## SUPPLEMENTARY MATERIAL

# Primary Afferent Depolarization and the Gate Control Theory of Pain: a tutorial simulation

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# Getting Neurosim

If you want to try out the tutorial simulations described in the article but do not have Neurosim, a fully-functional trial version is available to faculty on request to the author. This will also give access to a large number of other tutorials available on the Neurosim support website:
https://www.st-andrews.ac.uk/~wjh/neurosim/tutorial-base.html.

A permanent copy of the program for individual use can be purchased from the Microsoft store:
https://apps.microsoft.com/store/detail/neurosim-5/9NC7XNPKMSZM?hl=en-us&gl=us.

A volume discount is available for multi-seat institutional purchase (permanent licence). Please contact the author for details.

**Mac/Linux users**: Information for Mac or Linux users is available on the Neurosim support website here:
https://www.st-andrews.ac.uk/~wjh/neurosim/mac-users.html

# Student Tutorial

The target audience for JUNE articles is college faculty and tutors in tertiary education, and consequently the article is not intended for direct student use. A ready-built student-focussed tutorial presenting similar material is available here:
https://www.st-andrews.ac.uk/~wjh/neurosim/PAD-pain-drr/

Teachers can use this with their own students as is, or can adapt it as necessary.

# JUNE Instructions

The following instructions show how the simulations described in the JUNE article were run. Indented sub-sections labelled Aside show some additional features that tutors might want to incorporate if they use the simulations in their teaching, but which were not used in the article.

The links in the text below are to Neurosim parameter files that you can access individually from this site. You can also download the full set of parameter files if you wish:
https://www.st-andrews.ac.uk/~wjh/neurosim/JUNE-pain/files-for-JUNE.zip

- You can start Neurosim with a particular parameter file by clicking a link in the text, but be aware that this will probably store the file in your default download folder. This will start a new instance of the program.

- If Neurosim is already running and you have the file stored locally, you can load it by drag-and-drop from Windows Explorer to the Setup or Results view within Neurosim (you could also use the File: Open menu command). This will not start a new instance.

If you just want to see the finished simulations that were used to construct the figures in the article, then load the appropriate file from the list below:

fig 1 2
fig 3
fig 4
fig 5 6
fig 7a
fig 7b

If you want to carry out the experiments described in the manuscript, follow the instructions below.

# Pre-Synaptic Inhibition through PAD (Figs 1, 2)

- Start Neurosim, and load the parameter file pain 1 2.
  Note: You may be able to combine these steps just by clicking the link, as described above.

The **Setup** view on the left shows Fig. 1.

- Click the **Start** button.
- Note that there are two **Start** buttons with identical functionality, one located at the top-left of the **Experimental Control** panel to the left of the **Setup** view, the other at the top-left of the **Results** view. You can click either.

The simulation runs with no pre-synaptic inhibition (Fig. 2a).

Aside: The **Setup** view can be colour-coded to show the membrane potential as the simulation progresses. If you want to see this in action, either follow the instructions below, or load and run the file pain 1 2 a.

- Click the **Clear** button.
- Select the **Neuron: Colours: Colours from voltage** menu command.
- Select a **Slow down** factor of **5** from the drop-down list in the main toolbar (this is a suitable value on my computer – yours may vary),
  The **Slow down** factor is just a cosmetic change that gives you more time to appreciate the changing colours.
- Click **Start**, and watch the **Setup** view.

You can see the spike progress in the afferent axon as colour changes in the view.

- Reverse the steps above (it may be simpler just to reload and run the file pain 1 2 – you can probably do this from the **File** menu MRU list).

Now initiate pre-synaptic inhibition.

- Click the **up** spin arrow for the **ampltiude** of **stimulus 2** (already selected) in the **Experimental Control** panel to the left of the **Setup** view.
  This increases the stimulus amplitude to **6** nA, which generates a spike in the pre-synaptic inhibitor.

A new sweep runs because **Run-on-change** is pre-selected in the **Results** view. The new sweep superimposes on the original. Note that the size of the EPSP in the post-synaptic neuron (bottom trace, blue) is substantially reduced (Fig. 2b).

- Select **stimulus 1** by clicking on it in the **Stimulus** list of the **Experimental Control** panel, or by clicking the square box 1 in the **Setup** view (attached to the distal terminal of the afferent axon).
- Click the **down** spin arrow for the **amplitude** to remove the stimulus to the afferent.

Now the afferent central terminal just receives the dIPSP, there is no spike (Fig. 2c).
At this point 3 sweeps are superimposed in the **Results** view, which can be confusing.

- Click the **up** spin arrow of the **Hilight sweep** edit control in the **Results** view to highlight each of the 3 sweeps in turn. This facility was used to generate the three panels in Fig. 2.

Aside: The **Results** view can be configured to show the conductance underlying the EPSP in the ascending interneuron (neuron 15 in the **Setup** view) if desired. If you want to see this in action, load the file pain 1 2 b and carry out the steps described in the sequence described above. The bottom trace now shows the conductance. To see how this was set up:

- Click **Clear**.
- Click **Traces**.

The section on the right of the dialog shows traces associated with specific neurons/compartments. The **Common neuron ID** is **15**, which is the ascending interneuron. The **Show** box in the **Conductance** section is checked, and so is the **Chemical synapse** box. This means that the trace shows the summed conductance of all chemical synapses impinging on the selected neuron (but in this case there is only one). The axis **size** is set to **2**, to match the main display.

## Shunting or Inactivation/Activation (Fig. 3)

- Load the parameter file pain 3.
- Click **Start**.

This generates the control sweep with no pre-synaptic inhibition (Fig. 3a). The bottom axis has 2 traces which show conductance in the central afferent terminal (compartment 14) . The purple trace is the conductance generated by the inhibitory synaptic input ‘b’, but since the pre-synaptic inhibitor is not stimulated, this trace is flat. The khaki trace shows the total membrane conductance in this compartment, which is dominated by the spike-related conductances.

  - Click **Traces** if you want to see how this was set up.
- Click the **up** spin arrow for the **ampltiude** of **stimulus 2** (already selected) in the **Experimental Control** panel to the left of the **Setup** view.

This activates the pre-synaptic inhibitor, and generates a dIPSP in the afferent terminal (Fig. 3b). This reduces the terminal spike height, and consequently the amplitude of the EPSP in the ascending interneuron.

- Select the **Synapses: Spiking Chemical** menu command to open the **Spiking Chemical Synapse Types** dialog. This dialog is non-modal, and can be left open while changes are made and tested.
- Select the second row in the synapse list: **'b: pre-synaptic inhibition'**.
- Change the **Equilibrium potential** to **-70** mV, which is the resting potential of the afferent.

This immediately generates a new sweep, but now the inhibition in the afferent terminal is “silent” – there is no change in membrane potential associated with synaptic activation (Fig. 3c). However, the conductance change generating the silent IPSP is the same as that generating the dIPSP (the purple trace in the bottom axis).

- Change the **Equilibrium potential** to **-80** mV, which is negative to the resting potential of the afferent.

This generates a new sweep, but now the inhibition is hyperpolarizing (Fig. 3d).

- Click **OK** to close the **Spiking Chemical Synapse Types** dialog.

At this point 4 sweeps are superimposed in the **Results** view, which can be confusing.

- Click the **up** spin arrow of the **Hilight sweep** edit control in the **Results** view to highlight each of the 4 sweeps in turn. This facility was used to generate the four panels in Fig. 3.

## Effect of Polarity of Inhibition on Gate Variables (Fig. 4)

- Load the parameter file pain 4.
- Click **Start**.

This generates the control sweep with no pre-synaptic inhibition (Fig. 4a). The second axis shows the conductance in the central afferent terminal (compartment 14) for the voltage-dependent sodium and potassium channels, and the chloride conductance of the inhibitory synapse. The bottom trace shows the *m*, *h* and *n* activation probability variables for the voltage-dependent sodium and potassium channels.

    - Click **Traces** if you want to see how this was set up.
- Click the **up** spin arrow for the **ampltiude** of **stimulus 2** (already selected) in the **Experimental Control** panel to the left of the **Setup** view.

This activates the pre-synaptic inhibitor, and generates a dIPSP in the afferent terminal (Fig. 4b).

- Select the **Synapses: Spiking Chemical** menu command to open the **Spiking Chemical Synapse Types** dialog. This dialog is non-modal, and can be left open while changes are made and tested.
- Select the second row in the synapse list: **'b: pre-synaptic inhibition'**.
- Change the **Equilibrium potential** to **-80** mV.

This immediately generates a new sweep, but now the inhibition in the afferent terminal is hyperpolarizing (Fig. 4c). The conductance change generated by the IPSP (the purple trace in the middle axis) is unchanged.

# Gate Control of Pain (Figs. 5, 6)

- Load the parameter file pain 5 6.
- Click **Start**.
- Add the “pain stimulus” and “touch stimulus” annotations to the **Results** view using the **View: Annotation: Add** command.
    - Or right-click in the **Results** view and select **Add annotation** from the pop-up context menu.
- Use the mouse to drag them to the correct location over the traces.

Aside: The **Setup** view can be colour-coded to show the membrane potential as the simulation progresses. If you want to see this in action, either follow the instructions below, or load and run the file pain 5 6 a. WARNING: this involves flashing colours on the computer screen.

- Click the **Clear** button.
- Select the **Neuron: Colours: Colours from voltage** menu command.
- Click **Start**, and watch the **Setup** view.

You can see the spike progress in the afferent axon as colour changes in the view.

# Dorsal Root Reflex (Fig. 7)

- Load the parameter file pain 7.
- Click **Start**.
- Right-click in the **Results** view and select **Add annotation** from the pop-up context menu.
  - Enter “touch stimulus” in the annotation edit dialog, and click OK.
  - Drag the annotation to an appropriate location.

The touch stimulus activates the inhibitor interneuron (18), but the dIPSPs in the afferent terminal are subthreshold, and nothing happens.

- Select the **Synapses: Spiking Chemical** menu command to open the **Spiking Chemical Synapse Types** dialog. This dialog is non-modal, and can be left open while changes are made and tested.
- Select the second row in the synapse list: **'b: pre-synaptic inhibition'**.
- Change the **Equilibrium potential** to **-56** mV.

The dIPSPs are now larger, and elicit spikes in the central terminal compartment of the afferent. These propagate antidromically towards the periphery. The central spikes are reduced in amplitude and do not elicit significant EPSPs in the ascending interneuron. As the afferent spikes propagate to the periphery, they recover in amplitude (top axis, central and peripheral traces superimposed).