nature portfolio

Peer Review File

Border-ownership tuning determines the connectivity between V4 and V1 in the macaque visual system

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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This paper brings together figure-ground segmentation and border-ownership selectivity in the early visual cortex. They propose a model in which figure enhancement and suppression of the background in visual area V1 is driven by feedback from border-ownership neurons in the extrastriate cortex, in particular, V4. Specifically, the model assumes excitation of V1 neurons of which the receptive field lies on the figure by V4 border-ownership neurons that encode the location of the figure and inhibition of V1 neurons with receptive fields at the opposite side of the figure border by those border-ownership neurons. They test this model by examining noise correlations, computed during the baseline period before stimulus onset, between V1 and V4 neurons and find higher noise correlations between V4 neurons of which the border-ownership direction points towards the receptive field of the V1 neurons compared to other locations.

This paper provides a simple feedback model of figure-ground segmentation in which border-ownership neurons play an important role. This model assumes a particular pattern of connectivity between border-ownership V4 neurons and V1 neurons, depending on the relative location of the preferred figure direction of the V4 neurons and the location of the V1 receptive field. They take noise correlations as a proxy of such connectivity, showing the hypothesized difference in noise correlations between V1 and V4. This is a novel and exciting finding and the results appear to be solid (but see main comment 4 below). However, noise correlations can depend on many factors and also do not provide information about the direction of the effect. Also, the evidence for the proposed inhibitory connectivity is rather indirect, relying on a comparison between border-ownership neurons and mere orientation-selective neurons. Despite these limitations, this paper shows for the first time that noise correlations between V1 and V4 neurons depend on the relative position of the V1 receptive field and V4 border-ownership direction. This is an interesting finding but that it reflects feedback is not demonstrated.

Main comments.

1. The dependency of the noise correlations on the border-ownership direction and relative RF location does not imply feedback from border-ownership neurons to V1 neurons. The authors show that the V1 figure-ground response occurs later than the V4 border-ownership signal. However, this does not imply that the V4 border-owner signal is influencing V1. In this context, it would be

informative to show the evolution of noise correlations during the presentation of the stimulus. One may expect that the difference between the noise correlations for different directions increases after the onset of the border-ownership signal and has a larger size at the onset of the V1 figure-ground response. Please show the evolution of the noise correlations in small bins during the stimulus presentation and this for the conditions of Figure 5C.

2. I found the data of Figure 5C not easy to interpret since one compares noise correlations when there is a stimulus/figure on the RF of the V1 neurons (good condition for the < 45 deg) with noise correlations when there is no stimulus/figure on their receptive field (bad condition for the < 45 deg). Please discuss this.

3. I expect that the potential contribution of the V4 border-ownership neurons to figure-ground segmentation in V1 will be less for small stimulus sizes and highly irregular figures, because of the spatial range in which its influence is supposed to work (3 deg according to Figure 4). I would like the authors to test their model for a range of smaller figure sizes and more irregular figures. This is to test how well that model generalizes across figure size and shape. Related to this issue of stimulus size: is it possible that V2 plays a more important role than V4, especially for figures with a small size, given that a large proportion of V2 neurons show border-ownership selectivity?

4. The authors combine all simultaneously recorded sites of V1 and V4 to obtain the MUA site pairs to compute the noise correlations. Given that neurons within an area and for an array show noise correlations and thus are not independent, the noise correlations for the different pairs are expected to be not independent, which will affect the statistical test outcome (statistical tests assume independent data; the dependency of data affects the degrees of freedom). Were the noise correlations computed for a particular V1 array more similar than for different V1 arrays? This might explain the clustering of the data points in e.g. Figure 4B. How many arrays were employed? Please address this potential concern of data dependency.

5. The evidence for the proposed inhibitory connectivity is rather indirect, based on a comparison between neurons with different selectivities. This should be stated more explicitly.

Minor comments.

1. Lines 430-431: please rewrite this sentence. Something is missing.

2. To compute noise correlations, the authors concatenated the z-scored response across days. Why and how many days were pooled?

3. The authors perform preprocessing of the data before computing the noise correlations. To what extent did this affect the results, e.g. the specifics of the smoothing using robust loess regression? Results of noise correlations can depend on such specific preprocessing steps (e.g. see for discussion Lange et al, J. Neurophysiol., 129, 1021, 2023). The average inter-areal noise correlations appear to be rather high to me, even after detrending.

Reviewer #2 (Remarks to the Author):

This is an excellent paper by a leading lab with a distinguished record for groundbreaking contributions to primate and mouse visual neuroscience.

Using implanted Utah arrays to record multiunit responses in macaque monkey V4 and V1, the study shows that V1 neurons are synchronously active with border-ownership tuned neurons in V4. The findings provide evidence for ownership-selective functional connectivity between V4 and V1. The results suggest that feedback inputs from border-ownership neurons are the source for excitatory and inhibitory modulatory inputs by which perceptual figures can be distinguished from the background. Linking border-ownership tuning to figure/background segregation identifies a class of V4 neurons involved in the construction of an internal model, that organizes responses in V1 to enable scene segmentation and object recognition. This is a significant insight into the role of cortical feedback connections in predictive coding of natural images.

If the investigation has any weakness, it is using noise correlation as measure of connectivity. That said, the authors are very much aware of the issues and discuss the limitation of the approach in detail. Clearly, anatomical tracing approaches will be necessary to support the conclusions drawn for the present study directly. I wonder, though, whether the authors have tried to use stimulation of border-ownership clusters in V4 to interfere with the correlation structure of responses in V1. While this is doable, it not entirely clear how much the results would strengthen the conclusion that figure/background modulation is due to feedback from border-ownership clusters in V4.

The model proposed in Figure 6D suggests that excitatory and inhibitory influences in V1 may be distributed in spatially distinct non-random fashion. Figure 3E which shows cell clusters with vastly different agreement angles at greater between-pair-distances may capture a hint of this. Is it correct that clustering is more frequent at greater distances? If so, what does this tell about the diversion of feedback projections?

May be I missed it. But I have a hard time to find evidence for Petreanu's finding in mouse visual cortex that feedback from LM to V1 spreads more widely in the direction perpendicular to the optimal orientation tuning of inputs. If I understand the comment on line 184 correctly, the findings in monkey are counter to the organization in mice. If this is correct, why then is this organization highlighted in the introduction (line 67) and the discussion (line 463)?

In Figure 1B, it may be helpful for the non-specialist to explain that d.v.a. stands degree of visual angle.

In Figure 2B provide scale of responses.

Line 217 Revise the sentence to…"to the upper right of…".

In Figure 4, what is the reason for the low noise correlations for cells with excellent agreement angles? Does it mean that noise correlation is a poor measure of connectivity?

In relation to Figure 5A and 5B (line 324 and 329) it is stated that the latencies for BO and FBM was 47 vs 122 ms., respectively. Why is this not evident in Figures 5A and 5B?

Reviewer #3 (Remarks to the Author):

Jeurissen et al. investigate the relationship between Border Ownership cells in V4 and Figure-Ground-Modulation in V1. Using simultaneous recordings with multielectrode arrays from V4 and V1 they demonstrate that noise correlations between pairs of V4/V1 neurons are stronger when the V1 neuron's RF is on the preferred side of the V4 neuron's border. This is consistent with the interpretation that V4 neurons provide excitatory input to those V1 neurons and less excitation or inhibition to neurons on the non-preferred side of the border. A computational model using the observed functional coupling between the areas replicates key features of FGM in V1.

The paper provides novel insight into the computations underlying FGM and BO, and provides a first explicit link between these two subfields of investigation. The explicit formulation in terms of a model is a big plus as it moves ideas of processing from the abstract "top-down feedback" to the specifics implemented in the model.

The paper is well written and easy to follow. I have a few comments and requests for clarification:

The choice of "good" and "bad" as labels for the agreement between V4 and V1 cells is unfortunate and potentially confusing. There is nothing bad about the "bad" agreement. Preferred side of the border and non-preferred side of the border? Congruent/ incongruent spatial arrangement?

The agreement angle is circular on pi , but used as a linear variable in the regression analysis. Please explain how this was addressed.

Line 307. Why suppressive? Couldn't it be just less excitation? If it was suppressive shouldn't the noise correlations be negative (at sufficiently large distance)? Please discuss.

Line 311. This seems an important point (i.e., the arrow of

causality) that should be made more explicit in the Discussion.

Line 388. This could use some unpacking; I see that the refined model does better, but can you give an intuitive explanation?

Line 935. As written, this is not a test of significance.

Presumably FBM and BO labels were shuffled in the significance

test and not in the bootstrap estimate of the mean. Reword or explain.

Line 945. The model code should also be made available.

Supplementary materials were not available online. The video for 4A is probably helpful. It is not clear what the other supplementary figures are.

Reviewer #4 (Remarks to the Author):

Jeurissen et al. examine if the connectivity of cortical feedback inputs from border-ownership (BO) neurons in V4 could underlie figure/ground modulations (FGM) in V1. The manuscript proposes a model with a connectivity scheme between BO V4 neurons and V1 that would be consistent with V4 inputs mediating the experimentally observed FGM in V1.

This model predicts V4 influences to be excitatory or inhibitory depending on the V4 neurons' BO tuning and the position of of the V1 neurons' receptive fields. To test these predictions, the authors describe how noise correlations relate to the receptive field and the BO tuning of V1/V4 neurons in multi area recording from two monkeys. They find evidence that noise correlations during spontaneous and evoked activity depend on the relation of the BO tuning and receptive field of V1

neurons with a structure that is like the one proposed by the model. Finally, they modify the original model to more faithfully map to the measured V4-V1 connectivity, as measured by the noise correlations, and find that it provides a better explanatory power over the original one.

There is evidence that cortical feedback is involved in FGM. However, the neural mechanisms by which cortical feedback inputs would implement FGM are unknown. The manuscript proposes and interesting simple mechanism for FGM involving connectivity rules that depend on the BO tuning and the retinotopic position of the V1 neurons. This is an important contribution to our understanding FGM and, more generally, on the circuit mechanisms underlying visual perception. The manuscript is clearly written, and the data is well presented. A weakness of the approach is that the connectivity kernel proposed by the model is tested using noise correlations, a metric that only indirectly relates to direct feedback connectivity. Importantly, several connectivities not involving direct feedback connections from V4 to V1 could also give rise to the same noise correlation structure. While this is acknowledged in the discussion, it remains a limitation, nevertheless. If the connectivity pattern could also be backed with a more direct method, it would greatly strengthen the manuscript. Besides this, it have some concerns regarding the core measurements of the noise correlations and other suggestions for improvements.

Major:

1) Noise correlations increase as a function of the firing rate of the neurons . Thus, differences in joint firing rates in V1-V4 neurons might explain some of the observed differences. This confound might contribute to the differences in noise correlations in Figure 5 , as firing rates are expected to be lower when the stimulus is away from the preferred orientation than when it is not. In addition, neurons in bad agreement with the preferred border orientation are likely to be suppressed because they are on the background, while those with good agreement are expected to increase their firing (at least when borders are close to the preferred BO). The confounds of these expected firing rate differences on the noise correlations should be addressed for the analyses in both Figures 4 and 5.

2) Noise correlations are known to also depend on tuning similarity. Is the relations of noise correlations with BO preference and V1 RF position independent of their orientation tuning similarities?

3) It is unclear why a linear regression model is not used to analyze both spontaneous and evoked activities. The manuscript would benefit if similar analyses were used in both conditions.

4) Lines 272-275. Some of the main analyses of Figure 4 , specifically those in panels 4D and 4E seem not to have associated statistics backing the claims.

Minor.

5) In a previous study the same lab found that the suppression of the ground segment in u o n shaped figures depends on learning a task (Self et al 2019). As the connectivity kernel used in Figure 6 was obtained in non-trained monkeys, it would make sense to fit the model to the modulation observed in naïve animals. Yet, the modulations modeled in Figure 6C,D seem to correspond to the trained monkey from Self et al. 2019.

6) Line 183, do the authors meant “parallel” instead of perpendicular?

7) Line 330, “suggesting” would be more appropriate than “indicating” as the observation is purely correlational.

8) Line 344-245 . The number of good and bad pairs included seems to be missing.

Reply to Reviewers

We would like to thank all four reviewers for their time and effort in reviewing the manuscript, their enthusiasm for the findings and their constructive suggestions to improve the manuscript. Before addressing each reviewer's points in turn we will first highlight the major change in the manuscript which addresses points shared by all reviewers.

Multiple reviewers raised the issue that noise correlations do not give any indication of directionality of connectivity, because feed-forward and feedback connections can both give rise to increased noise correlations. The results in the original manuscript did not distinguish between these forms of connectivity. To address this issue, and in accordance with a suggestions of reviewer #2, we have recorded a new dataset from a third monkey (the original two monkeys are no longer in the lab), in which we applied electrical micro-stimulation to electrodes situated in V4 and measured the effects on spiking activity in V1.

Firstly, we replicated the pattern of noise correlations. The relationship between border-ownership agreement angle, RF distance and V1-V4 noise correlations in this monkey was very similar to that in the first two monkeys, confirming the consistency of the effect across animals.

To gain insight into directionality, we stimulated V4 electrodes with weak stimulation pulses while recording activity in V1. Specifically, we applied 5 pulses of 5μA to individual V4 electrodes with a frequency of 200Hz and examined the V1 MUA response in a time window from 35-100ms after the pulses (excluding the electrical stimulation artifact). Stimulation of V4 electrodes caused increased spiking responses of V1 neurons that were in good agreement with the V4 unit's BO tuning relative to the activity of simultaneously recorded neurons that were in bad agreement (Figure R1). We examined the relationship between the effect of micro-stimulation on V1 activity and the agreement angle and RF distance between the stimulated V4 site and V1 sites with the same linear model that we used to examine noise correlations. We found a remarkably similar pattern of regression coefficients (compare Figure R1B and Figure R1F).

The strong similarity between the pattern of noise correlation and the micro-stimulation results suggests that they reflect the same underlying process, namely feedback from V4 to V1 that is arranged according the border-ownership tuning of the V4 cells. Hence, this result strongly supports the proposed feedback connectivity scheme and provides direct causal evidence for the directionality of the effect. We have added the results of this experiment to the manuscript as the new Figure 6 (shown here as Figure R1).

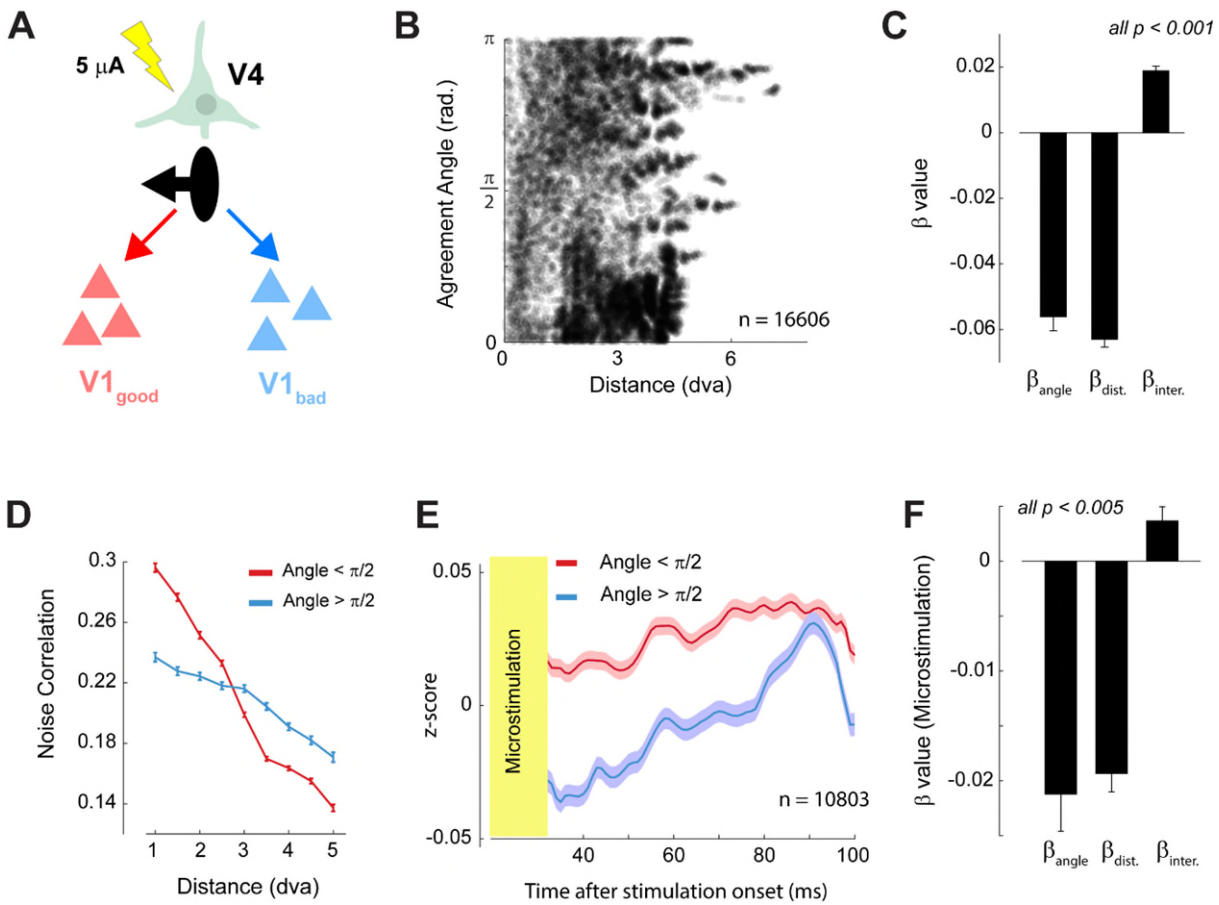


Figure R1. Micro-stimulation of V4 drives V1 responses in accordance to the agreement angle. A) *We micro-stimulated individual V4 recording sites in monkey N. The pictured V4 site prefers figures to the left of its RF (black arrow). We hypothesized that weak V4 stimulation would cause increased activity of V1 neurons with RFs situated to the left of the V4 cell's RF (red cells, good agreement) and suppress the activity of neurons in bad agreement (blue cells)* ***B)*** *Distribution of agreement angle and RF Euclidean distance from V1-V4 pairs recorded in the third monkey N.* ***C)*** *The beta coefficients from the linear model that relates noise correlations to agreement angle and RF distance in monkey N (conventions as in Figure 4C).* ***D)*** *Noise correlations were higher for good agreement pairs within 3 dva. All post-hoc comparisons at individual distance bins were significant at the $p < 0.05$ level (Bonferroni correction applied)* ***E)*** *Average responses in V1 aligned by the onset of micro-stimulation in V4. V1 sites were split into sites that were in good (red) and bad (blue) agreement with the V4 site where neurons were stimulated.* ***F)*** *Beta coefficients from a linear model in which the V1 MUA response after micro-stimulation (35-100ms) was the dependent variable.*

We will now address each reviewer's points in turn. The reviewer's questions are displayed in black font and our replies in blue font.

Reviewer #1:

This paper brings together figure-ground segmentation and border-ownership selectivity in the early visual cortex. They propose a model in which figure enhancement and suppression of the background in visual area V1 is driven by feedback from border-ownership neurons in the extrastriate cortex, in particular, V4. Specifically, the model assumes excitation of V1 neurons of which the receptive field lies on the figure by V4 border-ownership neurons that encode the location of the figure and inhibition of V1 neurons with receptive fields at the opposite side of the figure border by those border-ownership neurons. They test this model by examining noise correlations, computed during the baseline period before stimulus onset, between V1 and V4 neurons and find higher noise correlations between V4 neurons of which the border-ownership direction points towards the receptive field of the V1 neurons compared to other locations.

This paper provides a simple feedback model of figure-ground segmentation in which border-ownership neurons play an important role. This model assumes a particular pattern of connectivity between border-ownership V4 neurons and V1 neurons, depending on the relative location of the preferred figure direction of the V4 neurons and the location of the V1 receptive field. They take noise correlations as a proxy of such connectivity, showing the hypothesized difference in noise correlations between V1 and V4. This is a novel and exciting finding and the results appear to be solid (but see main comment 4 below). However, noise correlations can depend on many factors and also do not provide information about the direction of the effect. Also, the evidence for the proposed inhibitory connectivity is rather indirect, relying on a comparison between border-ownership neurons and mere orientation-selective neurons. Despite these limitations, this paper shows for the first time that noise correlations between V1 and V4 neurons depend on the relative position of the V1 receptive field and V4 border-ownership direction. This is an interesting finding but that it reflects feedback is not demonstrated.

We agree with the reviewer that noise-correlations do not convey information about the directionality of connectivity. As was described in the introduction of the rebuttal (Fig. R1), we have now added a causal manipulation of activity in V4 in a third monkey. We used electrical micro-stimulation of individual electrodes in V4 and measured the effect on activity in V1. Weak electrical stimulation increased the activity of V1 neurons with RFs at a good agreement angle and suppressed the activity of V1 neurons at a bad agreement angle. These results indicate that feedback from V4 to V1 that is arranged according the border-ownership tuning of the V4 cells. We have added the results of this experiment to the manuscript as the new Figure 6.

Main comments.

1. The dependency of the noise correlations on the border-ownership direction and relative RF location does not imply feedback from border-ownership neurons to V1 neurons. The authors show that the V1 figure-ground response occurs later than the V4 border-ownership signal. However, this does not imply that the V4 border-owner signal is influencing V1. In this context, it would be informative to show the evolution of noise correlations during the presentation of the stimulus. One may expect that the difference between the noise correlations for different directions increases after the onset of the border-ownership signal and has a larger size at the onset of the V1 figure-ground response. Please show the evolution of the noise correlations in small bins during the stimulus presentation and this for the conditions of Figure 5C.

The most direct evidence for a role of feedback connections comes from the new electrical micro-stimulation experiment, which was described above and in the introduction of this rebuttal.

We also examined how the noise correlations developed over time (Figure R2). We calculated noise correlations in a series of sliding windows of 20ms duration. Before the onset of the stimulus, the noise correlation is higher for V1-V4 pairs with RFs in good agreement than for pairs with RFs in bad agreement (see also Figure 4). Previous studies demonstrated that the presentation of a visual stimulus initially reduces the strength of noise correlations, because the feedforward propagation of information briefly dominates the neuronal activity levels (Churchland et al., 2010; Semedo et al., 2022; Smith and Kohn, 2008). Here we also observed that the onset of the visual stimulus caused an initial reduction in noise correlations, which was most pronounced for the V1-V4 pairs that were in good agreement. The activity and tuning of the V4 cells during this early response phase is driven by feedforward input, including input from V1 cells. The correlations during this initial response phase depend less strongly on the agreement of RFs. After the onset of figure-background modulation (FBM) in V1, however, the difference between the noise correlation between good and bad pairs is reestablished. We hypothesize that V4 and V1 now engage in recurrent interactions so that agreement angle and figure-ground segregation influence the noise correlations (see below). In the revision, we describe the time-course of the noise correlations on lines 372-376 and discuss them on lines 602-611 and we have added the Figure R2 as Supplementary Figure 5B.

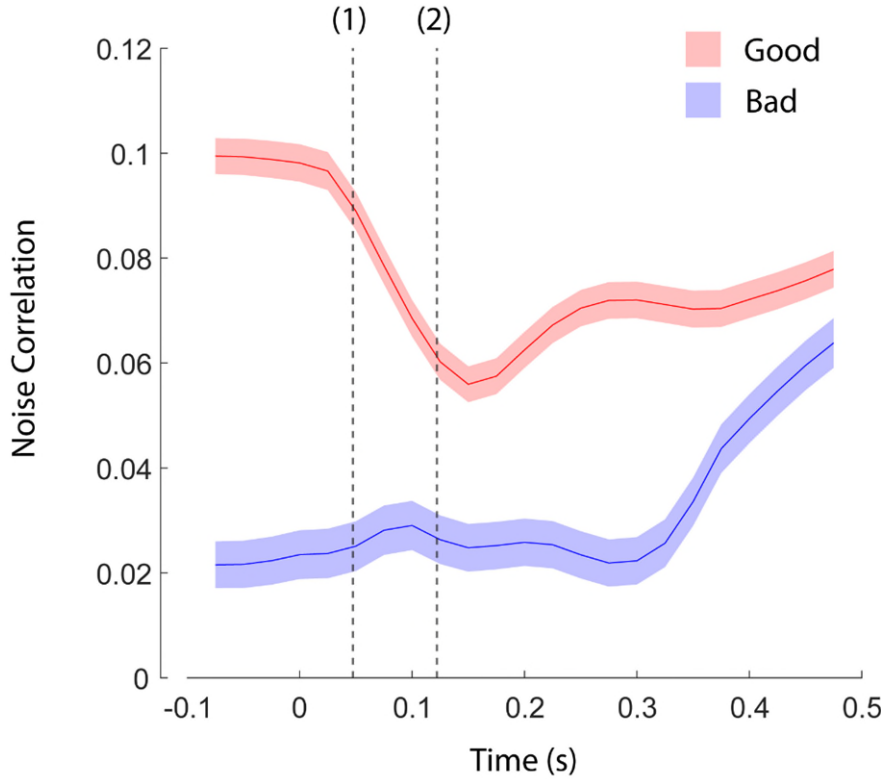


Figure R2. Development of noise correlations over time. *We measured the noise correlations observed during responses to the figure-ground stimuli (Figure 5) in sliding time-windows of 50ms duration. The shorter time-window leads to lower correlations overall (Cohen and Kohn, 2011). We limited the analysis to trials in which the V4 cell was well driven by the border-orientation (difference between preferred orientation and border-orientation < 90°). The dashed lines mark (1) the onset of BO-tuning and (2) the onset of FBM. The onset of the visual stimulus caused reduced the noise correlation for good-alignment pairs and also noise correlation difference between the V1-V4 pairs with RFs in good and bad alignment. After the onset of FBM the difference in noise correlation*

between good and bad pairs became stronger again, presumably because V1 and V4 now engaged in recurrent interactions.

2. I found the data of Figure 5C not easy to interpret since one compares noise correlations when there is a stimulus/figure on the RF of the V1 neurons (good condition for the < 45 deg) with noise correlations when there is no stimulus/figure on their receptive field (bad condition for the < 45 deg). Please discuss this.

It is to be expected that the noise correlations during responses to visual stimuli depend both on the connectivity and the activity that is driven by the visual stimulus. Our hypothesis was that V4 cells that are well driven by a preferred border should have a larger influence on the activity of V1 cells than V4 cells that are weakly driven by a non-preferred border. Hence, we predicted that a preferred border amplifies the difference in noise correlation strength between good and bad pairs. Figure 5C confirmed this prediction and a further confirmation was given by the micro-stimulation experiments described in the above. We have rewritten the description of the results in Figure 5C to clarify the logic of the analysis and our interpretation.

The reviewer correctly points out that when a V4 and V1 pair are in good agreement and the V4 cell is responding to its preferred border, then the V1 cell's RF is more likely to be on the figure. Conversely, the V1 RF is more likely to be on the background when the V4 cell responds to its non-preferred border. The opposite is true for V4 and V1 cells with RFs in bad agreement. If the V4 cell responds to its preferred border, the V1 cell is more likely to be driven by the background and vice versa. Interestingly, these correlations will also occur in most images that the animal perceives.

The results presented in Fig. 5C indicate that there is no difference between the noise correlations between good and bad pairs when the V4 cells respond to their non-preferred border (the >135° condition). In this configuration, the RFs of the V1 cells of the good pairs are usually on the background and those of the bad pairs on the figure, which implies that the strength of the V1-V4 noise correlation does not strongly depend on whether V1 neurons respond to figure or ground.

We reanalyzed the data to further disentangle these three factors: (1) V4 cells responding to either their preferred or non-preferred border, (2) V1 and V4 RFs in good or bad agreement and (3) V1 RFs on the figure or background (Reviewer Figure 3, included in the revision as Figure 5D).

If the V4 cell responded to its preferred border (left in Fig. R3), the noise correlation was higher for good-agreement pairs and if the V1-RF falls on a figure. This result, suggests that V4 cells responding to their preferred border engage in recurrent interactions with V1 cells that encoded the figure to which the border belongs. When the V4 RF fell on a non-preferred border, the noise correlations for good and bad agreement pairs were similar overall, with a slightly higher value for good-agreement pairs when the V1 RF was on the background.

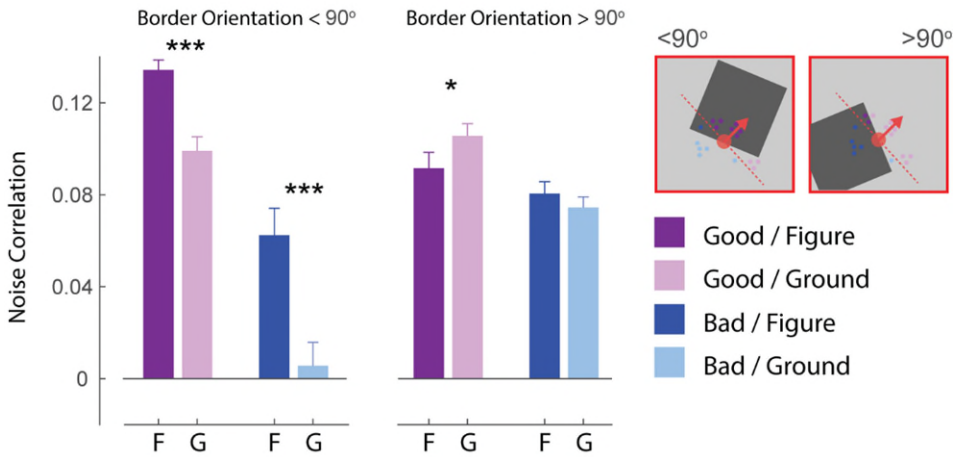


Figure R3 (Figure 5D of the revision). The effect of figure-ground organization on the noise correlations between V1 and V4. The noise correlations in the sustained period (100-500ms after stimulus onset) split according to whether the neurons at the V4 site responded to their preferred border or not, the agreement angle of the V4-V1 pair and whether the V1 RF was situated on the figure or the background. *** = $p < 0.001$, * = $p < 0.05$ paired t-test. Error-bars are 1 s.e.m.

3. I expect that the potential contribution of the V4 border-ownership neurons to figure-ground segmentation in V1 will be less for small stimulus sizes and highly irregular figures, because of the spatial range in which its influence is supposed to work (3 deg according to Figure 4). I would like the authors to test their model for a range of smaller figure sizes and more irregular figures. This is to test how well that model generalizes across figure size and shape. Related to this issue of stimulus size: is it possible that V2 plays a more important role than V4, especially for figures with a small size, given that a large proportion of V2 neurons show border-ownership selectivity?

We agree with the reviewer on these points. The model was purposefully simple, containing only four 'directions' of border-ownership preference, so it could not deal with more irregular figure shapes. We also agree that V2 is likely to play a very important role in refining the figure-ground modulation structure in V1 for small shapes (see also Jehee et al., 2007). To examine these points, we extended the model to include a larger range of border-ownership preferences (24 values in 15° steps) and examined the response to a range of figure sizes and shapes (Reviewer Figure 4). In line with the reviewer's prediction, the resolution of the figure-ground pattern observed in V1 has a lower limit because the patterns of figure-ground modulation elicited by 1° and 2° squares are very similar. We also included model predictions for more complex shapes. It can be seen that some residual figure-ground modulation remains for larger shapes (arrow in Reviewer Figure 4, lowest row).

We have added these additional results to the manuscript as Supplementary Figure 6. Future work could extend the model, by including feedback from 'V2' and 'IT' layers with different spatial precisions, for a more complete set of interactions between levels of the visual hierarchy. However, we prefer to keep the model simple for the present manuscript, so that we can maintain an emphasis on the principles of the proposed connectivity scheme.

[REDACTED]

4. The authors combine all simultaneously recorded sites of V1 and V4 to obtain the MUA site pairs to compute the noise correlations. Given that neurons within an area and for an array show noise correlations and thus are not independent, the noise correlations for the different pairs are expected to be not independent, which will affect the statistical test outcome (statistical tests assume independent data; the dependency of data affects the degrees of freedom). Were the noise correlations computed for a particular V1 array more similar than for different V1 arrays? This might explain the clustering of the data points in e.g. Figure 4B. How many arrays were employed? Please address this potential concern of data dependency.

We agree with the reviewer that correlations between neurons have to be taken into account in the statistical comparisons. Within-array noise correlations are higher than those across arrays, because the correlation strength generally depends on the distance between electrodes. To control for the higher correlations within arrays, we reran the linear models using a hierarchical approach with electrodes grouped per array. Specifically, we now included a random intercept term for 'array ID' in a Linear Mixed Effects (LME) model, both for the V1 array and for the V4 array. The results of these refined LMEs were very similar to the results of the original Linear Models. The fixed-effect coefficients for Agreement Angle, RF Distance and their interaction remained significant at the $p < 0.001$ level (compare Figure 4C and Figure S3C).

This results indicates that the correlations within arrays did not account for the relationship between Agreement Angle, RF Distance and V1-V4 noise correlations. We now report the results of the LMEs in Supplementary Figure 3C and discuss them on lines 289-291.

5. The evidence for the proposed inhibitory connectivity is rather indirect, based on a comparison between neurons with different selectivities. This should be stated more explicitly.

We agree with the reviewer and we have acknowledged this explicitly in the text on lines 325-328 and 588-591 and highlighted potential other explanations.

Minor comments.

1. Lines 430-431: please rewrite this sentence. Something is missing.

We have rewritten the sentence

2. To compute noise correlations, the authors concatenated the z-scored response across days. Why and how many days were pooled?

The BO tuning stimuli were shown in sessions on 4 days in both monkeys to obtain a sufficient number of trials. We pooled the data across days to calculate the tuning of the neurons and we therefore also pooled the baseline data to calculate the noise correlations. To account for the possibility that different recording days can have subtle differences in noise level and responsivity, we separately z-scored the data of each day before pooling the data.

3. The authors perform preprocessing of the data before computing the noise correlations. To what extent did this affect the results, e.g. the specifics of the smoothing using robust loess regression? Results of noise correlations can depend on such specific preprocessing steps (e.g. see for discussion

Lange et al, J. Neurophysiol., 129, 1021, 2023). The average inter-areal noise correlations appear to be rather high to me, even after detrending.

We have now examined the effect of using different smoothing windows on the noise correlation and on the results from the linear models. We reran the analyses with robust LOESS smoothing windows varying from 10 trials to 400 trials, to detrend the baseline level. *A priori* we expected the average values of noise correlations to decrease as the smoothing window length decreased and fluctuations at shorter and shorter time-scales were removed from the baseline data. However, we found that the average noise correlation only changed from 0.0895 for a 400 trial window to 0.0832 for a 10-trial window. The result suggests that the V1-V4 noise correlations are dominated by correlations at relatively short time-scales. The different window-lengths did not affect the regression coefficients from the linear models (or LMEs) as can be seen in Figure R5.

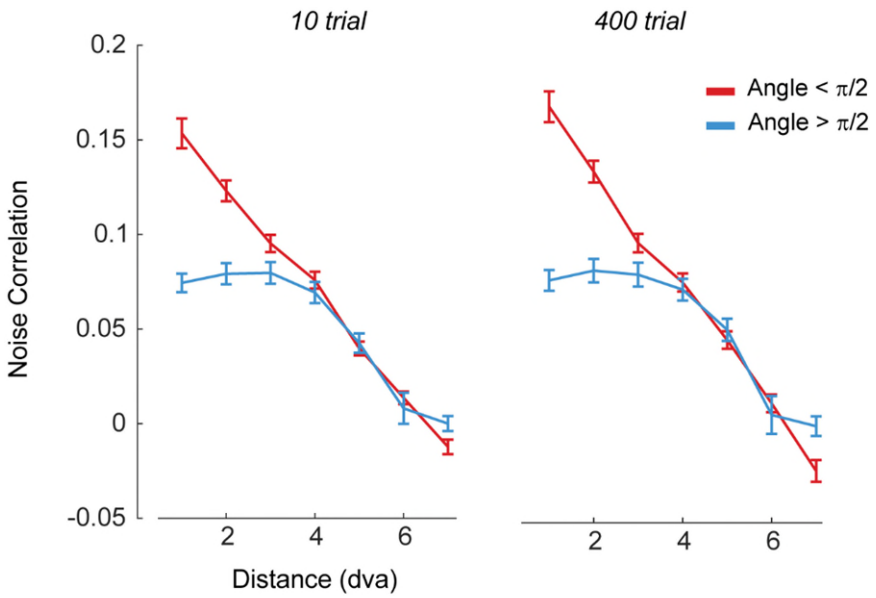


Figure R5. Replication of Figure 4D for different smoothing windows. *The 10 trial smoothing window (left panel) slightly reduced noise correlations but had no effect on the relationship between Agreement angle, RF distance and noise correlation.*

Noise correlations in previous studies varied widely, from close to zero (Ecker et al., 2010) to values above 0.3 (Gutnisky and Dragoi, 2008). The average value of ~0.09 lies within this range, although the noise correlations reported by us are between different brain areas, whereas most previous studies focused on intra-areal correlations. We note that we measured multi-unit signals in our study, which give rise to higher noise correlations than single units (Cohen and Kohn, 2011; Schulz et al., 2015).

Reviewer #2

This is an excellent paper by a leading lab with a distinguished record for groundbreaking contributions to primate and mouse visual neuroscience.

We would like to thank the reviewer for their positive feedback, suggestions and careful reading of the manuscript.

Using implanted Utah arrays to record multiunit responses in macaque monkey V4 and V1, the study shows that V1 neurons are synchronously active with border-ownership tuned neurons in V4. The findings provide evidence for ownership-selective functional connectivity between V4 and V1. The results suggest that feedback inputs from border-ownership neurons are the source for excitatory and inhibitory modulatory inputs by which perceptual figures can be distinguished from the background. Linking border-ownership tuning to figure/background segregation identifies a class of V4 neurons involved in the construction of an internal model, that organizes responses in V1 to enable scene segmentation and object recognition. This is a significant insight into the role of cortical feedback connections in predictive coding of natural images.

If the investigation has any weakness, it is using noise correlation as measure of connectivity. That said, the authors are very much aware of the issues and discuss the limitation of the approach in detail. Clearly, anatomical tracing approaches will be necessary to support the conclusions drawn for the present study directly. I wonder, though, whether the authors have tried to use stimulation of border-ownership clusters in V4 to interfere with the correlation structure of responses in V1. While this is doable, it not entirely clear how much the results would strengthen the conclusion that figure/background modulation is due to feedback from border-ownership clusters in V4.

This is an excellent suggestion and we have implemented the proposed experiment with V4 stimulation in a third monkey. We have added a short description of these new results in the introduction to this rebuttal. In short, we replicated the pattern of noise correlations that we had observed in the first two monkeys and we observed that V4 micro-stimulation increased the activity of V1 neurons with a good agreement angle relative to the activity of V1 neurons with a bad agreement angle. The similarity between the noise correlation and micro-stimulation results supports our hypothesis that feedback from V4 to V1 is arranged according the border-ownership tuning of the V4 cells. The data have been added to the manuscript as the new Figure 6.

The model proposed in Figure 6D suggests that excitatory and inhibitory influences in V1 may be distributed in spatially distinct non-random fashion. Figure 3E which shows cell clusters with vastly different agreement angles at greater between-pair-distances may capture a hint of this. Is it correct that clustering is more frequent at greater distances? If so, what does this tell about the diversion of feedback projections?

The clustering observed in Figure 3E is caused by the distribution of V1 receptive fields in monkey Bo (Reviewer Figure 6). In monkey Bo there was one functional V1 array with nearby receptive fields. The clusters in Figure 3E come from 8 V4 units with RFs that were relatively far away from the V1 array with different BO tuning preferences and Agreement Angles (causing vertical shifts in the graph). Note that the distribution of agreement angle and distance was more uniform in Monkey Bu

(Fig. R6) and particularly in monkey N (Fig. R1A), with a larger number of electrodes (7 arrays in V1 and 4 in V4, with 8x8 electrodes per array). To account for the effect of clustering on the statistics, we included the arrays as an additional factor in the Linear Mixed Effects model and reproduced the results (Figure S3C).

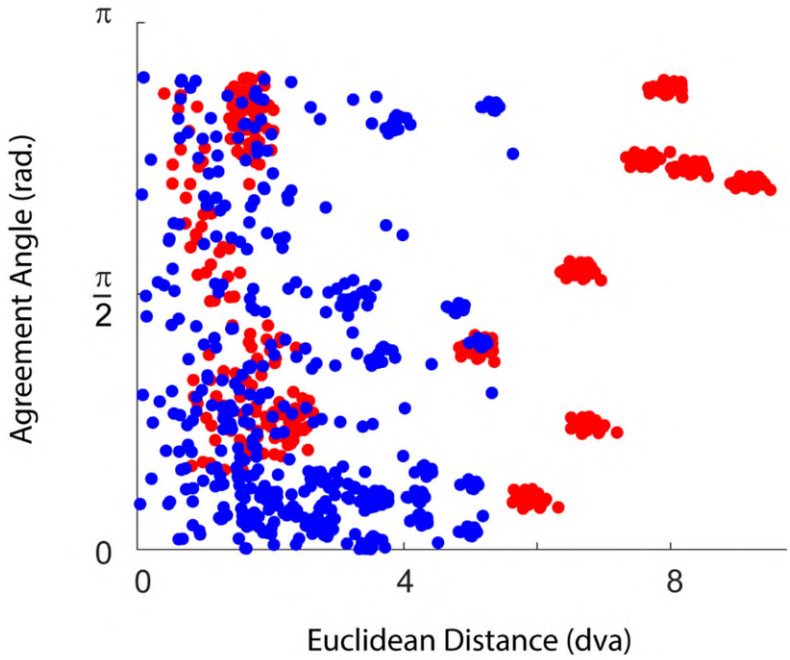


Figure R6. RF Distance vs. Agreement Angle (conventions as in Figure 3E)*. Red data points are from monkey Bo and blue data points from monkey Bu.*

May be I missed it. But I have a hard time to find evidence for Petreanu's finding in mouse visual cortex that feedback from LM to V1 spreads more widely in the direction perpendicular to the optimal orientation tuning of inputs. If I understand the comment on line 184 correctly, the findings in monkey are counter to the organization in mice. If this is correct, why then is this organization highlighted in the introduction (line 67) and the discussion (line 463)?

The study by Marques et al. (2018) from the group of Petreanu reported that cells in LM predominantly project to V1 along an axis that is orthogonal to their preferred orientation. In monkeys, border-ownership tuning is predominantly orthogonal to the preferred orientation of the V2/V4 cell. A cell that responds well to a vertical edge will prefer a figure to the left or right of the RF and the pattern of connectivity that was suggested by Marques et al. (2018) is therefore in accordance with our model. However, Marques et al. did not examine border-ownership and it is therefore unknown whether the projection pattern of LM cells is related to border ownership. Nevertheless, our model provides aligns with the feedback projection pattern from LM to V1 in mice.

In Figure 1B, it may be helpful for the non-specialist to explain that d.v.a. stands degree of visual angle.

This information has been added to the figure legend of Figure 1B.

In Figure 2B provide scale of responses.

We have added scale-bars to Figure 2B and E

Line 217 Revise the sentence to…"to the upper right of…".

We think this sentence was correct as originally stated in the manuscript.

In Figure 4, what is the reason for the low noise correlations for cells with excellent agreement angles? Does it mean that noise correlation is a poor measure of connectivity?

Noise correlations are an indirect measure of connectivity because they depend on several uncontrolled factors including fluctuations in the brain state. Furthermore, there is some noise in the measurement of the RFs in V1 and V4, and in the determination of border-ownership tuning of the neurons at V4 recording sites. These factors add unexplained variability around the regression plane. In spite of these sources of variability, the model explained more than half of the variance in the noise correlations in monkey Bo and more than a quarter in monkey Bu.

In relation to Figure 5A and 5B (line 324 and 329) it is stated that the latencies for BO and FBM was 47 vs 122 ms., respectively. Why is this not evident in Figures 5A and 5B?

We have added the latency estimates and s.e.m. to the graphs. The latency of BO and FBM is measured as the difference between the activity in two conditions, and it is difficult to see during phases when the neuronal activity changes rapidly, which happens at the onset of the visual response in V4. Fig. R7 illustrates the difference between the average V4 activity elicited by borders with preferred and non-preferred border-orientation, with the latency marked.

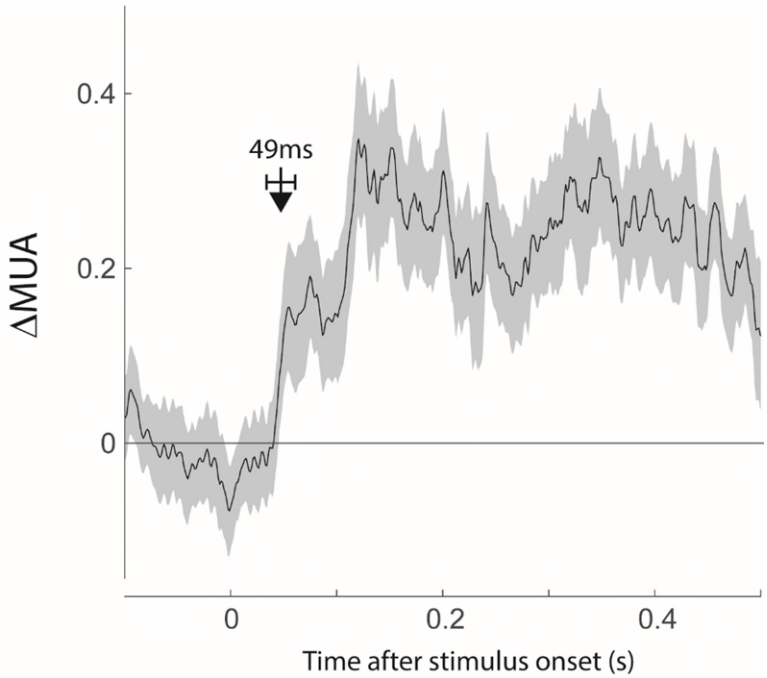


Figure R7. The difference in V4 MUA elicited by borders with the preferred and non-preferred orientation. *The latency is marked. The shaded region is +/-1 s.e.m across V4 recording sites.*

Reviewer #3 (Remarks to the Author):

Jeurissen et al. investigate the relationship between Border Ownership cells in V4 and Figure-Ground-Modulation in V1. Using simultaneous recordings with multielectrode arrays from V4 and V1 they demonstrate that noise correlations between pairs of V4/V1 neurons are stronger when the V1 neuron's RF is on the preferred side of the V4 neuron's border. This is consistent with the interpretation that V4 neurons provide excitatory input to those V1 neurons and less excitation or inhibition to neurons on the non-preferred side of the border. A computational model using the observed functional coupling between the areas replicates key features of FGM in V1.

The paper provides novel insight into the computations underlying FGM and BO, and provides a first explicit link between these two subfields of investigation. The explicit formulation in terms of a model is a big plus as it moves ideas of processing from the abstract "top-down feedback" to the specifics implemented in the model.

The paper is well written and easy to follow. I have a few comments and requests for clarification:

We would like to thank the reviewer for their positive feedback, suggestions and careful reading of the manuscript.

The choice of "good" and "bad" as labels for the agreement between V4 and V1 cells is unfortunate and potentially confusing. There is nothing bad about the "bad" agreement. Preferred side of the border and non-preferred side of the border? Congruent/ incongruent spatial arrangement?

We understand the reviewer's point, however we have tried various alternative terminologies and we find that the writing becomes very convoluted. We prefer the terms 'good' and 'bad' as they are very intuitive. We thank the reviewer for the suggestion but we prefer to keep the terminology as stated in the original manuscript. We have elaborated more on our choice of terminology in the results section on lines 224-226.

The agreement angle is circular on pi , but used as a linear variable in the regression analysis. Please explain how this was addressed.

Thanks for pointing out this possible source of confusion. We improved our writing, stating that the agreement angle θ_{BO} is the absolute value of the angle after it has been mapped onto the interval [-π,π]" so that values close to 0 implies that RFs are in agreement and values close to π are for RFs in non-agreement. Agreement angle is therefore not circular, but bounded by [0, π]. Linear regression with such a bounded predictor (independent variable) is standard.

Line 307. Why suppressive? Couldn't it be just less excitation? If it was suppressive shouldn't the noise correlations be negative (at sufficiently large distance)? Please discuss.

We agree with the reviewer that we cannot conclusively state whether the effect of V4 feedback at bad agreement angles is suppressive or less excitatory. Inhibitory connections do not necessarily result in negative noise correlations because noise correlations typically have a positive offset, which may be caused by non-specific sources related to variations in e.g. arousal. Suppressive connections might work to cancel out this positive offset, reducing the magnitude of the noise correlations

compared to excitatory connections that increase the magnitude. We have adjusted our description of the results and acknowledge the reviewer's point on lines 325-328 and 588-591.

Line 311. This seems an important point (i.e., the arrow of causality) that should be made more explicit in the Discussion.

We have now tested the causal influence of feedback connections in a new experiment in a third monkey, as was outlined in the introduction of this rebuttal above. In short, we found that weak electrical micro-stimulation of V4 neurons increase activity of V1 neurons with RFs in agreement with V4 border-ownership relative to the activity of V1 neurons in bad agreement. The similarity between the noise correlation and V4 micro-stimulation results is in accordance with our proposal that feedback connections from V4 to V1 are arranged according the border-ownership tuning of the V4 cells. We have added these new data to the paper as the new Figure 6.

Line 388. This could use some unpacking; I see that the refined model does better, but can you give an intuitive explanation?

The refined model differs from the initial model in that the spatial profile of excitatory feedback on the side of agreement is not the mirror image from the profile of inhibitory feedback on the side of non-agreement. Specifically, the overall feedback influence from V4 to V1 in the refined model is excitatory. Whereas the *a priori* model did not respond to a checkerboard stimulus because the excitatory and inhibitory influences cancelled, units of the refined model responded to the borders of the checkerboard because of the net excitatory feedback influence.

Line 935. As written, this is not a test of significance.
Presumably FBM and BO labels were shuffled in the significance test and not in the bootstrap estimate of the mean. Reword or explain.

Thanks for pointing out this typo in the description of the test, which has been corrected. The test described on line 344 does not involve label shuffling but instead follows the logic of a bootstrap t-test as explained here: https://garstats.wordpress.com/2019/07/25/boott/. Under the assumptions of the null distribution, the mean difference between the FBM and BO latencies is zero. We therefore derive a surrogate null distribution by subtracting the individual condition means from the FBM and BO resampled distributions. This yields a null-distribution with a mean of zero, but captures the variance of the distribution that would be expected by chance sampling. We then compared the observed latency difference to this null distribution. We improved the description of the test on lines 1066-1075. We have also used simulations to verify that this test has a Type I error-rate of 5%.

Line 945. The model code should also be made available.

We now provided the model code on GitHub at:

https://github.com/fattsmellf/BorderOwnership

Supplementary materials were not available online. The video for 4A is probably helpful. It is not clear what the other supplementary figures are.

Our apologies, the SI was sent through later by the editor but it was apparently not received by all reviewers. The SI for the revised manuscript should now be available.

Reviewer #4:

Jeurissen et al. examine if the connectivity of cortical feedback inputs from border-ownership (BO) neurons in V4 could underlie figure/ground modulations (FGM) in V1. The manuscript proposes a model with a connectivity scheme between BO V4 neurons and V1 that would be consistent with V4 inputs mediating the experimentally observed FGM in V1.
This model predicts V4 influences to be excitatory or inhibitory depending on the V4 neurons' BO tuning and the position of of the V1 neurons' receptive fields. To test these predictions, the authors describe how noise correlations relate to the receptive field and the BO tuning of V1/V4 neurons in multi area recording from two monkeys. They find evidence that noise correlations during spontaneous and evoked activity depend on the relation of the BO tuning and receptive field of V1 neurons with a structure that is like the one proposed by the model. Finally, they modify the original model to more faithfully map to the measured V4-V1 connectivity, as measured by the noise correlations, and find that it provides a better explanatory power over the original one.

There is evidence that cortical feedback is involved in FGM. However, the neural mechanisms by which cortical feedback inputs would implement FGM are unknown. The manuscript proposes and interesting simple mechanism for FGM involving connectivity rules that depend on the BO tuning and the retinotopic position of the V1 neurons. This is an important contribution to our understanding FGM and, more generally, on the circuit mechanisms underlying visual perception. The manuscript is clearly written, and the data is well presented.

We would like to thank the reviewer for their careful reading of the manuscript.

A weakness of the approach is that the connectivity kernel proposed by the model is tested using noise correlations, a metric that only indirectly relates to direct feedback connectivity. Importantly, several connectivities not involving direct feedback connections from V4 to V1 could also give rise to the same noise correlation structure. While this is acknowledged in the discussion, it remains a limitation, nevertheless. If the connectivity pattern could also be backed with a more direct method, it would greatly strengthen the manuscript. Besides this, it have some concerns regarding the core measurements of the noise correlations and other suggestions for improvements.

We agree with the reviewer's concerns about the directionality of the effect. As was described in the introduction to this rebuttal above, we have carried out an experiment in third monkey, in which we causally manipulated activity in V4 using electrical micro-stimulation. In short, we found that V4 micro-stimulation increased the activity of neurons at V1 sites with RFs that were in good agreement with the border-ownership tuning of the stimulated V4 neurons relative to the activity of neurons at V1 recording sites with RFs in non-agreement. The similarity between the spatial pattern of noise correlations and the new micro-stimulation results is in accordance with our proposal that feedback from V4 to V1 is arranged according the border-ownership tuning of the V4 cells. The micro-stimulation data have been added to the manuscript as the new Figure 6.

Major:
1) Noise correlations increase as a function of the firing rate of the neurons . Thus, differences in joint firing rates in V1-V4 neurons might explain some of the observed differences. This confound

might contribute to the differences in noise correlations in Figure 5 , as firing rates are expected to be lower when the stimulus is away from the preferred orientation than when it is not. In addition, neurons in bad agreement with the preferred border orientation are likely to be suppressed because they are on the background, while those with good agreement are expected to increase their firing (at least when borders are close to the preferred BO). The confounds of these expected firing rate differences on the noise correlations should be addressed for the analyses in both Figures 4 and 5.

We agree with the reviewer that it is important to consider the effects of firing-rate of noise correlations, as noise correlations have been shown to be higher for cells with high firing-rates (Cohen and Kohn, 2011; Smith and Kohn, 2008). We note that differences between firing rates might have contributed more to the stimulus-evoked noise correlations in Figure 5 than to the correlations in spontaneous activity of Figure 4. In Figure 4, the neurons are not being driven by visual stimuli and firing-rates are expected to be low. Furthermore, the data was de-trended and z-scored before the correlation analysis.

We have now examined how the noise correlations relate to the firing-rate of the V1 neurons in more detail (the new Figure 5D, replicated below as Fig. R8). We split trials according to whether the V1 RF was situated on the figure (high average firing-rate) or the background (low average firing-rate). When the V4 RF was well-driven by its preferred border, the noise correlation depended on the activity at the V1 recording site because it was higher for V1 responses elicited by figures than responses elicited by backgrounds. These results are compatible with a general effect of firing rates on the strength of noise correlations, but they could also reflect a stronger functional interaction between border ownership and the representation of the figure. Interestingly, when the V4 neurons responded to a border with a non-preferred ownership, there was no effect of V1 figure-ground modulation on the noise correlation.

If the strength of noise correlations only depends on the activity level of the V1 and V4 neurons, it should be lowest between responses of V4 neurons to their non-preferred border and responses of V1 neurons to the background. Instead, the lowest correlations occurred between V4 cell responding to the preferred border, and the V1 neurons with RFs in bad-agreement falling on the background.

These analyses, taken together, suggest that the strength of V1-V4 noise correlations depends on the agreement angle and that it is modulated by figure-ground assignment when the V4 cell is encoding its preferred boundary.

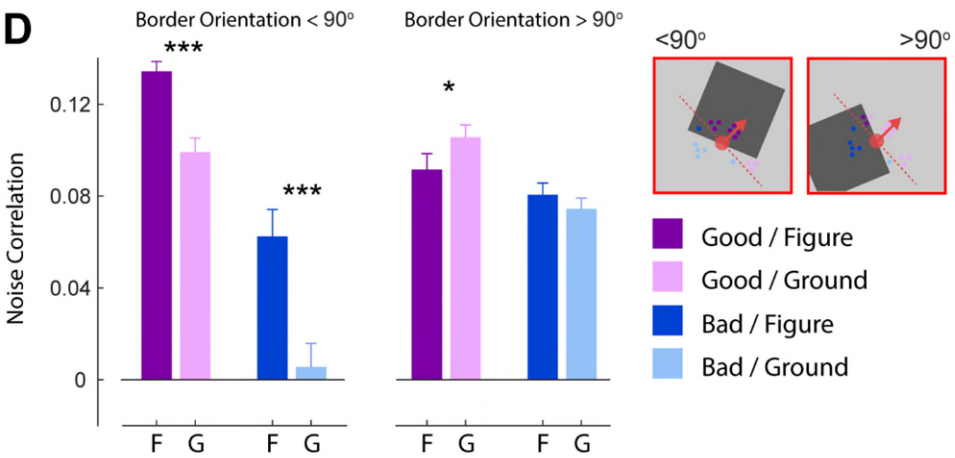


Figure R8. The effect of figure-ground modulation on the noise correlations between V1 and V4. We split trials according to whether the V1 RF center was on the figure or background, according to

whether the V4 neurons responded to their preferred orientation (Border orientation < 90°) or not (Border orientation > 90°), and the Agreement Angle of the pair.

2) Noise correlations are known to also depend on tuning similarity. Is the relations of noise correlations with BO preference and V1 RF position independent of their orientation tuning similarities?

Our main finding is that V4-V1 pairs with RFs in good agreement have stronger noise correlations than those with RFs in non-agreement. We don't believe that this difference can be explained by differences in tuning similarity.

We note that V1 multi-unit activity contains responses from nearby single cells with varying orientation tuning, which causes less sharp tuning than that of some single units. Unfortunately, we did not measure orientation tuning of the V1 cells and we can therefore not further investigate this question.

3) It is unclear why a linear regression model is not used to analyze both spontaneous and evoked activities. The manuscript would benefit if similar analyses were used in both conditions.

We have now implemented a linear model to also analyze the evoked noise correlations as suggested by the reviewer. We implemented two models, first a simple model replicating the model used for analyzing the spontaneous noise correlations with Agreement Angle and RF Distance as the predictors. This model replicated the results observed using spontaneous activity as would be expected (Figure R9, Figure S5A).

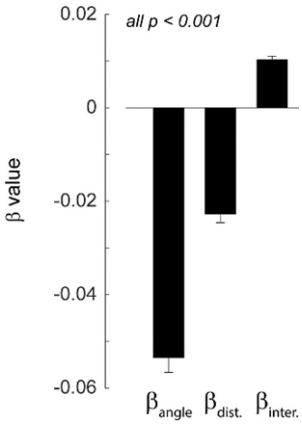


Figure R9. *Beta co-efficients from the simple linear model run on activity during the sustained period of the response (100-500ms). The beta-coefficients are for agreement angle, RF-distance and their interaction.*

We next implemented a more complex model, including the relationship between the border of the figure and the V4 cell's tuning preference ('Border') and whether the V1 RF fell on the figure or background as extra predictors in the model ('FG'). With four predictors there are 6 two-way interactions, 4 three-way interactions and a four-way interaction to consider, which makes interpretation of the model coefficients complex and potentially confusing. We observed a significant 4-way interaction between the predictors ($p<10^{-8}$), which indicates that we could not simplify the model. Because of this complexity, we believe that the reader would appreciate it if we break this model into its components and focus on the more relevant and interesting comparisons in the revised Figure 5C,D (Fig. R8 above). The results of the more complex model are as follows:

Effect	Estimate	p-value
Angle	-0.07	$<10^{-12}$
Distance	-0.01	0.2
Border	0.007	0.5
FG	0.12	$<10^{-4}$
Angle x Distance	0.007	0.01
Angle x Border	0.02	0.0004
Angle x FG	-0.02	0.21
Distance x Border	-0.004	0.17
Distance x FG	-0.03	0.001
Border x FG	-0.08	$<10^{-7}$
Angle x Distance x Border	0.0001	0.93
Angle x Distance x FG	0.018	$<10^{-5}$
Angle x Border x FG	0.017	0.03
Distance x Border x FG	0.024	$<10^{-6}$
Angle x Distance x Border x FG	-0.012	$<10^{-8}$

The strong two-way interaction between agreement angle and border orientation is presented in Figure 5C. This interaction relates to how the difference in the strength of noise correlations between good and bad pairs depends on the whether the V4 cell is being driven by its preferred or non-preferred orientation. The strong border orientation x FB effect is captured by the new Figure 5D, showing that the influence of figure/ground in V1 on the noise correlations is only pronounced when V4 neurons are well-driven by their preferred border orientation. These results are now presented in Table 1 of the Supplementary Information.

4) Lines 272-275. Some of the main analyses of Figure 4 , specifically those in panels 4D and 4E seem not to have associated statistics backing the claims.

We have added post-hoc statistical comparisons to Figures 4D and 4E, the data in each bin were compared using an independent samples t-test. We included the Satterthwaite correction for unequal variances and a Bonferroni correction to correct for multiple comparisons.

Minor.

5) In a previous study the same lab found that the suppression of the ground segment in u o n shaped figures depends on learning a task (Self et al 2019). As the connectivity kernel used in Figure 6 was obtained in non-trained monkeys, it would make sense to fit the model to the modulation observed in naïve animals. Yet, the modulations modeled in Figure 6C,D seem to correspond to the trained monkey from Self et al. 2019.

This is an interesting observation and the reviewer is correct, the connectivity kernel predicts a modulation profile that is more similar to the trained monkey response in our previous study. One possibility is that the monkeys of our previous study failed to completely process the interior boundaries of the U shape before they were trained. The weaker border-ownership response in V2/V4 for this interior boundary may have caused to weaker suppression of the V1 representation of the center of the figure. Future studies could examine how familiarization with a shape influences

the activity of neurons tuned to border-ownership.

6) Line 183, do the authors meant “parallel” instead of perpendicular?

Thank you for spotting this, we have corrected this to ‘parallel’.

7) Line 330, “suggesting” would be more appropriate than “indicating” as the observation is purely correlational.

We have implemented the change.

8) Line 344-245 . The number of good and bad pairs included seems to be missing.

These numbers have been added, there were 492 good pairs and 315 bad pairs.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors did an excellent revision and replied to all my comments, providing more data. Especially, I applaud the new microstimulation data that supports their interpretation of the noise correlations. Regarding the new results, I have a couple of comments that the authors should address.

1. The development of the noise correlations across time (Figure S5B) shows a strong increase after 300 ms for the bad alignment pairs. Any explanation? Please discuss.

2. The new microstimulation data are interesting, but more details are needed about the procedure and data analysis. Could the authors show some spike trains before and after microstimulation (I assume that they do not have interleaved trials without microstimulation), so that the reader can evaluate better the size of the microstimulation effects? What was the monkey doing (fixating?) and was there a visual stimulus? Also, what was z-scored and entered the linear model: baseline-subtracted firing rate, i.e. difference between stimulation and no-stimulation? I assume n in the figure refers to the number of V4 stimulation– V1 recording pairs. Are these independent because the same V1 site will contribute more than once and this can create dependencies if the effect of microstimulation depends on the site, irrespective of other variables? The latter should be considered when doing the statistics. Please provide more details about this interesting experiment and the data analysis.

Reviewer #2 (Remarks to the Author):

This is a successful revision in which the authors have addressed all the comments raised by the reviewer. In particular, adding the new Figure 6 strengthens the conclusions. I have no further comments.

Reviewer #3 (Remarks to the Author):

The authors have done an excellent job addressing concerns raised in the previous round of review. The new microstimulation experiment clearly adds powerful evidence in support of their hypothesis.

Reviewer #4 (Remarks to the Author):

The manuscript has greatly improved from the previous version with the inclusion of microstimulation experiments and new analyses. The authors have addressed all my comments and I recommend the article for publication.

Our reply in blue.

Reviewer #1 (Remarks to the Author):

The authors did an excellent revision and replied to all my comments, providing more data. Especially, I applaud the new microstimulation data that supports their interpretation of the noise correlations.

We thank the reviewer for their positive assessment.

Regarding the new results, I have a couple of comments that the authors should address.

1. The development of the noise correlations across time (Figure S5B) shows a strong increase after 300 ms for the bad alignment pairs. Any explanation? Please discuss.

This is an interesting observation. We note that the strength of border-ownership modulation begins to reduce towards the end of the trial (See Figure 5A), suggesting that there is a reduction in the strength of the representation of the object. This may reduce the level of feedback to V1 allowing the bad agreement pairs to become more correlated. We feel however that this is rather speculative and we have not added this to the paper.

2. The new microstimulation data are interesting, but more details are needed about the procedure and data analysis. Could the authors show some spike trains before and after microstimulation (I assume that they do not have interleaved trials without microstimulation), so that the reader can evaluate better the size of the microstimulation effects? What was the monkey doing (fixating?) and was there a visual stimulus? Also, what was z-scored and entered the linear model: baseline-subtracted firing rate, i.e. difference between stimulation and no-stimulation?

We have added the requested extra details to the methods section of the manuscript. The monkey was fixating on a central red dot on a grey background during the micro-stimulation experiment, there were no other visual stimuli present. Z-scoring was done on the data in the period following micro-stimulation (35-100ms). The methods section now reads as follows:

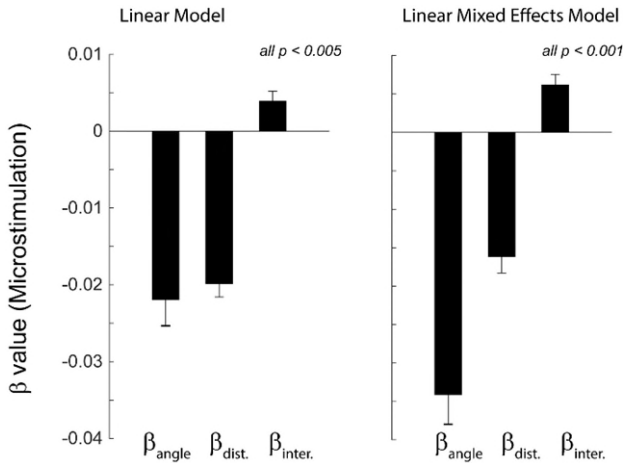
"In monkey N we applied micro-stimulation to a subset of V4 recording sites and measured the effects on MUA in V1 (n = 448 sites) in a single recording session. We first measured the RFs of V1 and V4 and the border-ownership tunings as described above. We then selected 62 V4 sites with RFs that were well fit by the Gaussian function described above (R2 > 0.6) to be micro-stimulated. We connected a stimulation device (Cerestim - Blackrock microsystems) to one bank of V4 electrodes (32 recording sites) and then randomly selected on each trial which site was stimulated from the pre-selected sites, while recording from V1. After a total of 50 trials at each selected site, we switched the stimulator to a different bank of V4 sites and repeated the experiment. We applied 5

bipolar pulses of 5 µA at a frequency of 200Hz (pulse-width = 170 µS, interphase-delay = 60 µS). The return electrode was a subdural wire. The procedure was divided into trials, the monkey initiated the trial by fixating on a red circle of 0.3^0 radius on an otherwise uniform grey screen. After 200ms the micro-stimulation train was triggered and the monkey had to carry on fixating for a further 200ms to receive a juice reward. The micro-stimulation pulses caused large artifacts in the recorded signal and we therefore focused on V1 activity 35-100ms after the onset of the stimulation train. We removed trials containing outlying values in this time period using the iterative procedure described above. We then z-scored the V1 data of each channel in the time period 35-100ms by subtracting the mean activity of the recording site across all trials and samples and then dividing by the standard deviation. We examined the difference between good and bad agreement pairs use a linear mixed effects model with random intercept terms for the V1 and V4 channel IDs, this is because each recording site appeared multiple times in the dataset due to the pairing with other sites. The mean z-scored data on each trial was also entered into a linear model as described above with the agreement angle and distance between the V1 and V4 RFs as predictors. Linear mixed effects models were also run using the array identity of the V4 and V1 electrodes as random intercept terms."

We note that it is not possible to show pre- and post-stimulation spike trains as we recorded the envelope of the multi-unit activity rather than individual spikes.

I assume n in the figure refers to the number of V4 stimulation– V1 recording pairs. Are these independent because the same V1 site will contribute more than once and this can create dependencies if the effect of microstimulation depends on the site, irrespective of other variables? The latter should be considered when doing the statistics. Please provide more details about this interesting experiment and the data analysis.

The reviewer is correct that n was the number of V1-V4 pairs we recorded from. Each V4 and V1 site contributed multiple data-points due to the pairing between the sites, we therefore also ran a linear mixed effects model with random intercept terms for the V1 and V4 channel IDs. This approach models dependencies of the effect of micro-stimulation on recording site. We found the same result as in the original t-test and we have replaced the t-test with the linear mixed effects model in the manuscript on line 335. We have also analyzed the relationship between the effect of micro-stimulation and agreement angle/RF distance using a linear mixed effects model (see Reviewer Figure below). The results of this model was in good agreement with the basic linear model in the original manuscript and all effects were highly significant at the $p < 0.001$ level. This analysis has now been reported in the manuscript on line 339.



Reviewer Figure. Beta-coefficients were very similar for a linear model and a linear mixed effects model. Conventions as in Figure 6F.