Student version

Title: "The Biomedical Blunder"

Summary: In this project, you will carry out an experiment to reveal the identity of three drugs after a mix up in the lab. In carrying out this project, you will emphasize the importance of membrane potential and how to carry out calculations. You will use this information to identify the drug you have been developing for the previous several months to correct a condition that presents itself in the form of muscle spasms. You will use the Goldman-Katz equation to calculate the membrane potential of three drugs and compare them against control measurements to identify the drug you have been developing.

Case study passage: You are a researcher at a non-profit biomedical company, "Ethical Biomedicals", a company that develops drugs and distributes them to individuals who need them most, free of charge. You are the lead researcher attempting to develop a drug that is primarily used to treat muscle spasms that are the result of nerve hyperactivity. Ethical Biomedicals is also responsible for developing several other drugs as well. One day you were in a hurry to leave work and were running down the hall to put your drug samples into the freezer. You collided with your lab mate, knocking the samples out of your hands. The samples fell to the ground mixing with the drug samples he was developing. The drug ID's were wiped clean from the containers during the commotion and neither of you knew which tube contained what drug. This was your only sample, which took six-months to produce, and you did not know whether the drug worked or not. You put all the samples in the freezer and decided to tackle the problem the next day.

The next day you design an experiment to test the actions of each drug by simply applying them to somatic nerves excised from African clawed frogs (*Xenopus laevis*). In order to complete the experiment to identify your drug, you will place the nerves into a bath containing a solution that reflects ion concentration found in the interstitial fluid of humans, but it will have elevated [K^+]. You test each tube in a separate experiment: Experiment 1 will test drug "A", Experiment 2 will test drug "B", and Experiment 3 will test drug "C". You will conduct a control trial in each experiment, that tests the neuronal response to the bath without the drug. You will apply 10 mL of drug A, B, or C depending on which drug trial you are conducting. You will use ion selective electrodes to measure intracellular and extracellular ion concentrations. Measuring the ion current through patch clamping allows you estimate permeability of the membrane to specific ions. After you have collected these measurements, you will calculate membrane potential using the Goldman-Katz equation. You collect two measurements for each nerve. 1: One hour after nerve placement in the control bath with elevated ECF [K^+]. 2: One hour after 10 mL of the unknown drug (Drug "A", Drug "B", or Drug "C") has been added to the bath. You gathered a sample size of 100 per drug; in total you utilized 100 femoral nerves per experiment.

Instructions for students:

Project guidelines: Students will work together in pairs to complete the project.

Data: Students are provided a unique data set in a Microsoft Excel spreadsheet, where the analysis should be carried out according to the instructions below.

Duration: The project should require 1-2 weeks to complete and require one 50-minute classroom period. Students will be required to turn in their assignments two weeks after the assigned date.

Procedure for project completion: Students should approach each experiment separately. For each experiment, the ion concentration for extracellular (ECF) and intracellular fluid (ICF) and the membrane permeability are provided. You will need to calculate membrane potential using the Goldman-Katz equation for each sample belonging to the experiment. Once values have been calculated, separate figures should be generated for ion concentrations, membrane permeability, and membrane potential for each experiment. Combining the control trial and the drug trial (Either A, B, or C). Statistical comparisons will be completed to compare the effect of the drug on membrane potential against control conditions and membrane permeability.

Deliverables:

Students will turn in a report in lab report format.

With the project write up, you must turn in the associated spreadsheets with the calculations (See below in data analysis for more detail regarding calculations to be performed).

Data analysis:

In this project, you will identify the drug that you have developed to reduce the prevalence of muscle spasms. In the spreadsheet provided, you will analyze the data. You must turn in your spreadsheet showing your work with your final product. You must complete the following:

1. In Microsoft Excel, calculate membrane potential for each control trial and drug trial.
    a. You will need to calculate the membrane potential for each sample according to the Goldman-Katz equation:
        - Experiment 1: Control membrane potential and drug “A” membrane potential
        - Experiment 2: Control membrane potential and drug “B” membrane potential
        - Experiment 3: Control membrane potential and drug “C” membrane potential

- To calculate membrane potential, use the formula:

$$V_{\text{memb}} = (61\text{ mV}) \log\left(\frac{[\text{K}]_{\text{ECF}} * \text{PK} + [\text{Na}]_{\text{ECF}} * \text{PNa} + [\text{Cl}]_{\text{ICF}} * \text{PCl}}{[\text{K}]_{\text{ICF}} * \text{PK} + [\text{Na}]_{\text{ICF}} * \text{PNa} + [\text{Cl}]_{\text{ECF}} * \text{PCl}}\right)$$

2. In Microsoft Excel, calculate the following descriptive statistics
    a. Averages and standard deviations for each experiment for each ion and ion permeability
        - Experiment 1 example calculations:
            - Control averages for ICF and ECF ion concentration, membrane permeability, and membrane potential
            - Drug "A" averages for ICF and ECF ion concentration, membrane permeability, and membrane potential
        - Repeat for each experiment
        -
3. Prepare 3 charts for each experiment
    a. **Charts:** Data presented in graphical format – bar charts recommended
        - ICF and ECF ion concentration, ion cell permeability, and membrane potential
        - Example:
            - Experiment 1: ICF and ECF ion concentrations for control and after drug: K, Na, and Cl
                - These can be included on one figure but need to be represented by separate bars.
            - Experiment 1: Neuron ion permeability for control and after drug: K, Na, and Cl
                - These can be included on one figure but will need to be represented by separate bars.
            - Experiment 1: Membrane potential for control (before drug) and after drug
            - Repeat for each experiment
    b. **Error bars:** Charts need to include the standard deviation as error bars.
    c. **Axis labels**: Properly labeled x and y axes with units
4. Run statistics on membrane permeability and membrane potential.
    a. Carry out a paired t-test comparing control and drug data.
        - This is a two-tailed paired t-test.
        - Repeat for each experiment
        - To calculate a t-test, use the Excel function: "=t.test(array1,array2,2,1)"
            - Array 1 = control values
            - Array 2 = drug values
            - Tails = 2 – Two tails

- Type = 1 – Paired t-test
- If the t-test returns values <0.05, there is a statistically significant difference between means.

Write up:

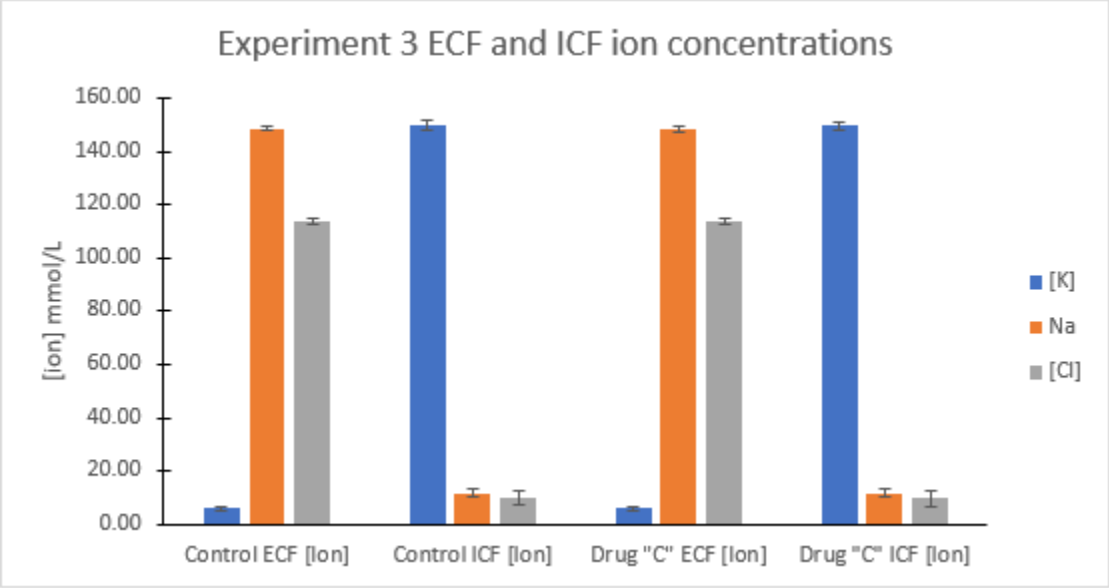
Introduction: Include the following questions and components in your write up.

1. What is membrane potential and why is it important for the application stated in the development of the drug?
2. How is membrane potential different from membrane ion equilibrium? How is resting membrane potential generated?
3. How does the neuronal resting membrane potential lead to increased excitation of skeletal muscle?
4. How could the drug potentially act to correct neuronal excitability?
5. Hypothesize the response to the drug.

Results:

1. Generate averages for ECF and ICF ion concentrations, ion membrane permeability, and membrane potential. Present this data in bar charts.
    a. Each figure needs to have the following:
        i. Error bars
        ii. Figure caption
        iii. Legend
        iv. Properly labeled axes

Example figure:



2. Explanation of figures in text.
    a. Summarize the effect of each drug on membrane potential in text.
        i. How each drug affected membrane potential.
        ii. You need to reference figures in text.

Discussion: (Figures should be referenced throughout discussion)

1. Identify which of the drug samples was the drug you have been developing. Explain how the results support your conclusion.
    - The effect on membrane potential should indicate this.
2. How does your drug reduce somatic nerve excitation?
    - You need to integrate information from fluid concentrations, membrane permeability, and membrane potential in order to do this.
3. What did the other drugs do? How did they carry this out?
4. Why did you use an elevated $[K]_{ECF}$ and how did this affect resting membrane potential?
5. Why does the resting membrane potential lead to increased excitation of skeletal muscle?
6. How else could a drug reduce the excitability of a neuron?

Evaluation:

Students will be evaluated based on the completeness and accuracy of their project. All projects must be polished and well written. If errors are present in the analysis or write up, the evaluation of your project will be negatively affected. Refer to the provided rubric for more detail regarding grading.

Evaluation rubric:

Grades will be assigned as plus or minus based on quality.

	A: Great, project meets or exceeds expectations 100-90%	**B: Good, minor improvements needed 89-80%**	**C: Sufficient, multiple aspects could be improved 79-70%**	**D: Inadequate, multiple aspects require improvement 69-60%**	**F: unacceptable, Major inadequacies in project. <50%**
Introduction **Out of 5 pts**	1. Question being tested is included AND 2. Questions to be addressed are fully answered AND 3. Hypothesis stated toward end of introduction.	1. Question being tested is not included OR 2. Questions are not fully answered, room for expansion on response is evident. OR 3. Hypothesis not clearly stated.	1. Question being tested is not included AND/OR 2. Questions are not fully answered, room for expansion on response is evident. AND/OR 3. Hypothesis not clearly stated.	1. Question being tested is not included AND/OR 2. Questions are not fully answered, room for expansion on response is evident. AND/OR 3. Hypothesis not clearly stated.	No attempt was made to include required items
Results **Out of 10 pts**	1. Membrane potential for experiments correctly calculated. AND 2. Averages and standard deviation have been correctly calculated for all experiments AND	1. Membrane potential is calculated incorrectly for one drug. AND/OR 2. Averages and standard deviation have not been correctly calculated for one experiment. AND/OR 3. All required figures are present, one is not	1. Membrane potential is calculated incorrectly for two drugs. AND/OR 2. Averages and standard deviation have not been correctly calculated for two experiments. AND/OR 3. All required figures are present, two are not	1. Membrane potential is calculated incorrectly all three drugs. AND/OR 2. Averages and standard deviation have not been correctly calculated for all three experiments. AND/OR 3. All required figures are present, three are not	1. Errors in calculations are abundant. AND/OR 2. Averages and standard deviation have not been correctly calculated for all three experiments. AND/OR 3. At least one required figure is absent, none

	3. All required figures are present, and they are summarized and referenced in the results section text. AND 4. Standard deviation is included on figures AND 5. Statistics are indicated through use of symbols on figures. AND 6. Figures are formatted correctly and contain proper title and descriptions. AND 7. Data analysis must be turned in to receive a grade higher than "C".	discussed or referenced in text. AND/OR 4. Standard deviation is absent on one figure. AND/OR 5. Statistics are not indicated on one figure through use of symbols. AND/OR 6. One figure has formatting errors or does not contain proper title and description. AND/OR 7. Data analysis must be turned in to receive a grade higher than a "C".	discussed or referenced in text. AND/OR 4. Standard deviation is absent on two figures. AND/OR 5. Statistics are not indicated on two figures through use of symbols. AND/OR 6. Two figures have formatting errors or do not contain proper titles and descriptions. AND/OR 7. Data analysis must be turned in to receive a grade higher than a "C".	discussed or referenced in text. AND/OR 4. Standard deviation is absent on three figures. AND/OR 5. Statistics are not indicated on three figures through use of symbols. AND/OR 6. Three figures have formatting errors or do not contain proper titles and descriptions. AND/OR 7. Data analysis turned in late.	of the figures are referenced in text. AND/OR 4. Standard deviation is absent on all figures. AND/OR 5. Statistics not performed. AND/OR 6. Three figures have formatting errors or do not contain proper titles and descriptions. AND/OR 7. Data analysis turned in late.
Discussion **Out of 10 pts**	1. Data explained correctly in the discussion AND 2. Questions are addressed thoroughly. AND 3. Figures referenced in discussion. AND	1. Minor errors explaining data. OR 2. Questions do not address thoroughly. OR 3. Figures not referenced consistently in discussion. OR	1. Major errors explaining data. AND/OR 2. Some questions not addressed. AND/OR 3. Figures not referenced consistently in discussion. AND/OR	1.Little attempt to explain Data. AND/OR 2. None of the questions addressed AND/OR 3. Statement regarding hypothesis either affirmed or rejected is absent.	1.No attempt to explain Data. AND/OR 2. None of the questions addressed AND/OR 3. Statement regarding hypothesis either affirmed or rejected is absent.

	4. Statement regarding hypothesis either affirmed or rejected present.	3. Statement regarding hypothesis either affirmed or rejection is not clear.	3. Statement regarding hypothesis either affirmed or rejection is not present.		
Mechanics and grammar **Out of 5 pts**	1. Lab report is well written with minimal errors present. AND 2. Spelling errors are absent.* *If present the highest grade that can be earned is a "C".	1. Some grammatical errors are present, nothing major. OR 2. Spelling errors are absent.* *If present the highest grade that can be earned is a "C".	1. Several grammatical errors present. AND/OR 2. 1-3 spelling errors present.	1. Grammatical errors are abundant and obvious. AND/OR 2. 4-5 spelling errors present.	1. Grammatical and spelling errors are abundant. 2. More than 5 spelling errors present.
Out of 30 pts total					