the individual's psychophysiological state. Importantly, a parameter called EDASymp, which could be extracted from the EDA signal employing frequency spectral analysis, corresponds to sympathetic dynamics in non-exercise conditions and has also been reported as a reliable index to sympathetic activation in stress elicitation studies [353]. For instance, previous studies reported a significant increase in tonic component and EDASymp when subjects are submitted to the Stroop test or other mental stress tasks [330, 354].

e) **Skin temperature (ST)** is also associated with changes in ANS, as the body temperature is controlled in response to stress. Acute stress provokes peripheral vasoconstriction, directing the blood into the core to protect vital organs, increasing core body temperature, and also decreasing distal temperature, thus the direction of temperature changes in a stress-inducing task depends on the site of measurement. For instance, on distal regions, like fingertips, previous studies revealed a significant decrease in temperature and, conversely, when sensors were placed on proximal sites, such as the neck, the temperature showed an increase [355, 356]. In our study, we will investigate whether the changes in peripheral temperature occur in response to the Stroop test, taking into account possible differences in basal temperature between healthy and those diagnosed with mental health disorders.

Appendix 13. Warwick-Edinburgh Mental Well-being Scale (WEMWBS)

A continuación, aparecen algunas afirmaciones sobre sentimientos y pensamientos. Por favor señale la casilla que mejor describa cómo se ha sentido durante las últimas 2 semanas.

Afirmaciones	Nunca	Muy pocas veces	Algunas veces	A menudo	Siempre
Me he sentido optimista respecto al futuro	1	2	3	4	5
Me he sentido útil	1	2	3	4	5
Me he sentido relajado/a	1	2	3	4	5
He afrontado bien los problemas	1	2	3	4	5
He podido pensar con claridad	1	2	3	4	5
Me he sentido cercano/a a los demás	1	2	3	4	5
He sido capaz de tomar mis propias decisiones	1	2	3	4	5

Appendix 14. Generalized anxiety disorder – 7 items questionnaire (GAD7)

En los últimos 14 días, ¿con qué frecuencia le han supuesto una molestia los siguientes problemas? (Para indicar su respuesta rodee el número con un círculo)

	No, en absoluto	Algunos días	Más de la mitad de los días	Casi todos los días
1. Sentirse nervioso/a, angustiado/a o muy tenso/a	0	1	2	3
2. Ser incapaz de dejar de preocuparse o de controlar la preocupación	0	1	2	3
3. Preocuparse demasiado por diferentes cuestiones	0	1	2	3
4. Tener problemas para relajarse	0	1	2	3
5. Estar tan inquieto/a que le resulta difícil permanecer sentado/a	0	1	2	3
6. Enfadarse o irritarse con facilidad	0	1	2	3
7. Sentir miedo de que algo terrible pueda ocurrir	0	1	2	3