A cost effective non-invasive method to measure the cardiac and respiratory rate for rodents

A Project Report by
Sanket B. Mashakhetri

Master of Technology

in

Electrical Engineering (EE6)
(Integrated Circuits and Systems)

at

Indian Institute of Technology, Madras
Department of Electrical Engineering
April 2019

Project Guide: Dr. Ganapathi Krishnamurthi

Table of Contents

ACKNOWLEDGMENTS

Special thanks to Dr. Ganapathi Krishnamurthi for supporting and offering your expertise and accountability. It was a pleasure working over the course of the project duration.

Special thanks to Ph.D Research Scholar, Ms. Swathi Lakshmi for meeting weekly, guiding and supporting throughout the project duration as well as providing weekly feedback on the progress and results.

Special thanks to Lab assistant of Mechatronics Laboratory, Mr. Ashok for assisting with encouragement as well as some of the breadboarding, providing components, soldering for the surface-mount IC’s on the PCB’s.

ACRONYMS AND ABBREVIATIONS

ECG	Electrocardiogram
ADC	Analog to Digital Converter
MATLAB	Matrix Laboratory (Software)
AD	Analog Devices
INA	Instrumentation Amplifier
Hz	Hertz (Frequency unit)
mV	milli Volts (Voltage units)
BPM	Beats Per Minute
dB	decibels
OPAMP	Operational Amplifier
DAQ	Data Acquisition
AI	Analog Input
GND	Ground
kΩ	kilo Ohms
CMRR	Common Mode Rejection Ratio
LCD	Liquid Crystal Display
RLD	Right Leg Drive

ABSTRACT

Animal testing plays a vital role in biomedical research. Some of the common studies using the small animals are drug delivery, disease progression, pathology treatment. Monitoring the animal is important in preclinical studies. In vivo electrophysiology remains a suitable method to monitor cardiac activity; however, surface electrocardiogram (ECG) monitoring remains complicated in the case of small animals. Here, we designed a low cost non-invasive method for monitoring the heart rate and breath rate of non-restrained, non-sedated laboratory mice. The design has been tested under different scenarios to capture the heart activity of mouse and rat. The beating heart generates an electric signal that can be used as a diagnostic tool for examining some of the function of the heart. The electric potentials generated by the heart appear throughout the body and on its surface. We determine potential differences by placing electrodes on the surface of the body and measuring the voltage between them. Different pairs of electrodes at different locations generally yield different voltages because of the spacial dependence of the electric field of the heart. In conclusion, this restrained ECG monitoring technique is a well-suited tool for exploring various aspects of cardiac electrophysiology in a wide variety of small animals including very young mice.

List of Figures

List of Tables

Chapter 1

Motivation:

The goal of the project was to develop an electrocardiogram (ECG) demonstration board for checking the health condition of the anesthatised rodents like rats and mice. An electrocardiogram (ECG) is a piece of electronic medical equipment that measures and displays the electrical activity associated with the heart. The measured cardiac signals are commonly used for diagnosis and understanding of patient conditions in the medical and research fields. One of the challenges in developing ECG systems lies in the fact that bio-potentials measured at the surface of the skin have low amplitude and are mainly low in frequency (fundamental below 3 Hz with spectral content up to 200 Hz). This requires precise filtering and low-noise amplification. Another challenge lies in the fact that the ECG bio-potentials measured from the heart are differential in nature as they are measured on opposite polarities of the cardiac muscles. A typical QRS-complex ECG waveform is a superposition of many physical actions in the body's cardiac muscles (See Figure 1).

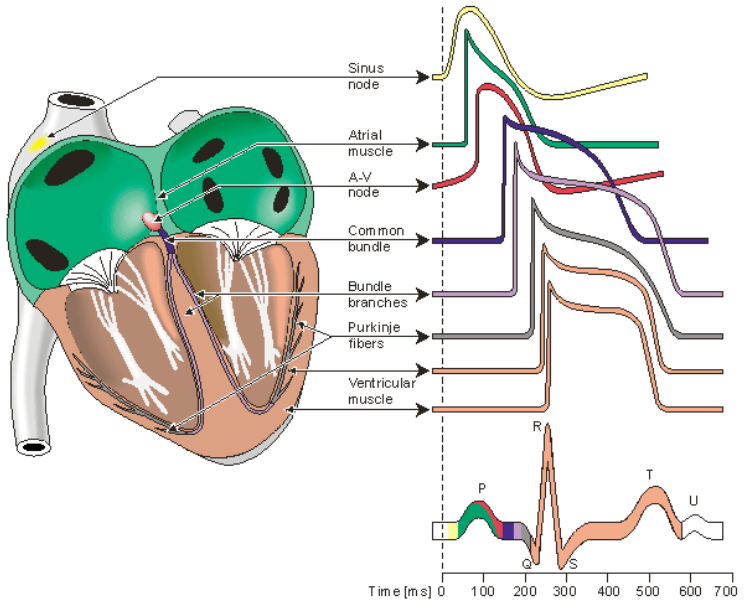


Figure 1: Superposition of action potentials that produce ECG signal

The composition of these activities occurring in time, relate to a variety of valuable ECG frequency spectrum across the lower frequency bands. The usable spectral content in a typical ECG signal falls between 0.3 Hz and 100 Hz. Because of this, most commercial ECG systems are designed with that bandwidth. I had been asked

to design the ECG monitoring circuit to measure the health condition of the anesthetized rodent.

Objective:

The desired specifications and main objective was to create a reliable analog-front end circuit. The circuit was specified to run from a portable battery as well can use the system (computer) to power it from arduino board. The signal integrity for the output signal needed to be good to have a clear enough ECG signal to show up on the screen after storage of the ECG data.

System configuration:

Anesthetized gently and firmly restrains the subject

- 3 electrode pads record up to 3-lead ECG
- Integrated amplifier
- USB link to computer through Arduino

Introduction:

The first phase of the study is done with the simulation of the circuitry needed to measure the ECG signal. The second phase included the layout of circuit performance on the breadboard to confirm practical circuit performance and function. The third phase included the analysis and interpretation of the obtained data and plotted ECG graphs. During each phase, came across many difficulties and necessary changes were made to steer the strategy and direction of the project. Tried out various designs to make the circuit, firstly with basic operational amplifier LM741 (OPAMP) and then using precision instrumentation amplifier (AD624), also check with the AD8232 which is designed for humans works well with rodents also. The AD8232 was chosen to provide high common-mode rejection of noise present in ECG applications. Another feature of the AD8232 is its low power operation, which includes quiescent currents under 150uA. To provide a low power voltage supply for the analog circuit, another. This report has been written for the purpose in documenting the detailed steps taken throughout the design of the ECG monitoring circuit for small animals.

It was discussed that, because the circuit is being used to monitor the health condition of the rodent it was crucial that the circuit was reliable and measured ECG signals accurately and clearly. In order for the overall system to work well, the signal integrity needed to be very high as well which required using low-noise

components such as AD624 precision instrumentation amplifiers. Using the AD8232 for appropriate bandwidth, the circuit's output contained very little noise when subsequent testing was performed. Other design considerations for cost optimization were also included while making design decisions.

Data analysis:

Acquisition is performed using PuTTY SSH client USB recording software and Post-acquisition analysis are performed in MATLAB software:

- Basic heart rate analysis
- Respiratory rates and duration, breathing depth

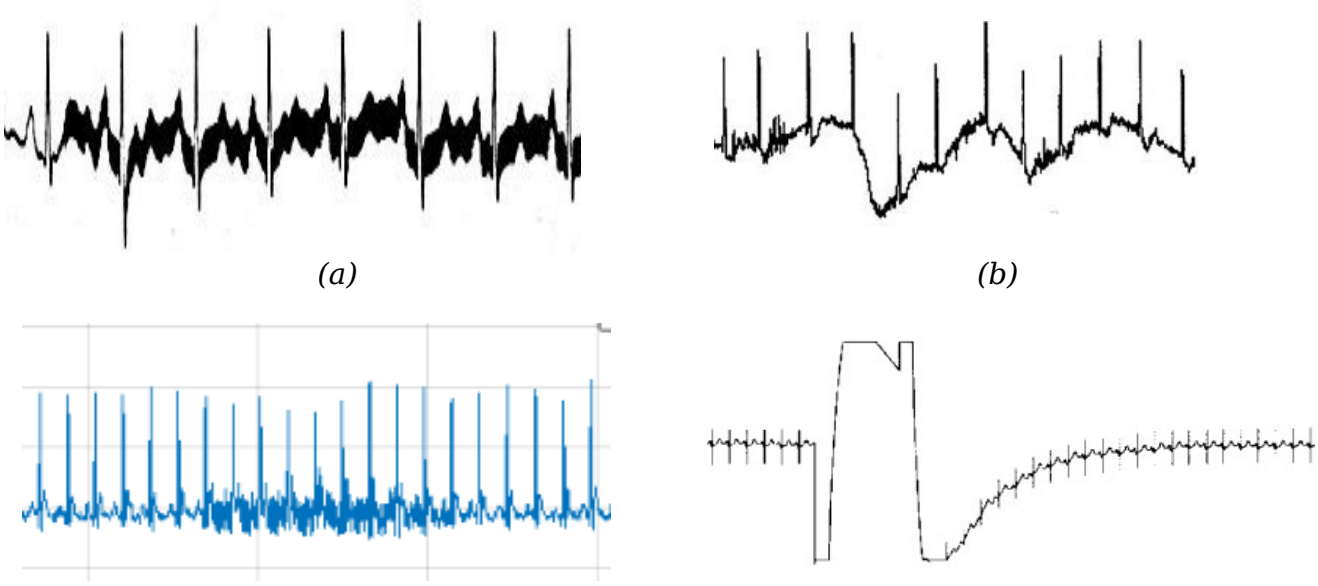
Common Problems in ECG:

There are many factors that must be taken into consideration in the design and application of the electrocardiograph. So, we must know few of the common problems encountered and its causes.

Baseline Wandering, as shown in Figure 2(b). This occurs due to motion of the electrodes or of the patient during respiration. The mean axis of the wave drifts because of the motion. Third, the trembling of the signal caused by muscle tremors. This noise has a varied frequency and generally occurs in older animals.

High-frequency distortion rounds off the sharp corners of the waveforms and diminishes the amplitude of the QRS-complex. And for low-frequency distortion, baseline is no longer horizontal, especially immediately following any event in the tracing.

High offset voltages at the electrodes or improperly adjusted amplifiers in the electrocardiograph can produce saturation or cutoff distortion that can greatly modify the appearance of the ECG. The combination of input-signal amplitude and offset voltage drives the amplifier into saturation during a portion of the QRS-complex. The peaks of the QRS-complex are cut off because the output of the amplifier cannot exceed the saturation voltage.



(a) *(b)*

(c) *(d)*

Figure 2: (a) A 50 Hz power-line interference. (b) Baseline Wandering. (c) Electromyographic interference on the ECG. (d) Effect of a voltage transient

A major source of interference when one is recording or monitoring the ECG is the electric-power system. Electric-field coupling between the power lines and the electrocardiograph and/or the rodent under test is a result of the electric fields surrounding main power lines and the power cords connecting different pieces of apparatus to electric outlets. It is illustrated in figure 2(a).

Chapter 2

Circuit Simulations (Trials on different designs approaches):

In the beginning of the project various designs were tried out before going for the final ECG design such as using,
1) Simple 1-operational amplifier (op-amp) ECG design, LM741 with the digital filtration method.(see figure)
2) Precision Instrumentation amplifier (AD624) which has variable and high gain with very high common mode rejection ratio. Connected to the noise filtration circuit.(See figure 3)
3) Signal conditioning integrated circuit AD8232 with the Arduino-UNO board (has been described separately in details in **Chapter 3** as it is a main design).

After analysis of the requirements, I began to develop and the first step in the design process was to simulate the system sub-circuits to understand their function and impact towards the total system behavior. I used the LTspice IV circuit simulation software to do so.

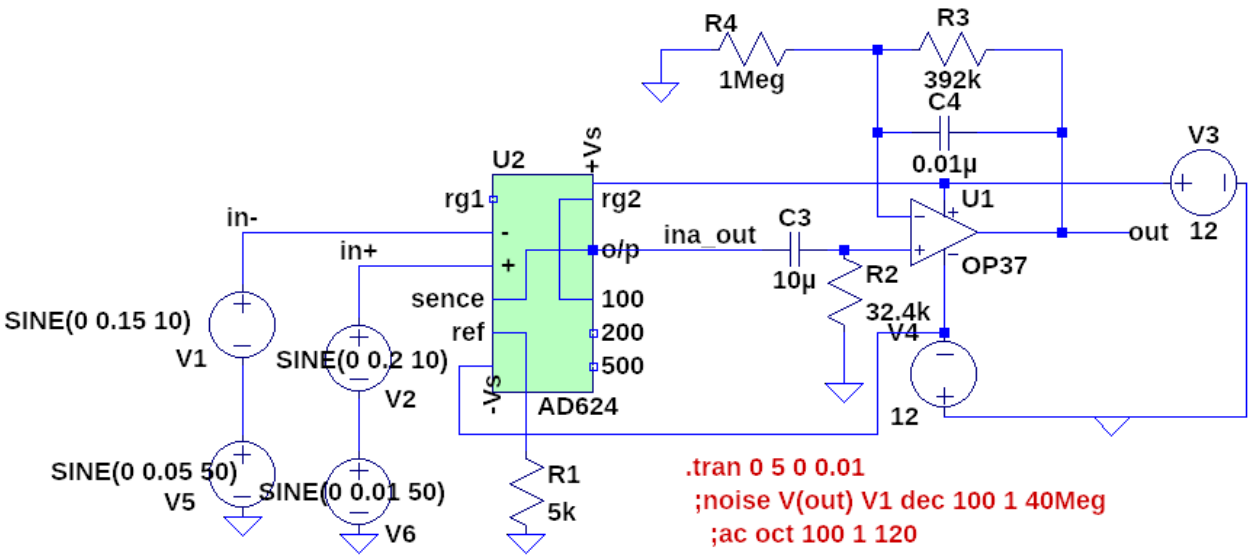


Figure 3: Circuit setup to see the gain, CMRR performance of AD624, a precision instrumentation amplifier and the output filter performance as well.

The sub-circuits simulated towards the beginning of the project were the input low-pass RC filter network, the AD624 gain and transient performance, and the output filtering. Figure 4 shows LTspice simulation circuit for the AD624 used to get the

high gain and high common mode rejection ratio (CMRR). Output filter performance is also tested in this.

The raw bio-signal that is directly extracted from the rodent's body is very small in amplitude (~1-4 mV) and hence amplification becomes crucial. An Instrumentation Amplifier (AD624) is used for this purpose. AD624 has high CMRR of 130dB (cancels any common-mode signals efficiently). It also has low internal noise, Pin programmable gains, and is primarily designed for low level transducers, hence is suitable for the purposes of this project. Figure 3 shows the circuit configuration.

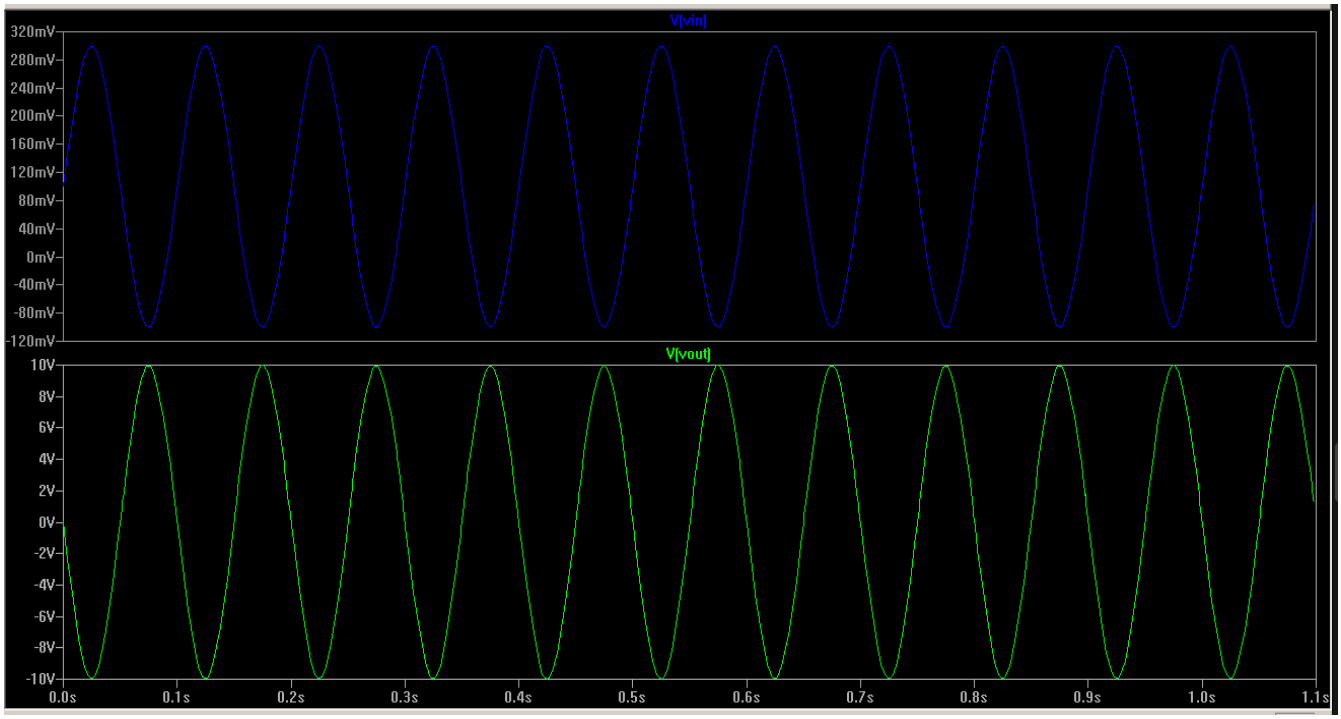


Figure 4: Input and output voltage waveform of the AD624 representing the very high gain and high CMRR

Another trial circuit explored was the simple one Op-amp ECG circuit (Figure 5). This circuit does not have any output filter circuit. Whereas the filtering is taken care by the digital filter (it is basically python code run on the windows software).

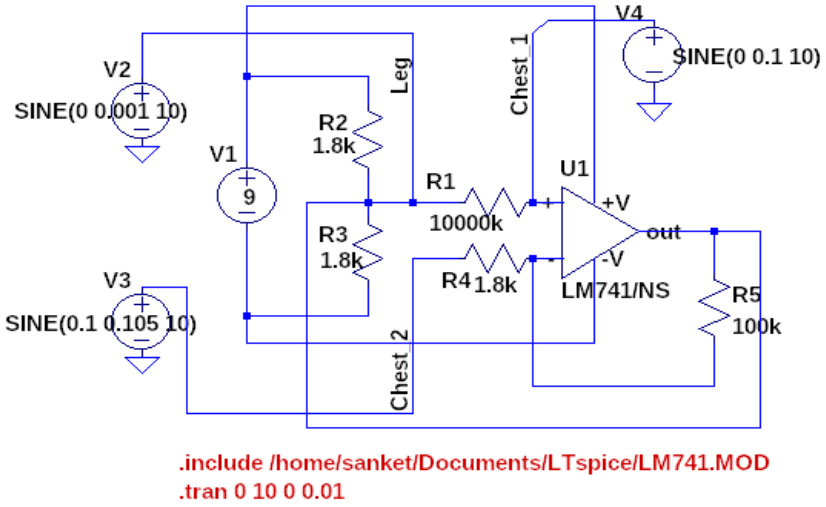


Figure 5: Simple ECG circuit with only one operational amplifier.

The ECG signal at the output is actually interfered with the power supply frequencies (50Hz mains). The simulation schematic and its ECG result after digital filtration of the 50Hz signal is shown below in Figure 5 and 6 respectively.

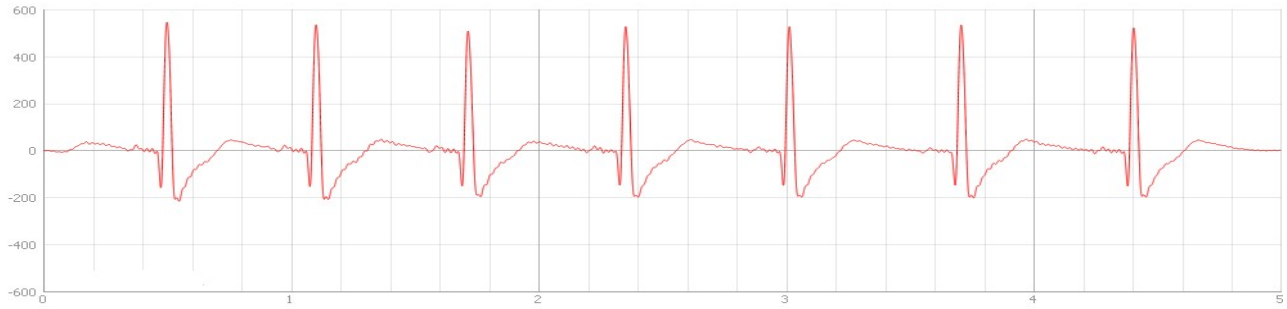


Figure 6: The Digitally filtered (50Hz) electrocardiograph output of the simple one op-amp ECG circuit for human.

This shows that QRS-complex is clear but the P-wave and T-wave are barely visible and that to for human, so we decided not to use it for the purpose of taking ECG of the small animals. This circuit has one more problem, it is very much sensitive to the external noise and motion artifacts.

Bread-boarding the Circuit:

Prior to decide which design would be suitable, we built and tested as much of the sub-circuits as possible using a breadboard in the labs. The AD642 was placed on a DIP adapter as shown in figure 8 to allow the surface mount IC to be placed in circuit and tested using through-hole passive components. In the circuit on the breadboard, the OP37 op-amps were replaced with readily available LM741 op-amps. The goal in putting the circuit together was to test and confirm the functionality of the reference circuits. The circuit was powered using a 9V external battery. The circuit was tested and measured to confirm the lab measurements with the results and performance seen in the LTspice simulations. Figure 7 shows the result of the breadboard test circuit. The sub-circuits placed on the breadboard included the input RC network, the AD624 precision instrumentation amplifier, active low-pass output filter.

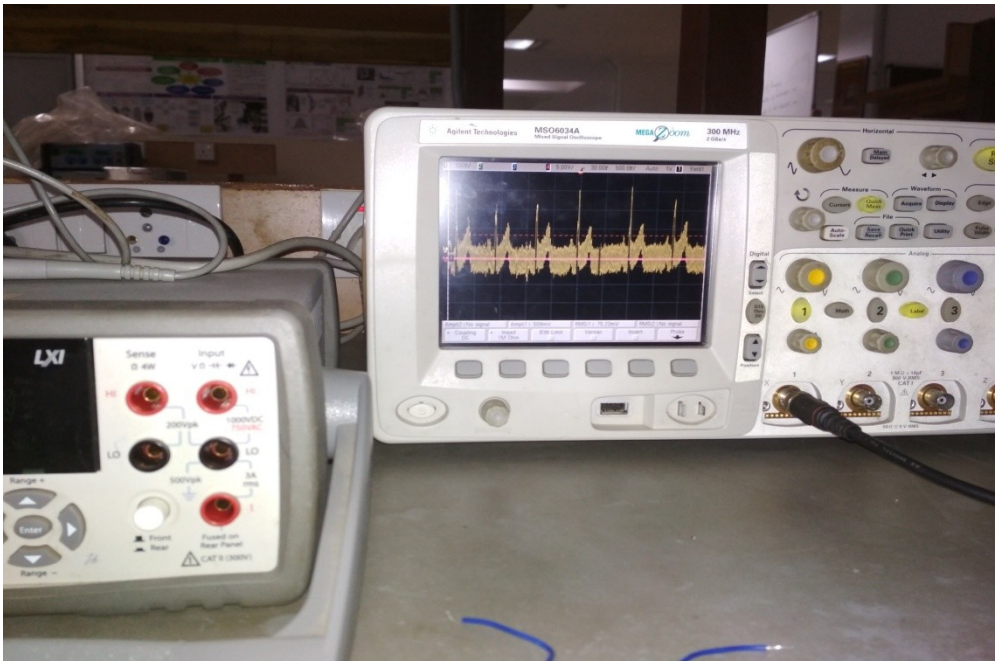


Figure 7: Result of the ECG circuit (AD624) on breadboard, testing on the CRO

A bandwidth from 0.7 Hz to at least 20 Hz was necessary to retain a clear and recognizable ECG output signal. This bandwidth confirmed the theoretical analysis and research of the required bandwidth for the ECG circuit. The gain was also adjusted using the adjustable RG terminal (see circuit in figure 3) on the AD642 circuit and by adjusting the pass band gain of the active output LP filter. After a clear output signal was found, the output signal integrity was then observed while tuning the gain of the system to approximately 54dB, which allowed the signal to

swing fully positive and negative between the rails without saturating the op-amps throughout the signal path. Figure 7 shows the result of the breadboard test setup using the oscilloscope (CRO). The ECG signal quality is shown on the oscilloscope and which clearly shows line frequency interference to a large level. The main disadvantage of this design is, it is not immune to external noise.

Figure 8: IC AD624 on board and whole ECG circuit with electrodes

Study Limitations:

Mice are sensitive to even modest handling and transport. The ECG indices we measured may reflect physiologic responses to the experimental environment relative to its home cage. It is encourage about 5 min acclimation period prior to recording data to attenuate effects consequent to handling and transport. Usually mice establish contact between 3 electrodes and 3 limbs within 5 minutes after acclimation to record a continuous ECG for approximately 2 seconds. The duration of the ECG recording should be considered in the interpretation of heart rate variability. The age, gender and strain variations in heart rate may reflect age, gender and strain variations in physiologic responses to transport, handling, and adaptation to repeated measurements. Yet, the inter-strain differences in heart rate we non-invasively obtained after ECG recordings of short duration are in agreement with invasive experimental techniques intended to monitor heart rate in mice as caged. Our measurements were made in daytime hours, disrupting the less active phases of the mouse circadian cycle. Future innovation might incorporate an array of conductive electrodes into the animals' cages to eliminate the effects of handling and transport, and perhaps enable timed recordings.

Chapter 3

Materials and Methods:

a) Experimental Setup:

Following figure 9 shows the experimental setup for the acquisition of the ECG signals. The ECG signals generated from the cardiac movement in the rodent is sensed by the transducers in the form of electrodes, the electrode pair are made up of Ag-AgCl. We use 3-lead ECG system and the 3-electrodes are connected at the paws of the rodent as shown in the figure below. The first electrode is connected to the right arm(RA), second is connected to the left arm (LA) and the third is connected to the right leg (RL). To have better electrical contact of the electrodes to the body pads with the transparent electrolytic gel is placed in between electrodes and the paws. This arrangement of the electrodes carries the ECG signal with some added noise coming from the various sources of the noise like motion artifact, mains power supply, voltage transients etc. Now this signal is proceed through the signal processing ECG circuit which consist of the amplifier with high gain and high CMRR, filters to pass the required frequency components only which is related to the cardiac motion and suppress other. Then this filtered and processed signal is given to the Arduino board as analog input through Ao pin and this allows us to see the data amplitude with respect to time and also the real time plot of this data on the monitor/laptop itself.

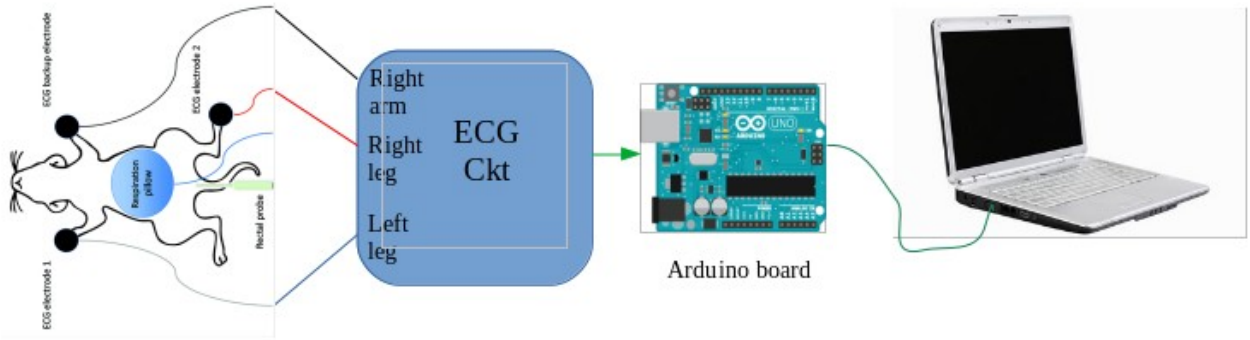


Figure 9: Flowchart of the Working of the proposed ECG monitoring method

But to further process and analyze this data, it is stored in the computer with help of the one USB serial data acquisition software, PuTTY SSH client. This software continuously record the data at the rate 230400 which is quite sufficient for the good resolution and thereby further analysis using MATLAB software tool.

b) Circuit Diagram (main design):

Here we are basically using amplifier and filtration circuit. Firstly we amplify the RFI filtered signal coming from the electrodes LA and RA using the instrumentation amplifier(IA) which has very high gain (100) and high CMRR (80dB) as well.

It is designed to extract, amplify, and filter small bio-potential signals in the presence of noisy conditions, such as those created by motion or remote electrode placement. This design allows for an ultra-low power analog-to-digital converter (ADC) or an embedded microcontroller to acquire the output signal easily. The circuit can implement a two-pole high-pass filter for eliminating motion artifacts and the electrode half-cell potential. This filter is tightly coupled with the instrumentation architecture of the amplifier to allow both large gain and high-pass filtering in a single stage, thereby saving space and cost.

To improve common-mode rejection of the line frequencies in the system and other undesired interferences, the AD8232 includes an amplifier for driven lead applications, such as right leg drive (RLD). It consists of a specialized instrumentation amplifier (IA), an operational amplifier (A1), a right leg drive amplifier (A2), and a midsupply reference buffer (A3). In addition, it includes leads off detection circuitry and an automatic fast restore circuit that brings back the signal shortly after leads are reconnected.

The AD8232 contains a specialized instrumentation amplifier that amplifies the ECG signal while rejecting the electrode half-cell potential on the same stage. This is possible with an indirect current feedback architecture, which reduces size and power compared with traditional implementations.

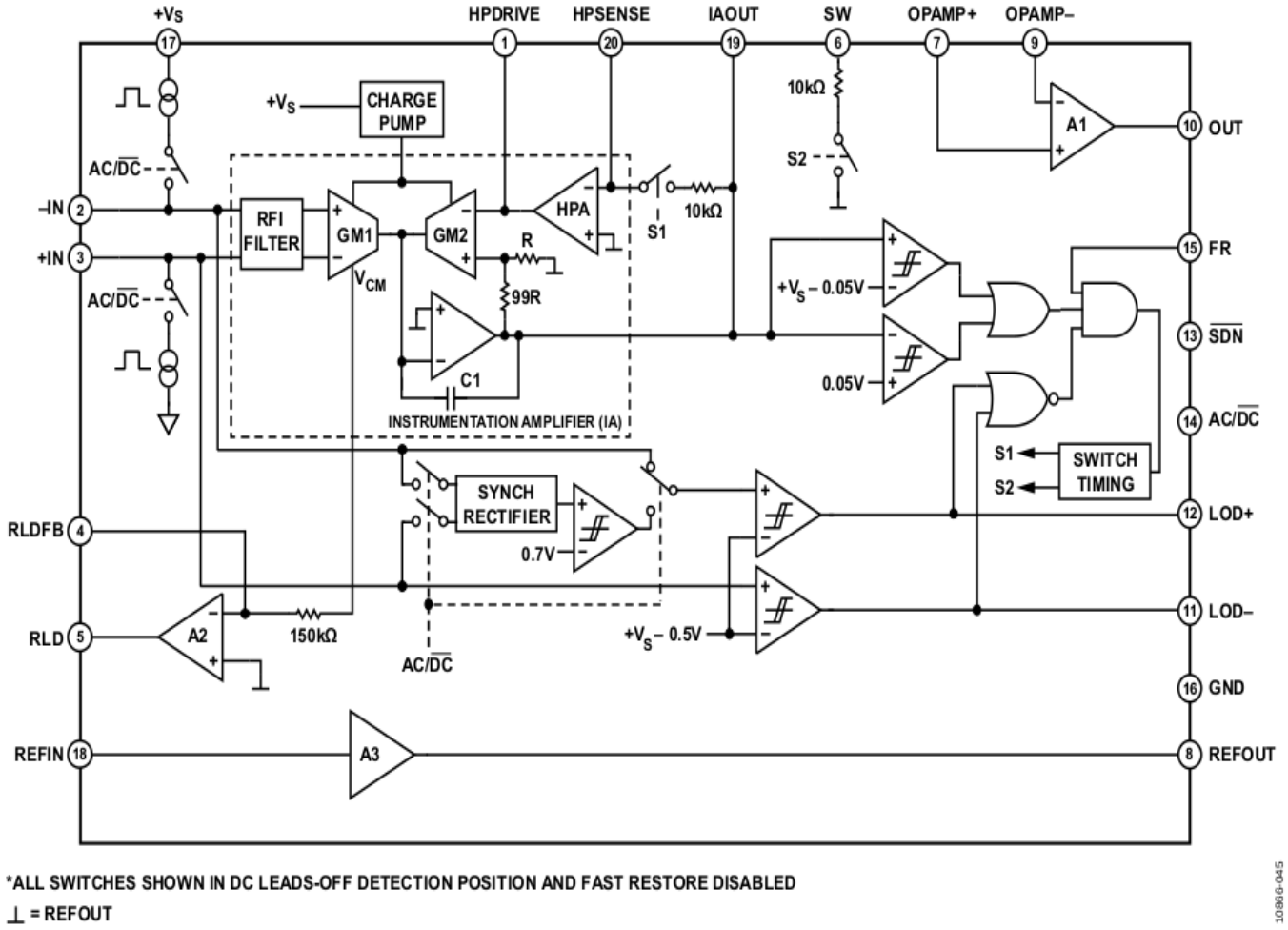


Figure 10: The circuit diagram of the bio-potential signal amplifier and noise filtration

The instrumentation amplifier is shown in Figure 0 as comprised by two well-matched transconductance amplifiers (GM1 and GM2), the dc blocking amplifier (HPA), and an integrator formed by C1 and an op amp. The transconductance amplifier, GM1, generates a current that is proportional to the voltage present at its inputs. When the feedback is satisfied, an equal voltage appears across the inputs of the transconductance amplifier, GM2, thereby matching the current generated by GM1. The difference generates an error current that is integrated across Capacitor C1. The resulting voltage appears at the output of the instrumentation amplifier.

The feedback of the amplifier is applied via GM2 through two separate paths: the two resistors divide the output signal to set an overall gain of 100, whereas the dc blocking amplifier integrates any deviation from the reference level. Consequently,

dc offsets as large as ±300 mV across the GM1 inputs appear inverted and with the same magnitude across the inputs of GM2, all without saturating the signal of interest.

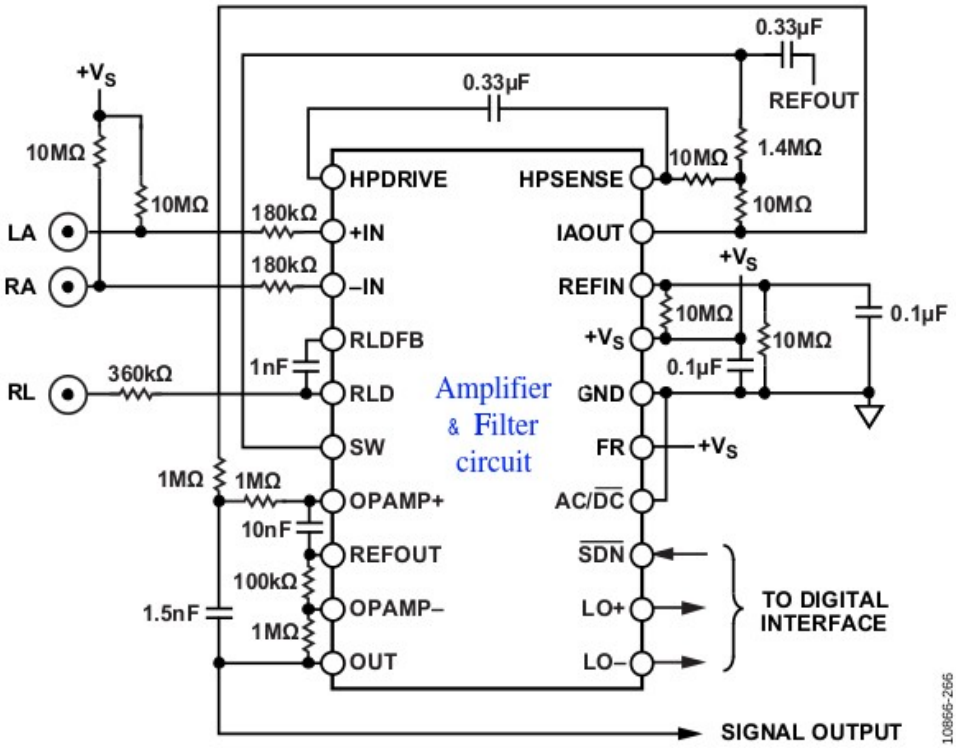


Figure 11: Circuit diagram for the ECG and Respiratory rate measurement using above bio-potential signal amplifier circuit

To increase the common-mode voltage range of the instrumentation amplifier, a charge pump boosts the supply voltage for the two transconductance amplifiers. This further prevents saturation of the amplifier in the presence of large common-mode signals, such as line interference. The charge pump runs from an internal oscillator, the frequency of which is set around 500 kHz.

This general-purpose operational amplifier (A1) is a rail-to-rail device that can be used for low-pass filtering and to add additional gain. The following sections provide details and example circuits that use this amplifier.

The right leg drive (RLD) amplifier inverts the common-mode signal that is present at the instrumentation amplifier inputs. When the right leg drive output current is injected into the subject, it counteracts common-mode voltage variations, thus improving the common-mode rejection of the system. The common-mode signal

that is present across the inputs of the instrumentation amplifier is derived from the transconductance amplifier, GM1. It is then connected to the inverting input of A2 through a 150 kΩ resistor.

An integrator can be built by connecting a capacitor between the RLD FB and RLD terminals. A good starting point is a 1 nF capacitor, which places the crossover frequency at about 1 kHz (the frequency at which the amplifier has an inverting unity gain). This configuration results in about 26 dB of loop gain available at a frequency range from 50 Hz to 60 Hz for common-mode line rejection. Higher capacitor values reduce the crossover frequency, thereby reducing the gain that is available for rejection and, consequently, increasing the line noise. Lower capacitor values move the crossover frequency to higher frequencies, allowing increased gain. The trade-off is that with higher gain, the system can become unstable and saturate the output of the right leg amplifier.

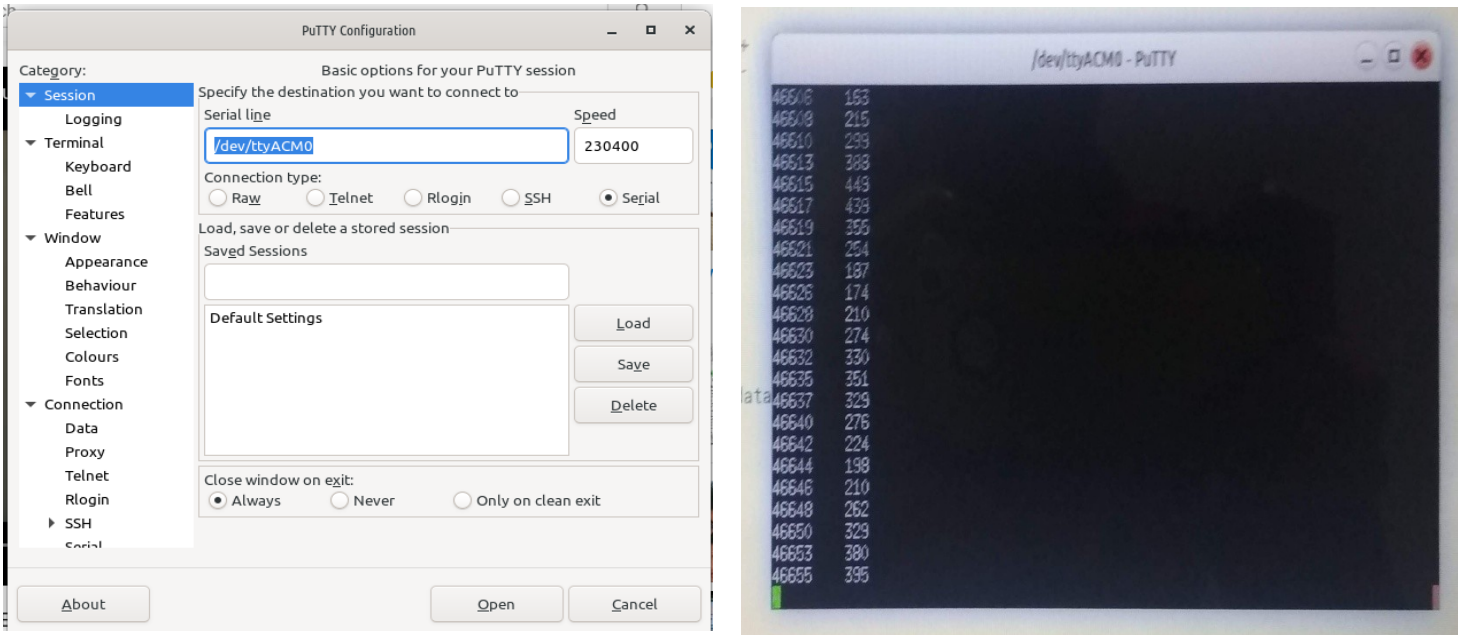
Note that when using this amplifier to drive an electrode, there should be a resistor in series with the output to limit the current to be always less than 10 µA even in fault conditions. For example, if the supply used is 3.0 V, this resistor should be greater than 330 kΩ to account for component and supply variations.

Experimental Studies:

a) ECG recording:

Mice were gently removed from their cages and positioned on the ECG recording platform. An array of gel-coated ECG electrodes were embedded in the floor of the platform and spaced to provide contact between the electrodes and animals' paws. For adults, the spacing between electrodes was 3 cm, and for nurslings, the spacing was reduced to 2.5 cm. Filter paper, with openings for the electrodes, prevented mouse urine from short-circuiting the signals. The electrodes were connected to an amplifier by a shielded 3-electrode lead set. Since even modest handling of mice may induce alterations in heart rate, each mouse was permitted to acclimatize for 10 min prior to collection of baseline data. The signals were digitized at a sampling rate of 230400 (baud rate). When mice were sitting or otherwise positioned such that the paws were not in contact with three electrodes, the output from the amplifier was discarded. Only data from continuous recordings of 20-30 ECG signals (sometimes more) were used in the analyses. This data is being stored in the system using USB recording software called PuTTY SSH client. Then the data is transmitted to the MATLAB software for further manipulation and analysis. Figure 12(a) shows the PuTTY software interface and 12(b) shows the

software recording the ECG signal amplitude in 2nd column with respect to the time in 1st column (in mili seconds) at rate of 230400. That is maximum possible.



(a) (b)

Figure 12: (a)PuTTY SSH client software interface. (b) Software while recording ECG data.

b) Data Analysis:

Here from the obtained data we are claiming that this ECG circuit for rodent is able to find both BPM (heart beats per minute) and Respiratory rate of the rat/mice (rodent in general). Following flowchart shows the algorithm used to calculate the BPM and the respiratory rate of the mice (See Figure 13).

Each signal was analyzed using MATLAB software. The software uses a peak detection algorithm to find the peak of the R-waves and to calculate heart rate. Heart rate variability was calculated as the mean of the differences between sequential heart rates for the complete set of ECG signals. Since the T-wave is not separate from the QRS in rodent ECGs, there have been discrepancies in the definition of the QT interval and reported values. While the breath rate is obtained by tracing the envelope of the ECG signal. As it is claimed that the envelope or the sinusoidal variation in the ECG is actually belongs to the breathing rhythm of the rodent. So, we call the envelope as respiratory signal.

The MATLAB code used for data analysis that is heart rate and respiratory rate calculation is attached at the end in the appendix-I.

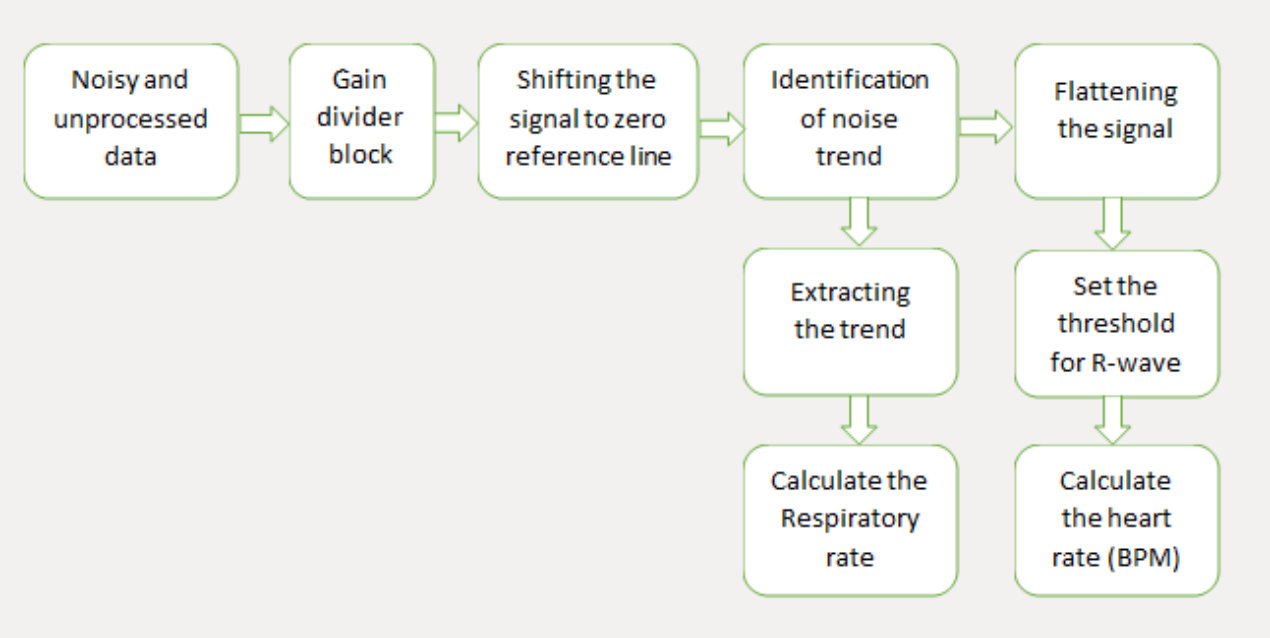


Figure 13: Algorithm used to calculate the BPM and Respiration rate

Actually the ECG signal is on the top of the respiratory signal. So, we abstract both the signal using the signal processing techniques in MATLAB software. Firstly the stored acquired data is taken in then(fig A) the Gaussian curve fitting polynomial is used to trace the envelop over which the ECG signal is seating. This envelop corresponds to the Respiratory rate(Fig B). Now the signal is divided by the gain of the amplifier and the offset is removed so that you will get the fig C. Finally the envelop is subtracted from the data to get the flattened ECG signal(fig D). So, now to measure the heart BPM the R-waves are marked and calculate the number of R-waves per minute. Which will corresponds to the heart rate (BPM) of the anesthetized rodent.

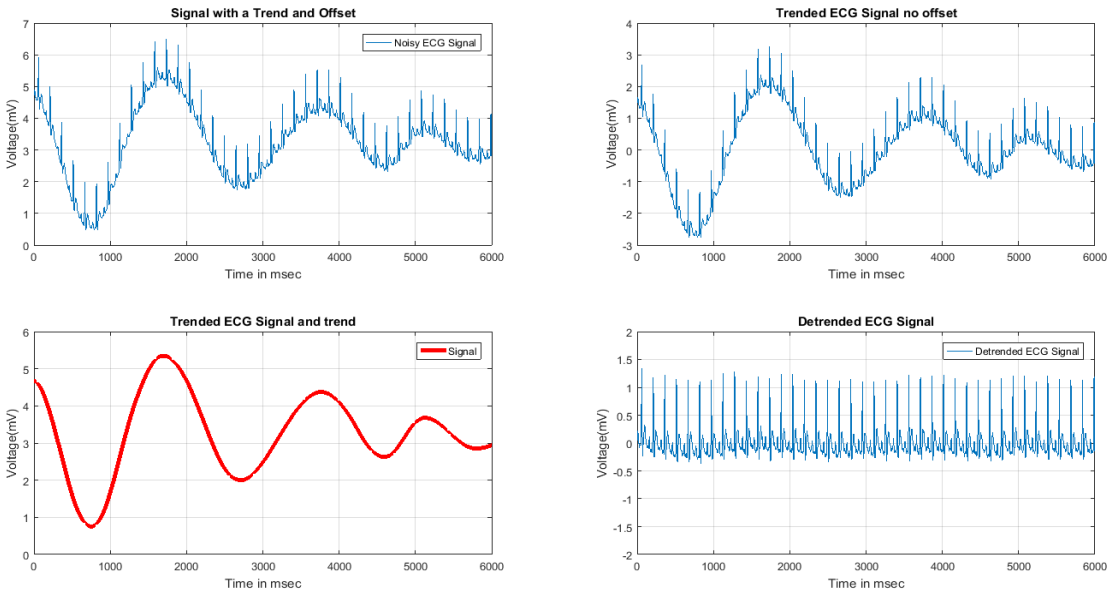


Figure 14: MATLAB signal processing of the data obtained in the excel format. (A) Noisy and unprocessed signal directly from the obtained data, (B) finding the trend of the noise(red), (C) off-setting and dividing the amplitude by the gain of the amplifier(100), (D) flattened final data to calculate the BPM.

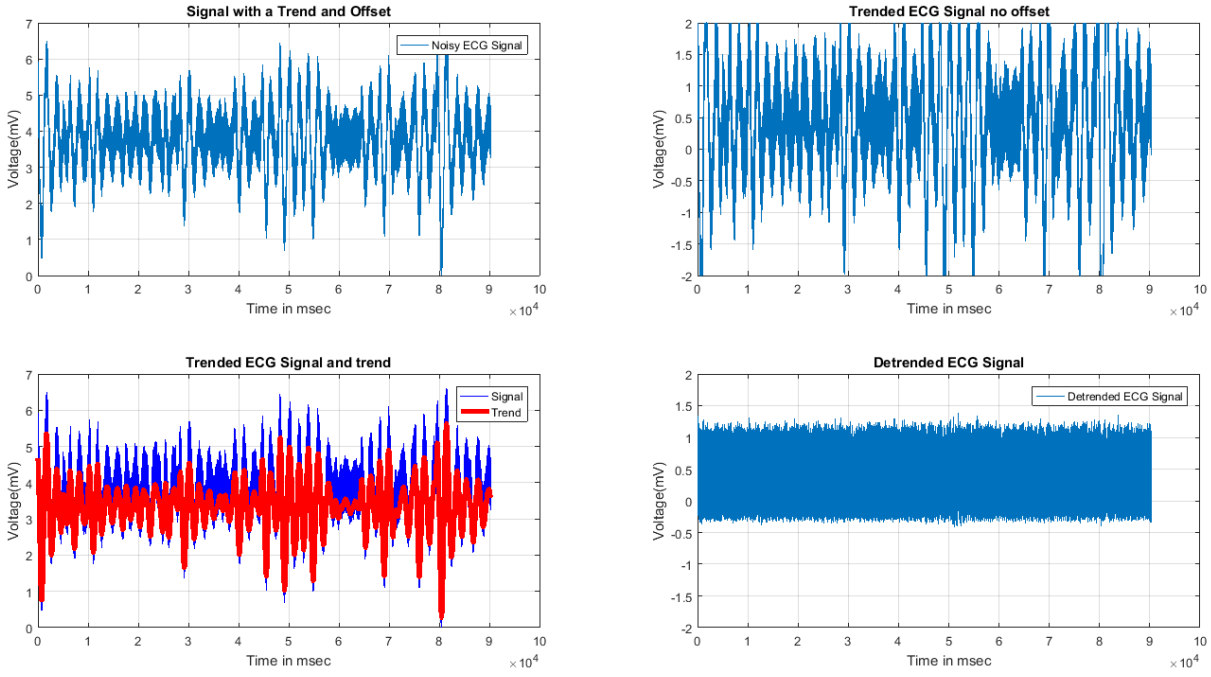


Figure 15: Various algorithm performed Noisy ECG signal on MATLAB software to get the final flattened ECG signal for 90 seconds of data

If ECG signal is being plotted for the data taken directly from the ECG circuit that is being designed the plot will look like figure 16.

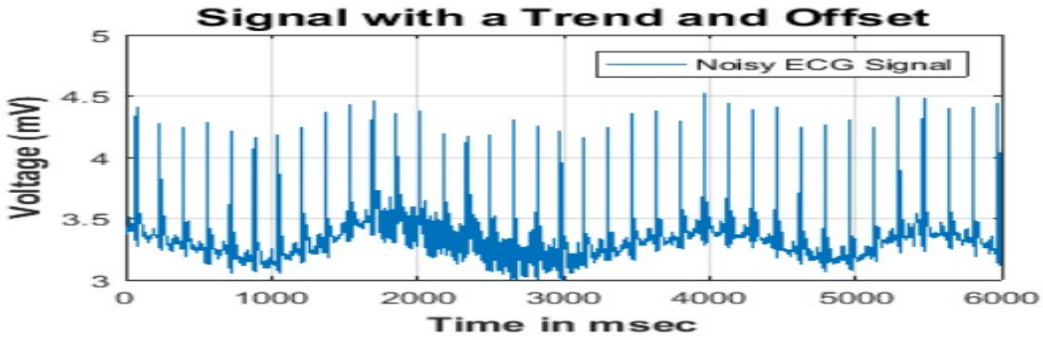


Figure 16: Original ECG signal

Then we perform the trend/envelope detection algorithm on the original signal to get the trend of the motion artifact which is basically the result of respiration, that is movement of the chest due inhalation and exhalation of the rodent. Following figure 17 shows this trend on the top of original signal in red color.

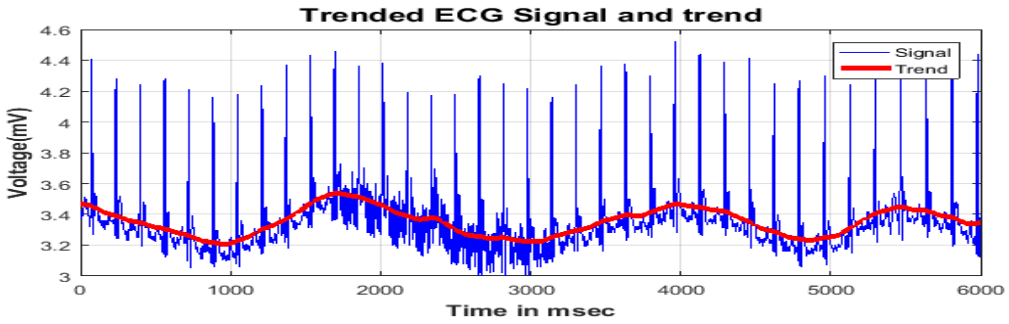


Figure 17: Trend of noise signal on the top of original signal

Now we remove the offset of the original signal and remove the gain by dividing the amplitude by the gain of the ECG circuit which is 100. The following figure shows the same.

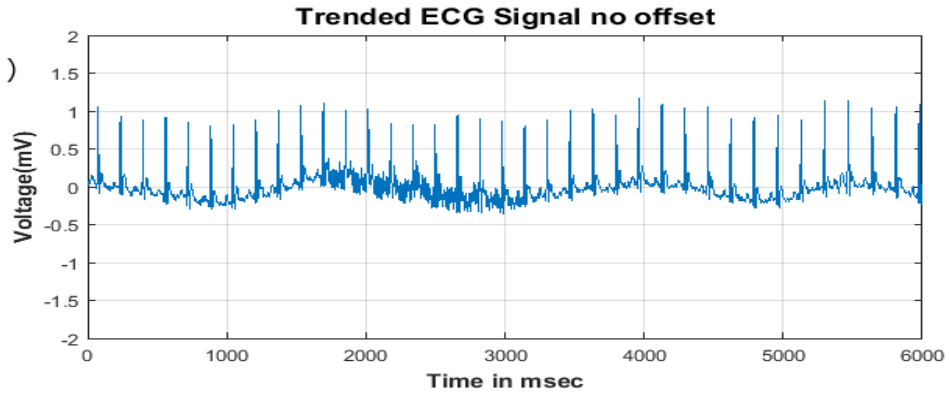


Figure 18: Zero offsetting of the original signal with gain adjustment

Now we make the above signal flatten by removing the trend we obtained by literally subtracting it the trend from the original signal to get the signal as shown in figure 19.

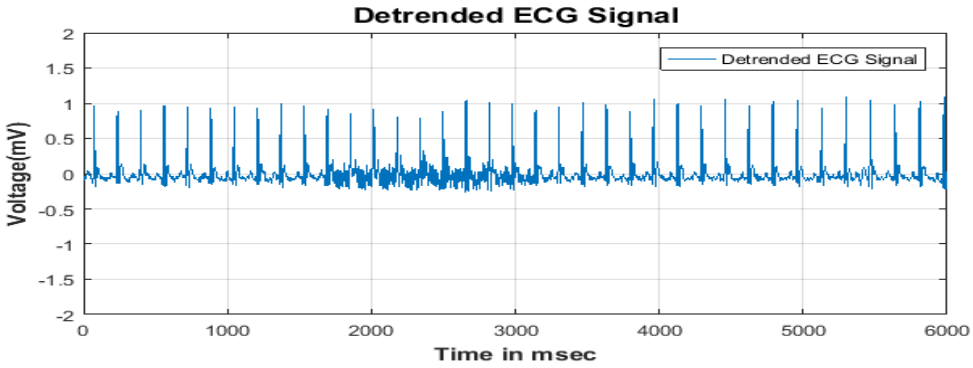


Figure 19: Flattening of the signal to apply the R-wave detection algorithm

As we got the data flatten now R-wave detection algorithm can be applied on it. From this we can calculate the breath rate of the rodent by directly calculating the number of the R-waves in the 60 seconds/ 1 minute of data and can be expressed in bits per minute (BPM). Following figure 20 shows the detected R waves. It is basically sees the how many times the wave crosses the threshold value.

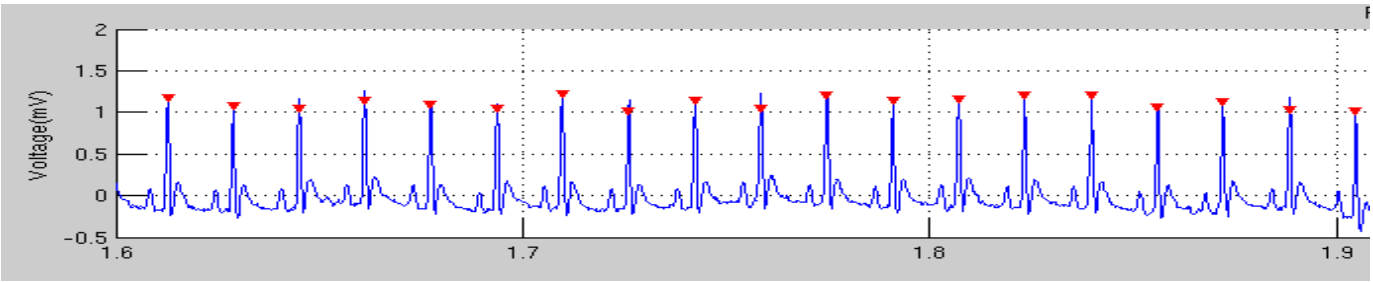


Figure 20: R-wave detection

Result:

These are the results tabulated (shown in Table 1) for various condition of the rodent like for mice and rat in the anesthetized, controlled and burn medical conditions. These states are recorded and the respective heart rate and respiration rate are measured using MATLAB tool post-recording of the ECG signal.

Figure 21 shows the various aspects of the ECG signal obtained in one of experiment. From this we can see PQRST-wave clearly and thereby calculate the duration of the different intervals like P-wave, PR-wave, QRS-complex, QT-wave, T-wave, etc. This intervals gives different information about the health condition of the rodent or human being. Here the P-wave corresponds to the atrial contraction, QRS-complex corresponds to contraction of the ventricles and T-wave corresponds to the relaxation of the ventricles. If the duration of these waves goes far away from the normal it may lead to some cardiac health issues. Not only the duration but also the relative amplitudes of the P-wave, R-wave and T-wave gives some health information of heart. For example the relative increase of the P-wave amplitude (compared with T-wave) indicate hypokalemia. It can also indicate right atrial enlargement. A P wave with decreased amplitude can indicate hyperkalemia.

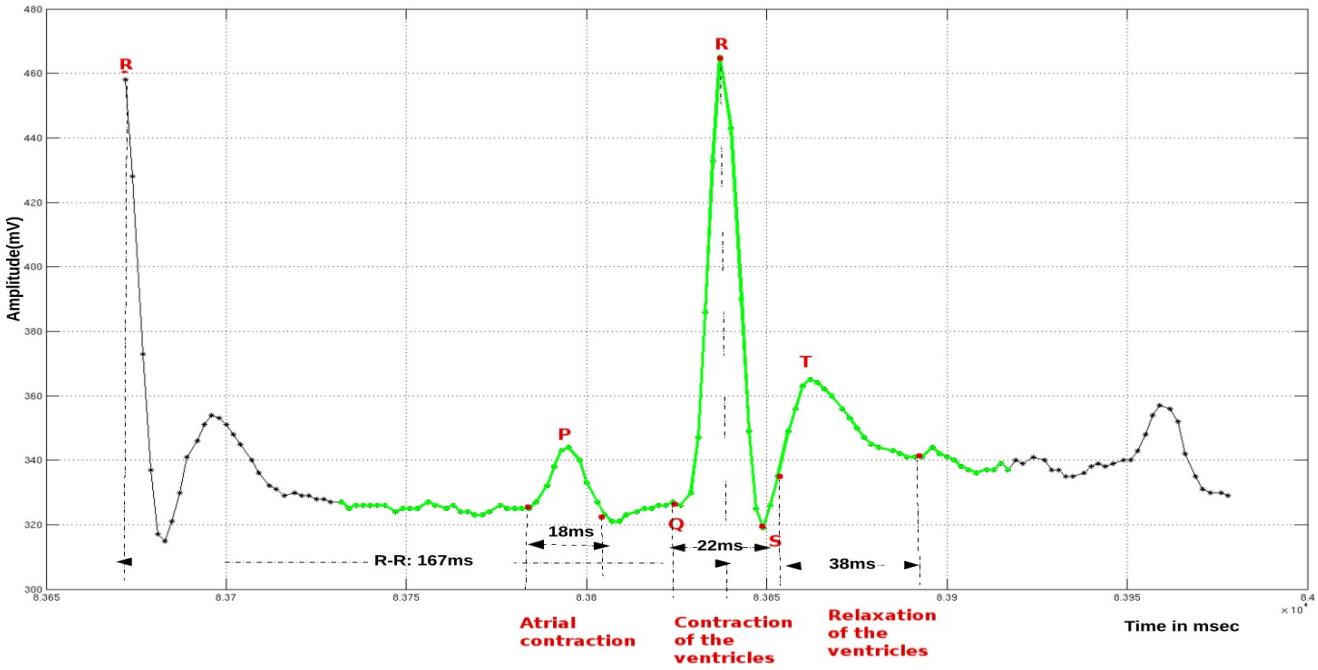


Figure 21: Zoomed in representation of a ECG signal of a rat for better understanding of various time intervals

PR interval: 0.045 sec, QRS-complex: 0.022 sec, QT interval: 0.064 sec

The following figure 22 shows the resulting ECG signal and various signal waveform after various signal conditioning performed on the original ECG signal. The 1st signal is the original ECG signal for more than a minute, 2nd one is trace of the original signal from 16 sec to 22 sec. While 3rd is one on which the R-wave detection algorithm is performed to measure the heart rate (BPM).

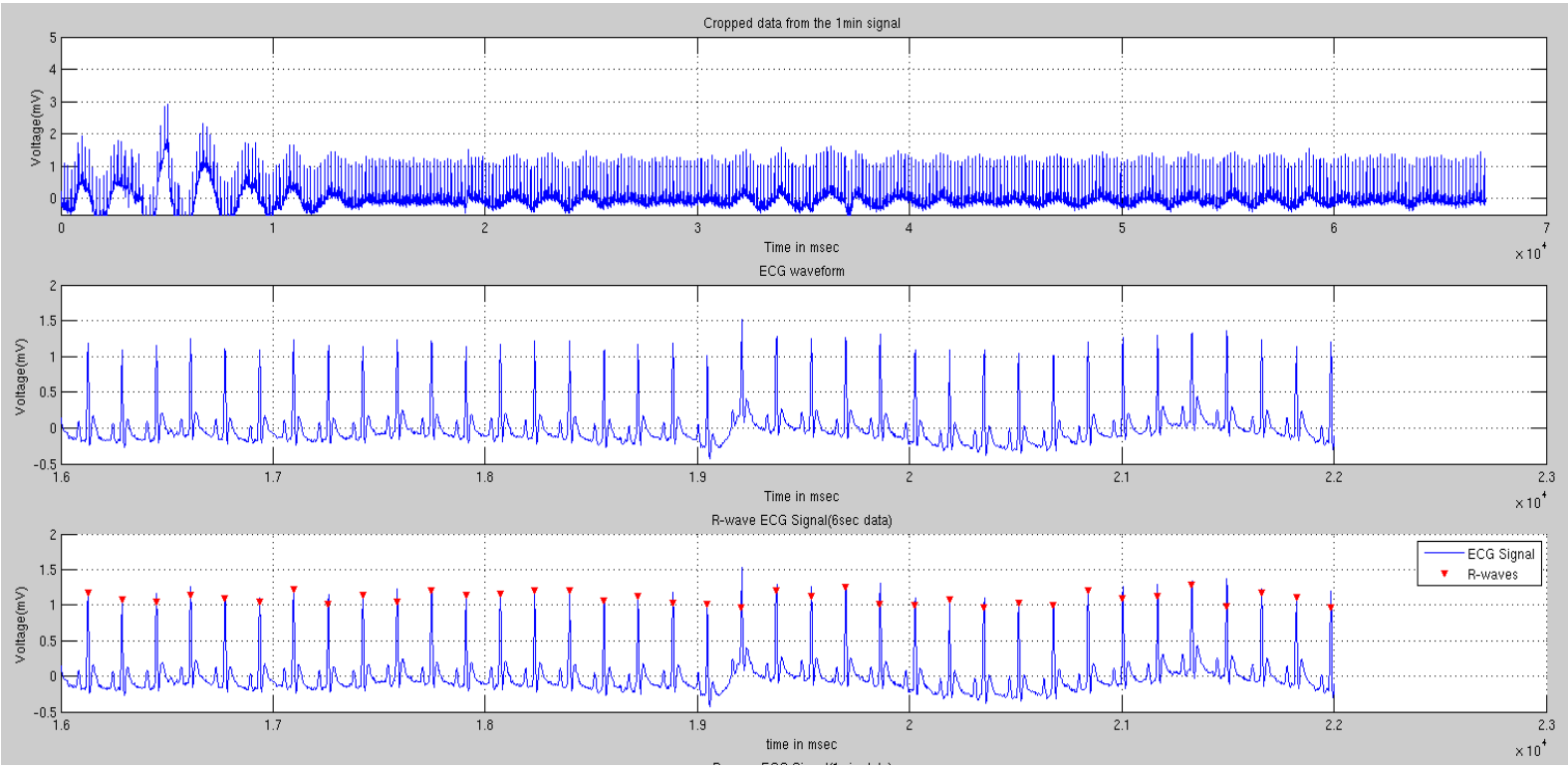


Figure 22: Result obtained from the ECG recorded data, signal processed using MATLAB software. Third graph shows the R-wave detection

The figures for different ECG signals and after MATLAB signal processing will be shown separately at the end of this report.

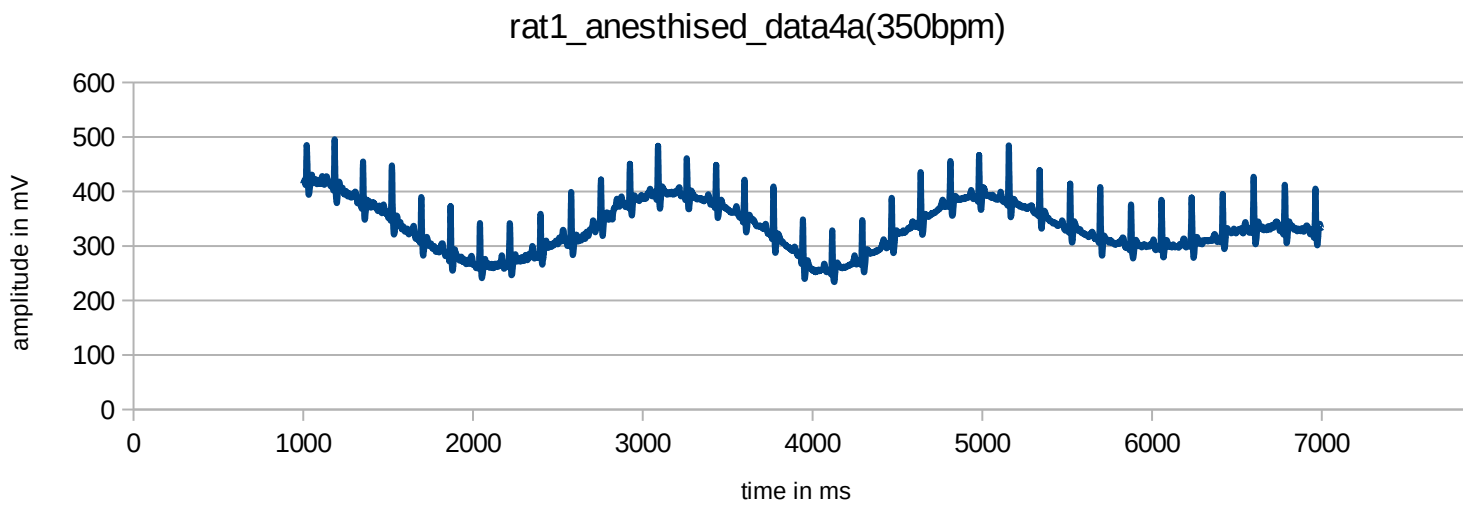


Figure 23: Baseline wandering with some other noise observed when data plotted for a rat

Rodent	Weight (gram)	ECG Parameters (ms)			R-R interval (ms)	Beat Rate (bpm)	Breath Rate (bpm)
		P (ms)	QRS (ms)	T (ms)			
Rat1	339	22	15.5	12.5	162	369	35
Rat2	351	17.5	16	24	158	363	31
Rat3	342.8	11	15	19	167	356	34
Rat4	368.3	14.5	15	23.5	196	304	31
Rat5	312	10	11	9	171	417	28
Rat6	347.2	24	17	19	172	346	31
Rat7	353	24.5	15	28.5	176	339	32
Rat8	356	16	20	33.5	151	396	34
Rat9	371	18	22	38	167	357	34
Mice1	23.6	13	15.5	26	173	345	33
Mice2	24	12	17	18	177	334	31
Mice3	24.2	14	8	23	169	353	30
Mice4	25	10	22	11	108	548	40

Table 1: Tabulation of the results obtained in all the experiments performed on mice and rats

Chapter 4

Project Testing:

At the beginning of the design process measurements were taken from the LTspice simulation and breadboard testing to understand the signal amplitude and noise levels present at the differential output from the simulator. The major tests and verification work done was to measure system bandwidth, gain, and signal integrity. The measurements taken and observed allowed to effectively shape the performance and success of the final solution.

Testing System Gain & Bandwidth:

The system bandwidth was an important measurable feature that directly impacted the ECG signal integrity present at the output of the analog front-end circuitry. The ECG analog filtering used in the circuitry created the total system effect of a band pass filter. The two main sub-components, that created the poles and zeros required to roll off the frequency content outside the desired pass band, were the input RC network filtering and the output filtering. The desired bandwidth of the ECG demonstration board was researched and defined to be from 0.3 - 100 Hz. After experimenting with the bandwidth on the breadboard circuit by altering the corner frequencies of the three circuits, acceptable signal integrity was found when using a bandwidth of 0.5Hz - 40 Hz. It was later decided that increasing the bandwidth above 40 Hz increased noise levels present on the signal but also included meaningful spectral content present in the faster occurring events such as the very recognizable R wave ECG spike. The system's bandwidth was measured to compare the actual board measurements with the theory used to select the circuit components. The technique for measuring the bandwidth was to use a function generator to generate a 2 mVpp input sine wave. Because of the differential nature of the inputs to the board, one lead (RA) was connected with the signal generator, while the other (LA) was set to a DC level that matched the level of the RA signal (2.5VDC). This eliminated the common mode offset between the two inputs from being amplified by the precision instrumentation amplifier (IA). The signal was placed at 2.5 V to allow maximum signal swing between the 5V power supply rail and the ground reference. The output amplitude (peak-to-peak) was measured using an oscilloscope (CRO) and the gain (dB) was calculated and recorded. The input signal frequency was swept from 0.01 Hz to 1 kHz and the gain was recorded at intervals along the sweep. Figure 18 shows the bode plot (logarithmic x- axis) of the gain (dB) vs. the frequency swept (Hz). As shown in Figure 24, the -3dB corner

frequencies were measured to be 0.5 Hz and 40 Hz. This was a success in confirming the theoretical system design with the actual results of the circuit.

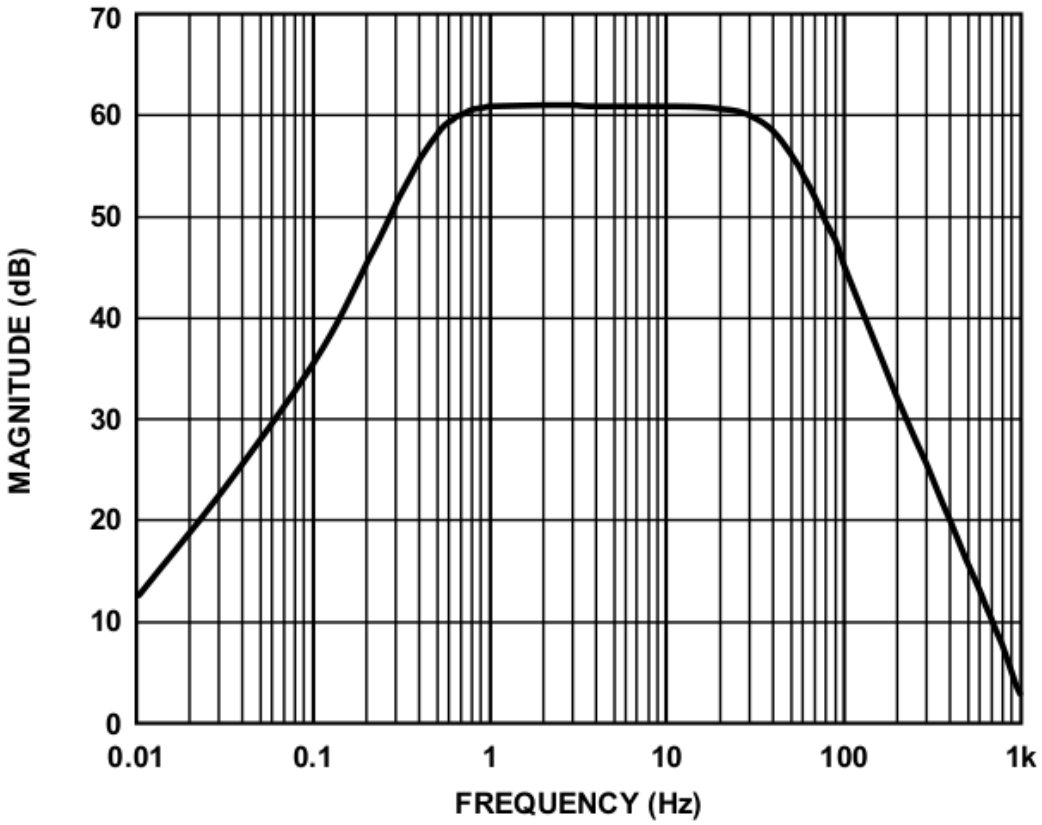


Figure 24: Frequency Response of Cardiac Monitor Circuit (0.5-40 Hz Bandwidth)

In addition to 40 Hz filtering, the op amp stage is configured for a gain of 11, resulting in a total system gain of 1100. To optimize the dynamic range of the system, the gain level is adjustable, depending on the input signal amplitude (which may vary with electrode placement) and ADC input range. The theoretical gain of the circuit was set by the gain of the INA instrumentation amplifier (can be set externally) and by the output filter gain. The gain was set to this value to set the amplitude of the output signal.

Testing the Input electrodes (Sensors/Pads):

A way that the ECG can be taken was by implementing input sensors to allow a rodent's ECG signals to be measured from the chest or paws. Two methods were experimented with and tested. Figure 25 shows one of the way i.e. electrodes placed on the paws of the mice. The electrodes can also be placed on the chest but it has some issue like improper contact. So, we adhere to the electrode placed on the paw which worked well and solved the purpose. To have good electrical contact

of the skin with the electrodes pads are used in between with transparent electrolyte gel put on it. This electrolyte contains Cl- as a principle anion to maintain good electrical contact.

Figure 25: Electrodes placed on the paws of the mice to test the electrodes-skin interface and the electrolyte containing pads and electrodes

Alternatively, we may use an electrode cream which contains Cl- and has a consistency of hand lotion. The electrolyte contributes to the series resistance associated with interface effects of the gel between the electrode and the skin. We can consider the epidermis, or at least the stratum corneum, as a membrane that is semipermeable to ions, if there is a difference in ionic concentration across membrane.

Chapter 5

Summary:

Throughout the semester, we were challenged to design and fabricate a portable ECG for the rodents. We were able to successfully develop a working ECG measurement board that meets and exceeds the specified project requirements. The defined project requirements were to develop the low cost battery-powered analog front-end circuitry. The overall scope of the project included precise amplification and filtering of low amplitude and low frequency bio-potentials. The actualized in the design, layout, and analysis of the analog circuitry needed to do this. We studied the ECG application, and went on to successfully design, test, and analyze the ECG circuit. The major results, found during this iterative design process, helped the us improve the design throughout the semester and ultimately helped the project to obtain quality ECG measurements. We were able to take the specifications and list of requested deliverables development from a theoretical concept to a reliable working product. The major success was in implementing the circuitry and hardware needed to take ECG measurements from paws of rodents which is quite easy than taking from chest. Figure 26 shows the final solution setup that we made.

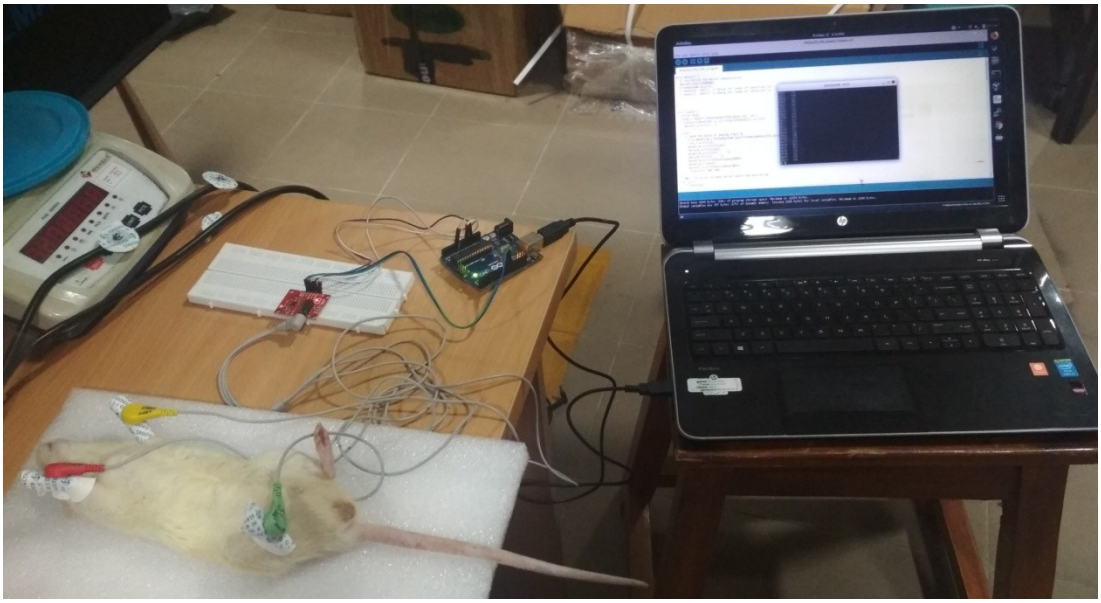


Figure 26: Final ECG measurement setup

Conclusion:

We were able to successfully develop a cost effective non-invasive method to measure the cardiac and respiratory rate for rodents. The requested functionality of the circuit was to measure the ECG signal of the rodent at a real time and store for further analysis of this ECG signal to check the health condition of the rodent while various medical experiment performing on it. We met this requirement and was able to condition, analyses and display the simulator waveforms on the portable laptop monitor with MATLAB software. We exceeded the requested functionality by implementing a solution to allow ECG measurements along with the heart rate and respiration rate measurement. We went through several design iterations throughout the semester was able to successfully simulate, design, test, and analyses the final ECG measurement circuit. The technology we developed enables non-invasive screening of large numbers of mice and rats for ECG changes resulting from genetic, pharmacological, or pathophysiological alterations. This report describes the development of a system for non-invasively recording ECGs in anesthetized rodent; surgical implantation of devices is not required.

Suggested Future Work:

Future work that could be performed to improve the functionality of the demo board includes the following:

- Implementing an FFT based beats/minute calculation of the signal
- Implementing digital filtering using the Stellaris microcontroller
- Designing the analog system using higher-order filters
- Implementing the ECG circuit for Anesthetized rodent
- Integrating the Stellaris display board and AFE board into one PCB
- Implementing live analysis and displaying system

Appendix I:

References:

[1] Motoshige Sato, Nobuyoshi Matsumoto, Asako Noguchi, Toya Okonogi,Takuya Sasaki, Yuji Ikegaya. Simultaneous monitoring of mouse respiratory and cardiac rates through a single precordial electrode. 137 (2018) 177-186

[2] Brüllmann G, Fritsch K, Thurnheer R, Bloch KE: Respiratory monitoring by inductive plethysmography in unrestrained subjects using position sensor-adjusted calibration. Respiration 2010; 79: 112–120.

[3] Hoymann HG: Invasive and noninvasive lung function measurements in rodents. J Pharmacol Toxicol Methods 2007; 55: 16–26.

[4] Phillipa L. Milton, Hayley Dickinson, Graham Jenkin, Rebecca Lim : Assessment of Respiratory Physiology of C57BL/6 Mice following Bleomycin Administration Using Barometric Plethysmography, 2012; 83: 253–266

[5] Piotr Konopelski, Marcin Ufnal : Electrocardiography in rats: a comparison to human.

[6] J. B. Farmer and G. P. Levy : A simple method for recording the electrocardiogram and heart rate from conscious animals. 1968: 32, 193-200.

[7] Johnson, K., “Introduction to rodent cardiac imaging,” ILAR journal 49(1), 27–34 (2008).

[8] Ho, D., Zhao, X., Gao, S., Hong, C., Vatner, D. E., and Vatner, S. F., “Heart rate and electrocardiography monitoring in mice,” Current protocols in mouse biology 1(1), 123–139 (2011).

[9] Badea, C., Hedlund, L., and Johnson, G., “Micro-ct with respiratory and cardiac gating,” Medical physics 31(12), 3324–3329 (2004).

[10] Zehendner, C. M., Luhmann, H. J., and Yang, J.-W., “A simple and novel method to monitor breathing and heart rate in awake and urethane-anesthetized newborn rodents,” PloS one 8(5), e62628 (2013).

[11] Geddes, L. and Baker, L., “The relationship between input impedance and electrode area in recording the ecg,” Medical and biological engineering 4(5), 439–450 (1966).

[12] El Bcharri, O., Latif, R., Elmansouri, K., Abenaou, A., and Jenkal, W., “Ecg signal performance de-noising assessment based on threshold tuning of dual-tree wavelet transform,” Biomedical engineering online 16(1), 26 (2017).

[13] Tanase, H., Yamori, Y., Hansen, C. T., and Lovenberg, W., “Heart size in inbred strains of rats. part 1. genetic determination of the development of cardiovascular enlargement in rats.,” Hypertension 4(6), 864–872 (1982).

[14] Dash, S., Shelley, K. H., Silverman, D. G., and Chon, K. H., “Estimation of respiratory rate from ecg, photoplethysmogram, and piezoelectric pulse transducer signals: a comparative study of time–frequency methods,” IEEE Transactions on Biomedical Engineering 57(5), 1099–1107 (2010).
[15] Cao, H., Zhao, Y., Kouki, A. B., Tai, Y.-c., and Hsiai, T. K., “A wireless ecg recording system for small animal models of heart regeneration,” in [2015 IEEE MTT-S International Microwave Symposium], 1–3, IEEE (2015).

Software:
MATLAB Software
http://www.mathworks.com/products/matlab.html
LTspice IV Software
http://ltspice-iv.en.lo4d.com
Arduino Software
www.arduino.cc/en/Main/Software

Datasheets:
LM741 Operational Amplifier
www.ti.com/lit/ds/symlink/lm741.pdf
AD624 Instrumentation Amplifier
www.analog.com/media/en/technical-documentation/data-sheets/AD624.pdf

.....

- Link of all the data acquired can be found here.

- Link of all the programs/algorithms used in the project is attached here.
  - It contains not only the programs but the data files and some results and their figures.

.....

Appendix II:

Programs:

Even though I have provided with the program file and all data files in the link above, attaching the one of the code to detect the envelope/trend of the noise signal interfered due to respiration into the ECG signal. Here is the code called gaussian kernel function. This is the curve fitting polynomial function.

```
function [ys]=gaussian_kern_reg_function(xs,x,y,h)

% Gaussian kernel function
kerf=@(z)exp(-z.*z/2)/sqrt(2*pi);

z=kerf((xs-x)/h);
ys=sum(z.*y)/sum(z);
```

Figures:

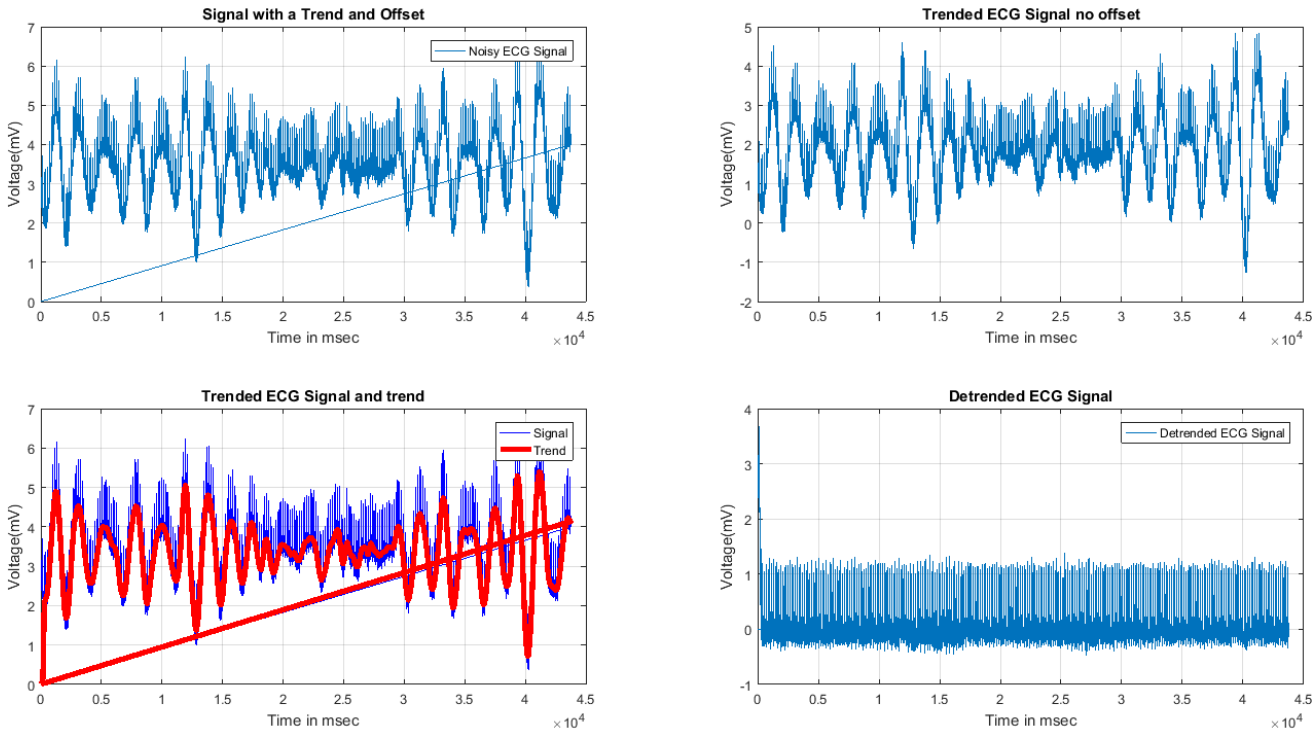


Figure 27: Various algorithm performed on the ECG signal for a rat