EPSIM

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Strathclyde Pharmacology Simulations

EPSIM V1.0.6

Epilepsy Neuron Simulation

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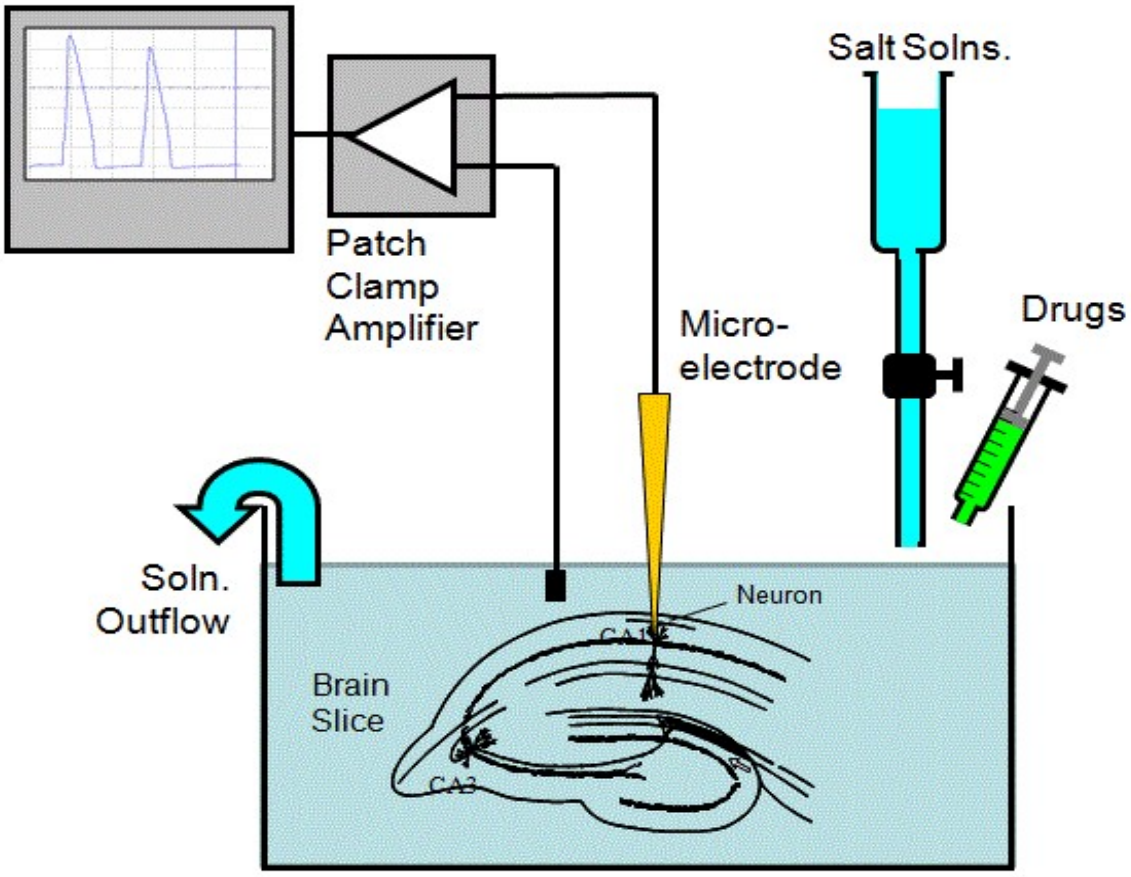
Introduction

EPSim is a simulated experiment for investigating the effects of anti-epileptic drugs on the action potential firing of a neuron within a brain slice.

The intracellular membrane potential of the neuron is recorded using a patch clamp amplifier via a glass micropipette electrode attached to the cell body of the neuron and connected to an oscilloscope recording device. The neuron can also be stimulated via this route.

Drugs can be applied to the to bath and the concentration of the Na, Ca and K ions in the bathing medium changed.

Neuronal Action Potential Recording Setup



AED Mechanisms of Action

An epileptic seizure is caused by repetitive bursts of abnormal electrical activity within one or more brain areas initiated by damage to the brain, genetic or other factors, leading to excess neuronal excitability. Anti-epileptic drugs (AEDs) terminate or prevent epileptic seizures by reducing neuronal excitability within the brain thus compensating for this excess excitability.

AEDs may reduce excitability by one or more different mechanisms:

Inhibition of Voltage-gated Sodium Channels

The voltage-gated Na ion channel is primarily responsible for the generation of the action potential in neurons. Reduction in the Na channel conductance leads to an increase in the action potential firing threshold and a reduction in the neuron's excitability in response to excitatory synaptic stimuli. Na channel inhibiting drugs which are effective as AEDs, selectively bind to, and prolong, the **inactivated** state of the Na channel, produced after it opens during an action potential. This causes them to display **use-dependent inhibition** where the degree of inhibition increases during rapid bursts of action potential, allowing them to selectively terminate the rapid bursts of activity while sparing the normal lower rate activity.

Enhancement of Inhibitory Neurotransmission

Inhibitory neurotransmitters such as GABA act by opening chloride (Cl) ion channels in the neuron membrane, increasing the Cl trans-membrane conductance. This leads to a hyperpolarization of the neuron resting potential, as the resting potential is shifted towards the Cl reversal potential. Neuronal excitability is therefore reduced because larger excitatory stimuli must are now required to reach the action potential firing threshold. AEDs may act by a number of mechanism to enhance GABA neurotransmission, such as prolongation of the open time of the GABA-activated Cl ion channel or by increasing the amount of GABA available at the synapse by inhibiting GABA reuptake.

Inhibition of Excitatory Neurotransmission

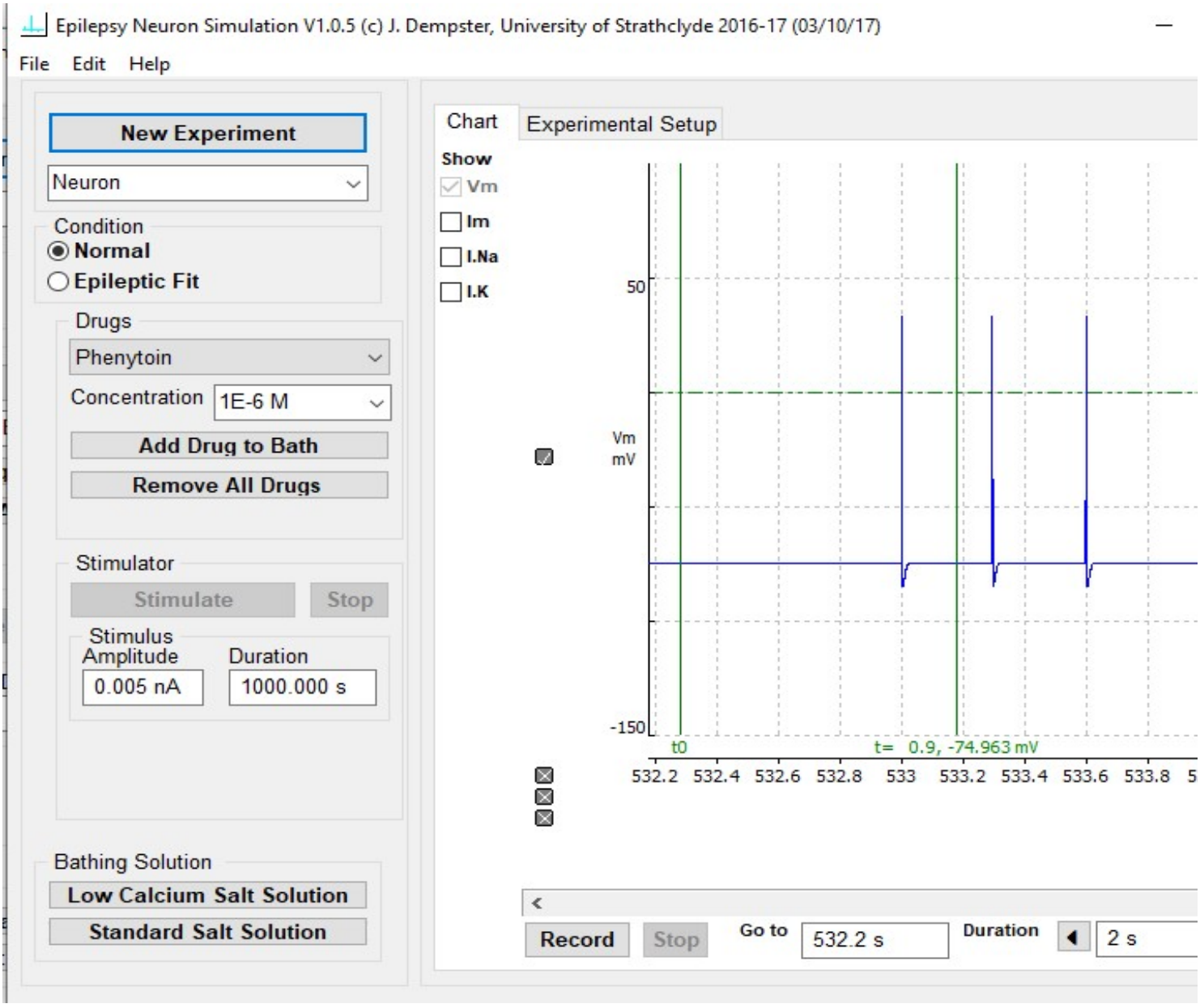
Neuronal excitability can be reduced by reducing the amount of excitatory neurotransmitters such as Glutamate released the presynaptic nerve terminal. Some AEDs act by inhibiting a class of high voltage activated calcium (Ca.HVA) located in the terminal and involved in regulating transmitter release.

Starting an Experiment

To start a new experiment:

a) Click the **New Experiment** button.

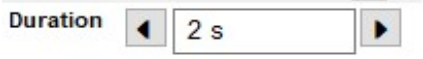
b) Click the **Record** button to start the chart recorder running. The membrane potential of the neuron is displayed in the **Vm** channel of the chart window.



Under normal non-epileptic conductions, action potentials can be seen to occur at random intervals every few seconds due to interactions between the neuron and the other synaptically connected neurons within the network of neurons in the brain slice.

Chart display duration

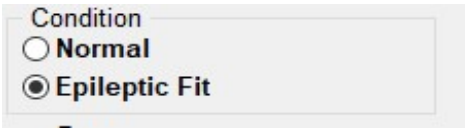
The duration of the chart display window can be changed by entering a new value into the **Duration** box or clicking the arrow buttons to increase or decrease the duration.


Duration
2 s

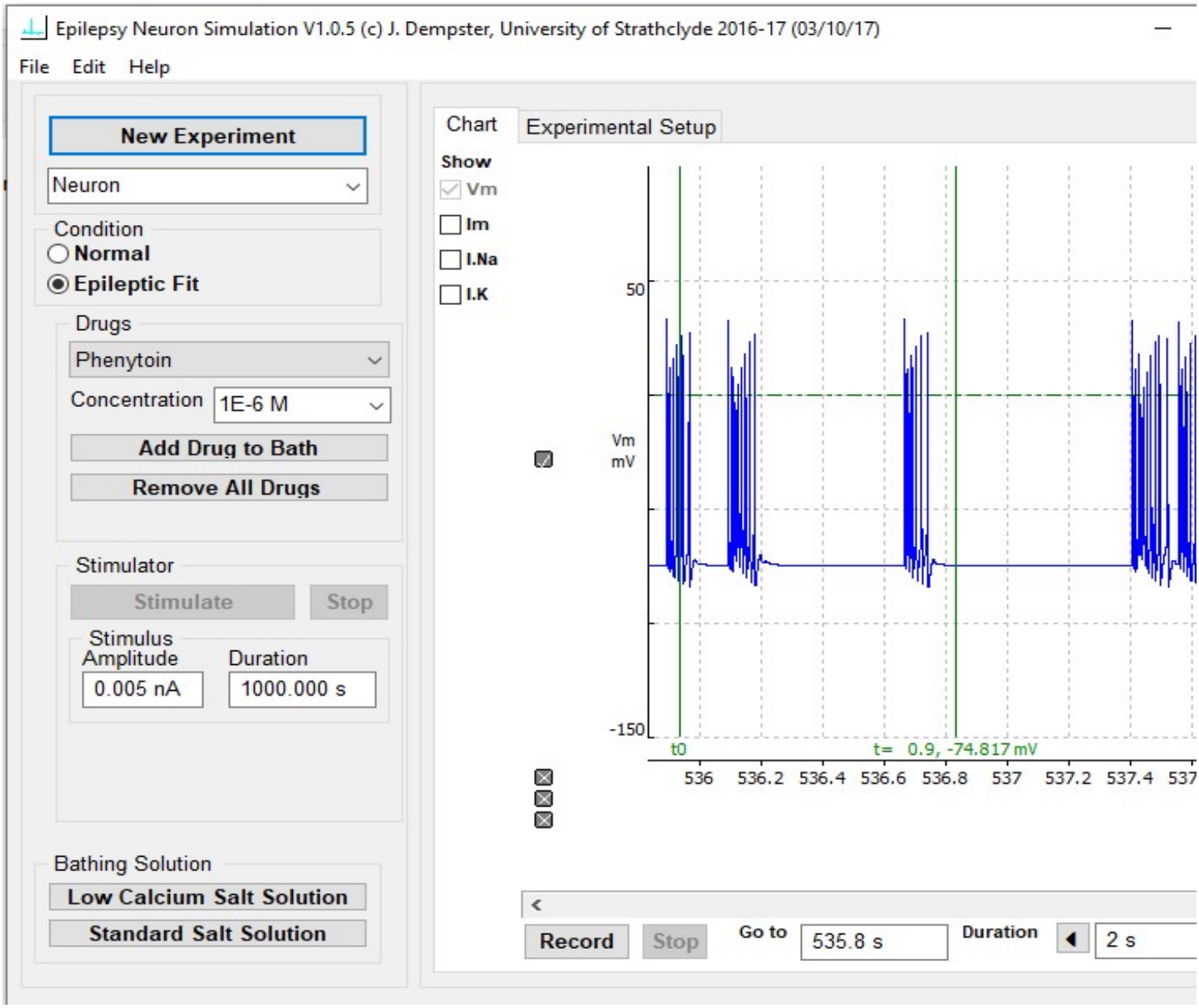
Inititating Epileptic Activity

Electrical activity similar to that observed during a human epileptic fit can be produced artificially in brain slice by adding a number of drugs (the GABA channel antagonist picrotoxin, for instance) or by slightly increasing the concentration of potassium ions in the bathing solution, reducing the resting membrane potential and making the network of neurons in the brain slice more excitable.

To initiate epileptic activity in the brain slice, select the **Epileptic Fit** option as the brain slice Condition.



High frequency bursts of action potentials can now be seen replacing the normal activity.



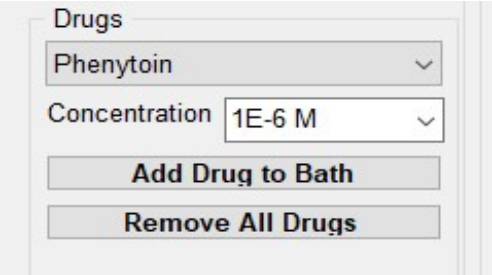
The slice can be returned to normal functioning by reselecting the **Normal** option.

Adding Drugs

To add a drug to the bath:

a) Select the type of drug to be applied from the **Drugs** list.

b) Select the concentration (in M/litre) of the drug to be added from the **Concentration** list.



c) Click the **Add Drug to Bath** button to add the drug to the bath.

Note.

You can remove the drug (and any others that have have been previously applied) by clicking the **Remove All Drugs** button.

Measuring Action Potential Frequency

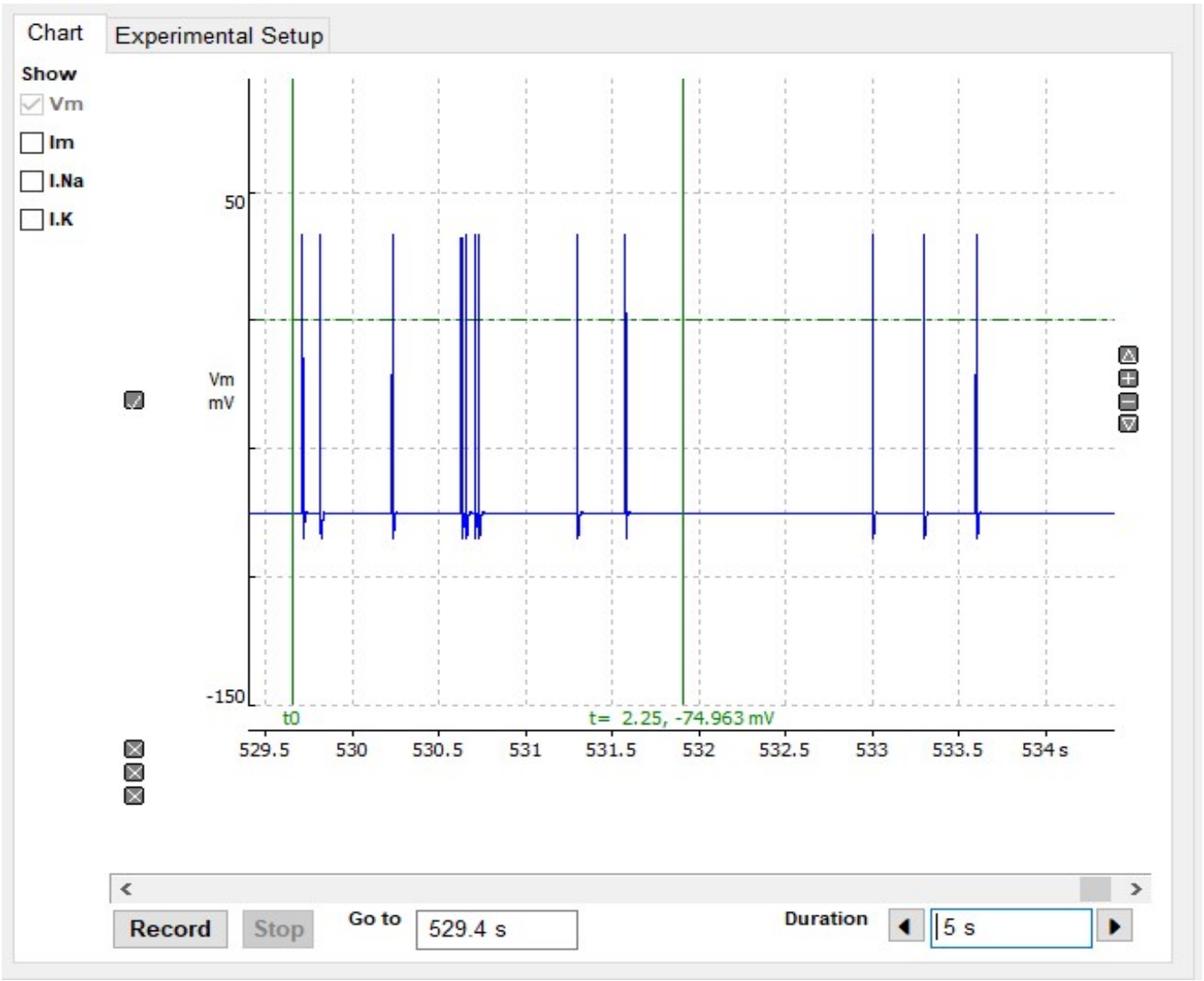
The average frequency of action potential (AP) production (in APs/second or Hz) is measured by counting the number of action potentials that occur over a fixed time period.

a) Select a chart display duration which contains at least 10 action potentials (but not too many to count accurately), by entering a value into the **Duration** box.

b) Count the number of action potentials visible in the display window. (Note. To be counted as an action potential, the peak of the observed 'spike' must exceed the dashed green zero level.)

c) The average AP frequency is obtained by dividing the observed number of action potentials by the chart display duration.

In the example below 12 APs have been observed in a 5 second window, giving a frequency of 12/5 = 2.8 Hz.



Measuring the Membrane Potential

To measure the membrane potential in the recording:

a) Click the **Stop** button to stop recording.

b) Using the scroll bar at the bottom of the chart recorder display, select a section of the recording containing the tissue contraction to be measured.

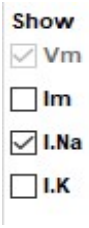
c) Drag the measurement cursor on the chart display to the point on the recording trace to be measured. The membrane potential at the cursor point (in units of mV.) is displayed below the cursor.

a)

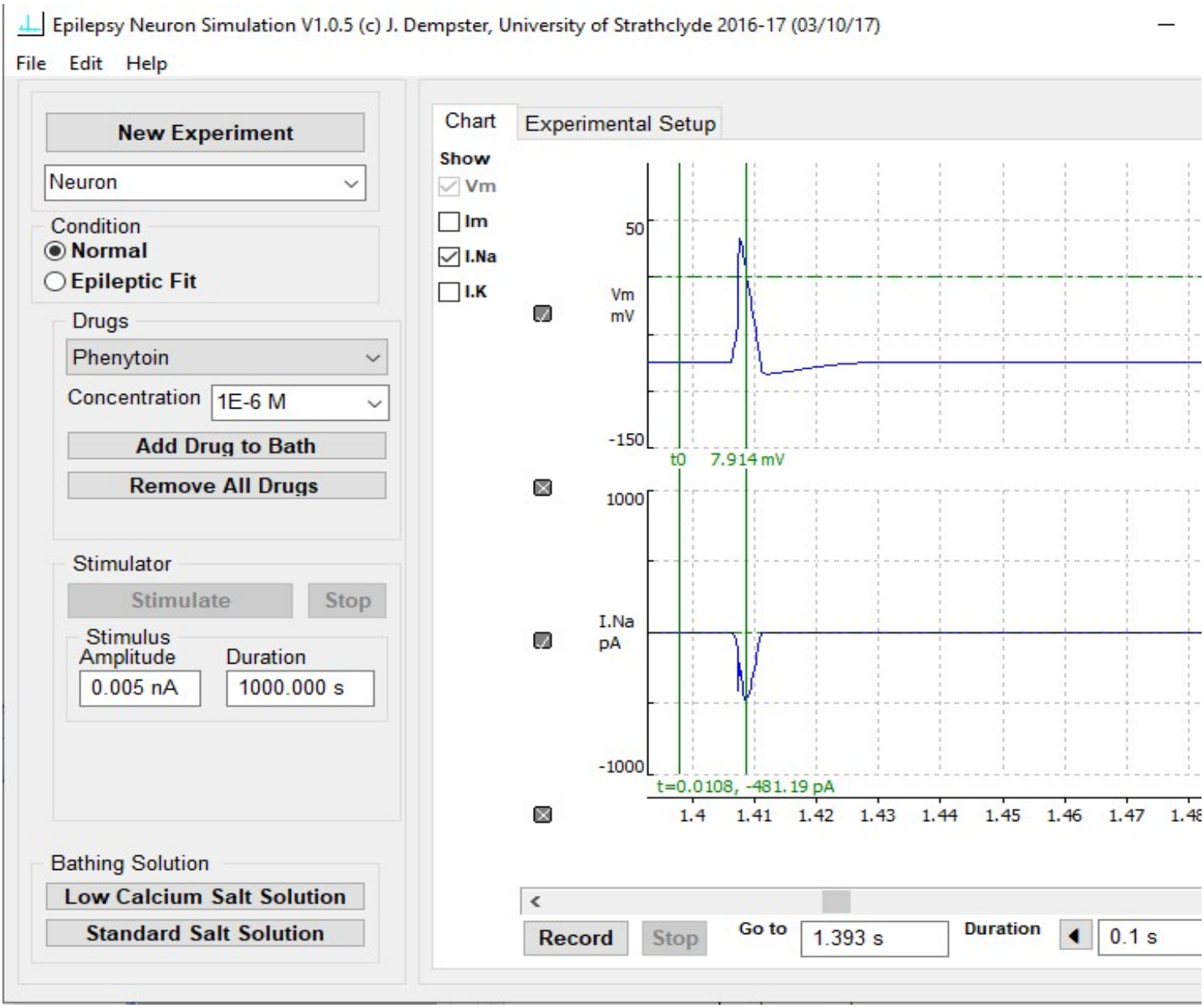
Measuring the Peak Sodium Current

To measure the peak sodium current during an action potential:

a) Tick the **I.Na** option to add the neuron's transmembrane sodium current channel to the chart.



b) Place the green measurement cursor over the peak of the I.Na current during the action potential.



The current is displayed beside the cursor at the bottom edge of the display channel.

Note. When measuring the peak sodium current, you may find it useful to stretch out the action potential and I.Na by reducing the duration of the display window to 0.1 seconds, as in the example here.

Menu Options

File > Print

To print a copy of the displayed recording on a printer, select **Print** from the **File** menu.

To select a specific printer or change printer settings, select **Printer Setup** from the **File** menu.

Edit > Copy Data

To copy the data points of the displayed recording to the Windows clipboard for pasting into a spreadsheet or graph plotting program, select **Copy Data** from the **Edit** menu.

Edit > Copy Image

To copy a picture of the displayed recording to the Windows clipboard for pasting into a Word document or a PowerPoint presentation, select **Copy Image** from the **Edit** menu.

File > Save Experiment

To save the recording for the current experiment to a data file, select **Save Experiment** from the **File** menu and enter the name of a new data file in the dialog box.

File > Load Experiment

To load a previously saved recording from a data file, select **Load Experiment** from the **File** menu and select the data file from the list displayed in the dialog box.

Acknowledgements & References

EPSIM was developed in the Strathclyde Institute for Pharmacy and Biomedical Sciences in 2016 by the author, in collaboration with Dr. Trevor Bushell. The simulation is loosely based on the experimental methods used to study AED mechanism of action, in vitro, using brain slices. An example of such as study can be found in West et al. (2018) and Ching-Huei et al (2017).

EPSIM was created using the Embarcadero Delphi XE software development environment. The neuronal model used is the classic Hodgkin-Huxley squid axon action potential model, modified to use mammalian internal and external ion concentrations with excitatory and inhibitory neurotransmitter conductances added. The Hodgkin-Huxley simulation code was generated using a CellML H-H model, translated into Delphi Pascal code using the Cellular Open Resource program (Garny et al., 2009). Drug-receptor pharmacodynamics are implemented using a simple single site binding model.

References

Garny A, Noble D, Hunter PJ & Kohl P. Cellular Open Resource (COR): current status and future directions. Phil Trans R Soc A 367: 1885-1906 (2009).

West P.J., Saunders G.W., Billingsley P., Smith M.D., White S., Metcalf C.S., Wilcox K.S. (2018) Recurrent Epileptiform Discharges in the Medial Entorhinal Cortex of Kainate-Treated Rats are Differentially Sensitive to Anti-Seizure Drugs. Epilepsia 59(11):2035-2048.

Ching-Huei Lin, Shih-Pin Hsu, Ting-Chun Cheng, Chin-Wei Huang, Yao-Chang Chiang, I-Han Hsiao, Ming-Hsueh Lee, Mei-Lin Shen1, Dong Chuan Wu & Ning Zhou Effects of anti-epileptic drugs on spreading depolarization-induced epileptiform activity in mouse hippocampal slices Nature Scientific Reports 7: 11884.