

# [Action potentials in squid axon](https://assignbuster.com/action-potentials-in-squid-axon/)

In 1952, Hodgkin and Huxley published a series of four papers in the Journal of Physiology (London) reporting their experiments to investigate the underlying events of the action potential. In their final paper, they derived a series of equations that describe the relationship between sodium conductance (gNa+), potassium conductance (gK+) and the membrane potential in a squid axon following electrical stimulation. Hodgkin and Huxley were awarded the Nobel Prize for this work.

In this practical, you will use a computer program based on the Hodgkin and Huxley equations to show what is happening to the membrane potential, gNa+ and gK+ during and after electrical stimulation. An example of the output from the program is illustrated in figure 1. It can be seen that the electrical stimulation depolarises the membrane. Once a depolarisation of 30mV has occurred, the conductance to sodium ions increases rapidly and the membrane potential rises to +20mV. The rise in gK+ is slower in onset and lasts for longer than the increase in gNa+. The fall in gNa+ and the associated rise in gK+ returns the membrane potential towards the resting value.

Figure 1: Simulation of changes in membrane potential, Na+ and K+ conductances following the application of a single electrical stimulus of 50 ­A/cm2 for 1 ms. The peak height, amplitude, latency and threshold of the action potential are shown.

## Methods and Results

Run the Squid Giant Axon simulation from the Start menu, HHX.

## Experiments using a single electrical stimulus

In the first series of experiments, you will use a single electrical stimulus to initiate an action potential. Run a simulation with the following parameters:

## Stimulus 1 Amplitude (­A/cm2)

## Stimulus 1 Duration (ms)

## Delay (ms)

## Stimulus 2 Amplitude (­A/cm2)

## Stimulus 2 Duration (ms)

50

1

0

0

0

A trace similar to figure 1 will be obtained. From this trace, you can measure the peak height, amplitude, latency and threshold of the action potential:

## Peak Height

## (mV)

## Amplitude

## (mV)

## Latency

## (ms)

## Threshold Voltage (mV)

+19

109

0. 46

-66

Q1 and 2. Investigate the effects of varying stimulus amplitude and duration by running all the simulations shown in the matrix below in Table 1: Enter a ‘ X’ in the Table 1 matrix for experiments that produce an action potential, and record the peak height, amplitude, latency and threshold of any action potentials in Table 2 overleaf. For experiments that fail to elicit an action potential, enter a ‘ O’ in the matrix below, and record a value of ¥ (infinity) for the latency and ‘-‘ for the other parameters in the table overleaf.

## Table 1. Success/failure matrix

## Stimulus Strength (­A/cm2)

## Stimulus Duration (ms)

## 0. 1

## 0. 5

## 1

## 2

## 5

## 50

## O

## X

## X

## X

## X

## 20

## O

## X

## X

## X

## X

## 10

## O

## O

## X

## X

## X

## 7

## O

## O

## X

## X

## X

## 5

## O

## O

## O

## X

## X

## 2

## O

## O

## O

## O

## O

## Table 2: Action potential characteristics

## Stimulus

## Response

## Strength

## (­A/cm2)

## Duration

## (ms)

## Peak Height

## (mV)

## Amplitude

## (mV)

## Latency

## (ms)

## Threshold Voltage (mV)

2

0. 1

## –

## –

## ¥

## –

0. 5

## –

## –

## ¥

## –

1

## –

## –

## ¥

## –

2

## –

## –

## ¥

## –

5

## –

## –

## ¥

## –

5

0. 1

## –

## –

## ¥

## –

0. 5

## –

## –

## ¥

## –

1

## –

## –

## ¥

## –

2

14

104

2. 89

-61

5

15

105

2. 74

-59

7

0. 1

## –

## –

## ¥

## –

0. 5

## –

## –

## ¥

## –

1

12

102

4. 38

-57

2

15

105

2. 16

-58

5

16

106

2. 16

-57

10

0. 1

## –

## –

## ¥

## –

0. 5

## –

## –

## ¥

## –

1

15

105

2. 01

-61

2

16

106

1. 62

-64

5

16

106

1. 62

-64

20

0. 1

## –

## –

## ¥

## –

0. 5

15

105

1. 58

-64

1

16

106

1. 02

-63

2

17

107

0. 97

-66

5

17

107

1. 04

-61

50

0. 1

## –

## –

## ¥

## –

0. 5

17

107

0. 59

-61

1

19

109

0. 54

-60

2

19

109

0. 52

-62

5

19

109

0. 57

-58

Q3. Plot two graphs to show the relationship between: (i) Stimulus strength and latency and (ii) Stimulus duration and latency.

How these graphs should be plotted is not immediately obvious, and information on how to complete this task will not be explicitly given! The optimal solution to the problem is for you to find, but the following points are provided for guidance:

It is not legitimate to plot infinity on graphs

It is not appropriate to extrapolate beyond data points

It is not legitimate to plot average latencies. The graphs must be plotted so that every value of latency (except ¥) is represented.

Use the blank sheet on the proforma, there is no need to use graph paper.

## Graph 1 : Stimulus strength and latency

Remember you need to distinguish different stim durations in this gr

## Graph 2: Stimulus Duration and Latency

Make sure you distinguish different strengths as well

These can be plotted accurately using excel for your submitted report.

## Experiments with dual stimuli

Q4. Run a simulation with the following parameters to demonstrate the absolute refractory period:

## Simulation

## Stimulus 1 Amplitude (­A/cm2)

## Stimulus 1 Duration (ms)

## Delay (ms)

## Stimulus 2 Amplitude (­A/cm2)

## Stimulus 2 Duration (ms)

A

50

0. 5

4

50

0. 5

B

50

0. 5

4

100

0. 5

Briefly describe the responses obtained in simulations A and B in the space below:

In A the first and second stimulus is equal. The first stimulus causes an action potential whilst the second stimulus does not. The delay is only 4ms. The membrane is at the absolute refractory period when the second stimulus is sent. Therefore an action potential cannot be produced. The first stimulus for A causes the gK value to change from -0. 36 to 6. 0. The gNa, 0. 01, does not increase for the second stimulus and the peak reached is -92mV for the second stimulus and the threshold is -52mV.

In B the second stimulus is larger than the first one but the delay remains the same at 4ms. The increase of the stimulus does not cause an action potential. This suggests it must be in the absolute refractory period because a larger stimulus should be able to generate an action potential if it is in the relative refractory period. The value of gK changes from -0. 36 to -5. 87. The peak was -83mV

Q5. Repeat the simulations, but with a longer delay between stimuli:

## Simulation

## Stimulus 1 Amplitude (­A/cm2)

## Stimulus 1 Duration (ms)

## Delay (ms)

## Stimulus 2 Amplitude (­A/cm2)

## Stimulus 2 Duration (ms)

C

50

0. 5

7

50

0. 5

D

50

0. 5

7

100

0. 5

Compare and contrast the responses obtained in simulations C and D with those of A and B.

Stimulation C and D has a longer delay between the first and second stimulus than stimulation A and B. Stimulations C has a lower second stimulus than D but the same as A. Likewise for Simulation A which has a lower second stimulus than B. Stimulation B and D have got the same amplitude for the second stimulus. The second stimulus, like A, for simulation C did not generate an action potential. Whilst with simulation D, unlike B, an action potential was generated. This is because in the absolute refractory period it is not possible for an action potential to be generated hence why simulation B did not produce an action potential. The delay in stimulation C and D is longer therefore the membrane is in the relative refractory period. This is suggested by the action potential produced in D. The extra delay in D enables more inactivation gates to open generating an action potential. The larger amplitude in D caused the membrane to reach threshold.

## Discussion

Answer the questions below in the spaces provided. This will provide the basis of your report discussion

Q6. Briefly justify why a latency of ¥ was recorded if an action potential was not produced.

Latency is the time from the start of the stimulus to threshold. If no action potential is produced then it is not ever possible for it to reach threshold, -59mV, therefore it has to be labelled as infinity because no matter how long you wait you will never reach threshold.

Q7. What evidence from your results suggests that action potentials are threshold phenomena?

Only the experiments which reached threshold value produced an action potential, refer to table one. For example when the strength of the stimulus is 2mA/cm2 no action potential was produced but the membrane potential did change however it did not reach threshold. When the strength of the stimulus was increased the, for example to 5 mA/cm2, and the duration of the stimulus as increased to 2ms then an action potential was reached. This is because the membrane must depolarise to the threshold level therefore generating an action potential with the same amplitude. This is the all or nothing principle.

Q8. Comment briefly on the amplitude of the action potentials generated in these experiments.

In all the experiments, table 2, which an action potential was generated, the amplitude was always similar even though the stimulus strength and duration had changed. This is part of the all or nothing principle. The amplitude was always around 106mV showing that action potentials are not graded. The frequency of the action potential is determined by the intensity of the stimulus. The frequency of action potential is caused during the relative refractory period. Graded potentials can be larger and last longer than action potentials. Therefore during the relative refractory period if the graded potential is stronger than the threshold at resting then it will produce another action potential. If the graded potential last longer than the relative refractory period an action potential will also be generated. Both these factor effect the frequency of action potentials.

Q9. From Graph 1, describe the effect of increasing stimulus strength on the latency of the action potential.

The graph shows that the strength of the stimulus increases as the latency decreases. For example, when the stimulus strength is 5mA/cm2 and has duration of 2ms the latency is 2. 89ms. When the stimulus strength is increased to 50mA/cm2 for the same duration of 2ms the latency decreased to 0. 52ms. This shows that the latency has decreased by 2. 37ms. Latency is the time from the start of the stimulus to the threshold. Therefore as the strength of the stimulus increases, the time for an action potential to be generated decreases.

Q10. From Graph 2, describe the effect of increasing stimulus duration on the latency of the action potential.

The graph shows a larger effect with the lower stimulus strength. For example if the stimulus strength is 50mA/cm2 and the duration is 0. 5 the latency is 0. 59ms and if the duration is 5ms the latency is 0. 57. However, if the stimulus strength is 10mA/cm2 and the duration is 1ms the latency is 2. 01ms and if the duration increases to 3ms the latency is 1. 62ms. Latency is the time from the start of the stimulus to the threshold. Therefore as the duration of the stimulus increases, the time for an action potential to be generated decreases.

Sodium permeability increase in membrane

Number of sodium channel open increaseQ11. Draw a simple flow diagram to illustrate the positive feedback cycle that results in the rapid depolarizing phase of the action potential.

Activation gates open

Membrane depolarises

Stimulus causing to reach threshold

Positive feedback

Charge of cell increases causing depolarisation

Influx of sodium into cell increase

Q12. What event at the ion channel level terminates the above cycle?

1ms after the activation gate open the inactivation gate closes. This is a delay response of the depolarisation. The channel is now incapable of opening until it reaches near resting potential; this is when the inactivation gate opens. Therefore the sodium channels closes and sodium ions can’t enter the cell. Also the opening of the potassium channels helps terminates this cycle.

Q13. What physiological mechanism is responsible for the absolute refractory period?

Absolute refractory period is during the depolarisation and most of the repolarisation phase. At this point the sodium channels inactivation gates are closed and the activation gates are open. Therefore the channel is closed and incapable of opening so an action potential cannot be generated by another stimulus in this period.

Q14. Explain your observations to simulations C and D in the Methods and Results section.

Stimulations C have a lower second stimulus than D. The second stimulus, for C did not generate an action potential but simulation D did. The delay in stimulation C and D is long therefore the membrane is in the relative refractory period. This is suggested by the action potential produced in D because the larger stimulus amplitude. The extra delay in D, compared to B, enables more inactivation gates to open allowing. Also the larger stimulus allows another action potential to be generated.

Q15. Briefly summarise two effects that refractory periods impose on the behaviour of neurones (N. B. restatement of the definitions of refractory periods is not what is asked here)

There are two types of refractory period absolute and relative. During the absolute refractory period no action potential can be produced. In the relative an action potential can only be produced depending on the strength of the stimulus. Therefore there is a minimum delay required before a second action potential can be generated. Also it controls the frequency of the action potential generated. This period also helps ensure action potential can only move in one direction.

## Questions to answer after the practical.

Q 16 . Most Local anaesthetics are Sodium channel blockers. Describe how these compounds work, the side-effects and what their main clinical uses are. ( max 300 words).

Local anaesthetics are weak bases which are used for loss of pain and muscle power so that a particular area of the body becomes numb. When sodium channel blockers, like lidocaine, enter the body it will be equilibrium with the tissue fluid. The anaesthetic will be in its ionised and non-ionised form. The non-ionised form will be able to pass through. It will be become partially ionised and can’t leave, ion trapping. The ionised form will bind to the sodium channel. This will prevent sodium ions from entering the cell and therefore it cannot be depolarised. As a result it does not reach threshold and an action potential is not generated. Consequently the nerve cells can’t signal to the brain so pain can’t be felt or muscle can’t be moved. (Tuckley, 1994).

There are many different local anaesthetic available with the side effects differing for each drug and. The general side-effects can be, for example, numbness, sickness, lower blood pressure, light headedness and drowsiness. Not all of these are felt by the patient. (Joint Formulary Committee (2010).

The anaesthetic can be administered in by several methods, for example, a dentist will use an injection to the mouth. The effect of the anaesthetics will only be felt by the area in which it is injected in. Dentist will use local anaesthetic so that their patient will have loss of pain only in their mouth. Therefore the patient will not be able to feel any pain whilst the dentist carries out the procedure. It is also used for some eye surgery and minor skin surgery. (Tuckley, 1994).

Referencing

Tuckley, J, M. (1994). The pharmacology of local anaesthetic agents, Pharmacology, 4, 7.

Joint Formulary Committee (2010). British National Formulary. (59th ed.). London: Pharmaceutical Press.

Q17. Will these compounds work if they don’t block all the Na channels ? Why ?

(Use your experimental data to help answer this question)

During the relative refractory period some channels are open allowing a second action potential to be generated. For example for stimulation D an action potential was produced for the second stimulus because the cell was in its relative refractory period. However for stimulation C an action potential was not produced for the second stimulus, even though the delay was the same. However the second stimulus was larger for D than C. Therefore if the compound does not block all the sodium channels then an action potential may be generated depending on the number of sodium channels blocked and the strength of the stimulus because the concept is very similar to the relative refractory period as some of the channels are not be open but in this case some channels are blocked. In both cases, relative refractory period and local anaesthetic, some channels allow sodium ions to enter the cell. As a result the compound will not work.