Manual

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1 Structure of the repository

This repository contains data from 10 sessions of multi-electrode array recordings (252 channels) from whole-mount salamander retinas that were stimulated with patterns of light. The data accompany the manuscript by Kühn and Gollisch: "Activity correlations between direction-selective retinal ganglion cells synergistically enhance motion decoding from complex visual scenes".

Each .rar file (named by recording date and Re/Le for right or left eye: YYYY-MM-DD-XX.rar) comprises one recording session including following files and folders:

goodChannels.txt	Table of identifiers for good units of spike-sorted data
**_frametimings.mat*	Matlab files containing time stamps of specific frames for each stimulus
CellStats_RF-SVD_DS-CircVar.mat	Matlab file containing properties of each unit in *goodChannels.txt*
spike times	Spike times of all spike sorted units (also containing bad units)

A minimal example on how to extract spike times and frame times in Matlab can be found on Github: https://github.com/gollischlab/AnalysisForDSPopulationCodes. The Github repository also contains functions for analysing the retinal responses to complex texture motion.

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

1.1 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 “spike times” contains the spike times of each spike-sorted unit in seconds. Good units can be found in “goodChannels.txt” where the first column is the first channel of a 4-channel grouping and the second is the number of the cluster. The third column indicates the goodness of each cluster (1: excellent, peaks > 5 SD, cluster distance > 10; 2: good, peaks > 5 SD, cluster distance > 7; 3: ok, peaks > 2 SD, cluster distance > 5). 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

1.2 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 container “ftimes” of the file

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

Frame times were saved in milliseconds. An offset of 25 ms has to be added for alignment with the spike times because of an internal delay between the graphics card and the display device.

2 Stimulus details and reconstruction

Six visual stimuli (*driftinggratings*, *OMSpatches*, *onoff30bl*, *checkerflicker*, *Gaussianfff* and *OMB*) were used to characterize and analyse the responses of retinal ganglion cells to motion and contrast changes and to assess the potential to decode complex motion trajectories from the responses of direction-selective ganglion cells in the salamander retina.

For identifying direction-selective cells, we used the stimulus *driftinggratings*. The stimulus *OMSpatches* was used to assess how well the direction-selective cells respond to global motion. From the responses to these stimuli, a direction-selectivity index (DSI) and an object-motion-sensitivity index (OMSI) were calculated (see also Section 3).

For each cell, the receptive field was determined from responses to a spatio-temporal white-noise stimulus (*checkerflicker*). For probing contrast sensitivity, a temporal white-noise stimulus (*Gaussianfff*) and flashes of dark and bright contrast (*onoff30bl*) were used.

For probing the encoding of complex texture motion, a static texture (usually a smoothed white-noise pattern) was moving in a random walk (*OMB* - only moving background).

For the stimuli *checkerflicker*, *Gaussainfff* and *OMB*, .cpp files are provided in the folder *functions* for the reconstruction of brightness values and motion steps in Matlab. Use the Matlab *mex* command, to build a binary mex file. These mex files can then be called in Matlab; their usage is detailed below.

2.1 Driftinggratings

We used drifting square-wave gratings of 100% contrast, 600 µm spatial period, and a temporal frequency of 0.75 Hz to identify direction-selective cells [2]. Gratings were shown in a sequence of eight equidistant directions θ = 0°, 45°, …, 270°, 315° for 6.67 s per direction (length of 5 cycles), separated by 1.67 s of gray screen (homogeneous illumination at mean intensity). Frame times mark the beginning of a new cycle of 1.33 s length. The sequence of eight directions was repeated five times. A direction-selectivity index (DSI) was calculated from the mean firing rates f_θ in response to the eight directions θ, leaving out the onset response to the first second of each direction:

$$\text{DSI} = \frac{\left|\sum_\theta f_\theta e^{i\theta}\right|}{\sum_\theta f_\theta}$$

Cells with DSI > 0.3 and a mean firing rate above 1 Hz for this stimulus were considered as direction-selective cells. The preferred direction of each direction-selective cell is then given by the angle of $\sum_\theta f_\theta e^{i\theta}$.

2.2 OMSpatches

For probing sensitivity to object motion, we applied a stimulus consisting of circular patches of 750 µm diameter, arranged in a hexagonal pattern on a mean-luminance background. The patches contained square-wave gratings of 300 µm period in vertical orientation [2], which were jittered by selecting motion steps of 15 µm randomly to either side at 30 Hz. Stimulus segments of 23.33 s were presented repeatedly (frame times marking the occurrence of each of the 700 motion steps), separated by 1.67 s of gray screen (onset marked by frame time), with all gratings jittering either coherently with the same

trajectory or with independent, differential trajectories for each patch. Each sequence started with differential motion. An object-motion-sensitivity index OMSI $= (f_d - f_c)/(f_d + f_c)$ was calculated from the firing rates in response to coherent and differential motion, f_c and f_d, respectively. Direction-selective cells with an OMSI > 0.7 were considered object-motion-sensitive and not further analysed.

2.3 Checkerflicker

A spatio-temporal white-noise stimulus of black and white squares (100% contrast) of 75 x 75 µm^2 was 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 30 Hz, frame times indicating an update of the stimulus. Brightness values of each square for each stimulus frame can be reconstructed by

CheckerFrames(<number x pxls>, <number y pxls>, <number of frames>)

Providing a three-dimensional output matrix of dimensions *(<number x pxls>, <number y pxls>, <number of frames>).*

Usage: *brightnessValues = CheckerFrames(80, 60, numel(ftimes)).*

2.4 Gaussianfff

Temporal full-screen noise of Gaussian-distributed brightness values with a standard deviation of 30% contrast from mean luminance (gray) and an update rate of 30 Hz, frame times indicating an update of the stimulus. The function GaussainFrames() provides brightness values between 0 to 1 and takes following input arguments:

GaussianFrames(<number x pxls>, <number y pxls>, <number of frames> [, <seed>])

Usage: *brightnessValues = squeeze(GaussianFrames(1, 1, numel(ftimes))).*

2.5 OMB – complex texture motion

This was the main stimulus that was used to analyse responses of direction-selective ganglion cells to complex texture motion. Complex texture motion was simulated by shifting a static texture in a two-dimensional random walk. The random shifts (motion steps) were Gaussian-distributed with 22.5 µm standard deviation ("Gsteps3SD" in the file name corresponding to 3*7.5 µm SD) and occurred every 33 ms. The two-dimensional motion trajectory can be reconstructed using *GaussianFrames()* where the seed of the random number generator has to be set to -10000. If not indicated in the file name, the stimulus was running continuously with frame times indicating the stimulus updates. Otherwise, a 15-min trajectory was repeated several times (indicated by "Nx15" in the file name) with 2 s of gray screen. During these trials, the trajectory was either simply repeated or offset by 1500 µm from the center ("addXYoffset200pxl"). The stimuli where the trajectory of the second trial was flipped in x- and y-direction are indicated by "flipDir_2x15".

During stimulation with complex texture motion, different textures were used. The use of the standard texture is indicated by "bg4x4corr8_C150", a smoothed white-noise pattern (pixel width 4*7.5 µm, Gaussian smoothing of 8*7.5 µm SD) with maximum contrast values scaled to 150% and then cropped at 100%. The use of other textures is indicated by "bgPinkNoise" (pink noise) and "bgNatImage-<name>"

(natural image). These were also scaled up to 150% contrast and then cropped at 100%. The standard texture can be regenerated by using the following function, yielding a square texture in resolution of the defined square pixel size:

bgTexture(<square size in pxl>, <smoothing width in pxl>)

Usage: *motionSteps = squeeze(GaussianFrames(2, 1, numel(ftimes), -10000)-.5)*2*22.5*

brightnessValues = bgTexture (4, 4)

The time course of the texture can be derived from the texture position by summing the motion steps and shifting the texture accordingly. The texture is centered in the middle of the screen.

Functions and a minimal example of how to reconstruct the motion steps from the spike responses of direction-selective cells are provided on Github:

https://github.com/gollischlab/AnalysisForDSPopulationCodes

2.6 Onoff30bl

We used flashes of 500 ms of increased or decreased light level at 40% from mean luminance, interleaved by 1.5 s of mean luminance. Sequences started with 1.5 s of mean luminance, followed by 500 ms of decreased contrast, and so forth, repeated for two minutes. Frame times mark the changes of light level. To assess the degree of ON vs. OFF responses, an ON-OFF index was calculated from the spike rates f_{on} and f_{off}, measured in a time window of 50 to 550 ms after the onset of the ON- and OFF-flash, respectively, such that the ON-OFF Index is $(f_{\mathrm{on}} - f_{\mathrm{off}})/(f_{\mathrm{on}} + f_{\mathrm{off}})$.

3 Analysed cell properties

The file *CellStats_RF-SVD_DS-CircVar.mat* contains analysed properties of each unit in *goodChannels.txt*, calculated from the responses to the stimuli *driftinggratings*, *OMSpatches*, *onoff30bl*, *checkerflicker* and *Gaussianfff*. Most variables (except for *Angles* and *offset*) are organized in columns where each row corresponds to the property of a good unit (rows in *goodChannels.txt*).

goodCells	Channel, cluster and rating of good units from *goodChannels.txt*
Angles	Tested motion directions θ in *driftinggratings*
TuningDS	Mean responses f_θ to *Angles* in *driftinggratings*
IndexDS	Direction-selectivity index from *Angles* and *TuningDS*
DScells	Units with *IndexDS* > 0.3
DSdir	Preferred directions of *DScells* from *Angles* and *TuningDS*
DSdirDiff	Differences between preferred directions in *DSdir*
IndexOMS	Object-motion-sensitivity index from *OMSpatches*
OMScells	Units with *IndexOMS* > 0.7
OMSDScells	Units with *IndexOMS* > 0.7 and *IndexDS* > 0.3
IndexONOFF	ON-OFF index from *onoff30bl*

RFparam	Parameters of Gaussian fit of spatial receptive field from *checkerflicker* (standard deviation (SD) in x; and y dimension; angle in rad; mean in x; and y dimension) with x- and y-dimension in units of 75 µm
RFsize	Receptive field diameter at 2 SD from *RFparam* in µm
offset	Center of recording area measure from stimulus center in x- and y-dimension
latency	Latency of temporal STAs from *Gaussianfff*
biphas	Biphasicness index of temporal STAs from *Gaussianfff*
IndexOS	Orientation-selectivity index from *Angles* and *TuningDS*

4 References

[1] C. Pouzat, O. Mazor, G. Laurent, J. Neurosci. Methods 122 (2002) 43–57.

[2] N.K. Kühn, T. Gollisch, J. Neurosci. 36 (2016) 12203–12216.