

Topic 3: Action Potentials

1 Required background:

1.1 Science

To complete this project you will need to access a substantial amount of additional information about action potentials and the particular experiment you will be undertaking. Some of this information will be presented in an optional lecture whose date will be advised in class (and which will also be recorded on Lectopia), and other material is available in two additional handouts: one is a document titled “The responses of mechanoreceptors of the tibial and femoral segments of the woodroach leg”, and the other is the experiment running sheet. You should also read the information in the next section on Experimental Work.

1.2 Experimental work

An important component of this project is an experimental investigation of action potentials in a woodroach leg. In the experimental component you will be working in small groups, but **everyone must complete all other work independently, write their own analyses, and submit an individual report**. We will be checking very carefully for plagiarism.

Your experimental work measures voltages due to action potentials in the woodroach leg. Measurements are taken at a number of equally-spaced discrete time steps (say 1/10000 of a second; you can select the frequency). Your experimental apparatus displays a graph of the measured voltages against time as the experiment proceeds. This allows you to make adjustments to your experiment, so that you obtain some “good” data.

When you have obtained some data which you wish to record, you can select a subset of that data and save it to a file. The data file will contain a large number of lines, with one line for each time step at which the voltage was sampled. Each line contains two values: the time at which the data was sampled, and the voltage which was measured at that time. If you load the data file into arrays in Python and plot a graph, you will see an equivalent plot to the one displayed during your experiment.

2 Questions:

- (0) (0 marks) A number of the following questions vary between students. Let T be the two digit number corresponding to the **last two digits of your student number**, so T is a number between 00 and 99 (inclusive). For example, if your number were 42136702, then $T = 02$. (It is important that you use the correct value of T for your student number; if you do not, you will lose a large number of marks. If you have any questions, please ask a tutor.)

The Blackboard site contains the file “APindividual.txt”, which includes 100 small datasets representing fabricated measurements from an idealised experiment measuring electrical signals in a woodroach leg at each of 30 consecutive time steps. Each line gives the time step (between 0 and 29 inclusive), and the measured value in volts V. Use the dataset with number T , where T is the value you calculated above. Call this dataset D_1 .

- (2 marks) Plot D_1 on a graph. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)
- (2 marks) Assume that for the experimental data above, an action potential occurs at a given time step if the measured voltage rises from **below** a threshold of $p = 0.00004$ V in **the previous** time step, to **above** that threshold in the **current** time step. Create a new dataset D_2 with 30 values, in which each entry is:

- 0, if an action potential **did not** occur in the corresponding time step; or
- 1, if an action potential **did** occur in the corresponding time step.

Plot D_2 on a graph. Rather than plotting the graph as a curve, you should mark each value with a cross, “×”. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)

3. (2 marks) Create another new dataset D_3 with 30 values, in which each entry is the **cumulative** number of action potentials which have occurred up to and including the corresponding time step.

Plot D_3 on a graph. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)

4. (2 marks) Create another new dataset D_4 with 5 “binned” values, in which each value contains the **total** number of action potentials which occurred in the corresponding group of 6 time steps. (So the first value is the number of action potentials which occurred during Time Steps 1 to 6, the second value is the number which occurred during Time Steps 7 to 12, and so on.)

Plot D_4 as a histogram. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)

5. (a) (2 marks) Create another new dataset D_5 with 30 values, in which each entry is defined by:

- * if there was no action potential at this time step, then the value is 0; or
- * if there was an action potential at this time step, then the value is the number of time steps since the most recent action potential prior to this one.

Plot D_5 on a graph. Rather than plotting the graph as a curve, you should mark each value with a cross, “×”. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)

- (b) (1 mark) Find the largest number of time steps which occurred between any two consecutive action potentials.

6. (2 marks) Create another new dataset D_6 with 30 values, in which each entry is the total number of action potentials that occurred in the 6 most recent time steps, including the current step. (For each time value less than 6, the entry should be the total number of action potentials that have occurred so far.)

Plot D_6 on a graph. Rather than plotting the graph as a curve, you should mark each value with a cross, “×”. Choose appropriate scales for your axes, and include a title and labels on your axes. (You may draw the graph in any way that you like, by hand or using a computer.)

7. (a) (0 marks) Write a Python program that analyses data recorded during an action potential experiment. You may assume that every line of the data file is in the correct form (that is, contains a time measurement followed by a voltage measurement), so you do not need to do any error checking of the file contents. Your program must:

- Prompt the user to enter the following:
 - the threshold voltage for an action potential; and
 - the name of the file that contains the data.
- For each time step, calculate whether or not an action potential occurred at that time step.
- For each time step, calculate the **cumulative** number of action potentials which have occurred up to and including that time step.
- Have variables with meaningful names, and be appropriately commented.
- On completion, print each time step number, whether an action potential occurred at that time step, and the cumulative number of action potentials that have occurred up to and including that time step.

Hint(s):

- You may assume that all input values are valid.
- The SCIE1000 Blackboard site contains a file called “biomed.py” which contains some useful commands. To use these commands, place a copy of that file in the same folder as the programs that you are writing, and import it into your program using the command

```
from biomed import *
```

after the other import statements. The following command which is defined in “biomed.py” may be useful:

readFile: This command asks the user for the name of a data file with lines in the correct form for this project, and reads all of the data values into arrays. The command may be used as follows:

```
(times, voltages, smoothed, numData) = readFile()
```

After calling, `times` will be an array containing the times at which voltage measurements were taken, `voltages` will be an array containing the measured voltages, and `numData` will be the number of values that were successfully read and placed in the arrays. For the moment, ignore the array `smoothed`.

- As an example, here is some Python that uses this command:

```
(times, voltages, smoothed, numData) = readFile()
print "The number of data values read is", numData
print "The full set of times read is", times
print "The voltage values read are", voltages
```

- Note that you must **not** change the contents of `biomed.py` under *any* circumstances. We suggest that you **do not** read the code in `biomed.py`, as it uses some programming constructs which you have not learned.

(b) (4 marks) Print and submit a copy of your program. Marks will be awarded for:

- Adherence to the program specifications given above
- Appropriate programming style, structure and logic
- Appropriate print statements, with helpful text explanations of the output
- Use of comments and meaningful variable names

(c) (2 marks) Test your program in the following ways. You must include a printed copy of the output from your program in your submission; in each case, verify the output against your hand calculations. (Hint: in order to test the program, you will need to copy and paste the dataset with number T from the file “APindividual.txt” into a file in the same folder as your Python program. You calculated the value of T in Question 0. When copying the dataset, only copy the 30 lines containing pairs of numbers.)

(i) Use your program to repeat Question 2.

(ii) Use your program to repeat Question 3.

(d) (1 mark) The SCIE1000 Blackboard site contains a file called “APsmall.txt”. Use the output from your program to answer the following questions. In each case, use an action potential threshold of 0.00004 V.

(i) How many action potentials occurred overall in this dataset?

(ii) At what timestep did the cumulative number of action potentials reach 20?

(iii) During which period of 15 consecutive timesteps did most action potentials occur, and how many were there?

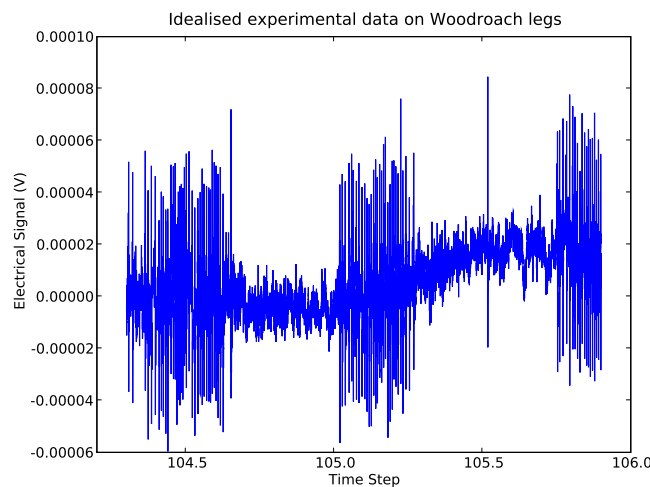
- (iv) What is the longest sequence of timesteps during which there was a consecutive sequence of alternating action potentials and no action potentials? At what timestep did this sequence commence?

All questions above this line must be completed as part of your initial project submission.

3 Science

3.1 Baseline shift

One source of error when experimentally measuring voltages is *baseline-shift*, where the base level of “background” voltage changes over time. Consider the following graph, showing data obtained from an experiment similar to yours:



The graph shows that for times from about 104 s to 105.2 s, the measured voltage is roughly centred around 0 V (which is what we would expect). However, for times greater than about 105.2 s, the baseline voltage has shifted up, and is no longer centred around 0 V. As a result, almost all voltage measurements after 105.2 s are above an action potential threshold $p = 0.00002$ V, but it is clear from the graph that action potentials are not occurring in many of those cases. Instead, the ‘baseline voltage’ has shifted.

A common way of handling such variations is to *smooth* the data. One way of doing this is to calculate, for each time step, a *rolling baseline average* value over a number of measurements before and after that time step, and then subtract this average from the measured value at that time step. Later, when writing your Python program to process the data from your experiment, you will use a command which we have written to read the data from the file. This command will automatically smooth the data for you if you request it to.

3.2 Python

If you wish to plot multiple graphs within a Python program then you need to use the `figure` command **before** each call to `plot()`. Also, **do not** include the `show()` command until you have drawn the final graph. Here is an example with two graphs; you can plot more graphs in a similar way:

```
figure()
plot(x,y1)
figure()
plot(x,y2)
show()
```

To plot a bar graph (or histogram) where the first x value for each bar is in the array `bins`, the height of each bar is in the array `heights`, and the width of each bar is a constant `binWidth`, use the following commands:

```
figure()
bar(bins, counts, width = 0.9*binWidth)
show()
```

4 Questions

You can do Question 9 whether or not you have done the experimental work in Question 8.

8. (6 marks) Complete the experimental component of the project. Details on how to do this are on a different handout, and there will be tutors available to help you. The goal of the experiment is to produce a number of datasets that can be analysed with your program. There are also some questions on the experiment sheet; your answers to those questions must be written on that sheet, and will be marked by your tutor during the laboratory work. If you do not do the experiment, or receive less than half marks on the laboratory questions, then you will **lose 20 marks from your project total**.

9. Note that we have not covered how to write *functions* in Python. You **may not** write functions in the following program. (If you do not know what this means then you don't need to worry about it.)

(a) (0 marks) Modify your Python program from Question 7 so that it now:

- Prompts the user to enter the following:
 - the threshold voltage for an action potential;
 - whether they want to perform baseline smoothing of the data;
 - the “bin size”, which is the number of time steps whose values should be included in each “bin” in a frequency histogram; and
 - the name of the file that contains the data;
- Produce the following output:
 - A graph of the data read from the file (equivalent to the graph in Question 1 above).
 - A graph showing when action potentials occurred (equivalent to the graph in Question 2 above).
 - A graph of the cumulative number of action potentials which have occurred up to and including each time step (equivalent to the graph in Question 3 above).
 - A graph of the total number of action potentials which occur in each consecutive “bin” of time steps (equivalent to the graph in Question 4 above).
 - A graph of the number of time steps since the most recent action potential, at any time (equivalent to the graph in Question 5 above).
 - A graph of the total number of action potentials that occurred in the “bin size” most recent time steps, including the current step. For time values less than the “bin size”, the values should be the total number of action potentials that have occurred so far. (This graph is very similar to the one in Question 6.)
- On completion, print the largest consecutive number of time steps during which no action potential occurred.

Hint(s):

- You have already copied the file “`biomed.py`” into your Python folder. The file defines the following commands:
readFile: You have already used this command to read the data into your program, in the following way:

```
(times, voltages, smoothed, numData) = readfile()
```

The array `voltages` will contain the voltage values read from the file, and the array `smoothed` contains those values but with the baseline smoothed. The choice of which values to use is governed by whether the user chooses baseline smoothing or not.

binIt: This command takes three input parameters: an array `times` of time values, an array `D2` of 0s and 1s indicating whether an action potential occurred at the corresponding time step (0 means no, 1 means yes), and the number of entries `numInBin` that should be “binned” into each group. The command may be used as follows:

```
(binTimes, binHeights, binWidth) = binIt(times, D2, numInBin)
```

After calling, `binTimes` will be an array containing the time values at which each bin commences, `binHeights` will be an array containing the number of 1s in each bin, and `binWidth` will be the (constant) time width of each bin. This command will be very useful for creating the data used to draw your “binned” histogram.

- As an example, here is a sample program that uses this command and then draws a histogram:

```
figure()
```

```
(bins, D4, binWidth) = binIt( ts, D2, binSize)
```

```
bar(bins, D4, width = 0.9*binWidth)
```

- As before, that you must **not** change the contents of `biomed.py` under *any* circumstances. We suggest that you **do not** read the code in `biomed.py`, as it uses some programming constructs which you have not learned.

(b) (14 marks) Print and submit a copy of your program. Marks will be awarded for:

- * Adherence to the program specifications given above
- * Appropriate programming style, structure and logic
- * Appropriate print statements, with helpful text explanations of the output
- * Use of comments and meaningful variable names

(c) First test your program in the following ways. (You must include a printed copy of the output from your program in your submission.) In each case, use the dataset you used in Questions 1 to 6, and a threshold value of $p = 0.00004$ V. At this stage, do **not** use baseline smoothing.

(i) (1 mark) Print the first graph and verify that it matches your answer to Question 1.

(ii) (1 mark) Print the second graph and verify that it matches your answer to Question 2.

(iii) (1 mark) Print the third graph and verify that it matches your answer to Question 3.

(iv) (1 mark) Print the fourth graph, using a “bin size” of 6 time steps per bin, and verify that it matches your answer to Question 4.

(v) (1 mark) Print the fifth graph and the largest number of time steps between action potentials, and verify that the graph and value match your answers to Question 5.

(vi) (1 mark) Print the sixth graph, using a “bin size” of 6 time steps, and verify that it matches your answer to Question 6.

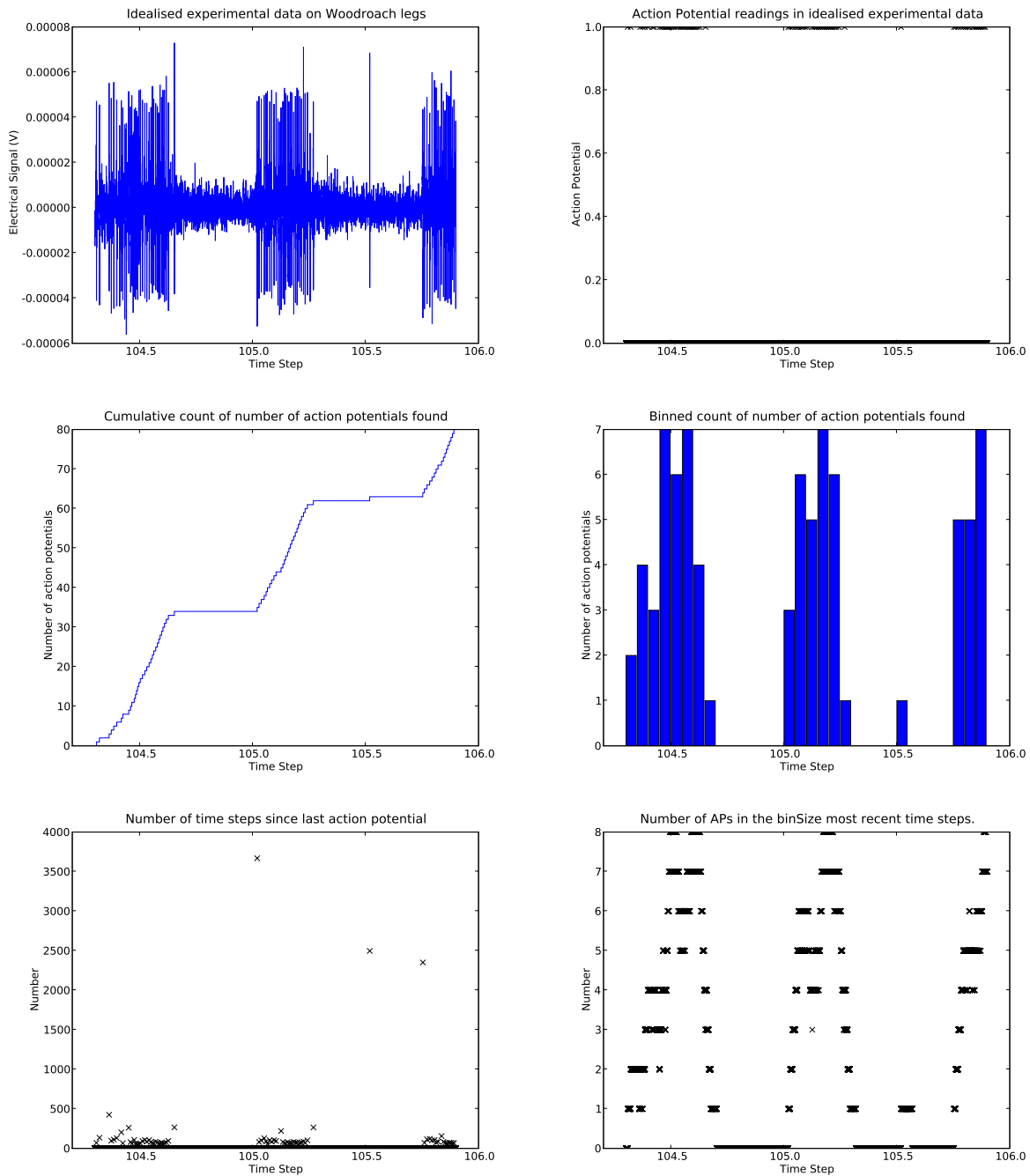
(d) Now test your program in the following ways. (You must include a printed copy of the output from your program in your submission.) In each case, use the dataset in the file called `APbig.txt` on the SCIE1000 Blackboard site. Choose an action potential threshold value of $p = 0.00004$ V, **do use** baseline smoothing, and choose a “bin size” of 500.

(i) (1 mark) Print the first graph from your program and visually verify that it matches the top-left graph below. (From now on we will call the first graph G1.)

(ii) (1 mark) Print the second graph G2 from your program and verify that it visually matches the top-right graph below.

(iii) (1 mark) Print the third graph G3 from your program and visually verify that it matches the middle-left graph below.

- (iv) (1 mark) Print the fourth graph G4 from your program (with a bin size of 500) and visually verify that it matches the middle-right graph below.
- (v) (1 mark) Print the fifth graph G5 from your program and the largest number of time steps between action potentials, and visually verify that the graph matches the bottom-left graph below. (You should have found that the largest number of time steps between action potentials is 3668.)
- (vi) (1 mark) Print the sixth graph G6 from your program (with a bin size of 500) and visually verify that it matches the bottom-right graph below.



(e) (5 marks) Write a brief user guide which explains how to use the program. (Your user guide should **not** assume that the user has read this assignment question sheet.) The guide should contain all necessary information about:

- What the program does.
- What input is requested by the program, and what valid input it can take.
- What output the program gives.

- Any assumptions you have made, or any special cases.

This is a user guide, not a programmer's manual. Do not describe the algorithm you have used or internal details of the program. Instead, if someone with a basic understanding of computers (and a good understanding of the science relevant to your project topic) wanted to run your program, what would they need to know?

10. When studying action potentials, biologists are often interested in the *rate* at which action potentials occur. Rather than directly discussing the frequency, it is more common to consider the average *interspike interval* or *ISI*, which is the average time between action potentials, averaged over a short time period (to smooth out fluctuations).
- (2 marks) In 60 words, describe how the features of G1 relate to the average ISI, including how a **small** average ISI would be reflected in G1?
 - (2 marks) In 60 words, describe how the features of G2 relate to the average ISI, including how a **small** average ISI would be reflected in G2?
 - (2 marks) In 60 words, describe how the features of G3 relate to the average ISI, including how a **small** average ISI would be reflected in G3?
 - (2 marks) In 60 words, describe how the features of G4 relate to the average ISI, including how a **small** average ISI would be reflected in G4?
 - (2 marks) In 60 words, describe how the features of G5 relate to the average ISI, including how a **small** average ISI would be reflected in G5?
 - (2 marks) In 60 words, describe how the features of G6 relate to the average ISI, including how a **small** average ISI would be reflected in G6?
11. (2 marks) The Python graphing window allows you to “zoom in” on a graph. Zoom in on the graph G1 until you can see two action potentials. Print a copy of the zoomed graph, and compare what you saw with the graph of the single action potential on your background information sheet. In 100 words, describe the key similarities and differences.
12. In Question 13, you need to write an extensive report based on your experimental data and the program output. In this question you need to choose the two datasets you will use in Question 13, and answer some introductory questions on them. **Do not** include any output from your program here; instead, all output will be included in Question 13.
- (2 marks) Select **one** of your datasets recorded when you stimulated part of the woodroach leg five times in succession; you should choose the dataset that most clearly shows the action potentials during each of the five stimulations. Call this dataset *Data 1*. Run your program on this dataset, **using baseline smoothing**, with an appropriate choice of action potential threshold and bin size. What values did you choose for your action potential threshold and bin size? In a total of about 60 words, justify your choices.
 - (2 marks) Select **one** of your datasets recorded when you stimulated part of the woodroach leg for an extended period; you should choose the dataset that most clearly shows the action potentials over time. Call this dataset *Data 2*. Run your program on this dataset, **using baseline smoothing**, with an appropriate choice of action potential threshold and bin size. What values did you choose for your action potential threshold and bin size? In a total of about 60 words, justify your choices.
13. (25 marks) When answering this question, if relevant you may include some graphs, diagrams, tables of values, equations or mathematical calculations (which will not be included in the word limit), but your response should be predominantly text-based. The report must be typed, written in a professional style,

and be within 10% of 2000 words in length. Marks will be deducted for any report with length outside this range.

It is **not** appropriate to use other sources or references in your report; all of the discussion and recommendations should arise solely from your own work, including output from your program. The course Blackboard site contains a Criteria Sheet for this report, showing how marks will be allocated. **(The answer to this question must be submitted in hardcopy and also via Turnitin; see the project overview document.)**

An insecticide company called *Bug begone* (BB) employs you to investigate habituation to stimuli in woodroaches. They pose the following broad question: is it possible to observe habituation in a simple experiment?

Write a report for BB. The report must meet the following criteria:

- It must be understandable by BB’s management, who have some knowledge of simple mathematics, but are not experts.
- It should **not** describe any of the experimental, computational or mathematical details of your work; instead, BB wants you to answer their specific questions.
- It must include five sections:
 1. An “Executive Summary” of around 150 words, giving a very brief overview of your findings, and listing the answers to some of their key questions. The rest of the document will expand on the content of the executive summary.
 2. A detailed answer to the following questions regarding your results using the dataset called *Data 1* (see Question 12). You must include copies of all six graphs.
 - (a) **Interpret** the results you obtained, with reference to your graphs.
 - (b) Explain how you could calculate the **approximate** slopes of the five oblique line segments in G3 **from the graph**, and then calculate the slopes of each of the segments. What do these slopes represent?
 - (c) Calculate the average (mean) of the five slopes.
 3. A detailed answer to the following questions regarding your results using the dataset called *Data 2* (see Question 12). You must include copies of all six graphs.
 - (a) **Interpret** the results you obtained, with reference to your graphs. (At this stage, do not compare your results to those obtained using *Data 1*.)
 - (b) Derive an equation which approximates the graph G3 from this dataset. You must submit a copy of your final equation, its graph, and a discussion about the process you used to estimate the equation. (Hint: Your equation will obviously not **exactly** match the graph; instead, aim for a reasonable match of the general shape and size of the graph. You may derive this in any way you like; one approach is to write a Python program that plots the graph of an equation, and then you can experiment with repeatedly changing the equation, plotting the graph, seeing how the graph compares with what you want, then changing the equation and repeating. For simplicity, you may assume that G3 starts at $t = 0$.)
 4. Did your experiment identify evidence of habituation? In your answer, compare and contrast each of the six pairs of corresponding graphs from *Data 2* and *Data 1*. In each case, explain how the graph from *Data 2* illustrates adaptation (or why it does not, if your data does not show adaptation).
 5. A detailed answer to the following questions.
 - (a) In Section 2(c) you calculated the average slope of five line segments, which gives the average rate at which action potentials occurred during those stimuli. Use this figure to estimate roughly the number of action potentials that would occur if the stimulus lasted for 200 seconds, assuming no adaptation.

- (b) In Section 3(b) you derived an equation for the number of action potentials that occurred when adaptation is present. Use this equation to estimate roughly the number of action potentials that would occur if the stimulus lasted for 200 seconds, assuming that adaptation occurs.
- (c) Compare your results from Parts (a) and (b). What is the biological significance of this?

The end