Identify a retinal ganglion cell based on its visual-evoked electrophysiological responses and morphology

Virtual experiment:

This virtual Retinal Electrophysiology Laboratory is designed to reproduce, record and download responses of different types of retinal ganglion cells (RGCs) to stimulation with a variety of visual stimuli. The response records will then be used for further analyses. The response properties and morphology of RGCs simulated in this virtual laboratory are modelled on those found in the retinas of old-world primates.

Aims

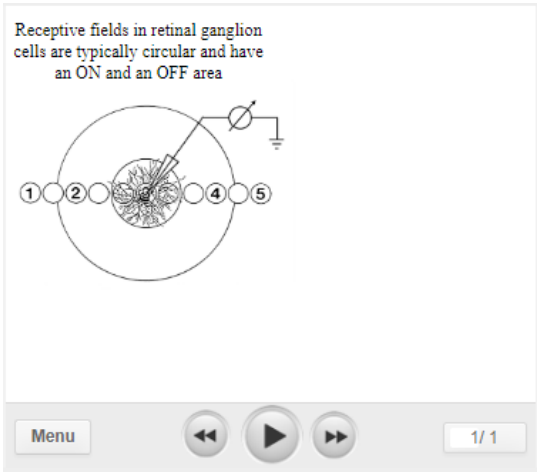
1. Probe a retinal ganglion cell with visual stimuli whose size, contrast, chromatic properties and direction of motion can be modified and observe its response dependence on these properties.

2. Download the data for analyses and use it to generate plots illustrating the stimulus-response relationship for different variables. Gather all possible experimental evidence to identify the cell type.

3. Complete an online quiz (go to Canvas> quizzes) to assess what you have learnt from this *in silico* experiment.

Starting the Prac: Open Google Chrome Go to the URL:
https://darioprotti.github.io/virtual_retina_electrophys/ and click on the **Introduction** tab.

This section provides a general description of some aspects of the early stages of visual processing in the retina and of the RGCs that carry visual signals from the retina to higher visual centres. The panel at the right illustrates the concept of receptive field using an RGC with classical antagonistic, centre-surround organisation as example.



Click on the Play button (▶) to advance slides.

Once this section is completed, you can move to the following section by clicking on the **Virtual Experiment** tab.

Virtual Experiment

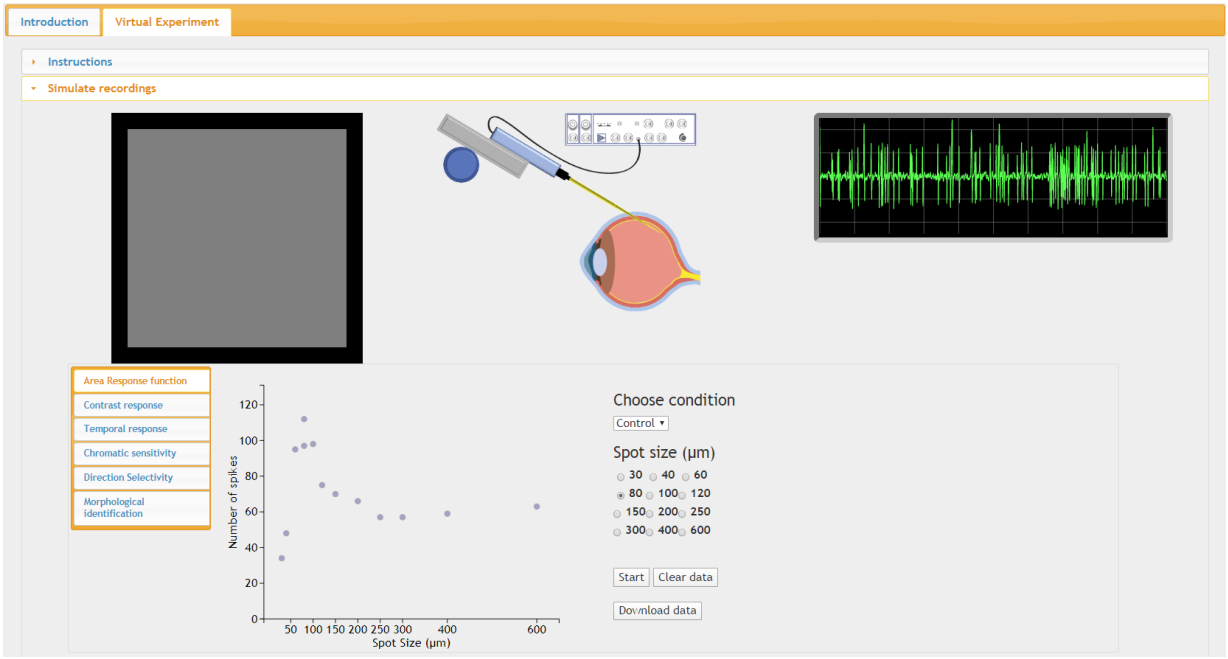
Click on the Cell Selection tab

Select the Cell type B from the pop up menu.

Enter a unique name (identifier) that you will use for this and any other session that you do in the box and then press the **Initialise** neuron button. Every time you return to the program and enter the same identifier, the program will generate a neuron with the same properties, so that your results can be consistent over different sessions. Once the neuron has been *"initialised"*, click on the **Simulate recordings** tab.

Simulate recordings

This section allows you to study the response properties of the RGC you selected by probing it with stimuli of different characteristics. The upper panel displays some of the basic elements present in real experiments, such as a stimulus monitor, biological preparation (eye), recording electrode, amplifier and oscilloscope window, where the electrical recordings will be displayed.



The lower panel contains 6 tabs, each of which designed to study different RGC properties (area response function, contrast sensitivity, temporal response, chromatic sensitivity, direction selectivity and morphological identification) as well as a graph where cell responses will be plotted and a panel to change stimulus parameters.

Once the desired value for a particular variable is selected, press the **Start** button to run the simulation, namely how the cell you selected reacts in response to stimulation with that specific stimulus. The stimulus will be displayed in the monitor and the electrical response of the RGC will be shown in the oscilloscope window. The oscilloscope shows the amplified voltage trace measured with an extracellular electrode in close apposition to the body of the neuron under recording. The vertical lines represent individual action potentials. You will also notice that together with the traces you can hear some popping sounds (make sure that the sound is unmuted). Historically,

neurophysiologists have taken advantage of the exquisite sensitivity of our auditory system to detect patterns of sound by converting the electrical signals originating from nerve cells into sounds and listening to them. *"Listening"* to the nerve signals can help us determine whether neural activity is correlated to the stimulus.

You are now ready to start characterising the properties of the selected RGC and to download the data for further analyses. It is advisable to characterise the properties as they are listed in the tabs.

Area Response function:

This section allows you to characterise the receptive field organisation of the retinal ganglion cell under recording.

Click on the **Choose condition** pop up menu and select "Control" to start the simulation. Select a Spot size and start the simulation by pressing the **Start** button. This will display the stimulus in the monitor for one second whilst the electrical activity of the neuron under recording will be displayed in the oscilloscope window. The total number of spikes will be plotted in the graph as a function of spot size.

***Repeat** the simulation for the same spot size. Is the response (number of spikes in the graph) the same as before?*

As you may have noted, presentation of the same stimulus does not always generate the same response. In biological systems there are several sources of variability. RGC response variability is caused by a number of different factors; which include photoreceptor noise, the stochastic nature of synaptic transmission, changes related to neuronal adaptation, etc., that act at the single cell and network level.

In order to reduce the impact of these factors and to obtain an accurate estimate of the biological response, experimenters perform several repeats of a given stimulus and then calculate the average response (mean) and its standard deviation and/or error.

Repeat the simulation for all twelve stimulus sizes. Repeat each size 4-6 times to obtain the mean response.

To download the data, enter how many presentations you did for each size and then press the **Download Data** button. A ".csv" (comma separated values) file containing information about each stimulus presentation (spot size and total number of spikes) will be downloaded. This file can be opened in Excel for further analyses. During the course of this prac, you will have to download several ".csv" files, therefore you should use meaningful filenames (in this case it could be "AreaResponse_spikecount_1") for easy identification.

Test the effect of pharmacological agents on receptive field organisation of RGCs

Before this step, you should remove from memory all data recorded in control conditions by pressing the button **Clear Data**.

Repeat the simulation for all twelve stimulus sizes, presenting each size 4-6 times to obtain the mean response.

You will have to download the data for stimulation in the presence of a pharmacological agent in a separate file. To download the data, enter how many presentations you did for each size and then press the **Download Data** button.

Before progressing to the next section, **return** the condition to Control.

Contrast response:

This section allows you to characterise the contrast sensitivity of the RGC under recording.

In this section, you can vary the contrast of a spot of fixed size and record ganglion cell responses to stimulation with different percentage contrasts.

In order to accurately characterise contrast sensitivity, low contrast values are highly represented in this experiment. Use the slider control labelled "**Contrast**" to change the contrast value (1, 3, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100) of the spot.

As in the previous section, you should do several repetitions of each stimulus in order to obtain a close estimate of the response.

To download the data, enter how many presentations you did for each contrast and then press the **Download Data** button. A ".csv" (comma separated values) file containing information about each stimulus presentation (stimulus contrast and total number of spikes) will be downloaded.

Temporal response:

This section allows you to characterise the time course of response of the RGC under recording.

Different cell types respond with different characteristic temporal patterns (i.e. transient vs sustained). In this section, you can display a spot (size = 80 µm, contrast = 100%) and observe the RGC response plotted in a peri-stimulus time histogram (PSTH). In this type of plot, spikes are quantified and sorted into bins of a defined size, typically 25ms. A PSTH provides an easy visualization of the time course of the RGC response.

The stimulus used to characterise the time course has fixed parameters (size = 80 µm, contrast = 100%). You can start the stimulation by pressing **Start**.

As in the previous section, you should do several repetitions in order to obtain a close estimate of the response. Before repeating a stimulus presentation you will have to remove the PSTH on display by pressing the **Clear Data** button.

To download the data, enter how many presentations you did and then press the **Download Data** button. A ".csv" (*comma separated values*) file containing information about the time in milliseconds of each action potential in each stimulus presentation will be downloaded. These data can be used to build a PSTH plotting average spike number (or frequency) per bin vs time.

Chromatic sensitivity:

This section allows you to characterise the chromatic sensitivity of the RGC under recording by displaying stimuli that contain the same luminance information but vary in their hue.

In the primate retina, responses to broadband and colour-opponent stimuli are processed by different channels.

Record responses to stimulation with all different stimuli available from the pop up menu (*achromatic, isoluminant ML-cone isolating, isoluminant S-cone isolating*). Stimuli are sinusoidally modulated gratings containing 3 cycles displayed during 1 second. Isoluminant stimuli have been carefully equated in luminance so that they stimulate only post-receptoral pathways that are wavelength-sensitive and not luminance-sensitive mechanisms. These stimuli are useful to distinguish cells that respond only to luminance from those that respond to luminance as well as to hue (colour).

As in the previous section, you should do several repetitions of each stimulus in order to obtain a close estimate of the response.

You will have to process and download data from each chromatic stimulus separately. Enter how many presentations you did for each condition and then press the **Download Data** button. A ".csv" (comma separated values) file containing information about each stimulus presentation (stimulus condition and total number of spikes) will be downloaded. Be sure to record on your downloaded spreadsheet the chromatic conditions for each download.

Direction selectivity:

This section allows you to characterise the direction selectivity of the RGC under recording by using a bar as stimulus.

Rotate the knob in the right panel to set and control the direction of movement of the bar.

Press the "**Start**" button to initiate the movement of the bar and the recording of the neuronal response. The bar will be displayed for one second.

Characterise direction selectivity sensitivity by repeating the presentation of the bar in different directions to obtain a close estimate of the response.

To download the data, enter how many presentations you did for each direction and then press the **Download Data** button. A ".csv" (comma separated values) file containing information about each stimulus presentation (stimulus direction and total number of spikes) will be downloaded.

Morphological identification:

In this section you can simulate an anatomical experiment in which a fluorescent dye is injected into the cell body using an electrode, to obtain information about the morphological properties of the cell under study.

Move the mouse cursor to the electrode, left click on the mouse and drag the electrode so that the tip of the electrode is near the asterisk in the retinal slice.

Click on the button corresponding to the cell you are recording from (A, B or C) and wait ~20 seconds for the morphology of the cell to be revealed.

Analysis

You should now have collected data on 5 different response properties of your cell and have filled a cell with fluorescent dye to gain an idea of its morphology. Your goal is now to undertake the quantitative analysis of the data you have generated and use that information to develop a hypothesis about the type of RGC you have been studying. This might include a few graphs and/or statistics (mean +/- SD) for those properties. You should assemble your evidence as a series of graphs (you can do this in a Word or OneNote file), interpreting each graph in terms of what it implies about the properties of the cell and the likely RGC type it belongs to. This will be tested in the quiz at 4:45pm.

About this program

This virtual Retinal Electrophysiology Laboratory was designed and developed by Dr Dario Protti, Department of Physiology, The University of Sydney. It builds upon "Visual Neuroscience" developed by Dr Mar Quiroga and Dr Nicholas Price, Monash University, under CC-BY-NC 4.0.

This virtual Retinal Electrophysiology Laboratory is designed to reproduce, record and download responses of different retinal ganglion cell (RGC) types to stimulation with a variety of visual stimuli for further analyses. The response properties and morphology of RGCs simulated in this virtual laboratory are modelled on those of Old world primate retinas using algorithms that describe their physiological responses.