

Manual

Mohammad Khani, February 2021

1 Structure of the repository

This repository contains data from 31 sessions of multi-electrode array recordings (60 and 252 channels) from half or whole-mount mouse retinas that were stimulated with different chromatic patterns of light. The data accompany the manuscript by Khani and Gollisch: "Linear and nonlinear chromatic integration in the mouse retina".

Each .rar file in the **Raw Data** folder contains the data from one experiment and is named in the following format: YYYY-MM-DD_XX_YY_ZZ.rar

- YYYY-MM-DD defines the recording date in year-month-day format.
- XX is either *fr* or *sr* : referring to either first retina or second retina.
- YY is either *fp* or *sp* : referring to first piece or second piece of retina used from the eye.
- ZZ defines the eye side (left or right) and retina region (ventral, dorsal or whole mount):
 - lv: left eye, ventral retina.
 - rv: right eye, ventral retina.
 - ld: left eye, dorsal retina.
 - rd: right eye, dorsal retina.
 - lw: left eye, whole mount.
 - rw: right eye whole mount.

For example the file 20180212_fr_fp_lw refer to the recording done as a whole mount with the first retina (used for experiment) from the left eye of the mouse on 12th of February 2018.

Each .rar file comprises of the following files and folders:

- *list_of_good_cells.txt* Table of identifiers for good units of spike-sorted data
- *frametimes* Folder containing text files with time stamps of specific frames for each stimulus.
- *spiketimes* Spike times of all spike sorted units (also containing bad units).

- *Stimulusparameters* Folder containing text files that shows the parameters used for each stimulus that was displayed during each experiment. These text files can be selected by the functions available in the **Stimulation** folder to recreate or visualize each stimulus presented to the retina.
- *stimuli_names* List of the names of the stimuli used in each experiment.

A minimal example on how to extract spike times and frame times in Matlab can be found on Github:

[GITHUB LINK HERE](#)

The Github repository also contains functions for analysing the retinal responses to Chromatic integration stimulus.

Details of the applied stimuli and their reconstruction can be found in Section 2.

1.1 Frame times

In order to align recorded spike times and the timing of the stimulus, the time of appearance of specific stimulus frames for each stimulus is saved in the folder “frametimes”. For each recorded stimulus inside this folder there is a .txt. The name of the file is defined as:

<stimulus number>_<stimulus name and parameters>_frametimings.txt

Frame times were saved in seconds. An offset of 35 ms has already been added for alignment with the spike times because of an internal delay between the graphics card and the display device. Stimulus names could be found in the “stimuli_names.txt” file.

1.2 Spike times

The spike responses of retinal ganglion cells to a visual stimulus were extracted with a custom-made spike sorting algorithm [1] from the multielectrode-array recorded data. The folder “spiketimes” contains the spike times of each spike-sorted unit in seconds. Good units can be found in “list_of_good_cells.txt” where the first column is the first channel of a 4-channel grouping and the second is the number of each cluster assigned to that channel. All the units included in the “list_of_good_cells.txt” file are the ones with clear refractory period and a well-separated cluster of voltage traces. The spikes of each unit in response to a certain stimulus can then be found in the file:

<stimulus number>_SP_C<channel number><2-digit cluster number>.txt

1.3 Stimulus Parameters

To recreate and visualize each stimulus and access detailed parameters used in each experiments, a text (.txt) file with a designated name per stimulus is saved in the stimulus parameters folder. This text file can be used to access parameters needed to analyse the response of the cells to a specific stimulus. Moreover each text file could be called by functions provided in the **Stimulation** folder (see below) to recreate and visualize each stimulus. The name of the stimulus parameters file define as:

<stimulus number>_<stimulus name and parameters>_parameters.txt

2 Stimulus details and reconstruction

Six visual stimuli:

- *Chromatic Integration Stimulus*
- *Chromatic Grating*
- *Local Chromatic Integration*
- *Spatio-temporal Binary White Noise*
- *Contrast Reversing Grating*
- *Drifting Grating*

were used to characterize and analyse the linear and nonlinear chromatic integration in the mouse retinal ganglion cells.

The receptive field of each cell were analysed using *Spatio-temporal Binary White Noise* stimulus.

The *Contrast Reversing Grating* stimulus was used to investigate spatial integration in the mouse retinal ganglion cells. This is then used to compare spatial and chromatic integration in the recorded ganglion cells.

For identifying direction-selective and orientation selective cells, we used the stimulus *Drifting Grating* stimulus. From the responses to this stimulus, a direction-selectivity index (DSI) and an Orientation-selectivity index (OSI) were calculated (see also Section 2.6).

2.1 Chromatic Integration Stimulus

The chromatic integration stimulus consisted of 22 different contrast combinations of UV and green light, which were presented in a step-like fashion for 500 ms each, separated by either 1.5 or 2 s of background illumination. Each contrast combination was presented on average 50 times, and the order was determined by a Fisher-Yates random permutation algorithm [2] to ensure randomized, unbiased sampling of all contrast combinations. The contrast combinations were categorized into two sets of green-On-UV-Off and green-Off-UV-On, and contrast values refer to Weber contrast $C = (I - I_b)/I_b$, where I is the applied stimulus intensity and I_b is the background intensity. The green-On-UV-Off set consisted of 11 different combinations, ranging from 20% green and 0% UV to 0% green and -20% UV in steps of -2% for both colors. The green-Off-UV-On set contained the contrast-reversed combinations. The presented contrast values of the two set were as follows:

green-On-UV-Off =

Green contrasts:	[20	18	16	14	12	10	8	6	4	2	0]
UV contrasts:	[0	-2	-4	-6	-8	-10	-12	-14	-16	-18	-20]

green-Off-UV-On =

Green contrasts:	[-20	-18	-16	-14	-12	-10	-8	-6	-4	-2	0]
UV contrasts:	[0	2	4	6	8	10	12	14	16	18	20]

The design of the stimulus aimed at being analogous to shifting a black/white edge across the receptive field, as used to study spatial integration. This is the main stimulus used to assess the chromatic integration properties of mouse ganglion cells.

To visualize this stimulus, it is possible to call the function `Chromatic_Integration_Stimulus()` provided in the **Stimulation** folder. To use this function, first, add the stimulation folder or this function to the MATLAB path. To run this function and check the chromatic integration stimulus type `Chromatic_Integration_Stimulus()` in MATLAB command window. This command automatically displays the stimulus with a set of default parameters. To check the list of available parameters for this function call the help option with the following command: `Chromatic_Integration_Stimulus('help',true)`. To recreate any chromatic integration stimulus used during the experiments provided in the **Raw Data** folder, it is possible to select an experiment specific stimulus parameter text file. To do so, use the 'selecttextfile' option in the stimulus function. For example: `Chromatic_Integration_Stimulus('selecttextfile',true)`. This call will open a prompt window to select the stimulus parameter text file provided in the **stimulusparamters** folder in each experiment. Beyond these

options, it is possible to use this stimulus function with a list of independent parameters inserted as series of string and values to probe other possible variation of the chromatic integration stimulus. For example:

`Chromatic_Integration_Stimulus('maxcontrast,0.4, 'mincontrast',-0.4, 'contrastdiff', 0.04)` displays a variation of the stimulus were the contrasts are shown from 40% to -40% ranges with 4% steps in between the values. Note that the `Chromatic_Integration_Stimulus()` is provided for the purpose of visualizing and recreating the chromatic integration stimulus. For this purpose and also for simplicity, operations such opsin-isolation and gamma-correction of the contrast values were removed from this function. Additionally, the time-scale of the stimulus presentation may vary due to variations in MATLAB performance in different machines or operating systems. This list of parameters used in this function is as follows:

Parameter	Default	Usage
selecttextfile	false	Browse prompt to select the parameters text file
stimduration	30	Stimulus presentation duration screen refresh rate unit (30 = 0.5 sec)
preframes	120	Duration of background light presented in between the stimulus flashes
maxcontrast	0.2	Maximum Weber contrast value (ranges from -1 to 1)
mincontrast	-0.2	Minimum Weber contrast value (ranges from -1 to 1)
contrastdiff	0.02	Contrast steps from mincontrast to maxcontrast e.g. -20 : 2 : 20
seed	-1000	Starting value for random number generator (should be always negative)
redmeanintensity	0	Mean intensity for red gun of the screen
greenmeanintensity	0.5	Mean intensity for green gun of the screen
bluemeanintensity	0.5	Mean intensity for blue gun of the screen
screensize	864 x 480	Screen resolution, default value is the resolution of the lightcrafter projector
refreshrate	60	Screen refresh rate in Hz
fullscreen	false	Option to display the stimulus in full-screen mode
help	false	Option to check the list of available parameters
lmargin	0	Margins from left side of the screen (not available for this stimulus)
rmargin	0	Margins from right side of the screen (not available for this stimulus)
tmargin	0	Margins from top of the screen (not available for this stimulus)
bmargin	0	Margins from bottom of the screen (not available for this stimulus)
conisolating	false	Option to activate opsin-isolation (excluded for simplicity)

2.2 Chromatic Grating Stimulus

The chromatic grating stimulus was a slowly moving UV-green sinusoid with opposite spatial phases of the two colors. We used 11 different contrast combinations, matching the contrast combinations of the spatially homogeneous chromatic integration stimulus (see above, 2.1). The spatial period of the grating was set to 480 μm , and the temporal period was 1 second. Each contrast combination was presented for 10 seconds continuously before the next UV-green combination was selected randomly (using Fisher-Yates random permutation algorithm) with no intervals between the stimuli. The stimulus was presented for 30-60 repetitions per contrast combination. The contrast ranges of the sinusoids were identical to the ranges of contrast values used in the chromatic integration stimulus (see above).

To visualize the chromatic grating stimulus, call the function *Chromatic_Grating_Stimulus()* provided in the **Stimulation** folder in MATLAB command window. This automatically displays the stimulus with a set of default parameters. To check the list of available parameters for this function call the help option with the following command: *Chromatic_Grating_Stimulus('help',true)*. To recreate any chromatic grating stimulus used during the experiments provided in the **Raw Data** folder, it is possible to select an experiment specific stimulus parameter text file. To do so, use the 'selecttextfile' option in the stimulus function. For example: *Chromatic_Grating_Stimulus('selecttextfile',true)*. This call will open a prompt window to select the stimulus parameter text file provided in the **stimulusparamters** folder inside each experiment folder. Additionally, it is possible to use this function with a list of independent parameters inserted as series of string and values to check other possible variations of the chromatic grating stimulus. For example:

Chromatic_Grating_Stimulus('period',120, 'gratingwidth',180) displays a variation of the stimulus where the duration of one period of the stimulus is 120 frames (2 seconds on 60 Hz monitor) and the width of each grating is equal to 180 pixels of the screen. Note that the *Chromatic_Grating_Stimulus()* is provided for the purpose of visualizing and recreating the chromatic grating stimulus. For this purpose and also for simplicity, operations such opsin-isolation and gamma-correction of the contrast values were removed from this function. Additionally, the time-scale of the stimulus presentation may vary due to variations in MATLAB performance in different machines or operating systems. This list of parameters used in this function is as follows:

Parameter	Default	Usage
selecttextfile	false	Browse prompt to select the parameters text file
stimduration	600	Stimulus presentation duration in refresh rate unit (600 = 10 sec).
period	60	Duration for one period of the grating in refresh rate unit (60 = 1 sec).

gratingwidth	60	Grating widths in pixels
squarewave	false	Option to switch between sinusoid and square-wave gratings
maxcontrast	0.2	Maximum Weber contrast value (ranges from -1 to 1)
mincontrast	-0.2	Minimum Weber contrast value (ranges from -1 to 1)
contrastdiff	0.02	Contrast steps from mincontrast to maxcontrast e.g. -20 : 2 : 20
seed	-1000	Starting value for random number generator (should be always negative)
redmeanintensity	0	Mean intensity for red gun of the screen
greenmeanintensity	0.5	Mean intensity for green gun of the screen
bluemeanintensity	0.5	Mean intensity for blue gun of the screen
screensize	864 x 480	Screen resolution, default value is the resolution of the lightcrafter projector
refreshrate	60	Screen refresh rate in Hz
fullscreen	false	Option to display the stimulus in full-screen mode
help	false	Option to check the list of available parameters
lmargin	0	Margins from left side of the screen (not available for this stimulus)
rmargin	0	Margins from right side of the screen (not available for this stimulus)
tmargin	0	Margins from top of the screen (not available for this stimulus)
bmargin	0	Margins from bottom of the screen (not available for this stimulus)
coneisolating	false	Option to activate opsin-isolation (excluded for simplicity)

2.3 Local Chromatic Integration Stimulus

Local chromatic integration stimulus is a spatially local version of the chromatic integration stimulus (see above) which was used to probe for involvement of the receptive field surround in nonlinear chromatic integration. This stimulus aimed at presenting the same contrast combinations of UV and green light as used in the full-field chromatic integration stimulus, but spatially restricted to small regions roughly inside individual ganglion cell receptive fields. To make this stimulus efficient while covering the entire recording area, multiple randomly selected locations were stimulated simultaneously. Concretely, the stimulus was structured into step-like contrast presentations of 500 ms duration with no interval between successive presentations. For every 500-ms presentation, stimulus locations were randomly selected from a square grid (44 X 24 vertices, 160 μ m between neighboring vertices) in a way so that, for every chosen vertex, 24 vertices in a square area around it (2 vertices or 320 μ m in each of the four directions along the grid) were avoided. Centered on each selected vertex, a spot with diameter of 160 μ m was presented with a UV/green contrast combination, randomly chosen (using again Fisher-Yates randomization) from the same set of 22 combinations as used in the full-field chromatic integration stimulus. The rest of the screen remained at

background illumination. Note that the simultaneously presented contrast combinations at different locations were chosen independently of each other. On average, around 80-90 locations were selected for simultaneous display (until all vertices lay within the square areas of 5x5 vertices around selected locations), and each location was chosen on average every 6.4 ± 1.2 seconds (mean \pm SD). We recorded responses under this stimulus for 65 minutes on average, which led to each location being chosen 595 ± 164 times, providing 27 ± 7 trials for each contrast combination.

To visualize the local chromatic integration stimulus, call the function *Local_Chromatic_Integration_Stimulus()* from in the **Stimulation** folder in MATLAB command window. This automatically displays the stimulus with a set of default parameters. To check the list of available parameters for this function call the help option with the following command: *Local_Chromatic_Integration_Stimulus('help',true)*. To recreate the same local chromatic integration stimulus that was used during the experiments provided in the **Raw Data** folder, it is possible to select an experiment specific stimulus parameter text file. To do so, use the 'selecttextfile' option in the stimulus function. For example: *Local_Chromatic_Integration_Stimulus('selecttextfile',true)*. This call will open a prompt window to select the stimulus parameter text file provided in the **stimulusparamters** folder inside each experiment folder. Additionally, it is possible to use this function with a list of independent parameters inserted as series of string and values to check other possible variations of the local chromatic integration stimulus. For example:

Local_Chromatic_Integration_Stimulus('gapwidth,4, 'gapheight',3) displays a variation of the stimulus where 4 locations along the x-axis and 3 locations along the y-axis around each selected stimulus location is avoided. Note that the *Local_Chromatic_Integration_Stimulus()* is provided for the purpose of visualizing and recreating the local chromatic integration stimulus. For this purpose and also for simplicity, operations such opsin-isolation and gamma-correction of the contrast values were removed from this function. Additionally, the time-scale of the stimulus presentation may vary due to variations in MATLAB performance in different machines or operating systems. This list of parameters used in this function is as follows:

Parameter	Default	Usage
selecttextfile	false	Browse prompt to select the parameters text file
stimduration	600	Stimulus presentation duration in refresh rate unit (600 = 10 sec).
maxcontrast	0.2	Maximum Weber contrast value (ranges from -1 to 1)
mincontrast	-0.2	Minimum Weber contrast value (ranges from -1 to 1)
contrastdiff	0.02	Contrast steps from mincontrast to maxcontrast e.g. -20 : 2 : 20
stixelwidth	20	Width of each local stimulus in pixels

stixelheight	20	Height of each local stimulus in pixels
gapwidth	2	Number of locations to be avoided around each locally selected stimulus area
gapheight	2	Same as gapwidth but for the y-axis of each selected location
seedlocation	-1000	Seed for random number generator for locations' list (should be negative)
seedonoroffpixels	-10000	Seed for random number generator of binary contrasts (not used here)
seedcolorcontrast	-2000	Seed for random number generator for color contrast list (should be negative)
redmeanintensity	0	Mean intensity for red gun of the screen
greenmeanintensity	0.5	Mean intensity for green gun of the screen
bluemeanintensity	0.5	Mean intensity for blue gun of the screen
screensize	864 x 480	Screen resolution, default value is the resolution of the lightcrafter projector
refreshrate	60	Screen refresh rate in Hz
drawcircle	true	Option to display stimulus as circle or squares
drawannulus	false	Option to display the stimulus as annulus (not available for this stimulus)
fullscreen	false	Option to display the stimulus in full-screen mode
help	false	Option to check the list of available parameters
lmargin	0	Margins from left side of the screen (not available for this stimulus)
rmargin	0	Margins from right side of the screen (not available for this stimulus)
tmargin	0	Margins from top of the screen (not available for this stimulus)
bmargin	0	Margins from bottom of the screen (not available for this stimulus)
conisolating	false	Option to activate opsin-isolation (excluded for simplicity)

2.4 Spatio-temporal Binary White-Noise Stimulus

A spatio-temporal binary white-noise stimulus of black and white squares (either 75% or 100% contrast), which was displayed on a checkerboard layout with squares of either 48 or 60 μm to the side were used to estimate a cell's receptive field. Each square was randomly assigned to black (0) and white (1) with a probability of 50% each. Spatial patterns were updated with a frequency of either 30 or 60 Hz, frame times indicating an update of the stimulus.

To visualize or recreate the Spatio-temporal binary white-noise stimulus, call the function [SpatioTemporal_Binary_White_Noise_Stimulus\(\)](#) provided in the **Stimulation** folder in MATLAB command window. This automatically displays the stimulus with a set of default parameters. To check the list of available parameters for this function call the help option with the following command: [SpatioTemporal_Binary_White_Noise_Stimulus\('help',true\)](#). To recreate any Spatio-temporal binary

white-noise stimulus used during the experiments provided in the **Raw Data** folder, it is possible to select an experiment specific stimulus parameter text file. To do so, use the 'selecttextfile' option in the stimulus function. For example: `SpatioTemporal_Binary_White_Noise_Stimulus('selecttextfile',true)`. This call will open a prompt window to select the stimulus parameter text file provided in the **stimulusparamters** folder inside each experiment folder. Additionally, it is possible to use this function with a list of independent parameters inserted as series of string and values to check other possible variations of the spatio-temporal binary white noise stimulus. For example:

`SpatioTemporal_Binary_White_Noise_Stimulus('stixelwidth',10, 'stixelheight',10)` displays a variation of the stimulus where each square of the checkerboard is 10 pixels by the side.. Note that the `SpatioTemporal_Binary_White_Noise_Stimulus ()` is provided for the purpose of visualizing and recreating the spatio-temporal binary white-noise stimulus. For this purpose and also for simplicity, operations such opsin-isolation and gamma-correction of the contrast values were removed from this function. Additionally, the time-scale of the stimulus presentation may vary due to variations in MATLAB performance in different machines or operating systems. This list of parameters used in this function is as follows:

Parameter	Default	Usage
selecttextfile	false	Browse prompt to select the parameters text file
stixelwidth	6	Width of each pixel of the checkerboard defined in monitor pixels unit
stixelheight	6	Height of each pixel of the checkerboard defined in monitor pixels unit
blackwhite	true	Option to switch between Binary and Gaussian white-noise (Not available)
contrast	1	Contrast of black & white stimulus. 1 is 100% and -1 is -100% contrast.
meanintensity	0.5	Mean intensity value used to define contrast
seed	-1000	Starting value for random number generator (should be always negative)
secondseed	-10000	Same as seed but for green color (only used if color is true).
thirdseed	-2000	Same as seed but for blue color (only used if color is true).
nblinks	2	Number of frames to hold the stimulus on screen (2 is 30 Hz stimulus)
color	false	Option to switch between black & white and chromatic white-noise
redmeanintensity	0	Mean intensity for red gun of the screen (only used if color is true).
greenmeanintensity	0.5	Mean intensity for green gun of the screen (only used if color is true).
bluemeanintensity	0.5	Mean intensity for blue gun of the screen (only used if color is true).
redcontrast	0	Contrast of red stimulus (only used if color is true).
greencontrast	0.75	Contrast of green stimulus (only used if color is true).
bluecontrast	0.75	Contrast of blue stimulus (only used if color is true).

screensize	864 x 480	Screen resolution, default value is the resolution of the lightcrafter projector
refreshrate	60	Screen refresh rate in Hz
fullscreen	false	Option to display the stimulus in full-screen mode
help	false	Option to check the list of available parameters
lmargin	0	Margins from left side of the screen (not available for this stimulus)
rmargin	0	Margins from right side of the screen (not available for this stimulus)
tmargin	0	Margins from top of the screen (not available for this stimulus)
bmargin	0	Margins from bottom of the screen (not available for this stimulus)
coneisolating	false	Option to activate opsin-isolation (excluded for simplicity)

2.5 Contrast-reversing Grating Stimulus

We measured the spatial integration properties of ganglion cells using monochromatic square-wave grating stimuli (100% contrast) with spatial periods of 32, 64, 128, 224, and 448 μm , and we also included a spatially homogeneous full-field stimulus for comparison. For spatial periods of 64 and 128 μm , two different spatial phases (separated by 90°), and for spatial periods of 224 and 448 μm , four spatial phases (separated by 45°) were applied. The polarity of each grating was reversed every second for a total of 30 reversals.

2.6 Drifting Grating Stimulus

We used drifting grating stimulus (100% contrast) in eight equidistant directions to identify direction-selective (DS) cells and orientation-selective (OS) cells. The gratings were either square-wave with 600 μm spatial period and 0.75 Hz temporal frequency or sinusoidal with 250 μm spatial period and 0.6 Hz temporal frequency. Each direction was presented for 6.67 sec, separated by 3 to 5 sec of background illumination. To identify orientation-selective (OS) cells, we used square-wave drifting gratings with 240 μm spatial period and 4 Hz temporal frequency presented with eight equidistant directions (3 sec per direction and 2 sec gray screen between). All stimulus sequences were repeated for 4 times.

We measured the direction tuning for all the cells by calculating the mean firing rates R_θ in response to each direction θ ($0 \leq \theta < 2\pi$), leaving out the onset response during the first period of the stimulus. For each cell, we computed a direction-selectivity index as the normalized vector sum:

$$DSI = \frac{|\sum_{\theta} R_{\theta} e^{i\theta}|}{\sum_{\theta} R_{\theta}}$$

In addition, we assessed the statistical significance of direction tuning, using a permutation test^{47, 89}. We created surrogate data by shuffling the spike counts randomly across all angles and repetitions of the stimulus for 1000 times. For each shuffling, we calculated a DSI to generate a null distribution of DSIs and used the percentile of the true DSI as a p-value of direction tuning. As a criterion for direction selectivity, we required $DSI > 0.3$ with a p-value < 0.05 . Cells with mean firing rates < 1 Hz across different directions of the drifting grating stimuli were excluded from this analysis.

To classify orientation-selective cells, we defined an orientation-selectivity index:

$$OSI = \frac{|\sum_{\theta} R_{\theta} e^{2i\theta}|}{\sum_{\theta} R_{\theta}}$$

Cells that were not classified as DS and that had $OSI > 0.3$ with a p-value < 0.05 and an average firing rate > 1 Hz were considered as OS cells.

3 References

- [1] Pouzat C, Mazor O, Laurent G. Using noise signature to optimize spike-sorting and to assess neuronal classification quality. *J Neurosci Methods* 122, 43-57 (2002).
- [2] Fisher RA, Yates F. *Statistical tables for biological, agricultural and medical research*. Oliver and Boyd (1948).