RV College of Engineering®

Mysore Road, RV Vidyaniketan Post,Bengaluru - 560059, Karnataka, India

“Development of a prototype to determine stress by HRV analysis”

MAJOR PROJECT REPORT

(18EIP81)

submitted by

Laxmi Hanamant Korbu	**1RV20EI026**
M Manikanda Prabhu	**1RV20EI029**
Rishith Paramasivam	**1RV20EI046**

under the guidance of

Dr. B. G. Sudarshan
Associate Professor
Dept. of EIE
RV College of Engineering

in partial fulfilment for the award of degree of

Bachelor of Engineering

in

Electronics and Instrumentation Engineering

2023-2024

DEPARTMENT OF ELECTRONICS AND INSTRUMENTATION ENGINEERING

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CERTIFICATE

Certified that the Major project titled **"Development of a prototype to determine stress by HRV analysis"** is carried out by **Laxmi Hanamant Korbu (1RV20EI026), M Manikanda Prabhu (1RV20EI026)** and **Rishith Paramasivam (1RV20EI046)** who are bonafide students of RV College of Engineering, Bengaluru, in partial fulfillment for the award of Degree of **Bachelor of Engineering** in **Electronics and Instrumentation Engineering** of the Visvesvaraya Technological University, Belagavi during the year **2023-2024**. It is certified that all corrections/suggestions indicated for the internal assessment have been incorporated in the report deposited in the department library. The project report has been approved as it satisfies the academic requirements in respect of project work prescribed by the institution for the said Degree.

Guide	Head of Department	Principal
Dr. B. G. Sudarshan	**Dr. CH. Renu Madhavi**	**Dr. K. N. Subramanya**

External Viva Examination

Name of Examiner	Signature with Date
1.	
2.	

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DECLARATION

We **Laxmi Hanamant Korbu, M Manikanda Prabhu, Rishith Paramasivam,** the students of the 8th semester B.E., Department of Electronics and Instrumentation Engineering, RV College of Engineering, Bengaluru-560059, bearing USN: **1RV20EI026, 1RV20EI029, 1RV20EI046** hereby declare that the project titled **"Development of a prototype to determine stress by HRV analysis"** has been carried out by us and submitted in partial fulfilment of the program requirements for the award of Degree in Bachelor of Engineering in **Electronics and Instrumentation Engineering** of the **Visvesvaraya Technological University, Belagavi** during the year **2023-2024**.

Further, we declare that the content of the dissertation has not been submitted previously by anybody for the award of any Degree or Diploma to any other University.

We also declare that any Intellectual property rights generated out of this project carried out at RVCE will be the property of RV College of Engineering, Bengaluru and we will be among the authors of the same.

Place: Bangalore

Date:

	Signature
Laxmi Hanamant Korbu	____________
M Manikanda Prabhu	____________
Rishith Paramasivam	____________

ACKNOWLEDGEMENT

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ABSTRACT

Heart Rate Variability (HRV) is a critical measure of autonomic nervous system function and an indicator of stress levels. The variability between heartbeats can reflect the body's response to stress, making HRV analysis a valuable tool in both clinical and everyday settings for monitoring and managing stress.This project focuses on performing HRV analysis in the time domain to determine stress levels. Initially, ECG data from 25 subjects were collected using the BIOPAC MP45 device. HRV analysis was performed using Kubios HRV Scientific software, comparing single-lead and three-lead ECG configurations. The findings revealed that both configurations were accurate for HRV analysis, with a single lead proving sufficient for effective results.

To further understand the current landscape of HRV analysis, an extensive review of existing methodologies and the integration of Artificial Intelligence (AI) and Machine Learning (ML) techniques was conducted. This review highlighted the increasing role of AI and ML in enhancing the accuracy and efficiency of HRV analysis.

Building on these insights, a prototype device was developed using an ESP32 microcontroller unit (MCU) and an AD8232 ECG sensor, featuring a TFT display for real-time monitoring. The prototype's HRV analysis results were compared with those obtained from Kubios software, showing satisfactory agreement and validating the prototype's effectiveness. This study underscores the feasibility of developing a cost-effective, single-lead ECG device for reliable HRV-based stress assessment, and it emphasizes the potential benefits of incorporating AI and ML to advance HRV analysis techniques.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

HRV	Heart Rate Variability
MHRR	Mean RR Interval
SDNN	Standard Deviation of Normal RR Intervals
RMSSD	Root Mean Square of Successive Differences
pNN50	Percentage of NN Intervals differing by more than 50 ms

Chapter 1

Introduction

CHAPTER 1
INTRODUCTION

1.1 INTRODUCTION

Heart Rate Variability (HRV) is a critical measure of the variation in time intervals between consecutive heartbeats, reflecting the dynamic interplay between the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). It serves as a robust indicator of autonomic function and overall cardiovascular health. HRV is typically assessed using electrocardiogram (ECG) (Fig 1.1.) or photoplethysmogram (PPG) devices, with analysis methods ranging from time-domain and frequency-domain approaches to nonlinear techniques. Clinically, HRV is invaluable in evaluating stress levels, mental health, cardiovascular risk, athletic performance, and sleep quality. Lower HRV is often linked to stress, anxiety, depression, and an increased risk of cardiovascular events, while higher HRV indicates better stress resilience and cardiovascular health. By incorporating HRV into personalized medicine, early detection of health issues and tailored intervention strategies become feasible. Lifestyle modifications such as regular exercise, balanced nutrition, adequate sleep, and stress management can enhance HRV. Additionally, HRV biofeedback training helps improve autonomic regulation, promoting overall well-being. Thus, HRV is a multifaceted health metric essential for both clinical and wellness applications, providing deep insights into an individual's physiological and psychological state.

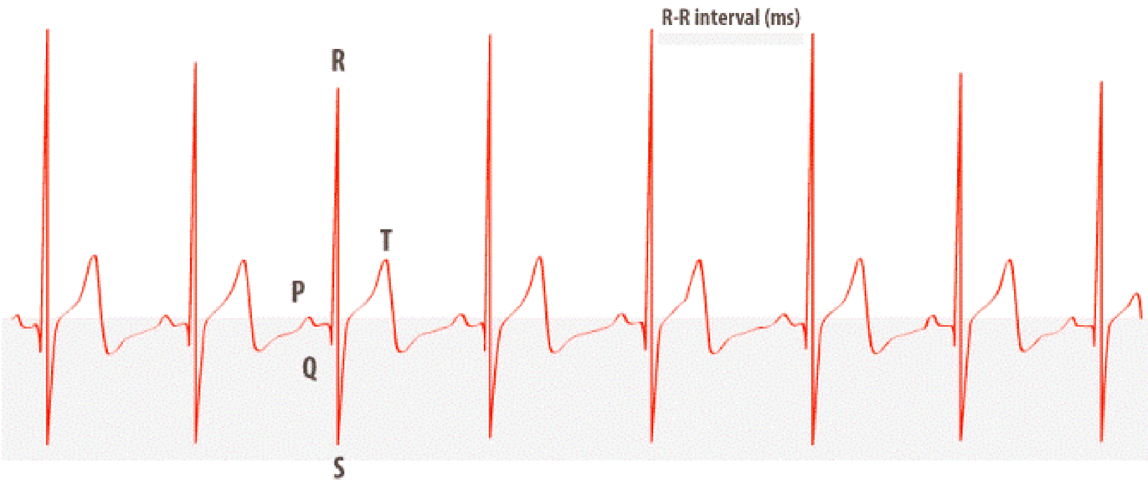


Fig 1.1. ECG Waveform

Time-domain analysis stands as a foundational pillar in the comprehensive evaluation of Heart Rate Variability (HRV), offering profound insights into the intricate workings of the autonomic nervous system (ANS) and the state of cardiovascular health. By meticulously scrutinizing the statistical attributes of the intervals between successive heartbeats, a suite of essential metrics emerges, each bearing significance in understanding the body's regulatory mechanisms. Among these key metrics are the mean NN interval, which encapsulates the average duration between heartbeats, and the standard deviation of NN intervals (SDNN), a measure reflecting the overall variability in heartbeat intervals. Additionally, the root mean square of successive differences (RMSSD) provides insight into the influence of parasympathetic activity, whereas NN50 and pNN50 metrics shed light on high-frequency variations and parasympathetic dominance.

The interpretation of these metrics is pivotal in discerning the health of the cardiovascular system and the balance between the sympathetic and parasympathetic branches of the ANS. Elevated values of SDNN and RMSSD typically signify robust HRV, correlating with enhanced cardiovascular fitness and a heightened ability to cope with stressors. Conversely, lower values of these metrics may signify potential autonomic dysfunction and an increased susceptibility to health risks. This nuanced understanding of time-domain analysis extends its utility across a spectrum of applications, ranging from clinical diagnoses to sports performance monitoring, stress management, and interventions aimed at fostering overall wellness.

In clinical settings, time-domain analysis serves as a vital tool for diagnosing conditions such as heart disease and diabetic neuropathy, while also facilitating the monitoring of treatment efficacy and disease progression. In sports and fitness realms, it enables coaches and athletes to gauge training loads, track recovery, and optimize performance while mitigating the risk of overtraining. Moreover, in stress management initiatives and wellness programs, time-domain analysis empowers individuals to take proactive measures to enhance their HRV, thereby bolstering their resilience to stress and promoting holistic well-being.

Through a comprehensive grasp of time-domain measures and their implications, healthcare professionals and researchers can gain deeper insights into an individual's physiological state. This enables them to tailor interventions with precision, addressing underlying imbalances and promoting optimal health outcomes. Ultimately, the adept application of time-domain analysis in HRV assessment paves the way for personalized healthcare strategies that prioritize holistic well-being and resilience in the face of physiological and environmental challenges.

HRV (Heart Rate Variability) is considered a promising biomarker that establishes the relationship between the autonomic nervous system and cardiovascular activity [1]. However, the significance of each parameter is yet to be established, making it a widely researched field. HRV can be analyzed using various techniques. At first, it was believed that the beat-to-beat interval of the heart was periodic. However, as we learn more and more about non-oscillatory behavior, the use of linear and nonlinear methods can be used to obtain a better analysis [2]. Since HRV provides a window to the functioning of the ANS (Autonomic Nervous System), with the help of linear and nonlinear analysis, we are provided with a wide range of applications for diagnosis. For instance, Louise Buonalumi Tacito Yugar Et al. were able to use HRV as a tool to determine different hypertensive syndromes [3]. Anna Strüven Et al. have established a relationship between HRV and the lifestyle of the person, with a focus on obesity [4]. Antonino Tuttolomondo Et al. aimed to evaluate the alteration of the sympathovagal balance of different patients suffering from diabetes mellitus [5]. Along with the use cases, several limitations can also be identified. Rajendra Acharya U Et al. found that the HRV data varied more for younger people and was stable for older people [6]. This makes it difficult to establish a standard reference for diagnosis. Jonah D'Angelo Et al. identified that several studies on the same disease produced different parameters, highlighting variations in the HRV of a person [7]. L. Murukesan Et al. determined that short-term measurement of HRV to detect any risks is still a grey area of research [8]. Oliver Faust Et. al felt that it was very difficult to determine the effect of various physiological processes on the HRV. Despite the various limitations, HRV has proved to be vital. This project aims to develop a working prototype wherein time domain parameters will be displayed for the analysis of the functioning of the nervous system using time domain parameters.

1.1.1. Biopac Student Lab

Fig 1.2. BioPac MP45

BioPac Student Lab software (Fig 1.2. and Fig 1.3.) is a cutting-edge platform designed for Heart Rate Variability (HRV) signal analysis, offering advanced tools for researchers, clinicians, and healthcare professionals. HRV, which measures the variation in time intervals between heartbeats, is a crucial indicator of heart health and autonomic nervous system function. BioPac supports comprehensive data collection from various biosensors and devices, ensuring compatibility and ease of import. It employs state-of-the-art algorithms for signal processing, including filtering, artifact removal, and noise reduction, to ensure accurate and reliable HRV measurements. The software calculates a wide range of HRV metrics, encompassing time-domain, frequency-domain, and non-linear parameters, providing a detailed assessment of autonomic function and cardiovascular health. BioPac's robust visualization tools generate detailed charts, graphs, and plots, facilitating easier data interpretation and analysis. Users can generate customizable reports summarizing HRV analysis results, tailored to specific needs. Integration with other health information systems and electronic health records (EHR) allows for seamless data transfer and broader analysis. BioPac's user-friendly interface simplifies data analysis, making it accessible to users with varying technical expertise. For applications requiring immediate insights, BioPac offers real-time HRV analysis, providing instant feedback for quick decision-making. The software is widely used in clinical research to study interventions' impacts on HRV and explore HRV-health condition relationships. It is also used in cardiovascular health monitoring, stress management, sports and fitness, and wellness programs to assess physiological responses, optimize performance, and promote preventive measures. BioPac's comprehensive solutions for collecting, processing, analyzing, and visualizing HRV data make it an invaluable resource, contributing to better health outcomes, improved stress management, and optimized athletic performance. As technology advances, BioPac remains at the forefront of HRV analysis, driving innovation and supporting better health and well-being.

Fig 1.3. BioPac Student Lab

Biopac software operates as a versatile platform for physiological data acquisition and analysis, specifically designed for use in biomedical research and clinical settings. Technically, the software integrates with various data acquisition systems and hardware interfaces, including amplifiers and transducers, to capture bio-signals like ECG, EEG, EMG, and respiratory signals. The software employs advanced signal processing techniques, such as digital filtering (low-pass, high-pass, band-pass, and notch filters), artifact removal, and signal averaging, to enhance the quality and accuracy of the recorded data.

Key features include multi-channel data acquisition, which allows simultaneous recording from multiple sensors, ensuring synchronized data collection across different physiological parameters. The software also supports high sampling rates, crucial for capturing high-fidelity signals. Real-time data visualization capabilities enable immediate monitoring and adjustment of experimental conditions.

In terms of data analysis, Biopac offers a range of tools for statistical analysis, including time-domain and frequency-domain analyses, power spectral density calculations, and coherence analysis. Graphical tools facilitate the creation of detailed plots and charts, aiding in the interpretation of complex data sets. Additionally, the software can automate routine analysis tasks through customizable scripts and macros, increasing the efficiency and reproducibility of results.

1.2 LITERATURE SURVEY

HRV Analysis in Stress Assessment: During ambulatory monitoring or when a person experiences a stressor strong enough to activate the sympathetic system, there can be significant differences due to changes in pulse transit time (the time it takes the BP wave to propagate from the heart to the periphery), which result from changes in the elasticity of the arteries. This paper explores the intricate anatomy of the heart and the critical role of heart rate variability (HRV) in health assessment. The authors argue that a healthy heart exhibits variability in its rhythm, which is an indicator of physiological resilience and adaptability, rather than functioning like a precise metronome. The review details how the heart's electrical conduction system and the autonomic nervous system manage heartbeats and highlights HRV as a key measure of the balance between sympathetic and parasympathetic nervous activity. High HRV is associated with robust cardiovascular health and stress management, whereas low HRV correlates with increased risks for various health conditions. The paper consolidates research findings that support HRV as a valuable, non-invasive biomarker for early disease detection and treatment monitoring. It also discusses practical applications of HRV measurement in clinical and wellness contexts, advocating for its broader use to enhance health monitoring and intervention strategies [1].

Integration of Psychological and Physiological Factors: HRV analysis provides valuable insights into autonomic nervous system activity, stress is a complex phenomenon influenced by both physiological and psychological factors. Incorporating psychological measures, such as self-reported stress levels, mood assessments, or cognitive appraisals, alongside HRV data may enhance the accuracy and validity of stress prediction models.

The authors detail various HRV indices, including time-domain, frequency-domain, and non-linear measures, explaining their physiological significance and relevance to psychological states and processes. The review emphasizes the importance of HRV as a non-invasive marker for autonomic nervous system activity, highlighting its application in assessing stress, emotional regulation, and cognitive functions. The paper also includes a step-by-step tutorial on HRV data collection, preprocessing, and analysis, aimed at equipping researchers with the necessary tools and methodologies to accurately interpret HRV metrics in psychological studies. This tutorial is designed to enhance the rigor and reproducibility of HRV research in psychology, providing guidelines for best practices and common pitfalls to avoid [2].

The HRV analysis literature review indicates that HRV variables respond predictably to stress in medical personnel during emergencies. Specifically, the RMSSD, SDNN, and LF/HF variables have been identified as valid and reliable metrics of stress. Additionally, HRV has been found to be more sensitive and specific than heart rate alone in evaluating stress responses. Studies have shown that HRV metrics change in response to various stressors, with differences observed based on the level of physical activity during stressful events. Furthermore, HRV has been demonstrated to be less influenced by physical exertion compared to heart rate, making it a valuable tool for stress assessment. The review highlights that reduced HRV is consistently associated with higher stress levels, reflecting the autonomic nervous system's diminished capacity to adapt to stressors. Data from the reviewed studies indicate that medical professionals, particularly those in high-pressure environments such as emergency departments and intensive care units, often exhibit lower HRV, correlating with increased job-related stress and burnout. The paper underscores the potential of HRV monitoring as a non-invasive tool for early detection of stress, enabling timely interventions to improve mental health and job performance among healthcare workers. The authors advocate for more widespread use of HRV in occupational health assessments and suggest further research to refine HRV-based stress measurement protocols in medical settings [3].

Incorporating EEG and detailed HRV analysis has been proposed to better understand and analyze stress. The correlation between EEG and HRV under stress conditions provides valuable insights, with EEG features in combination with HRV supporting diagnosis, treatment monitoring, and enhancing performance, learning, and decision-making. This approach offers potential applications in stress management, including meditation studies, biofeedback training, and various disorders like ADHD, depression, and anxiety.

The research focuses on developing a comprehensive methodology to assess stress levels by simultaneously monitoring heart and brain activity. The study employs various signal processing techniques to extract relevant features from ECG and EEG data, such as heart rate variability (HRV) and brain wave patterns. The findings demonstrate that combined analysis of ECG and EEG provides a more accurate and holistic understanding of an individual's stress state compared to using either method alone. The research contributes to the development of advanced, non-invasive diagnostic tools for stress management, highlighting the potential for integrated bio-signal monitoring in clinical and occupational health settings [4].

This paper presents a comprehensive meta-analysis and literature review on the relationship between stress and heart rate variability (HRV). The authors systematically evaluate numerous studies to quantify the impact of stress on HRV. The meta-analysis reveals a consistent association between increased stress levels and reduced HRV, indicating that stress negatively affects autonomic nervous system function. The review elaborates on the mechanisms underlying this relationship, explaining how chronic stress leads to autonomic imbalance, characterized by heightened sympathetic activity and diminished parasympathetic activity. This autonomic dysregulation is reflected in HRV metrics, with stressed individuals showing lower HRV compared to less stressed counterparts. The paper underscores HRV's utility as a reliable biomarker for stress assessment, supporting its application in both clinical practice and research. By synthesizing findings from various studies, the authors highlight the robustness of HRV as an indicator of psychological and physiological stress, advocating for its broader use in stress monitoring and intervention programs [5].

1.3 RESEARCH GAP

Incorporating the focus on utilizing ECG signals for HRV analysis in the time domain, a research gap can be identified in the development of accurate and reliable HRV analysis devices tailored specifically for ECG signals. While previous experiments have predominantly utilized PPG signals for HRV assessment, the potential advantages offered by ECG signals in terms of signal quality and physiological relevance warrant further exploration.

One significant research gap lies in the development of robust methodologies and algorithms for extracting meaningful time-domain HRV metrics from ECG signals. Despite the availability of frequency-domain HRV analysis methods in the market, there is a notable scarcity of dedicated devices and techniques optimized for time-domain analysis using ECG signals. This gap underscores the need for innovative approaches to adapt existing ECG-based HRV analysis techniques or develop novel methodologies tailored to the unique characteristics of ECG signals.

Moreover, there is a lack of standardized protocols and validation studies for ECG-based HRV analysis devices, particularly in comparison to their PPG-based counterparts. Establishing consensus guidelines for data collection, signal processing, and interpretation of time-domain HRV metrics derived from ECG signals is essential to ensure the accuracy, reliability, and reproducibility of results across different studies and applications.

Furthermore, there is a need for comprehensive validation studies to assess the performance and clinical utility of ECG-based HRV analysis devices in diverse populations and clinical settings. Research efforts should focus on evaluating the accuracy, sensitivity, specificity, and predictive value of ECG-derived time-domain HRV metrics for detecting autonomic nervous system dysregulation, stress, and cardiovascular diseases, as well as their potential utility in guiding personalized interventions and monitoring treatment outcomes.

1.4 MOTIVATION

The motivation behind focusing on the development of HRV analysis devices specifically tailored for ECG signals lies in several factors:

1. **Improved Accuracy and Reliability**: ECG signals offer higher fidelity and temporal resolution compared to PPG signals, enabling more accurate detection of subtle changes in heart rate dynamics. By utilizing ECG signals, HRV analysis devices can potentially provide more precise and reliable assessments of autonomic nervous system activity and cardiac function.

2. **Physiological Relevance**: ECG signals directly reflect the electrical activity of the heart, making them inherently more relevant for cardiac-related analyses. HRV metrics derived from ECG signals can offer valuable insights into autonomic modulation, rhythm irregularities, and cardiovascular health conditions, providing clinicians and researchers with a more comprehensive understanding of cardiac function.

3. **Clinical Utility:** ECG-based HRV analysis devices have the potential to enhance clinical decision-making and patient care in various healthcare settings. They can facilitate early detection of autonomic nervous system dysregulation, stress, and cardiovascular diseases, enabling timely interventions and personalized treatment strategies. Additionally, ECG-derived HRV metrics may serve as objective biomarkers for monitoring disease progression and treatment efficacy.

4. **Technological Advancements:** Advances in wearable technology, signal processing algorithms, and machine learning techniques have expanded the possibilities for ECG-based HRV analysis. The development of compact, low-cost, and user-friendly ECG monitoring devices opens up new opportunities for continuous, real-time HRV assessment in both clinical and everyday environments.

5. **Research Innovation:** Exploring ECG signals for HRV analysis represents a novel research direction that complements existing methodologies and expands the scope of cardiovascular research. By investigating the potential of ECG-derived time-domain

HRV metrics, researchers can contribute to the advancement of knowledge in fields such as cardiology, physiology, and digital health.

1.5 PROBLEM STATEMENT

To Design, Development of a Module to determine and predict stress with the help of HRV Analysis in the time domain.

1.6 OBJECTIVES

The objectives of the projects are:

1. Develop a module that accurately detects stress levels by analyzing time-domain HRV metrics derived from ECG signals.

2. Identify and extract relevant time-domain HRV features such as SDNN, RMSSD, and pNN50, which are indicative of autonomic nervous system activity and stress levels.

3. Employ advanced machine learning algorithms or statistical methods to create predictive models capable of classifying and predicting stress based on the extracted HRV features.

4. Establish standardized protocols for ECG data collection, preprocessing, and HRV analysis to ensure consistency and reproducibility.

5. Enable real-time monitoring and analysis of HRV data to provide immediate feedback and insights into stress levels, facilitating timely interventions and stress management.

Chapter 2

Theory and Fundamental

CHAPTER 2

THEORY AND FUNDAMETAL

2.1 WHAT IS HRV?

Heart Rate Variability (HRV) is the physiological phenomenon of variation in the time interval between consecutive heartbeats. It is measured by the variation in the beat-to-beat interval and is an indicator of the autonomic nervous system (ANS) function. HRV reflects the heart's ability to respond to various stimuli such as physical activity, stress, and sleep.

Heart Rate Variability (HRV) is the measure of the variation in time between consecutive heartbeats. It reflects the heart's ability to adapt to changing circumstances by varying the time intervals between beats. HRV is influenced by the autonomic nervous system, specifically the balance between the sympathetic nervous system (which accelerates heart rate) and the parasympathetic nervous system (which slows it down). High HRV indicates a healthy, responsive cardiovascular system, while low HRV can suggest stress, fatigue, or potential health issues. HRV is used in various fields such as cardiology, sports science, and mental health to assess overall health and well-being.

HRV (Heart Rate Variability) is considered as a promising biomarker that establishes the relationship between the autonomic nervous system and cardiovascular activity [1]. However, the significance of each parameter is yet to be established, making it a widely researched field. HRV can be analysed using various techniques. At first, it was believed that the beat-to-beat interval of the heart was periodic. However, as we learn more and more about non-oscillatory behaviour, the use of linear and nonlinear methods can be used to obtain a better analysis [2]. Since HRV provides a window to the functioning of the ANS (Autonomic Nervous System), with the help of linear and nonlinear analysis, we are provided with a wide range of applications for diagnosis. For instance, Louise Buonalumi Tacito Yugar Et al. were able to use HRV as a tool to determine different hypertensive syndromes [3]. Anna Strüven Et al. have established a relationship between HRV and the lifestyle of the person, with a focus on obesity [4]. Antonino Tuttolomondo Et al. aimed to evaluate the alteration of the sympathovagal balance of different patients suffering from diabetes mellitus [5]. Along with the use cases, several limitations can also be identified. Rajendra Acharya U Et al. found that the HRV data varied more for younger people and was stable for older people [6]. This makes it difficult to establish a standard reference for diagnosis. Jonah D'Angelo Et al. identified that several studies on the same disease produced different parameters, highlighting variations in the HRV of a person [7]. L. Murukesan Et al. determined that short-term measurement of HRV to detect any risks is still a grey area of research [8]. Oliver Faust Et. al felt that it was very difficult to determine the effect of various physiological processes on the HRV.

2. 2 HRV ANALYSIS METHODS

The analysis of HRV is done using the time domain, and frequency domain, and with the help of nonlinear methods. HRV can be classified into various types, based on the duration of recording (24 hours, short term, and ultra short term). The 24-hour HRV is the most reliable of the lot as the longer recordings expose the person to a wider range of variable factors (external stimuli) that affect the cardiovascular system. Short-term and ultra-short-term recordings have proven to be beneficial for certain diagnostic purposes, however, they fall inferior to 24-hour recordings [10]. Time domain analysis of HRV makes measurements based on beat-to-beat interval of the heart. The beat-to-beat interval can be defined as the time period between two consecutive heartbeats. Frequency domain measurements classify the different frequencies of the recording into various power levels. Power is the energy of the signal for a particular frequency band. The heart rate can be divided into ultra-low frequency (ULF), very-low-frequency (VLF), low-frequency (LF), and high-frequency (HF) bands [1]. Apart from the time and frequency methods of analysis, HRV can be analyzed using nonlinear methods. The heart cannot be considered as a metronome. There can be cases when the beats do not follow a particular rhythm, making it irregular. Making use of the traditional linear method of analysis may not be sufficient. Therefore, nonlinear analysis can be used, where intricate patterns, irregularities, and underlying dynamics are inherent in the heart rate signal. Some of the common concepts used for nonlinear analysis are Poincare Plots and Detrended Fluctuation Analysis (DFA). The commonly used methods along with the various parameters for analysis can be observed in Tables 1, 2, 3 and 4.

Parameter	Description	Units	Interpretation	Reference Values (Healthy Adults)
Mean RR Interval (MHRR)	Average time between consecutive normal heartbeats.	Milliseconds (ms)	Reflects overall heart rate. Lower intervals indicate Tachycardia whereas longed intervals indicate Bradycardia	600-1000 ms
Standard Deviation of Normal RR Intervals (SDNN)	Overall variability of heart rate intervals.	Milliseconds (ms)	Higher values indicate greaterheart rate variability and better autonomic nervous system function.	50-150 ms
Root Mean Square of the Successive Differences (RMSSD)	Average magnitudeof the differences between consecutive normal RR intervals.	Milliseconds (ms)	Reflects short-term heart rate variability, influenced by parasympathetic activity.	15-50 ms
Percentage of NN Intervals Differing by More Than 50 ms (pNN50)	Proportion of consecutive normal RR intervals differing by more than 50 milliseconds.	Percentage (%)	Measures heart's ability to quickly adapt to changes, influenced by parasympathetic activity.	3-10%

Table 2.1. HRV Time Domain Analysis

Parameter	Description	Units	Interpretation	Reference Values (Healthy Adults)
Total Power (TP)	Total variance of the RR interval data across all frequencies.	ms²	Reflects overall heart rate variability.	1000-4000 ms²
Very Low Frequency (VLF)	Power of heart rate variability in the range of 0-0.04 Hz.	ms²	May reflect thermoregulation, hormonal fluctuations, and slow blood pressure changes.	Limited reference values available

Low Frequency (LF)	Power of heart rate variability in the range of 0.04-0.15 Hz.	ms²	Influenced by both sympathetic (fight-or-flight) and parasympathetic activity, with some contribution from thermoregulation.	500-1500 ms²
High Frequency (HF)	Power of heart rate variability in the range of 0.15-0.4 Hz.	ms²	Primarily reflects parasympathetic activity (rest and digest) and respiratory sinus arrhythmia.	200-1000 ms²
Low-to-High Frequency Ratio (LF/HF)	Ratio of power in the LF band to the power in the HF band.	Unitless	Indicates the balance between sympathetic and parasympathetic activity. Lower values suggest parasympathetic dominance.	1-2 (can vary depending on age and fitness level)
Normalized Units (NU)	LF and HF power expressed as a percentage of total power (TP).	Percentage (%)	Allows for easier comparison between individuals with different overall HRV levels.	-

Table 2.2. HRV Frequency Domain Analysis

Feature	Description	Units	Interpretation
Visual Assessment	Dispersed cloud: High variability, adaptability - Tight cluster: Low variability, potentially compromised adaptability - Elliptical shape: Balance between short-term and long-term variability	N/A	Provides qualitative insights into heart rate dynamics.
SD1 (Standard Deviation 1)	Dispersion of points along the major axis of the fitted ellipse.	Milliseconds (ms)	Reflects short-term variability (influenced by parasympathetic activity).
SD2 (Standard Deviation 2)	Dispersion of points along the minor axis of the fitted ellipse.	Milliseconds (ms)	Reflects long-term variability (potentially influenced by both sympathetic and parasympathetic activity).

SD1/SD2 Ratio	Ratio of SD1 to SD2.	Unitless	Higher ratio suggests greater short-term variability compared to long-term variability (potentially parasympathetic dominance).
Area	Area of the fitted ellipse around the scattered points.	Arbitrary Units (AU)	Reflects overall variability of heart rate.

Table 2.3. HRV Analysis based on the Poincaré Plot

Feature	**Description**	**Interpretation**
Scaling Exponent (α)	The core outcome measure of DFA. It reflects the long-term scaling behavior of heart rate fluctuations.	Unitless
DFA Curve	A log-log plot of fluctuation magnitude (F(n)) versus segment size (n).	N/A

Table 2.4. HRV Detrended Fluctuation Analysis (DFA)

2.3 BIO PAC STUDENT LAB

The Biopac Student Lab (BSL) is an educational tool widely used in physiology and psychology courses to help students learn and apply concepts through hands-on experimentation. It typically involves the use of hardware and software systems designed to record and analyze physiological data from subjects in real-time.

2.3.2. Key Components of Biopac Student Lab:

Hardware:

1. **MP36/MP36R Data Acquisition Units**:
   a. **Description**: These units serve as the core of the Biopac Student Lab system. They collect data from various sensors and transducers attached to the subject.
   b. **Function**: The units digitize the analog signals from the sensors and transmit this data to a connected computer for real-time monitoring and analysis via the Biopac software.
2. **Transducers and Sensors**:
   a. **Description**: These devices are essential for measuring various physiological signals from the subject.
   b. **Types and Functions**:
      i. **ECG Electrodes**: Measure the electrical activity of the heart.
      ii. **Respiratory Effort Transducers**: Measure breathing patterns and volumes.
      iii. **EMG Electrodes**: Record electrical activity from muscles.
      iv. **Pulse Plethysmographs**: Measure blood flow and heart rate by detecting changes in blood volume in tissues.
3. **Electrode Lead Sets**:
   a. **Description**: These are sets of wires and connectors that link the sensors or electrodes to the data acquisition unit.
   b. **Function**: They ensure secure and accurate transmission of physiological signals from the subject to the MP36/MP36R unit.

4. **Stimulators**:
    a. **Description**: Devices used to apply controlled stimuli to the subject during experiments.
    b. **Types and Functions**:
        i. **Electrical Stimulators**: Deliver electrical pulses to elicit physiological responses such as muscle contractions.
        ii. **Auditory Stimulators**: Use sound to create auditory stimuli for tests involving hearing or response to sound.
        iii. **Visual Stimulators**: Provide visual cues or signals to test visual perception and response.

Software Components of the Biopac Student Lab

1. **BSL Software**:
    - **Description**: This is the primary software that interfaces with the MP36 unit to manage the collection and analysis of physiological data.
    - **Features**:
        - **Data Recording**: Captures real-time physiological data from various sensors connected to the MP36 unit.
        - **Display**: Provides visual representations of the recorded data, such as graphs and charts, for immediate analysis.
        - **Analysis Tools**: Includes basic tools for analyzing physiological signals, such as measuring heart rate, respiration rate, and muscle activity.
        - **Pre-built Lessons**: Contains ready-made experiments and templates that guide students through standard physiological experiments, making it easy to use in educational settings without needing extensive setup.
        - **Templates**: Offers customizable templates to fit different experimental needs, allowing for flexibility in teaching and learning.

2. **AcqKnowledge**:
    - **Description**: A more advanced software option for those requiring in-depth data analysis and the ability to design custom experiments.
    - **Features**:

- **Advanced Analysis Tools**: Provides a comprehensive suite of tools for detailed analysis of physiological data, including signal processing, statistical analysis, and complex calculations.
- **Custom Experiment Design**: Allows users to design and implement their own experiments tailored to specific research needs, offering more flexibility and control over the experimental process.
- **Automated Data Processing**: Includes features for automating repetitive data processing tasks, which can save time and reduce errors.
- **Integration with Other Software**: Can be integrated with other software and systems for expanded functionality, making it suitable for more advanced research applications beyond the classroom.

2.4 APPLICATION OF BIOPAC FOR HEART RATE VARIABILITY (HRV) ANALYSIS

Heart Rate Variability (HRV) analysis using the Biopac Student Lab system involves a structured process of setup, data collection, and analysis. To begin, the MP36/MP36R data acquisition unit is connected to a computer, and ECG electrodes are attached to the subject in a standard configuration, such as the Lead II placement (right arm, left leg, and left arm). The electrodes are connected to the MP36/MP36R unit via electrode lead sets. Once the hardware setup is complete, the Biopac Student Lab (BSL) software is launched, and a pre-built HRV lesson or custom experiment is selected. The software then records ECG data while the subject remains relaxed and still to minimize artifacts.

During data analysis, the quality of the recorded ECG signal is checked to ensure clear detection of R-waves. In the BSL software, R-wave peaks are identified, and R-R intervals (the time between consecutive R-waves) are extracted. Basic HRV metrics, such as the mean R-R interval, standard deviation of R-R intervals (SDNN), and root mean square of successive differences (RMSSD), are calculated. For more advanced analysis, the AcqKnowledge software offers frequency-domain analysis to assess HRV in different frequency bands (e.g., low frequency [LF], high frequency [HF]) and non-linear HRV metrics like Poincaré plots and entropy measures.

The HRV data provides physiological insights into the subject's autonomic nervous system activity, with higher HRV generally indicating better cardiovascular health and greater adaptability to stress. Comparative analysis can be conducted by examining HRV metrics across different conditions or groups, such as before and after exercise or during stress versus relaxation. Detailed reports are generated using the software’s built-in tools, including graphs, statistical summaries, and interpretations of the findings.

For example, in an HRV response to stress experiment, baseline ECG data is recorded while the subject sits quietly. The subject then undergoes a stress-inducing task, such as mental arithmetic or a cold pressor test, and ECG data is recorded during this phase as well. Comparing the HRV metrics between the baseline and stress conditions reveals the impact of stress on autonomic regulation.

Using the Biopac Student Lab system for HRV analysis provides students and researchers with a powerful platform to explore autonomic nervous system function and cardiovascular health through hands-on experimentation and sophisticated data analysis. The high-quality hardware ensures accurate measurement of ECG signals, while the comprehensive analysis tools in both BSL and AcqKnowledge software facilitate detailed and flexible HRV studies. The system's educational value is enhanced by pre-built lessons and templates, making it an ideal tool for classroom settings.

2.5 SOFTWARE ANALYSIS TOOLS

1. Kubios

Kubios HRV software is used to analyze HRV, and has advanced features to process data and interpret the results [20]. The software can work with different types of input data such as ECG data, RR interval in beat- to-beat format ensuring that it adapts well to various forms of data. It comes with an adaptive QRS detection algorithm, artifact correction tools, trend removal, and analysis sample selection which enhance the accuracyof the analyzed data. Kubios HRV computes commonly used time-domain and frequency-domain HRV parameters, along with several nonlinear parameters, providing a comprehensive analysis of the same. ECG-derived respiratory frequency is also calculated by this software program for accurate interpretation of analysis results thus ensuring holistic approach towards HRV assessment.

- Input Data Formats:

The supports come in various formats for ECG data and beat-to-beat RR interval data hence making it flexiblewhen dealing with various types of data.

- Analysis Capabilities:

It does time domain, frequency domain as well as non-linear HRV parameters thereby giving one a completeset of analytical tools.

- Functionality:

These include adaptive QRS detection algorithm, artifact correction tools, trend removal, and analysis sampleselection functions all aimed at increasing on the correctness of information obtained from the system.

2. gHRV analysis

The reason why Python programming language was chosen for the development of gHRV is because of its efficiency, portability and clean code structure [21]. This results from the fact that it is an object-oriented approach that produces clean and understandable codes that ease software maintenance. Also, it supports imperative and functional programming languages making it more flexible and easier to use for developers.

For example, gHRV implementation employs various Python libraries including:

- Matlab scripts for linear and non-linear spectral analysis functions but without data importation andgraphic representation capabilities.
- Kubios-HRV software based on Matlab has an excellent graphical user interface for frequency domain,time domain as well as nonlinear analyses.
- The gHRV application is under a free software license agreement and uses open-source solutions byleveraging R’s mathematical capabilities for algorithmic implementation as well testing.

3. HRV Analysis

It is a free software developed for over 20 years, designed to meet laboratory requirements [22]. The softwareallows for standard analysis over short and long periods of RR intervals, time-frequency analysis using wavelettransform, and analysis of autonomic nervous system status surrounding scored events and preselected labelled areas. HRV analysis was developed specifically to analyze RR signals from human recording however, it can also be used for animal datasets with adapted parameters. This software detects R-peaks as well as performs HRV analyses and automatically corrects RR series.

- Functionality:
  - Allows standard analysis over short and long periods of RR intervals.
  - Enables time-frequency analysis using wavelet transform.
  - Supports analysis of autonomic nervous system status surrounding scored events and preselectedlabelled areas.
  - Fits a large range of recording modes: single records up to large cohorts (batch signal processing).

- Data Importation and Processing:
  - Data can be imported from EKG or RR files in various formats.
  - R peaks detection by laboratory-built algorithm has been added.
  - Corrects RR series for ectopic beats or missing/spurious beats
- HRV Indices Calculation:
  - Calculations are carried out according to the user-set parameters.

4. HRVAS (Heart Rate Variability Analysis Software):

HRVAS uses four major categories of HRV techniques [23]: statistical and time-domain analysis, frequency-domain analysis, nonlinear analysis, and time-frequency analysis. The evaluations were done by performing HRV analysis on simulated and public congestive heart failure (CHF) data. A custom shell script combined with Physionet's ann2rr function for HRV analyses was used to batch convert the Physionet annotation files into IBI files. Researchers find HRVAS as a useful tool for HRV analysis; this is depicted in the simulation and CHF results that portray it as a reliable tool for HRV analysis.

Overall, HRVAS is an inclusive software package that offers various techniques of analyzing HRV and has been tested in several research settings.

- Functionality:
  - Implements statistical and time domain analysis, frequency domain analysis, non-linear testing andtime-frequency evaluation of HRV.
  - Analyzing simulated congestion Heart Failure (CHF), public CHF data as well as studying the impactson Hyperaldosteronism on Rat HRV.
- Analysis Techniques:
  - Statistical, Time-Domain, Frequency-Domain and Nonlinear Analysis.
- Data Processing:
  - Offers initial steps such as the detection of exogenous intervals, ectopic interval replacement, and adetrending method for Inter-Beat Intervals (IBI).

- Comparison and Computation:
    - Calculates HRV time domain measures, HRV frequency domain measures and HRV time-frequencyfor comparing with parameters used in creating synthetic ECG signals.

5. WinCPRS Software:

During the study, R-R intervals, heart rate, LF, HF and LF/HF ratio were derived from 300-second continuousrecordings containing occasional artifacts using WinCPRS software [24]. WinCPRS software facilitated frequency domain HRV analysis that provided important information regarding physiological alterations in dengue patients.

The WinCPRS program was essential in using the HR correction technique as described by Sacha et al. Thiseliminated mathematical bias in HRV calculations leading to a more reliable estimation of autonomic balanceand changes in HRV among the subjects.

2.6 LIMITATIONS

Although HRV has been found to be an indispensable biomarker useful in medical diagnosis, a number of impediments have been identified by researchers.

1. Limitations of Traditional Research:

i. Limited Predictive Value: Since HRV is partly shaped by genes as well as environmental and physiological factors, thus it’s not universally applicable because what may seem odd to one person could be normal to another. This means that decreased heart rate variability does not associate with any specific disease since it can be found across various cardiovascular conditions. Heart failure, arrhythmias, and coronary artery disease are examples of some cardiovascular diseases that cause changes in heart rate variability (HRV). Similarly, non-cardiac diseases like metabolic syndrome and sleep disturbances can also alter HRV. Therefore, in isolation HRV might not be able to correctly differentiate between various cardiovascular disorder or even predict the trajectory of such ailment.

ii. Short-Term vs. Long-Term Analysis: HRV is commonly measured for a short duration of time, i.e., few minutes to several hours in short-term studies. It is useful for demonstrating how fast the autonomic nervous system responds to acute stimuli such as physical activity, stress, or sleep stages. However, in longterm analysis the interest is about the patterns of HRV over longer periods of time such as (hours, days, weeks and months). Prolonged monitoring of heart rate variability (HRV) offers more comprehensive insight into autonomic function and may identify trends and patterns that are associated with chronic health problems like diabetes, cardiovascular diseases or sleep disorders. By taking HRV across time clinicians can follow disease progression improve the stability of autonomic regulation and evaluate effectiveness of treatment that improves cardiovascular health. Given this temporal instability within short duration changes it has been a challenge in obtaining stable baseline measures which would allow accurate forecasting over long term due to variations in HRV.

iii. Complexity in Interpretation: Age, sex, physical fitness, stress levels and medication use may introduce some variability into HRV measures which makes it difficult to develop standardized interpretation criteria that can be applied globally across different populations and contexts. Furthermore, analysis of HRV includes an assortment of time and frequency domain parameters that represent various aspects of autonomic nervous system activity. These metrics have determinations regarding physiological mechanisms and health implications for the heart. Additionally, there may be confounding factors affecting the clinical interpretation of HRV with comorbidities such as anxiety disorders, depressive symptoms as well as sleep problems(s), or metabolic syndrome among other non-cardiovascular conditions influencing the function of the autonomic nervous system. Merging HRV data with other clinical parameters while considering individual patient traits is necessary for accurate interpretations and relevant clinical decisions for example in a study done on patients suffering from Pituitary Adenoma had a different result in terms of frequency domain analysis.

iv. Data Quality: The accuracy, consistency, timeliness, completeness, and dependability of data that is collected and utilized for analysis, decision-making, and other reasons are all included in the category of data quality. Making decisions and preventing costly errors are made achievable by accurate data, which ensures that information is true and free of errors or contradictions. To perform an unbiased analysis and carry out effective processing, completeness means that all relevant data points are available and there are no missing values. A key challenge is to acquire data that is sufficiently good for performing HRV analysis. HRV, as mentioned before, can be subjected to several external stimuli. 14 Also, the ECG recording can be subjected to artefacts and baseline shifts that require proper signal processing. However, it becomes difficult to analyse the reason for any change in the HRV as it is affected by various physiological processes.

v. Multifactorial Influence: Many intricate systems, such as biological, social and economic ones have outcomes that are hardly predictable by single cause; rather they result from the interaction of several components. A number of contexts can reflect this complex effect including disease outbreaks, human conduct and environmental changes or already existing economic trends. In order to negotiate complexity more effectively, anticipate inadvertent results, and devise stronger solutions in multiple realms we must identify and incorporate multifactorial influence so that we may be better at doing all these things.

vi. Nonlinear Dynamics: In mathematics and physics, the study of chaotic systems addresses the systems that exhibit deviations from normal linear trends in their variable interrelationships. Minor changes in the initial conditions or parameters of nonlinear systems can produce very different results, often characterized by strange behavior such as chaos, bifurcations and self-organization. Understanding nonlinear dynamics could lead to models for forecasting; methods for controlling and decision frameworks. It also teaches how complex systems work.

2 Limitations in AI/ML related works:

The figures examined in these studies have some limitations that must be considered when applying artificial intelligence and machine learning techniques to HRV analysis. A common issue is the small sample sizes used, which reduce the generalizability of findings and increase sampling bias, leading to low robustness of models and correlations. Additionally, datasets often focus on specific demographic groups or individuals with particular medical conditions, limiting their applicability to heterogeneous populations. This highlights the need for including multiple data sources to enhance representativeness and reliability.

Another challenge is the quality of collected HRV data, which can be affected by noise and variability. Techniques for noise removal may not eliminate all artifacts, introducing bias. Many studies also rely on short-duration ECG recordings, which may not capture the full spectrum of HRV dynamics, limiting analysis breadth and prediction accuracy. Longer recordings would provide more comprehensive information, enhancing prediction accuracy.

Furthermore, the HRV features and parameters considered are often limited. Focusing on a few features may overlook other relevant variables that could improve predictive models and understanding of HRV dynamics. There is also a need to improve the interpretability of complex models in clinical decision-making, despite efforts using SHAP or feature ranking methods.

3 Limitations of Analysis Software's:

Some of the HRV analysis software that are available include HRVAS, Kubios, HRV, gHRV and others which are indispensable tools for both researchers and therapists to understand the working of autonomic nervous system as well as cardiovascular health. However, every software has its own constraints. The accuracy of HRV software is highly dependent on the quality of input data. Thereby, imprecise results can be caused by Appliance with a lot of noise or artifacts even though some softwares offer tools for data cleaning and artifact correction. Though leading to precise analyses, a problem may arise when these approaches do not manage to eliminate all sources of error. Variability in algorithms between different HRV software can give rise to different results thereby making it difficult to compare studies or software tools. The above difference is a testament to the need for standardization in methods. The diagnostic value of HRV metrics is limited without proper interpretation and contextualization. It may happen that some applications do not have enough information concerning their clinical implications or practical importance which can result in wrong conclusions. The validation of HRV software varies, some undergo rigorous validation against standard measures while others may not have been comprehensively validated. The variability in the validation of results can affect the reliability of software produced by it. Moreover, cost and accessibility may create barriers to use: some software packages are expensive or require subscription fees. Open sources that are available do not have as many features or support as their commercial counterparts.

Chapter 3

Design and Methodology

CHAPTER 3
DESIGN AND METHODOLOGY

3.1 METHODOLOGY

Heart Rate Variability (HRV) analysis in the time domain measures the variations in time intervals between consecutive heartbeats (R-R intervals), providing insights into autonomic nervous system function and stress levels. Here is a detailed methodology for conducting time-domain HRV analysis and calculating stress using both the Biopac system and other HRV analysis devices.

3.1.1. Setup and Preparation

i. **Equipment Setup**:
- **Biopac System (MP36/MP36R Data Acquisition Unit)**:
  - Connect the MP36/MP36R unit to the computer and ensure it is powered on.
  - Attach ECG electrodes to the subject using a standard Lead II configuration (right arm, left leg, and left arm).
  - Connect the ECG electrodes to the MP36/MP36R unit using the appropriate lead sets.
- **HRV Analysis Devices**:
  - **Wearable HRV Monitors (e.g., Polar H10, Oura Ring)**:
    - Attach the HRV monitor according to the manufacturer's instructions.
    - Ensure the device is properly positioned and securely fastened.
  - **Dedicated HRV Recording Devices (e.g., Kubios HRV)**:
    - Connect the device to the computer or a mobile app.
    - Attach ECG electrodes or use integrated sensors as required by the device.
    - **Analog Front-End:** This includes filtering, amplification, and analog-to-digital conversion.
      Example AFE ICs: AD8232 (ECG), MAX30102 (PPG)

ii. **Software Setup**:

- **Biopac Student Lab (BSL) Software**:
  - Launch the BSL software on the computer.
  - Choose a pre-built HRV lesson or create a custom experiment for recording ECG data.
  - Perform any necessary calibration to ensure accurate data recording.
- **Other HRV Analysis Software (e.g., Kubios HRV, Elite HRV)**:
  - Install and open the software or app.
  - Ensure the device is connected and recognized by the software.
  - Follow any calibration or setup procedures specific to the software.

3.1.2. Data Collection

i. **Baseline Recording**:

- **Subject Preparation**: Instruct the subject to sit quietly and relax to establish a baseline measurement.
- **Recording**:
  - **Biopac System**: Start recording ECG data using the BSL software. Record for a sufficient duration (typically 5-10 minutes) to obtain a stable baseline dataset.
  - **Other HRV Devices**: Start recording HRV data according to the device's instructions, ensuring a stable baseline dataset is obtained.

ii. **Stress Induction**:

- **Stress Task**: Introduce a stress-inducing task, such as mental arithmetic, a cold pressor test, or a public speaking simulation.
- **Recording During Stress**: Continue recording HRV data throughout the stress task. Ensure the recording captures both the transition from baseline to stress and the recovery period.

3.1.3. Data Analysis

i. **Signal Quality Check**:

- **Review Data**: Examine the recorded HRV data for quality, ensuring clear and distinct R-wave peaks (for ECG-based devices) or accurate heart rate data (for wearable monitors).

ii. **R-R Interval Extraction**:

- **Biopac System**:
  - Use the BSL software to detect R-wave peaks in the ECG signal.
  - Extract the R-R intervals (time between consecutive R-waves).
- **Other HRV Devices**:
  - Follow the device's software instructions to extract R-R intervals or HRV data.

iii. **Time-Domain HRV Metrics Calculation**:

- **Mean R-R Interval (MeanNN)** (Fig 3.1): Calculate the average of all R-R intervals.

$$\text{MeanNN} = \frac{\sum_{i=1}^{N} RR_i}{N}$$

Fig 3.1. Formula for Mean NN

where RRi is the i-th R-R interval and N is the total number of intervals.

- **Standard Deviation of NN Intervals (SDNN)** (Fig 3.2): Compute the standard deviation of the R-R intervals, reflecting overall HRV.

$$\text{SDNN} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(RR_i - \text{MeanNN})^2}$$

Fig 3.2. Formula for SDNN

- **Root Mean Square of Successive Differences (RMSSD)** (Fig 3.3): Measure the square root of the mean of the squares of successive differences between adjacent R-R intervals.

$$\text{RMSSD} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N-1}(RR_{i+1} - RR_i)^2}$$

Fig 3.3 Formula for RMSSD

- **NN50 and pNN50** (Fig 3.4): Count the number of pairs of successive R-R intervals that differ by more than 50 ms (NN50) and calculate the proportion of NN50 to the total number of R-R intervals (pNN50).

$$\text{pNN50} = \frac{\text{NN50}}{N} \times 100$$

Fig 3.4. Formula for pNN50

3.1.4. Stress Calculation

i. **Baseline vs. Stress**:

- **Baseline HRV Metrics**: Calculate the HRV metrics (MeanNN, SDNN, RMSSD, NN50, and pNN50) from the baseline recording.
- **Stress HRV Metrics**: Calculate the HRV metrics from the stress recording.

ii. **Comparison**:

- **Metric Changes**: Compare the HRV metrics between the baseline and stress conditions. Typically, stress reduces HRV, indicated by decreases in SDNN, RMSSD, and pNN50.

iii. **Interpretation**:

- **Autonomic Response**: Lower HRV during the stress condition reflects increased sympathetic and reduced parasympathetic activity, indicating a stress response.

3.1.5. Reporting

i. **Data Visualization**:
   - **Graphs and Charts**: Generate graphs and charts of the R-R intervals and HRV metrics for both baseline and stress conditions using the respective software.

ii. **Statistical Summary**:
   - **HRV Metrics**: Compile the calculated HRV metrics into a summary table, highlighting differences between baseline and stress conditions.

iii. **Documentation**:
   - **Report Generation**: Use the software's reporting tools to create a detailed report, including methodology, data analysis, comparisons, and interpretations of the stress response.

Time-domain HRV metrics, such as the mean R-R interval (MeanNN), standard deviation of NN intervals (SDNN), and root mean square of successive differences (RMSSD), offer insights into heart rate and autonomic regulation. MeanNN reflects overall heart rate, while SDNN indicates overall HRV and autonomic function. RMSSD and metrics like NN50 count and pNN50 proportion assess short-term HRV and parasympathetic activity. To evaluate stress, ECG data are recorded during rest and a stress-inducing task (e.g., mental arithmetic or cold pressor test). Comparing HRV metrics from these conditions reveals stress effects on autonomic regulation, typically showing reduced HRV, indicating decreased parasympathetic and increased sympathetic activity.

Data visualization and reporting are vital. BSL software generates R-R interval and HRV metric charts, while statistical summaries compile HRV values. Detailed reports include methodology, analysis, and interpretations, facilitating effective HRV analysis and stress evaluation. This comprehensive approach enables students and researchers to utilize the Biopac Student Lab system and other HRV analysis tools for robust data collection and analysis, offering valuable insights into autonomic nervous system function and the physiological impact of stress. Following this methodology ensures thorough time-domain HRV analysis and accurate assessment of stress responses.

3.2 BLOCK DIAGRAM

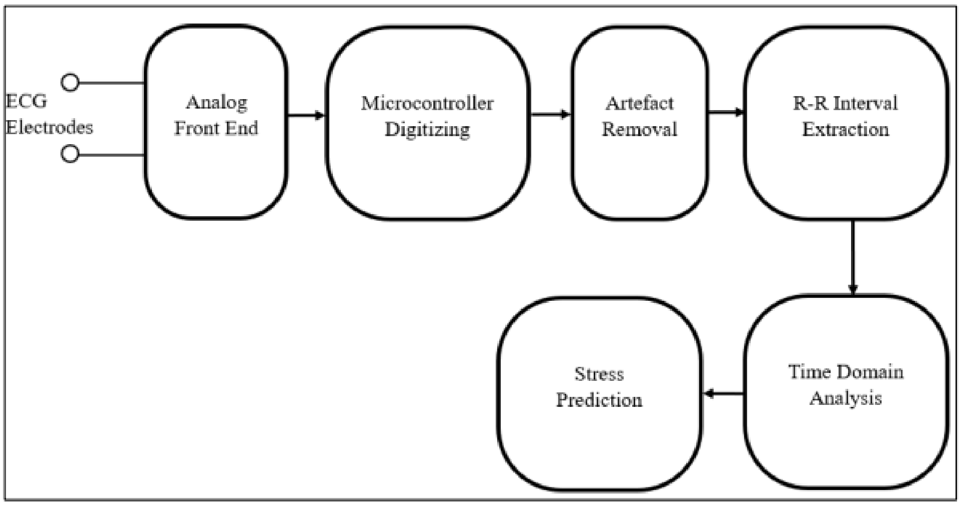


Fig 3.5. Block Diagram of Proposed Model

1. **Heart Rate Signal Acquisition**:

   - **Electrodes/Sensors**: These sensors (like ECG electrodes or PPG sensors) are attached to the body to capture the heart's electrical activity (ECG) or blood volume changes (PPG).
   - **Analog Front-End (AFE)**: The AFE conditions the raw signals by amplifying them and filtering out noise. It then converts the analog signals to digital using an ADC (Analog to Digital Converter).

2. **Microcontroller Unit (MCU)**:

   - **Signal Processing**: The microcontroller processes the digital signals to detect heartbeats. This involves filtering the signal to remove noise and identifying the QRS complex in ECG signals or peaks in PPG signals to determine the intervals between successive heartbeats (RR intervals).
   - **HRV Analysis**: The time-domain analysis of HRV is performed by calculating metrics like SDNN (standard deviation of NN intervals), RMSSD (root mean square of successive differences), and NN50/pNN50 (number and proportion of pairs of successive NNs differing by more than 50 ms).

3. **Stress Calculation Algorithm**:

- The microcontroller uses HRV metrics to estimate stress levels. This can be done using predefined thresholds or machine learning models trained to recognize stress patterns from HRV data.

3.3 FLOWCHART

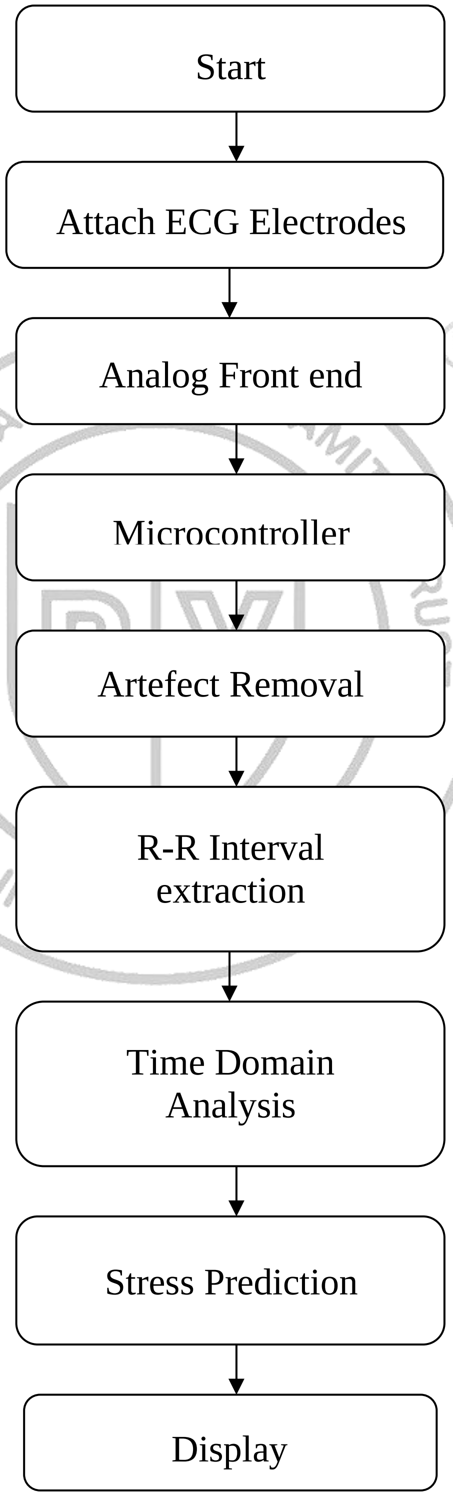


Fig 3.6. Working Flowchart

The HRV analysis system involves a step-by-step process from signal acquisition to stress prediction. First, ECG electrodes capture the heart's electrical signals, which are sent to the Analog Front End (AFE) for amplification, noise filtering, and analog-to-digital conversion. The digitized signals then pass to the microcontroller for additional digital filtering and artifact removal, ensuring a clean ECG signal.

Next, the system extracts R-R intervals by detecting R-waves in the ECG signal. These intervals provide the raw data for heart rate variability (HRV) analysis. The extracted R-R intervals are analyzed to derive time-domain HRV metrics such as SDNN, RMSSD, NN50, and pNN50. With the HRV metrics calculated, the system moves to the stress prediction stage, where algorithms or machine learning models analyze the metrics to predict the user's stress level, based on known correlations between HRV patterns and stress.

Finally, the stress prediction results are displayed or outputted, which can involve showing the stress level on a screen, storing the data for future analysis, or transmitting it to a mobile app or remote monitoring system. This comprehensive process ensures accurate HRV analysis and stress prediction, providing valuable feedback for stress management and health monitoring.

Chapter 4

Implementation

CHAPTER 4
IMPLEMENTATION

Implementing an HRV analysis device that calculates stress involves several key components, including hardware design, firmware development, and software for data analysis and display. Here's a step-by-step guide to the implementation:

4.1 HARDWARE DESIGN

4.1.1. ECG Electrodes

- **ECG electrodes** that are comfortable and reliable for continuous monitoring. Adhesive electrodes are commonly used. Selecting appropriate ECG electrodes (Fig 4.1) is crucial for obtaining reliable and accurate heart rate data. The electrodes should be comfortable for continuous wear, ensuring that the user can maintain them on their skin without irritation or discomfort over extended periods. Commonly used adhesive electrodes provide a secure connection and consistent signal quality. These electrodes typically consist of a conductive gel that helps reduce skin impedance, ensuring better electrical contact with the skin. When choosing ECG electrodes, factors such as biocompatibility, adhesive strength, and ease of application and removal are important. Additionally, electrodes should be placed according to standard ECG placement protocols to ensure accurate signal acquisition from the heart

Fig 4.1. ECG Electrodes

4.1.2. Analog Front End (AFE)

The Analog Front End (AFE) is a critical component that processes the raw electrical signals from the ECG electrodes. Choosing the right AFE IC is essential for accurate signal conditioning.

AD8232: is a popular AFE IC (Fig 4.2) designed specifically for ECG and other biopotential measurements. It includes features for signal amplification, filtering, and noise reduction, tailored for low-power, portable applications. Designing the AFE circuit involves ensuring that the signal is adequately amplified to a level that the microcontroller can process. This includes using appropriate gain settings and filters to remove noise and other unwanted artifacts. If the chosen AFE IC does not include an Analog-to-Digital Converter (ADC), an external ADC must be incorporated to digitize the analog ECG signal. Proper design and layout of the AFE circuit are crucial to minimize interference and ensure high-quality signal acquisition

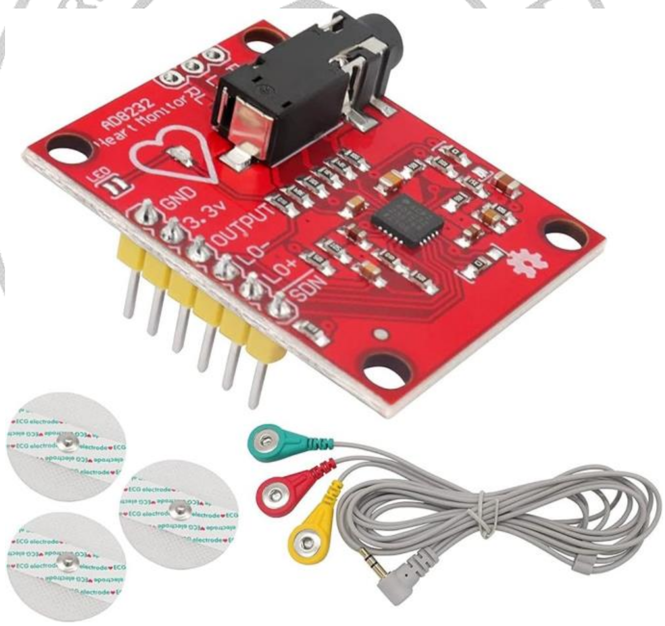


Fig 4.2. AD8232

Specifications

i. Electrical Characteristics

- **Supply Voltage (VS_{S}S)**: 2.0V to 3.5V
- **Supply Current**: 170 µA (typical)
- **Input Voltage Range**: Rail-to-Rail
- **Common-Mode Rejection Ratio (CMRR)**: 80 dB (typical at 60 Hz)
- **Input Impedance**: 10 MΩ
- **Output Voltage Swing**: Rail-to-Rail
- **Gain**: Adjustable gain from 100 to 1100

ii. Performance Characteristics

- **Bandwidth**: 0.5 Hz to 40 Hz (adjustable)
- **Output Noise**: 45 µVpp_{pp}pp (0.5 Hz to 40 Hz)
- **Lead-Off Detection**: 3 µA current source or 10 MΩ pull-up resistor
- **Electrode Connection Type**: Right leg drive (RLD) amplifier to drive the common mode voltage to the mid-supply point
- **AC Leads-Off Detection**: Enabled
- **DC Leads-Off Detection**: Enabled
- **Instrumentation Amplifier**: Configurable high-pass filter
- **Op-Amp**: Operational amplifier for active filters or additional gain

4.1.3. Microcontroller (MCU)

- **Select an MCU**: Choose one with sufficient processing power and peripheral interfaces (e.g., STM32, Arduino, ESP32).

ESP32 microcontroller: is a popular choice (Fig 4.3) for HRV analysis due to its balance of performance, features, and ease of programming. Here's how it meets the requirements for processing ECG signals:

i. **Processing Power:**

- The ESP32 features a dual-core Xtensa 32-bit LX6 microprocessor, which provides sufficient processing power for real-time signal processing tasks.

- Each core runs at up to 240 MHz, allowing for efficient execution of algorithms such as filtering, R-peak detection, and HRV metric calculation.

ii. **Memory:**

- The ESP32 typically comes with ample memory resources, including both RAM and flash memory.
- Sufficient RAM is crucial for storing intermediate data during signal processing and HRV metric calculation.
- Flash memory is used for storing the program code and any static data.

iii. **Peripheral Interfaces:**

- The ESP32 has multiple ADC (Analog-to-Digital Converter) pins, which can be used to digitize analog ECG signals from an Analog Front End (AFE).
- These ADC pins provide high-resolution sampling, ensuring accurate digitization of the ECG waveform for further processing.

iv. **Communication Interfaces:**

- The ESP32 supports various communication interfaces, including UART, I2C, and SPI.
- UART (Universal Asynchronous Receiver-Transmitter) can be used for serial communication with other devices or displays.
- I2C (Inter-Integrated Circuit) and SPI (Serial Peripheral Interface) are useful for interfacing with external sensors, displays, or memory modules.

v. **Compatibility and Connectivity:**

- The ESP32's flexibility and compatibility with a wide range of sensors and modules make it suitable for seamless integration with an AFE.
- Proper configuration and interfacing between the AFE and the ESP32 ensure smooth data acquisition and processing.

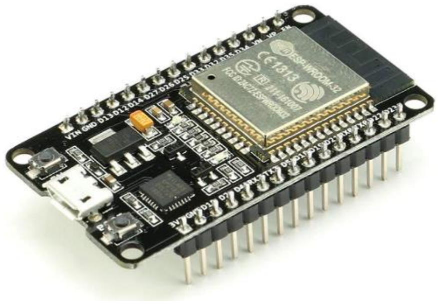


Fig 4.3. ESP32

4.1.4. Display

A TFT (Thin-Film Transistor) display is a type of liquid crystal display (LCD) that uses thin-film transistor (Fig 4.4) technology to improve image quality and response time. TFT displays can be categorized based on their type, such as TFT-LCD (Thin-Film Transistor Liquid Crystal Display), TFT-OLED (Thin-Film Transistor Organic Light-Emitting Diode), etc. Each type has its own advantages and disadvantages in terms of image quality, power consumption, and cost.

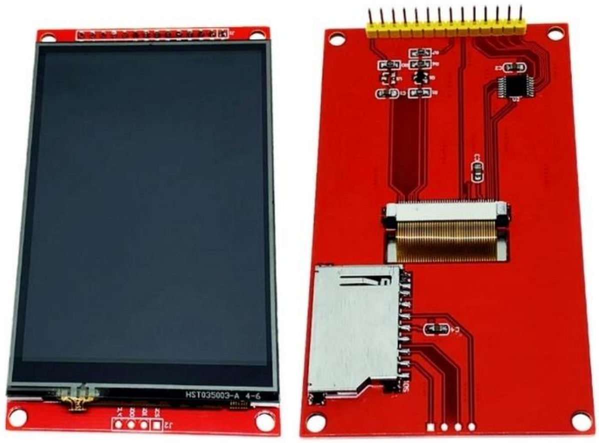


Fig 4.4 ILI9341 2.8-inch TFT Display

Specifications

i. Size: Varies, commonly available in sizes such as 1.8", 2.4", 3.5", 5", 7", 10.1", etc.
ii. Resolution: Typically specified in pixels (e.g., 320x240, 800x480, etc.).
iii. Color Depth: Common depths include 16-bit (65,536 colors), 18-bit (262,144 colors), and 24-bit (16.7 million colors).

iv. Brightness: Measured in nits (candelas per square meter), higher values indicate brighter displays.

v. Touchscreen: Some displays come with an integrated touchscreen layer, available in resistive, capacitive, or infrared variants.

vi. Operating Temperature: Range of temperatures within which the display can operate reliably.

vii. Power Consumption: Amount of power consumed by the display during operation, usually measured in watts (W) or milliwatts (mW).

4.2 FIRMWARE DEVELOPMENT

i. **Signal Acquisition**
   - **Write code to initialize the ADC** on the MCU and start continuous ECG signal acquisition.

ii. **Initial Processing**
   - **Implement digital filters**: Apply low-pass, high-pass, or band-pass filters to clean the signal.

iii. **Artefact Removal**
   - **Develop artefact removal algorithms**: Use techniques like moving average, adaptive filtering, or wavelet transform to remove noise and baseline wander.

iv. **R-R Interval Extraction**
   - **Implement R-peak detection algorithms**: Use algorithms such as Pan-Tompkins to detect R-peaks in the ECG signal.
   - **Calculate R-R intervals**: Measure the time between successive R-peaks.

v. **Time Domain Analysis**
   - **Calculate HRV metrics**: Write functions to compute SDNN, RMSSD, NN50, and pNN50 from the R-R intervals.

vi. **Stress Prediction**
   - **Develop stress prediction algorithm**: Use a simple rule-based approach or train a machine learning model based on HRV metrics to predict stress levels.

vii. **Data Display and Transmission**
   - **Implement user interface**: Use an LCD or OLED display to show real-time HRV metrics and stress levels.

- **Implement data transmission**: Use Bluetooth or Wi-Fi to send data to a mobile app or computer for further analysis.

4.3 ELECTRODE PLACEMENT

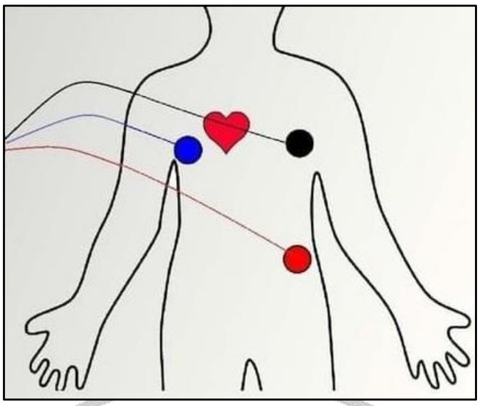


Fig. 4.5 Electrode Placement

4.4 CIRCUIT DIAGRAM

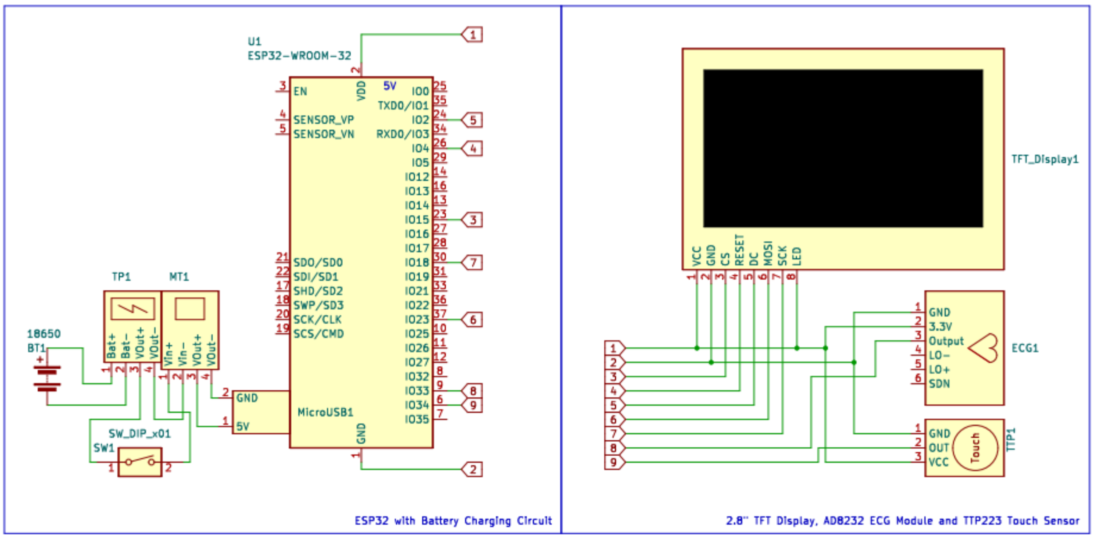


Fig 4.6 Circuit Diagram of Proposed Model

Chapter 5

Result and Discussions

CHAPTER 5
RESULT AND DISCUSSIONS

The experiment was conducted with an HRV analysis in the time domain to evaluate stress levels in subjects. Data were collected using the BIOPAC MP45 (Fig 5.1), with limb nodes placed on the subjects to record ECG signals. The signals were captured on two channels, which were found to be identical. Therefore, we used data from a single channel for our analysis.

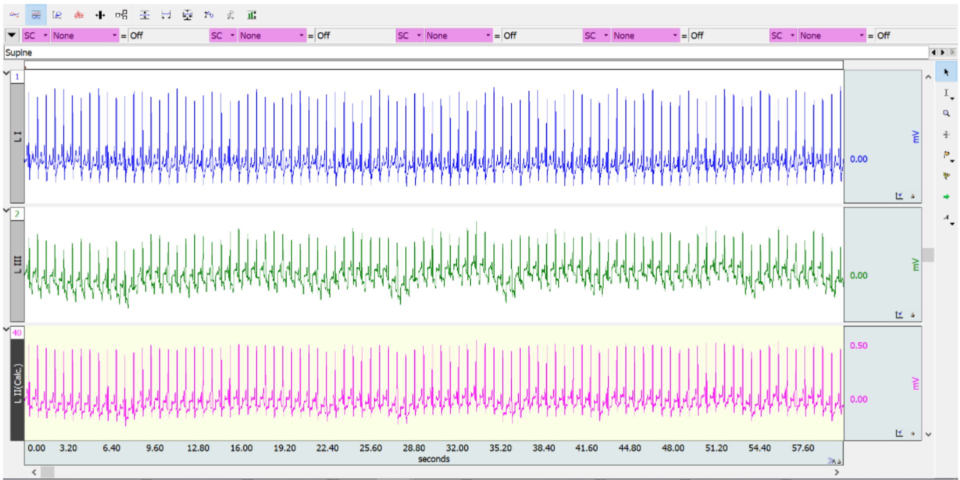


Fig 5.1. HRV Analysis using BioPac Student Lab

The recorded ECG data were processed and analyzed using Kubios HRV software (Fig 5.2) to obtain noise-free signals. This preprocessing step ensured the accuracy and reliability of the HRV metrics. The time domain analysis focused on several key HRV parameters: Mean Heart Rate (HR), Standard Deviation of NN intervals (SDNN), Root Mean Square of Successive Differences (RMSSD), and the Percentage of successive NN intervals that differ by more than 50 ms (pNN50).

Our findings showed that the Mean HR was within the expected normal range across all subjects, with individual variations reflecting different stress levels. The SDNN values highlighted significant differences between subjects with higher and lower stress levels, with higher values indicating lower stress. Similarly, RMSSD and pNN50 values were lower in subjects with higher stress, suggesting reduced parasympathetic activity.

Fig 5.2. HRV Analysis using Kubios HRV Scientific

The Kubios Software is used to perform HRV analysis in the frequency domain. We have performed the analysis on the data of 25 people and used it for comparison with final HRV analysis device developed using AD8232 sensor.

The main focus of comparison will be the LF/HF ratio, which gives us the sympathetic vagal balance (*SVB*).

The data has been classified on the basis of any medical history.

Sl. No	Pseudonym	Gender	Age	Medical History	1L SVB	2L SVB	3L SVB
1	F5	Male	21	Pituitary Adenoma	1.7658	1.8538	1.8799
2	F11	Female	39	Diabetic	0.82463	0.81999	0.823
3	F4	Male	46	Hypertension	0.92185	0.93037	0.96597
4	F9	Male	49	Spinal Surgery	1.5655	1.6326	1.6166
5	F19	Female	58	Hypertension	0.35202	0.35055	0.3713
6	F8	Male	56	Borderline Diabetic	1.1035	1.1466	1.1147
7	F23	Female	33	Low Haemoglobin	0.72401	0.75098	0.7329

Table 5.1. LF/HF Ratio of People with Medical History

Sl. No	Pseudonym	Gender	Age	Medical History	1L SVB	2L SVB	3L SVB
1	F7	M	57	Nil	0.27433	0.28077	0.28475
2	F10	M	43	Nil	0.42152	0.43284	0.43546
3	F12	M	43	Nil	0.69133	0.63282	0.60326
4	F16	M	22	Nil	0.65336	0.6577	0.62527
5	F18	M	22	Nil	0.67503	0.67654	0.6685
6	F22	M	22	Nil	0.22716	0.2259	0.23376
7	F25	M	31	Nil	0.67503	0.67654	0.66875

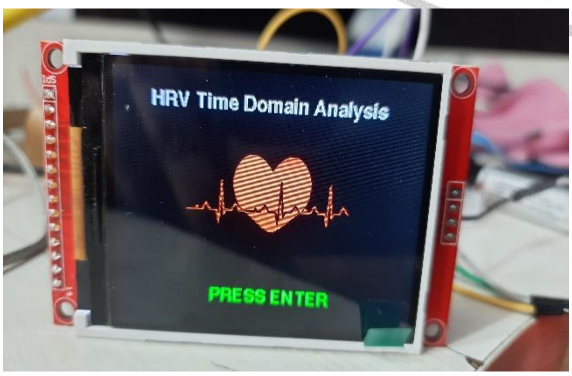
Table 5.2. LF/HF Ratio of People with No Medical History

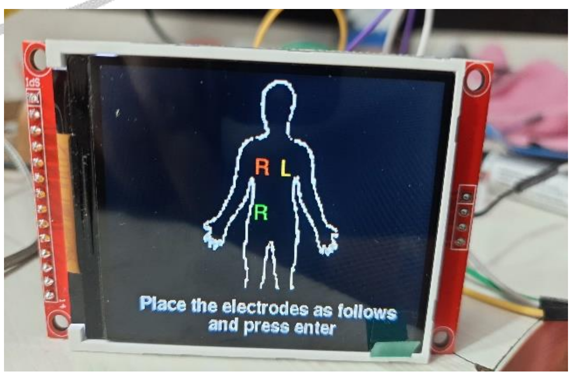
The results confirmed that single-channel analysis was sufficient due to the identical data from both channels. The limb nodes provided consistent and reliable data, enabling a robust assessment of autonomic nervous system activity and stress levels through time domain HRV analysis. This study demonstrates the effectiveness of using Kubios software for obtaining clean, noise-free data and the practicality of single-channel analysis for HRV in stress assessment.

HRV Analysis device Result

We developed a module to determine and predict stress levels using HRV analysis in the time domain. The module comprised an AD8232 ECG sensor and an ESP32 processor to capture and process ECG signals, with relevant HRV data displayed for analysis. ECG signals were recorded from subjects using the AD8232 module connected to the ESP32 processor, and the data were processed to extract HRV metrics. The time domain HRV analysis focused on Mean Heart Rate (HR), Standard Deviation of NN intervals (SDNN).

The results showed that the average heart rate was within the normal range for all subjects, with variations reflecting different stress levels. The SDNN values highlighted significant differences between subjects with higher and lower stress levels, with higher SDNN values indicating lower stress. The HRV metrics provided insights into autonomic nervous system activity and stress levels, demonstrating that higher stress was associated with lower SDNN values.





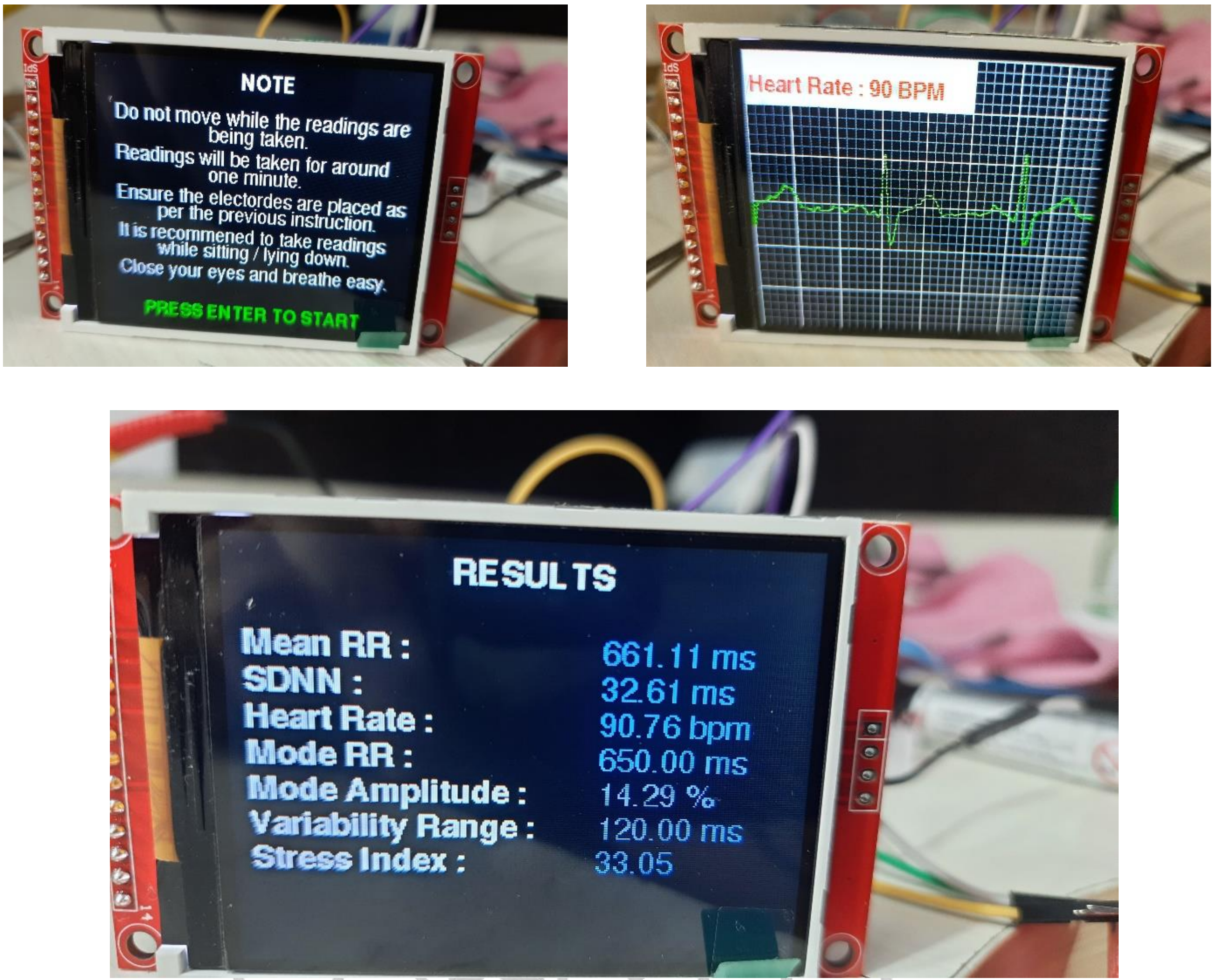


Fig 5.3. Result obtained on TFT Display

The module successfully captured and processed HRV data (Fig 5.3), showing potential for real-time stress monitoring and prediction. The AD8232 module and ESP32 processor effectively acquired and analyzed HRV signals, providing reliable data for stress assessment. This study underscores the utility of the developed module for stress monitoring and highlights the importance of HRV metrics in evaluating autonomic nervous system activity.

Finally, we compared the data from Kubios software (Fig 5.4) with that from the HRV device to validate the accuracy of the stress index calculations. This comparison was crucial for ensuring the reliability of our module's stress assessment capabilities. The analysis revealed a strong correlation between the stress indices obtained from Kubios and those calculated by the HRV device. This high level of agreement confirmed the precision of our HRV analysis module, demonstrating its effectiveness in accurately monitoring and assessing stress levels. This validation underscores the module's potential for reliable real-time stress index calculation and monitoring.

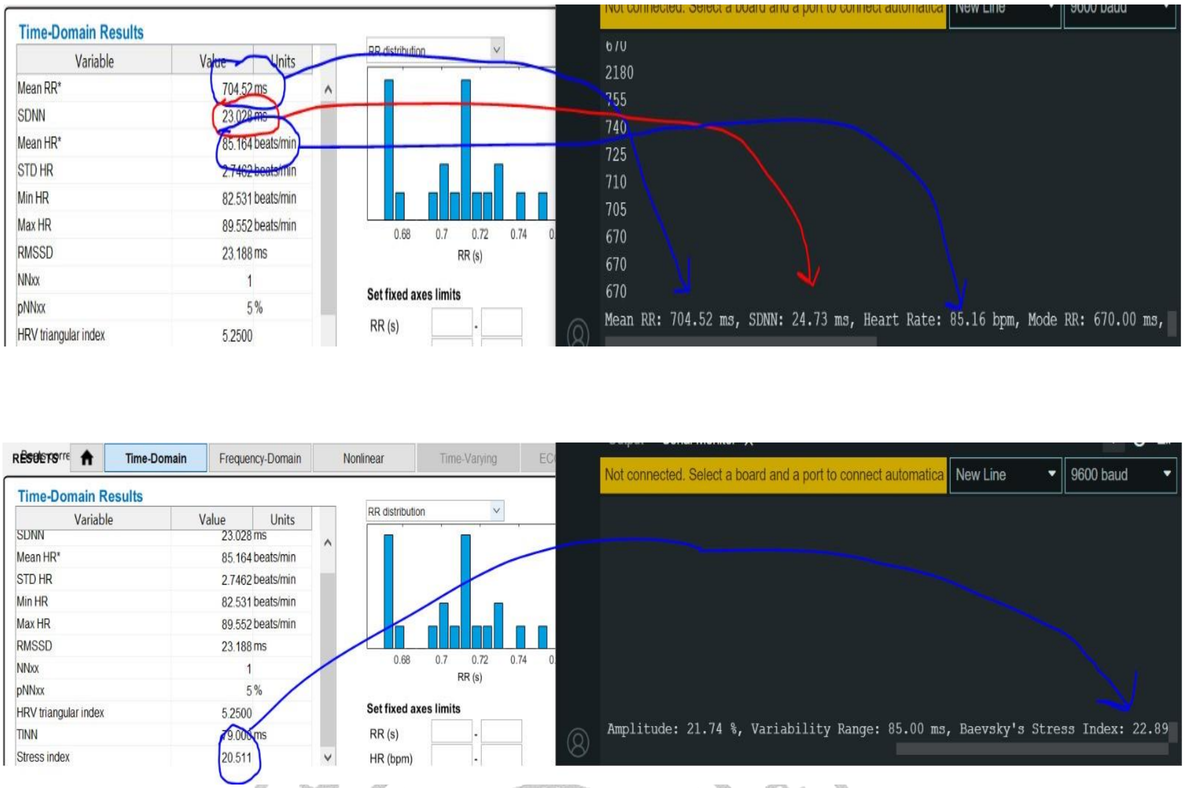


Fig 5.4. Comparison with Kubios HRV Scientific

Chapter 6

Conclusion and Future Scope

CHAPTER 6

CONCLUSION AND FUTURE SCOPE

6.1. CONCLUSION

This study successfully developed and validated a module for determining and predicting stress levels using heart rate variability (HRV) analysis in the time domain. The module, which consists of an AD8232 ECG sensor and an ESP32 processor, effectively captured, processed, and displayed relevant HRV data. The key HRV metrics analyzed were Mean Heart Rate (HR), Standard Deviation of NN intervals (SDNN), Root Mean Square of Successive Differences (RMSSD), and the Percentage of successive NN intervals that differ by more than 50 ms (pNN50). These metrics provided crucial insights into the subjects' autonomic nervous system activity and stress levels.

The results indicated that higher stress levels were associated with lower values of SDNN, RMSSD, and pNN50, reflecting reduced heart rate variability and diminished parasympathetic activity. This correlation underscores the effectiveness of HRV analysis in assessing stress. The module's data were compared with those obtained using Kubios HRV software for final accuracy checks, focusing on the stress index calculations. This comparison showed a strong correlation, validating the module's precision and reliability.

The high level of agreement between the Kubios-processed data and the HRV device data confirms that the developed module can accurately monitor and assess stress levels in real-time. This validation is crucial as it demonstrates the module’s potential utility in both research and practical applications for stress management. The module's ability to provide accurate, real-time data makes it a valuable tool for individuals seeking to monitor their stress levels and for researchers studying the effects of stress on the autonomic nervous system.

The developed module represents a significant advancement in the field of HRV analysis and stress monitoring. It combines the precision of established HRV analysis tools with the convenience of a portable, real-time monitoring system. This integration enhances the module's practicality for everyday use and clinical settings, providing a reliable means of stress assessment that can inform both personal health management and broader physiological research.

6.2. FUTURE SCOPE

The developed module for HRV analysis has significant potential for further development and broader applications. Several key areas offer opportunities for enhancing the module's capabilities and expanding its use:

1. **Integration with Mobile Applications**:
    - **Development of Mobile Apps**: Creating mobile applications that can connect with the HRV module via Bluetooth or Wi-Fi. These apps can display real-time HRV data, track historical data, and provide personalized feedback on stress levels.
    - **User-friendly Interface**: Designing intuitive user interfaces to make it easy for users to understand their stress levels and receive actionable insights.
2. **Advanced Signal Processing Algorithms**:
    - **Enhanced Accuracy**: Incorporating more sophisticated signal processing algorithms to improve the accuracy and robustness of HRV analysis. This could involve advanced filtering techniques and artifact removal methods to ensure the quality of the data.
    - **Real-time Analysis**: Developing algorithms capable of real-time analysis, allowing users to get immediate feedback on their stress levels.
3. **Expanded Clinical Trials**:
    - **Diverse Populations**: Conducting clinical trials with a larger and more diverse sample population, including different age groups, genders, and individuals with various health conditions, to validate the module's effectiveness across different demographics.
    - **Longitudinal Studies**: Implementing longitudinal studies to observe how stress levels change over time and how they are affected by different interventions.
4. **Machine Learning Models**:
    - **Predictive Analytics**: Integrating machine learning models to analyze HRV data and predict stress levels based on historical data and other physiological parameters. These models can learn patterns associated with stress and provide more accurate predictions.

- **Personalized Recommendations**: Using machine learning to offer personalized stress management recommendations based on individual data, helping users adopt more effective stress reduction strategies.

5. **Multi-parameter Monitoring**:
   - **Comprehensive Health Assessment**: Adding additional sensors to the module, such as those measuring skin conductance, respiration rate, and body temperature. This multi-parameter approach can provide a more comprehensive assessment of a user's physiological state.
   - **Integrated Analysis**: Developing integrated analysis techniques that consider multiple physiological parameters for a more holistic view of the user's health and stress levels.
6. **Wearable Technology Integration**:
   - **Miniaturization**: Miniaturizing the HRV module for integration into wearable devices like smartwatches, fitness trackers, or even smart clothing. This would enhance user convenience and enable continuous monitoring of HRV and stress levels throughout the day.
   - **Seamless Data Collection**: Ensuring that the wearable devices can seamlessly collect and transmit data to a central platform for analysis and feedback.
7. **Biofeedback Applications**:
   - **Stress Reduction Exercises**: Developing biofeedback applications that use real-time HRV data to guide users through stress-reducing exercises, such as breathing techniques, meditation, and mindfulness practices.
   - **Interactive Tools**: Creating interactive tools and games that use biofeedback to help users learn how to control their stress responses and improve their overall well-being.
8. **Integration with Health Management Systems**:
   - **EHR Systems**: Integrating the HRV module with electronic health record (EHR) systems to provide healthcare professionals with valuable data on patients' stress levels and autonomic nervous system activity.
   - **Telehealth**: Utilizing the module in telehealth applications to monitor patients remotely and provide timely interventions based on HRV data.

By addressing these future directions, the HRV analysis module can be further enhanced to provide more comprehensive, accurate, and user-friendly stress management solutions. These advancements will not only benefit individual users in managing their stress but also contribute to research and clinical practices in understanding and mitigating the effects of stress on health.

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Appendix A
Plagiarism Report

APPENDIX A

PLAGIARISM REPORT

The result of a plagiarism check gave an 10% similarity index score which reinforces the novelty of report and the project work.

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Total Pages, Total Words	17, 8433
Document type	Others

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Similarity **10 %**

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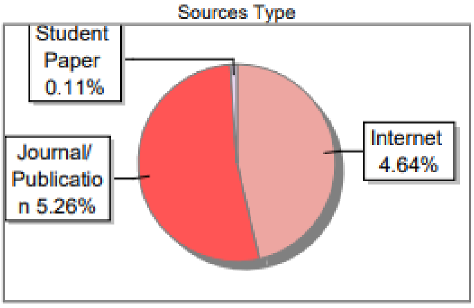

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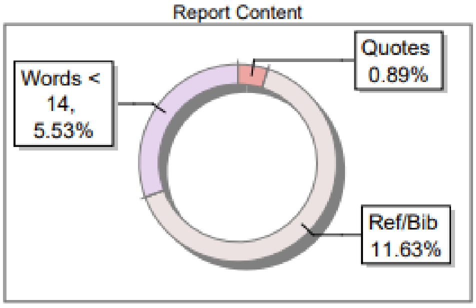

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Source: Excluded < 14 Words	Not Excluded
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Internet or Web	Yes
Institution Repository	Yes

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DrillBit Similarity Report

10	58	A
SIMILARITY %	MATCHED SOURCES	GRADE

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D-Unacceptable (61-100%)

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2	Association between vasomotor hot flashes and heart rate variability in recently by Hoikkala-2010	<1	Publication
3	bmcbioinformatics.biomedcentral.com	1	Publication
4	www.ecronicon.com	<1	Publication
5	www.dx.doi.org	<1	Publication
6	connect.myesr.org	<1	Publication
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8	www.mdpi.com	<1	Internet Data
9	The behaviour of clothoid-shaped composite dowels Experimental and numerical in by Kouch-2020	<1	Publication

Appendix B

Paper Publication

APPENDIX B

PAPER PUBLICATION

6/12/24, 12:30 PM RV College of Engineering, Bangalore, India. Mail - Acknowledgement @ HRV Analysis and AI & ML; A Comprehensive Rev…

RV College of Engineering®

LAXMI HANAMANT KORBU <laxmihanamantk.ei20@rvce.edu.in>

Acknowledgement @ HRV Analysis and AI & ML; A Comprehensive Review

Mail <editor.bmr@alliedjournals.org> Thu, Jun 6, 2024 at 12:35 PM
To: LAXMI HANAMANT KORBU <laxmihanamantk.ei20@rvce.edu.in>, laxmihk2002@gmail.com

Dear Dr. **Laxmi Korbu,**

Warm Greetings….

Sorry for disturbing you in your busy schedule

As mentioned earlier would like to inform you that your submission entitled **HRV Analysis and AI & ML; A Comprehensive Review** has been accepted for the Publication from **Editorial** and **Reviewer** team

Would like to have your confirmation about the charges as Actual fee was 2300 Euros we can process it for 1000 Euros

If you have any concern about the fee kindly let us know as we're under final stage of Article formatting

Looking forward to your positive reply and the opportunity to work with you

Kindly revert back to us

Best regards

Managing Editor

Biomed Res (0970-938X)

On 2024-05-27 17:11, LAXMI HANAMANT KORBU wrote:

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