

Version History

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This page contains a detailed description of how the Nested RF Protocols have evolved over time. It includes detailed descriptions of the contents of the different protocol versions, a timeline of changes and tabular comparisons of the different parameters used in each version.

1 Descriptions of Protocol 1 Versions

1.0.1 P1 protocol (Dec 2024 - January 2025) - RNAi experiments

- Area of arena presented: Left half of the arena screen (LHS). 17:112 pixels in x (width) and 1:48 pixels in y (height). [96 x 48 pixels area]
- Background intensity: (6/15) - $gs_val = 4$.
- Number of repetitions: 4
- Flash sizes: 12 x 12 pixels and 6 x 6 pixels
- Flash contrasts: both ON (15/15) and OFF (0/15)
- Flash duration: 200ms
- Flash overlap: 0% (non-overlapping grid)
- Interval between flashes: 50ms
- Location: protocols/LHS within the nested_RF_stimulus GitHub repository.
- Protocol: `protocol1_10kHz_4reps_12px_6px_LHS_2sbkg_200msfl_50msint_12-13-24_14-33-03`

i Results file:

“Spring2025_RNAi_Protocol2_Analysis”. Data: 2024_12_12 to 2025_01_23. See the file “RNAi_exp_log”.

1.0.2 P1 protocol (April 2025 - May 2025) - TmY3, TmY18, TmY5a, Tm5Y

- Area of arena presented: Right half of the arena screen (LHS). 97:196 pixels in x (width) and 1:48 pixels in y (height). [96 x 48 pixels area]
- Background intensity: (6/15) - $gs_val = 4$.
- Number of repetitions: 4
- Flash sizes: 12 x 12 pixels and 6 x 6 pixels
- Flash contrasts: both ON (15/15) and OFF (0/15)
- Flash duration: 200ms
- Flash overlap: 0% (non-overlapping grid)
- Interval between flashes: 50ms
- Location: protocols/RHS within the nested_RF_stimulus GitHub repository.
- Protocol: `protocol1_10kHz_4reps_12px_6px_RHS_2sbkg_200msfl_50msint_04-08-25_08-08-42` and `protocol1_10kHz_4reps_12px_6px_RHS2_2sbkg_200msfl_50msint_81_180_05-05-25_16-18-66`

i Results file:

“Exp_Log_NestedRF_v2”. Data: 2025_04_08 to 2025_05_09

1.0.3 P1 protocol (July 2025 - Nov 2025) - T4/T5 experiments

- Area of arena presented: Left half of the arena screen (LHS). 17:112 pixels in x (width) and 1:48 pixels in y (height). [96 x 48 pixels area]
- Background intensity: (4/15) - gs_val = 4.
- Number of repetitions: 4
- Flash sizes: 12 x 12 pixels and 6 x 6 pixels
- Flash contrasts: both ON (15/15) and OFF (0/15)
- Flash duration: 200ms
- Flash overlap: 0% (non-overlapping grid)
- Interval between flashes: 150ms
- Location: protocols/bkg4/LHS within the nested_RF_stimulus GitHub repository.
- Protocol: protocol1_10kHz_4reps_12px_6px_LHS_2sbkg_200msfl_150msint_07-10-25_15-45-60

i Results file:

“Summer2025_MARCM_p2_analysis” and “Autumn2025_MARCM_p2_analysis”.
Data: 2025_07_12 to 2025_11_13

1.0.4 P1 protocol (Sep 2025 - Dec 2025) - pharmacology experiments

- Area of arena presented: Left half of the arena screen (LHS). 17:112 pixels in x (width) and 1:48 pixels in y (height). [96 x 48 pixels area]
- Background intensity: (4/15) - gs_val = 4.
- Number of repetitions: 4
- Flash sizes: 6 x 6 pixels
- Flash contrasts: both ON (15/15) and OFF (0/15)
- Flash duration: 200ms
- Flash overlap: 0% (non-overlapping grid)
- Interval between flashes: 150ms
- Location: protocols/bkg4/LHS within the nested_RF_stimulus GitHub repository.

- Protocol: protocol1_10kHz_4reps_6pxONLY_LHS_2sbkg_200msf1_150msint_11-14-25_13-47-30

i Results file:

“Autumn2025_T4T5_Pharma”. Data: 2025_11_18 to 2025_12_22

2 Parameter Overview

2.1 Protocol 1 Parameter Comparison

Side-by-side comparison of all Protocol 1 versions, highlighting the key parameter differences.

Parameter	Early Testing	RNAi (Dec-Jan)	DS (Apr-May)	T4/T5 (Jul-Nov)	Pharmacology (Nov-Dec)
Date range	Nov 27 - Dec 12, 2024	Dec 13, 2024 - Jan 23, 2025	Apr 8 - May 9, 2025	Jul 10 - Nov 2025	Nov 14 - Dec 2025
Screen side	LHS	LHS	RHS	LHS	LHS
Pixel range (x)	17:112	17:112	97:196/81:180	17:112	17:112
Flash sizes	12px + 6px	12px + 6px	12px + 6px	12px + 6px	6px only
Flash duration	200ms	200ms	200ms	200ms	200ms
Inter-flash interval	50ms	50ms	50ms	150ms	150ms
Background intensity	gs_val=4 (6/15)	gs_val=4 (6/15)	gs_val=4 (6/15)	gs_val=4 (4/15)	gs_val=4 (4/15)
Flash contrasts	ON + OFF	ON + OFF	ON + OFF	ON + OFF	ON + OFF
Repetitions	4	4	4	4	4

Parameter	Early Testing	RNAi (Dec-Jan)	DS (Apr-May)	T4/T5 (Jul-Nov)	Pharmacology (Nov-Dec)
Sampling rate	20 kHz	10 kHz	10 kHz	10 kHz	10 kHz

! Key Protocol 1 Changes Over Time

1. **Dec 2024:** Changed from 20kHz to 10kHz sampling rate
2. **Apr 2025:** Switched from LHS to RHS for DS Probe experiments
3. **Jul 2025:** Increased inter-flash interval from 50ms to 150ms; changed background from 6/15 to 4/15
4. **Nov 2025:** Removed 12px flashes for pharmacology experiments (6px only)

3 Descriptions of Protocol 2 Versions

3.0.1 P2 protocol (Dec 2024 - Jan 2025) - RNAi experiments

- Area of arena presented: [30 x 30 pixel area]
- Background intensity: (6/15) - $gs_val = 4$.
- Number of repetitions: 3
- Only ONE contrast - either ON (15/15) or OFF (0/15)
- Square flashes presented on 50% overlapping grid.

Sequence of stimuli in the protocol:

- 4 pixel square flashes - 160ms duration - 340ms interval
- 4 pixel square flashes - 80ms duration - 170ms interval
- 4 pixel wide bar sweeps - 28 dps
- 4 pixel wide bar sweeps - 56 dps

i Results file:

“Spring2025_RNAi_Protocol2_Analysis”. Data: 2024_12_12 to 2025_01_23. See the file “RNAi_exp_log”.

3.0.2 P2 protocol (April 2025 - May 2025) - TmY3, TmY18, TmY5a, Tm5Y

- Area of arena presented: [30 x 30 pixel area]
- Background intensity: (6/15) - gs_val = 4.
- Number of repetitions: 3
- Only ONE contrast - either ON (15/15) or OFF (0/15)
- Square flashes presented on 50% overlapping grid.

Sequence of stimuli in the protocol:

- 10s background at the start and between repetitions (added 2025_05_06 onwards)
- 4 pixel square flashes - 160ms duration - 340ms interval
- 4 pixel square flashes - 80ms duration - 170ms interval
- 4 pixel wide bar sweeps - 28 dps
- 4 pixel wide bar sweeps - 56 dps

i Results file:

“Exp_Log_NestedRF_v2”. Data: 2025_04_08 to 2025_05_09

3.0.3 P2 protocol (July 2025 - Sep 2025) - T4/T5 experiments - SUMMER

- Area of arena presented: [30 x 30 pixel area] The 6 x 6 pixel flashes are presented in a 33 x 33 pixel square area - larger than the 30 x 30 pixel square area for the 4 x 4 pixel flashes - to get an even number of flashes on a 50% overlapping grid.
- Background intensity: (4/15) - gs_val = 4.
- Number of repetitions: 3
- Only ONE contrast - either ON (15/15) or OFF (0/15)
- Square flashes presented on 50% overlapping grid.
- 1s interval between each bar sweep

Sequence of stimuli in the protocol:

- 10s background at the start and between repetitions
- 4 pixel square flashes - 160ms duration - 440ms interval
- 6 pixel square flashes - 160ms duration - 440ms interval
- 4 pixel wide bar sweeps - 28 dps
- 4 pixel wide bar sweeps - 56 dps

i Results file:

“Summer2025_MARCM_p2_analysis”. Data: 2025_07_12 to 2025_09_12

3.0.4 P2 protocol (Oct 2025 - Nov 2025) - T4/T5 experiments - AUTUMN

- Area of arena presented: [30 x 30 pixel area] The 6 x 6 pixel flashes are presented in a 33 x 33 pixel square area - larger than the 30 x 30 pixel square area for the 4 x 4 pixel flashes - to get an even number of flashes on a 50% overlapping grid.
- Background intensity: (4/15) - $gs_val = 4$.
- Number of repetitions: 3
- Only ONE contrast - either ON (15/15) or OFF (0/15)
- Square flashes presented on 50% overlapping grid.
- 1s interval between each bar sweep

Sequence of stimuli in the protocol:

- 10s background at the start and between repetitions
- 3s background interval between square flashes and sweeps, between sweep speeds and between bar flash speeds. (Added 2025_10_21).
- 4 pixel square flashes - 160ms duration - 440ms interval
- 6 pixel square flashes - 160ms duration - 440ms interval
- 4 pixel wide bar sweeps - 28 dps
- 4 pixel wide bar sweeps - 56 dps
- **4 pixel wide bar sweeps - 168 dps**
- **4 pixel wide bar flashes - 80ms duration - 920ms interval**
- **4 pixel wide bar flashes - 14ms duration - 486ms interval**

The bar flashes are presented in different random orders for each repetition. The bar flash stimuli contains 11 different positions. From 2025_10_15 - 2025_10_23, the “central” bar flash is positioned in the middle of the 30 x 30 pixel area, then the 5 positions either side are this bar moved by 1 pixel increments and decrements from this position in the 8 different orientations. From 2024_10_23 onwards, these 5 other positions are the central bar moved by 2 pixel increments and decrements.

i Results file:

“Autumn2025_MARCM_p2_analysis”. Data: 2025_10_15 to 2025_11_17

3.0.5 P2 protocol (Nov 2025 - Dec 2025) - T4/T5 pharmacology experiments

- Area of arena presented: [30 x 30 pixel area]
- Background intensity: (4/15) - gs_val = 4.
- Number of repetitions: 3
- Only ONE contrast - either ON (15/15) or OFF (0/15)
- 1s interval between each bar sweep
- NO SQUARE PIXEL FLASHES PRESENTED IN P2.

Sequence of stimuli in the protocol:

- 10s background at the start and between repetitions
- 3s background interval between sweep speeds and between bar flash speeds.
- 4 pixel wide bar sweeps - 28 dps
- 4 pixel wide bar sweeps - 56 dps
- 4 pixel wide bar sweeps - 168 dps
- **4 pixel wide bar sweeps - 250 dps**
- **4 pixel wide bar sweeps - 500 dps**
- 4 pixel wide bar flashes - 80ms duration - 920ms interval
- 4 pixel wide bar flashes - 14ms duration - 486ms interval

There are 88 bar flash stimuli. 8 different orientations. Bar centred on the 30 x 30 pixel square and then moved by 2 pixel steps in either direction along this axis.

i Results file:

“Autumn2025_T4T5_Pharma”. Data: 2025_11_18 to 2025_12_22

3.1 Protocol 2 Parameter Comparison

Side-by-side comparison of all Protocol 2 versions.

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacology
Date range	Dec 12, 2024 - Jan 23, 2025	Apr 8 - May 9, 2025	Jul 12 - Sep 12, 2025	Oct 15 - Nov 17, 2025	Nov 18 - Dec 22, 2025
Back- ground intensity	6	6	4	4	4

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacology
Repetitions	3	3	3	3	3
Stimulus area	30x30 px	30x30 px	30x30 px (33x33 for 6px)	30x30 px (33x33 for 6px)	30x30 px
Contrast	ON or OFF	ON or OFF	ON or OFF	ON or OFF	ON or OFF

3.2 Square Flash Parameters

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacology
4px flashes	Yes	Yes	Yes	Yes	No
4px duration	160ms, 80ms	160ms, 80ms	160ms	160ms	-
4px interval	340ms, 170ms	340ms, 170ms	440ms	440ms	-
6px flashes	No	No	Yes	Yes	No
6px duration	-	-	160ms	160ms	-
6px interval	-	-	440ms	440ms	-

3.3 Bar Sweep Parameters

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacology
14 dps	No	No	No	No	No
28 dps	Yes	Yes	Yes	Yes	Yes
56 dps	Yes	Yes	Yes	Yes	Yes
168 dps	No	No	No	Yes	Yes
250 dps	No	No	No	No	Yes
500 dps	No	No	No	No	Yes
Inter-sweep interval	-	-	1s	1s	1s

3.4 Bar Flash Parameters

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacology
80ms bar flashes	-	-	-	Yes (920ms interval)	Yes (920ms interval)
14ms bar flashes	-	-	-	Yes (486ms interval)	Yes (486ms interval)
Number of positions	-	-	-	11	11
Position spacing	-	-	-	1px (Oct 15-23), 2px (Oct 23+)	2px

3.5 Timing Parameters

Parameter	RNAi	DS Probe	T4/T5 Summer	T4/T5 Autumn	Pharmacol- ogy
10s background at start	No	Yes (from May 6)	Yes	Yes	Yes
10s background between reps	No	Yes (from May 6)	Yes	Yes	Yes
3s background between stimuli	No	No	No	Yes (from Oct 21)	Yes

! Key Protocol 2 Changes Over Time

1. **May 6th 2025:** Added 10s background intervals between repetitions and at the start of the protocol.
2. **July 2025:** Changed the background intensity from 6/15 to 4/15.
3. **Oct 2025:** Added 168 dps sweeps and bar flashes (80ms and 14ms) to the protocol.
4. **Oct 2025:** Introduced a 3s background interval between stimulus blocks for easier data parsing.
5. **Oct 23rd 2025:** Changed bar flash position spacing from 1px to 2px increments.
6. **Nov 2025:** Removed square flashes for pharmacology experiments, focusing solely on bar sweeps and flashes. Added 2 faster speeds.

4 Timeline Overview

The following table provides a chronological overview of all protocol versions used, linking dates to experiment types and data locations.

4.1 Protocol 1 Timeline

Date Range	Experiment Type	Key Changes	Data Location
Nov 27 - Dec 12, 2024	Early testing	Initial version, 20kHz	protocol1/LHS/protocol1_200msf1_50msint_11-27-24_15-02-95/
Dec 13, 2024 - Jan 23, 2025	RNAi experiments	10kHz sampling, 50ms interval, LHS	protocol1/LHS/protocol1_200msf1_50msint_12-13-24_14-33-03/
Apr 8 - May 4, 2025	DS Probe (TmY3, TmY18, TmY5a, Tm5Y)	Switched to RHS	protocol1/RHS/protocol1_200msf1_50msint_04-08-25_08-08-42/
May 5 - May 9, 2025	DS Probe (continued)	RHS v2 (pixels 81-180) shifted to more central part of arena	protocol1/RHS/protocol1_200msf1_50msint_81_180_05-25_16-18-66/
Jul 10 - Nov 2025	T4/T5 MARCM	150ms interval, bkg4	protocol1/bkg4/protocol1_200msf1_150msint_07-10-25_15-45-60/
Nov 14 - Dec 2025	Pharmacology	6px flashes only	protocol1/bkg4/protocol1_200msf1_150msint_11-14-25_13-47-30/

4.2 Protocol 2 Timeline

Date Range	Experiment Type	Key Changes	Data Location
Dec 12, 2024 - Jan 23, 2025	RNAi experiments	160ms/80ms flashes, 28/56 dps sweeps	protocol2/data/RNAi_ttl_2025/
Apr 8 - May 5, 2025	DS Probe	Same as RNAi version	protocol2/data/DS_v2/
May 6 - May 9, 2025	DS Probe	Added 10s background between reps	protocol2/data/DS_v2/
Jul 12 - Sep 12, 2025	MARCM summer	Added 6px flashes, 440ms intervals	protocol2/data/T4T5_Summer2025/
Oct 15 - Nov 17, 2025	MARCM autumn	Added 168 dps sweeps, bar flashes	protocol2/data/T4T5_autumn2025/
Nov 18 - Dec 22, 2025	Pharmacology	No square flashes, 5 sweep speeds	protocol2/data/Octopamine/, protocol2/data/CGP54626/

i How to verify the version if unsure

For definitive confirmation, check the `metadata.mat` or `currentExp.mat` files within the experiment folder, which contain the exact parameters used.