Animal Lab Module 3

Bishoy Abdelmalek, Blake Bragaw, Andrew Sifferlen

BME 3111 Team 10, Worcester Polytechnic Institute

All authors contributed equally to the report

***Abstract*— Rats were used in this laboratory setting to understand the changes in heart rate and blood pressure due to separate administrations of epinephrine and pentobarbital with potassium. This lab module allowed our team to demonstrate the skills learned in previous modules by handling live animals and collecting data. In this laboratory module students performed an ECG and inserted a catheter into the carotid artery of a rat. A transducer measured the blood pressure and a labview virtual instrument, designed by our team, collected the timed data.**

I. Introduction

The circulatory system is the key to supplying the body's tissues with essential molecules. The four-chambered heart self-rhythmically pumps blood throughout the body functioning as if it was one cell. Septums separate the ventricles and atriums in order to efficiently send oxygenated blood to deprived cells and send deoxygenated blood to exhibit gas exchange in the lungs.

As the right and left atria contract, blood fills the right and left ventricles through the tricuspid and bicuspid valves, respectively. Following ventricular contraction, deoxygenated blood leaves the right ventricle through the pulmonary artery leading to the lungs. Pulmonary capillaries exchange gases through simple diffusion with alveoli as oxygen enters the bloodstream while carbon dioxide withdrawals. The oxygenated blood travels through pulmonary veins and fills the left atrium as the right ventricle contracts. The right ventricle transports the previously oxygenated blood through the Aorta and systemic arteries to be distributed to the rest of the body's systemic capillaries. In these instances, blood releases oxygen into tissues and gains carbon dioxide. Deoxygenated blood moves through veins including the Superior or Inferior vena cava until it reaches the right atrium to begin the process again.

The carotid artery supplies blood from the heart to the brain, neck, and face. Blood passes through the Aorta and enters the Common carotid artery, eventually branching off at the Carotid sinus [1]. Baroreceptors line this widened area, sensing the change in blood pressure depending on how greatly they stretch as blood passes through. These sensors use the vasomotor center in the medulla oblongata to integrate this signal into the excitation or parasympathetic and sympathetic axons. These axons act as effectors to the heart and blood vessels. As the carotid artery is the natural spot of blood pressure sensory and is not far from the heart, it is the perfect artery to insert a catheter to measure the exact change in metrics after injections.

Secreted by the adrenal medulla, catecholamine hormones, such as epinephrine, "stimulate glycogenolysis and the release of glucose from the liver" [1]. Epinephrine is released in response to the "flight-or-flight reaction to stress" [1]. The body anticipates "intense physical activity" and "provides circulating energy substrates" [1]. These substrates, cAMP, stimulate the heart to beat faster. Beta-adrenergic receptors in the heart are sensitive to epinephrine and produce a faster heart rate and greater contractility [1]. The blood pressure of an artery is a function of total peripheral resistance (TPR), which represents the frictional resistance to blood flow in the arteries. An increase in cardiac rate and strength of ventricular contraction exhibits a greater volume of blood pumped per beat, increasing blood pressure.

In the central nervous system (CNS), pentobarbital binds to "gamma-aminobutyric acid (GABA) A subtype receptors" [2]. This reaction causes a change in chloride transport receptors, leading to "an increase in the duration that the chloride channels remain open" [2]. GABA depresses the CNS and prolongs "the time the channels remain open" which "intensifies the depressant effects on the CNS" [2]. Adding on to the CNS depression, pentobarbital inhibits glutamate, "which is responsible for nerve depolarization in the voltage-activated calcium currents" [2]. In the myocardial action potential, a slow intake of calcium and excretion of potassium leads to repolarization from about +20 mV to about -80 mV. Due to sodium pentobarbital, the voltage-gated calcium channels can not activate, therefore calcium induced calcium release of the sarcoplasmic reticulum will not occur. The heart muscles will be unable to beat as there is not enough calcium to bind with troponin, which usually leads to contraction. This lack of calcium also leads to diminished contractibility. A decrease in cardiac rate and weakness of ventricular contraction exhibits reduced cardiac output and lower blood pressure.

Similar to laboratory modules 1 and 2, the ECG was performed using three leads (negative, ground, positive) punctured in the rat's skin. In this specific module, the lead areas were not shaven. After calibration of the pressure transducer using water, the system was flushed out and filled with heparinized saline solution. As the catheter is inserted into the rat's carotid artery, it is connected to the pressure transducer with tubing.

For both rats and humans, "direct measurement of blood pressure requires an intra-arterial assessment" [3]. In humans, the measurements are taken "on the arm over the brachial

artery" [3]. Blood pressure can be collected in noninvasive methods such as "auscultation of the brachial artery with a stethoscope," which allows a physician to "detect the appearance and muffling… of the Korotkoff sounds, which represent" systolic blood pressure and diastolic blood pressure [3].

In the laboratory module, the program AqcKnowledge allows teams to read voltages of two pressures. This information is necessary to calibrate the pressure transducer in the second program, Labview. The pre-designed virtual instruments, created by each group, collects information from the Data Acquisition Device (DAQ), which is displayed on the labview vi. The vi also computes the data into spectral analysis and separates maximum/minimum data.

II. Materials and Methods

A. LabVIEW VI

We utilized a VI made in LabVIEW to graphically display and record live ECG and blood pressure data from the rat. The VI was designed to read in raw ECG and blood pressure data and display it graphically over time on the Front Panel of the VI (Fig 1).

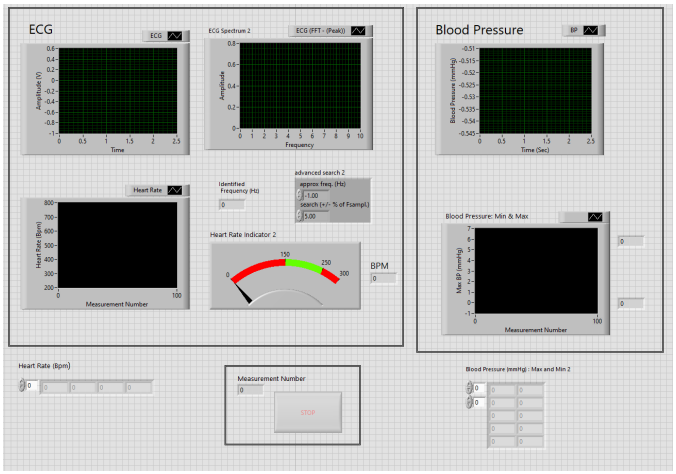

Figure 1: Front Panel of finished VI

By using a Fast Fourier Transform (FFT) in our VI we were able to generate a frequency spectrum of the data which allowed us to identify peaks in the ECG reading and calculate the instantaneous heart rate of the rat. This heart rate was also indicated in real time on the Front Panel on both a graph and the numerical indicator dial which was color coded to help ensure that the rat was living for the duration of the experiment. The VI also reads in data from a pressure transducer that is connected to a catheter in the rat's carotid artery. This data indicates the blood pressure of the rat and is displayed graphically on the Front Panel. The VI also tracks the maximum and minimum (diastolic and systolic) blood pressure values and displayed them on another graph. Upon completion of the experiment, both blood pressure and ECG raw data as well as maximum and minimum blood pressure values are saved by the VI.

B. Experimental and Surgical Procedures

Firstly, the rat was anesthetized using a mixture of ketamine and xylazine. The stock concentration of ketamine is 100mg/ml and it was dosed at 100mg/kg. The stock concentration of xylazine is 20mg/ml and it was dosed at 10mg/kg. The following formula was used to calculate the dosages of each:

$$weight(kg) \times \frac{(\frac{100mg}{kg})}{(\frac{100mg}{mL})} = ketamine\ dose\ (mL)$$

$$weight(kg) \times \frac{(\frac{10mg}{kg})}{(\frac{20mg}{mL})} = xylazine\ dose\ (mL)$$

While the anesthesia took effect, the pressure transducer that would be used to track blood pressure was calibrated. The first calibration point was found by holding the tubing at a height equal to the column of water in the tube. The second point was found by holding the tubing as close to the base of the pressure transducer as possible. The height difference between these two points was measured and recorded and the pressure transducer was calibrated using the following formulas:

$$Pressure\ (mmHg) = \frac{water\ column\ height\ (cm)}{10(cm)} \times 7.356(mmHg)$$

$$Slope = m = \frac{y_2 - y_1}{x_2 - x_1}$$

$$Y = mx + b$$

This linear calibration was entered into the VI and the tubing of the pressure transducer was filled with a saline solution.

Once the rat appeared to be completely anesthetized and the calibration of the pressure transducer was complete the reflexes of the rat were tested in order to gauge the depth of the anesthesia. The rat was pinched between the toes and a saline solution was dropped into the rat's eye and the rat remained unresponsive through both tests. The rat was then shaved at the neck to make for easier removal of the skin.

The rat was secured to the table by taping down all its limbs and hooking a suture loop around the front teeth and taping the suture to the table to stabilize the head. All team members were wearing the proper PPE. The ECG leads were then placed in the positions shown below (Fig 2).

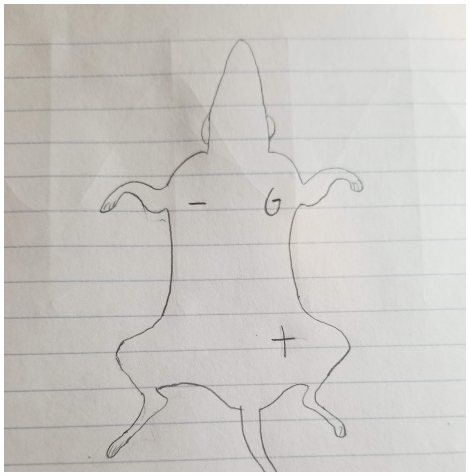

Figure 2: Placement of ECG Leads

A quarter-sized section of the skin was cut away at the shaven part of the rats neck. Blunt dissection was used to separate the layers of fat and muscle surrounding the trachea. One the the trachea was located the left carotid artery was found and isolated from the rest of the surrounding tissue and the vagus nerve using a pair of fine tip forceps. Once isolated, two sutures were tied around the artery. One knot was tied tightly at the cranial end of the artery to arrest the blood flow. A second knot was left loosely tied at the caudal end of the artery to act as a means to further arrest blood flow in the case of bleeding.

To place the catheter in the artery, the caudal loop was lifted slightly upward to restrict the flow of blood into the vessel. The first attempt to place the catheter failed and resulted in bleeding. The second attempt had the same outcome. The third attempt was successfully inserted into the artery and the catheter was advanced further into the artery until the full catheter tip was inserted. A small flow of blood exited the catheter which indicated that the catheter had been properly placed. The catheter was then attached to the pressure transducer and the collection of blood pressure data began and could be clearly seen in the LabVIEW VI.

After 1-2 minutes of data collection 0.1mL of 1mg/ml epinephrine was injected into the thoracic cavity of the rat. The epinephrine was not injected directly into the heart to prevent the animal from going into shock. Changes in the heart rate and blood pressure of the animal could be seen in the LabVIEW VI. After an additional minute of data collection 0.5mL of sodium pentobarbital euthenasia was injected directly into the heart of the rat and an immediate decrease in blood pressure was observed. After euthanization, the lab equipment was removed from the rat and cleaned and a double takedown was performed by removal of the heart.

III. Results

To begin this lab, the neck of our anesthetized rat was shaved to prepare for the removal of its skin. Next the three leads of the ECG monitor were inserted into the rats left underarm (ground), left inner hip (positive), and the right underarm (negative).

The next step of the lab was to calibrate the pressure sensor that would be used to measure the rats blood pressure using a catheter. The sensor was calibrated using

Pressure (mmHg)	Voltage (V)
-0.01495	0
0.585	58.85

These values were used for calibration by solving for the formula

$$Y = mx + b$$

to solve for the slope (m) and y-intercept (b). The pressure also needed to be converted from cmH_2O to mmHg by using the conversion (7.356 mmHg / 10 cmH_2O). The slope (m) was found to be 100.6 V/mmHg by using the formula

$$Slope = m = \frac{y_2 - y_1}{x_2 - x_1}.$$

Then using the formula y = mx + b, the y-intercept was found to be 1.50397 mmHg.

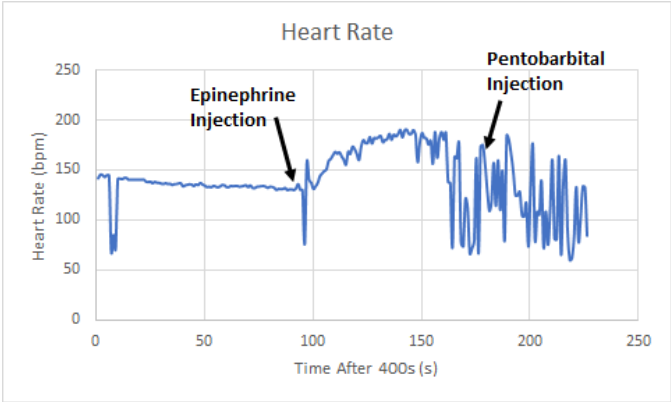

Figure 3: Heart Rate During Injections

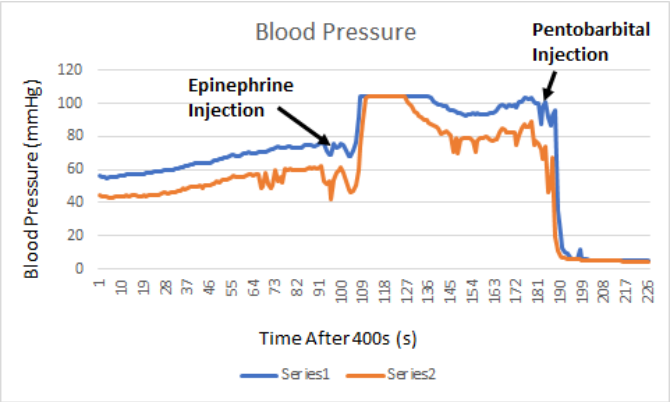

Figure 4: Blood Pressure During Injections

The average blood pressures over the course of the two injections are:
Baseline Heart Rate: 133.85 bpm
Baseline Systolic: 65.37 mmHg
Baseline Diastolic: 51.13 mmHg

After Epinephrine Heart Rate: 157.20 bpm
After Epinephrine Systolic: 96.33 mmHg
After Epinephrine Diastolic: 81.80 mmHg

After Pentobarbital Heart Rate: 114.98 bpm
After Pentobarbital Systolic: 11.37 mmHg
After Pentobarbital Diastolic: 7.18 mmHg

The average heart rate was found to be 193.91 bpm.

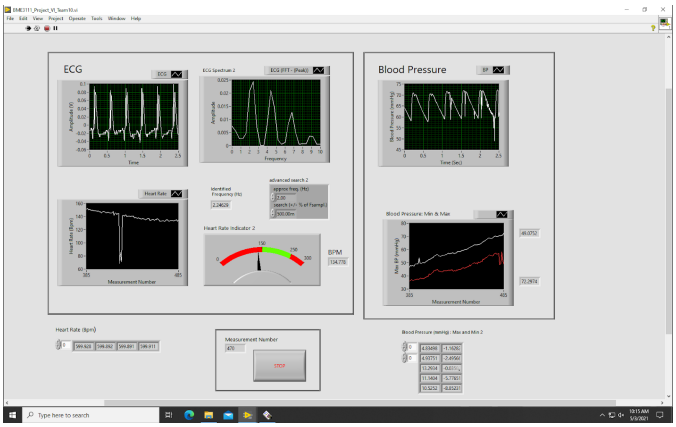

Figure 5: VI Front Panel After Catheter Is Inserted

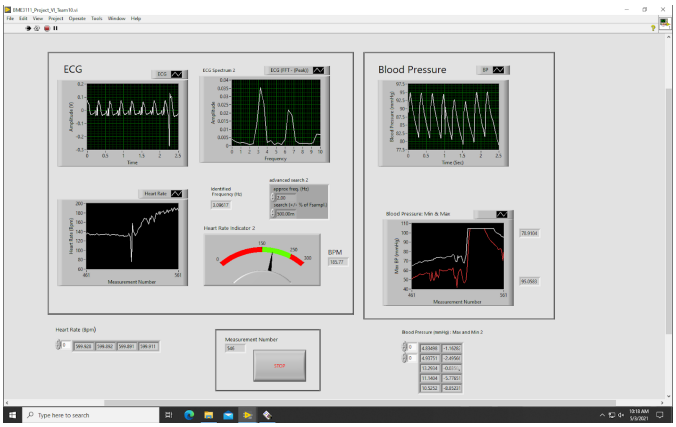

Figure 6: VI Front Panel After Epinephrine Injection

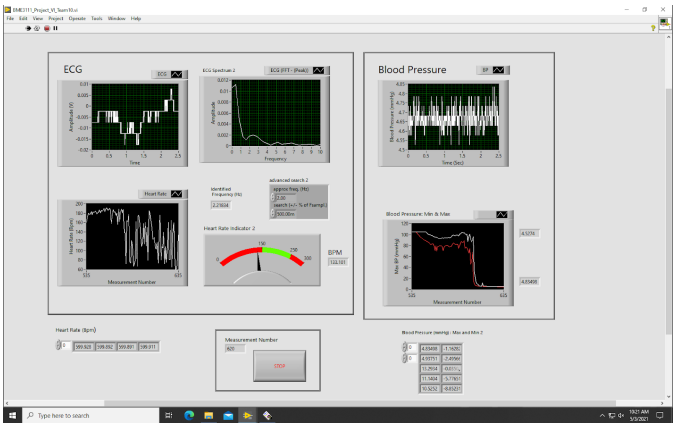

Figure 7: VI Front Panel After Euthanized

IV. Discussion

The carotid artery is a blood vessel taking freshly oxygenated blood from the heart to the brain. Since the artery is in close proximity to the heart and is coming directly from it, the measurement of blood pressure at this location is accurate to the readings in the heart. The carotid is also a large artery allowing for more ease while inserting the catheter to obtain data.

The epinephrine injection occurred at 494 s and the pentobarbital injection occurred at 585 s. All data represented in the figures starts at 400 s, so the injections occur at 94 s and 185 s on all figures. The heart rate data recorded showed very little noise and was as expected until the pentobarbital was injected. After this injection there was significant noise in the reading and the heart rate data is random rather than descending towards zero as expected. The baseline of the heart rate is rather steady and increases rather slowly at a fairly consistent rate after the epinephrine is injected. The blood pressure reading showed a little noise during the baseline period. After the epinephrine injection the blood pressure increases rapidly until it levels out because it exceeds

the maximum reading. After the pentobarbital injection, the blood pressure descends towards zero as expected with little noise.

The data shown for both the blood pressure and the heart rate begin at 400 s because the catheter fell out during the first attempt of insertion, so there was significant time between closing off the carotid artery to prevent bleeding and re-inserting the catheter. It does not appear that the loss of blood from the rat from this mistake affected the data in any way after the catheter was placed in correctly.

Both sets of data show a steady period during the baseline readings with an increase after the epinephrine was injected and then a decrease to zero after the pentobarbital is injected. This is expected because epinephrine is a form of adrenaline that will increase the rat's heart rate and both systolic and diastolic blood pressures. Pentobarbital is used to euthanize the rat, so a steady decrease to zero shown in the blood pressure data is expected. The randomness in the heart rate data was unexpected, but we believe this occurred because of errors in the sensor as the heart was stopping.

V. Conclusion

Overall, the lab shows how to take an ECG reading on a rat using subcutaneous leads and measure the arterial blood pressure resulting from injections of both epinephrine and sodium pentobarbital. The graphs representing the heart rates and blood pressures after each solution administration showed little noise and displayed expected results from our understanding of physiology and previous knowledge of the lab. The ECG test was run using a positive, negative, and ground lead to measure the voltage potential across the anesthetized rat. The blood pressure of the carotid artery was measured using a pressure transducer connected to a DAQ, which obtained the data. The ECG reading and blood pressure information were displayed and calculated through a labview virtual instrument, designed by the lab group. The experiments performed allowed the student groups to gain experience with handling an anesthetized rat, collecting an ECG reading, inserting a catheter, measuring blood pressure, and programming in Labview.

VI. References

[1] Fox, S. (2016). *Human Physiology* (14th ed.). McGraw-Hill Education.

[2]Johnson AB, Sadiq NM. Pentobarbital. [Updated 2020 May 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545288/

[3]Muntner, P., Shimbo, D., Carey, R. M., Charleston, J. B., Gaillard, T., Misra, S., Myers, M. G., Ogedegbe, G., Schwartz, J. E., Townsend, R. R., Urbina, E. M., Viera, A. J., White, W. B., & Wright, J. T., Jr (2019). Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association. *Hypertension (Dallas, Tex. : 1979)*, *73*(5), e35–e66. https://doi.org/10.1161/HYP.0000000000000087