ECG Lab Report

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October 27th, 2024

*Abstract*

This report presents the development and validation of an ECG biopotential amplifier using the Arduino Nano Every to explore heart rate variability influenced by exercise, breathing techniques, and emotional stimuli. Experiments were conducted to test hypotheses on heart rate responses to varying physical and psychological conditions, utilizing statistical methods like ANOVA and mixed-effects models. Significant findings include heart rate increases with exercise intensity and distinct spikes from acute emotional stress (jump-scare), validating the device’s effectiveness in capturing cardiac activity, using cheap and accessible electronic parts.

**I. Introduction**

Electrocardiograms (ECGs) are vital diagnostic tools used to analyze the heart’s electrical activity and can effectively monitor heart rate and identify various heart diseases, such as arrhythmias, myocardial infarction, and cardiomyopathy, which manifest as irregularities in the ECG waveform. The electrical activity is captured from the skin's surface, where it presents an amplitude range from 0.5 mV to 5 mV and a frequency of 0.5 to 100 Hz. Due to the weak nature of this signal, ECG devices incorporate gain and filtering components to amplify electrical activity and minimize noise interference.

A diagram of a circuit

Description automatically generatedThe objective of this lab is to construct an ECG device capable of detecting the electrical activity of the heart, using a microcontroller and a biopotential amplifier circuit. The amplifier circuit was initially tested with the Texas Instruments TechPoint Cardio ECG Simulator to ensure safety and functionality. Following verification, the device was employed to collect real heart signal data from participants. Additionally, hypothesis testing was conducted under various conditions, including different exercise durations, breathing methods, and a jump-scare test, to evaluate their effects on heart rate (HR). We hypothesize that subjects’ exercise levels have a significant and negative impact on HR (slower HR), while subjects’ sleep level has a significant and positive impact on HR (faster HR).

**II. Methods**

a. *Biopotential Amplifier Circuit Design*

Refer to the lab manual for an in-depth description of the circuit design [1]. In this lab, we use the Arduino Nano Every microcontroller, INA126 instrumentation amplifier, LF353 operational amplifier, and various capacitors and resistors for filtering. The biopotential amplifier circuit was constructed by combining these circuit components in series: the INA126 set-up as a differential amplifier, a high-pass filter to block DC noise, an active low-pass filter using the LF353, and another inverting active low-pass filter. The final inverting low-pass filter was constructed with resistors and capacitor values such that the cutoff frequency ( was as close to 320Hz and with a gain of approximately 33. Eq. 1 shows the equations used to solve component values:

Eq. 1. (a) Cutoff frequency equation to derive the capacitance of the filter, . (b) Gain equation determined by the ratio of over

Based on the cutoff frequency and Eq. 1 (a), a resistor-capacitor pair of 10nF and 47kΩ was selected, which yielded an actual cutoff frequency of: . From there, was chosen based on and the desired gain. According to Eq. 1. (b), . The closest value resistor available to the lab was 1.5kΩ and was thus chosen. This meant that the gain of our active low-pass filter was not 33, but 31.33 instead.

The schematic of the final low-pass filter with a cutoff frequency of 338.6 Hz and a gain of 31.33, according to the component values, is shown in Fig. 2.

Fig. 1. Circuit diagram of the final active low-pass filter

The circuit diagram of the entire biopotential amplifier circuit is shown below:

A diagram of a circuit

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Fig. 2. Circuit diagram of the entire bio amplification circuit, annotated to specify the differential amplifier, high-pass filter, and active low-pass filters components used in the circuit.

The input signal going into the circuit is typically 1mV, while the desired amplified signal is 7V, requiring a gain of the circuit to be approximately 7000. The gain of the entire biopotential amplifier circuit is equivalent to the gain of the separate components of the circuit, multiplied, and is described by Eq. 2.

Eq. 2. (a) Total gain of the circuit, where G1 is the gain of the instrumentation amplifier, G2 is the gain of the middle active low-pass filter, and G3 is the gain of the final active low-pass filter. (b) G2 is calculated using a non-inverting gain equation and with the specified component values. (c) G1 is derived from Eq. 2. (a) and (b) and found to be 6.79 to achieve a total gain of 7000.

Setting the gain, G1, of the instrumentation amplifier is dependent on RG, and is described by Eq. 3., found in the INA126 specification sheet.

Eq. 3. Gain setting equation found in the INA126 specification sheet

RG was calculated to be 44.7kΩ, however, the closest resistor value available in the lab was 47kΩ and was used instead, giving a , and the total gain of the circuit is then: .

b. *ECG Data Collection*

After fabricating the circuit and performing safety testing and verification on the ECG device, HR data was collected on participants. First, the effect of exercise was tested by using the ECG to measure HR during 6 different exercise durations: resting, 5, 15, 30, 60, and 120 seconds. Next, the effect of breathing behavior on HR was tested under 4 different physiological states, induced by: resting, box breathing, withholding breath, and a jump scare. Additional variables of interest that were hypothesized to affect heart rate were recorded for each participant, including day-of caffeine intake, weekly exercise level, and amount of sleep over the past 72 hrs. For each experimental condition, a sample was taken long enough to record 6 distinct peaks in the ECG signal. HR for each sample was calculated by measuring 6 individual peak-to-peak distances and then averaged. Data was stored in .xlsx files and long-formatted to allow for Python-based statistical analysis. Files can be found in the Data section of the Appendix.

c. *Statistical Analysis*

Repeated measures ANOVA was conducted on exercise\_data.xlsx (found in the Data section of the Appendix) to determine the effect of the different exercise durations on subjects’ heart rate. To incorporate co-factor analysis, exercise and sleep level responses from subjects were divided into discrete categories: 0-4 hrs/week was described as “low” fitness, while 4-8 hrs/week was described as “high” fitness. For sleep, 15-18 hrs, 18-21 hrs, and 21-24 hrs over a 72hr period was described as “low”, “moderate”, and “high” for sleep levels. Assumptions of sphericity, normality, and homogeneity of variance of the data were tested using the Mauchly’s Test, Shapiro-Wilk, and Levene’s, respectively. Bonferroni corrected pairwise t-tests within exercise duration groups were conducted post-hoc to determine significant relationships. In addition, two separate mixed-model ANOVAs to test between-subject factors were conducted, including one for weekly exercise levels and one for sleep levels.

For the next experiment, a mixed-effects linear model was used to fit heart rate data with co-factors: treatment (box breathing, holding breath, or jump-scare), exercise level, and sleep level, using breathing\_data.xlsx. For this statistical analysis, exercise and sleep responses were kept continuous. Assumptions testing of the linear model was performed, including a Shapiro-Wilk test for normality, plotting residuals for homoscedasticity, and checking for multicollinearity. To evaluate the effect of co-factors specifically on subjects’ resting HR, additional linear regression models comparing the relationship between exercise level and HR, and sleep level and HR, were performed and plotted.

All statistical analysis was done using statsmodels, pingouin, and scipy.stats libraries in Python. Refer to the Code Section in the Appendix to learn more about the exact methods used.

**III. Results**

a. *ECG Sampling*

After safety validation, the ECG was tested on a human subject and a snapshot of the ECG signal was taken on the oscilloscope:

A close-up of a graph

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Fig. 3. Snapshot of the ECG signal, showing the PQRS and T characteristics of the peak. PR Interval, PR Segment, ST Segment, and QT Interval are also depicted.

b. *Effect of exercise duration on HR*

For repeated measures ANOVA testing effect of exercise duration on HR, sphericity (p-value= 0.06), normality (p-value = 0.,10, 0.20, 0.44, 0.61, 0.60, 0.98 for resting, 5s, 15s, 30s, 60s, 120s groups, respectively, and homogeneity (p-value = 0.06) was confirmed. Though independence of observations cannot be verified due to the nature of our experimental design, we will assume that criteria are met for our statistical analysis.

Repeated measures ANOVA results (F Value =23.90, degrees of freedom, numerator DF=5, denominator DF = 55, p-value < 0.00001) indicate a significant effect of exercise duration on HR. Post-hoc pairwise t-testing:

|  |  |  |  |
| --- | --- | --- | --- |
| Duration A | **Table of Significant Pairwise Comparisons** | | |
| Duration B | T-stat | Corrected p-value |
| 120s | 15s | 4.365 | 0.0169\* |
| 120s | 5s | 5.523 | 0.0027\*\* |
| 120s | Rest | 6.484 | <0.001\*\*\* |
| 15s | 5s | 4.142 | 0.0246\* |
| 15s | 60s | -4.327 | 0.0180\* |
| 30s | 5s | 4.552 | 0.0124\* |
| 30s | Rest | 7.917 | <0.001\*\*\* |
| 5s | 60s | -5.718 | 0.0020\*\* |
| 60s | Rest | 7.975 | <0.001\*\*\* |

Table 1. Pairwise comparisons from repeated measures ANOVA results that had a significant relationship, all other pairwise comparisons were omitted from the table.

Mixed-model ANOVA for exercise levels: (SS2 = 10192, df1 = 1, df2 = 8, MS = 10192, F = 6.89, p-value = 0.03). and Mixed-model ANOVA for sleep levels: (SS2=9189, df1=2, df2=7, MS=4594.7, F=3.11, p-value= 0.108)

c. *Effect of breathing patterns and jump-scare on HR*

HR data across different treatment groups was visualized with a box plot:

A diagram of a heart rate distribution by treatment

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Fig. 4. Box plot of each treatment group (resting, box breathing, holding breath, and jump-scare), showing minimum, maximum, lower and upper quartile. Circle in the holding breath group indicates a lower bound outlier, a star in the jump-scare group indicates a significant effect on HR compared to resting.

A mixed-effect linear regression model was obtained:

A screen shot of a computer

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Fig. 5. Model results from Python terminal, (y-intercept = 70.51, p-val < 0.001). Coefficients: (Tresting = -0.404, p-val = 0.77, Thold\_breath = -1.75, p-val = 0.396, Tjump\_scare = 9.23, p-val = 0.041), (exercise = -2.97, p-val = 0.045, other co-factor model effects are non-significant)

After performing the mixed-effect linear regression model, residuals were plotted to test for normality of the model:

A graph showing a line with blue dots

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Fig. 6. Residuals of the model’s predicted values to actual values, as a test for normality of the model’s predictive behavior.

**IV. Discussion**

The primary objective of this lab was to construct an ECG device capable of effectively capturing and amplifying the heart's electrical signals using an Arduino Nano Every and a custom biopotential amplifier circuit. The findings from our experiments provide significant insights into the impacts of various exercise and breathing treatments, as well as external patient factors such as fitness levels and sleep levels, on heart rate, supporting our hypotheses with statistically significant results.

Our experiments began by evaluating the effect of exercise on heart rate. As hypothesized, heart rate increased with the duration of exercise, which was confirmed by a highly significant p-value (<0.00001) from the repeated measures ANOVA (see Fig. 2) for multiple pair-wise comparisons, most notably comparisons between resting HR and 30s, 60s, 120s exercise durations. These results align with the established knowledge that increasing durations of exercise increases HR, indicating that our ECG device was effective in capturing the expected increases in heart rate due to increased cardiac demand through exercise.

Interestingly, the mixed-model ANOVA revealed that variations in weekly exercise levels also significantly affected heart rate (SS2 = 10192, F = 6.89, p-value = 0.03), suggesting that greater exercise levels of a patient can influence heart rate responses to acute exercise. This underscores the importance of considering individual fitness levels in monitoring and predicting HR and heart health in patients.

The second part of our analysis focused on the effects of different breathing patterns and a jump-scare on heart rate. Our mixed-effects linear model indicated that the jump-scare significantly increases heart rate compared to the resting condition (y-intercept = 70.5, coefficient = 9.23, p-value = 0.041), which was visually supported by the data shown in Fig. 4. This means that, from a baseline of 70.5 BPM (y-intercept), the jumps-scare effect increases heart rate by 9.23 BPM. This finding supports our knowledge that the sympathetic nervous system can trigger physiological stress upon activation, including increased HR, as reflected in our statistical analysis. Other interesting inferences from our model include the effect of exercise (coefficient = -2.97, p-value = 0.045), which means that exercise has a significant and negative effect on HR, affecting HR by -2.97 for each hour increase in a patient’s weekly exercise. Interestingly, the effect of sleep suggest a positive effect on HR (coefficient = 0.882), which contradicts our hypothesis that decreases in sleep would decrease patients’ HR. However, p-value for this statistic is: p-value = 0.223, meaning that this is not a significant effect. More tests with more subjects should be done to increase our statistical power to explain this factor.

Furthermore, our ECG circuit design, which incorporated multiple stages of amplification and filtering, showed a robust ability to handle wide ranges of signals with minimal noise interference, as evidenced by the clear ECG signal depicted in Fig. 3. However, the gain achieved (31.33 instead of the targeted 33) highlighted a limitation in the precision of component values and availability of materials in the lab.

Challenges in the lab included ensuring consistent skin contact for signal acquisition and managing the variability of biological responses across different subjects, as well as maintaining a consistent ECG signal that was without heavy spikes or fluctuations. Despite these issues, our device successfully met the experimental goals, and the hypotheses were supported by the analyses provided.

In summary, the experiments conducted, and the analyses performed have not only validated our ECG device's functionality but also enhanced our understanding of heart rate and possible co-factors affecting it. Further research could explore additional variables such as patient response of stress levels, dietary influences, or quantitative reporting of caffeine levels, on HR potentially using more mixed-effect models to analyze interactions between multiple factors affecting HR.

**IV. Conclusion**

In this lab, we developed and tested an ECG biopotential amplifier, hypothesizing that exercise and sleep levels would significantly impact heart rate variability. Our findings confirmed that heart rate increased with exercise duration but was inconclusive sleep levels’ effect on HR. Additionally, our tests revealed that HR showed variance with different breathing techniques and spiked significantly in response to a jump-scares. This also demonstrates the device's effectiveness in capturing nuanced changes in cardiac activity. Notably, the analysis also revealed that individuals' habitual exercise levels significantly moderate the effect of exercise duration on HR, illustrating how personal fitness can influence HR responses and heart health. This experiment not only validated our custom-built ECG device as a valuable educational and experimental tool but also highlighted the critical role of precise component selection and experimental setup in obtaining reliable data and heart physiology insights.

**References**

[1] Duke BME 354 Lab 3 Protocol. Overview, Spring, 2019.

**Appendix**

Code

Code can be accessed through my BME354L repo: <https://github.com/chef-spf/BME354L>

Data

Exercise\_data.xlsx:

<https://d.docs.live.net/a55b1ce7a7a8bace/Documents/BME354L/EKG%20Lab%203/exercise_data.xlsx>

Breathing\_data.xlsx: <https://d.docs.live.net/a55b1ce7a7a8bace/Documents/BME354L/EKG%20Lab%203/breathing_data.xlsx>