Manual for a Tool that Simulates an Experiment to Determine the Contribution of Rods to the Retinal Signal

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1 Motivation

In mice, rods contribute to the visual experience not only under scotopic, but photopic conditions as well (Peirson et. Al., 2018 [1]). When designing a silencing stimulus, any contribution of the rods can be silenced in conjunction with the cones or individually, as long as the contribution is constant. Because the contribution of the rods is controlled by melanopsin-containing intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) (Allen et. Al., 2014 [2]), it would probably not be constant under a cone/rod silencing stimuli. Under these conditions, a cone isolating stimuli would require another light source to silence melanopsin as well, which in turn would increase the complexity of the setup and execution of the experiment, especially the fine tuning of the signal.
Therefore, it is desirable to determine if and for which illumination levels the rods contribute to the total visual response of the mouse retina. In order to obtain these informations, we propose an experiment that makes use of the relatively low temporal resolution of the ipRGCs and a flickering dichromatic light stimulus.
The simulation tool discussed in this manual is designed as an aid in setting up and tuning of the actual experiment. It can not provide actual values or exact results, but will hopefully give a helpful overview over which values are experimentally accessible and how altering these will affect the measurable output in dependence of biological variables that are unknown.

2 Program

The tool is designed as a python code made interactive through the Jupyter Notebook Application. Each code block can be executed and will return a graphical display of different values. Interactive widgets make it possible to alter various variables which in turn will alter the the graphical output. The variables adjusted by the user will be stored and used for calculations in the rest of the script. Each code block should be executed after the one preceding it, when a variable in a section above is re-adjusted, the subsequent code blocks should be re-executed to make sure the latest variables are used for the calculations. Each plot produced by the script is stored in the folder "output" in the directory containing the Jupyter Notebook script.

2.1 Spectral sensitivity of various opsin types

The first code block returns a plot of the spectral sensitivity of four opsin types. The curves are drawn using the formula proposed by Govardovskii, 2000 [3]. The presets are chosen so that the curves represent the opsins found in mice (Jacobs et. Al., 2004 [4]), but can be altered to make the tool usable for other species as well. The upper and lower boundaries of the plot can be adjusted, this will not only change the window frame, but also the boundaries for integration of the sensitivity curves and emission spectra, they should therefore be adjusted to not only show the necessary areas for observation but for calculations as well. The horizontal and vertical plot size can be adjusted globally at this point, these values will not only change the size of the display, but also the resolution of the output figures.

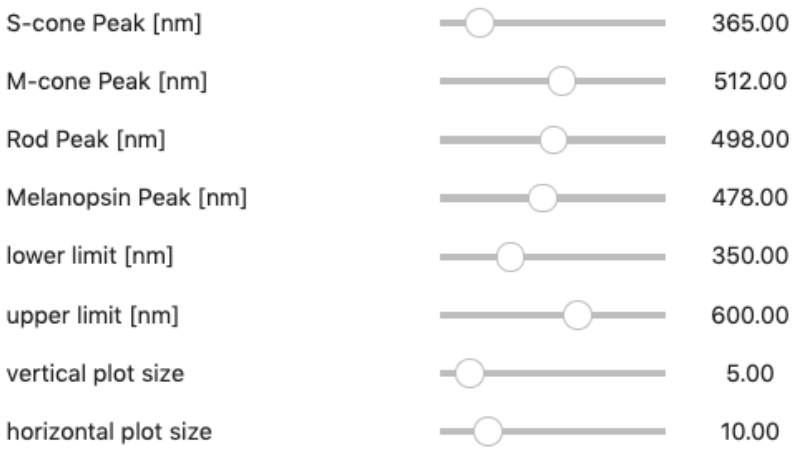

Figure 1: Available controls for the initial code block

2.2 Defining the light sources

This code block introduces the two lights sources used for the experiment into the calculations. The light sources are approximated by a normal distribution which can be altered in the location of its peak spectral intensity and the width of the shape of the curve. Figure 2 shows the default output of this code block. The curves are normalized by height, values for relative intensity will therefore be representative for the relative intensity of the total emission spectrum.

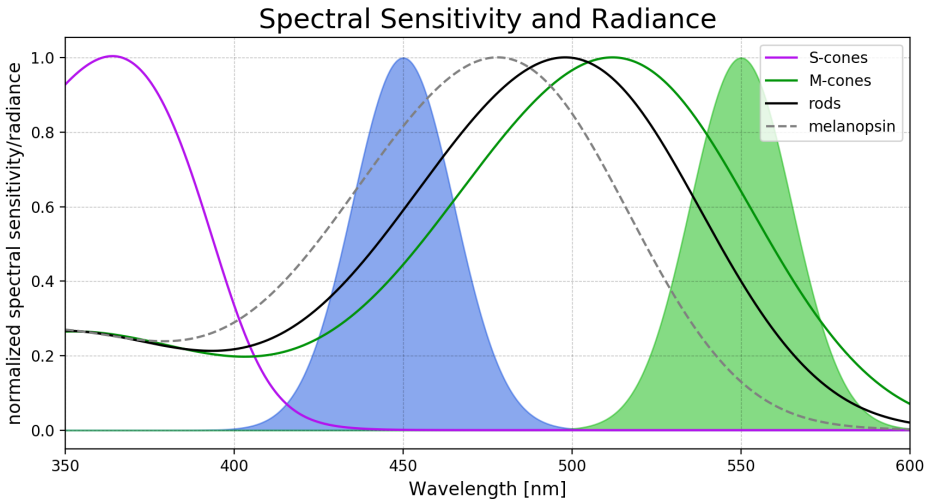

Figure 2: Plot of the spectral opsin sensitivity and emission spectra

2.3 Relative contributions of cones and rods

In this section, the first slider can be used to define the contribution of the S-cones relative to the M-cones. The second slider will define how much contribution the rods should have relative to the M-cones for a high temporal frequency of the signal. The first returning plot shows the contribution of S-cone, M-cone and rods to the ERG response for a tuned signal, where the sum of all visual opsins is equally high for both channels. The second plot displays the relative excitation of the opsins, especially interesting is by how much the excitation of melanopsin differs between both channels. The intensity of channel 2 relative to channel 1 is printed below the second plot, and the difference in melanopsin excitation for both channels is printed below that as can be seen in figure 3.

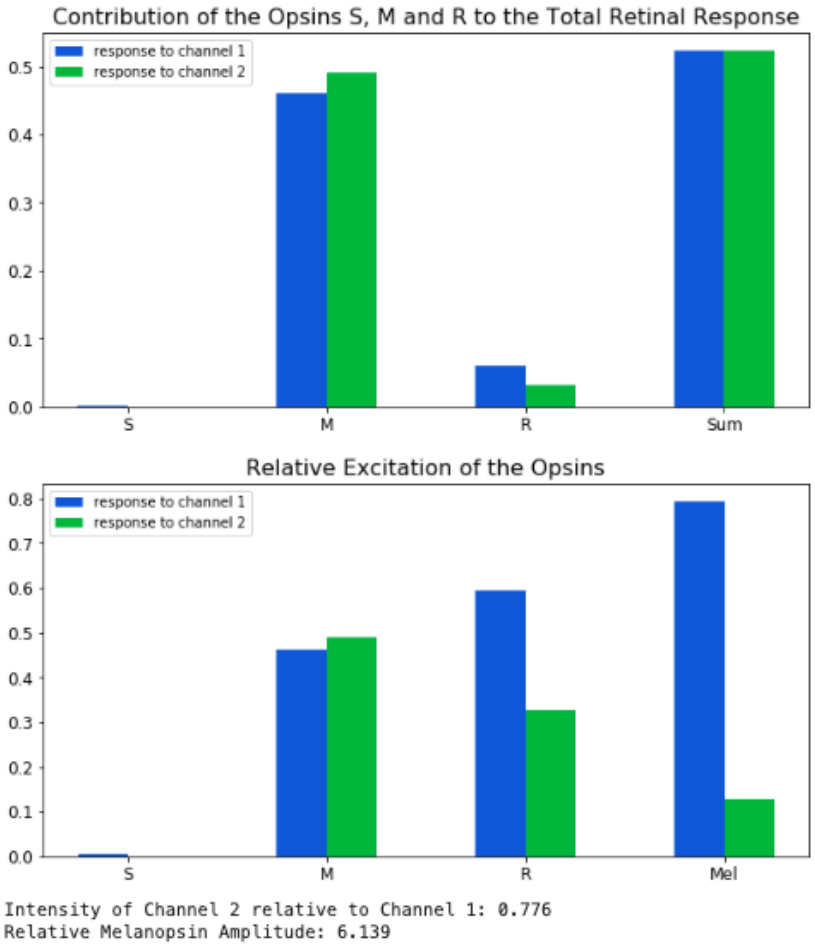

Figure 3: Display of the relative contribution and excitation of the opsins. Relative intensities of both channels and melanopsin amplitudes are printed below

2.4 Simulation of the retinal signal

The final section of code will provide a qualitative display of of the expected retinal signal and the excitation of the underlying melanopsin circuit. The first control element provides the user with control over the number of displayed phases. Control element 2 alters the ipRGC threshold. Please note that this number can only be considered to be a hypothetical value, the actual value of the ipRGC threshold can hopefully be obtained once the experiment is carried out. The design of the experiment presumes that the melanopsin containing ipRGCs are temporally more sluggish than the

cones and rods. The code implements this behavior by applying a first order Butterworth lowpass filter to the melanopsin excitation. Figure 4 shows a filter of such form applied to different signals.

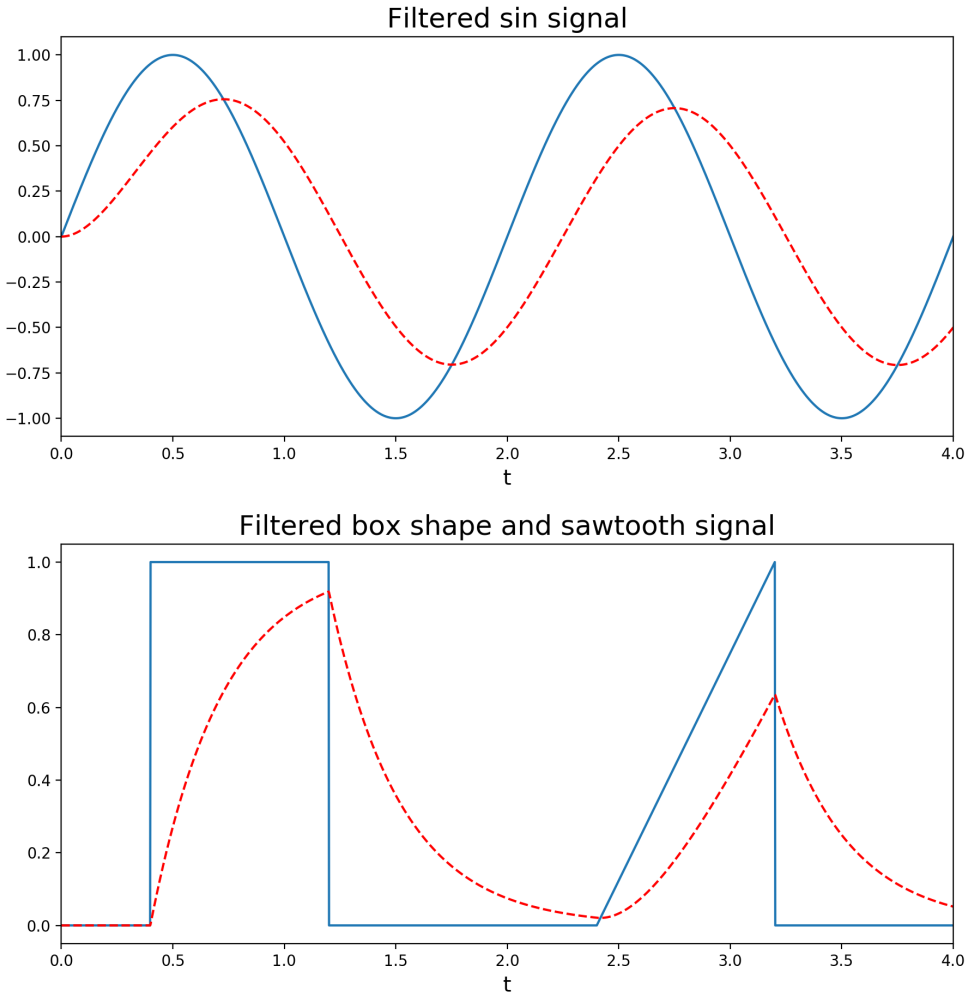

Figure 4: A Butterworth lowpass filter applied to sin, box and sawtooth signals

The third control element gives access to the critical frequency of the lowpass filter. For this frequency, a filter of the Butterworth type returns a signal with $\frac{1}{\sqrt{2}}$ times the intensity of the input signal. The filter is implemented as described by the SciPy community [5], the parameters are defined as listed in table 1.
Control element 4 is implemented to make the total luminance adjustable. In the actual experiment, this value will be adjusted until the retinal output will become invariant under a change of the signal frequency. The last control element alters the frequency with which the light stimulus is switching from one color to the other and back again. A change in the frequency will result in a change in the response of the ipRGCs. Figure 5 shows the plots that are returned by this section of code. The first figure displays the input signals, the set ipRGC threshold used for the calculations, the current melanopsin excitation resulting from the input signals and the ipRGC response defined by melanopsin excitation, signal frequency and integration interval. The second figure is the expected retinal output. In the experiment the input signal will be adjusted to deliver a constant retinal output for a high temporal frequency, this frequency is then lowered which may result in the ipRGC response dropping under the threshold and in return cause an upregulation of the rod contribution.

Parameter	description	value
N	Order of the filter	1
Wn	Critical frequency	User definable
btype	Type of filter	Lowpass
analog	Analog or digital filter	Digital
output	Type of output: numerator/denominator (‘ba’) or pole-zero (‘zpk’).	‘ba’

Table 1: Parameters and their values for the SciPy Butterworth filter

This contribution is considered to be a linear function of the difference between ipRGC threshold and the ipRGC response. If the response drops under the threshold, the retinal response, which was constant before, will now show peaks that arise from the increased rod contribution.

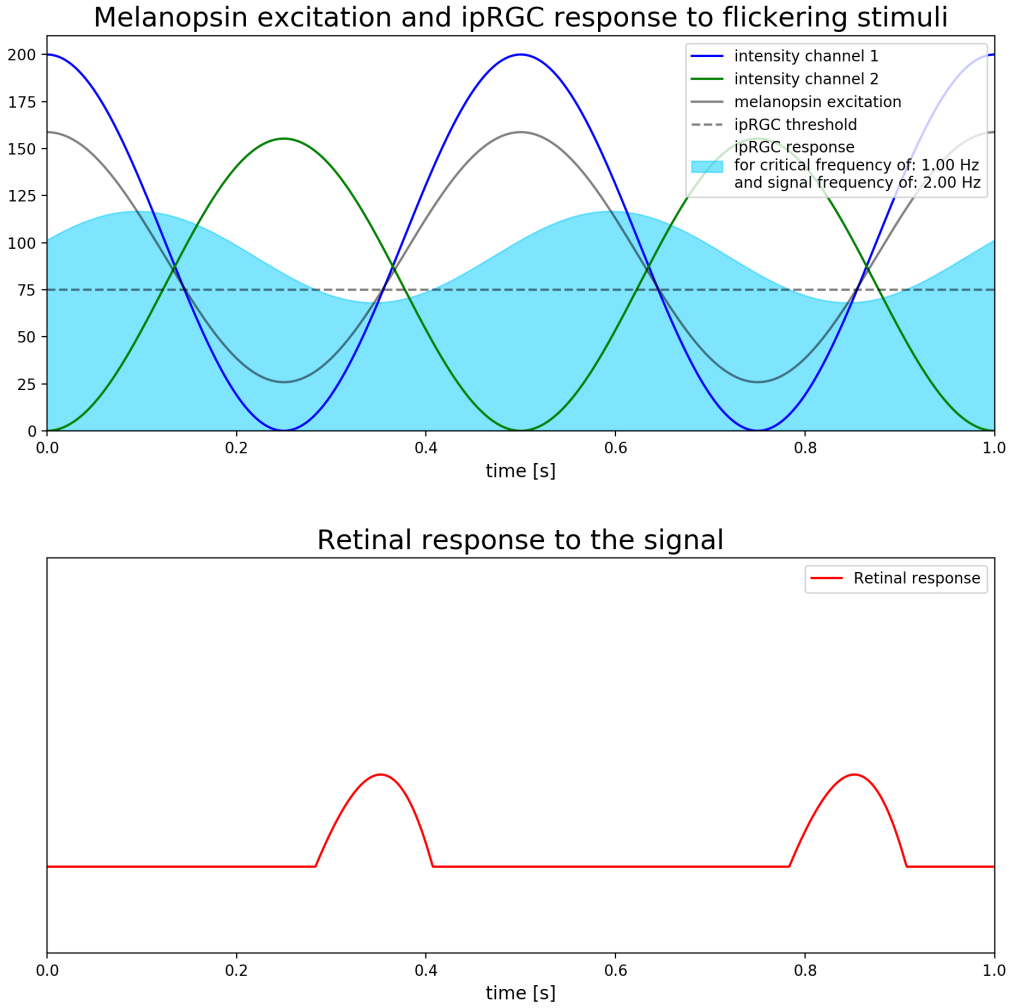

Figure 5: The ipRGC response and expected retinal output for a specific set of variables

References

[1] Peirson, Stuart, Brown, Laurence, A. Pothecary, Carina, Benson, Lindsay, Fisk, Angus. (2017). Light and the laboratory mouse. Journal of Neuroscience Methods. 300. https://doi.org/10.1016/j.jneumeth.2017.04.007.

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[5] Scipy.signal.butter - SciPy v1.3.0 Reference Guide, The SciPy Community, 17 May 2019, https://docs.scipy.org/doc/scipy/reference/generated/scipy.signal.butter.html