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Materials and Methods

Electrophysiology. Two adult male monkeys (*Macaca mulatta*) were implanted with a head holding device and recording chamber, positioned over area V4 using structural MRIs and stereotaxic coordinates. Area V4 was identified by assessing receptive field sizes, topographic organization, and feature selectivity. Electrodes were advanced via multielectrode drives (Mini05, Thomas Recording Inc; EPS, Alpha-Omega Engineering; minimicrodrive, 3NRMD-3A, Crist Instruments). Neuronal signals were recorded using a Multichannel Acquisition Processor (Plexon, Inc). Single neurons were isolated on-line with Rasputin software and off-line with Offline Sorter (Plexon, Inc). Spike times were determined with 1 ms accuracy. Receptive fields were plotted based on responses to a manually controlled flashing bar. Receptive field diameters ranged from 2.2-7.0° of visual arc ("dva", mean: 4.7 dva; standard deviation 1.4 dva). All neurons were tested with a minimum of 20 stimulus repetitions in each experimental condition.

Stimuli and Task. Stimuli were presented on a computer monitor (Sony Trinitron Multiscan, 640 × 480 resolution, 120 Hz) placed 57 cm from the monkey. Eye position was monitored via infrared eye tracking (ISCAN Model ETL-400, 240 Hz). Experimental control was maintained with Cortex software.

To receive a juice reward, the monkeys had to maintain gaze within 0.75 dva of a 0.25 dva fixation point throughout the entire trial. After maintaining fixation for 200 ms, one of the two surfaces appeared (dot field: 2.75 dva radius, density: 5 dots/dva^2, dot size: 0.05 dva, speed of rotation: 50° of rotation/s). Each surface was defined by dots of one of two photometrically equiluminant colors: red, green, or blue. Colors were chosen based on color selectivity, attempting to optimize selectivity across multiple neurons and electrodes. Similarly, the positioning of the stimulus was optimized to cover as much of the CRFs of the multiple neurons as possible. The color and direction of rotation of the first surface to appear were selected at random, with equal probability. The second surface appeared 750 ms after the first surface. This delayed surface rotated in the direction opposite to that of the first surface and was of the other color. The two surfaces continued rotating for another 750 ms before disappearing.

Experiment 2 also included conditions in which only a single surface moved through the CRF. The responses evoked by this single surface were used to compute the neuron's color selectivity necessary for computing the Normalized Modulation Index (NMI). Variance in the sizes and locations of neuronal receptive fields resulted in the pair entering different neuron's receptive fields at different delays following addition of the second stimulus.

Data Analysis. For all three experiments, responses were divided into two periods: single surface and pair. A neuron was included in the analyses if it exhibited a significant increase in response from baseline for each single surface, (one-tailed *t* test, $P < 0.05$) and a significant difference in responses to the individual colored surfaces, (two-tailed *t* test, $P < 0.05$). The colored surface eliciting the greater response was designated the preferred surface and the other, the nonpreferred surface. Three neurons were excluded because they were selective for direction of rotation (two-tailed *t* test, $P < 0.05$). The single cell responses appearing in Fig. 1 were smoothed with a 20 ms Gaussian window. The population average responses appearing in Figs. 2-4 were computed on smoothed neuronal responses. All other analyses were computed on raw (1 ms. resolution) data.

In experiments 1 and 3, where the rotating surfaces did not move relative to the CRF, the selectivity index was computed over the interval 100-600 ms after single surface onset. The MI was computed over the interval 100-600 ms after delayed surface onset, the time period indicated by the vertical lines in Figs. 2 and 3*A*. The NMI was computed by dividing the MI by the selectivity index. In experiment 2, we computed indices as in experiment 1, but used a different time window for each neuron so that it included the period over which the stimuli passed through the CRF. Analysis windows ranged in duration from 210-560 ms. Specifically, the window began when the leading edge of the stimuli reached the edge of the CRF (Fig. 4*A*, III) and lasted until it reached the other edge of the CRF (Fig. 4*A*, IV), offset by a response latency of 75 ms.

We quantified each neuron's susceptibility to adaptation using a standard metric, the transient-sustained ratio (1, 2): TSR = $R_{\mathrm{max}}/R_{\mathrm{sus}}$, where R_{max} is the peak response in a 32 ms sliding window from 0-150 ms after onset of the first surface, and R_{sus} is the mean response over the final 100 ms before the onset of the second surface. Two neurons were excluded from this analysis because TSR was undefined ($R_{\mathrm{sus}} = 0$).

To determine the latency of the population response, we computed, for each neuron, the mean baseline rate for the 50 ms before first surface onset. We then computed neuronal responses following first surface onset, using a sliding 10 ms window, shifted at 1 ms intervals over the 150 ms postonset period. For each shift of the window, we tested whether the distribution of differences across the population was significantly greater than zero (one-tailed *t* test, $P < 0.05$). We defined the population response latency to be the midpoint of the first 10 ms window for which (1) the difference was significant and (2) remained significant for the following 20 ms. To determine the latency of the response to the second surface, we used the identical method, except that we compared the firing rate after second surface onset (within a 10 ms sliding window) with responses during the 50 ms before its onset. These latencies indicate when the elevation and reduction elicited by addition of the preferred and nonpreferred surfaces, respectively, reached significance. These analyses revealed that adding the nonpreferred surface to the already present preferred surface led to a significant dip in the response.

The approach we have taken provides a conservative measure of the actual strength of the delayed onset effect. We selected neurons based on significant single-surface selectivity and then designated the stimuli that yielded the larger and smaller responses as the preferred and nonpreferred stimuli respectively. We then compared this single surface selectivity (SI) with the delayed onset effect (as expressed by the MI and NMI). Some fraction of the single-surface selectivity was due to response noise rather than to intrinsic selectivity. Thus some of these cells may have not been selective at all and others were less selective than our sample of single surface responses indicated. For this reason, our estimate of single-surface selectivity is presumably greater, on average, than the true selectivity of those neurons. This is an example of "regression to the mean" (3) and it implies that the magnitudes of our MI and NMI measures are smaller than they would have been had we perfectly estimated each neuron's "true" selectivity.

Results

Analysis of Spike-Dependent Adaptation's Contribution to Results of Experiment 1. In experiment 1, the initial presentation of the preferred surface elicited more spikes than did the initial presentation of the nonpreferred surface. If this difference in the responses to the single surfaces led to differential adaptation, this could have contributed to the differential response to the pair.

While spike-dependent adaptation could not account for the response suppression that was observed upon addition of a nonpreferred surface in experiment 1, it could have led to a stimulus-dependent reduction in responsiveness, thereby accounting for a portion of the observed modulation in experiment 1. If so, we would expect to find a stronger response modulation among cells that adapt more readily. To examine this, we computed a standard adaptation metric (1, 2) for each neuron and determined whether it correlated with the NMI across the population. Our index of adaptation was the "transient-sustained ratio" (TSR): the ratio of a neuron's peak transient response to its sustained response. The correlation between the NMI and the TSR was very small and not statistically significant ($r^2 = 0.026$, $n = 97$, $t = 1.602$, one-tailed, $P = 0.06$). Thus, the data do not indicate that spike-dependent adaptation contributed strongly to the modulations observed in experiment 1. This conclusion is validated in experiment 2, below, which found that the cueing effect persists under conditions that rule out spike-dependent adaptation.

Analysis of Surround Modulation's Contribution to Experiment 2. Stimuli outside the CRF can modulate neuronal responses. Schein and Desimone (4) found that "surround" modulation in area V4 is usually silent, meaning that it only occurs when a stimulus is present within the CRF. They also found that typically this modulation is chromatically selective and antagonistic. Surround modulation would not, therefore, be expected to lead to a change in response before the time when the two stimuli entered the CRF. Despite this, it is nevertheless possible that the presence of the first surface alone outside the CRF could have modulated neuronal activity. If this modulation differed across conditions, this different response history could potentially have impacted how the neuron responded when the stimulus pair later entered the CRF.

To test this possibility directly, we examined the population average firing rate during the 750 millisecond period immediately before the addition of the second surface. We observed no effect of the first surface during this period: the firing rate with the preferred surface in surround (3.82 spikes/second) was not significantly different (two-tailed paired t test, $P = 0.81$) than that for the nonpreferred surface in the surround (3.79 spikes/second) and neither was significantly different from the baseline rate of 3.77 spikes/second (two-tailed paired t test, $P = 0.86$). The same result held over the 100 millisecond period immediately before addition of the second surface, demonstrating that the single surface did not alter firing rate as it moved toward the CRF border. Thus, response history before the addition of the second surface cannot account for response differences seen after the stimulus pair entered the CRF.

Ruling Out Low-Level Mechanisms. It is important to consider whether there are previously characterized neuronal mechanisms that might account for the stimulus selection effects observed in the present study. An obvious candidate is spike-dependent adaptation. Prolonged presentation of a neuron's preferred stimulus leads to reduced responsiveness (5-9). In our paradigm, activating a V4 neuron with its preferred color surface might be expected to reduce its responsiveness, thereby reducing its firing rate during subsequent presentation of the pair of stimuli. However, we did not find a significant correlation between degree of adaptation and the modulation of the pair response. Furthermore, adaptation alone cannot account for the reduction in response that occurred shortly after appearance of the nonpreferred stimulus. This suppression and its time course are, however, consistent with that seen for competitive interactions previously observed in area V4 (10) and in other areas (11-14), suggesting that this reduction results from the same underlying competitive mechanism. Finally, spike-dependent adaptation cannot explain the results of experiment 2, where we found that the effect of delayed onset persisted even when the delayed surface appeared well outside of the CRF. The response bias persisted even when the stimulus history within the CRF was held constant and there was no differential activation before the presentation of the pair.

One mechanism that could potentially contribute to effects like those observed in experiment 2 is center-surround antagonism. It is well established that presentation of a stimulus in the surround of a V4 neuron can suppress responses evoked by a simultaneously presented stimulus in the CRF. This surround suppression is strongest for surround stimuli that are of the neuron's preferred color (4), presumably reflecting inhibitory input from neurons with similar color selectivity that are activated by the surround stimulus. In experiment 2, the addition of a surface of the recorded neuron's preferred color in the surround would be expected to preferentially activate neurons preferring that color, resulting in suppression of the recorded neuron. If this suppression lasted long enough to reduce responses when the pair later entered the CRF, this would have led to a reduced response when the preferred color surface was added outside the CRF, relative to when the nonpreferred color surface was added. Thus, the predicted effect of surround suppression is the opposite of what we observed in experiment 2.

It is unlikely, in any case, that surround suppression persisted long enough to influence the response evoked by the pair when it later entered the CRF. To our knowledge, only one study has examined the effect of sequentially presenting stimuli first in the surround, then in the CRF of V4 neurons (15). This study found that an annulus appearing in the surround suppressed the response evoked by a subsequently appearing center stimulus. The duration of this suppressive effect (mean duration 77 ms) is too brief to account for the effect observed in experiment 2, which occurred even when the second surface was added hundreds of milliseconds before the pair moved into the CRF.

Although spike-dependent adaptation and center-surround antagonism seem insufficient to account for the findings presented here, it should be emphasized that the biophysical mechanisms underlying these effects are not yet fully understood. Seemingly simple mechanisms have been found to contribute in unexpected ways to complex phenomena. For example, Macknik and Martinez-Conde (16) have studied another type of temporal interaction, visual masking. They have found evidence that suggests that mechanisms underlying lateral inhibition can give rise to the seemingly complex phenomena of forward and backing masking.

Relationship to Previous Studies of Attention in LIP. The present findings have interesting parallels with a study by Gottlieb, Kusunoki, and Goldberg (17), which found, similar to the current study, that an abruptly appearing stimulus could elicit a stronger response than an older stimulus even when the newer stimulus was brought into a neuron's receptive field after stimulus onset. In Gottlieb *et al.*'s experiments, the entry into the receptive field occurred as a result of a saccadic eye movement rather than the motion of the stimulus itself. The present findings complement and extend Gottlieb *et al.*'s findings in several ways. First, our new results show that the processing benefit elicited by abrupt onset occurs even when the new stimulus is superimposed on a second older stimulus. Second, the present findings demonstrate that abrupt onset can cause either an increase or a reduction in response, depending on whether the new stimulus is a preferred or a nonpreferred stimulus for the neuron. Third, the present experiments demonstrate an effect among color-selective neurons that would not be expected to occur in the color-insensitive parietal cortical area studied by Gottlieb and colleagues. Thus, while there are interesting parallels between the effects of delayed onset in V4 and LIP, it seems unlikely that the effects in V4 are the result of the spatially selective enhancements that were documented in LIP.

Depth-Based Selection. We found that stimulus selection was specific to the color and location of the delayed surface. The V4 neurons that were recorded in the present study were color-selective and had spatially restricted receptive fields. The stimuli in our experiment, as with most natural visual stimuli, were defined by multiple features. The motion of each of these stimuli was presumably encoded by neurons selective for direction of motion. Such neurons are far more prevalent in areas MT and MST than in area V4. Previous single-unit recording studies of attention have found evidence of competitive selection among spatially separate stimuli in motion-selective areas, including MT and MST (14, 18). Psychophysical experiments using the type of superimposed stimuli used in the current study have found that the motion of a delayed stimulus enjoys a temporary processing advantage (19). In concert with our new results, this suggests that selection operates on an integrated representation of features and location. The precise nature of this representation is not yet clear.

One intriguing possibility is that integrated stimulus selection may rely, in part, on the mechanisms underlying perceptual segregation of stimuli into separate depth planes (20-22). Neurons in area V4 have been found to be selective for depth based on binocular disparity (23). The elements that defined each surface in the present paradigm were at the same distance from the monkey and they could not, therefore, be selected on the basis of actual depth differences. However, the overlapping surfaces used in our study may well have been perceptually segregated in depth despite the absence of depth-ordering cues. Indeed, the responses of disparity selective neurons in area MT have been found to correlate with the perceived depth ordering of transparent motion stimuli that, like those used in the present study, were devoid of depth cues and hence were ambiguous (24, 25). Assuming that this connection between perceived depth-order and neuronal responses holds for V4 neurons, this would provide a means by which the selection of an object's different attributes (e.g., its location, color, and motion) could be coordinated across multiple areas.

Although appealing, this suggestion does not explain how the brain achieves integrated stimulus selection but rather offers a formulation of the problem in terms of depth-order. This explanation would pose a number of key questions. First, how are the individual texture elements that define a surface assigned to the same depth plane? Second, how are the features (color and rotation in our experiment) of each surface assigned to a common depth plane? Third, how are the various features that are perceived to lie at a particular depth plane, presumably defined by neuronal activity lying within multiple areas, selected as a unit? Finally, how, in the case of ambiguous depth, would these depth order relationships be maintained over time as different populations of neurons are activated when stimuli move across the visual field (as was observed in experiment 2)? If depth order is found to be involved in stimulus selection, a full understanding of stimulus selection will require that these various mechanistic issues be addressed.

Relationship to Previous Studies of Selection in Area V4. The present results demonstrate stimulus selection in area V4 under conditions that rule out purely spatial or feature-specific selection. This does not imply that V4 cannot be modulated by spatial or featural attention. There are several clear examples of spatially selective forms of response modulation in V4, including attention-dependent changes in the shape of the receptive field (26, 27), spatially selective elevations in baseline activity (28), and spatially selective elevations in contrast sensitivity (53). Subthreshold electrical stimulation of the Frontal Eye Fields (FEF) leads to a spatially selective increase in contrast sensitivity (29) and neuronal responsiveness in area V4 (30). These findings demonstrate that feedback, including signals originating in FEF, can modulate V4 responses in a spatially selective manner. Likewise, single-unit recordings have clearly established that spatially global feature-specific attention modulates responses in area V4 (31-35).

The present findings are similar in several respects to findings of earlier studies in which a preferred and a nonpreferred stimulus were placed at separate locations in the CRF of extrastriate cortex neurons. These studies have found that the response evoked by the stimulus pair typically falls between the responses evoked by either stimulus alone (10, 14, 36). The pair response can be biased in favor of one of the two stimuli either by endogenously directing attention to that stimulus (18, 37-40) or by introducing a stimulus-driven bias such as elevating its contrast relative to the other stimulus (10). As in the present study, manipulations favoring the nonpreferred stimulus resulted in reductions in response, whereas manipulations favoring the preferred stimulus resulted in increased pair responses. The most parsimonious explanation for the similarities between the present results and those from previous studies is that the present results reflect the operation of competitive mechanisms that are also involved in selecting a target from among spatially separate distracters. The present results thus suggest that these mechanisms are involved in both spatial and stimulus-specific selection.

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