Microsaccadic eye movements and firing of single cells in the striate cortex of macaque monkeys

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When viewing a stationary object, we unconsciously make small, involuntary eye movements or 'microsaccades'. If displacements of the retinal image are prevented, the image quickly fades from perception. To understand how microsaccades sustain perception, we studied their relationship to the firing of cells in primary visual cortex (V1). We tracked eye movements and recorded from V1 cells as macaque monkeys fixated. When an optimally oriented line was centered over a cell's receptive field, activity increased after microsaccades. Moreover, microsaccades were better correlated with bursts of spikes than with either single spikes or instantaneous firing rate. These findings may help explain maintenance of perception during normal visual fixation.

Our interest in microsaccades and burst firing grew out of an attempt to study the activity of single cortical cells in an awake monkey during free viewing of a visual scene. We made continuous recordings of eye position while recording spikes from a cortical cell. We marked on a video screen the position of the eyes a suitable length of time before each spike (eye-position-corrected reverse correlation). If the scene viewed by the monkey consisted, for example, of a large bright circle on a dark background, we expected the cell to fire whenever its receptive field was crossed by an appropriately oriented contour[1]. To sample the scene evenly, we had the animal fixate on a spot whose position changed at random every few seconds. For some cells, we could see a clear correlation between the stimulus and the eye positions that were spike related, but for other cells, the constellation of spots marking spike-associated eye positions showed little or no relationship to the scene (**Fig. 1a** and **b**). During our recordings, we noticed that for certain gaze locations, cells tended to fire in bursts rather than trains of random spikes. When we filtered our records to examine only burst firing, we saw a much clearer correlation between the cells' activity and the stimulus (**Fig. 1c** and **d**). Evidently, any tendency for cells to fire in high-frequency bursts was enhanced by the contours of the stimulus. This project stemmed in part from our suspicion that the bursts were produced largely in response to microsaccades that occurred during the intermittent periods of visual fixation. Here we examine the relationship between microsaccades and various firing patterns of V1 cells, specifically, single spikes, instantaneous firing rates and spike bursts of various lengths.

RESULTS

We stimulated each V1 cell with an optimally oriented bright, stationary bar centered over the cell's receptive field while the monkey fixated on a spot of light on another part of the screen. From simply listening to the cell's firing while watching the eye position on the computer screen, it immediately

became clear that the cell's firing and the monkey's microsaccades were correlated, and that the eye movements tended to be associated with bursts of spikes.

This paper is devoted to analyzing this relationship between microsaccades and spikes. The results are based on recordings from 258 cells in 3 monkeys. We asked how often, with or without a stimulus, a microsaccade was followed by spike activity and whether any suppression of activity accompanied or followed a microsaccade. We also asked the converse question: how often were spikes preceded by microsaccades, and how would the correlation be influenced by whether one looks at single spikes, instantaneous firing rates or bursts of spikes. To ask these questions meaningfully, we began by defining the terms 'microsaccade' and 'burst'.

Analysis of microsaccades

In most studies of longer-range eye movements (movements outside the scope of this study), determining length, direction and velocity of the movements is relatively simple. Large saccades reach velocities in the hundreds of degrees per second, and so a straightforward velocity detector can be used to determine when and where each saccade begins and ends. Moreover, the starting and end positions of the eye movements are generally determined in advance. With microsaccades, starting and end positions and timing are relatively random, and their velocities are low. Previous studies identify microsaccades with instantaneous velocity thresholds[2,3] set to fairly high levels (10° per second) to protect the analysis from noise. By using the fastest portion of the microsaccade to categorize them in terms of size and speed, these studies neglected portions of the microsaccade at speeds of less than 10° per second, a considerable portion of a microsaccade because of its slow speed and small size. In addition, these studies measured either the horizontal or vertical directional component of microsaccades, but not both. Thus the size of obliquely directional microsaccades was underestimated by as much as √2 (or 41% of the actual size).

The problem is made more difficult by confusion as to what, exactly, constitutes a microsaccade. Our new algorithm depends on both freedom from bias as to microsaccade direction and accurate assessment of total size and speed of eye movements to help separate out drifts and blinks. Our algorithm therefore expresses eye position in polar coordinates, uses low velocity thresholds and is free of directional bias (see Methods). We defined microsaccades to be those eye movements that had a total angular subtense between three minutes and two degrees of arc, had an instantaneous velocity (measured each millisecond) that never fell below 3° per second and never varied from a straight trajectory by more than 15° in any direction. Microsaccades were recorded from two monkeys during several two-second intervals (**Fig. 2**).
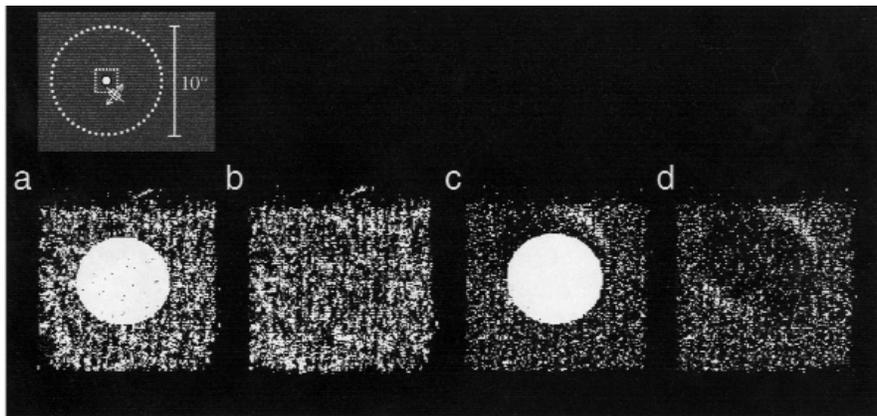
Fig. 1. Responses of a cell located within the operculum of striate cortex to a white 8° circle. The upper left inset shows the fovea (small circle) in relationship to the cell's receptive field. The fixation spot (fovea) is surrounded by a fixation window, $2° \times 2°$. (**a, b**) Each pixel represents the eye position associated with a single spike. (**c, d**) The same data, filtered so that each pixel represents a burst of 4 or more spikes occurring within an interval of 20 ms at a delay of 35 ms. (**b, d**) Same data as (**a, c**), except that the circle is removed to reveal the pixels. Duration of the recording was about 40 min.

### Probability of discharges following microsaccades

We set out first to determine whether microsaccades were correlated with modulations in neural activity, and if the modulations were stimulus driven or caused by the microsaccades themselves and unrelated to the visual stimulus.

In the main portion of our analysis, we examined 246 cells from 2 monkeys. After the beginning of a microsaccade when an optimal stimulus was centered over the cell's receptive field, average spike probability showed a clear but small increase of about 0.5%, peaking at about 70 ms (**Fig. 3a**, upper curve). The average did not show suppression of activity associated with the onset of microsaccades. We examined the individual

records to see if the effect of microsaccadic suppression had been drowned out by the averaging process, and found that only 6 of the 246 cells showed clear suppression of firing associated with microsaccade onset. In all six cells, the suppression preceded a period of excitation.

Average probability of spikes recorded from 45 cells after microsaccades without any visual stimulus (the monitor was black except for the fixation point) revealed no correlation between the microsaccades and modulation of neural activity (**Fig. 3a**, lower curve), indicating that microsaccade-induced activity in V1 was caused by microsaccades driving V1 neurons by moving their receptive fields over stationary stimuli, and not by direct excitation from the motor system.

We found similar increases in neural firing correlated with the end (as opposed to the beginning)
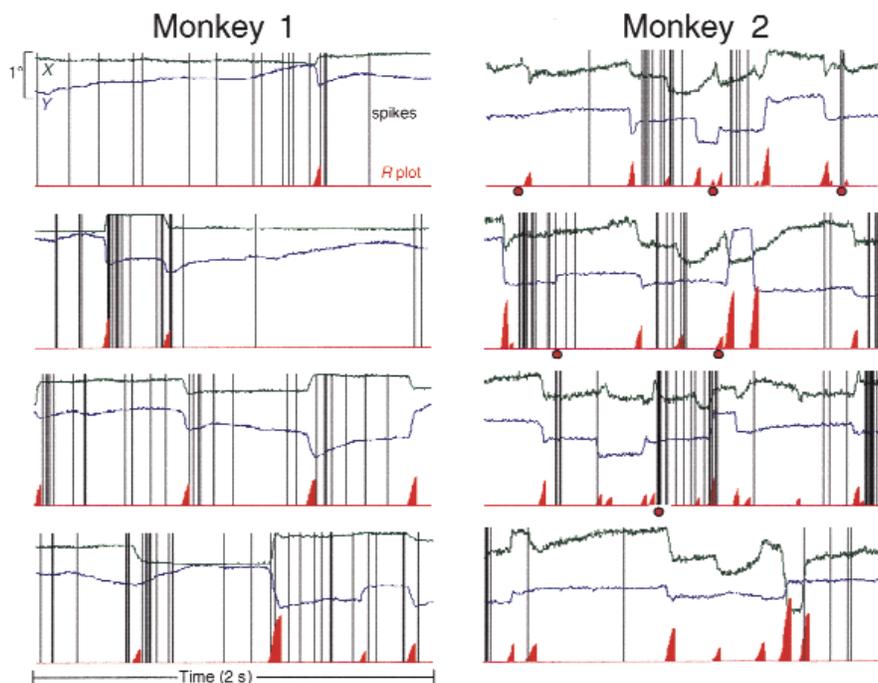
Fig. 2. Spikes and eye positions recorded during several two-second trials from two monkeys. In each panel, the upper (green) and middle (blue) traces represent *x* and *y* eye position (raw data); the lowermost record (red) represents amplitude of each microsaccade after processing to the same scale as the *x* and *y* plots. Each peak in the lower record thus represents a microsaccade. (Its height indicates the size of the microsaccade.) On the whole, there is a good agreement between the deflections in the eye position traces (green and blue) and the deflections in the microsaccade plot (red). Where possible discrepancies occur, we placed a red dot under the panel. Notice that frequency of eye movements can vary between monkeys. Vertical black lines represent spikes.

Fig. 3. Correlation between microsaccades and spikes. (**a**) Probability of a spike after the start of a microsaccade. Starts of all microsaccades were aligned (vertical line). The probability of spikes after microsaccades increased in the presence of the stimulus, whereas it did not change if the stimulus was absent. (**b**) Probability that a microsaccade preceded any given spike. (All spikes were aligned at the vertical line.)
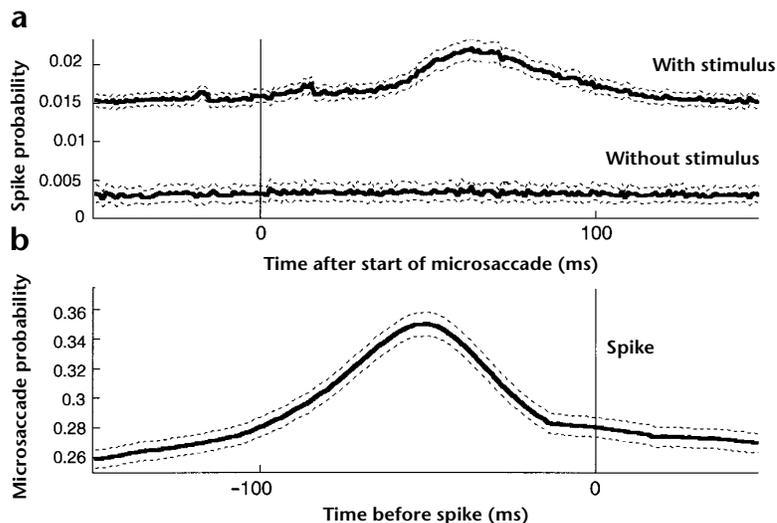
of microsaccades. The result held true even when we correlated neural activity with each individual millisecond of each microsaccade in flight (data not shown).

We ruled out the possibility that the observed excitation after microsaccades was caused by flicker on the stimulus monitor by recording from 12 additional neurons from a third monkey using a bar projected from a tungsten incandescent bulb as a visual stimulus. We again found excitation after microsaccades, ruling out the possibility that microsaccade-related activity was due to flicker in our video display.

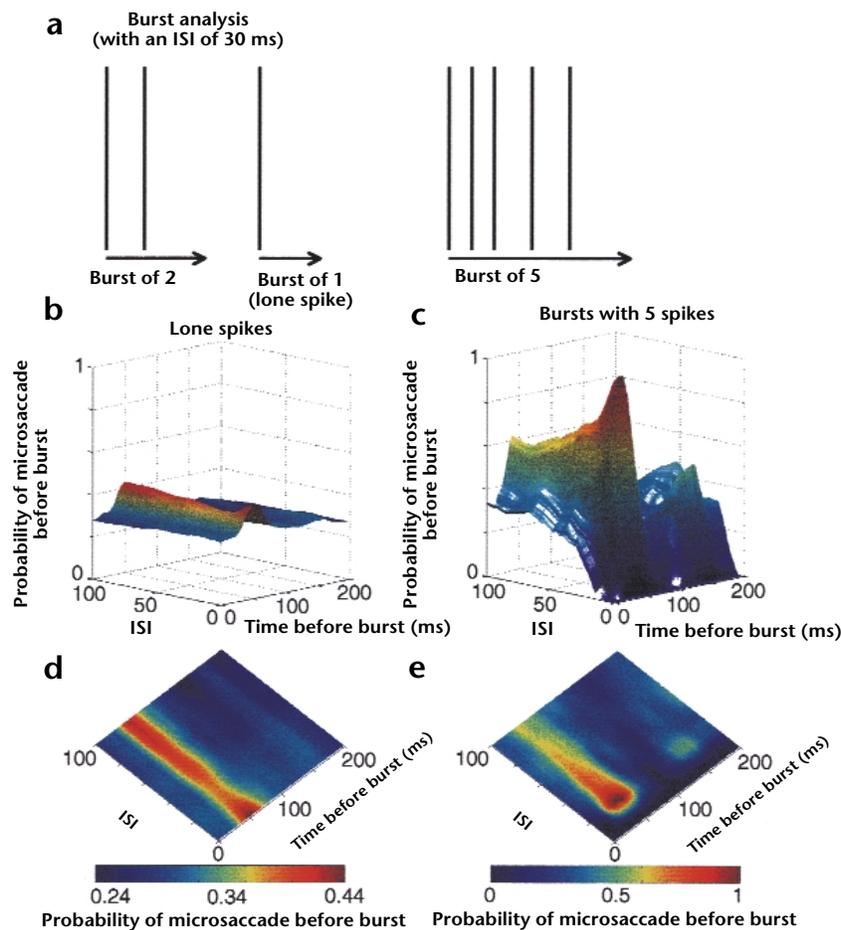### Probability that a spike was preceded by a microsaccade

We next computed the reverse correlation between spikes and preceding microsaccades, that is, the probability of a microsaccade preceding the discharge for the 246 cells from the main portion of our study (**Fig. 3b**). The peak probability of spikes correlated with previous microsaccades was enhanced by 9%, many times the peak probability seen with the forward correlation (compare with **Fig. 3a**).

We presume that the difference arose because the reverse correlation (**Fig. 3b**) considered only those microsaccades that were effective, whereas the forward correlation (**Fig. 3a**) included all microsaccades, regardless of their effectiveness. We considered whether a microsaccade would be rendered ineffective when its direction was parallel to the stimulus orientation. Microsaccades

perpendicular to the orientation of the cell's receptive field might then preferentially activate the cell. We found, however, no clear relationship between microsaccade direction and response magnitude. This finding may have been due to the aperture problem: a V1 cell cannot distinguish between a slow microsaccade that moves the stimulus perpendicularly across its receptive field and a fast microsaccade that moves the stimulus in an oblique direction to the receptive field. Thus two unlikely events would need to occur simultaneously for microsaccade direction to be involved: the stimulus would need to be perfectly aligned with the angle of the receptive field, and the microsaccade direction would need to be perfectly parallel to the angle of the receptive field. In addition, it may be that only a fraction of microsaccades activated area V1 cells effectively because of imperfectly main-
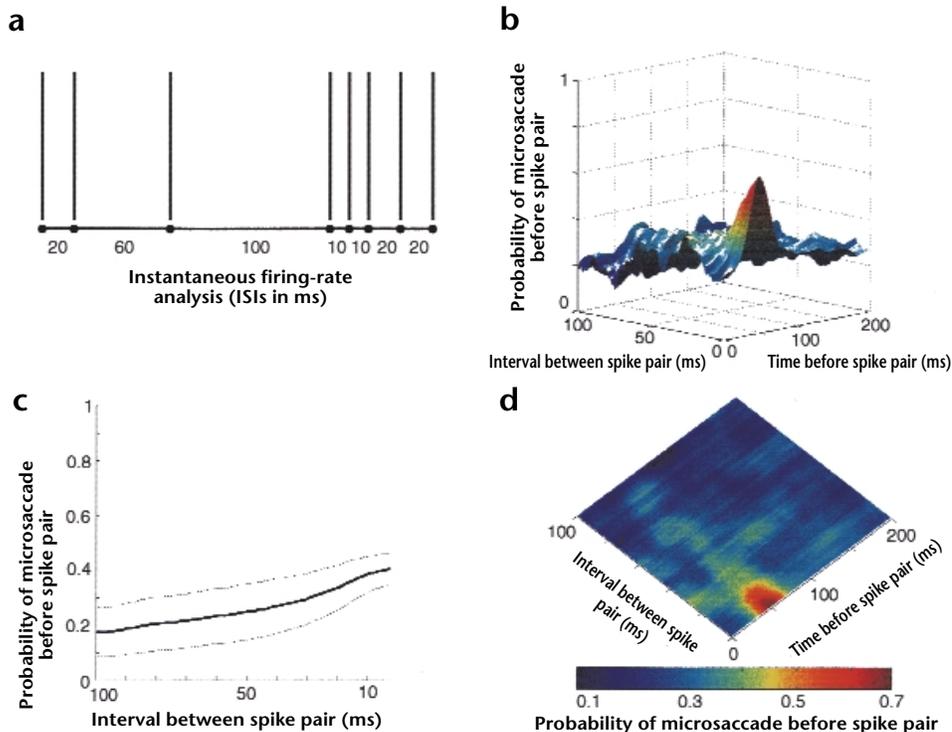
Fig. 4. Instantaneous firing-rate analysis. (**a**) Hypothetical train of spikes. The interspike intervals (ISIs) were calculated for each successive pair of spikes and expressed in milliseconds (below each pair). Spikes are represented as vertical lines. (**b**) Three-dimensional plot of the probability of a microsaccade before a pair of spikes, as a function of latency and ISI, for a single V1 cell. (**c**) Average probability of a microsaccade before a pair of spikes for all cells, as a function of ISI, at the average peak latency for the population of cells (65 ms). (**d**) Normalized contour plot of the data in (**b**).

articles

a Burst analysis (with an ISI of 30 ms)

Burst of 2   Burst of 1 (lone spike)   Burst of 5

b Lone spikes

Probability of microsaccade before burst

ISI   Time before burst (ms)

c Bursts with 5 spikes

Probability of microsaccade before burst

ISI   Time before burst (ms)

d

ISI   Time before burst (ms)

0.24   0.34   0.44
Probability of microsaccade before burst

e

ISI   Time before burst (ms)

0   0.5   1
Probability of microsaccade before burst

Fig. 5. Burst analysis. (**a**) The same train of spikes as in Fig. 4a, grouped into bursts. An ISI of 30 ms has been chosen arbitrarily for this example. Because the first spike in the train was separated from the second one by 20 ms, and this interval was smaller than 30 ms, both spikes were assigned to the same burst. The third spike occured 60 ms after the second spike, and so was part of another burst, and so on. (**b**) Three-dimensional plot of burst correlations using bursts of one spike (lone spikes) for the same V1 cell as in Figs. 4b and d. (**c**) Three-dimensional plot of burst correlations using bursts of five spikes for the same cell. The probability of a microsaccade before a burst was plotted as a function of latency (time before the first spike in each burst) and ISI. (**d, e**) Normalized contour plots of the data in (**b, c**). For this cell, bursts of five spikes were best correlated with previous microsaccades.

Bursts of spikes

Because previous experiments (**Fig. 1**) suggested that bursts may convey more reliable information about the visual scene than single spikes[1], we were especially interested in studying the relationship between bursts (of one or more spikes) and preceding microsaccades. We assigned each spike uniquely to a burst by determining the interval between it and the spikes preceding and following: two spikes with a given ISI (or less) were thus considered members of a single burst, and two spikes with an interval greater than the given ISI were considered elements of separate bursts (S. M. Smirnakis, D. K. Warland, M.J. Berry and M. Meister, *Neural Information Coding Conference*, Snowbird, Utah, 1997; **Fig. 5a**). The ISI chosen is crucial in determining the overall distribution of burst lengths (number of spikes per burst). Because we could not know in advance the most appropriate ISI for any given cell, we compared, for all the records and for all the cells, all ISIs between 1 and 100 ms and measured the probability of a microsaccade occurring some time (latency; ≤ 200 ms) before the first spike in each burst. We then sorted all bursts according to length (numbers of spikes per burst), which ranged from 1 (lone spike) to 20. We then constructed 20 (for each burst length) three-dimensional arrays; from each of these we plotted the probability of a microsaccade preceding a burst against latency (≤ 200 ms) and ISIs (≤ 100 ms). These plots allowed us to assess the probability of a microsaccade as a function of burst size, latency and ISI.

We show three-dimensional plots and normalized contour plots for bursts of one (**Fig. 5b**) and five (**Fig. 5c**) spikes for the same cell as in Figs. 4b and d. Burst of one, or lone spikes, were relatively poor indicators of previous microsaccades. Bursts of 5 were more closely correlated with previous microsaccades in this cell, and had a peak correlation of almost one. Peak probability varied with latency in all cells, whereas peak latencies remained stable as burst size varied and were consistent with V1 responses. For this cell, bursts of 5 spikes were the best indicators that a microsaccade had occured about 44 ms before.

We next compared the strengths of the associations for all the different burst lengths examined, regardless of ISI or latency

tained fixation: some microsaccades may have begun and ended with the stimulus outside the receptive field, without ever crossing it. Such occurrences would diminish the average enhancement of firing by microsaccades but not affect the incidence of microsaccades preceding cell firing.

Instantaneous firing rate

We examined the relationship between the interspike intervals (ISIs) that separated pairs of successive spikes (within the range of 1–100 ms) and the microsaccades that preceded these spike pairs by up to 200 ms (**Fig. 4a**). We define instantaneous firing rate as the inverse of interspike interval (1/ISI)[4]. Plots of the probability of a microsaccade preceding the first of two successive spikes with a given ISI (**Fig. 4b** and **d**) demonstrate that microsaccades were more closely linked to high instantaneous firing rates (small ISIs) than to slow rates. The probability of a microsaccade was maximal about 44 ms before a spike pair. At higher ISIs, the probability of a microsaccade before a pair of spikes did not drop to zero, presumably because of spontaneous firing.

We plotted average microsaccade probability against ISI for the cell population by averaging all three-dimensional graphs and slicing the result parallel to the probability–ISI plane at the average peak latency, 65 ms (**Fig. 4c**). Pairs of spikes were most closely related to preceding microsaccades when the interspike interval was short. Averaged across all cells, the peak probability of a microsaccade preceding a pair of spikes was 0.42.

Fig. 6. (**a**) Peak probability that a microsaccade occurred before bursts, as a function of burst size, for all latencies and ISIs. Same cell as in Figs. 4 and 5. (**b**) Probability that a burst was preceded by a microsaccade, as a function of ISI, at the peak latency (44 ms; same cell as in **a**). The two-dimensional plot was obtained from the three-dimensional graphs in Figs. 5b and c.

a

b

(**Fig. 6a**; same cell). The optimal burst size was clearly five, with a fall-off for shorter and longer bursts. But why did the correlation decrease for bursts longer than five? The occurrence of longer bursts during the experiment that were not well correlated with microsaccades led us to wonder if larger eye movements omitted from our analysis were associated with longer bursts. To test this, we examined all eye movements longer than three minutes of arc and found a clear correlation with all bursts measured (data not shown).

For the same cell, we then plotted probability against ISI for bursts of 1 and 5 spikes at a latency of 44 ms (the cell's optimal latency; **Fig. 6b**). This amounted to cutting Fig. 5b and c by planes normal to the latency axis at 44 ms. For single spikes, it showed an almost constant probability of about 0.4. For bursts of five spikes, the probability variations were presumably explained by the tendency of spikes to occur in clumps separated by periods of relative quiescence, often lasting hundreds of milliseconds: when ISIs that defined bursts were small, they tended to cut these clumps into fragments that were poorly correlated with microsaccades. ISIs greater than the optimum value did not affect the probabilities unless sufficiently large that successive clumps coalesced. To sum up, burst size and latency were highly correlated with previous microsaccades, whereas the ISI used to define bursts was less crucial, at least for values greater than 20–30 ms.

The correlation with preceding microsaccades was always higher for bursts, defined in terms of ISIs, than for pairs of spikes that determined instantaneous firing rates. This is demonstrated by plotting for all ISIs and latencies the peak probability of a microsaccade before the optimum burst size against the peak probability of a microsaccade before the optimum instantaneous firing rate (**Fig. 7**). The difference in the probabilities obtained by the two methods was significant ($p < 0.001$; t-test). Thus for every cell tested, burst configuration indicated a previous microsaccade with more reliability than the best possible instantaneous spike rate.

From Fig. 6a, it is clear that a burst size of five supplied by far the best indicator of a previous microsaccade for the cell under consideration. We were curious to learn the variability of such a preference across our sample population. We therefore plotted
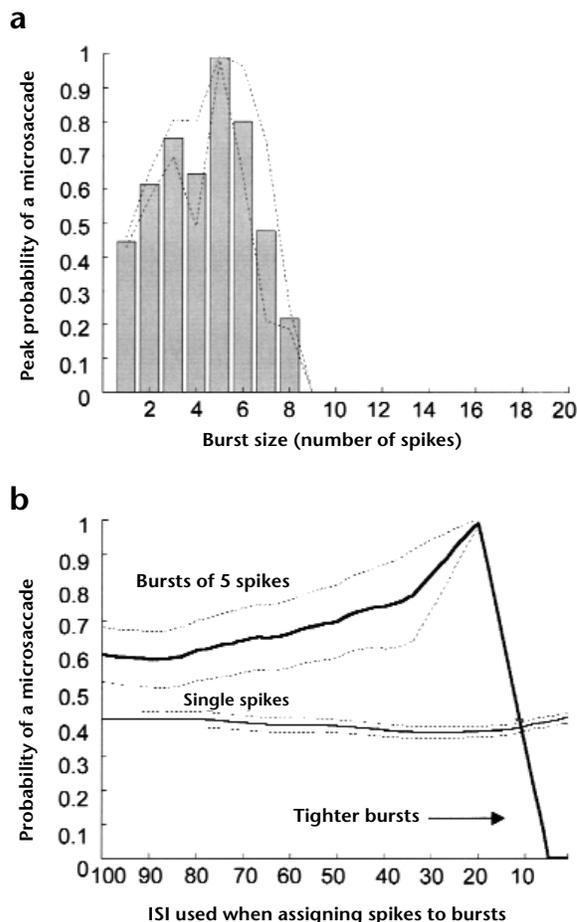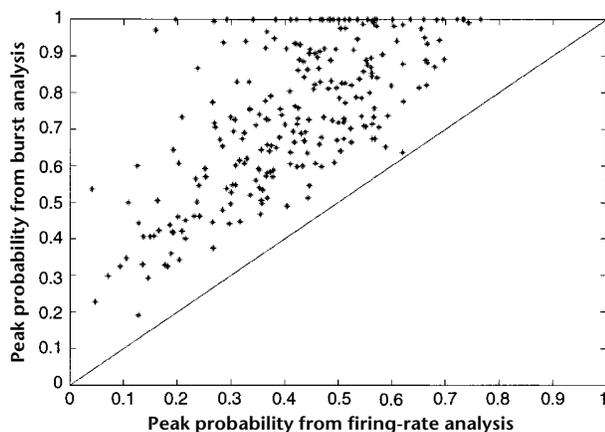

for all ISIs and latencies the distribution of burst sizes associated with peak probabilities (**Fig. 8**). Peak burst sizes showed a wide spread, with an average of 11 ($\pm$ 5, s.d.) spikes per burst and a substantial fall-off for short bursts. The average latency associated with microsaccade activity was 65 $\pm$ 46 ms, and the average peak ISI was 56 $\pm$ 29 ms.

Discussion

The phenomenon of fading of a stabilized image comes as a great surprise to someone who learns of it for the first time; when first discovered[5,6] in the early 1950s, it was little short of astonishing. Determined attempts to reproduce the fading by careful fixation often fail, especially for objects in or near the fovea, and observers are quite unaware of the microsaccades whose occurrence largely accounts for failure to perceive fading of stationary images.

It was perhaps equally surprising to learn, in the late '50s, that cells in the mammalian primary visual cortex respond far more vigorously to moving stimuli than to stationary ones[7,8]. These first studies of visual cortex pointed out the possible relationship between this and the fading of stabilized images. Our sensory

Fig. 7. Peak probability that a spike was preceded by a microsaccade for each cell. Peak probabilities determined by instantaneous firing rate were plotted against determinations using optimum burst sizes, across all latencies and ISIs. For every cell, analysis from the burst pattern predicted preceding microsaccades better than determinations based on instantaneous firing rate.
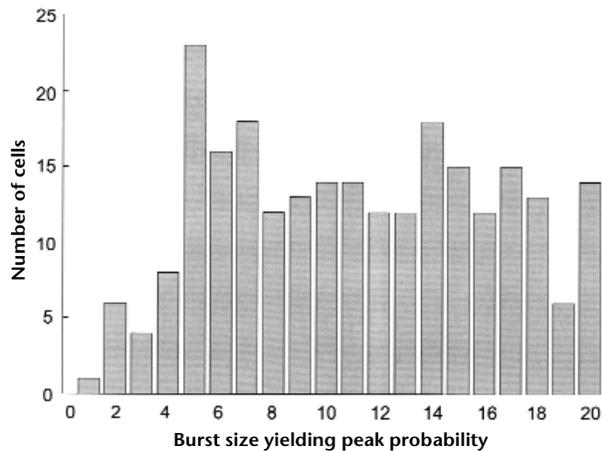
Fig. 8. Distribution of burst sizes yielding peak probabilities across the population of cells. Optimal burst sizes tended to be greater than three spikes.

systems seem to be organized on the principle that, whether we are predators or prey, changing stimuli are essential for survival and that sensory adaptation—the decline with time of a response to a maintained stimulus—guarantees that a novel stimulus will stand out, especially if it moves. The normal inability to see one's own retinal blood vessels or optic disc is presumably also related to this process[9]. Ironically, the process works so well that some further mechanism seems to be required to overcome the fading, so that we can see a stationary scene despite adaptation.

If involuntary eye movements during fixation are crucial role in maintaining the visibility of stationary scenes, we should be able to observe the relationship in awake alert animals by recording eye movements while observing the firing of single cells in the presence of an adequate visual stimulus. This was the purpose of the present study.

Quantitatively correlating microsaccades and bursts of spikes has been a challenging problem. Under the term 'bursts,' we included both 'clustered activity' (short, high-frequency trains of two to eight or more spikes) and 'bursty' firing, consisting of highly irregular longer-lasting periods of rapid activity separated by irregular silent periods. Both types of firing were described in 1958 using these terms[7]. Here, although recognizing that their origins may differ, we lumped the two together, simply referring to both as 'bursts'.

We found an excitatory effect of microsaccades on V1 cells. Such effects were seen in all 258 cells we examined. Correlating a cell's activity with the beginning of the microsaccades (or its end, or any point between) showed that the probability of spike production increased when a stimulus, a stationary bar, was centered over the receptive field. No such correlation was seen in the absence of the stimulus (**Fig. 3a**), and we conclude that microsaccade-related activity is a direct product of visual-sensory mechanisms rather than

a byproduct of oculomotor mechanisms. Further, its overall effect is excitatory. Our results are consistent with previous findings that much of the variability of responses in striate cortex of awake behaving monkeys during visual stimulation is due to the effects of microsaccades[10]. This is not to say that visual responses driven by microsaccades are likely to be restricted to V1 cells: one would expect to find similar results both earlier and later in the visual pathway.

We were surprised to find so little evidence for microsaccade-related suppression, as there is ample evidence of suppression in the much faster long-range saccades[11–16]. Visual responses in primate area V1 are suppressed when the receptive field of a single cell crosses a stationary bar during a saccade[12–14]. This suppression occurs only in cells that do not respond during fixation to stimuli moving at saccadic velocities. The difference between these results and ours is probably related to the ability of most neurons to respond to the stimulus velocities evoked by microsaccades (~30° per s) while failing to respond to stimuli moving at large saccadic velocities (typically, over 300° per s). Hypothetically, suppression associated with microsaccades could block awareness of fixational eye movements, which produce displacements of images across the retina sufficient to be easily seen if produced by a moving object. We saw microsaccade-related suppression in only 7 of the 258 cells we studied, and the suppression was always followed by excitation. It is possible that our experimental protocol made excitation easier to detect than inhibition. In any case, we found no conclusive mechanism to explain unawareness of microsaccades, at least at the level of the striate cortex.

The enhancement of firing in V1 cells by microsaccades that we observed is at odds with similar recordings of cells in monkey V1 during fixation with a small grating covering the receptive field[3]. This study revealed enhancement of firing after microsaccades in only 6 of 35 cells, suppression of firing in 13 cells and no changes in the remaining 16 (but enhancement of firing downstream, in areas V2, V4 and IT). The failure to see microsaccade-related activation in V1 surprised us, given the marked elevation of firing rate we consistently saw whenever a stationary bar was placed in the receptive field and the clear asso-
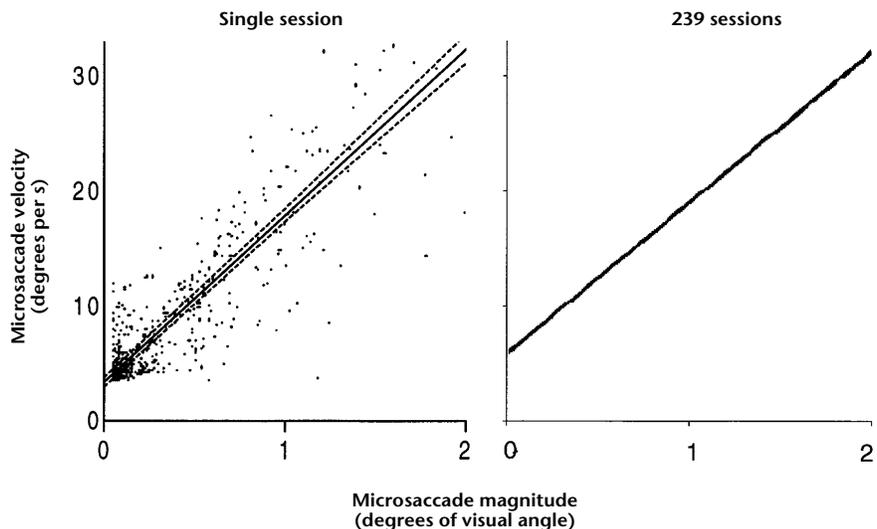
Fig. 9. The main sequence analysis. The left panel represents 523 microsaccades measured while studying a single neuron; it plots the linear regression of these microsaccades and 95% confidence intervals. The right panel shows the linear regression of the main sequence from **691,899** microsaccades recorded from 239 cells from a single monkey (99% confidence intervals were obscured by the regression line).

ciation, to simple inspection, between eye movements and firing. The discrepancy in results may be due to differences in methods. We stimulated our cells with stationary bars of light, whereas they used circular patches of oriented gratings that sometimes were binocularly rivalrous, with orthogonal orientations in the two eyes. Their cell population in V1 was much smaller, and they sampled eye position at 200 Hz, interpolating 80% of their eye movement data to simulate a sampling rate of 1 KHz. In addition, our monkeys fixated continuously during the entire recording session (nothing in our experimental protocol signaled the beginning or end of a trial to the monkey), so the visual stimulus remained on the screen throughout. Their monkeys, on the other hand, performed a visual discrimination task with discrete trials that were separated by blank screens.

In the present analysis, we did not look for effects of slow drifts in eye position on the firing of cells, and cannot rule out a possible role for them in the prevention of image fading. The relative role of microsaccades and drifts has been a subject of considerable discussion in the literature. Several studies[17,18] address both types of movement but do not isolate microsaccades and drifts from each other. The strongest claim against a role of microsaccades in preserving visual perception[19] is mainly based on two ideas. First, visual thresholds may be elevated during saccades. This is true of long-range saccades, but the evidence for suppression during the much slower microsaccades is controversial, and we found little physiological evidence for it in the present study. Second, subjects can be trained to suppress their microsaccades without experiencing fading of images. The possibility that drifts alone can prevent fading does not prove that microsaccades play no part. Moreover, if one fixates steadily on a point while paying attention to a small (and, preferably, low contrast) object in the periphery of the visual field, the object will disappear almost immediately[20]. If such precise fixation eliminates microsaccades, this would seem to support their role in preventing fading. A careful analysis of the physiological effects of slow drifts in eye position would be useful, and we are currently attempting such an analysis.

Having determined the probability that a microsaccade is followed by spike activity, our next step was to ask whether, conversely, neural discharges were associated with an increased probability of previous microsaccades. By doing so, we could concentrate on those microsaccades that had been effective in driving the cells but ignore ones that were ineffective. (The stimulus can at times drift away from the receptive field, among other possibilities.) We found that the probability of microsaccades before a spike (**Fig. 3b**) was much higher than the probability of a spike after microsaccades (**Fig. 3a**). The instantaneous firing rate of each cell, defined as the inverse of the interval between the two members of a pair of successive spikes, was still better than single spikes in indicating a preceding microsaccade.

Furthermore, bursts of spikes were better correlated with preceding microsaccades than single spikes or instantaneous firing rates. For most cells, bursts of fewer than four spikes were less effective than longer bursts, and on average there was no sharp optimum size.

Why did V1 cells show such a marked tendency to fire in bursts when microsaccades made their receptive fields move across a visual stimulus? Here we will not discuss the intrinsic properties of neurons that cause them to fire in bursts, but will instead consider the teleological significance of such firing. Are bursts useful in terms of enhancing the information conveyed by one cell to a postsynaptic cell? Specifically, are spikes arriving at a synaptic terminal more or less likely to evoke spikes in a postsynaptic cell if they occur in bursts? One can begin by asking whether the probability that a microsaccade precedes a single burst of arbitrary size (say of three spikes) is greater than the sum of the probabilities associated with three spikes considered separately. In our results, this seemed, on average, not to be so. For example, if a given spike was related to a previous microsaccade with a probability of over 0.3 (**Fig. 3b**), simple addition should predict that a rapid succession of three spikes should give a probability of three times 0.3, and on average this is roughly what we found. There may thus be nothing magic about bursts: by definition, several spikes in quick succession necessarily constitute a burst.

We cannot, on the other hand, rule out the existence of presynaptic facilitation in some synapses. At a presynaptic level, both synaptic facilitation and depression are described *in vitro*[21,22]. Regardless of these effects, however, rapidly sequential EPSPs should sum postsynaptically, so that even when synaptic efficacy is reduced in size, as in presynaptic depression, multiple EPSPs should improve the chances of postsynaptic signaling through classical temporal summation. For a pair of spikes, a clear example of paired synaptic facilitation has been shown *in vivo* for retinogeniculate synapses[23]. Thus, although we have no direct evidence pertaining to cortical synapses, given the simplest assumptions of temporal summation, it seems reasonable to expect a burst of spikes to be more reliable than single spikes in influencing a postsynaptic cell.

Equally interesting is the expectation that each microsaccade will produce synchronous bursts in many cells. Receptive fields of neighboring cells in striate cortex overlap extensively and share the same orientation preference. Consequently, as a stimulus is swept across their receptive fields, many cells should fire more or less synchronously, in bursts that can be expected to overlap in time. The result should be strong spatial summation of messages carried by axons converging at the next stage. Microsaccades would thus seem to represent an ingenious mechanism for enforcing and refreshing information coming from stationary visual stimuli.

It is furthermore possible that microsaccades may be important for allowing us to use latency in our visual discriminations. Latency differences in neural responses are proposed as an encoding mechanism for changes in the magnitude of contrast[24–26]. But it has remained unclear how the brain could use latency information, as the brain cannot know the latency *a priori*. Microsaccades could theoretically be used by the visual system to measure latency, because the brain knows both when the microsaccade motor signal starts and when the visual signal arrives. Relative latencies of responses to stimuli could then be used to indicate contrast.

METHODS

We used three rhesus monkeys, *Macaca mulatta*. A midline stainless-steel head post was mounted by screws over the skull, and a steel recording chamber was mounted over the occipital operculum. We recorded from single neurons in area V1 with lacquer-coated electropolished tungsten electrodes. A visual search coil attached to the sclera of one eye recorded the animals' eye movements. Standard sterile surgical techniques and animal care methods are described[1,27]. The Harvard Medical Area Standing Committee on Animals (protocol #02935) approved all electrophysiological experimentation.

Stimuli and receptive-field mapping. Monkeys were trained to fixate on a small spot displayed on a monitor at a distance of 58 cm. A monkey was required to keep fixation within a 2° window to receive a drop of juice; eye movements exceeding the window's limits were also recorded. The stimuli displayed on the monitor had a luminance of 24.3 cd per m^2, and the monitor background was 3.84 cd per m^2. We recorded V1 over the occipital operculum (eccentricity, 6°–8°) or from the folds of calcarine cortex immediately beneath. Receptive fields were about

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$0.33° \times 0.5°$, and thus the same size or larger than many eye movements during fixation. We first mapped the receptive field roughly by hand, using computer-generated moving slits whose orientation, size and rate of movement were controlled with a computer mouse. We then used a bar of optimum length, width and orientation that flashed in quick succession in various parts of the visual field to more precisely determine the position and size by reverse correlation. A stationary bar with these optimal parameters of width, length and orientation was then positioned over the center of the receptive field, and collected spikes were subsequently correlated with the monkey's microsaccades. The bar turned on before data collection and remained on throughout the recording session for each cell. Within total recording times of 100–800 s (mean, 334 s per cell), we recorded eye movements and spikes in consecutive 2-s trials. The beginning and end of each trial were unknown to the animal, and the fixation point persisted between trials.

Analysis of microsaccades. We developed an algorithm for determining when the eye was stopped and when it was moving. We regarded an eye movement as ended when either its speed dropped below some threshold or its direction changed by more than some predetermined angle. First, we digitally sampled the horizontal (x) and vertical (y) eye tracker voltages (with 11-bit resolution at a sampling rate of 1 kHz, each A/D unit corresponding to 0.982 arcmin of visual angle); we then created a table of change in x and y (dx and dy) computed as the difference between values at successive intervals. After differentiating the data, so that each element then represented the instantaneous velocity of the eye in horizontal and vertical space, we smoothed the processed data with a 31 ms-wide unweighted boxcar filter to reduce noise. Varying the width of this filter, we found that averaging each value of dx and dy over a period up to ±15 ms surrounding each sampling interval gave results consistent with categorization of microsaccades by inspection of raw data from the eye-movement monitor, the method used in most previous studies. We prepared an array of vectors representing the instantaneous direction (θ) and size (**r**) of motion of the eye at each 1-ms interval of the recording. Thus r may be thought of as the instantaneous velocity of the eye in degrees of visual space per ms. In addition to θ and **r**, we applied a velocity threshold of r < 3° per s as a measure of when the eye had stopped, giving a third datum for each interval: a value of 1 (stopped) or 0 (not stopped) was recorded to generate the 'eye-stopped array'.

At each interval, a rate-of-turn indicator checked whether θ had changed by >15°; if so, we considered the eye to have stopped between intervals and amended the eye-stopped array appropriately. This indicator was important for accuracy of the eye-stopped array, as the eye seldom had zero velocity even after the data had been smoothed—probably because of noise and drifts in eye position. During a true saccade, successive measurements of θ were similar (aligned in the direction of the overall eye movement). If θ continually changed, we considered saccades to be absent.

To determine the overall distance and direction over an entire microsaccade (average, 29 ms), we used simple vector addition to generate cumulative distance and directions of the movement (integrated **r** and θ) at each interval between eye stops as registered in the eye-stopped array. At the final 1-ms interval of each microsaccade, values of integrated **r** and integrated θ were therefore equal to the final distance and direction of the entire eye movement. To distinguish microsaccades from small artifacts or large voluntary saccades or eye blinks, we applied lower and upper limits of 3 arcmin and 2°, to the final value of integrated **r**. Plots of the microsaccadic main sequence (size versus velocity) showed a linear regression (**Fig. 9**).

Data processing. We calculated the reverse correlation for microsaccades preceding either pairs of spikes or bursts of spikes and plotted our results in three dimensions (**Figs. 4** and **5**). For particularly rare combinations of burst length and ISI, small sample size resulted in an artifactually high probability of the burst being preceded by a microsaccade; for instance,

if a particular burst size and ISI occurred only once and followed a microsaccade, the probability of such a unique event was thus 1. We therefore smoothed every three-dimensional array with a boxcar filter whose width was 15 elements in the ISI-latency plane; thus every probability was averaged with those corresponding to ±7 ms latency and ±7 ms ISI. (At the array edges, this boxcar was reduced to fewer elements.) This smoothing removed virtually all the random peaks of probability associated with rare bursts.

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