**Lab: Understanding Action Potential and Frequency Coding**

Pre Lab Activity:

Clinical Healthcare Connection

Early diagnosis of diabetic peripheral neuropathy is important for the successful treatment of diabetes mellitus. Electrophysiology studies of neuronal axons in the extremity can help in facilitating early diagnosis of diabetic peripheral neuropathy, reducing treatment costs and improving therapeutic success. Diabetic peripheral neuropathy develops with various clinical manifestations. In the early stages, diagnosis is difficult as there are no symptoms. Fortunately, increasing use of electrophysiological techniques that allow the identification of sub-clinical pathological changes has made early diagnosis of diabetic peripheral neuropathy possible.

Based on the action potential activity that you have completed. Has this activity changed your opinion about the importance of understanding neurophysiology in determining proper diagnosis and treatment options for diseases such as peripheral neuropathy. Explain your reasoning.

Have you heard of diabetic peripheral neuropathy? If yes, explain. If no, please take the time to ask someone at your healthcare workplace to start a conversation.

Scientist Spotlight

https://[scientistspotlights](https://scientistspotlights.org/).org/

Primary Literature:

[A low-cost computational approach to analyze spiking activity in cockroach sensory neurons](https://journals.physiology.org/doi/abs/10.1152/advan.00034.2020)

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**In Lab:**

**Background**

An understanding of the electrical properties of the neurons is essential in unraveling the enormous complexity of the central nervous system and peripheral nervous system of the human body. Understanding the important features of the neuronal action potential can be helpful in understanding how the nervous system encodes information based on frequency. Deeper appreciation and insight into the conduction and transmission process of the neuronal network is essential many disease models such as Parkinson’s, Epilepsy and Peripheral Neuropathy.

In neurons, action potentials play a central role in neuronal cell to cell communication by assisting the propagation of signals along the axon towards the axon synaptic terminal situated at the end of the neuronal axon. Action potentials in neurons are also known as "**nerve impulses**" or "**spikes**". A neuron that emits an action potential, or nerve impulse, is often said to "fire".

As the nerve impulse travels down an axon there is a change in the polarity across the membrane of the axon. In response to a signal, gated ion channels open and close as the membrane reaches its threshold potential.

There are many models that describe nervous impulse as an electromechanical wave that travels along nerve fibers, explaining many concepts about how the brain works.

The initial experiments carried out by Alan Hodgkin and Aldous Huxley in 1930’s explained the mechanism of nervous impulse as an electrical phenomenon. Their experiments were carried out in giant squid axons in salt water solution using microelectrodes. They injected electrical current into the nerve fiber and recorded its voltage. This enabled them to control the voltage across the membrane and also measure the movements of current responsible for producing the impulse. These initial studies led to the description of action potential as a wave of electrical discharge that travels along the axonal membrane.

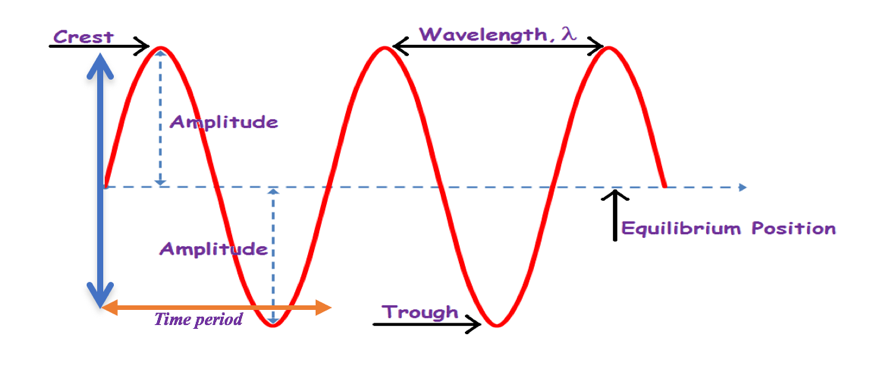
 Squid giant axon

By NIH History Office from Bethesda - Giant Axon of Squid, Public Domain, https://commons.wikimedia.org/w/index.php?curid=82867221

# **Anatomy of a wave:**

Before we start talking about the nervous system and flow of electric signal between the neuronal membrane, we must understand the anatomy of a wavefunction.

Figure 1



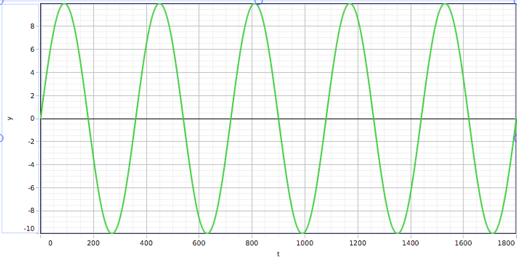
Wavelength (λ): is the distance between one point on a wave and the exact same place on the next wave. The units are meter (m).

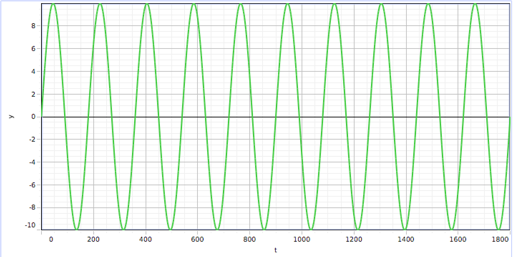
Amplitude: is how far high is the crest from rest position (equilibrium position). The units of the amplitude depend on the type of wave.

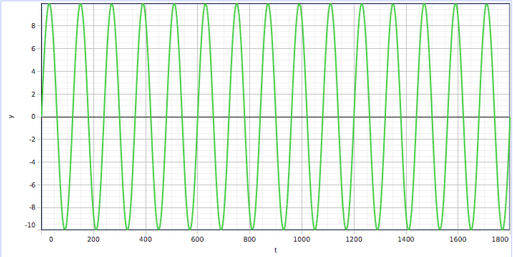
Frequency (f): is the number of cycles that pass a reference point per second; frequency is just measured in "inverse seconds", or Hertz, (Hz).

* + If 10 waves go past in 1 second, frequency is 10 Hz
* Higher the frequency greater is the intensity of the signal
* As the wavelength decreases frequency increases as seen in the three images below (Figure 2)
  + The orange line denotes the time that runs through the three different scenarios.
  + Note in Part A: There is one complete wave within the timeframe denoted by the orange line.
  + Note in Part B: There are two complete waves within the timeframe denoted by the orange line. This suggests that the frequency is doubled.
  + Note in Part C There are three complete waves within the timeframe denoted by the orange line. This suggests that the frequency is tripled.

Figure 2

 A

 B

 C

# **Electrical Changes during an Action Potential:**

The working of the human nervous system depends on the flow of electric charge; the electric signal passes along the basic elements of the nervous system, the neurons. A neuron before transmitting an electric signal is in the so called “resting state”. The neurons have a net positive charge on the outer surface of the cell membrane and a negative charge on the inner surface. This difference in charge implies that if using a voltmeter, we measure the potential across the cell membrane a potential difference will exist. Just like if you put the leads of the voltmeter to the two ends of a AA battery it would read a potential difference of 1.5 V. The potential difference across a neuron when it is not transmitting; the resting potential (Vinside -Voutside) is typically in the -60mV to -90mV range.

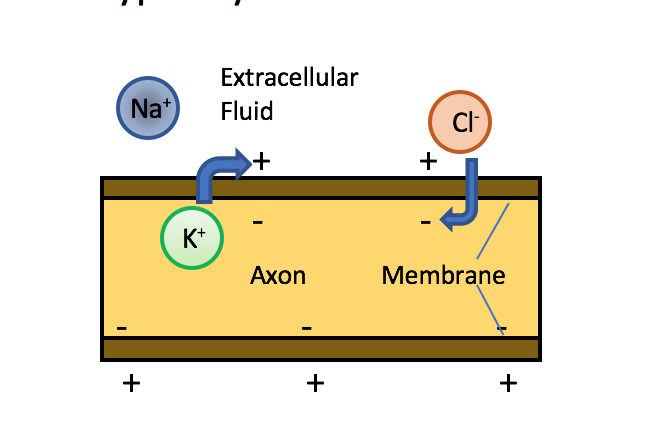
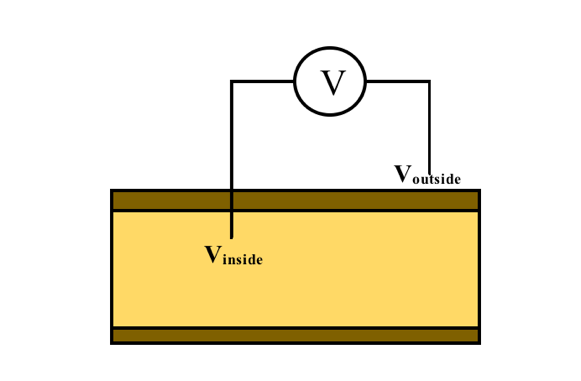
 

Figure 3: Dipole layer of charge across the cell membrane Figure 4: Measuring the potential difference across the cell membrane.

The most important aspect of neurons is not this potential difference. Neurons can respond to a stimulus; the stimulus can be thermal, coming from a hot stove or chemical as in your taste buds; it can be pressure on your skin when something pokes you or it can be the light falling on your eyes. In the laboratory this signal is generally electrical and is applied using a tiny probe on some point on the neuron. If this stimulus exceeds some threshold, a voltage pulse will travel down the axon and can be measured using a voltmeter or oscilloscope connected across the cell membrane. This voltage pulse has the shape as shown below and is called an action potential. Here as we see the potential increases from the resting potential of -70 mV to +40 mV. The action potential lasts for about 1 ms and travels down the axon at a speed of 30 m/s to 150 m/s that is somewhere between 60.1 mph to 335.54 mph.

Role of ions in Action Potential:

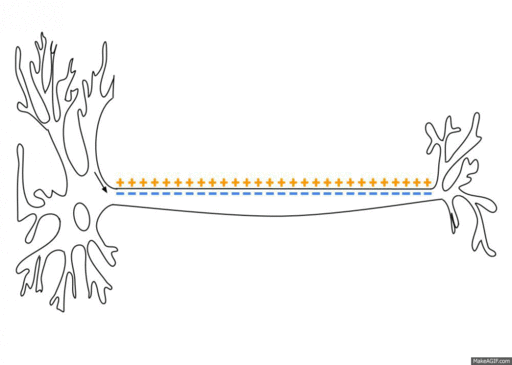


Figure 5: Role of ions in neuronal action potential transmission

[By Laurentaylorj - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=26311114](file:////Users/Sheela/Downloads/By%20Laurentaylorj%20-%20Own%20work,%20CC%20BY-SA%203.0,%20https:/commons.wikimedia.org/w/index.php%3fcurid=26311114)

An action potential is a rapid rise and subsequent fall in voltage across the cellular membrane with the characteristic pattern as shown above. At the point where the stimulus is applied the membrane suddenly alters its permeability, becoming much more permeable to Na+ ions than to K+ and Cl-. Thus Na+ ions rush into the cell and the inner surface of the wall becomes positively charged and the potential difference quickly becomes positive. Just as suddenly, the membrane returns to its original characteristics; and starts pumping out Na+ and the original resting potential is restored. Sufficient current (stimulus) is required to initiate a voltage response in a cell membrane; if the current is insufficient to depolarize the membrane to the threshold level, an action potential will not fire.

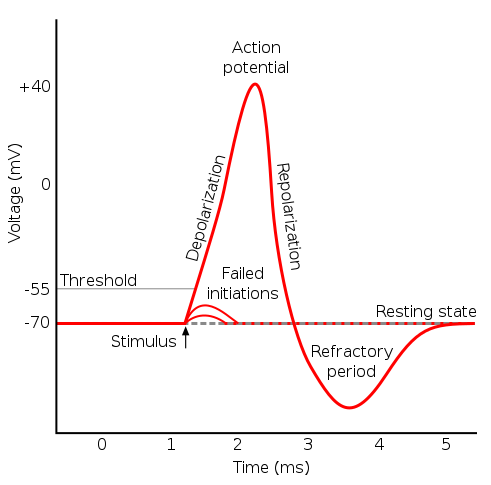
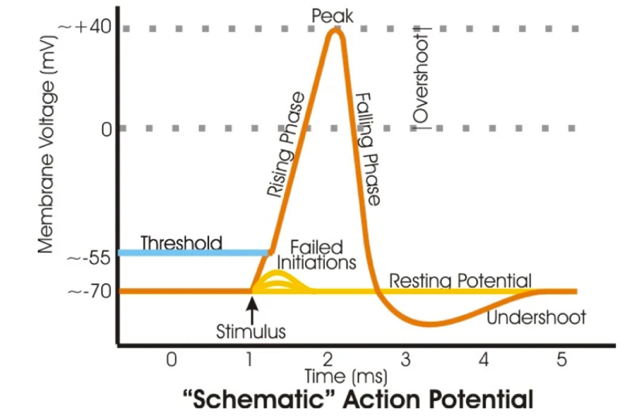


Figure 6: Schematic Action Potential plots

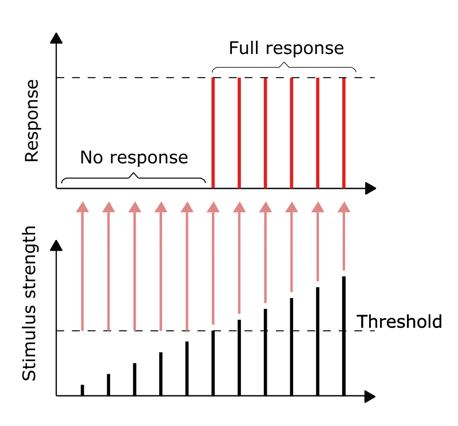
Source: <https://psychology.wikia.org/wiki/Action_potential>

Approximate plot of a typical action potential shows its various phases as the action potential passes a point on a cell membrane. The membrane potential starts out at approximately −70 mV at time zero. A stimulus is applied at time = 1 ms, which raises the membrane potential above −55 mV (the threshold potential). After the stimulus is applied, the membrane potential rapidly rises to a peak potential of +40 mV at time = 2 ms. Just as quickly, the potential then drops and overshoots to −90 mV at time = 3 ms, and finally the resting potential of −70 mV is reestablished at time =5ms. [CC BY-SA 3.0](file:///Users/Sheela/Downloads/CC%20BY-SA%203.0)

How does the nervous system differentiate between the various intensities of the stimulus especially when the main characteristic of the Action Potential is the all or nothing concept?

The all or none principle of action potential says that the neuronal axonal fiber can give maximum response or none at all. When the single axon is stimulated, above the threshold level it will always give the maximum response and produces spikes of the same amplitude. Even if the stimulus strength is increased, the size (i.e., amplitude) of the action potential will not change. This is called the "all or none" principle.

How does the nervous system differentiate between the intensity of the stimulus? The frequencies of the action potential that is generated is an important indicator in coding the strength of the stimulus. When the stimulus is strong, it leads to the generation of higher frequency action potentials. We can conclude that the frequency of action potentials generated is directly related to the intensity of the stimulus that is applied on the neuronal axon membrane. Therefore, it is described that our nervous system is frequency-modulated and not amplitude-modulated.



As long as the stimulus reaches the threshold, the full response would be given. Larger stimulus does not result in a larger response,

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Quantitative Reasoning:

Part A: Using mathematical reasoning to understand a biological concept

**Question 1**

Let us now look at the list of stimuli provided and sort the stimuli that will pass the threshold of -55 mV and are able to create an action potential.

Choose from the following choices:

* Yes,
* No,
* Maybe,
* Cannot decide based on the information.

Table 1:

|  |  |
| --- | --- |
| Stimulus applied (mV) | Will it create an action potential? |
| -65 |  |
| -40 |  |
| -20 |  |
| -45 |  |
| +5 |  |
| -52 |  |
| -57 |  |
| -30 |  |
| -26 |  |
| -42 |  |
| -60 |  |
| +16 |  |
| -39 |  |
| -15 |  |
| -10 |  |
| -68 |  |
| -55 |  |
| -27 |  |
| -59 |  |
| 30 |  |

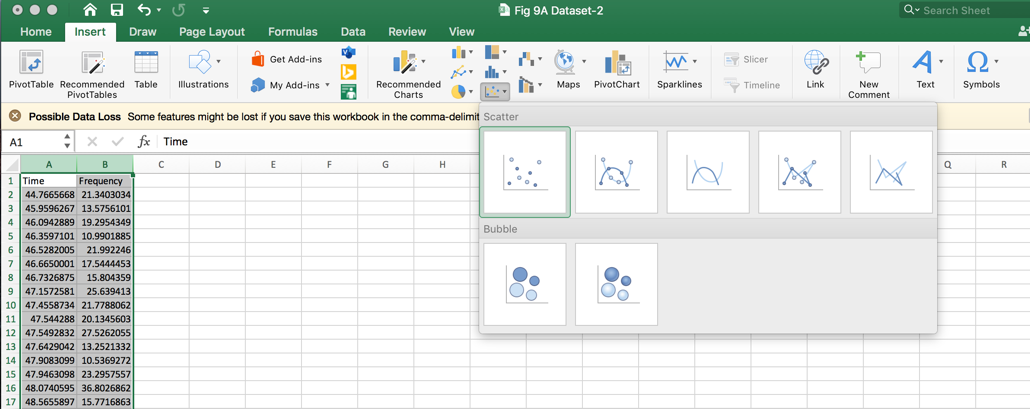
**Question 2 Explain** your rationale for choosing the options

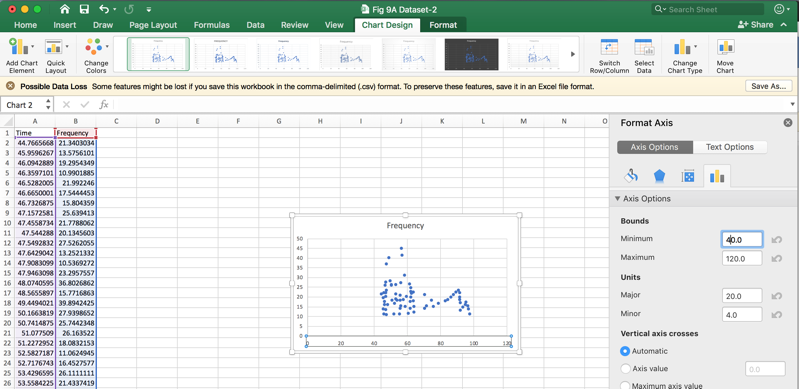
Part B: Plotting and Analysis of Raw Experimental data

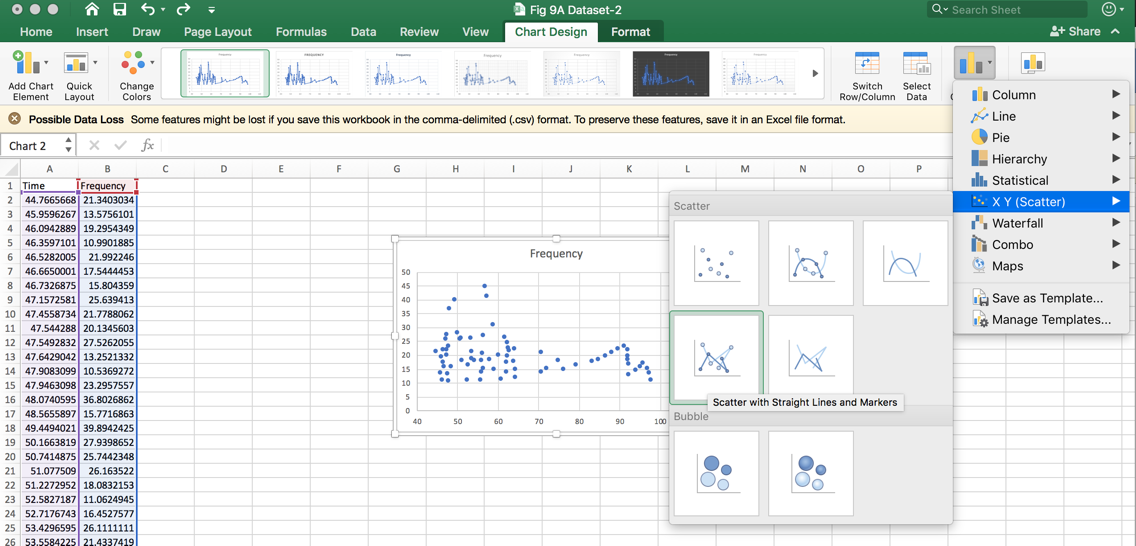
Use the data in the attached excel sheet to plot the firing frequency (y-axis) vs time (x-axis) for the mechanical stimulation of the first spine of the hissing cockroach.

Specific instructions on creating a graph with a spreadsheet:

1. Open Excel sheet attached.
2. Highlight all the data in both the columns.
3. Choose “Insert” on the top ribbon.
   * Select “Scatter Chart”. Some versions of Excel will call it an X-Y scatter plot.



1. Click on the horizontal axis and under Format Axis-> Axis Options set Bounds minimum to 40.0
2. At the top ribbon on you excel sheet click on Change Chart Type (1); go the menu for XY (Scatter) (2) and choose Scatter with Straight lines and markers (3).



3

2

1

1. Add appropriate Axis labels and Chart Title to your XY scatter plot

**Question 3**

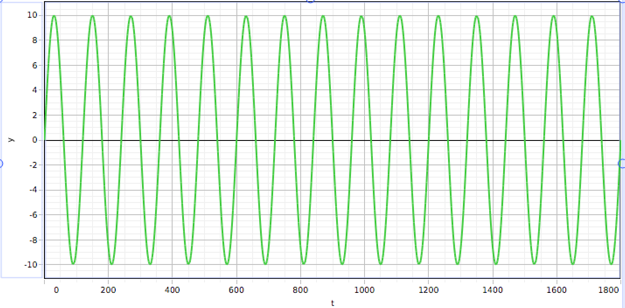
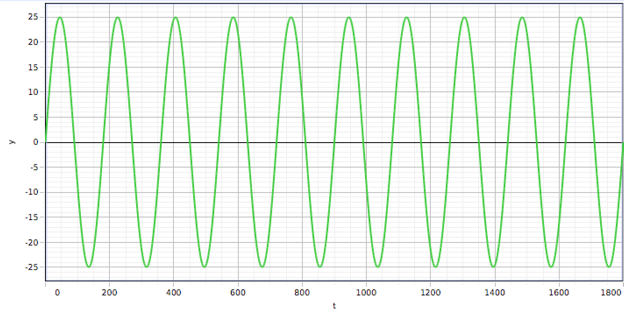
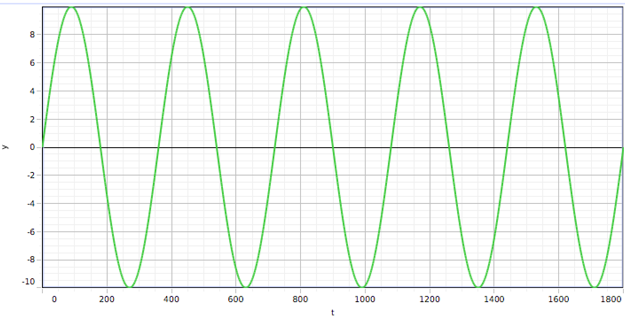
How does the firing frequency change with time in the graph you plotted in Step e?

**Question 4**

Describe in your own words, how the process of analyzing the data sheet using Excel helped you clarify some of your own misconceptions about the firing of the neuronal action potential.

1. What did you learn new about the firing of the action potential while working on this analysis?
2. What areas did you find difficult during the process of analysis and how did you overcome the challenges?

Part C Analysis of data presented in a graphical format



B

C

A

**Question 5**

Look at the **abov**e three wave-signals and pick your choice

1. Which of the wave signal has maximum amplitude?
2. Which of the wave signal has maximum frequency?
3. Which of the wave signal has maximum wavelength?

**Question 6**

You must explain the rationale for each of your choices. You must also explain why the other two choices are not part of your answer.

**Question 7**

Using the scatter plot you created in Excel what is the frequency range with maximum data?

1. 10 Hz – 30 Hz
2. 25 Hz – 45 Hz
3. 0 Hz – 20 Hz

**Question 8**

Explain your rationale for the choice in Q7.

**Question 9**

How does our nervous system distinguish between weak and strong stimulus?

**Question 10:** How will the action potential generated differ with the changes in stimulus strength?

**Reflection Questions based on Scientist Spotlight Initiative**

Based on what you know now, describe the types of people that do science. If possible, refer to specific scientists and what they tell you about the types of people that do science.

Please reflect on the statement below.

Choose and write the number and phrase that reflects your level of agreement.

Then, write 200 or more words about your reflections on this statement and your level of agreement.

**“I know of one or more important scientists to whom I can personally relate.”**

Strongly Disagree Disagree Agree Strongly Agree Don’t Know

**1 2 3 4 DK**

***Explain whether your views changed on either of these prompts, and if so, why did this change.***