Supplementary Material

Primary visual cortex represents the difference between past and present.

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Contents of Supplementary Information

1. Supplementary Figures

Figure S1: Time course of difference representation.
This Figure shows the correlation between population orientation tuning over time and a standard VH-orientation map. The purpose of this Figure is to also show the time course of the main effect beyond the window used for analysis throughout the paper (50-90 ms after switch). For the single switch paradigm correlation over time is shown in Fig. 6.

Figure S2: Stimulus-triggered versus switch-triggered averages.
A. Illustration of the stimulus paradigm and the averaging procedure for analysis. B. The purpose of this Figure is to show how the differences in switch-triggered responses that we find for the different stimulus durations (30 ms vs. 100 ms) lead to differences in stimulus-triggered responses.

Figure S3: Switches to blank, sustained orientation, and from superposition to a single orientation.
The purpose of this Figure is to show responses to a switch from a single orientation to blank, as a supplement to the data provided in Figure 4 of the main text.

2. Supplementary Tables

Table S1: Statistical evaluation of population tuning, step 1.
Table S2: Statistical evaluation of population tuning, step 2.
Table S3: Statistical evaluation of population tuning, step 3, orientation-tuning amplitudes.
The purpose of these three tables is to give a comprehensive report of the statistical results.

Table S4: Response decomposition (results): contributions of adaptive and off-components to change responses.
The purpose of this table is to show the results from the comparison of responses to constituent stimuli (off-switch of one orientation and sustained presentation of the orthogonal orientation) with the response to their composite stimulus (switch from superimposed orientations to a single orientation).

3. Supplementary Methods

Response decomposition (methods): evaluation and fitting procedure

Here we describe the method that was used to compute the results in Table S4, which are referenced in the Discussion section of the main text. The response to the switch from superposition to single orientations is approximated using responses to the two constituent stimuli.

1. Supplementary Figures

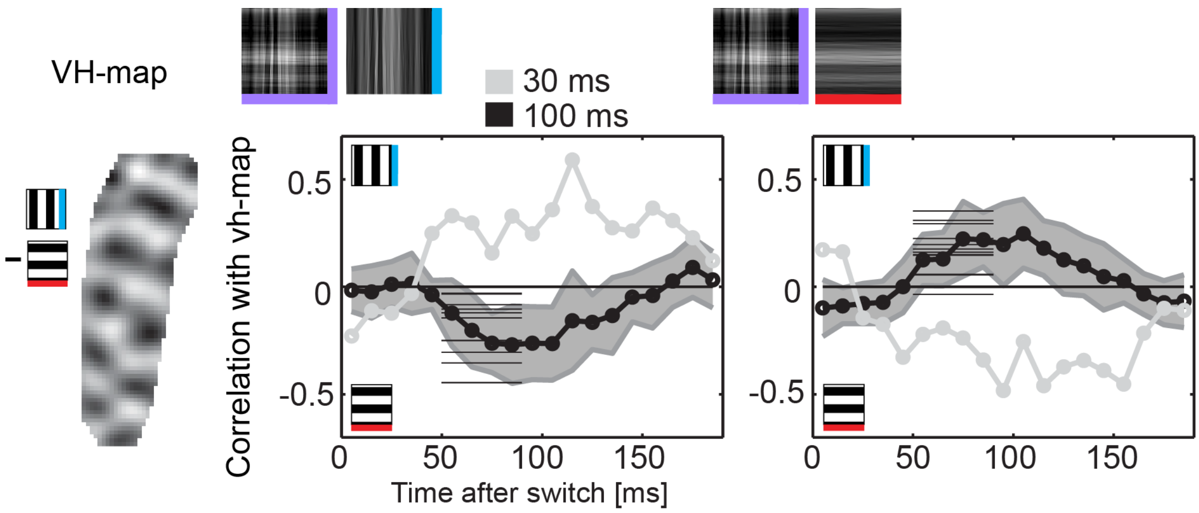


Figure S1: **Time course of difference representation.** Correlations between responses to a switch from superposition to single orientation (measured within sequences of narrowly filtered natural images) and VH-map (independently measured differential horizontal-vertical activity pattern, see Methods) over time. Positive values indicate similarity with orientation pattern evoked by a vertical grating (corresponding to white regions in VH-map), and negative correlation indicates similarity with the horizontal pattern (dark regions in VH-map). When presented within 30-ms time sequences, increase in correlation showed tuning for the orientation that was actually present (thin grey traces; average correlation from 50-90 ms after switch, 1 experiment: 0.28 for switch to vertical and -0.25 for switch to horizontal). For 100-ms stimulus time sequences (black traces, grey shaded area mark standard deviation across 12 different experiments; black lines show average values from 50-90 ms for the individual experiments), correlation values reversed, indicating tuning for the orientation that had disappeared (time window 50-90 ms: -0.21±0.15 (*std*), left tailed t-test against zero $p<0.001$ for switch to vertical; 0.17±0.11 (*std*), right tailed t-test $p<0.001$ for switch to horizontal).

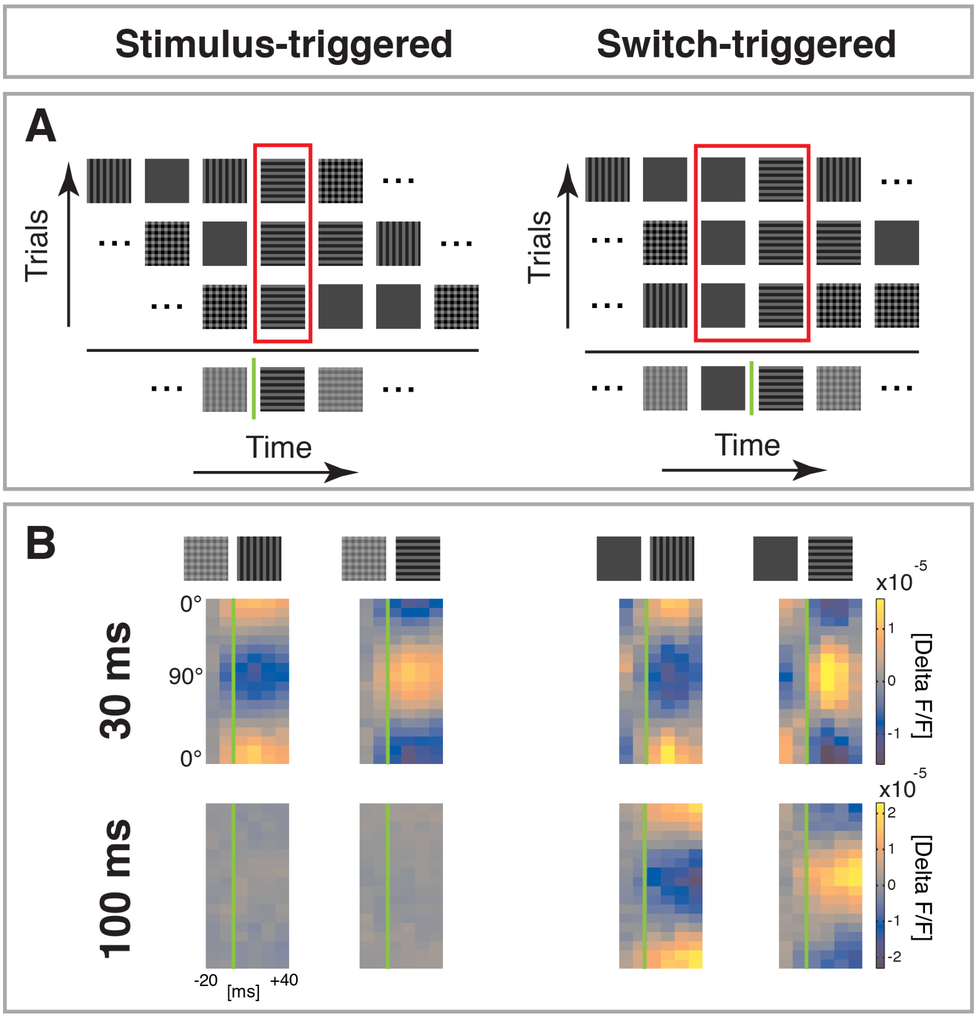


Figure S2: Stimulus-triggered versus switch-triggered averages. A: Illustration of averaging procedures. Exemplified are three different stimulus sequences (5 stimuli from 17-stimulus-long sequences are shown; in the experiments we used at least 64 different sequences of at least 17 images, see Methods). On the left, sequences are aligned at a specific stimulus (horizontal in this example, red box) and averaged. Note that the different stimuli before and after are averaged. For *stimulus-triggered averaging*, this procedure is used to average the cortical responses to a particular single stimulus across all sequences. On the right, sequences are aligned at switches between specific pairs of stimuli (blank to horizontal in this example) and averaged. In this case the pair is fixed, while different stimuli before and after are averaged. For *switch-triggered averaging*, this procedure is used to average cortical responses to a particular stimulus pair (i.e. switch) across all sequences.
B: Stimulus-triggered versus switch-triggered responses. The left side shows average population responses triggered by either the vertical or horizontal stimulus. Here we averaged over all preceding stimuli (see **A**). This is usually seen as the pure response to the single orientation stimulus. When measured within 30-ms sequences, there is clear population tuning to the currently resented stimulus. However, when measured within 100-ms sequences, the stimulus-triggered response is flat, indicating that the different underlying switch-triggered responses (change responses), comprising switches in which the orientation was removed as well as switches in which is was added, average each other out. The right side shows example switch-triggered responses for switches from blank to a single orientation. Here, we find orientation tuning for both sequence speeds. Altogether this illustrates that at 30-ms presentation duration, population-tuning responses are evoked by individual stimuli in a sequence, while at 100-ms presentation duration responses are highly dependent on specific pairs of stimuli. The window shows 30-90 ms after onset of the oriented stimulus, the green line is at 50 ms (the approximate response latency).

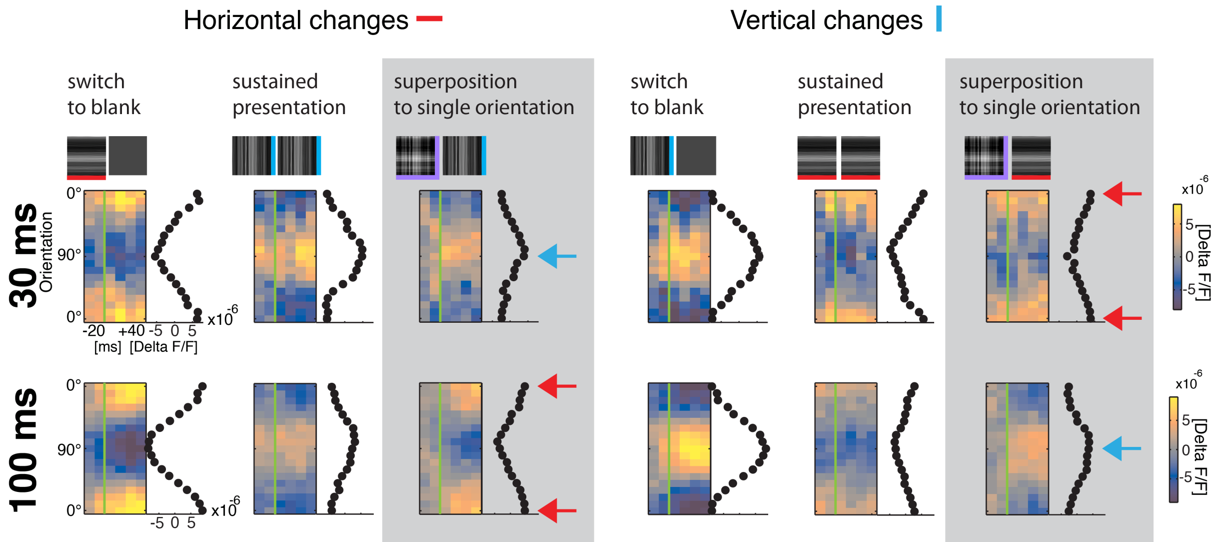


Figure S3: Switches to blank, sustained orientation, and from superposition to a single orientation. We find that population responses were faithfully tuned to both turned-off and sustained orientations (first and second column, respectively) after both 30 ms (first row) and 100 ms (second row) presentation duration. When one orientation was turned-off from superimposed orientations (grey columns), the direction of population tuning was dependent on stimulus timing: Whereas in fast sequences the actual (i.e. the sustained) orientation after the switch was represented, slower sequences resulted in a representation of stimulus difference (i.e. the removed orientation). Conventions as in Fig. 4, main text.

2. Supplementary Tables

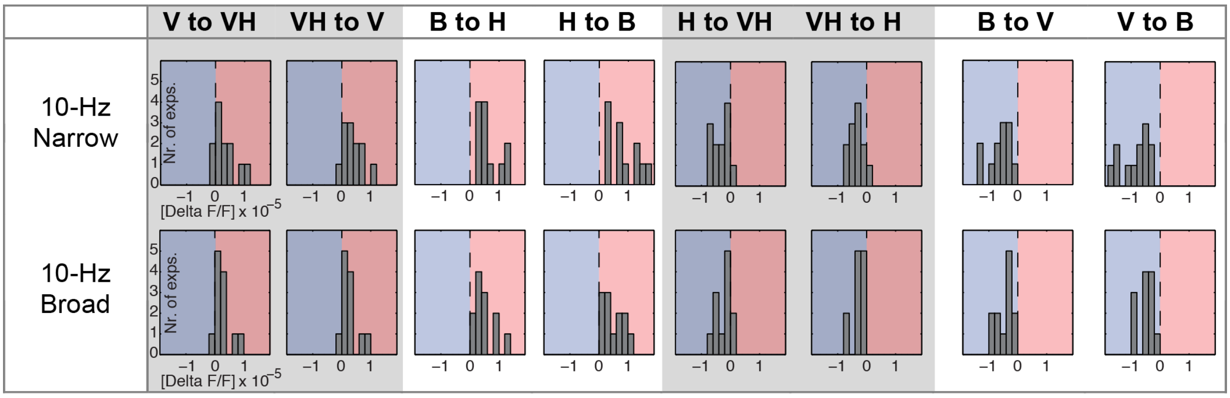
	V to VH	VH to V	B to H	H to B	H to VH	VH to H	B to V	V to B
33-Hz Gratings	56***	339***	350***	727***	8 (p=0.97)	123***	471***	618***
33-Hz Narrow	6 (p>0.99)	37**	34**	114***	5 (p>0.99)	33*	47***	151***
10-Hz Narrow	563***	671***	1180***	2144***	422***	443***	1463***	2663***
10-Hz Broad	388***	370***	704***	810***	324***	306***	728***	1051***
10-Hz Gratings	280***	184***	372***	450***	149***	179***	276***	518***

Table S1: Statistical evaluation of population tuning, step 1. In the first step, we evaluated the difference to zero (or flat response). Shown are the X^2-statistic and p-values (*** = p<0.001, ** = p<0.01, * = p<0.02). For the 33-Hz data, we find significant differences to zero for switches with a single orientation present after the switch (columns 2, 3, 6, and 7) and off-switches from a single orientation to the grey screen (columns 4 and 8). For the transition from a single orientation to the superposition, we find no significant deviation (columns 1 and 5), as is expected from a representation of both orientations present after the switch, with the exception of one switch (V to VH, column 1, row 1). For the 10-Hz data, the population tuning curves deviate significantly from zero in all cases (lower three rows).

	V to VH	VH to V	B to H	H to B	H to VH	VH to H	B to V	V to B
33-Hz Gratings	4 (p>0.99)	5 (p>0.99)	5 (p>0.99)	12 (p=0.73)	3 (p>0.99)	10 (p=0.87)	6 (p=0.98)	19 (p=0.26)
33-Hz Narrow	3 (p>0.99)	6 (p=0.99)	4 (p>0.99)	7 (p=0.97)	5 (p>0.99)	10 (p=0.88)	5 (p>0.99)	5 (p>0.99)
10-Hz Narrow	101 (p>0.99)	139 (p>0.99)	119 (p>0.99)	143 (p>0.99)	52 (p>0.99)	126 (p>0.99)	105 (p>0.99)	120 (p>0.99)
10-Hz Broad	76 (p>0.99)	116 (p>0.99)	97 (p>0.99)	86 (p>0.99)	96 (p>0.99)	107 (p>0.99)	66 (p>0.99)	85 (p>0.99)
10-Hz Gratings	7 (p=0.96)	13 (p=0.69)	9 (p=0.93)	21 (p=0.16)	13 (p=0.65)	3 (p>0.99)	33 (p<0.01)	14 (p=0.62)

Table S2: Statistical evaluation of population tuning, step 2. In the second step, we fit sinusoids to the population tuning curves of each experiment and repeat the test against zero on the residuals. Shown are X^2-statistics and p-values. Now there are no significant differences, except in one case (10-Hz-Gratings B to V). In all cases the X^2-values decreased substantially, thus a large part of the variance in the data can be explained by the fitted function.

	V to VH	VH to V	B to H	H to B	H to VH	VH to H	B to V	V to B
33-Hz Gratings	-0.33	-0.88	0.89	1.32	-0.10	0.53	-1.02	-1.21
33-Hz Narrow	-0.08	-0.28	0.27	0.53	0.01	0.26	-0.33	-0.62
10-Hz Gratings	1.29	0.99	1.46	1.62	-0.90	-1.05	-1.19	-1.79
10-Hz Narrow	0.30 (0.21*)	0.39 (0.34**)	0.61 (0.45***)	0.84 (0.77***)	-0.32 (-0.32**)	-0.34 (-0.32**)	-0.62 (-0.56***)	-0.84 (-0.67***)
10-Hz Broad	0.25 (0.20**)	0.30 (0.22**)	0.48 (0.43***)	0.49 (0.35***)	-0.24 (-0.14*)	-0.31 (-0.25***)	-0.45 (-0.37***)	-0.52 (-0.49***)



All values: x10^{-5} ΔF/F; Sign test: ***p<0.001, **p<0.01, *p<0.05

Table S3: Statistical evaluation of population tuning, step 3, orientation-tuning amplitudes.

Black values indicate switches where population-tuning curves did not significantly deviate from zero. Amplitudes for switches were population-tuning curves deviated significantly are indicated in either red or blue, depending on the sign of the fitted functions. Positive values indicate horizontal tuning and are indicated in **red**; negative values indicate vertical tuning and are indicated in **blue**. Values were fitted for each experiment individually. For the 33-Hz data and the 10-Hz grating data, the fitted values are shown directly (n = 1 in both cases). For the 10-Hz data (derived from narrowly and broadly filtered natural images), averages and medians (in brackets) over experiments (n = 12) are shown, asterisks indicate results from a sign test against zero over n = 12 values. Histograms detail the individual values for the 12 experiments (blue side negative, red side positive amplitudes), corresponding to the last two rows of the above table.

33-Hz data: Amplitudes are negative (indicating vertical tuning) when vertical orientation is presented after the switch (columns 2 and 7) and positive (indicating horizontal tuning) when the orthogonal (horizontal) orientation is presented after the switch (columns 3 and 6). We confirm persistent tuning to previous orientation after a switch from a single orientation to blank for horizontal (column 4) and vertical orientation (column 8). When we examine the case where transition to the superposition led to significant differences from zero before the fitting, we find that the population tuning represents the vertical orientation presented before the switch and not the horizontal orientation that was turned on (column 1, row 1), most likely reflecting a delayed response during transition.

10-Hz data: Responses were tuned to a single orientation switched on from blank for both horizontal (column 3) and vertical orientation (column 7). Corresponding to the 33-Hz data, we find a persistent response to the previous orientation when switched-off to blank (off-component), for both horizontal (column 4) and vertical orientation (column 8). For switches from a single orientation to the superposition, positive amplitudes indicate horizontal population tuning when the horizontal orientation was added (column 1), and negative amplitudes indicate vertical population tuning when the vertical orientation was added (column 5). For switches from the superposition to a single orientation, amplitudes indicate population tuning to the removed orientation when horizontal is turned-off (column 2) and when vertical is turned off (column 6). In general, we find that the sign of the fitted amplitude is consistent with population tuning to the orientation present after the switch for the 33-Hz data and representative of the difference between two successive stimuli for the 10-Hz data.

#	Description	Adaptive comp.	Off-comp.	df	X^2	X^2/X^2 of Baseline	p-value
0	Baseline	0	0	863	1789	100%	<0.0001
1	Adaptive comp. alone	1	0	863	5101	285%	<0.0001
2	Off comp. alone	0	1	863	3283	183%	<0.0001
3	50-50-Average	0.5	0.5	863	1077	60%	<0.0001
4	Weighted Average	0.40± 0.03	0.60	839	909	51%	0.047

Table S4: Response decomposition (results): contribution of adaptive and off-component to change response.

Results for 10-Hz sequences, 12 experiments. Columns describe the models, which are characterized by the respective weights given to adaptive component (i.e. response to sustained orientation, third column) and off-component (response to orientation that is turned-off, fourth column). For model #4, mean and *sem* across fitted weights for experiments and both filter conditions (broad and narrow) is given. The fifth column shows degrees of freedom, the sixth column the $\boldsymbol{X}^2$-statistic (see Methods). The seventh column shows the ratio of $\boldsymbol{X}^2$-values between the respective model and the baseline. The eighth column shows the p-value for data-model comparison.

Neither of the constituent responses alone (#1 and #2) can explain the data better than the baseline model (X^2-values in #1 and #2 are higher than in #0), albeit the off-component alone is closer to the measured data than the adaptive component alone (X^2-values in #2 are smaller than in #1). The 50-50-average of both components (#3) provides a better fit than either of the components alone (#1 and #2). When constituent responses are combined as weighted average, we obtain significantly higher weights for the off-component (than for the adaptive component (see #4; two-tailed t-test against 0.5, $p<0.01$). The prediction resulting from such a combination reduces the X^2-values by 49%.

3. Supplementary Methods

Response decomposition (methods): evaluation and fitting procedure

To investigate the response to the switch from the superposition to a single orientation (VH to V/H) at 10 Hz in more detail, we approximated response to this complex switch through the combination of the responses to its constituents, a sustained presentation of a single orientation (V to V or H to H), and the off-switch of the other orientation (H to blank or V to blank). The data used in our models are time-averaged (50–90 ms after switch) population tuning curves.

To statistically evaluate the goodness of the fit, we first tested the response from the complex switch (VH to H/V) against a flat response at zero to obtain a baseline. In the next step, we repeated the test on the residuals between the predictions of models 1 to 4 and the data.

As test statistics, we computed one X^2-value across all experiments (e = 1,…,n; where n is the number of experiments), stimulus types (f = narrowly filter, broadly filtered naturals), orientation bins (θ = 1,…,18; bins of 10° each), and types of switches (c = 1,2; corresponding to VH to V and VH to H), taking the standard error over repetitions of a switch type (repetitions $r_e = 1,\ldots,m_e$; where r_e is a repetition in experiment e, and m_e is the overall number of repetitions in experiment e), as a normalization factor:

$$\mathrm{X}^2 = \sum_e \sum_f \sum_\theta \sum_c \left(\frac{a\langle u_{e,f,\theta,c,r_e} \rangle_{r_e} + b\langle v_{e,f,\theta,c,r_e} \rangle_{r_e} - \langle d_{e,f,\theta,c,r_e} \rangle_{r_e}}{\frac{1}{\sqrt{m_e}}\sigma} \right)^2$$

$$\sigma = \sqrt{\frac{1}{m_e - 1} \sum_{r_e} \left(d_{e,f,\theta,c,r_e} - \langle d_{e,f,\theta,c,r_e} \rangle_{r_e} \right)^2}$$

Here, $\langle \quad \rangle_{r_e}$ denotes the average response over repetitions in experiment e, and d_{e,f,θ,c,r_e} is the response that we wanted to model in experiment e, filter condition f, orientation bin θ, switch type c, and repetition r_e. Respectively, $\langle u_{e,f,\theta,c,r_e} \rangle_{r_e}$ is the average of responses to the sustained orientation, and $\langle v_{e,f,\theta,c,r_e} \rangle_{r_e}$ is the average of off-response over all repetitions in experiment e, in filter condition f, orientation bin θ, for switch type c.

We investigated three different models and the baseline response (model 0). The models differ in their weights for the sustained response, denoted with a, and the weight for the off-response, denoted with b. For the baseline (test against constant zero), m_0: $a = b = 0$. In model 1 (only sustained response), m_1: $a = 1$; $b = 0$, in model 2 (only off-response), m_2: $a = 0$; $b = 1$, in model 3 (50-50-average) m_3: $a = 0.5$; $b = 0.5$. In model 4 parameters were fitted to the data using the constraint $a + b = 1$. We fitted a_{ef} for each experiment e and filter condition f by minimizing all ε_{ef}:

$$\varepsilon_{ef} \coloneqq \sqrt{\sum_\theta \sum_c \sum_{r_e} \left(a_{ef}\langle u_{e,f,\theta,c,r_e} \rangle_{r_e} + (1 - a_{ef})\langle v_{e,f,\theta,c,r_e} \rangle_{r_e} - d_{e,f,\theta,c,r_e} \right)^2}$$

This way, one set of weights was fitted to each experiment and filter condition, with data-model comparison at 864 points: 12 experiments x 2 filter conditions x 18 bins of orientation preference x 2 versions of the switch. The models have different degrees of freedom, depending on the number of fitted parameters: $df_{nr} = 864 - 1 - p_{nr}$, with fitted parameters (p_{rn}): $p_0 = p_1 = p_2 = 0$, $p_3 = 24$.