

# Overview

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The **Nested RF Protocol** was designed by LE Burnett in 2024 for patch electrophysiology experiments ran by Jin Yong Park. It was based upon the protocol used in *Gruntman et al. 2018*<sup>1</sup>. All fly husbandry was carried out by Edward Rogers.

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<sup>1</sup>Gruntman, E., Romani, S. & Reiser, M.B. Simple integration of fast excitation and offset, delayed inhibition computes directional selectivity in *Drosophila* . Nat Neurosci 21, 250–257 (2018). <https://doi.org/10.1038/s41593-017-0046-4>

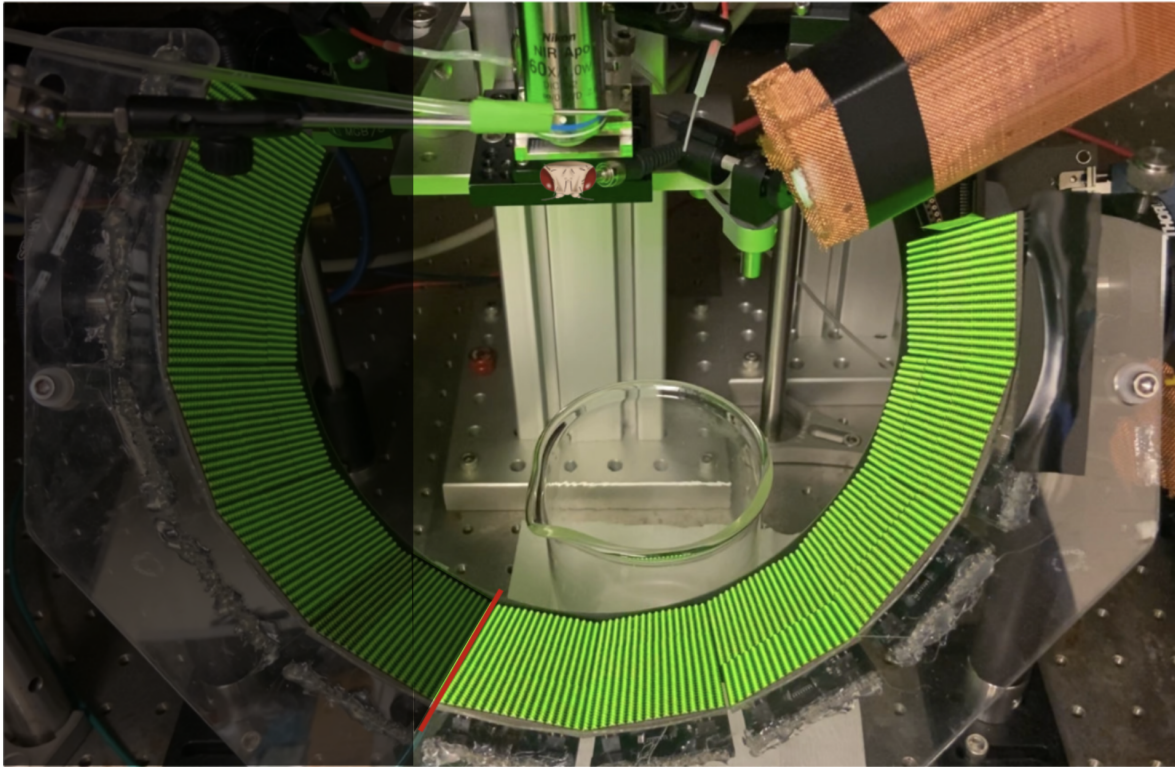


Figure 1: Experiment Rig: G4 LED - Ephys arena with a cartoon representation of the position of the fly within the arena. For many protocols, visual stimuli are only presented over 1/2 of the arena screen, ipsilateral to the location of the recorded cell.

## 0.1 Protocol overview

### **i** Quick summary:

This is a multi-step protocol consisting of a short first protocol (P1) that determines the rough receptive field location and preferred contrast of the cell, and a longer second protocol (P2) that is only presented at the location of the RF determined in P1. This follow-up protocol probes the direction selectivity of the cell using moving bar stimuli, as well as measuring the receptive field structure at higher spatial resolution using small flashing squares.

The first protocol is run through the `G4_experiment_conductor` GUI whereas the second protocol is generated and run through a custom MATLAB script called `generate_protocol2()`.

### **i** Aim:

To characterise the receptive field structure and direction selectivity of visual neurons in *Drosophila*, particularly T4/T5 cells. The experiments specifically aimed to understand the role of certain proteins linked to specific receptors that are localised to specific locations along the T4/T5 dendrites in shaping the receptive field properties of these cells<sup>2</sup>.

## **0.2 Protocol descriptions**

If you would like to see detailed descriptions of the contents of the two protocols, please see the following pages:

- [Protocol 1](#)
- [Protocol 2](#)

If you would like to see how the parameters of Protocol 1 and 2 have changed over time please see the following page:

- [Version History](#)

## **0.3 Creating and running the protocols**

- [How the protocols are made](#)
- [How to run the protocols](#)

## **0.4 Experiment log**

Find a log of the different experiments and fly strains here:

- [Experiment Log](#).

## **0.5 Analysing the data**

- [How to analyse P1](#)
- [How to analyse P2](#)

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<sup>2</sup>Sanfilippo, P., Kim, A.J., Bhukel, A., Yoo, J., Mirshahidi, P.S., Pandey, V., Bevir, H., Yuen, A., Mirshahidi, P.S., Guo, P., Li, H.S., Wohlschlegel, J.A., Aso, Y., Zipursky, S.L. (2024) Mapping of multiple neurotransmitter receptor subtypes and distinct protein complexes to the connectome. *Neuron*, <https://doi.org/10.1016/j.neuron.2023.12.014>.

## 0.6 Code availability

The code for generating and running these protocols is publicly available [here](#). This code was cloned locally onto the computer for the G4 electrophysiology rig here: `C:\matlabroot\G4_Protocols\nested_RF_stimulus\`.

Running these experiments requires the user to have the ‘G4\_Display\_Tools’ [repository](#) downloaded locally too.

## 0.7 Data availability

The data from all of these experiments can be found on the Reiser Lab prfs drive here: `smb://prfs.hhmi.org/reiserlab/burnett1/Ephys_experiments/nested_RF_stimulus/DATA`. The data is then further organised based on sub-type of experiment grouped in the same way as the Experiment Log above (e.g. RNAi ttl experiments, DS\_v2 experiments).

The data within the folder `2025_whiteplus` contains duplicates of data in other folders. This folder was created for ease of access to all experiments involving the white+ genotype.

The raw data is also found on the computer in the Ephys rig room within the folder `C:\matlabroot\G4_Protocols\nested_RF_protocol2\data`.

Please see the following [page](#) for details on how the data is saved and stored.

## 1 References