

Changes in Visual Receptive Fields with Microstimulation of Frontal Cortex

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Summary

The influence of attention on visual cortical neurons has been described in terms of its effect on the structure of receptive fields (RFs), where multiple stimuli compete to drive neural responses and ultimately behavior. We stimulated the frontal eye field (FEF) of passively fixating monkeys and produced changes in V4 responses similar to known effects of voluntary attention. Subthreshold FEF stimulation enhanced visual responses at particular locations within the RF and altered the interaction between pairs of RF stimuli to favor those aligned with the activated FEF site. Thus, we could influence which stimulus drove the responses of individual V4 neurons. These results suggest that spatial signals involved in saccade preparation are used to covertly select among multiple stimuli appearing within the RFs of visual cortical neurons.

Introduction

Only a small fraction of the myriad signals conveyed to visual cortex can be consciously perceived, remembered, or used to guide behavior. Incoming visual signals activate competing representations in extrastriate cortex, and attention provides a means to willfully select relevant objects. Neurophysiological studies in monkeys and functional imaging studies in humans have established that covert attention enhances representations in visual cortex (Kastner et al., 1998; Luck et al., 1997; Moran and Desimone, 1985; Rees et al., 1997; Reynolds et al., 1999; Reynolds and Desimone, 2003). For example, in the classic study of Moran and Desimone (1985), it was found that when two stimuli were presented simultaneously within the receptive fields (RFs) of single neurons in areas V2, V4, and the inferior temporal cortex, directing attention to the more effective stimulus increased neuronal responses as compared to when attention was directed to the less-effective stimulus. This effect has been interpreted within a framework in which attention provides a top-down signal that selects particular RF inputs to favor responses to attended stimuli (Desimone and Duncan, 1995; Reynolds et al., 1999). Although the effect of attention on extrastriate responses has been well characterized during the last twenty years, a signal capable of selecting particular RF stimuli has not been identified.

Several studies have provided evidence that brain areas with established roles in the programming of visually-guided saccadic eye movements, such as the

frontal eye field (FEF; Moore and Fallah, 2001; Moore and Fallah, 2004), the superior colliculus (SC; Cavanaugh and Wurtz, 2004; McPeck and Keller, 2004; Muller et al., 2005), and area LIP (Bisley and Goldberg, 2003; Bushnell et al., 1981), are causally involved in covert attention. We recently found that subthreshold microstimulation of the FEF enhances retinotopically corresponding V4 responses to isolated stimuli (Moore and Armstrong, 2003). This suggests that FEF stimulation drives covert attention and its neural correlates in visual cortex (Moore and Armstrong, 2003; Moore et al., 2003). However, a critical test of this interpretation is whether microstimulation changes visual RFs in a manner that reproduces the effects of voluntary attention.

We studied the influence of subthreshold FEF microstimulation on V4 responses in monkeys trained only to fixate (Figure 1). Recording, stimulation, and alignment procedures were as described previously (Moore and Armstrong, 2003). To test for changes in the weight of RF inputs, we examined the effect of microstimulation on V4 responses to oriented-bar stimuli presented to the RF both singly and in pairs. RF stimuli were presented at locations that were either spatially *aligned* or *misaligned* with the endpoint of a saccade that could be evoked from the FEF site, or at both locations simultaneously. We reasoned that if microstimulation simply amplifies responses uniformly across the RF, then its effects should be the same when single stimuli are presented at either location. However, if instead microstimulation drives the selection of stimuli located at the saccade endpoint, then it should only enhance responses in the aligned condition. Furthermore, FEF stimulation should also modulate pair responses in a manner similar to that observed during voluntary attention in trained monkeys (Luck et al., 1997; Moran and Desimone, 1985; Reynolds et al., 1999).

Results

Effects with Single RF Stimuli

The effect of FEF stimulation on V4 RF structure was examined in 49 neurons at 33 FEF sites in two monkeys (Monkey W—24 neurons, 18 sites; Monkey B—25 neurons, 15 sites). An example experiment is shown in Figure 2. Electrical stimulation of the FEF site using currents $>35 \mu\text{A}$ evoked saccades into the lower contralateral visual field and to the edge of the RF under study. Subthreshold microstimulation of this site did not evoke saccades but produced a transient enhancement of the V4 response to a stable RF stimulus located at a position aligned with the endpoint of the FEF saccade vector (Figure 2, top; paired t test, $p < 0.05$). When the same stimulus was presented at another location, still within the V4 neuron's RF but misaligned with the evoked saccade endpoint by $\sim 9^\circ$, FEF stimulation had no effect on the neuron's response (Figure 2, bottom, $p > 0.45$). Therefore, response enhancement depended not only on the presence of an effective stimulus within a RF that encompassed the saccade endpoint, but also on

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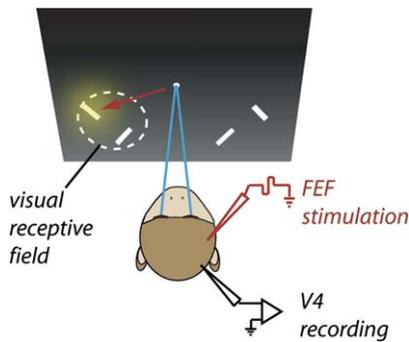


Figure 1. Covert Attention Alters Neuronal Responses to Multiple Receptive Field Stimuli to Favor the Attended Stimulus

When pairs of stimuli (oriented bars) are presented simultaneously in the receptive field (RF) of a neuron in extrastriate cortex, visual responses to the pair fall between the responses to each stimulus presented in isolation. Directing attention to one of two RF stimuli (yellow spotlight) increases the influence of that stimulus in determining the neuron's response (Luck et al., 1997; Moran and Desimone, 1985; Reynolds et al., 1999). This effect could reflect a plan to make a saccadic eye movement to the attended stimulus (red arrow). In this study, we tested whether subthreshold stimulation of sites within the FEF, an area with a known role in saccade planning, changes visual RFs in a manner that reproduces the effects of voluntary attention.

the alignment of the visual stimulus and the saccade endpoint *within* the RF.

The confinement of stimulation-driven enhancement to aligned stimuli indicates that the weight of visual inputs corresponding to the activated FEF site is selectively increased. We tested whether this effect held for the entire population of V4 neurons studied. During all experiments, the scatter of the evoked saccade endpoints was always considerably less than the size of the corresponding V4 receptive field (Figure 3A), thus allowing us to present visual stimuli at positions that were

clearly either aligned or misaligned with the evoked saccade endpoint. The mean separation between the visual stimulus and the endpoint of the evoked saccade was 0.8° and 7.1° for aligned and misaligned conditions, respectively. We quantified the relative responsiveness of each V4 neuron to aligned and misaligned stimuli and examined whether it was altered by subthreshold FEF stimulation. For each neuron, we computed a position selectivity index from its response to an effective stimulus (normalized response at aligned position minus normalized response at misaligned position; Figure 3B). Although there was a range of position selectivity indices across the sample of V4 neurons, on average the population was equally responsive to stimuli at aligned and misaligned positions during control trials (mean = 0.05; t test, $p > 0.2$). However, following microstimulation of the FEF site, the average position selectivity index showed a shift toward the aligned position (mean = 0.24; $p < 0.0005$). This resulted in a reliable difference in position selectivity indices between stimulation and control conditions (paired t test, $p < 0.0005$), with responses favoring the aligned position after FEF stimulation. This shift resulted from a response enhancement during the aligned condition ($p < 0.0001$) and an absence of a reliable effect during the misaligned condition ($p > 0.6$). In fact, only a subset of neurons, those that were stimulus selective at the misaligned location ($n = 17$), were significantly affected by microstimulation during the misaligned condition. In these cases, responses to preferred stimuli were suppressed ($p < 0.02$). Thus, the overall effect of microstimulation was to increase the weight of RF inputs at the aligned location.

Subthreshold microstimulation almost never evoked saccades during the task; however, it nonetheless measurably increased the probability that the monkey would break fixation, consistent with previous results (Schiller and Tehovnik, 2001). Whereas the probability of abortive saccades in the last half of the trial was only 1.4% during

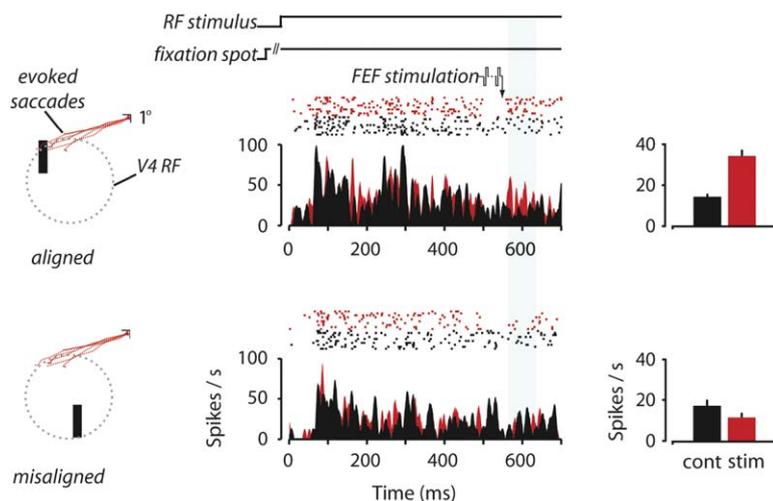


Figure 2. Effect of Subthreshold FEF Stimulation on the Response of a Single V4 Neuron to RF Stimuli that Were Spatially Aligned or Misaligned with the Evoked Saccade Endpoint

(Left) Electrical stimulation of the FEF site using currents $>35 \mu\text{A}$ evoked saccades (five dotted traces) into the lower contralateral visual field and to the upper edge of the RF of a single V4 neuron (dashed circle). (Center) Response histograms show average V4 neuron activity for control conditions (black) superimposed on stimulation conditions (red). Rasters show individual spikes for each trial. Subthreshold FEF stimulation (50 ms train, $18 \mu\text{A}$, 200 Hz) late in the trial did not evoke saccades but enhanced V4 responses to a visual stimulus presented at the aligned position (top). When the same stimulus was presented to the RF at the misaligned location, stimulation did not affect the neuron's response (bottom). Responses during FEF stimulation are omitted due to the stimulation artifact. The time window (70 ms) used for population analyses is shaded in blue. (Right) Bar graphs show the mean response during the analysis window for control (black) and stimulation (red) trials. Error bars denote SEM.

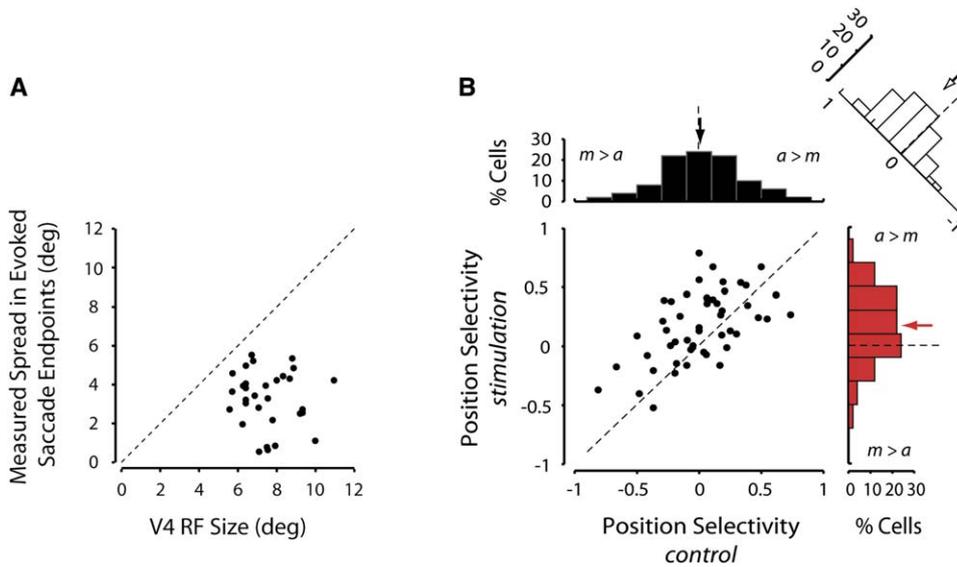


Figure 3. FEF Stimulation and V4 Position Selectivity

(A) Spread of evoked-saccade endpoints was smaller than the size of the corresponding V4 RF. The evoked-saccade endpoint scatter at each FEF site is plotted as a function of V4 receptive field size (calculated according to our observations and those of Gattass et al. [1988]). (B) Comparison of position selectivity during stimulation and control trials. Positive position selectivity values indicate a preference for the aligned location ($a > m$), whereas negative values indicate a misaligned location preference ($m > a$). During control trials, there was no preference in the population of V4 neurons toward either position (abscissa, black histogram). Subthreshold FEF stimulation shifted the position selectivity to favor the aligned position (ordinate, red histogram). When stimulation and control position indices are plotted against each other (black dots), the majority of points fall above the line of unity (open histogram) indicating that microstimulation shifted position selectivity toward the aligned location. Arrows denote means of black, red, and open distributions.

control conditions, it was increased to 3.3% following stimulation (chi-square test, $p < 0.005$). However, the increased frequency of abortive saccades following microstimulation was not uniform across visual stimulus conditions. The probability of evoking an abortive saccade was significantly greater during the aligned condition (5.5%) than during the misaligned condition (2.0%; $p < 0.005$). Thus, the behavioral effect of FEF stimulation was similar to the neural effect: in both cases the synergy between visual and electrical stimulation depended on the alignment of the visual stimulus with the activated FEF representation.

Although conditions with single RF stimuli were categorized as either aligned or misaligned with the evoked saccade endpoint, across experiments there was a considerable range in the separation between the two within each category (aligned, 0° – 4° ; misaligned, 3° – 13° ; Figure 4A). This variation allowed us to directly examine the effect of saccade endpoint-RF stimulus separation on the magnitude of response enhancement for the population of V4 neurons (Figure 4B). For each cell, we computed the stimulation-driven enhancement by subtracting the normalized control response from the normalized response following microstimulation. This revealed a negative correlation between enhancement and the separation between the saccade endpoint and the RF stimulus ($r = -0.33$; $p < 0.005$), indicating that enhancement decreased with increasing separation.

Effects with Pairs of RF Stimuli

The fact that FEF microstimulation alters responses to single RF stimuli to favor the aligned location suggests that stimulation reproduces the effect of voluntary

attention. However, there have been no studies examining how attentional modulation depends on the spatial alignment of the locus of attention *within* the RF and the visual stimulus. Moreover, the results with single RF stimuli fail to provide a parallel to the most classic effect of attention on V4 neurons, namely that attention alters responses to pairs of RF stimuli in favor of the selected stimulus (Moran and Desimone, 1985; Reynolds et al., 1999). Therefore, we tested the effect of FEF microstimulation on V4 responses to pairs of simultaneously presented RF stimuli. As observed previously (Moore and Armstrong, 2003), the stimulation-driven enhancement observed with single aligned stimuli depended on the effectiveness of the RF stimulus. That is, for the population of neurons, there was a positive correlation between the effect of microstimulation and the magnitude of the initial visual response to the aligned stimulus ($r = 0.27$; $p < 0.007$). However, for the same neurons, when two stimuli appeared in the RF, the effect of microstimulation was not predicted by the magnitude of the onset response to the aligned stimulus ($r = 0.17$; $p > 0.09$). Thus the impact of FEF stimulation on responses to pairs of RF stimuli was altered by the presence of the second RF stimulus. Nevertheless, the RF changes observed during single stimulus conditions suggest that FEF stimulation might modulate V4 responses to stimulus pairs in a manner similar to that observed during covert attention.

When two unattended stimuli appear together within a neuron's RF, one preferred and one nonpreferred, the response to the pair falls approximately between the responses elicited when the stimuli appear individually, revealing the suppressive influence of the

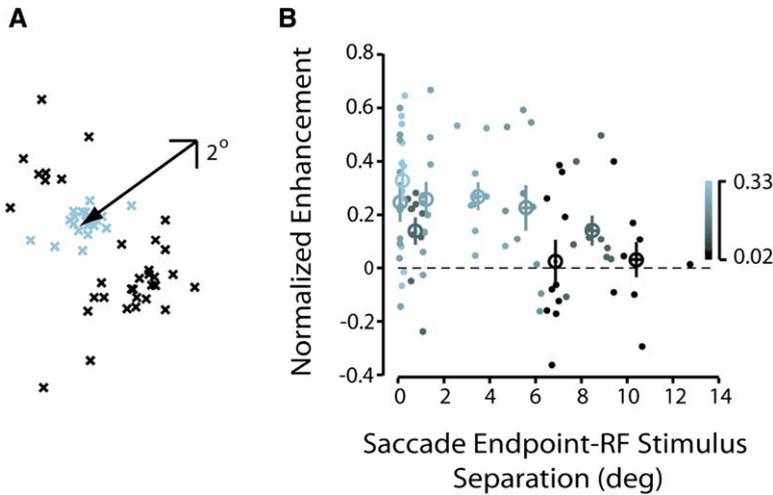


Figure 4. Relationship between Response Enhancement and RF Stimulus-Saccade Endpoint Separation

(A) The distribution of all visual stimulus positions categorized as spatially aligned (blue) and misaligned (black) is shown with respect to the average saccade evoked from all FEF sites (black arrow, mean amplitude = 9.8°; mean direction = 216°).

(B) V4 response enhancement decreased with increasing separation. Stimulation-driven response enhancement is plotted against the separation between the saccade endpoint and the visual stimulus (small solid dots). A running ten neuron average enhancement was calculated (open circles, horizontal and vertical error bars indicate the SEM separation and SEM enhancement, respectively). Data points for each neuron are colored according to the mean enhancement of the bin to which they belong.

nonpreferred stimulus on the preferred stimulus response (Miller et al., 1993; Reynolds et al., 1999; Zoccolan et al., 2005). We observed V4 responses to pairs of stimuli that confirm this finding. Late responses of an example V4 neuron to pairs of simultaneously presented stimuli are shown in Figure 5A. Control responses fell between the responses to preferred and nonpreferred stimuli presented individually. However, as observed during directed attention, FEF stimulation altered the pair response to reflect whether the preferred or nonpreferred stimulus was aligned with the saccade endpoint.

To study the effect of microstimulation on competitive interactions for the population, we employed the same analysis previously used to measure the impact of voluntary attention on V4 responses to multiple RF stimuli (Reynolds et al., 1999). It examines how well neural responses to pairs of RF stimuli are predicted by a weighted average of the singleton responses. Stimulus selectivity (SE) indices quantify how each neuron responds to two distinct test stimuli presented individually at aligned and misaligned RF locations and can range from -1 (prefers the misaligned stimulus) to $+1$

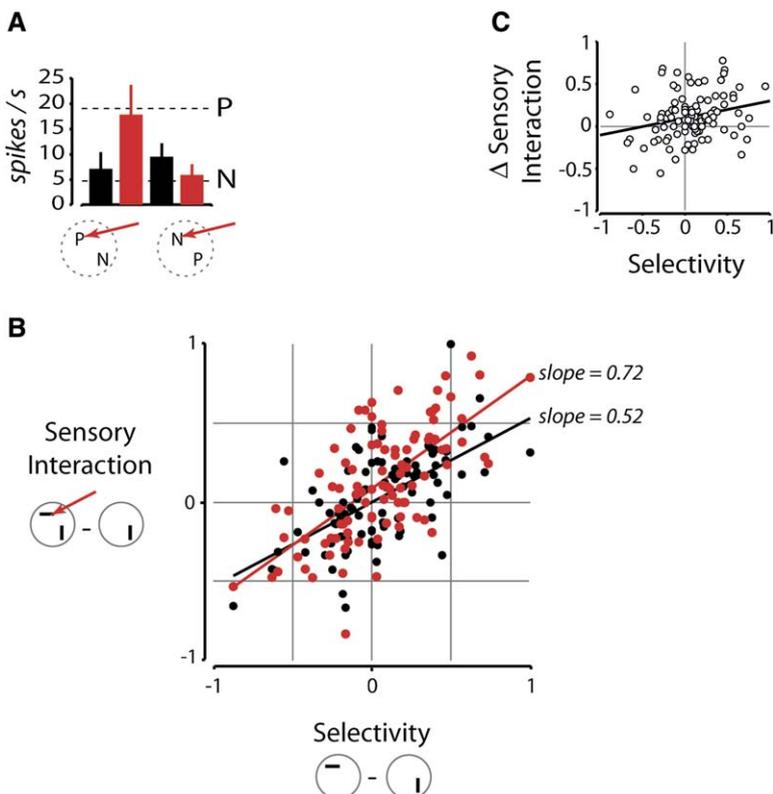


Figure 5. Effect of FEF Microstimulation on V4 Neuronal Responses to Pairs of RF Stimuli

(A) Responses of an example V4 neuron to pairs consisting of a preferred (P) and a non-preferred (N) stimulus. Control responses (black) typically fell between the responses evoked by each stimulus presented alone. Mean singleton responses, which are the average of responses at the aligned and misaligned location, are indicated by the dotted lines. FEF stimulation altered the pair response to reflect whether the preferred or nonpreferred stimulus was aligned with the saccade endpoint. Error bars denote SEM.

(B) Selectivity indicates whether responses are greater to the aligned or misaligned stimulus. Sensory interaction indices quantify how responses to the misaligned stimulus are affected by the addition of the aligned stimulus during the pair condition. During the control conditions (black), responses to the aligned and misaligned stimuli were averaged with equal weighting to yield the response to the pair, resulting in a linear relationship between sensory interaction and selectivity with a slope of 0.52 (black line). Following FEF stimulation (red), V4 responses to the pair of stimuli favored the spatially aligned stimulus, increasing the slope of the sensory interaction-selectivity relationship to 0.72 (red line).

(C) The difference in the sensory interaction index (microstimulation minus control) is plotted as a function of the selectivity index. There is a positive relationship between the effect of microstimulation during pair conditions and stimulus selectivity.

(prefers the aligned stimulus). Sensory interaction indices ($SI_{control}$) measure the added impact of the spatially aligned stimulus on the response to the misaligned stimulus during the pair condition. Like the SE index, the $SI_{control}$ index ranges from -1 to $+1$, with negative values indicating that the neuron's response to the pair was smaller than the response to the misaligned stimulus alone and positive values indicating that the pair response was greater. If responses to aligned and misaligned stimuli are equally weighted during the pair condition, the relationship between $SI_{control}$ and SE indices should be positive with a slope of 0.5 (Reynolds et al., 1999). A linear regression for the sample of V4 neurons (Figure 5B) showed a correlation between the $SI_{control}$ and SE indices ($r = 0.60$; $p < 0.0001$), and the slope of the best-fit line did not differ from 0.5 (slope = 0.52 ± 0.07 [SEM]; t test, $p > 0.5$). Furthermore, the intercept was not different from zero (intercept = 0.01 ± 0.03). Thus, in control conditions, responses to the aligned and misaligned stimuli appear to be averaged with equal weight to yield the response to the pair.

The effect of attention on responses to pairs of RF stimuli is to increase the weight of the attended stimulus and to decrease the influence of the distractor in driving the pair response (Moran and Desimone, 1985; Reynolds et al., 1999). In order to test whether FEF microstimulation has a similar effect, we computed an additional sensory interaction index for the pair condition following microstimulation (SI_{stim}) (Figure 5B). As with the control, there was a correlation between SI_{stim} and SE ($r = 0.70$; $p < 0.0001$). However, in contrast to the control condition, the slope of the linear fit was significantly greater than 0.5 (slope = 0.72 ± 0.08 , $p < 0.01$). This change in slope shows an increased weighting of the aligned RF stimulus in driving the pair response and closely matches the effect observed during voluntary attention (Reynolds et al., 1999). The increase in slope reflects the fact that the effect of microstimulation during the pair condition, as measured by the change in SI, was positively correlated with SE ($r = 0.25$; $p < 0.02$) (Figure 5C). There was also an increase in the y intercept above 0 (intercept = 0.09 ± 0.04 ; $p < 0.05$). Such an increase was also observed in V4 responses recorded in monkeys trained to selectively attend to one RF stimulus and is believed to reflect a global increase in firing rate under attention (Reynolds et al., 1999), or in this case, following FEF microstimulation. The increase in slopes observed with microstimulation and attention demonstrates that both alter visual responses to favor the selected stimulus. In fact, with stimulus pairs, we found that following microstimulation, the responses of significantly tuned neurons signaled whether the preferred or nonpreferred stimulus was at the aligned location (paired t test, $p < 0.002$; $n = 17$), whereas the control responses did not ($p > 0.19$). This observation parallels the effects of attention on V4 response as measured during covert attention tasks (Luck et al., 1997; Moran and Desimone, 1985; Reynolds and Desimone, 2003; Reynolds et al., 1999) as well as tasks in which monkeys make saccades (i.e., overtly attend) to one of two competing RF stimuli (Chelazzi et al., 2001). Our results suggest a mechanism by which interactions between multiple receptive field stimuli are modulated according to incipient saccade plans, whether or not those plans are carried out.

Fixational Stability Following Microstimulation

Several studies have reported an influence of fixational saccades, or microsaccades, on the responses of neurons in visual cortex, including area V4 (Bair and O'Keefe, 1998; Leopold and Logothetis, 1998). It is therefore important to consider the possibility that FEF microstimulation exerts effects on V4 responses indirectly via disturbances in fixational stability. Indeed, we have previously observed that even subthreshold FEF microstimulation destabilizes gaze (Moore and Fallah, 2004). Although the results expected from a simple destabilization of gaze following microstimulation are not consistent with the observed dependence of the enhancement on RF stimulus alignment or with the response changes seen with stimulus pairs (i.e., gaze destabilization should cause enhancement in all conditions), we nonetheless sought to determine its possible influence on the post-stimulation activity we studied. The frequency of microsaccades ($>0.1^\circ$ in amplitude) was measured before and after the time of microstimulation for both control and stimulation trials. We found a measurable increase in the frequency of microsaccades on stimulation trials (1.33 microsaccades/ s_{stim} versus 0.97 microsaccades/ $s_{control}$) that lasted for ~ 100 ms (see Figure S1 in the Supplemental Data available with this article online). However, this increase in microsaccade frequency began ~ 95 ms after stimulation onset. Given that the shortest visual latencies in area V4 are ≥ 50 ms (Nowak and Bullier, 1997), any influence of added microsaccades on V4 responses should occur >145 ms following microstimulation onset and beyond the extent of our analysis window (≤ 135 ms post-stimulation). Thus, the disruption in fixational stability could not have affected neural activity during the analysis window.

In addition, we also verified that differences in absolute gaze position could not have contributed to the microstimulation effects we observed. We compared the median fixational position difference between the stimulation and control conditions for both monkeys during a 120 ms time period beginning 100 ms before the start of the 70 ms V4 activity-analysis window and ending 20 ms into it. This offset was chosen to account for both the approximate maximum and minimum latencies of V4 visual responses, respectively (Nowak and Bullier, 1997). In both animals, the median difference in gaze position between stimulation and control conditions was $<0.02^\circ$ and thus could not have contributed to the observed effects of microstimulation.

Discussion

Microstimulating the FEF could recruit a number of different neural pathways. The FEF contains neurons exhibiting a range of functional properties (Bruce, 1990) and projecting to a diverse set of brain regions (Stanton et al., 1995). Stimulus selection might have resulted from electrically activating neurons in the FEF that project directly to area V4 (Stanton et al., 1995). However, the FEF also contains neurons that project to area LIP (Stanton et al., 1995), the SC (Sommer and Wurtz, 2000), and the pulvinar nucleus of the thalamus (Huerta et al., 1986), and there is a growing body of evidence implicating these areas in the control of covert attention (Bisley and Goldberg, 2003; Bushnell et al., 1981; Cavanaugh and

Wurtz, 2004; Kustov and Robinson, 1996; McPeck and Keller, 2004; Muller et al., 2005; Petersen et al., 1987). Therefore, it is plausible that visual selection resulted from the orthodromic activation of neurons in these areas, all of which project mono- or disynaptically to area V4 and, thus, are capable of influencing activity in that area (Andersen et al., 1990; Barbas and Mesulam, 1981; Ferraina et al., 2002; Lynch et al., 1994; Shipp, 2004). Because these visuoculomotor areas are reciprocally connected, a unique role of the FEF during voluntary attention is not easily inferred. Furthermore, electrical stimulation directly activates not only the cell bodies of FEF neurons but all other elements, including axon terminals (Tehovnik, 1996), so we cannot rule out a role of neurons in other structures with inputs to the FEF. This raises the question of whether the FEF itself is necessary for visual selection. The conclusion that the voluntary deployment of spatial attention originates in the FEF would be consistent with the behavioral effects of lesions and inactivation (Sommer and Tehovnik, 1997; Welch and Stuteville, 1958), with current views of prefrontal cortical function (Miller and Cohen, 2001), and with the demonstrated willful control of neural activity within motor cortices (Fetz and Finocchio, 1971). However, future experiments will need to determine if the activity of FEF neurons per se drives spatial attention and its correlates in visual cortex. Nonetheless, results to date show that FEF stimulation modulates RFs in a manner that, so far, is physiologically indistinguishable from voluntarily directed attention.

Experimental Procedures

General and Surgical Procedures

Two male rhesus monkeys (*Macaca mulatta*, 5 and 7 kg) were used in these experiments. All experimental procedures were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, the Society for Neuroscience Guidelines and Policies, and Stanford University Animal Care and Use Committee. General experimental and surgical procedures have been described previously (Graziano et al., 1997). Each animal was surgically implanted with a head post, a scleral eye coil, and two recording chambers. Surgery was conducted using aseptic techniques under general anesthesia (isoflurane) and analgesics were provided during postsurgical recovery. Two craniotomies were performed on each animal, allowing access to dorsal V4, on the prelunate gyrus, and FEF, on the anterior bank of the arcuate sulcus.

Visual Stimuli and Behavioral Task

Monkeys were trained to fixate within a 3° diameter error window surrounding a central spot. Two hundred and fifty milliseconds following fixation, oriented bar stimuli (1.4°–3.5° × 0.3°–0.9°) were presented for 1 s at locations both inside and outside the RF of a V4 neuron under study. Subthreshold microstimulation of an FEF site was applied 500 ms after the appearance of the visual stimuli on half of the trials. Monkeys were required to maintain fixation throughout the course of visual stimulus presentation and only correctly completed trials were included in the analyses. Throughout all experiments, eye position was monitored with a scleral search coil and digitized at 200 Hz. All visual stimulus and microstimulation conditions were pseudorandomly interleaved and were controlled by the Cortex system for data acquisition and behavioral control.

Responses to two oriented bar stimuli (0°, 45°, 90°, or 135°) were examined during each experiment, and on each trial visual stimuli were presented to the RF either individually or as a pair. Stimuli could be presented at two positions within the RF: either at the endpoint of the saccade that could be evoked with suprathreshold FEF stimulation (the *aligned* position), at another position (the *misaligned* position), or at both positions simultaneously. The misaligned posi-

tion was chosen to maximize the separation between the two stimuli, while still evoking a reliable response from the V4 neuron. Each RF stimulus was also presented at the mirror-image location in the ipsilateral hemifield on every trial, since the effects of FEF stimulation have been shown to be greatest in the presence of “distracter” stimuli outside the RF (Moore and Armstrong, 2003). Stimulus pairs were most often two grayscale bars of orthogonal orientation (~0.90 Michelson contrast), but occasionally the pair consisted of two bars of the same orientation but different colors (0.68–0.92 Michelson contrast). The experimenter attempted to select two test-stimuli that varied in their ability to evoke V4 responses, but stimulus tuning was not characterized before carrying out an experiment. All visual stimuli were displayed on an LCD monitor (52 cm vertical × 87 cm horizontal, 60 Hz) positioned 57 cm in front of the monkey, with a background illumination of 3.55 cd/m². Ambient illumination in the experimental room was 0.902 cd/m².

Single-Neuron Recording in V4

Single-neuron recordings in awake monkeys were made through a surgically implanted cylindrical titanium chamber (20 mm diameter) overlaying the prelunate gyrus. Electrodes were lowered into the cortex using a hydraulic microdrive (Narashige). Activity was recorded extracellularly with varnish-coated tungsten microelectrodes (FHC) of 0.2–1.0 M Ω impedance (measured at 1 KHz). Extracellular waveforms were digitized and classified as single neurons using both template-matching and window-discrimination techniques (FHC, Plexon). V4 neuron receptive fields were mapped in a separate behavioral paradigm in which oriented bars were swept across the display while the monkey fixated. The RFs of V4 neurons studied were in the lower contralateral visual field with eccentricities between 8° and 16°.

Electrical Microstimulation of the FEF

Electrical microstimulation consisted of a 30–50 ms train of biphasic current pulses (0.25 ms, 200 Hz) delivered with a Grass stimulator (S88) and two Grass stimulation isolation units (PSIU-6). Current amplitude was measured via the voltage drop across a 1 k Ω resistor in series with the return lead of the current source. All stimulation was delivered via varnish-coated tungsten microelectrodes of 0.2–1.0 M Ω impedance (measured at 1 KHz). In each monkey, the FEF was first localized on the basis of its surrounding physiological and anatomical landmarks and the ability to evoke fixed-vector, saccadic eye movements with stimulation at currents of less than 50 μ A (Bruce et al., 1985). During each experimental session, we mapped the saccade vector elicited at the cortical site under study with the use of a separate behavioral paradigm (Moore and Fallah, 2001). In this paradigm, the monkey was required to fixate on a visual stimulus (1.2° square) for 500 ms, after which time a 100 ms stimulation train was delivered on half the trials. For each trial, the visual stimulus was positioned at one of five positions, one at the center of gaze and one 10°–13° from center along each cardinal direction. The stimulating electrode was advanced until sites were localized from which saccades could be evoked into the RF of the V4 neuron under study and the current threshold for evoking saccades was measured for this site. Experimental currents were set to 50% of the site's threshold (Moore and Armstrong, 2003; Moore and Fallah, 2004; Moore and Fallah, 2001).

Analyses

All analyses of the effects of FEF stimulation on V4 responses were conducted on a 70 ms time window beginning 15 ms after the offset of stimulation. This window was chosen to avoid contamination with the stimulation artifact and to avoid any indirect effects of disturbances in fixation on visual responses that resulted from stimulation. Each neuron's activity was averaged during this window and normalized by dividing by the largest average response for that neuron. During the analysis of competitive interactions between visual stimuli, normalized V4 responses were computed in six stimulus configurations: (1 and 2) stimulus 1 and stimulus 2 appearing alone at the aligned position, (3 and 4) stimulus 1 and stimulus 2 appearing alone at the misaligned position, and (5 and 6) stimulus 1 and stimulus 2 presented simultaneously at the aligned and misaligned, or the misaligned and aligned positions, respectively, and these responses were used to compute selectivity and sensory-interaction

indices. SE indices were computed by taking the difference between normalized responses to the first test stimulus at the spatially aligned position and the second test stimulus at the spatially misaligned position (test 1_{aligned} – test 2_{misaligned}), and vice versa (test 2_{aligned} – test 1_{misaligned}), yielding two SE indices for each neuron. The sensory-interaction index (SI_{control}) is the difference between the normalized response to the pair and the normalized response to the misaligned stimulus alone during control conditions. SI_{stim} is the difference between the pair response following stimulation and the response to the spatially misaligned stimulus alone during control trials. All analyses were performed on the combined population of neurons from monkey W and monkey B, as stimulation-driven enhancement effects were statistically indistinguishable between the two animals (t test on response enhancement to preferred stimuli in the aligned condition). A criterion level of $p < 0.05$ was used in all statistical analysis.

Microsaccades

Microsaccades coinciding with microstimulation were detected off-line using an iterative algorithm based on the intersection of a velocity threshold, an amplitude threshold, and statistically significant deflections in the x or y position. The velocity threshold flagged time points at which the instantaneous velocity was above 10 deg/s for a minimum of 10 ms (Bair and O'Keefe, 1998). Two moving windows of 50 ms separated by 25 ms were iterated in 5 ms steps over the x and y components of eye position. At each step, a two sample Kolmogorov-Smirnov test ($p < 0.01$) compared the x and y components of the two periods. If either the x or y component differed significantly, the time point at the end of the first window was marked. The amplitude of each eye movement was approximated by the displacement of the median x and y components between the two windows. Points with amplitudes $>0.1^\circ$ were flagged (Bair and O'Keefe, 1998). The first of consecutive time points which passed all three criteria was considered a saccade start time. Successive saccades were constrained to start a minimum of 50 ms after any previous saccade.

Supplemental Data

Supplemental Data for this article can be found online at <http://www.neuron.org/cgi/content/full/50/5/791/DC1>.

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