Note

Surround modulation of neuronal responses in V1 is as stable over time as responses to direct stimulation of receptive fields

Sergiu P. Paşca [a,b], *Wolf Singer* [a,c] *and Danko Nikolić* [a,c,*]

[a] *Department of Neurophysiology, Max Planck Institute for Brain Research, Frankfurt (Main), Germany*
[b] *Center for Cognitive and Neural Studies (Coneural), Cluj-Napoca, Romania*
[c] *Frankfurt Institute for Advanced Studies (FIAS), Johann Wolfgang Goethe University, Frankfurt (Main), Germany*

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ABSTRACT

In the primary visual cortex (V1) the modulation of neuronal responses by surround stimuli displays considerable variability. At present, it is not known whether this variability across neurons is due to temporal instability or to neuron-specific differences. We explored this question in the cat visual cortex by making multi-channel recordings while repeatedly presenting surround gratings of collinear and orthogonal orientation to the centre stimulus for a period of 96 h. Our results indicate that surround modulation is temporally stable to about the same degree as the responses evoked by the centre stimuli. The results support the notion that the mechanisms of surround modulation exhibit a high degree of stability and play an important role in the modulation of cortical responses.

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1. Introduction

In the primary visual cortex (V1), responses evoked by stimuli presented in the (classical) receptive fields (RFs) of neurons (Hubel and Wiesel, 1962) are modulated by additional stimulation of surrounding areas (Fitzpatrick, 2000; Gilbert, 1998; Series et al., 2003). The strength and polarity of surround modulation depend on stimulus properties (i.e., size, contrast level, relative orientation) and in addition, exhibit considerable neuron-to-neuron variability and less spatial regularity than the responses to RF stimuli (Levitt and Lund, 1997; Sengpiel et al., 1997; Series et al., 2003). The properties of the classical RFs (i.e., location, size, preferred orientation) are rather stable over time (Crist et al., 2001; Gilbert, 1998), but little is known about the temporal stability of surround effects. It is not known whether the high variability of surround effects can be explained to a certain degree by temporal fluctuations of neuronal responses or whether these responses are temporally stable and reflect exclusively neuron-specific variability. This distinction is important for functional interpretations of surround effects because temporal stability is a prerequisite for their contribution to context dependent phenomena such as perceptual pop-out, contour integration and competition (Kastner et al., 1997; Li, 1999; Nothdurft et al., 1990).

This question can be addressed only with highly parallel multi-channel recordings because long-term tracking of

sequentially recorded individual units would sum up to a prohibitively long period of time. Hence, we addressed the question of temporal stability by recording in parallel from a cluster of neurons with overlapping RFs composed of 17 multi-unit (MU) signals in cat area 17 recorded by Michigan probes. The same sets of stimuli were presented repeatedly over a time span of 96 h. The centre stimulus consisted of a 7° wide disc containing a drifting sinusoidal grating that covered the entire cluster of RFs and was oriented such as to activate

a maximum number of neurons (Fig. 1A). This has as consequence that the centre stimulus was larger than each individual RF and hence, already when presented alone, straddled a narrow strip of the immediate surround, inducing a certain degree of modulation itself. The average distance between the border of individual classical RFs and the border of the centre stimulus was ∼2°. This design generated a confound between centre and surround effects but allowed for the required parallel recording of large numbers of neurons. Because
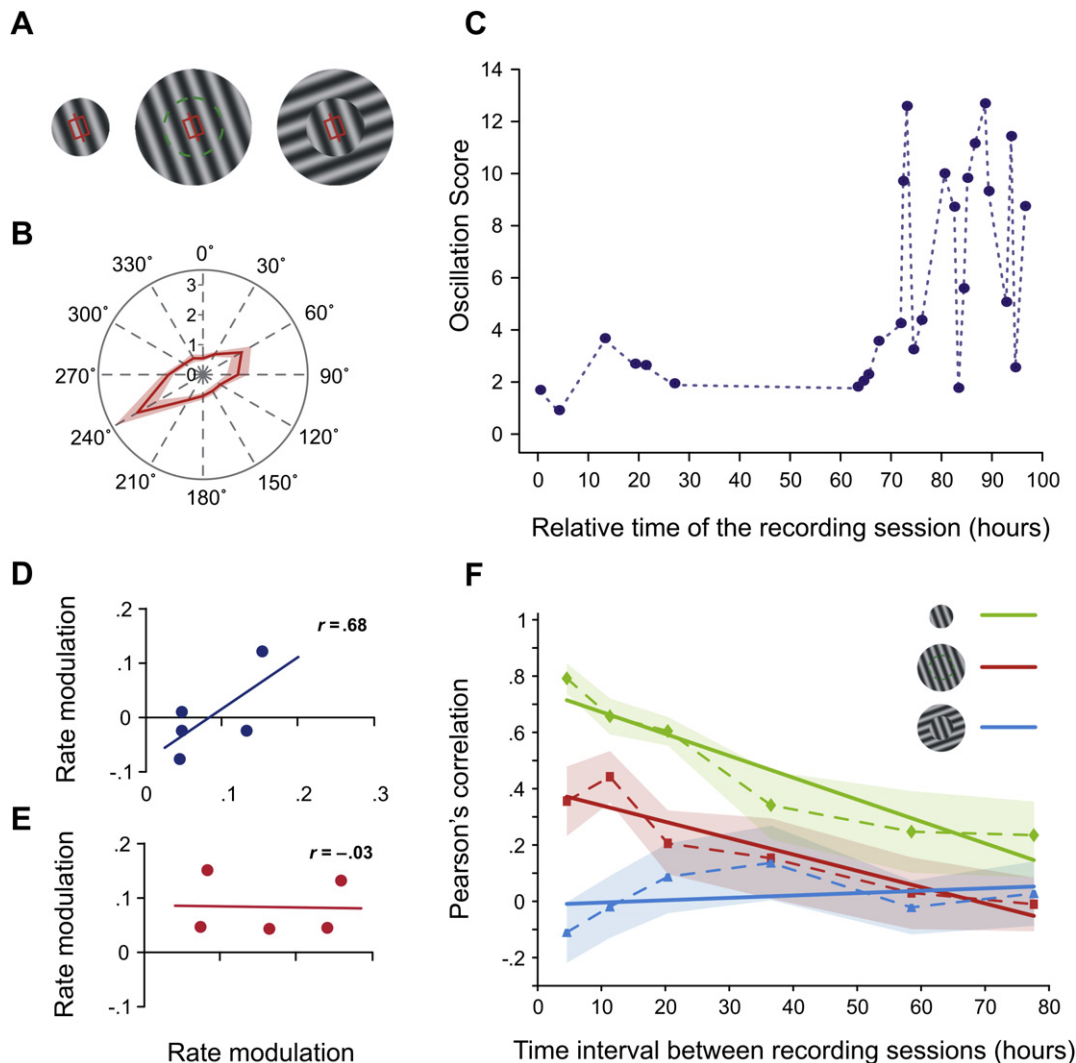

Fig. 1 – A, Visual stimuli. The presented visual stimuli were drifting sinusoidal gratings of circular shape. Left: only centre stimulus is presented. Middle: centre with a collinear surround. Right: centre with an orthogonal surround. The centre stimulus was oriented to match the orientation preference of the RF (schematized by an example RF shown in red). Not drawn to scale. B, Stability of direction selectivity. Temporal stability of responses to 12 grating directions is presented for a representative unit. Thick line: the average direction tuning curve computed across 23 measurements (in total, ∼96 h). The radial axis represents the strength of response normalized to the average across all 12 grating directions. Flanks: variability across measurements indicated as ±1 SD. C, Spontaneous changes in the oscillatory patterning of neuronal responses estimated by calculating Oscillation Score for each recording (see Methods). D and E, Representative examples of pairs of recordings for 5 units, one with short time difference and high correlation in modulation of rate responses to collinear surround (9.98 h, D) and one with long time difference and low such correlation (65.08 h, E). F, Stability of neuronal responses over time. Averaged Pearson's correlation coefficients shown as a function of time elapsed between a total of 115 pairs of recording sessions (up to ∼96 h) and computed as averages for all 17 MUs for rate responses to centre (green) and modulation of these responses by collinear (red) and orthogonal surround stimuli (blue). Dashed line: the group mean. Colored transparent surfaces: ±1 SEM. Solid line: best linear fit.

recording conditions were sable and the centre stimulus was kept constant throughout the recording period this confound can be considered as stable. Modulation effects were then induced by additional stimuli consisting of large circular gratings surrounding the centre stimuli and spanning in total 13.9° of visual angle (i.e., they extended the stimulated area for 3.45° on each side of the centre grating). Therefore, the study investigated modulation effects from distant rather than immediate surrounds of the RFs.

2. Methods

2.1. Preparation

In one adult cat anesthesia was induced with a combination of Ketamine (10 mg kg^{-1}, intramuscular) and maintained with a mixture of 70% N$_2$O and 30% O$_2$ supplemented with halothane (.5–1.0%). The animal was paralyzed by intravenous injection of Pancuronium bromide (.15 mg kg^{-1} h^{-1}). The experiment was conducted according to the guidelines of the Society for Neuroscience and the German law for the protection of animals, approved by the local government's ethical committee and overseen by a veterinarian. Further details of surgical and recording procedures are available in Nikolić (2007), Schneider et al. (2006), Schneider and Nikolić (2006).

2.2. Recordings

We recorded MU activity using 2 silicon-based 16-channel Michigan probes. The probes were inserted into area 17 of the left cortical hemisphere. The electrode contacts had an impedance of .3–.5 MΩ at 1000 Hz, and were organized in a 4 × 4 matrix on four shanks, with a distance of .2 mm separating the centers of the neighboring contacts. The probes were inserted perpendicularly to the surface of the cortex. For this study, we selected recording sites with vigorous and orientation selective MU responses (n = 17). Signals were amplified first 1000×, then band pass filtered at 500 Hz to 3.5 kHz, and sampled with a frequency of 32 kHz. Action potentials (spikes) were detected using a two-sided threshold discriminator. The probes were kept at the same position in the cortex for the entire duration of the experiment. The rate responses of MU signals to optimal centre stimuli were on average ~16 spikes/sec. We also isolated single-unit (SU) activity by an offline spike-sorting method but could not track a sufficient number of SUs for a sufficiently long period of time to allow for reliable analysis. Therefore, we needed to limit most of the analysis on MU activity.

2.3. Visual stimulation

Visual stimuli were presented binocularly on a 21-inch CRT monitor positioned at a distance of 57 cm from the eyes using ActiveSTIM software (www.ActiveSTIM.com). The pupils were dilated with atropine, and the nictitating membrane retracted with phenylephrine (Neo-Synephrine®). After refraction, the eyes were focused on the monitor by mounting correcting contact lenses. The optical axes of the eyes were aligned by positioning a prism in front of one eye. To confirm the stability of the optical axes, the positions of RFs were checked daily. The RFs of all signals recorded within one probe always overlapped, producing clusters spanning up to 7° of visual angle. The stimuli were centered over the centre of the RF cluster.

We determined the orientation tuning for each MU by presenting high-contrast drifting sinusoidal gratings (diameter 7°; spatial frequency 1 cycle/°; speed 1.5°/sec) with orientation varying in twelve steps of 30°, covering the entire circle of 360°. The spatial frequency of the centre and surround gratings was always the same and either .7 or 1.0 cycle/° and drift speed was either 1.0 or 1.5°/sec (18 and 9 recordings, respectively). These differences in stimulus parameters had no significant effect on the amplitude of the rate responses to the centre stimuli [paired t-test, $t(16) = .22$, $p = .82$] nor on the strength of surround modulation [two paired t-tests: all $t(16)$-values $\leq .49$, all p-values $\geq .62$]. Hence, to increase sample size we pooled the responses to the different stimuli. Each recording included three stimulation conditions: centre only, centre with collinear surround and centre with orthogonal surround (Fig. 1A). In each recording session, each stimulus was presented for 4 sec and repeated 20 times in randomized order, with an inter-trial interval of about 3 sec. The orientation preferences of MUs differed and, for the correlation analysis, we always chose the drift direction of the centre grating that stimulated the unit near optimally. Hence, each particular set of stimuli and each recording were used to correlate the responses of one subset of MUs, multiple recordings being required for a complete set of measurements. For the analysis of the stability of orientation tuning, repeated measurements of orientation preferences were made throughout the experiment (23× in total), which were interleaved with the recordings designed to investigate surround modulation.

2.4. Analysis

The degree of surround modulation was computed for each recording site as a relative modulation index (RMI) according to the following formula: RMI = $(R_C - R_{C+S})/R_C$, where R_C indicates the rate responses to centre stimuli alone and R_{C+S} rate responses to combined centre–surround stimulation. Thus, RMI indicates the proportion by which the responses to the centre stimulus change with the addition of the surround stimulus, positive values indicating suppression and negative values facilitation of rate responses. For the analysis of the stability of orientation tuning, responses were normalized and expressed as a change in firing rate relative to the mean firing rate across all 12 stimulation conditions. Correlations were computed as product-moment Pearson's coefficient of correlation, r, between variables x and y as, $r = \text{cov}(x,y)/[\text{SD}(x)\text{SD}(y)]$, where cov and SD indicate covariance and standard deviation, respectively. In Microsoft Excel, r is implemented as function CORREL. The sample size, N, was defined by the number of units whose responses were recorded simultaneously and included into computation. Oscillation Score is computed to indicate the power of beta/gamma oscillations (20–40 Hz) relative to the power in all other frequency bands (for details see Mureşan et al., 2008).

3.   Results

We first determined the orientation preferences of the MU responses. In Fig. 1B we illustrate the variability of relative direction preferences for one representative site. Units at this site preferred a stimulus drifting in the direction 240° and the lack of overlap between the variability indicator (±1 SD) for the preferred and all other directions indicates a high degree of tuning stability. The response variability across stimulation conditions was about 2.43 times larger than the variability of responses to the same stimuli across repeated recordings (SD = .56 and .23, respectively). For the entire population of 17 MUs, this ratio was 1.72 (on average, SD = .43 and .25, respectively). The units exhibited stable direction preferences despite the intermittent spontaneous changes in the strength of beta/gamma oscillations (Fig. 1C).

Collinear surrounds suppressed the responses to the centre stimulus on average by 6.66%, which was statistically significant [paired t-test, $t(16) = 4.63$, $p < .001$], while orthogonal surrounds produced less but still significant suppression of 3.66% [paired t-test, $t(16) = 3.81$, $p = .001$]. The difference in the degree of suppression between collinear and orthogonal surrounds was significant at the $p = .04$ level [paired t-test, $t(16) = 2.22$]. These results are consistent with previous reports (DeAngelis et al., 1994; Knierim and Van Essen, 1992; Sengpiel et al., 1997).

To investigate the stability of rate modulation induced by surround stimuli we i) calculated correlation coefficients, r, between pairs of recordings sessions, investigating whether the site with the strongest modulation in one recording remained the most strongly modulated in subsequent recordings; and ii) compared the magnitude of these correlation coefficients as a function of the time gap between the pairs of recordings. A high positive value of r indicates that a unit with the highest rate response (or its modulation) during one recording is also the unit with about the highest rate response in the other recording (see the example in Fig. 1D). Low values of r indicate that measures obtained in one recording do not predict those in the other recording (e.g., Fig. 1E). This analysis was performed for the two differently oriented surround stimuli and for the centre stimuli presented alone. Overall, across all 115 pairs of recording sessions (i.e., irrespectively of the time gap), the responses to the centre stimuli alone were correlated more strongly $r = .53$, than modulations of these responses by either collinear $r = .20$ or orthogonal surrounds $r = .03$. This was indicated by the significant main effect of stimulation condition [$F(2, 218) = 28.54$, $p < .001$] of a 2-way analysis of variance (ANOVA) stimulus × time gap. The smaller r-values in the two later cases were expected because the modulation (i.e., differences in rate responses, on average ~.80 spikes/sec) had usually smaller values than the raw rate responses (on average ~16.0 spikes/sec). Hence, the range of variation was smaller for the former than the latter: the average SD of surround modulation was .11 and .12 for collinear and orthogonal surrounds, respectively, and for the raw rate responses to centre stimuli it was 8.76 spikes/sec. Thus, the absolute values of the correlation coefficients cannot be used to directly compare the temporal stabilities of centre responses and surround effects, respectively. When we

excluded from the analysis all the weak correlations i.e., $.15 \le r \le -.15$ (in total, 42 out of 345 correlation coefficients excluded), the results did not change (not shown).

Therefore, we investigated the dependence of the decrease of correlations as a function of the time gap separating the pairs of recordings. In Fig. 1F average correlations are shown for all 17 MUs. For the raw rate responses to centre stimuli, the correlations dropped from $r = .79$ between the group of 25 pairs recorded in consecutive sessions (separated on average by 4.77 h) to $r = .23$ for the 16 pairs recorded at the largest time gaps (separated on average 77.65 h). For collinear surrounds, correlations decreased from $r = .35$ to $r = -.01$, while for the orthogonal surrounds, these correlations stayed consistently low, varying on average between $r = -.11$ and $r = .13$ (see Fig. 1F). These changes resulted in a significant main effect of time gap [$F(5, 109) = 3.40$, $p = .007$] and a significant interaction stimulus × time gap [$F(10, 218) = 2.34$, $p = .012$].

Importantly, the slope with which the correlations decreased as a function of the time gap (compare the fitted trend lines in Fig. 1F) was very similar for raw rate responses to centre stimuli and for their modulation by collinear surrounds ($a = -7.8 \times 10^{-3}$ and $a = -5.8 \times 10^{-3}$ 1/h, respectively). This was confirmed by the lack of a significant interaction between time and stimulation condition when only these two types of responses were included in a 2-way ANOVA [$F(5, 109) = .54$, $p = .74$]. As the correlations for the orthogonal surround stimuli were low for all time gaps, the corresponding slope had a much smaller value ($a = -.8 \times 10^{-3}$ 1/h). Thus, temporal stability of the modulation by collinear surrounds was similar to that of the responses to centre stimuli, while the temporal stability of the orthogonal surrounds could not be assessed reliably.

In the present study, single units could not be tracked for the entire period of the experiment (up to 96 h). Nevertheless, we were able to track four single cells for a period of up to 11 h, which enabled us to compare short-term stability of responses (107 min between recordings) to medium-term stability (671 min). Over these two time periods correlations of raw rate responses stayed constantly high ($r = .83$ and .92, respectively). The correlation of modulation by collinear surround was high over the short-term period ($r = .76$) but dropped significantly 10 h later ($r = -.63$). The correlation of the orthogonal surround was consistently low ($r = -.18$ and $-.47$, respectively). These results suggest that the stability of the responses of single units is similar to that of MUs.

4.   Discussion

Using a centre–surround paradigm we compared the temporal stability of neuronal responses evoked by centre stimuli and of surround effects with long-term, multi-channel recordings from the cat visual cortex. The size of the centre stimulus was adjusted to match the sizes of an entire cluster of RFs. Consequently, the centre stimuli covered always an area larger than individual RFs and, as a result, the centre stimuli also induced a certain degree of surround modulation themselves. Hence, in the present setup we investigated the modulation effects of surround stimuli that did not modulate responses from the immediate neighbourhood of the RFs, but

instead from more distant regions. This explains the relatively small average modulation effects (Bair et al., 2003).

Despite the fact that small effects tend to exhibit larger variability, our data indicate that the responses to surround stimuli are about equally stable over time as the responses to centre stimuli. More specifically, by computing correlations between repeated recording sessions, we showed that rate responses to the centre stimuli changed over time with about the same pace as the modulation of rate responses to collinear surround stimuli. Had we been able to investigate the stronger effects evoked from the near surround, the temporal stability of the surround modulation would, with all likelihood, have exceeded that found for the more remote effects.

The much lower correlations of the modulation to orthogonal surround gratings are probably due the much lower overall modulation effects with such stimuli, rather than due to low temporal stability of this interaction. Hence, we have no evidence that surround modulation evoked by gratings with orthogonal orientation is less constant.

We could not discern the degree to which the variability of MU responses was due to changes in their composition, or alternatively, due to changes in the response properties of the component neurons. However, one can expect that the two factors have similar impacts on both the responses to the centre and to the modulation by surround stimuli. Thus, the observed temporal stability of surround effects suggests two conclusions: first, the often reported variability of surround effects is due to site- or neuron-specific rather than to temporal, state-dependent variability. Second, surround modulation effects appear adequately stable over time to play a functional role in cortical computations, as proposed previously (Field et al., 1993; Kastner et al., 1997; Li, 1999; Nothdurft et al., 1990).

Our study does not explain the causes of the remaining temporal variability in neuronal responses. Reasons could be state dependent drifting in excitability or functional coupling of neurons. This possibility is currently under investigation.

5. Conclusions

Despite considerable neuron-to-neuron variability in responses to surround stimuli presented outside the RFs, we showed that, over long time periods, surround modulation by collinear stimuli displays considerable stability, similar to that of the responses to centre stimuli. These results suggest that the mechanisms of surround modulation play a significant role in cortical processing.

Acknowledgments

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