**The Big Picture:** In lab this week, you will use your fetal pig, a cow heart, and prepared histological specimens to explore the anatomy of the mammalian cardiovascular system. Further, you will examine the mechanisms that control the human heartbeat by recording and analyzing electrocardiograms (ECGs) taken from students within the class.

<table>
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<th>Before lab</th>
<th>During lab</th>
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| • Background Information:  
  o Smith and Schenk (dissection manual)  
    Chapter 5  
  o Russell et al., CH 42 | • Half of the class: ECG experiments (Part A)  
• Half of the class: continue fetal pig dissection, and examine histology slides (Parts B and C).  
• (Halfway through the lab period, the groups will switch activities.) |

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<th>Assignments (Due at the beginning of next week’s lab)</th>
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| • Worksheet 3: Veins and Arteries  
• Lab 3 Report: ECG |
Thought and discussion questions

- Is it true that the arteries always carry oxygenated blood, and the veins always carry deoxygenated blood? Explain.
- What is the function of the hepatic portal system? How does its function differ from that of the systemic or pulmonary circulatory systems?
- How do gases and nutrients pass between the arteries and the tissue beds?
- Define a cardiac cycle. Explain the functional significance of the delay between the P wave and the QRS wave in the ECG.
- What is happening in the heart between the T wave of one cardiac cycle and the P wave of the next cardiac cycle?
- How do you determine if a variable is independent or dependent?

Learning objectives

1. To identify the structure and function of the major arteries and veins of the fetal pig.
2. To identify the structure and function of the different chambers of a mammalian heart.
3. To understand differences between fetal and adult circulatory systems.
4. To learn how to record and interpret an electrocardiogram (ECG). Specifically, to be able to identify the P, QRS, and T waves within an ECG, and be able to relate each wave to the physiological events which occur during the cardiac cycle.
5. To be able to distinguish between histological sections of veins and arteries.
6. Be able to use statistics (ANOVA) to compare the effect of three levels of one variable on heart rate.
PART A: HUMAN CARDIOVASCULAR PHYSIOLOGY

- Background on cardiovascular anatomy and physiology
- Introduction to electrocardiograms

HYPOTHESIS TESTING AND CAUSALITY

Last week, you learned how to perform t-tests to determine if two different data sets (body lengths of male and female crayfish) were significantly different from each other. Recall that t-tests can only compare the means of two data sets, but in this week’s lab, you will examine ECG signals from one individual at three different levels, for example, after laying down, sitting down, and exercising or possibly after drinking water, coke, and coffee, etc. To be able to compare the means among three groups, you will then use a different type of statistical test, called an ANOVA (Analysis of Variance).

For this lab, it is important to understand what dependent and independent variables are, and the relationship or presumed causality that makes each variable dependent or independent. The terms independent variable and dependent variable are used to describe relationships of causality. An independent variable must have the potential to affect a second variable, termed a dependent variable. Independent variables thus do not ‘depend’ upon the value of another variable, while a dependent variable can be influenced by an independent variable. Therefore, the independent variable is assumed to be causative for the dependent variable. In most biology experiments, the scientist chooses the independent variable and then uses a method to measure the dependent variable. When making a graph to examine the relationship between dependent and independent variables, the independent variable is always graphed on the X-axis and the dependent variable is graphed on the Y-axis.

In today’s lab, we have two variables: experimental condition (activity level, consumption of caffeinated beverage, etc), and time between cardiac cycles, or ΔT (measured in seconds). Which variable is the dependent variable and which is the independent?

To be able to determine causality, one must know something about the nature of the relationship between the two variables being studied. For example, if we knew nothing about heart physiology, we could assert that the timing between each cardiac cycle detected by the electrodes influenced the type of activity an individual will do, meaning that we could claim that a very high ΔT would cause a person to exercise. This idea that an electrical detection system could affect the way a heart functions seems unlikely, knowing what we do about heart physiology. However, in experiments in which there is no prior knowledge about the relationship between two variables, we generally do not try to make conclusions about causality (although we can make conclusions about correlation – something you will learn more about in next week’s lab).

STATISTICAL ANALYSIS OF DATA: ANALYSIS OF VARIANCE (ANOVA)

ANOVA is a robust and powerful statistical method for comparing the response of a single dependent variable to two or more treatments of an independent variable, more commonly known as a factor. ANOVA is used to compare the difference between three (or more) groups, in contrast to a t-test which is used to compare two groups. In this week’s lab, you will measure an individual’s heart rate under three different conditions (for example, lying down, sitting down, and exercising).

ANOVA works by partitioning the total variance in the dataset into two types. The first is among-group variance, the amount of variation between the means of groups for one particular variable (or factor). The second is within-group variance, the pooled amount of variation within all groups for
that factor. After partitioning the variance, ANOVA calculates an **F statistic**, which is the ratio of the variance among-groups to the variance within-groups. The greater the difference between group means (among-group variance), relative to the variability within all groups (within-group variance), the greater the likelihood that there is a real difference between the groups.

Similar to the t-test, where the t-value provided a corresponding p-value, the F statistic provides a corresponding p-value as well (similarly, the computer software program, such as SPSS, will provide you with the corresponding p-value). For ANOVAs, as the F statistic grows (because among group variance increases relative to within group variance), the smaller the p-value becomes. This is similar to the t-test in which a large t-value produces a small p-value. As we have seen before, when p < 0.05, we can reject the null hypothesis and conclude that there is a difference in the means among the groups for a particular factor. The converse also occurs – the smaller the difference between group means becomes, and the greater variation within groups, the less likely there is any difference among the groups. The F ratio will be low, the p-value will be high, and we cannot reject the null hypothesis (and must conclude that there is no difference among the means of the groups for that factor).
PART B: HISTOLOGY

- Introduction to anatomy of arteries and veins

PART C: CARDIOVASCULAR SYSTEM OF THE FETAL PIG

- Instructions for fetal pig dissection
- Examination of arteries and veins

PROCEDURE PART A (work in groups of 3-4):

1. As a group, determine the experiment you wish to perform to investigate the effect of a variable on a subject’s heart rate. You may investigate the effect of any variable (within reason) such as exercise, meditation, drinking a caffeinated beverage, etc. However, for this study, we are asking you to design your experiment so that you can investigate the effect of three different levels of the same variable. For example, you may wish to investigate the effect that listening to music has on a subject’s heart rate. In this case, you may wish to compare a subject’s heart rate under the following three conditions: (a) not listening to music, (b) listening to soft music and (c) listening to very loud music. Or perhaps you wish to compare: (a) listening to music for 0 min, (b) 10 min, and (c) 20 min. As you design your experiment, think carefully about the rationale for your experiment. What is your variable? What is your hypothesis about how the different levels of the variable will affect the subject’s ECG? Use the space provided on your worksheet to explain the reasoning behind your predictions.

2. It is also important to think carefully about your experimental design. Consider the timing involved, such as will the subject exercise for 5 minutes or 30 minutes? Consider any other factors, such as what concentration of caffeinated beverage will the subject drink? Think about what condition will you measure first (it seems important to measure resting heart rate before exercising heart rate, etc.) Once you have determined the variable you wish to investigate and devised an experimental design, check with your lab instructor to make sure that you have proposed a feasible experiment. Record your experimental design on your ECG worksheet (note: you do not have to hand in your ECG worksheet but it will be an invaluable resource for you as you write your lab report).

3. Choose a subject to record from, and attach 3 surface electrodes and their associated leads to her. To this end, complete the following steps:
   • Ask the subject to sit down on a chair near the recording equipment.
   • Attach one electrode to the medial surface of each leg, just above the ankle, and the third electrode to the inside of the right wrist (see Fig. 5 for details).
   • Clip the 3 leads (via the pinch connectors) onto each of the surface electrodes as shown in Fig. 5. The pinch connectors on the leads work like a small
clothespin, but will only latch onto the nipple of the electrode from one side of the connector.

• You should notice a metal clip in the middle of the lead wires. Attach this clip to the subject’s clothing at a convenient location (e.g., along the waistline of her pants).

• You now need to calibrate the ECG program. On the computer, click on the desktop icon labeled “ECG Activity and EMG Activity 1”. A menu will appear; select “L05-ECG-1.” You will then be asked to type in a file name. Type in any name (it doesn’t matter what), but you must type in a name to be able to continue. Click “OK”.

• Next, a screen should appear with an empty window filling the top 2/3 of the screen. At the top left of the window, you should see a button labeled CALIBRATE. Before clicking this button, make sure that the subject is relaxed and sitting as still as possible, with her hands folded across her lap. Because the ECG device is extremely sensitive to small changes in voltage caused by contraction of the skeletal muscles, it will be corrupted by any movement of the arms or legs.

• Run the calibration procedure, which establishes the recording device’s internal parameters (e.g., gain, offset and scaling) and is critical for optimum performance. All you need to do is click the CALIBRATE button and wait 8 seconds. You should see a horizontal line move across the computer screen with small blips every half second or so. If the horizontal line shows any large spikes, jitter, or baseline drifts, then you should redo the calibration by clicking on the REDO CALIBRATION button and repeating the entire calibration sequence.

• Once this procedure is completed, you are ready to run the experiment. Your goal is to estimate the subject’s heart rate under 3 conditions:

1. after sitting down in a chair (baseline or control condition)
2. after one level of the experimental condition (have the subject sit down in a chair after performing the experimental variable decided on earlier).
3. after the other level of the experimental condition (have the subject sit down in a chair after performing the experimental variable decided on earlier).

• Sitting (control) condition: Ask the subject to sit down in a chair. Then, click on the RECORD button. Halt the recording process 10 seconds later by clicking on the SUSPEND button.

Note: For this and all subsequent tests, you will need to redo the recording if (i) the SUSPEND button was pressed prematurely; (ii) an electrode became detached from the subject, causing a large baseline drift, spikes, or loss of signal; or (iii) the subject has too much muscle artifact. If any of these problems occur, then you should redo the recording by clicking on REDO and repeating the 10 sec recording. Note that once you press REDO, the data you have just recorded will be erased.

• Level one of Experimental Condition: Remove the leads from the subject, and ask her to do the experimental procedure. Then, have the subject sit back in the chair, replace the electrodes as quickly as possible, and click on the RESUME button. Halt the recording 10 sec later by clicking on the SUSPEND button.

• Level two of Experimental Condition: Remove the leads from the subject and ask her to do the second level of the experimental procedure. Then, have the subject sit back in the chair, replace the electrodes as quickly as possible, and click on the RESUME button. Halt the recording 10 sec later by clicking on the SUSPEND button.
Then, click on the DONE button. Select “Yes” from the screen that asks “Are you sure you are finished with all the recording segments?”

A new window will appear. Select “Analyze current data file” and then “OK”.

Remove the leads from your subject, peel off the electrodes (and dispose of them), and then wash the electrode residue off the subject’s skin with soap and water.

To analyze your ECG recordings, set up your window display so that you are viewing the initial 5 seconds of your recording session (follow the written instruction posted by the computer or ask your instructor or TA). Record the relevant information on the ECG Worksheet. This information is required for writing your lab report.

Before you quit the program, print one copy of your ECG recordings for each group member as you will need to attach this figure to your lab report.

Make sure that you have answered all of the relevant questions on the ECG Worksheet; you will need this information for your lab report.

Follow the directions below to perform the statistical test, ANOVA, to determine if the means among the three treatment conditions are significantly different.

**ANALYSIS OF VARIANCE (ANOVA) USING SPSS**

To determine if the different activity levels affected heart rate, you will use SPSS to perform the statistical test, ANOVA. Follow the directions hanging above the computers in the lab to perform this ANOVA.

**OBTAIN HANDOUTS**

Before you leave lab today, make sure that you have a copy of three handouts: You will need the information in these handouts to write your lab report:

a. ECG Lab Report Guidelines
b. General Guidelines to Writing Lab Reports
c. Sample Lab Report

Also make sure that you have filled out all the information on the ECG Worksheet (located at the end of this lab handout). Information on that worksheet will be critical for writing your lab report.
This worksheet will be helpful when writing your lab report. Complete this worksheet as a group during lab. This worksheet will help you organize your raw data; do not turn in this worksheet. Instead, each group member should fill it out as it contains necessary information to write the lab report.

1. Describe the question that you are investigating. Include: (1) your hypothesis about how the frequency of the ECG waves (e.g. ∆T) will change after performing the experiment and (2) the biological rationale for your hypothesis.

2. Describe the experimental design. Include any important information you will need to include in the methods section of your lab report, including the timing and/or concentrations of all conditions.
3. After you capture ECGs under control (sitting), and experimental conditions, record the duration of the cardiac cycle ($\Delta T$) in the table below. Record this value for 10 cardiac cycles in each condition. Then calculate the means and standard deviations and record these values in the table below. You may use a calculator, Excel or SPSS for these calculations.

Table 1: $\Delta T$ for sitting and experimental conditions

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<th>$\Delta T$ (sec) for baseline condition (Describe)</th>
<th>$\Delta T$ (sec) for experimental condition, level one (Describe)</th>
<th>$\Delta T$ (sec) for experimental condition, level two (Describe)</th>
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<td>Standard deviation</td>
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4. Print out one copy of each of the three ECG printouts per individual in your group. Each group member must attach one copy of this ECG figure to her lab report. On the control (sitting) printout, label each part (T, QRS, and P wave) of one cardiac cycle on the ECG.