

BC1501 SKELETONS AND MUSCLES

OVERVIEW

The Big Picture: In this week's laboratory, you will investigate the vertebrate muscular-skeletal system from two perspectives. First, you will study the forelimb of 4 species of vertebrates (human, bat, cat, and bird), and attempt to identify the homologous bones. Second, you will examine how skeletal muscles are able to generate graded forces and move skeletal elements around joints.

Before lab	<ul style="list-style-type: none">• Background Information:<ul style="list-style-type: none">○ Russell et al., CH 41
During lab	<ul style="list-style-type: none">• Half of the class: Comparative anatomy of vertebrate forelimb skeletons and muscle histology (Parts A and B)• Half of the class: Electromyography (EMG) Activity. (Part C)• Halfway through the lab period, the groups will switch activities.
Assignments (Due at the beginning of next week's lab)	<ul style="list-style-type: none">• Worksheet 4A (Individual): Skeleton Homologies• Worksheet 4B (Group): EMG

Thought and discussion questions:

- What is the distinction between a homologous and analogous structure? What types of evidence are useful in judging homology?
- What are the functional similarities and structural differences between the wings of the bat and the pigeon?
- What is the source of the signal detected by the EMG electrodes?
- What physiological processes underlie the observed relationship between EMG response and force output?
- What does the term “motor unit recruitment” mean?
- When you are holding a heavy cup of coffee in your hand, would you expect your biceps and triceps muscle to be contracting vigorously? Explain.
- What is the difference between correlation and causation?

Learning objectives:

1. To compare, analyze, and be able to identify the structural equivalencies in the forearm bones of the human, bat, pigeon and cat.
2. To be able to distinguish histological sections of skeletal, cardiac, and smooth muscle.
3. To learn how to make and to interpret electromyography (EMG) recordings from your own muscles.
4. To understand the causal connection between motor unit recruitment, the magnitude of the EMG signal, and force generated during a skeletal muscle contraction.
5. To learn how antagonistic muscles control the movement of bones around joints.
6. To understand the difference between continuous and categorical variables.
7. To understand the difference between correlation and regression analysis.
8. To learn how to perform regression analysis to analyze data.

PART A: COMPARATIVE ANATOMY OF VERTEBRATE FORELIMBS

- Background on vertebrate anatomy
- Discussion of homology and analogy

PART B: MUSCLE HISTOLOGY

- Background on muscular histology

PART C: MUSCLE FUNCTION AND ELECTROMYOGRAPHY (EMG)

- Background on muscle physiology and use of EMG

CORRELATION AND REGRESSION ANALYSIS

Both correlation and regression analysis are statistical techniques that allow one to examine the relationship between two variables (X and Y). **Correlation** allows one to test if two variables have a relationship (i.e. is the change in one variable associated with a change in another variable?). This relationship, however, is not necessarily a causal relationship (i.e. the change in X may or may not cause the associated change in Y). For example, does an increase in crayfish claw size correlate with an increase in body length? You can probably guess that these two variables are correlated. But this does not mean that the increased claw size *caused* the increased body length, nor does it mean that the increased body size *caused* the increased claw size (probably both factors are influenced by age). This is a very important thing to keep in mind when interpreting correlations.

Regression analysis is a useful statistical technique for examining *causal* relationships between two variables. Specifically, regression enables one to test whether one variable causes systematic increases or decreases in a second variable. The knowledge of this relationship allows an individual to predict values of the second variable from the values of the first by fitting a line through the data points on a graph. One can only perform regression if there is knowledge about how one variable affects the other variable.

When one performs either correlation or regression analysis, the **r value, or correlation coefficient**, can be calculated. This r value indicates the degree to which X and Y vary together, and always varies between -1 and +1. A positive r-value indicates that X and Y change in the same way (as one increases, so does the other; similarly, if one decreases, so does the other). A negative r-value indicates that X and Y change in opposite ways (as one increases, the other decreases).

To use correlation or regression analysis, one must have clear independent and dependent variables. Recall from last week's lab that an independent variable must have the potential to

affect a second variable, termed a dependent variable. Generally, independent variables are chosen by experimenters, and dependent variables are measured.

For example, it is known that nitrogen availability affects plant growth rate. To test if specific concentrations of nitrogen affect plant growth rate, one could perform an experiment where different concentrations of nitrogen were added to soil, and plant growth rate in each type of nitrogen-boosted soil was measured. In this case, the independent variable would be the concentration of nitrogen in the plant soil (chosen by the experimenter), and the dependent variable would be the height of the plant (measured by the experimenter). For this experiment, regression analysis would be appropriate because it is known that the amount of nitrogen in the soil *causes* a change in plant growth rate. It is known that the opposite situation (an increase in plant growth rate *caused* more nitrogen to become available in the soil) does not make biological sense. If, however, there was no prior knowledge about the relationship between these two variables, correlation would be a more appropriate analysis to use.

Both correlation and regression analysis produce the familiar Y-intercept (a) and slope (b) equation for a line from the data, and the resulting equation provides a concise summary of the relationship between the two variables (Y and X) under study:

$$Y = a + bX$$

Let's go back to the example of how nitrogen availability affects plant growth rate. Figure 5 below describes the relationship between the amount of nitrogen added to soil and plant growth rate. The equation $Y = 5.14 + 5.43X$ of the line in the graph below describes the specific relationship found between nitrogen concentration and plant growth rate for this experiment. The slope of 5.43 predicts that for every gram of nitrogen added per cm^3 of soil, the plants will grow by approximately an additional 5 cm per week.

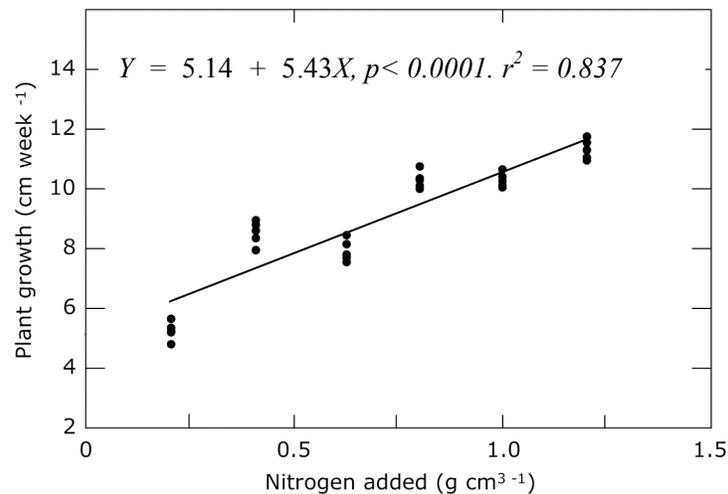


Figure 5: This figure depicts the relationship between nitrogen added to the soil and plant growth rate. There is a positive correlation: as more nitrogen is added, there is an increase in plant growth rate.

As with the t-test, the **null hypothesis** is tested by regression/correlation analysis. In the case of regression/correlation, the null hypothesis states that there is no relationship between the two variables. If this were the case, then the data, when graphed, would produce a line with a slope of 0 (i.e., a horizontal line). Similar to the t-test, regression/correlation analysis compares two scenarios: (1) relationship between two variables as determined in the experiment (slope of the line for the experimental data) and (2) relationship between the two variables assuming the null hypothesis is true (slope of 0).

Like the t-test, regression/correlation provides a **p-value** that indicates the probability that the difference between the two scenarios (slope generated by the experimental data set and the slope based on the null hypothesis) is due to chance. In the case of our nitrogen-plant growth data, $p < 0.0001$, indicating that the probability that the difference is due to coincidence is less than one in ten thousand. In this case, a scientist would reject the null hypothesis that there is no relationship between nitrogen added to soil and plant growth. The data support the hypothesis that nitrogen added to soil increase plant growth.

The other important statistic produced by regression/correlation is r^2 , which is based on the correlation coefficient, r . This value, r^2 , represents how much of the variation in the Y variable is explained by the X variable. In this example, r^2 is 0.837, meaning that nearly 84% of the variation in growth rates in this experiment are the direct result of the nitrogen treatments.

There is a very important distinction between the t-test and regression/correlation analysis. Unlike t-tests, in which two distinct groups of samples are compared to see if there is a significant difference, regression/correlation requires two continuous variables. **Continuous variables** can assume any conceivable value within the possible range. For instance, the timing of a cardiac cycle or ΔT (measured in seconds) that we measured last week is a continuous variable because it can take on any physiologically possible value. This is in contrast to **categorical variables** such as ECG subject activity level, which can take on only certain values or states (e.g., resting vs. exercising). Note that in the plant growth example above, both variables are continuous.

REGRESSION ANALYSIS AND EMG ACTIVITIES

In the first EMG activity in lab today, you will examine the relationship between the amount of force generated while clenching an object and the magnitude of the EMG signal from your forearm flexors. The forearm flexors are the muscles on the inside of your forearm. Contraction of these muscles causes your fingers to curl and, hence, clench an object in your hand. To release your grip on the same object, you would relax your forearm flexors *and* contract your forearm extensors (i.e., the muscles located on the outside of your forearm).

This is an appropriate data set to analyze by regression because the amount of force generated by clenching (the independent variable) *causes* your muscles to elicit a specific physiological electrical response (the dependent variable) which can be measured by EMG. In addition, each of these variables is continuous.

PROCEDURE PART A: COMPARISON OF TETRAPOD SKELETONS

- Visual comparison of four tetrapod skeletons, labeling bones and identifying equivalents in human forelimbs. Discussion of homology.

PROCEDURE PART B

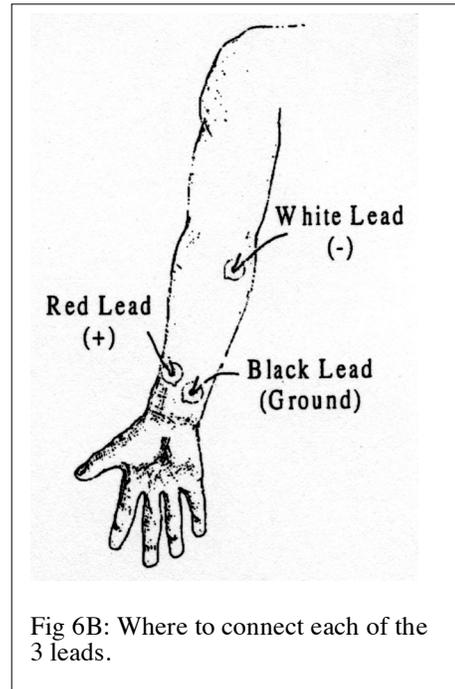
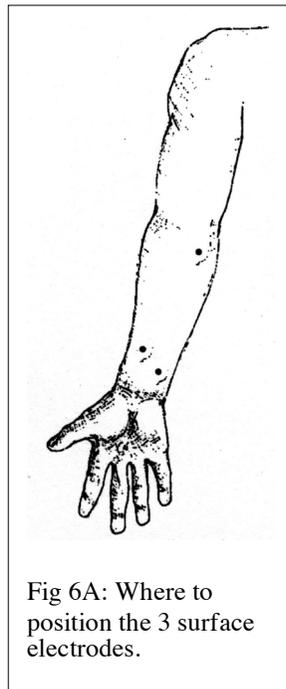
- Visual comparison of muscle histology slides.

PROCEDURE PART C (1 and 2): ELECTROMYOGRAPHY

C1: RELATIONSHIP BETWEEN FORCE OUTPUT AND EMG SIGNAL SIZE

1. **Work in groups of 3-4.** As a group, generate a hypothesis about the relationship between the magnitude of the EMG signal and the force output of your forearm flexors. Describe this hypothesis in the appropriate place on Worksheet 4B: EMG. Your prediction should be stated both in words and also graphically. To state your hypothesis graphically, construct a bivariate (X-Y) plot with the independent variable (amount of force exerted) on the X-axis, and the dependent variable (magnitude of the EMG signal) on the Y-axis. Indicate your prediction by drawing a line on this graph. For example, if you predict a direct linear relationship between the two variables, then draw a straight line with a positive slope.

2. Obtain one volunteer from your group. Place the electrodes on her dominant forearm by attaching 3 surface electrodes, and their associated leads, to the ventral (inside) surface of the subject's forearm. To this end, ask the subject to rotate the forearm of her dominant arm so that the palm of her hand is facing up. Then, attach the surface electrodes at the locations indicated in Fig 6A. Next, attach the 3 leads (via the pinch connectors) to each of the surface electrodes in the pattern shown in Figure 6B.



3. To open the program you need, click on the computer desktop icon labeled “ECG Activity and EMG Activity 1.” Choose “L01-EMG-1”. You will be asked to type in a file name. Type in anything here – it doesn’t matter what—but you will not be able to proceed without typing in something. Click “OK”.
4. Next, a new screen should appear containing an empty window and a button labeled “CALIBRATE” in the upper left corner. To calibrate the program, click on this button and follow the instructions having the student relax and then clench her fist. This calibration procedure lasts 8 seconds, and is critical for optimum performance. Basically, the computer needs to take an EMG reading while you clench your fist (i.e., activate your forearm flexors) maximally to set its internal parameters. You should see an EMG signal appear as soon as the subject begins clenching her fist—it should look like a cluster of vertical lines. Once this calibration procedure is completed, you should be ready to run the experiment.
5. Before beginning the experiment, have the subject practice clenching her fist to varying degrees of intensity. She should become proficient at generating 7 different levels of clench intensity (as measured by the dynamometer). While the subject becomes proficient at using the hand

dynamometer, another member of your group should become proficient at turning on and off the EMG recording (i.e., clicking the SUSPEND and RESUME buttons, respectively). After becoming proficient with these skills, you are ready to collect your data.

6. Begin your experiment by clicking on the RECORD button. At this point, the subject should make the weakest fist clench and hold for a short time (~1 sec). **Immediately after the clench, have one member of the group record the amount of force (in kg) generated on the hand dynamometer in the appropriate place on Worksheet 4B: EMG.** You must write down the value on the dynamometer to know how much force was generated by each clench.
7. Then, have the subject make a slightly more forceful clench (holding for the same amount of time), and record the amount of force generated on the hand dynamometer. Continue in this manner until you have recorded the EMG response and force output during an ascending series of successively more forceful clenches (7 clenches in total).

You will notice that there are two windows on the data collection screen. The upper window contains the raw EMG signal, and the lower window contains the integrated EMG signal (i.e., a moving average of the EMG signal over time). The vertical axis of each window represents the magnitude of the EMG signal, and the horizontal axis represents real time (in seconds).

8. Once you have recorded all 7 EMG responses, click on the “stop” button. Select “Done” and from the window that appears select “Analyze current data file” and click “OK”. At this point, you need to determine the highest integrated EMG value (in millivolts) obtained for each fist clench, and enter these values into the appropriate place on Worksheet 4B EMG. Print one copy per group to attach to Worksheet 4B. For instructions on how to do this, follow the written guide by the computers in lab or ask your instructor or TA.
9. As a group, answer the appropriate questions on Worksheet 4B.
10. Perform regression analysis by using SPSS and following the directions hanging above the computers in your lab room.

Group members _____

Time/Day/Section _____

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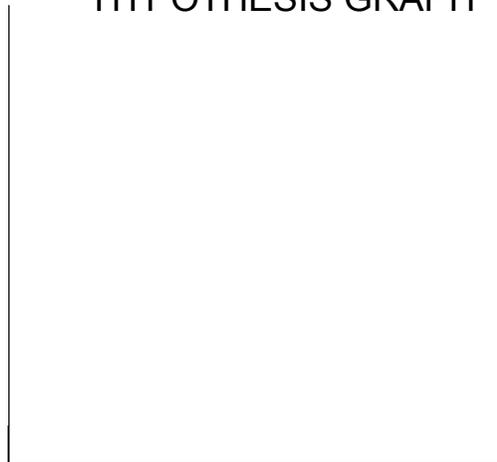
Fall 2009

WORKSHEET 4B (GROUP) EMG (10pts)

As a group, answer the questions on this worksheet and hand in **one copy per group before you leave lab today.**

1. (1 pt) As a group, generate a hypothesis about the relationship between the force output of your forearm flexors and the magnitude of the EMG signal. Use the space below to write out the hypothesis. Use the graph below to graphically depict the hypothesis. On the graph, label the x-axis with the independent variable and the y-axis with the dependent variable.

HYPOTHESIS GRAPH



WORKSHEET 4 (GROUP): EMG (10 pts)

2. (1 pt) Fill in the following table with the data you obtained from your EMG recordings. Be sure to record the amount of force from the dynamometer. Attach a copy of your EMG recordings to this printout.

Forearm Contraction	Force (kg)	EMG signal (mV)
Fist clench 1		
Fist clench 2		
Fist clench 3		
Fist clench 4		
Fist clench 5		
Fist clench 6		
Fist clench 7		

3. (1 pt) Using SPSS, graph the data you obtained, and perform regression analysis. Report the r^2 value and p-value below. Attach a printout of this graph to this worksheet.

r^2 value _____

p-value _____

4. (1 pt) Based on your r^2 -value and p-value, is there a significant correlation between the amount of force generated by your forearm and the EMG signal detected by the electrodes? Explain.
5. (1 pt) Did your data support your hypothesis described in question 1? Explain.

WORKSHEET 4 (GROUP): EMG (10 pts)

6. (1 pt) State your predictions of EMG activity in relation to muscle movement for the flexion movement (lifting a mortar) and each muscle (biceps and triceps).
7. (1 pt) State your predictions of EMG activity in relation to muscle movement for the extension movement (backwards push-up) and each muscle (biceps and triceps).
8. (1 pt) Record the EMG readings during these movements in the table below. Do each action, either flexion (lifting a mortar) or extension (backwards push-up), three times. Print out a copy of your EMG records and attach them to this worksheet.

Lifting a Mortar	Biceps (mV)	Triceps (mV)
Trial 1		
Trial 2		
Trial 3		
MEAN		
Backwards Push-up	Biceps (mV)	Triceps (mV)
Trial 1		
Trial 2		
Trial 3		
MEAN		

9. (1 pt) Were your hypotheses supported or refuted by your EMG data? Explain.
10. (1 pt) If you brought a coffee cup down from your lips to the table, which muscles would be activated? Explain.