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Distinct Feedforward and Feedback Effects of Microstimulation in Visual Cortex Reveal Neural Mechanisms of Texture Segregation

Highlights

- Microstimulation of visual cortex evokes local excitation followed by inhibition
- Microstimulation of V1 causes feedforward excitation and inhibition in V4
- Microstimulation of V4 only causes feedback-based reductions in V1 firing rates
- When V4 is suppressed by microstimulation, V1 figure-ground segregation is reduced

Authors

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In Brief

Klink et al. probe interactions between visual cortical areas V1 and V4 with electrical microstimulation. Microstimulation effects reliably propagated in the feedforward direction. In the feedback direction, they depended on visual stimulation and figure-ground segregation. These results reveal the driving and modulatory roles of feedforward and feedback connections, respectively.

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Distinct Feedforward and Feedback Effects of Microstimulation in Visual Cortex Reveal Neural Mechanisms of Texture Segregation

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SUMMARY

The visual cortex is hierarchically organized, with low-level areas coding for simple features and higher areas for complex ones. Feedforward and feedback connections propagate information between areas in opposite directions, but their functional roles are only partially understood. We used electrical microstimulation to perturb the propagation of neuronal activity between areas V1 and V4 in monkeys performing a texture-segregation task. In both areas, microstimulation locally caused a brief phase of excitation, followed by inhibition. Both these effects propagated faithfully in the feedforward direction from V1 to V4. Stimulation of V4, however, caused little V1 excitation, but it did yield a delayed suppression during the late phase of visually driven activity. This suppression was pronounced for the V1 figure representation and weaker for background representations. Our results reveal functional differences between feedforward and feedback processing in texture segregation and suggest a specific modulating role for feedback connections in perceptual organization.

INTRODUCTION

Visual stimuli elicit a complex pattern of neuronal activity that spans a large number of cortical areas. In primary visual cortex (V1), the first cortical stage of visual information processing, neurons encode elementary features such as the orientation of line elements. After V1, activity is propagated to higher visual areas that represent more complex aspects of the visual world (Felleman and Van Essen, 1991; Salin and Bullier, 1995). How do neurons across different areas interact with each other when we interpret what we see? Feedforward connections drive neurons in higher areas so they can combine information and construct more complex receptive field (RF) properties. Higher-level neurons are silent when upstream activity in lower areas is blocked (Schmid et al., 2010). There is a similarly dense set of feedback connections from higher areas down to the lower areas. These connections are thought to modulate visually driven activity but to be less capable of evoking activity in the absence of stimuli (Hupé et al., 1998; Moore and Armstrong, 2003; Nassi et al., 2013; Roelfsema, 2006), which implies a functional asymmetry between feedforward and feedback effects (Crick and Koch, 1998; Lamme and Roelfsema, 2000; Sherman and Guillery, 1998). Accordingly, top-down effects on V1 activity are stronger when a stimulus is visible than when it is kept in working memory (van Kerkoerle et al., 2016). Anatomical information from monkeys further supports such an asymmetry, with feedforward connections targeting neurons in input layer 4 of higher cortical areas and feedback connections avoiding layer 4 and instead targeting the superficial layers and layer 5 of lower cortical regions (Felleman and Van Essen, 1991; Salin and Bullier, 1995).

Here, we used electrical microstimulation to study the interactions between a lower and higher area of visual cortex by perturbing neuronal activity in one area and measuring its distal effects in feedforward or feedback directions. There is an extensive body of work investigating how intracortical electrical microstimulation affects neuronal activity in the vicinity of the electrode (Clark et al., 2011; Histed et al., 2013; Tehovnik, 1996; Tehovnik et al., 2006). The prevailing view is that microstimulation directly activates a pool of neurons near the tip of the stimulation electrode, probably through initiation of action potentials in their axons, which are highly excitable. There have also been many previous studies that examined how microstimulation influences behavior (Cicmil and Krug, 2015; Clark et al., 2011). Microstimulation of the V1, for example, can produce reportable phosphenes, artificial percepts of light at the location of the RF of the stimulated neurons (Bartlett et al., 2005; Schiller and Tehovnik, 2008; Schmidt et al., 1996; Winawer and Parvizi, 2016). Furthermore, microstimulation of small groups of neurons in sensory

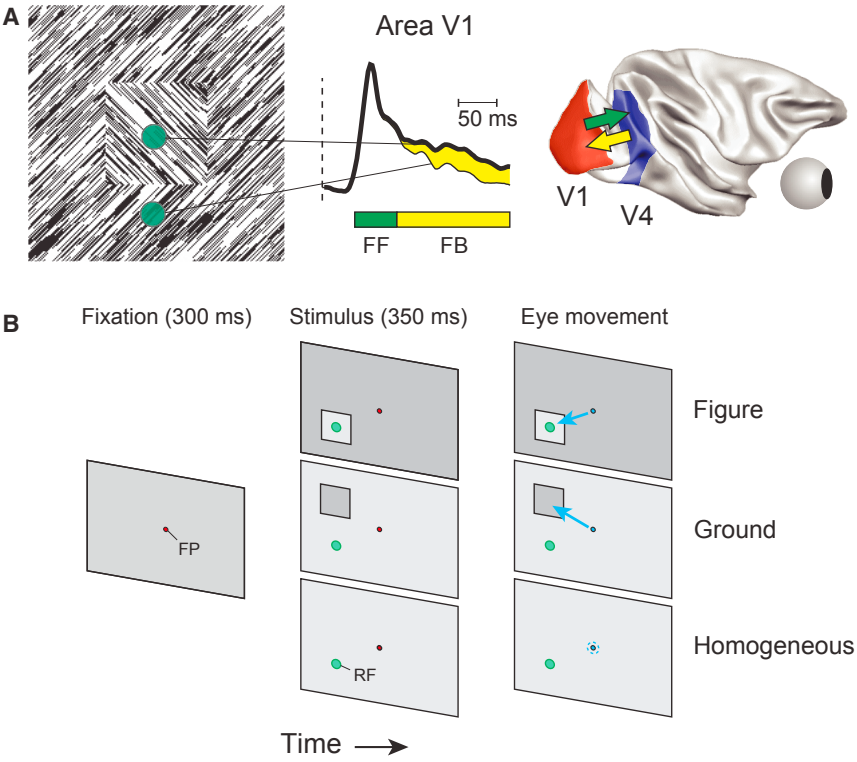

Figure 1. Testing the Effect of Microstimulation on Feedforward and Feedback Processing during Figure-Ground Segregation

(A) Left: texture-segregation stimulus where a square figure is superimposed on a background with an orthogonal texture. On average, the local features in the neurons' RF were held constant by using both orientations for the figure and the background. In area V1 and V4, responses elicited by a figure are stronger than responses elicited by the background, a difference called figure-ground modulation (yellow in the middle panel). The initial V1 response is driven by feedforward connections (FF; green bar), and the response modulation is thought to depend on feedback from higher visual areas (FB; yellow bar).

(B) The texture appeared when the monkeys had maintained their gaze on the fixation point for 300 ms, causing image elements of the figure, background, or a homogeneous texture to fall in the neurons' RF (green circle). The monkeys' task was to make an eye movement toward the figure (blue arrow), unless the texture was homogeneous, in which case they had to maintain fixation (dashed circle).

cortices, tuned to a particular feature, biases perception and makes the animal more likely to report perceiving that feature (DeAngelis et al., 1998; Salzman et al., 1990). The implication is that microstimulation of a sensory area must cause widespread effects on other brain areas, including areas that are involved in response selection.

The influence of microstimulation on distal brain areas has been investigated using fMRI (Ekstrom et al., 2008; Logothetis et al., 2010; Moeller et al., 2008; Sultan et al., 2011; Tolias et al., 2005). Some fMRI studies have reported that microstimulation causes excitation of neurons at the next cortical area but that this excitation does not propagate beyond one hierarchical level (Logothetis et al., 2010; Sultan et al., 2011). Such an isolated stimulation effect seems to be incompatible with the effect of microstimulation on behavioral choice, which implies an impact on high-level decision stages as well (Gold and Shadlen, 2007). However, the temporal resolution of fMRI is limited, and fMRI signals are only indirectly related to neuronal spiking activity. A direct comparison of the propagation of microstimulation-induced spiking activity in the feedforward and feedback directions has been lacking.

We tested the effects of electrical, intracortical microstimulation in macaque monkeys carrying out a texture-segregation segregation task (Figure 1A) in which they had to detect a square figure with line elements of one orientation on a background with line elements of the opposite orientation (Lamme, 1995). We recorded from and stimulated in V1 and visual cortical area V4. Area V4 is higher up in the visual cortical hierarchy and contributes to figure-ground segregation, as demonstrated by neurophysiological studies (Poort et al., 2012) and lesion work (Merigan, 1996). Visual cortical processing in the texture segregation task is characterized by an initial feedforward processing phase and a later recurrent phase (Lamme, 1995; Poort et al., 2012; Self et al., 2013; Supèr et al., 2001). In the initial phase, feedforward connections propagate visual information from the lateral geniculate nucleus to V1 and then onward to higher visual areas (Figure 1A, FF). At this time, both V1 and V4 neurons represent the local orientation of the contour elements that fall in their RFs. In the subsequent, recurrent phase, horizontal and feedback connections (Figure 1A, FB) come into play. They propagate activity from higher areas back to lower areas to increase the activity of neurons with a RF that falls on the figure. Now, the entire figural region is labeled with enhanced neuronal activity. The presence of two distinct processing phases in this task allows us to distinguish the influences of microstimulation-induced activity perturbations during different visual processing stages by simply varying the timing of microstimulation relative to the onset of a visual stimulus.

In the current study, we addressed the following questions: (1) Is there an asymmetry in feedforward and feedback microstimulation influences? (2) How does microstimulation interact with visually driven activity? (3) How do microstimulation effects interact with figure-ground organization? Our results reveal a clear asymmetry in feedforward and feedback microstimulation effects. In the feedforward direction, V1 microstimulation immediately drives V4 activity, even in the absence of a visual stimulus. This excitatory effect is followed by later phase of suppression. In the feedback direction, however, V4 microstimulation only decreased V1 activity in a late time window when V4 neurons themselves were also silenced. This suppressive feedback effect was particularly pronounced during the late phase of visual processing in V1, and it was stronger

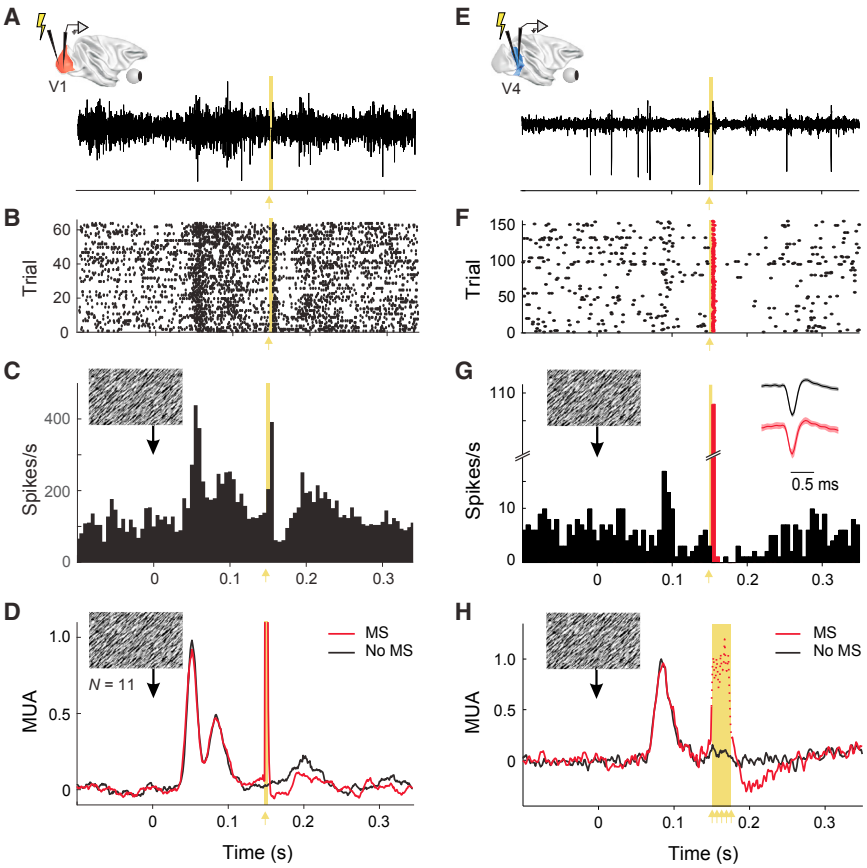

Figure 2. Microstimulation Causes Excitation followed by Inhibition

(A) V1 multi-unit activity (MUA) elicited by a full-screen texture and a single microstimulation pulse (yellow line) in a single trial.

(B) Raster plot showing spikes on consecutive trials (black dots). The full-screen texture appeared at time zero and evoked a visual response after ~40 ms. The single microstimulation pulse at 150 ms elicited spikes in almost every trial.

(C) Average response in trials with microstimulation. The spikes that were elicited by microstimulation were followed by a phase of inhibition.

(D) Average response of eleven unique combinations of stimulation (stimulation strength 32 ± 6 μA) and recording electrodes in V1. On microstimulation (MS) trials (red line), a single pulse of stimulation evoked a brief excitation, followed by a delayed phase of suppression that lasted ~70 ms.

(E) Activity of a well-isolated V4 neuron on a single trial elicited by a single microstimulation pulse (yellow line).

(F) Raster plot showing spikes on consecutive trials (black dots). The full-screen texture appeared at time zero and evoked a visual response after ~90 ms. The single microstimulation pulse at 150 ms elicited an extra spike (red dot) in almost every trial.

(G) Average response in trials with microstimulation. The spikes that were elicited by microstimulation on most trials (red bin, note interrupted y axis) were followed by a phase of inhibition. The inset demonstrates that the spikes elicited by microstimulation have the same shape as those fired spontaneously or in response to the visual stimulus.

(H) Influence of five microstimulation pulses within V4 on MUA at an adjacent electrode (red curve). The dashed part of the red curve represents a remnant of the stimulation artifact, which was difficult to remove when we stimulated an adjacent electrode of the same array. However, the delayed phase of inhibition, which lasted ~70 ms, can be seen. The black curve represents MUA on trials without microstimulation.

See also Figure S1.

for the representation of texture-defined figures than for backgrounds.

RESULTS

Local Effects of Intracortical Electrical Microstimulation

We implanted chronic electrode arrays in V1 and V4 of four monkeys (B, C, D, and M) that were trained to perform the texture-segregation task. Previous studies have demonstrated that the local effect of electrical microstimulation on neurons in the vicinity of the stimulated electrode consists of a brief phase of excitation followed by longer-lasting inhibition (Butovas and Schwarz, 2003; Histed et al., 2009; Houweling and Brecht, 2008; Seidemann et al., 2002). We replicated both phases in pilot experiments in which we stimulated bipolarly through two adjacent electrodes of one of the arrays, and we recorded single-unit activity (SUA) or multi-unit activity (MUA) from nearby electrodes of the same array. During these local microstimulation experiments, the monkeys maintained gaze at a fixation point while we presented a texture with a homogeneous orientation (Figure 1B). We developed a method to remove the electrical stimulation artifact from the data (Figure S1). Figures 2A–2C and 2E–2G illustrate the influence of a single pulse, 150 ms after the appearance of the visual stimulus delivered through adjacent electrodes on the array in V1 (40 μA) and V4 (60 μA), respectively. In the V1 example, we recorded MUA, while in the V4 example we also obtained data from a well-isolated single neuron. In both areas, microstimulation caused one or a few spikes on almost every trial, followed by ~70 ms of reduced activity. The average of 11 unique combinations of stimulation (32 ± 6 μA) and recording electrodes in V1 further illustrates this profile of microstimulation-evoked excitation followed by a period of suppression (Figure 2D). A similar profile was observed at another V4 MUA recording site in response to five microstimulation pulses at an adjacent electrode pair (Figure 2H), but in this case, we were unable to remove the microstimulation artifacts (due to the proximity of the stimulated electrodes), resulting in an occlusion of the early excitatory effect. These local stimulation results are in accordance with previous studies (Butovas and Schwarz, 2003; Histed et al., 2009; Houweling and Brecht, 2008; Seidemann et al., 2002) and provide a starting point for our main objective: investigating how microstimulation effects propagate in feedforward and feedback directions at different

phases of the neuronal responses elicited by a figure-ground stimulus.

Feedforward Microstimulation Effects: Influence of V1 Stimulation on V4 Activity

To examine the feedforward influence of the microstimulation pulse train, we stimulated neurons in V1 and recorded MUA in V4 during the texture-segregation task. In 67% of the trials, the stimulus contained a texture-defined square figure, which either fell inside the RF of the V1 and V4 neurons (33% of the trials) or outside of it, in which case there was a background inside the RF (33% of trials; Figure 1B). In these figure-ground trials, the monkeys made a saccade to the figure location after a 350-ms fixation delay. In the final 33% of trials, the monkeys saw a homogeneous texture without figure-ground organization and they maintained their gaze on the fixation point. The animals were highly proficient in this task. Their average accuracy in homogeneous texture trials without microstimulation was 98.8% for monkey B and 99.3% for monkey C. In figure trials, the accuracy was 97.5% for monkey B and 98.3% for monkey C. We aimed to cause a small perturbation of neuronal activity by using weak microstimulation amplitudes. Accordingly, microstimulation did not influence the monkeys' accuracy at any stimulus-onset asynchrony (SOA) between stimulus appearance and the microstimulation pulses (t tests; all p values > 0.05). Microstimulation did not influence the stability of gaze either, as quantified by the amplitude (ANOVA; $F_{1,53,981} = 0.003$, $p = 0.96$) or number ($F_{1,22,886} = 0.36$, $p = 0.55$) of microsaccades that the animal made during the fixation epoch (Figures S2A–S2D).

We first examined the influence of V1 stimulation on V4 activity when the monkeys viewed the homogeneous texture (Figure 3A). Figure 3C illustrates MUA for successive trials at an example V4 site. We applied five microstimulation pulses (15 μA, 200 Hz) to a V1 region where RFs overlapped with those of the V4 site (Figure 3B). Microstimulation occurring 50–30 ms before the appearance of the visual stimulus quickly increased V4 activity, with substantial variability in the precise response profile across trials (Figure 3C). Figure 3D shows the average response elicited at this V4 recording site and demonstrates that the microstimulation-induced response was even stronger than the activity evoked by the full-screen texture stimulus.

We recorded MUA from a total of 140 V4-recording sites (62 in monkey B and 78 in monkey C, average current: 89 ± 41 μA) and observed that V1 stimulation elicited a significant excitatory response in 91% of the recording sites ($p < 0.05$ for t tests contrasting the average V4 activity in a 50-ms window after stimulation onset with activity in non-stimulation trials). This excitatory microstimulation effect was stronger if the overlap between the V1 and V4 RFs was larger (Pearson's linear correlation; $r = 0.33$, $t_{152} = 4.4$, $p < 10^{-4}$) (Figure 4A). We next examined how the V4 response depended on the timing of the V1 pulse train relative to the onset of the homogeneous texture stimulus by applying microstimulation 50 ms before, 50 ms after or 150 ms after the onset of the visual stimulus (Figures 3E–3G). Both the early excitatory and the later inhibitory effects that were elicited locally in V1 (Figures 2B–2D) reliably propagated to V4 (Figures 3F and 3G), which is in accordance with a driving influence of V1 on V4. Excitation was strongest when microstimulation was applied before stimulus onset. Temporal overlap with the visual response decreased the microstimulation effect (one-way ANOVA with pulse-timing as factor; $F_{2,417} = 72.8$, Tukey, $p < 10^{-7}$ for all pairwise comparisons in both monkeys; Figure S3A). In some of the experiments, the visual response even completely abolished the microstimulation effect (Figure S4), suggesting that the connections that are responsible for propagating the V1 microstimulation effect to V4 overlap with the connections that drive the V4 visual response. In accordance with such an "occlusion" effect, the influence of V1 microstimulation was weaker for V4 neurons with stronger visually driven activity (Pearson's $r = -0.51$, $t_{138} = 6.99$, $p < 10^{-9}$ at SOA = 150 ms; Figure 4B).

Microstimulation activates axons in the vicinity of the electrode tip (Butovas and Schwarz, 2003; Histed et al., 2009), where it can cause both orthodromic and antidromic stimulation effects. Orthodromic effects require the activation of V1 neurons projecting in the feedforward direction. These effects in V4 are probably mediated indirectly, because the direct projection from V1 to V4 is weak and confined to the foveal representation (Markov et al., 2011; Nakamura et al., 1993; Zeki, 1978). The stimulated V1 cells are likely to activate V2 neurons (and possibly the pulvinar), which in turn activate V4 neurons. Antidromic effects would occur by stimulation of V4 axon terminals projecting to V1 so that action potentials can travel backward to the V4 cell bodies. Antidromic activation is highly efficient and can only be blocked if the antidromic action potential collides with an orthodromic one. Importantly, the occlusion effect (Figure S4) suggests that the antidromic contribution to V4 activation by V1 stimulation was relatively minor. Typical interspike intervals are longer than 20–50 ms, which would be sufficient time for antidromic spikes to reach the cell bodies. In contrast, an orthodromic, transsynaptic effect readily explains the occlusion effect if the electrical stimulation activates the same projection neurons that propagate visually driven activity from V1 to V4. The stimulation current levels used here indeed typically cause most neurons to be stimulated transsynaptically (Butovas and Schwarz, 2003; Douglas and Martin, 1991). The relatively large trial-to-trial variability of the interareal microstimulation effect (Figure 3C) also supports a transsynaptic, orthodromic influence, because antidromic stimulation effects are more stereotypical.

Interestingly, V1 microstimulation also interacted with figure-ground organization in V4. In accordance with previous work (Poort et al., 2012), the texture elements of figures elicited stronger activity in V4 than those of background stimuli (Poort et al., 2012) (Figure 5B) (time window 150–350 ms; t test; $t_{61} = 15.02$, $p < 10^{-21}$). V1 microstimulation caused V4 neurons to reach a relatively constant activity level, irrespective of whether the RF fell on the figure or on the background. As a consequence, the excitatory effect was stronger for backgrounds than for figures (Figures 5A, 5C, and 5D) (paired t test; $t_{60} = 2.36$, $p < 10^{-6}$), while the delayed suppression effect was more pronounced for figures (Figures 5C and 5D) (paired t test; $t_{60} = 6.87$, $p < 10^{-8}$).

These results demonstrate that both the early excitatory phase and the later inhibitory phase elicited by microstimulation in V1 reliably propagate in the feedforward direction to area V4. The strongest excitatory effects occur when V4 neurons are only

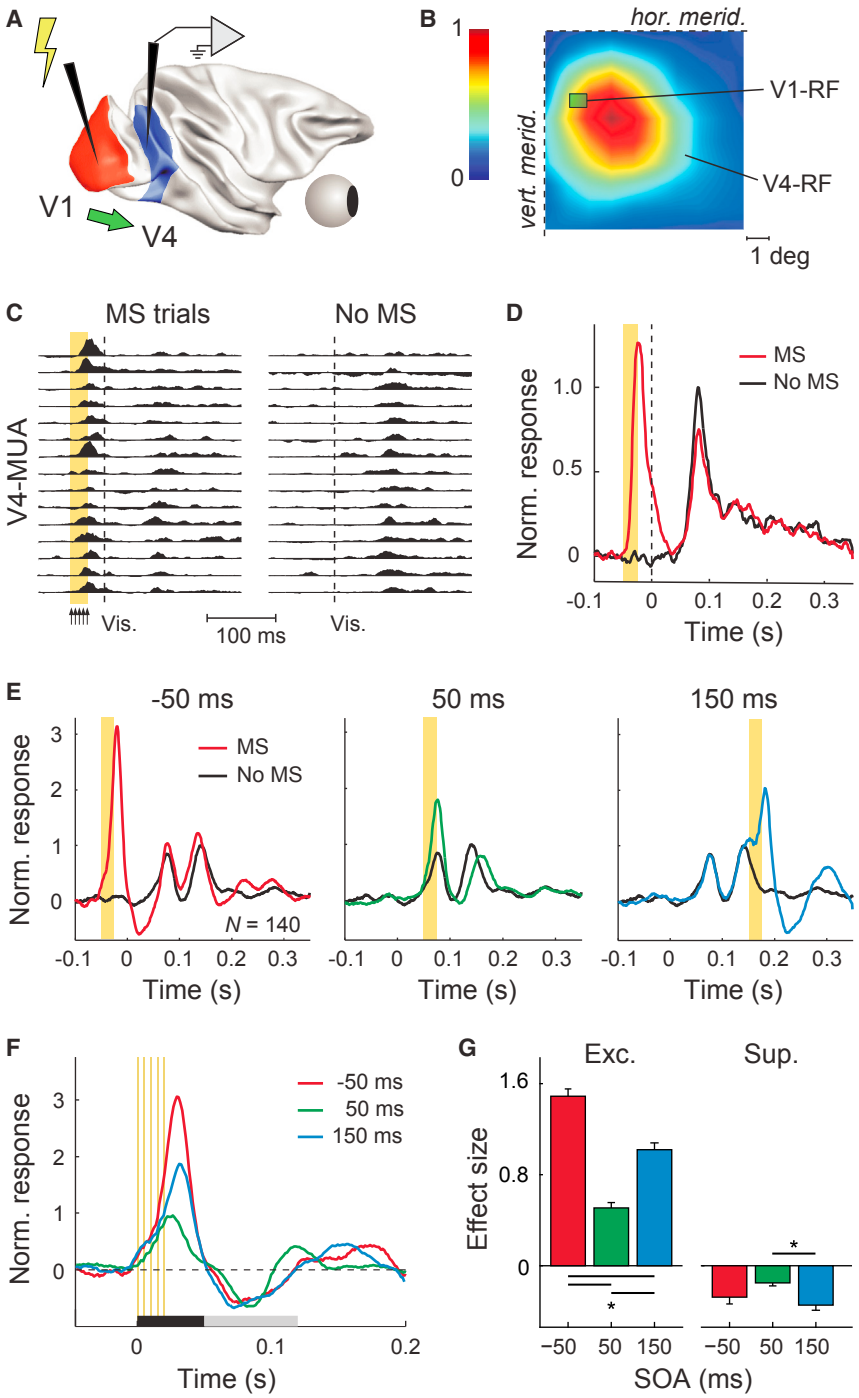

Figure 3. Effect of V1 Stimulation on Activity in Area V4

(A) Schematic of the experiment with microstimulation in V1 and recording in V4.

(B) Overlap between RFs in V1 (green rectangle) and V4 (color shows relative strength of visually driven activity).

(C) V4 MUA responses in single trials. Left: successive trials with five microstimulation (MS) pulses 50–30 ms before the appearance of the texture (arrows and yellow region). Right: visual stimulation only.

(D) Average response at the example V4 site elicited by the visual stimulus in the presence (red) and absence of microstimulation (black).

(E) Average effect of V1 microstimulation across all V4 recording sites with a significant stimulation effect (n = 140/154 sites) 50 ms before (left) and 50 (middle) and 150 ms after stimulus onset (right).

(F) Difference in activity between trials with and without microstimulation, aligned to the start of microstimulation.

(G) Average effect sizes of microstimulation at the three SOAs.

Excitation effects (left) were calculated in a 0–50 ms time window after stimulation onset (black line in F). Suppression effects (right) were determined in the 50–120 ms post-microstimulation time window. Error bars represent SEM (*$p < 0.01$, for pairwise comparisons calculated with Tukey's post hoc test). See also Figures S3 and S4.

weakly activated by a visual stimulus, supporting a driving effect of the feedforward connections.

Feedback Microstimulation Effects: V4 to V1

We next investigated the propagation of microstimulation-induced activity in the opposite direction by stimulating V4 and recording from sites with overlapping RFs in V1 (Figures 6A and 6B). Figure 6C illustrates the effect of microstimulation 150 ms after the presentation of a homogeneous texture at an example V1 recording site. V4 stimulation caused little excitation in V1, implying that the excitatory phase we saw locally in V4 (Figure 2E–2H) did not reliably propagate back to V1. In contrast, V4 stimulation caused a suppression of V1 activity at approximately the same time when V4 itself was also suppressed by V4 stimulation, starting ~30–40 ms after the onset of the pulse train. The suppression effect depended on the timing of microstimulation relative to the visual stimulus. An ANOVA comparing three SOAs (−20 ms, 50 ms, and 150 ms relative to stimulus onset) revealed that the effect was largest at 150 ms (ANOVA; $F_{2,354} = 53.89$, $p < 10^{-20}$; Tukey post hoc, $p < 10^{-9}$) (Figures 6D–6F; Figures S3B and S3C illustrate effects per monkey). Out of 289 V4-V1 combinations (148 in monkey B and 141 in C, average current 57 ± 23 μA), there were 109 cases of significant inhibition in V1 at the 150 ms SOA and only 13 cases of excitation. The excitatory influence was only visible if microstimulation preceded the visual stimulus (t tests; SOA −20 ms: $t_{118} = 6.53$, $p < 10^{-8}$; SOA 50 ms: $t_{118} = 0.43$, $p = 0.67$; SOA 150 ms: $t_{118} = 0.03$, $p = 0.97$). Thus, although microstimulation locally causes excitation followed by inhibition

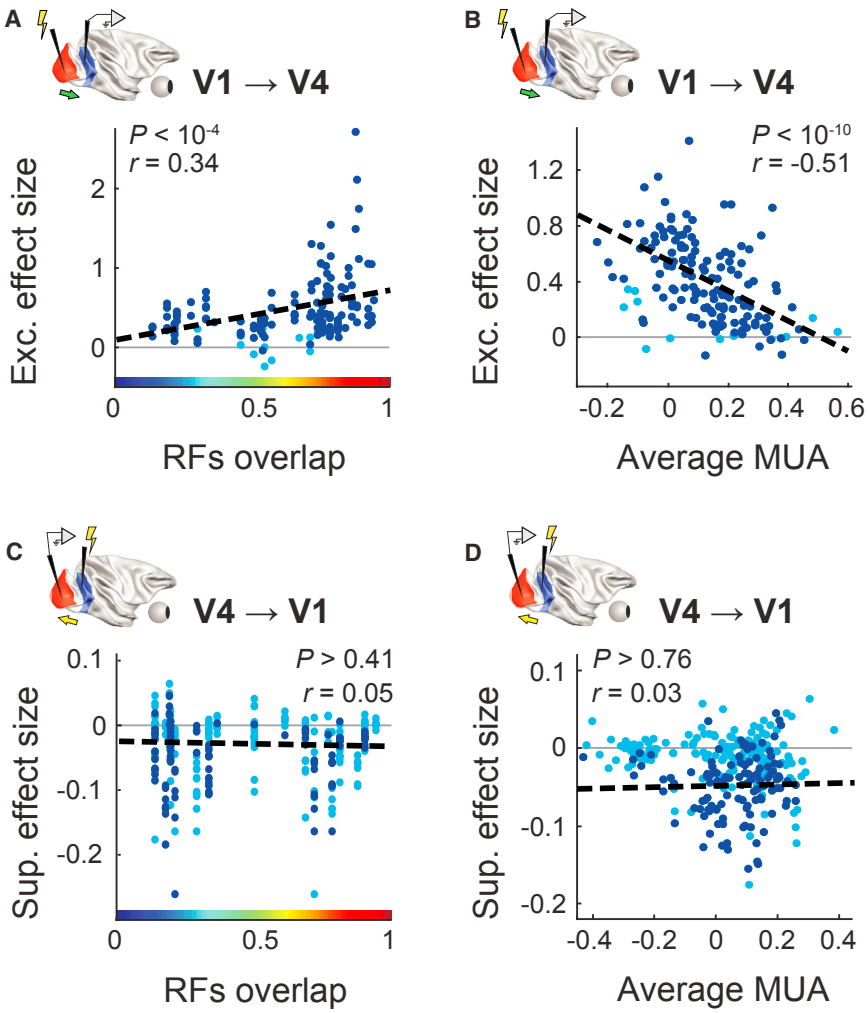

Figure 4. Effect Size Dependence on RF Overlap and Average Activity

(A) Influence of the overlap between V1 and V4 RFs (measured as the relative V4 excitability at the V1 RF location and plotted with a color scale matching that of the V4 RF maps as displayed in Figure 3B) on the microstimulation excitation effect (SOA = −50 ms). Dark blue (light blue) circles indicate recording sites with (without) a significant microstimulation induced response (Pearson's linear correlation, t test, $p < 0.05$).

(B) Correlation of the visually driven activity in V4 and the magnitude of the V1 microstimulation excitation effect, tested at an SOA of 150 ms.

(C) The inhibitory effect of V4 microstimulation (SOA = 150 ms) on V1 activity did not depend on the overlap between RFs. Dark blue (light blue) data points represent cases with a significant (non-significant) microstimulation effect.

(D) The level of V1 activity did not have a significant influence on the V4 microstimulation effect in V1 (SOA = 150 ms).

within V4 (Butovas and Schwarz, 2003; Houweling and Brecht, 2008; Seidemann et al., 2002; Figures 2E–2H), the predominant influence on V1 was a delayed inhibitory phase. When V4 stimulation was applied at an SOA of 150 ms, the amount of V1 suppression was relatively independent of both the level of V1 activity at the moment of microstimulation (Pearson's $r = 0.03$, $t_{116} = 0.30$, $p = 0.77$ at 150 ms SOA) and the overlap between the V1 and V4 RFs (Pearson's $r = 0.05$, $t_{245} = 0.81$, $p = 0.42$ (Figures 4C and 4D).

The later phase of the V1 response depends on figure-ground organization and is strongest if the RF falls on a figure (Figure 1A). To examine the influence of figure-ground modulation on the V4 microstimulation effect, we compared V1 activity elicited by a figure-ground display and a homogeneous texture (Figures 7A and 7B) across three groups of V1 recording sites. For the first group, the RF fell on the figure, and the responses elicited by the figure were stronger than those evoked by the homogeneous texture (Figure 7C) (t test; $t_{82} = 14.89$, $p < 10^{-24}$). For the second group, the RFs fell on the background near the figure (<7 degrees from the figure center). For these neurons, the responses were suppressed relative to those elicited by a homogeneous texture (t test; $t_{105} = -5.97$, $p < 10^{-7}$), as has been observed previously (Lamme, 1995; Poort et al., 2012, 2016). For the third group, the figure was far from the RF (> 7 deg. from the center), and its presence had little influence on neuronal activity (Figures 7B and 7C) (t test; $t_{138} = 0.29$, $p = 0.77$). We next compared the influence of V4 microstimulation across these three groups of sites. When the RF fell on the figure, V4 microstimulation caused strong suppression, comparable to the suppression for the homogeneous texture (t test; $t_{82} = 1.03$, $p = 0.31$) (Figures 7C–7E; Figure S5 shows data per monkey). This suppression was much weaker when the RF fell on the near background (t test; $t_{105} = -3.89$, $p < 10^{-3}$; ANOVA on the differences between responses to figure-ground stimuli and homogeneous textures, $F_{2,325} = 8.07$, $p < 0.001$; Tukey post hoc test, $p < 0.001$) and also mildly reduced when the RF fell on the far background (t test; $t_{138} = -2.94$, $p < 0.01$; Tukey, $p = 0.08$). Thus, during perception of a figure-ground display, the V1 representation of the figure is affected most during the phase that V4 activity decreases, suggesting that figure-ground modulation in V1 may originate in part from V4. Importantly, the weakening of microstimulation-induced suppression in the near surround was not caused by a floor effect, because we also observed it when we compared recording sites with RFs on the figure and near ground with a similar visual response amplitude (Figures 7F and 7G).

In the V4 stimulation experiments, antidromic stimulation of V1 terminals is unlikely given the relatively weak direct projection from V1 to V4 (Markov et al., 2011; Nakamura et al., 1993; Zeki, 1978). Indeed, we observed little direct excitation of V1 neurons by V4 microstimulation and mostly inhibition. It is therefore more likely that the suppression of V1 activity by V4 microstimulation is

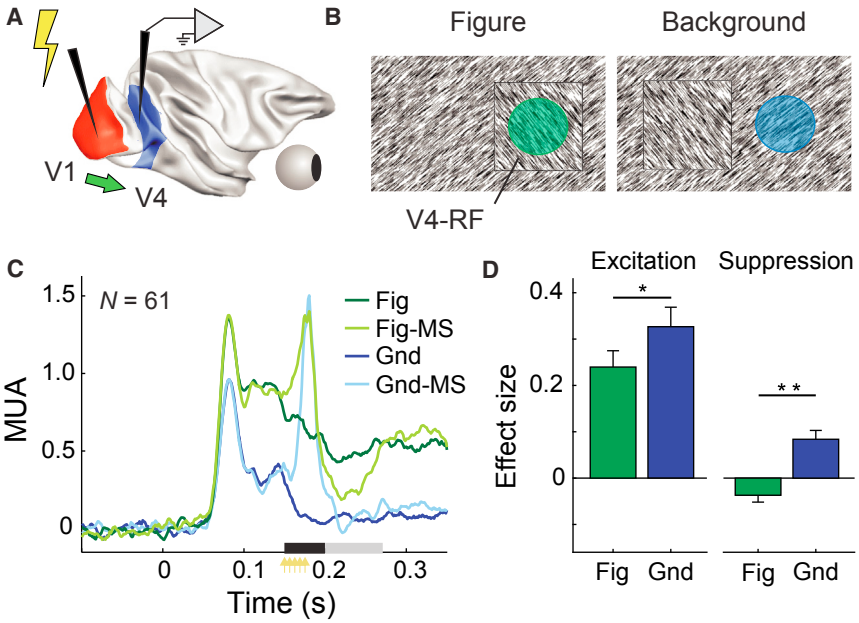

Figure 5. Interaction between V1 Microstimulation and Figure-Ground Organization in V4

(A) Schematic of the experiment with microstimulation in V1 and recording in V4.

(B) The V4 RF fell either on a figure or on the background texture.

(C) The response elicited by a figure (green) is stronger than that evoked by the background (blue). The excitatory effect (0–50 ms after microstimulation, black line) of V1 microstimulation (MS), 150 ms after stimulus onset, was stronger if the V4 RF fell on the background (cyan) than if it fell on the figure (light green). The suppression effect (50–120 ms after microstimulation, gray line), on the other hand, was stronger for figure responses. In this analysis, we only included V1-V4 combinations for which the V1-microstimulation effect was tested with the V4 RF on figure and background (n = 61).

(D) Comparison of the strength of the V1 microstimulation effect for V4 neurons with a RF on the figure (green) and background (blue). Error bars represent SEM (paired t tests; *$p < 0.05$, **$p < 10^{-8}$). See also Figure S6.

caused by the withdrawal of an excitatory (or disinhibitory) feedback influence. This interpretation is in accordance with a recent study in mouse S1 in which late, but not early, activity driven by whisker stimulation was shown to depend on feedback from motor cortex (Zagha et al., 2013).

Control Experiments and Analyses

The results of this study suggest an asymmetric interaction between V1 and V4, which depends on figure-ground organization. We performed control experiments to rule out a number of alternative explanations. First, we investigated if the local effects of microstimulation within a cortical area might also depend on SOA and figure-ground organization. To this end, we stimulated in V1 and recorded from adjacent V1 electrodes while varying the SOA and the visual stimulus (Figure S6). The stimulation artifact partially occluded the local excitation effect, but the strength of delayed suppression did not depend on SOA (ANOVA; $F_{2,183} = 0.28$, $p = 0.76$) or on whether the figure or background fell in the neurons' RF (ANOVA; $F_{1,78} = 0.39$, $p = 0.53$). Hence, the influence of SOA and figure-ground organization arises not locally but in the interaction between V1 and V4 (and with likely contributions from other brain regions that we did not record from).

Second, the average current with which we stimulated V4 (57 ± 23 μA) was lower than the average current used for V1 stimulation (89 ± 41 μA). To exclude the possibility that this difference was responsible for the weak excitatory microstimulation effects in the feedback direction, we performed a stratification analysis (Figure S7). The distributions of current strengths used in V1 and V4 overlapped substantially. We randomly excluded surplus cases of V1 and V4 microstimulation in order to equalize the distributions of stimulation strengths (Figures S7E and S7F, insets). In the remaining subset of recordings, V1 stimulation caused significant excitation ($t_{56} = -2.85$, $p < 0.01$) followed by suppression ($t_{56} = 9.71$, $p < 10^{-12}$) in V4, whereas V4 stimulation only caused suppression in V1 ($t_{56} = -10.94$, $p < 10^{-14}$) and no excitation ($t_{56} = -0.74$, $p = 0.46$) (Figures S7E and S7F). Moreover, even for the strongest microstimulation currents applied to V4 (100 μA), the excitation effect was absent from V1 (Figure S7H), whereas the cases with weakest V1 stimulation (15 μA) caused excitation in V4 (Figure S7G). The observed asymmetry in microstimulation effects in the feedforward and feedback directions is thus unlikely to be a consequence of a difference in stimulation strengths.

Finally, we examined whether the influence of V1 microstimulation on area V4 depends on the perception of phosphenes, artificial percepts of light at the RF location (Schmidt et al., 1996). In these experiments, we trained monkeys to detect pulse trains of V1 microstimulation (five pulses at 200 Hz) and compared V4 activity between stimulation trains of the same amplitude that were either seen or missed. Both the perceived and non-perceived V1 pulses elicited feedforward excitation in V4, with a very similar time course and amplitude (Figure S2E). The excitation was accompanied by relatively little inhibition, which is likely caused by the lack of visual drive of the V4 neurons that had no visual stimulus in their RF. These results demonstrate that excitation of V4 neurons by V1 microstimulation does not depend on phosphene perception.

DISCUSSION

Intracortical microstimulation is a valuable tool in dissecting the functional neural circuits that drive behavior (Cicmil and Krug, 2015; Clark et al., 2011; Histed et al., 2013). The results presented here provide new insights into the mechanisms of cortical microstimulation by revealing differences in the propagation of its effects in feedforward and feedback directions. A short train of microstimulation pulses in the cortex locally causes a brief phase of excitation followed by a longer phase of inhibition (Butovas and Schwarz, 2003; Histed et al., 2009; Houweling and Brecht, 2008; Logothetis et al., 2010; Seidemann et al., 2002).

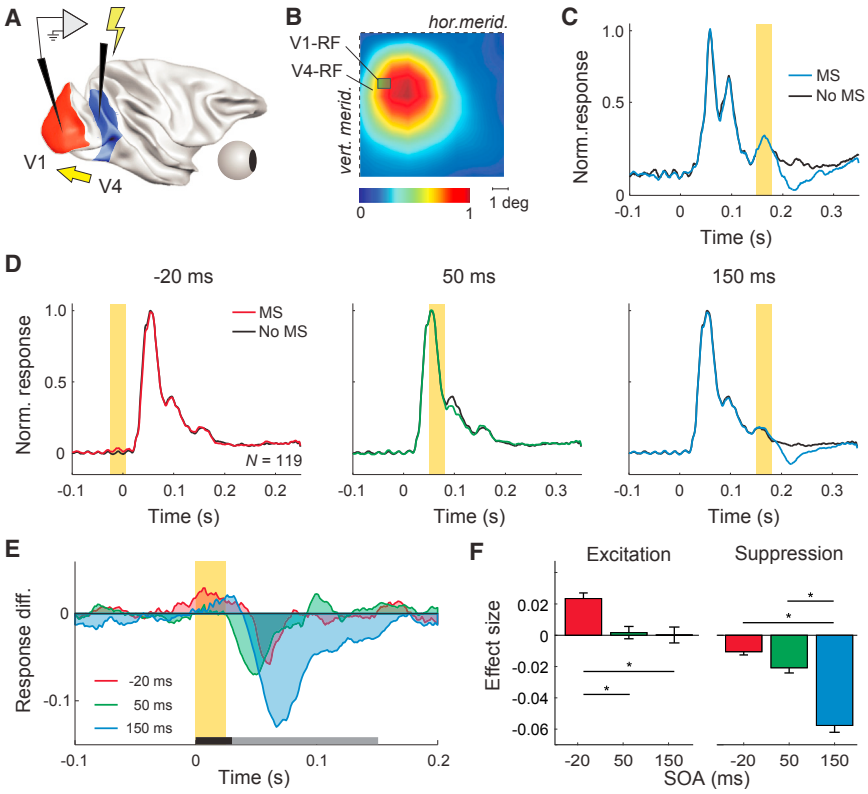

Figure 6. Effect of V4 Stimulation on Activity in Area V1

(A) Schematic of the experiment with microstimulation in V4 and recording in V1.

(B) Overlap between RFs in V1 (green rectangle) and V4 in an example experiment (color shows relative strength of visually driven activity).

(C) A train of V4 microstimulation pulses (40 μA) caused a pronounced inhibition of V1 activity during the late visual response.

(D) Average influence of V4 microstimulation applied 20 ms before, 50 ms after, or 150 ms after stimulus appearance on activity in V1. Only recording sites with a significant effect at any of the SOAs have been included in the average (n = 119/289).

(E) Difference in V1 activity between trials with and without microstimulation as a function of SOA.

(F) Average effect sizes at the three SOAs in time windows for excitation (0–30 ms after microstimulation, black line in E) and suppression (30–150 ms after microstimulation, gray line in E). Error bars represent SEM (*p < 0.001, for pairwise comparisons calculated with Tukey's post hoc test).

See also Figures S3, S6, and S7.

In the feedforward direction, the initial excitation and the later inhibition were both faithfully propagated from V1 to V4. The appearance of a visual stimulus reduced the excitatory V1 microstimulation effect on V4 and completely occluded it in a subset of experiments, implying that V1 microstimulation activates feedforward connections that overlap with those driving the visual responses in V4, and excluding the possibility that V4 activation was caused by an antidromic stimulation effect. At first sight, the results contradict previous fMRI studies in anesthetized monkeys that suggested that microstimulation causes excitation at the next hierarchical level only while it inhibits higher cortical levels (Logothetis et al., 2010; Sultan et al., 2011; but see also Matsui et al., 2012). However, the limited temporal resolution of fMRI makes it impossible to separate inhibitory from excitatory response phases if they are only tens of milliseconds apart. Furthermore, fMRI does not directly measure spiking activity. The present results demonstrate that microstimulation in visual cortex excites neurons in areas that are more than one hierarchical level higher, which is in accordance with the reported influence of microstimulation on perceptual decisions (DeAngelis et al., 1998; Salzman et al., 1990).

In addition to elucidating the functional effects of microstimulation in lower and higher visual areas, the present results are of importance for our understanding of the role of feedforward and feedback connections in visual processing. We found that during texture segregation, stimulation of V1 had a driving effect on the activity in V4 (feedforward), whereas the influence of V4 on V1 (feedback) was of a modulatory nature. The antidromic activation of projection neurons is a possible caveat in distinguishing between feedforward and feedback microstimulation effects. However, the absence of a clear excitatory V4 stimulation effect in V1 suggests that the observed effects were not antidromic. Furthermore, the V1 microstimulation effects in V4 were occluded by visually driven activity and exhibited substantial trial-to-trial variability (Figure 3C), adding support to the idea that antidromic activation played a minor role at most. Such weak antidromic effects are in accordance with previous studies that demonstrated that (1) orthodromic stimulation effects are several times stronger than antidromic effects, even between areas with strong direct projections (Bullier et al., 1988; Girard et al., 2001; Movshon and Newsome, 1996), and (2) a predominance of orthodromic influences in the laminar profiles of synaptic input caused by microstimulation (Domenici et al., 1995). V1 and V4 are areas with relatively sparse direct connectivity (Markov et al., 2011), so most of the interactions between neurons in these areas are presumably mediated by intermediate area V2 (and subcortical routes through, e.g., the pulvinar). Antidromic activation of neurons at these intermediate stages can only cause transsynaptic, orthodromic effects in the other area. For example, the antidromic activation of V2 neurons by V4 microstimulation would cause a genuine feedback effect in V1. The interareal microstimulation effects are thus predominantly orthodromic and transsynaptic, and it is safe to interpret the influence of V1 stimulation on V4 activity as a feedforward influence, and, vice versa, to interpret the influence of V4 stimulation on V1 activity as a feedback effect.

The visual cortex is a laminar structure in which each layer has its own characteristic set of input and output projections. Feedforward projections predominantly arrive in layer 4 and originate from layers 2/3 and 5. Feedback projections arrive in superficial

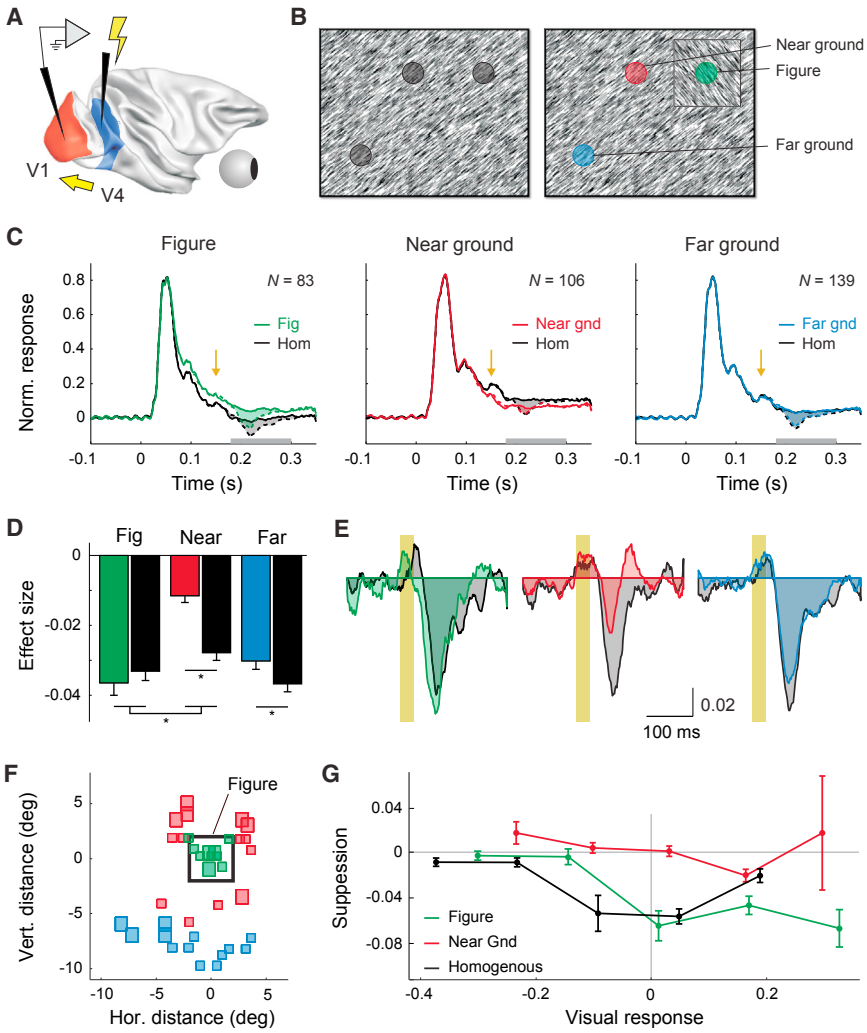

Figure 7. Influence of Figure-Ground Organization on the V4 Microstimulation Effect in V1

(A) Schematic of the experiment with microstimulation in V4 and recording in V1.

(B) We compared responses elicited by the homogeneous texture (left) to those evoked when the V1 RF fell on the figure (green), on the background near the figure (red), or on the background far from the figure (blue).

(C) V1 responses elicited by the figure (green), near background (red), and far background (blue) compared to activity elicited by a homogeneous texture (black) at the same recording sites. Continuous lines show activity in trials without microstimulation, and dashed lines represent trials with microstimulation. The use of both orientations for figure and background ensured that, on average, RF stimulation was the same.

(D) Strength of V1 suppression in a time window from 180 to 300 ms (thick line on x axis in C). Black, suppression at the same sites when a homogeneous texture fell in the RF (paired t test for figure/ground versus homogeneous texture; ANOVA with post hoc Tukey test on the differences; *$p < 0.01$).

(E) Time course of the microstimulation effect in the different conditions.

(F) RFs on the figure are shown in green, RFs in the near ground in red, and RFs in the far ground in blue.

(G) Dependence of the suppressive V4 microstimulation effect at 150 ms after stimulus onset on visual responsiveness of V1 neurons with a RF on the figure (green), near ground (red) and homogeneous background (black). We sorted the data into five bins based on the visually driven activity, in a time window from 175 to 350 ms, at the recording sites and measured the microstimulation effect in a 180–300 ms time window. Note that the suppression was pronounced at the representation of the figure and homogeneous texture but that the activity of neurons with a RF on the near ground was hardly affected, even if the strength of the visual response was similar. Error bars represent SEM.

See also Figures S5 and S7.

and deep layers, mostly avoid layer 4, and originate from the superficial and deep layers as well (Felleman and Van Essen, 1991; Markov et al., 2011). It is therefore conceivable that the depth of a stimulation electrode could influence the propagation of microstimulation effects in the feedforward and feedback directions. The electrode arrays we used in the current study had shafts of 1 or 1.5 mm, implying that their tips were located in layers 4 or 5. Importantly, previous results indicate that the precise positioning of the electrode arrays within this cortical depth range has relatively little influence on the qualitative effect of microstimulation (DeYoe et al., 2005; Murphey and Maunsell, 2007; Tehovnik and Slocum, 2009; Tehovnik et al., 2002). Neuronal activity during texture segregation is also qualitatively similar in the different layers of area V1 (Self et al., 2013). These results, taken together, indicate that it is unlikely that the microstimulation effects depended on the precise positioning of the recording and stimulation electrodes within layers 4 and 5. Nevertheless, future studies could use laminar electrodes with contacts at different depths of the same cortical column to create a fine-grained map of how the different cortical layers contribute to the propagation of microstimulation effects in the feedforward and feedback directions.

The large asymmetry in the feedforward and feedback influences of microstimulation is remarkable. V1 microstimulation efficiently activated V4 neurons, with strongest activation in the absence of a visual stimulus. In contrast, V4 microstimulation predominantly suppressed V1 neurons, and this effect was strongest in the presence of a visual stimulus. There are at least two possible explanations for this suppressive feedback effect. First, the excited V4 neurons might target inhibitory neurons in V1, thereby decreasing activity in the cortical column. Second, the late phase of the visual response in V1 might depend on excitatory (or disinhibitory: Zhang et al., 2014) feedback from V4, which is withdrawn during the late, inhibitory phase of the local V4 microstimulation effect. We favor the second explanation, because (1) V1 suppression coincided with the suppressive phase in V4; (2) the inhibitory microstimulation effect was weak during the early V1 response, which is driven by input from the lateral geniculate nucleus (LGN), and pronounced during the

late sustained response phase, when activity depends on feedback from higher visual areas (Poort et al., 2012); and (3) inhibition of feedback decreases sensory driven activity in mouse visual and somatosensory cortex (Pafundo et al., 2016; Zagha et al., 2013).

Theories on the neural mechanisms for texture segregation propose a number of processing phases with a differential involvement of feedforward and feedback connections (Lamme and Roelfsema, 2000; Roelfsema et al., 2002). The responses of visual cortical neurons indeed go through a number of phases during a texture-segregation task (Lamme, 1995). Initial responses are driven by feedforward input from the LGN and reflect the appearance of texture elements in the neurons' RFs. A phase of recurrent processing follows, which is thought to rely on feedback connections from higher cortical areas back to early areas that enhance the neuronal activity elicited by figural image elements and suppress the representation of background elements. In accordance with this view, texture-defined figures cause extra synaptic inputs into the superficial layers and layer 5 of area V1 (Self et al., 2013), which are the layers targeted by feedback connections. The enhancement of the figure representation in V1 is stronger when the monkey directs attention to the figure than when attention is directed elsewhere (Poort et al., 2012), and it can be completely absent when a monkey fails to perceive the figure (Supèr et al., 2001). The present results demonstrate that the figure enhancement in V1 is decreased when V4 neurons are silenced, which supports the view that it depends on feedback from area V4. A recent study suggests that the efficacy of feedforward microstimulation effects between V1 and MT might depend on attention (Ruff and Cohen, 2016). It would be an interesting topic for future studies to determine how the propagation of feedforward and feedback microstimulation influences depend on selective attention.

An understanding of the roles of feedforward and feedback connections is of crucial importance for general theories of visual cortical processing (Felleman and Van Essen, 1991; Roelfsema, 2006; Salin and Bullier, 1995). By directly comparing the effects of microstimulation over feedforward and feedback connections, the present study supports the view that feedforward connections drive neurons in higher areas and shape their RFs (green arrow in Figure 1A). Feedback connections, on the other hand, are modulatory: they do not drive neurons in lower areas but enhance the representation of behaviorally relevant image elements, which in texture segregation tasks are those that belong to figures (yellow arrow in Figure 1A).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.05.033.

AUTHOR CONTRIBUTIONS

P.R.R., B.D., and M.-A.G.-M. conceived the study. M.-A.G.-M. and P.C.K. performed experiments. P.C.K. and B.D. analyzed the data with advice from P.R.R. P.C.K., B.D., and P.R.R. wrote the paper.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Rhesus Macaque (*Macaca mulatta*)	Biomedical Primate Research Center, the Netherlands	N/A
Software and Algorithms		
OpenEx software	Tucker Davis Technology	http://www.tdt.com/openex.html
MATLAB	MathWorks	https://www.mathworks.com
Other		
Utah Array (Electrodes)	Blackrock Microsystems	http://blackrockmicro.com
Cereport connector	Blackrock Microsystems	http://blackrockmicro.com
Recording equipment	Tucker Davis Technology	http://blackrockmicro.com
RA16AC head-stage	Tucker Davis Technology	http://blackrockmicro.com
RA16SD or PZ2 preamplifier	Tucker Davis Technology	http://blackrockmicro.com
Master-8 Pulse generator	AMPI	http://www.ampi.co.il/master8cp.html
Custom-built microstimulator	Netherlands Institute for Neuroscience	
ET-49C eye-tracker	Thomas Recording	http://www.thomasrecording.com
Reward system	Crist Instruments	http://www.cristinstrument.com

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. P. Roelfsema (proelfsema@nin.knaw.nl).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Four healthy, male rhesus macaques (*Macaca mulatta*), aged 7-18 years, weighing between 7 and 15 kg, participated in this study. Two monkeys (B and C, aged 9 and 18 years) participated in the main experiments, while two others (D and M, aged 7 and 11 years) participated in pilot and control experiments. All animals had participated in similar experiments in the past. They were group-housed and fluid intake was controlled for the duration of the experiments. All animal procedures were performed during the daytime and approved by the Institutional Animal Care and Use Committee of the Royal Netherlands Academy of Arts and Sciences.

METHOD DETAILS

Surgical Procedures

In a first surgical procedure, a head holder was implanted. In a separate surgery, arrays of 4x5 or 5x5 electrodes (Blackrock) with a thickness of 80 μm and a length of 1 or 1.5 mm were chronically implanted in areas V1 and V4. The tips of these electrodes are likely to be located in layers 4 and 5. The surgical procedures were similar to those previously reported by our group (Poort et al., 2012; Supèr and Roelfsema, 2005). They were performed under aseptic conditions and general anesthesia, and complied with the US National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. General anesthesia was induced with ketamine (15 mg kg^{-1} injected intramuscularly) and maintained after intubation by ventilation with a mixture of 70% N_2O and 30% O_2, supplemented with 0.8% isoflurane, fentanyl (0.005 mg kg^{-1} intravenously), and midazolam (0.5 mg kg^{-1} h^{-1} intravenously).

RF Mapping

We measured the RF dimensions of every V1 recording site by determining the onset and offset of the response to a slowly moving light bar for each of four movement directions (Kato et al., 1978). V4 RFs were mapped by presenting white squares (1°x1°) on a gray background at different positions of a grid (1° spacing).

Figure-Ground Segregation Task

The monkeys performed the task while seated at a distance of 75 cm from a 21" CRT monitor with a refresh rate of 100 Hz and a resolution of 1024x768 pixels. The eye position was monitored with a video based eye tracker (Thomas Recording) and sampled at 250 Hz.

We presented a fixation point (a red circle of 0.3°) on a gray background and the monkey triggered the beginning of the trial by directing gaze to a 1° fixation window centered on the fixation point. After 300 ms of fixation the texture stimulus was presented. In 67% of the trials the texture contained a 4° square figure with texture elements with an orientation that was orthogonal to the orientation of the texture elements of the background. This figure appeared at one of two locations. After a further 350 ms, the fixation circle became blue, cueing the monkey to make a saccadic eye-movement into a target-window (2° diameter) centered on the figure position. Correct responses were rewarded with juice. Trials in which the animal broke fixation before the fixation point was extinguished were aborted. In 33% of trials we presented a homogeneous texture without a figure and the animals were rewarded for maintaining fixation for a further 400 ms. All stimulus conditions were presented in a pseudorandom order. We compared the distribution of eye positions between the figure and homogeneous conditions by measuring the projection of the eye position on the line connecting the fixation point to the possible figure location, and did not observe significant differences (paired t test across sessions, $p > 0.2$).

The figure-ground stimuli were full-screen bitmaps of textures consisting of black oriented line elements (45° and 135° orientation) on a white background. Two bitmaps of each texture orientation (i.e., two leftward oriented and two rightward oriented textures) were made with randomly placed elements. To make the figure stimuli, a square region of one bitmap of 4x4 degrees was copied onto the same position of a bitmap of the orthogonal orientation. We thus ensured that on average the same texture elements were presented at every location on the screen and that RF stimulation was identical in the figure and ground conditions. The V4 RFs were usually larger than the figure so that they also fell on the boundary between figure and background. In half of the trials microstimulation was delivered to the cortex but this brief perturbation of activity was irrelevant to the task.

Microstimulation

We applied negative-first biphasic pulses (200 μs per phase) at a frequency of 200 Hz, through one of the V1/V4 electrodes using a custom-made two-channel constant current stimulator. An adjacent electrode on the same array was used for current return because a close proximity of current source and sink decreases the magnitude of the stimulation artifact. In the main experiment, we delivered a train of 5 pulses at −20/-50 ms, 50 ms and 150 ms relative to stimulus onset (−58, 50, and 150 ms in the control experiment with both stimulation and recording in V1). For precise timing of the microstimulation pulse train, the stimulator was triggered by our electrophysiological data acquisition system (Tucker Davis Technologies) through a Master-8 pulse generator (AMPI). To study the propagation in the feedback direction we delivered microstimulation through the V4 electrodes and to study feedforward propagation electrodes we applied microstimulation to the electrodes in V1. For V1 stimulation the average current was 89 ± 41 μA (mean ± s.d.), and for V4 stimulation it was 57 ± 23 μA. Before every V1 stimulation session we measured the phosphene threshold using a 3-down/1-up staircase procedure. All the currents used in the V1 stimulation sessions were between 1.2 and 1.5 times the phosphene threshold. We did not measure the phosphene threshold in every V4 microstimulation experiment. However, the average current was 57 ± 23 μA, which is slightly larger than the average V4 threshold that we measured in our previous work with similar electrode arrays (51 ± 33 μA) (Dagnino et al., 2015). We investigated the effect of microstimulation on fixation stability by analyzing the number and amplitude of microsaccades during the period of fixation in trials with and without microstimulation. We detected microsaccades as eye movements with a minimum path length of 0.05 degrees, duration between 10 and 300 ms, speed over 8 deg/s, which started time at least 20 ms after a previous microsaccade, and had no direction changes larger than 30 degrees in consecutive 5 ms intervals (Herrington et al., 2009).

Data Acquisition and Artifact Removal

Recordings from the chronically implanted electrode arrays were made with TDT (Tucker Davis Technology) recording equipment using a high-impedance headstage (RA16AC) and a preamplifier (either RA16SD or PZ2). Data were sampled at 24.4 kHz, digitized, band-pass filtered (between 300 Hz and 9 kHz), full-wave rectified and low-pass filtered (300 Hz) to produce an envelope of the multi-unit activity (MUA) (Cohen and Maunsell, 2009; Legatt et al., 1980). This MUA signal measures spiking activity of neurons in the vicinity of the tip of the electrode and the population response obtained with this method is therefore expected to be identical to the population response obtained by pooling across single units (Cohen and Maunsell, 2009; Supèr and Roelfsema, 2005). Data from experiments in which we stimulated and recorded in the same area were more strongly affected by a stimulation artifact. Here, instead of using the envelope procedure to obtain a MUA signal, we detected spikes with a threshold amplitude window and calculated histograms.

We developed an offline procedure to remove the microstimulation artifact from the broadband data (24.4 kHz) after it had been stored to disk. Microstimulation pulses were synchronized to the clock of the data acquisition system, so that samples were always taken at identical time points relative to the microstimulation pulses. We computed the average shape of the stimulation artifact at each recording site and subtracted the artifacts from the raw signal (Figure S1). We then band-pass filtered and rectified the signal (as described above), and removed a period of 1ms (24 samples) centered on each pulse from the signal to remove remnants of the artifacts. We used linear interpolation to fill in the missing samples and low-pass filtered the signal to compute the MUA. As a control, we applied the same procedure to trials without microstimulation and found that it did not influence the shape or amplitude of the MUA signal.

QUANTIFICATION AND STATISTICAL ANALYSIS

Only electrodes with a signal to noise ratio (peak response divided by the standard deviation of spontaneous activity across trials) greater than one were included in the analysis so that the visual response was visible in almost every trial (e.g., Figure 3C). We normalized activity at every recording site to the peak response in trials without microstimulation, after subtraction of the spontaneous activity level. If a particular combination of V1 and V4 electrodes was tested in multiple sessions, we averaged the responses across sessions so that this combination entered only once into the statistics. To quantify the strength of excitatory microstimulation effects, we averaged activity in a time-window from 0-50 ms (V1 stimulation with V4 recording), or 0-30 ms (V4 stimulation with V1 recording, or V1 stimulation and recording) after the electrical stimulus. Inhibitory effects were investigated in time-windows from 50-120 ms (V1 stimulation with V4 recording), 30-150 ms (V4 stimulation with V1 recording), or 40-190 ms (V1 stimulation and recording) after the electrical stimulus.

All statistical analysis was performed in MATLAB (Mathworks). All tests are described in the corresponding figure legends and result section. Comparisons were two-sided. Student's t tests (paired where applicable), ANOVA's with post hoc Tukey tests for multiple comparisons, and Pearson's linear correlation analysis are used throughout. Summary data are presented as mean ± SEM.

DATA AND SOFTWARE AVAILABILITY

Analysis-specific code and data are available by request to the Lead Contact.