Differential temporal storage capacity in the baseline activity of neurons in macaque frontal eye field and area V4

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Abstract

Previous studies have suggested that spontaneous fluctuations in neuronal activity reflect intrinsic functional brain architecture. Inspired by these findings, we analyzed baseline neuronal activity in the monkey frontal eye field (FEF; a visuomotor area) and area V4 (a visual area) during the fixation period of a cognitive behavioral task in the absence of any task-specific stimuli or behaviors. Specifically, we examined the temporal storage capacity of the instantaneous discharge rate in FEF and V4 neurons by calculating the correlation of the spike count in a bin with another bin during the baseline activity of a trial. We found that most FEF neurons fired significantly more (or less) in one bin if they fired more (or less) in another bin within a trial, even when these two time bins were separated by hundreds of milliseconds. By contrast, similar long time-lag correlations were observed in only a small fraction of V4 neurons, indicating that temporal correlations were considerably stronger in FEF compared to V4 neurons. Additional analyses revealed that the findings were not attributable to other task-related variables or ongoing behavioral performance, suggesting that the differences in temporal correlation strength reflect differences in intrinsic structural and functional architecture between visual and visuomotor areas. Thus, FEF neurons probably play a greater role than V4 neurons in neural circuits responsible for temporal storage in activity.

Key words

Monkey, temporal integration, visual search, attention
Introduction

Neurons in separate cortical areas respond in different ways even when subjects perform the same cognitive–behavioral tasks. This differential activity depends on a number of factors, including sensitivity to sensory stimuli and the influences of cognitive processing or motor behavior, but also may be due to differences in intrinsic processing across brain regions. Evidence has begun to accumulate supporting this latter notion; fluctuations in spontaneous activity have been proposed to reflect the intrinsic functional architecture of the brain (Fox and Raichle 2007; Kenet et al. 2003; Luczak et al. 2009; Shmuel and Leopold 2008; Tsodyks et al. 1999; Vincent et al. 2007). Thus, region-specific differences in neural circuitry should produce different neuronal responses not only in task-related activity but also in baseline activity that is not directly associated with a task. In this study, we recorded neuronal activity in the frontal eye field (FEF; a visuomotor area) and area V4 (a visual area) of monkeys performing a cognitive–behavioral task and attempted to find area-dependent intrinsic differences in baseline activity.

We focused specifically on persistent neuronal activity. Neurons in visuomotor areas such as FEF or the lateral intraparietal area often exhibit sustained activation in the absence of visual stimulation, such as in memory-guided saccade tasks (e.g., Chafee and Goldman-Rakic 2000; Lawrence et al. 2005; Thompson et al. 2005). Conversely, neurons in V4 and other visual areas generally do not exhibit clear sustained activity without visual stimulation (e.g., Bisley et al. 2004; Chelazzi et al. 2001; Nakamura and Colby 2000). In addition, lateral intraparietal area neurons can show ramp-like changes in activity when monkeys perform a discrimination task. This ramping of activity in visuomotor areas is thought to reflect the temporal integration of sensory input (Huk and Shadlen 2005; Roitman and Shadlen 2002; Shadlen and Newsome 2001). Although previous studies have emphasized the area
dependence of persistent changes in task-related activity, these differences may be due at least in part to differences in intrinsic neural processing.

To assess this possibility, we compared to what extent modulations in neuronal activity at a given time persist in the baseline activity (i.e., “temporal storage”) of FEF and V4 neurons. Temporal storage capacity was evaluated by correlating the spike count in a bin with another bin during the baseline activity of a trial. Thus, if a neuron has a significant temporal storage capacity, then if it fires more (or less) in one bin, it will fire more (or less) in another bin within the same trial, even when these bins are separated by substantial amounts of time. We found that temporal correlations in baseline activity were significantly stronger in FEF neurons than in V4 neurons. The finding that there are region-specific differences in a neuron’s ability to store the history of its own activity in the absence of sensory or cognitive information processing suggests that there are differences in the intrinsic properties of the neural circuitry in these areas.
Materials and Methods

Subjects and surgery

Three Japanese macaque monkeys (*Macaca fuscata*) weighing 5.0–6.0 kg were used in this study. Surgery was performed while each animal was under deep anesthesia (intravenous sodium pentobarbital), and an eye coil, head holder, and recording chambers were implanted using sterile techniques. Recording chambers were placed over FEF and V4, identified using magnetic resonance images obtained prior to surgery, and anchored flat against the skull so that recording electrodes could be advanced nearly perpendicular to the cortical surface.

Following surgery, monkeys were allowed to recover for at least 2 weeks before the initiation of training and recording. During the training and recording periods, the monkeys were deprived of water on weekdays. Their weight and health status were carefully monitored, and they were given additional water or food when necessary. All procedures for animal care and all experimental protocols were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (1996) and were approved by the Institutional Animal Experimentation Committee.

Visual stimuli and behavioral tasks

Visual stimuli and behavioral tasks were described previously (Ogawa and Komatsu 2006; 2004). Briefly, the monkeys were required to perform a multidimensional visual search task (Fig. 1). In search trials (92.3% of trials), each visual stimulus array had two singletons, one unique in shape (shape singleton) and the other in color (color singleton), that were presented with four additional identical stimuli. Each stimulus had two possible shapes (bar or circle) and two possible colors (cyan or yellow). In the shape search, the shape singleton was the target, and the color singleton was the distractor. The converse was true for the color
search. The search condition was indicated by the color of the fixation spot (shape search, red; color search, blue). In a catch trial (7.7% of trials), all six stimuli had the same color and shape, and the monkeys were required to maintain fixation throughout the trial. Each trial began with the appearance of a fixation spot at the center of a monitor screen. The monkeys had to fixate on that spot within a window of ±0.5–0.75°. After monkeys fixated for the required time (typically 1200 ms, but occasionally varying between 800 and 3000 ms), the array was displayed. The monkeys were then required to make a saccade toward the target. For each isolated neuron, the shape search and color search conditions were applied sequentially in separate blocks (~100 trials).

**Data recording**

Eye position was monitored and recorded at 1 kHz (MEL-250UD; Enzanshi Kogyo, Tokyo, Japan) using the scleral search coil technique as described in the literature (Fuchs and Robinson 1966; Judge et al. 1980). Single neurons were recorded using stainless steel or tungsten electrodes (Frederick Haer & Co, Bowdoinham, ME) with an impedance greater than 2.0 MΩ measured at 1 kHz (Model IMP-1; Bak Electronics, Germantown, MD). Extracellular activity was amplified using a microelectrode AC amplifier (SS-202J; Nihon Kohden, Tokyo, Japan; Model-1800; AM-Systems, Carlsborg, WA) and stored on a computer equipped with a multichannel analog-to-digital converter at a sampling rate of 25 kHz (PCI-416L2A; Datel Japan, Tokyo, Japan). Because we used high-impedance electrodes and recorded only from well-isolated single units, spikes from a single unit were easily discriminated according to spike amplitude using a simple threshold method, from which online peristimulus-time histograms were constructed. If necessary, precise spike discrimination was performed offline using a template-matching method. Neurons were located by slowly advancing the recording electrode using an oil-filled hydraulic
micromanipulator (MO-95; Narishige, Tokyo, Japan) as the monkeys performed the visual search task. FEF and V4 were located based on their physiological properties and anatomical landmarks. FEF was also located using an electrical stimulator (SEN-7203; Nihon Kohden, Tokyo, Japan), such that the FEF was defined as the site where a 60-ms train of biphasic current pulses (amplitude, <50 µA; width, 0.2 ms; frequency, 333 Hz) evoked fixed-vector, saccadic eye movements during fixation (Bruce and Goldberg 1985; Bruce et al. 1985).

Data analysis

To confirm adequate spike isolation, we constructed interspike interval distribution histograms. Neurons were included in the analysis if they maintained stable spike isolation throughout a recording session and their distribution histograms showed an absence of interspike intervals shorter than 2 ms (the refractory period). In this study, we focused on baseline activity during the fixation period, before array presentation. Because the monkeys were required to keep their gaze directed toward the central spot on the display during this period, and they had no information about the forthcoming array, baseline activity was virtually free of any specific eye movements and visual stimulus. This allowed us to examine temporal storage capacity under conditions when visual sensory stimuli and eye movements were at a minimum. Unless otherwise indicated, only data from successful trials were analyzed in this study. To ensure that there were sufficient spike data for the analysis of baseline activity, we selected only neurons in which the mean activity during the 1000-ms interval before array presentation was greater than 3 spikes/s. Note that this threshold level was not essential to observe our main findings; the results were essentially the same when the spike rate varied from 1 to 10 spikes/s.

To illustrate temporal storage capacity in baseline activity in an intuitive manner,
we separated all trials into three groups (high, medium, and low activity) according to the
spike counts in the 100-ms interval extending from 800 ms to 700 ms (or from 100 ms to 0
ms) before array presentation (e.g., Fig. 2B). The three groups were created such that each
group consisted of at least 50 trials. To this end, we first delineated trials in which no spikes
occurred in the corresponding 100-ms window as the low activity group, which typically
involved the majority of trials (more than 50 trials). We next divided the remaining trials into
high and medium activity groups so that their numbers were balanced. If the number of trials
in either the high or medium activity group was less than 50, we combined them into one
group (high activity group). We then calculated the spike density function for each group of
trials. We expected that if a neuron participated in the processing network responsible for
temporal storage, the difference in spike density functions at a given time would persist for a
long duration; if not, the differences in spike density would diminish after a short interval. To
test this statistically, we examined whether the difference in the spike density functions
between the high and low activity groups was significant (Mann-Whitney U test, p < 0.01) at
1-ms intervals from 1500 ms before array presentation to 500 ms after array presentation.
Note that the selected intervals for analysis (the 100 ms intervals from 800 ms to 700 ms or
from 100 ms to 0 ms before array presentation) were not essential. The results were
substantially the same when other 100 ms intervals were used for this analysis.

To quantitatively characterize the nature of temporal storage in baseline activity, we
calculated the correlation of spike counts per bin within a trial (temporal correlation analysis).
We first divided the 1000-ms period before array presentation into 10 successive 100-ms time
bins and recorded the spike count in each bin. We then calculated Pearson’s correlation
coefficients for spike counts in pairs of bins separated by intervals of 100, 200, 300, 400, 500,
600, 700, 800, or 900 ms and plotted the correlation values as a function of the separation
time (e.g., Fig. 2E). Correlations were considered significant at $p < 0.001$. A shift predictor analysis was also performed to estimate the strength of the correlations resulting from intertrial changes in baseline activity. The shift predictor was constructed by correlating the spike count in one bin in a given trial with the bin occurring at the same separation time in the following trial.

We found that some FEF and V4 neurons exhibited persistent ramping of baseline activity throughout the fixation period (e.g., Fig. 3). Such ramp-like activity changes may represent the temporal integration of sensory input (Roitman and Shadlen 2002; Shadlen and Newsome 2001). If so, these neurons should display a strong temporal storage capacity. To examine this possibility, we separately analyzed the activity of these “ramping” neurons. We selected neurons in which the number of spikes in 10 successive 100-ms time bins before array presentation changed significantly (one-way analysis of variance, $p < 0.01$) monotonically (Spearman’s correlation: $r > 0.90$) and linearly (linear regression analysis, coefficient of determination: $R^2 > 0.81$) and the difference in the mean discharge rate in the first and last time bins (i.e., 1st and 10th bins) was substantial (>5 spikes/s). We then compared the strength of the temporal correlations between these ramping neurons in FEF and V4.

Average population activity was calculated by averaging the spike density functions of individual neurons. Significant differences in cell-averaged spike density functions between high and low activity groups were determined using a permutation test (Efron and Tibshirani 1993). In each permutation, trial data for each neuron were randomly shuffled across responses in high and low activity trials and were divided into the two groups (the number of trials in each group was the same as in the actual data). Shuffled cell-averaged
spike density functions were then calculated in the same way as were the actual data. This
procedure was repeated to produce 1000 total permutations. We moved across time points in
1-ms increments from 1500 ms before array presentation to 500 ms after array presentation
and conducted the permutation test at each time point. If the difference between high and low
activity groups in the actual data was larger than the permuted differences in more than 990
iterations, it was considered significant (at the p < 0.01 level).
Results

Neuronal database

Neuronal data were obtained from three monkeys. We recorded from visually responsive single neurons in FEF of one monkey, V4 of another monkey, and in both areas of the remaining monkey. Only neurons that maintained stable spike isolation throughout the recording session and showed interspike interval distribution histograms with an absence of interspike intervals shorter than 2 ms were included in the analysis. Visual responsivity was determined by testing whether activity occurring 50 to 150 ms after array presentation was significantly different from the 100 ms of activity immediately preceding array presentation (Mann-Whitney U test, p < 0.01). The eccentricity of array stimulus used in the recording sessions of FEF neurons ranged from 7.1 to 11.3 deg (9.7±1.1deg; mean ± SD) and that of V4 neurons from 4.0 to 11.3 deg (8.1±1.8deg). We examined the activity of 77 well-isolated single FEF neurons and 90 V4 neurons for which the data sets were large enough (>200 trials) and the fixation periods were long enough (>1000 ms). From this population of neurons, we selected neurons that exhibited appreciable activity (>3 spikes/s) during the 1000 ms preceding array presentation. In all, 34 FEF cells and 43 V4 cells fulfilled this criterion; however, a small portion of the neurons (1 in FEF and 3 in V4) were excluded because their baseline activity during the 1000-ms prior to array presentation differed significantly between the shape and color search conditions (Mann-Whitney U test, p < 0.01). Accordingly, data from 33 FEF cells and 40 V4 cells were analyzed.

Differential capacity for temporal storage in FEF and V4 neurons

There were marked differences between FEF and V4 neurons with regard to the ability to store the history of their own activity. This ability was assessed as the temporal
storage capacity of the instantaneous discharge rate. Data from a representative FEF neuron are shown in the left panel of Figure 2. During the recording of this neuron, the fixation period was 1200 ms. Neuronal activity was recorded in 496 successful trials of the visual search task. Figure 2A shows spike density functions (mean ± SEM) constructed by convolving spike trains with a Gaussian function (SD = 20 ms; Richmond and Optican 1987) under the shape search (black traces) and color search (gray traces) conditions. The mean discharge rates in the 1000 ms before array presentation were not significantly different between the shape search and color search conditions (Mann-Whitney U test, p = 0.29).

To illustrate temporal storage capacity in an intuitive manner, we separated trials into three groups—high (red in Fig. 2B), medium (orange), and low (green) activity—according to the spike count during the 100-ms interval from 800 ms to 700 ms before array presentation (gray rectangle in Fig. 2B). These three trial groups were evenly distributed across the entire recording session (Fig. 2C), and differences in the spike density functions obtained from the three groups were evident not only in the 100 ms used for trial separation but also during the remaining fixation period. The difference in neuronal firing between the high and low activity groups was significant throughout the entire fixation period (horizontal black line in Fig. 2B; Mann-Whitney U test, p < 0.01). Moreover, this effect was not unique to the particular time window used for grouping the trials. When the trials were grouped according to the spike count in the 100 ms immediately before array presentation, a similar result was observed (Fig. 2D).

These results were further confirmed by temporal correlation analysis (see Materials and Methods). Figure 2E shows the result of a temporal correlation analysis illustrating how the correlation of the spike count in two bins depended on the time interval between the bins.
Although the magnitude of the correlation coefficients gradually decreased as the separation time increased, the correlation coefficients were significantly larger than zero at all separation times (filled circles in Fig. 2E; Pearson correlation coefficient, p < 0.0001), indicating that baseline activity in a given bin correlated with activity in bins at other bins, even when bins were separated by almost 1 s. Thus, fluctuations in baseline activity were correlated across the entire fixation period, suggesting that this neuron possesses a high capacity for maintaining the instantaneous discharge rate. In addition, shift predictor analysis yielded correlation coefficients that were near zero and that were not significant at any separation time (open squares in Fig. 2E; Pearson correlation coefficient, p > 0.091). Because the shift predictor was computed as the correlation between the spike count in a given trial and that in the next trial, these results indicate that the observed temporal correlation was not the result of neural modulation across trials but rather within a trial.

By contrast, the temporal storage capacity of V4 neurons was rather weak. Data from a representative V4 neuron are shown in the right panel of Figure 2. For this neuron, the fixation period was 1200 ms, and baseline activity did not differ between the two search conditions (Mann-Whitney U test, p = 0.35; Fig. 2F). Neuronal activity was recorded in 423 successful trials of the visual search task. Figure 2G illustrates the spike density functions when trials were separated into three activity groups according to spike counts in the 100 ms from 800 ms to 700 ms before array presentation. The difference in activity between groups was restricted to the period in and around the 100 ms used for trial separation. Thus, activity differences between the high and low activity groups was significant only for a short period (at most 300 ms; horizontal black line; Mann-Whitney U test, p < 0.01), and a similar result was obtained when trials were separated based on activity in the 100-ms interval immediately before array presentation (Fig. 2I). Consistent with those results, temporal correlation
analysis revealed the correlation coefficients to be weak (<0.1) and not statistically
significant (open circles; Pearson correlation coefficient, p > 0.001) at all of the separation
times except 100 ms (Fig. 2J).

These results indicate that the temporal correlation in baseline activity was
considerably stronger in FEF neurons than in V4 neurons, suggesting that temporal storage
capacity is much greater in FEF than in V4.

**Temporal storage in ramp-like activity**

We observed persistently increasing or decreasing activity changes (i.e., ramp-like
changes) in both FEF and V4 neurons. Such persistent ramping of activity may reflect the
temporal integration of sensory input signals (Huk and Shadlen 2005; Roitman and Shadlen
2002). However, in the present study, no peripheral visual stimulus was available during the
fixation period and we examined the baseline activity of neurons representing the peripheral
visual field. Therefore we think that the observed ramp-like activity changes during fixation
were unlikely to reflect the temporal accumulation of external sensory inputs. We were
interested in determining whether these ramping neurons in FEF and V4 also exhibited
differences in their ability for temporal storage of firing rates.

Data from an activity-ramping FEF neuron are shown in the left panel of Figure 3.
The fixation period was 1200 ms. Activity began ramping up after the onset of fixation, and
this effect was sustained until array presentation. The average spike count in each of the 10
successive 100-ms time bins preceding array presentation increased monotonically
(Spearman’s rank correlation coefficient: $r = 0.99$, $p < 0.0001$) and linearly (linear regression
analysis, coefficient of determination: $R^2 = 0.87$). When trials were separated into two (Fig.
3B) or three (Fig. 3D) activity groups according to spike counts in a 100-ms bin, firing rate
differences in the high and low activity groups were significant for almost the entire fixation
period (horizontal black lines; Mann-Whitney U test, p < 0.01), suggesting a strong temporal
storage function. Consistent with these data, temporal correlation analysis (Fig. 3E) revealed
that the correlation coefficients were strong and significant at all separation times (Pearson’s
correlation coefficient, r = 0.31–0.58, p < 0.001).

Data from a V4 neuron with ramp-like changes in activity are illustrated in the right
panel of Figure 3. During recording sessions for this neuron, the fixation period was not
constant; instead, it varied randomly from 1200 ms to 1500 ms. As such, for the following
analysis, baseline activity was locked to array presentation (results were essentially similar
when the baseline activity was locked to the beginning of the fixation period). Baseline
activity in the 1000 ms preceding array presentation increased monotonically (Spearman’s r =
1.0, p < 0.0001) and linearly (regression analysis, coefficient of determination: $R^2 = 0.98$). At
first glance, it appeared that this activity ramping reflected integration over time. However, as
shown in Figures 3G and I, the spike density functions obtained from high, medium, and low
activity trials were nearly superimposable, except for the period in and around the 100 ms
used for trial separation (horizontal black lines; Mann-Whitney U test, p < 0.01). This
indicates that this neuron’s capacity for temporal storage was low. Consistent with this
finding, temporal correlation analysis demonstrated that the correlation coefficients were very
weak and not significant at any separation time (Pearson correlation coefficient, p > 0.001)
except for the separation times of 100 and 300 ms. Thus, although similar ramp-like changes
in activity were seen in both FEF and V4 neurons, the strengths of the temporal correlations
were substantially different between them.
We performed temporal correlation analysis of the bin activity of individual neurons across the entire population of 33 FEF cells and 40 V4 cells. Out of them, 10 cells in FEF (30%; 10/33) and 9 cells in V4 (23%; 9/40) exhibited monotonic (Spearman’s rank correlation coefficient, \( r > 0.90 \)) and linear (linear regression analysis, coefficient of determination: \( R^2 > 0.81 \)) changes in the baseline activity during the 1000 ms before array presentation. The percentages of the ramping neurons did not differ significantly between FEF and V4 (chi-square test, \( p = 0.82 \)). The temporal storage ability of ramping neurons was essentially the same as that of non-ramping neurons. The strength of temporal correlations was not significantly different between ramping and non-ramping neurons at any separation times ranged from 100 ms to 900 ms in FEF (ramping neurons, \( n = 10 \); non-ramping neurons, \( n = 23 \); Mann-Whitney U test, \( p > 0.131 \)) and V4 (ramping neurons, \( n = 9 \); non-ramping neurons = 31; \( p > 0.195 \)). Therefore, we combined the ramping neurons and the non-ramping neurons in the following population analyses, unless otherwise indicated.

Figures 4A and D show the strengths of the correlations in baseline activity bins as a function of separation time for FEF and V4 neurons, respectively. The data corresponding to the example neurons (Figs. 2 and 3) are indicated by highlighted lines. Note that the average correlation coefficient was significantly larger in FEF (thick line in Fig. 4A) than in V4 (thick line in Fig. 4D) for all separation times (Mann-Whitney U test, \( p < 0.001 \)). In FEF, correlation coefficients were significant in about half of the neurons (47%; 16/33) even at a separation time of 800 ms (Fig. 4C). In V4, by contrast, the correlation coefficients were significant in only 10% (4/40) of neurons (Fig. 4F). Thus, the degree of temporal correlation in baseline activity was much stronger in FEF neurons than in V4 neurons at the population level. Figures 4B and E show the results of the shift predictor analysis. Correlation strength
was greatly diminished in both areas. The differences between the raw temporal correlations and the shift predictors were significant at all separation times for FEF neurons (Fig. 4A vs. B, Mann-Whitney U test, p < 0.002) and they were significant at separation times ranged from 100 ms to 700 ms for V4 neurons (Fig. 4D vs. E, Mann-Whitney U test, p < 0.047). The differences in the shift predictors between FEF and V4 neurons were not statistically significant at all of the separation times (Fig. 4B vs. E, Mann-Whitney U test, p > 0.073), except for the marginal difference at a separation time of 700 ms (Mann-Whitney U test, p = 0.045). These results indicate that the temporal correlations observed in baseline activity were not due to neural modulation across trials but to modulation within individual trials.

These findings were preserved even when the population was restricted to neurons that exhibited ramp-like changes in their activity (FEF; n = 10, V4, n = 9). Most FEF neurons (80%; 8/10) exhibited significant temporal correlations at an 800 ms separation time (Pearson’s correlation coefficient, p < 0.001), while no V4 neuron exhibited significant correlation at separation times greater than 700 ms. Thus, even though similar ramp-like changes were observed in both FEF and V4 neurons, the temporal correlations observed in V4 neurons were considerably weaker compared to FEF neurons, indicating that the ramp-like activity is not necessarily related to the powerful temporal storage ability.

Influences of pre-fixation eye movements

In this study, we examined the activity of visually responsive neurons. So, differential responses could be elicited when the monkeys made saccades to the fixation point from different locations. If such activity lasted, this could yield false temporal correlations in baseline activity. However, if this was the case, the strength of temporal correlations would decrease as the fixation time increased, because the influences of pre-fixation eye movements...
on baseline activity should gradually diminish after the onset of fixation. To assess this
possibility, we examined how the strength of temporal correlations changed depending on the
time during the fixation period. The correlation strength at different times was calculated by
moving a pair of 100 ms bins for analysis in 100 ms steps within the 1000 ms period
preceding array presentation. A pair of bins were temporally separated by either 100, 200,
300, 400, or 500 ms. Figures 5A and B show the correlation coefficients as a function of the
time before array presentation for FEF and V4 neurons, respectively. In both areas, at any
separation times, the strength of temporal correlations did not significantly change depending
on the time before array presentation (one-way ANOVA, FEF, p > 0.672; V4, p > 0.341).
Thus, these results indicate that it is unlikely that pre-fixation eye movements were
responsible for producing the temporal correlations observed in baseline activity.

Temporal storage in baseline activity vs. stimulus-driven activity

So far, our analysis has been limited to baseline activity before array presentation.
However, in some FEF and V4 neurons, the temporal storage capacity in baseline activity
differed from that during stimulus-driven activity. This can be seen in data from the second
representative FEF neuron (Fig. 3D). Although the difference between high (red) and low
(green) activity trials was large (>30 spikes/s) before array presentation, that difference
disappeared following array presentation. However, in the first representative FEF neuron
(Fig. 2D), differences between the high and low activity trials before array presentation were
preserved after array presentation.

To determine whether the instantaneous discharge rates of FEF and V4 neurons were
maintained after array presentation at the population level, we calculated correlations
between the spike counts in the 100 ms immediately before array presentation and those
during the 100-ms period beginning 50 ms after array presentation (i.e., baseline activity vs. stimulus-driven activity). As a control measure, we also calculated correlations between the spike counts in the 100 ms beginning 250 ms before array presentation and immediately before array presentation (i.e., correlations within baseline activity). To eliminate the variance in visual responses across the different stimulus conditions, we subtracted the mean spike count in each of the stimulus conditions from the spike count in the individual trials. We then compared the correlations between baseline activity and stimulus-driven activity to those within baseline activity.

Figure 6 shows the comparison of temporal storage capacity in baseline activity and stimulus-driven activity. Thirty neurons in FEF (91%; 30/33, gray and black circles in top panel of Fig. 6) and 28 neurons in V4 (70%; 28/40, gray and black circles in Fig. 6 bottom) exhibited strong correlations within their baseline activity (Pearson’s correlation coefficient, p < 0.001). Out of them, 70% of FEF neurons (10/30) with baseline correlations exhibited correlations with stimulus-driven activity (61% of total; 20/33) and 46% of V4 neurons (13/28) with baseline correlations exhibited correlations with stimulus-driven activity (33% of total; 13/40). Thus, many neurons in both areas maintained temporal correlations during stimulus-driven activity.

**Stability of baseline activity**

Baseline activity changes according to a subject’s internal state (e.g., arousal level; Morrow and Casey 1992). If a subject’s internal state varies over the course of a recording session such that the mean discharge rate of the baseline activity drifts across trials, spurious quasi-significant correlations could be produced in the temporal correlation analysis. To assess this possibility, we divided the recording session into 50 trial blocks and then tested
whether mean baseline activity varied significantly across trial blocks. We found that only a
dominant of FEF (18%; 6/33) and V4 (15%; 6/40) neurons significantly changed their
baseline activity across trials (Kruskal-Wallis nonparametric one-way analysis of variance, p < 0.01), and the percentages of these neurons did not differ significantly between FEF and V4
(chi-square test, p > 0.1). This makes it unlikely that slow drifts in baseline activity were
responsible for the observed differences in the strength of temporal correlations in FEF and
V4 neurons.

**Influences of mean strength and trial-to-trial variance in baseline activity**

In this study we only analyzed neurons that had appreciable baseline activity (i.e., a
mean discharge rate >3 spikes/s in the 1000 ms before array presentation). If the degree of
temporal correlation varied with the absolute magnitude of baseline activity, then the
difference between FEF and V4 neurons could have been due simply to the mean difference
in baseline firing between these two areas. To assess this possibility, we compared the
baseline firing rates of FEF (n = 33) and V4 (n = 40) neurons. We found that the discharge
rate in FEF neurons (11.4 ± 10.5 spikes/s; mean ± SD) was not significantly different from
that in V4 neurons (9.3 ± 7.0 spikes/s; Mann-Whitney U test, p = 0.265). We also examined
the relationship between the mean temporal correlation strength and the mean baseline firing
rate (Fig. 7A). For each neuron, the mean temporal correlation strength was defined as the
average of the correlation coefficients computed for time separations ranging from 100 ms to
900 ms, and the mean discharge rate was defined as the average firing rate in the 1000 ms
before array presentation. An analysis of covariance (ANCOVA) for FEF and V4 neurons
showed no significant dependence of the mean temporal correlation strength on the mean
firing rate (ANCOVA, slope, p = 0.227), although the mean correlation strength in FEF
neurons was significantly larger than those in V4 neurons (ANCOVA, intercept, p < 0.001).
It is also possible that the strength of temporal correlation depended on the magnitude of trial-to-trial variance in baseline activity. To test this possibility, we compared the standard deviation of baseline activity of FEF and V4 neurons. There was no significant difference between FEF neurons (12.5 ± 6.2 spikes/s; mean ± SD) and V4 neurons (12.1 ± 6.0 spikes/s) on this measure (Mann-Whitney U test, p = 0.64). We found no significant dependence of the mean temporal correlation strength on the magnitude of trial-to-trial variance (ANCOVA, slope, p = 0.885), although the mean correlation strength was significantly different between two areas (ANCOVA, intercept, p < 0.001). These analyses confirm that the difference in the strength of temporal correlations in FEF and V4 neurons is not attributable to differences in either in the discharge rate or the trial-to-trial variability of baseline activity.

Influences of behavioral performance

Neuronal activity during the fixation period varies predictably according to the ongoing trial state (success/error) and impending saccade latency (Kobayashi et al. 2002; Okada et al. 2009). Consequently, there is a possibility that the difference in the strength of the temporal correlations in FEF and V4 neurons reflected differences in behavioral performance during recording sessions in these two cortical areas. To assess this possibility, we compared behavioral performance during recording sessions in FEF and V4. We found that the percentage of correct responses did not differ significantly by FEF (86.8 ± 5.9%) or V4 (85.3 ± 7.6%; Mann-Whitney U test, p = 0.67). In addition, we found that the mean discharge rate during the 1000-ms interval before array presentation did not differ significantly between the success and error trials for any neurons in FEF or V4 (Mann-Whitney U test, p > 0.01; Fig. 8A). Furthermore, we compared the mean correlation strength
in success and error trials in FEF and V4 neurons. We found no significant difference in either FEF (Mann-Whitney U test, p = 0.949) or V4 (p = 0.889) neurons.

When we compared saccade latency (time from array onset to saccade initiation) during recording sessions in FEF with that in V4, we found a marginal but significant difference (FEF: 293 ± 27 ms vs. V4: 331 ± 63 ms; Mann-Whitney U test, p = 0.0089). However, further examination of the relationship between saccade latency and baseline activity during the individual trials for each neuron revealed no substantial relationship. Figure 8B illustrates the distribution of correlation coefficients relating saccade latency and magnitude of the baseline activity obtained from FEF (top) and V4 (bottom) neurons. The correlation coefficients were generally small (<0.2) and, with the exception of two V4 neurons, nonsignificant (Pearson’s correlation test, p > 0.01).

Taken together, these findings indicate that the difference in the strength of the temporal correlations in FEF and V4 neurons cannot be explained by differences in behavioral performance during recording sessions.

**Influences of eye position and small eye movements**

In the present task, the monkeys were required to fixate on a small spot located in the center of the display screen throughout the fixation period. Despite this requirement, the eye-position window criteria allowed small eye movements within a window set at ±0.5–0.75°. If these small changes in eye position produced trial-to-trial variance in neuronal activity, this variance could have generated quasi-temporal correlations in the baseline activity. To test this possibility, we divided the data set for each neuron into high and low activity trials according to the magnitude of the baseline activity during the 1000-ms period before array presentation.
We then compared the horizontal and vertical eye positions between the high and low activity groups. When recording from FEF, the mean (±SD) horizontal position was 0.050 ± 0.076° and 0.049 ± 0.075° in the high and low activity trials, respectively, and the mean (±SD) vertical position was 0.12 ± 0.10° and 0.12 ± 0.097°, respectively. When recording from V4, the mean horizontal position was 0.036 ± 0.091° and 0.028 ± 0.079°, and the mean vertical eye position was 0.043 ± 0.096° and 0.045 ± 0.11°, in the high and low activity trials, respectively. No neurons in either FEF or V4 exhibited significant activity differences due to eye position between the high and low activity trials (Mann-Whitney U test, p > 0.01).

We further examined the influence of small eye movements (microsaccades) on activity during the fixation period by dividing the data set into high and low activity trials in the same way as described above, and then counted the number of small eye movements (absolute eye velocity > 20°/s, duration > 10 ms) in each of the high and low activity trials during the 1000 ms before array presentation. When recording from FEF, the frequency of small eye movements was 0.60 ± 0.59 and 0.61 ± 0.68 Hz (mean ± SD) in the high and low activity trials, respectively. When recording from V4, the values were 0.47 ± 0.59 and 0.48 ± 0.68 Hz, respectively. These parameters were consistent with those previously reported (Herrington et al. 2009). No FEF neurons showed a significant difference in activity reflecting the frequency of small eye movements in the high and low activity trials (Mann-Whitney U test, p > 0.01), and only one V4 neuron (1/40) showed a significant difference (Mann-Whitney U test, p < 0.01). These results confirm that neither eye position nor small eye movements during the fixation period substantially influenced the magnitude of baseline activity. These factors thus cannot account for the differences in the strengths of the temporal correlations seen in FEF and V4 neurons.
Influences of fixation time

In the present study, we occasionally varied the length of the fixation period from neuron to neuron (Table 1). If the strength of temporal correlations varied with the fixation time, then the difference in temporal correlations between FEF and V4 neurons could have been due to the difference in the fixation time. To test this possibility, we selected neurons that were recorded when the fixation time was fixed at 1200 ms and compared the correlation strength between FEF (n = 25) and V4 (n = 23) neurons. We found that the correlation coefficients were significantly larger in FEF than V4 neurons at all the separation times ranged from 100 ms to 900 ms (Mann-Whitney U test, p < 0.001), indicating that the difference in the temporal storage ability between FEF and V4 neurons was not attributable to differences in fixation time.
Discussion

In this study we focused on baseline activity recorded during the pre-stimulus fixation period while monkeys performed a cognitive–behavioral task. We compared the strength of temporal correlations in baseline activity between neurons in FEF (a visuomotor area) and in V4 (a visual area) by calculating correlations of the number of spikes per 100-ms bin in a 1000-ms period before visual stimulus presentation. We found that temporal correlations were much stronger in FEF than in V4, indicating that FEF neurons tend to store their instantaneous discharge rate for a longer time than V4 neurons. Additional analyses revealed that the modulation of baseline activity does not depend on either task-relevant variables (e.g., search dimension defining the target) or behavioral performance (e.g., small eye movements during fixation, saccade reaction time, success/error trial). This indicates that the ability of neurons to store histories of their activity level depends on the cortical areas where they are located. These results are consistent with previous reports suggesting that spontaneous neuronal activity is tightly linked to the cortical networks in which the neurons are embedded (Fox and Raichle 2007; Kenet et al. 2003; Shmuel and Leopold 2008; Tsodyks et al. 1999; Vincent et al. 2007). Thus, our results suggest that the crucial difference in the temporal correlations within FEF and V4 neurons is attributable to a difference in the intrinsic properties of the neural networks that comprise these two cortical areas. These analyses provide a new metric that can lend insight into the temporal properties of neurons hidden in baseline activity.

Relation to previous studies of visual and visuomotor areas

Previous studies have provided little evidence of a temporal storage function in the early and intermediate visual areas (e.g., V4, MT, V3A). Whereas neurons in the inferior temporal cortex showed sustained activity during the delay period in a memory-guided visual
A possible neural mechanism for persistent activity changes without temporal integration

When monkeys are required to make a discrimination based on stimulus motion, lateral intraparietal area neurons undergo ramp-like changes in their discharge rate that predict the monkey’s decision. This ramping of activity may reflect temporal integration of sensory input,
and this area is intimately involved in mediation of temporal integration by neural circuits (Huk and Shadlen 2005; Mazurek et al. 2003; Roitman and Shadlen 2002; Shadlen and Newsome 2001). In the present study, similar ramp-like changes were observed in the baseline activity of subsets of neurons in both FEF and V4 (Fig. 5). However, temporal storage capacity differed markedly between these two areas, and the strength of temporal activity correlations was considerably weaker in V4. This finding provides further evidence that neural mechanisms underlying temporal storage are substantially different in FEF and V4. This leads us to consider a neural mechanism other than temporal integration to explain the ramp-like changes in the activity of V4 neurons.

Under the experimental conditions of this study, the most salient event for the monkeys was the presentation of the target array. Prefrontal and parietal cortex neurons exhibit persistent increases or decreases in firing in the time leading up to such a behaviorally salient event, even in the absence of sensory input (Janssen and Shadlen 2005; Leon and Shadlen 2003; Mita et al. 2009; Tanaka 2007). This type of activity may be the origin of top-down attentional influences (Moore and Armstrong 2003; Moore and Fallah 2004) that produce persistent ramp-like changes in baseline activity of neurons in visual cortical areas (Ghose and Maunsell 2002). However, these modulatory top-down attentional influences would not elicit substantial temporal correlations in baseline activity. Even if top-down influences show ramping of activity, if input to a visual neuron generating spontaneous activity occurs in a constant but random sequence, the resultant output of the neuron generated by multiplying input signals by top-down modulatory signals would still exhibit random fluctuations in individual trials. This view might explain why there are only poor temporal correlations in V4 neurons even when they show substantial ramp-like changes in trial-averaged baseline activity.
Different roles in visual and visuomotor areas

Although integration over time is a powerful strategy for extracting and facilitating behaviorally relevant signals from a noisy background, one possible side effect of using integration over time is diminished sensitivity to high-frequency input signals. Because temporal integration works as a low-pass filter, low frequency signals are transmitted but high frequency signals are damped. If visual brain areas participate in the neural circuits for time integration, visual information in the high-frequency domain would be largely lost and unavailable for further downstream processing. This would be problematic in situations in which the brain is required to detect rapid changes in sensory input signals. To avoid this issue, the brain might use strategies other than temporal integration in visual sensory areas. Top-down modulation would be a reasonable strategy for enhancing behaviorally relevant signals in visual areas without degrading temporal resolution. It is thus conceivable that the present results reflect contrasting neural processing strategies in visual and visuomotor areas. Previous studies showed that neurons in visual areas have temporal precision up to about 100 Hz (Bair and Koch 1996; Mazurek and Shadlen 2002). We therefore speculate that although neurons in visual sensory areas are involved in real-time representation of the visual world, those in visuomotor areas more emphasis is placed on temporal accumulation of visual sensory input for extracting and facilitating behaviorally relevant signals and generating signals for use with top-down attention processes (Monosov et al. 2008).
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Figure legends

Figure 1. Visual search task design. A trial was initiated by presenting a fixation spot at the center of the display. Once the monkey fixated for the required time (typically 1200 ms), the fixation spot was extinguished and a search array appeared. The monkeys had to make a saccade (arrow) toward one of the singleton stimuli according to the instructed target-defining dimension. No delay period was imposed. In the shape search, the shape singleton was the target, and the color singleton was the distractor (as shown here). In the color search, the opposite was true. Identical sets of arrays were presented in both search conditions. The two search conditions were implemented sequentially in separate blocks of ~100 trials. The ongoing target-defining dimension was signaled by the color of the fixation spot (red, shape search; blue, color search). In most of the neurons studied, the color of the cue was given for only the first 10 trials in each block, and a white spot was presented in the rest of trials.

Figure 2. Differences in temporal correlations in baseline activity of FEF and V4 neurons. (A–E) Data from a representative FEF neuron. (A) Spike density functions (mean ± SEM) for shape searches (black trace) and color searches (gray traces). Traces are aligned to array presentation. The fixation period was 1200 ms for all trials with this neuron. Left and right vertical dashed lines indicate fixation onset and stimulus array onset, respectively. (B) Spike density functions obtained from high (red trace), medium (orange trace), and low (green trace) activity trials. Trials were separated according to the spike count in the 100-ms interval from 800 ms to 700 ms before stimulus onset (gray rectangle). Small vertical tick marks indicate action potentials, and each row of rasters indicates 1 trial. The horizontal black line indicates when the difference in high and low activity traces was statistically
significant (Mann-Whitney U test, p < 0.01). The numbers above this line reflect the start and end times of the interval in which a significant difference persisted for more than 100 ms. (C) Baseline activity during the selected 100-ms interval (800–700 ms prior to array presentation) in each trial is shown as a function of the cumulative successful trial count. Open and filled circles indicate data obtained from trials in shape and color search conditions, respectively. Red, orange, and green symbols indicate high, medium, and low activity trials. (D) The same analysis as in (B) but based on the activity during the 100-ms interval immediately before array presentation. (E) The strength of the temporal correlation plotted as a function of interval separation time. Circles indicate correlations of the spike counts in 100-ms time bins within a trial, whereas squares indicate correlations between one bin in a given trial and one in the following trial (i.e., shift predictors). Filled symbols indicate significant correlation values (Pearson’s correlation test, p < 0.001). (F–J) The same analysis as summarized in (A–E) but for a representative V4 neuron.

Figure 3. Examples of FEF and V4 neurons showing ramp-like changes in baseline activity. Data from a representative FEF neuron (A–E) and V4 neuron (F–J) showing that baseline activity increased monotonically and linearly. Conventions are the same as in Fig. 2.

Figure 4. Population analysis of temporal correlations in FEF and V4.

(A) Temporal correlation analysis of baseline activity in FEF neurons (n = 33). Circles connected with a thin gray line represent the correlation coefficients obtained from each neuron, and the thick line indicates the mean values across all neurons. Thin black lines indicate the data corresponding to the example neurons (Figs. 2 and 3). Filled circles indicate significant correlation coefficients (Pearson’s correlation test, p < 0.001). (B) Shift predictor analysis. Conventions are the same as in (A). (C) The percentage of FEF neurons exhibiting
significant correlations in (A) plotted as a function of separation time. (D–F) Results of the same analyses described in (A–C) but based for V4 neurons (n = 40).

Figure 5. Effect of fixation time on temporal correlations in FEF and V4.

(A) The strength of temporal correlations for FEF neurons (n = 33) is illustrated as a function of time before array presentation. Each circle indicates the correlation strength calculated from a pair of 100 ms bins and is plotted at the mean time of those two time bins. Temporal correlations at different times are calculated by moving the bins for analysis in 100 ms steps within the 1000 ms period before array presentation. Circles connected with a line represent the correlation coefficients obtained from the same separation time (either 100, 200, 300, 400 or 500 ms). (B) Result of the same analysis for V4 neurons (n = 40). Conventions are the same as in A.

Figure 6. Temporal correlations in baseline activity vs. stimulus-driven activity.

Comparison of temporal correlations in baseline activity with those in stimulus-driven activity in FEF (n = 33, top) and V4 (n = 40, bottom) neurons. The ordinate shows correlation coefficients relating the spike count in the 100 ms immediately before array presentation and the 100-ms interval extending from 50 ms to 150 ms after array presentation (i.e., the correlation between baseline activity and stimulus-driven activity). The abscissa shows correlations coefficients relating the spike count in the 100-ms interval extending from 250 ms to 150 ms before array presentation and the 100-ms interval immediately before array presentation (i.e., the correlation within baseline activity). Filled circles indicate that the correlation coefficient was significantly greater than zero on both axes. Gray circles indicate that the correlation coefficient was significant only with respect to the abscissa, whereas triangles indicate that it was significant only with respect to the ordinate. Open circles
indicate a lack of significance with respect to either axis.

Figure 7. Effects of the magnitude and variance of baseline activity on temporal correlations in FEF and V4.

(A) Relationship between mean correlation strength and mean magnitude of baseline activity in FEF (n = 33) and V4 (n = 40) neurons. Each symbol represents a neuron. Filled symbols indicate mean values across neurons for each area. An analysis of covariance for FEF and V4 neurons showed no significant dependence of mean correlation strength on mean firing rate (ANCOVA, slope, p = 0.227). (B) Relationship between mean correlation strength and trial-to-trial variance of baseline activity. There was no significant relationship between the two variables (ANCOVA, slope, p = 0.885).

Figure 8. Effect of behavioral performance on the strength of temporal correlations in FEF and V4.

(A) Comparison of baseline activity in success and error trials. No neurons showed a significant difference in activity according to performance (Mann-Whitney U test, p > 0.01). (B) Histogram showing the distribution of correlation coefficients representing the dependence of saccade reaction time on baseline activity of each FEF (n = 33) and V4 (n = 40) neuron. Filled bars indicate correlation coefficients significantly different from zero (Pearson’s correlation test, p < 0.01).
### Table 1. Summary of fixation-time conditions

<table>
<thead>
<tr>
<th>Fixation time (ms)</th>
<th>Number of neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>FEF</em> (n = 33)</td>
</tr>
<tr>
<td>1000-1200</td>
<td>2</td>
</tr>
<tr>
<td>1200</td>
<td>25</td>
</tr>
<tr>
<td>1200-1500</td>
<td>6</td>
</tr>
<tr>
<td>1200-3000</td>
<td>0</td>
</tr>
<tr>
<td>3000</td>
<td>0</td>
</tr>
</tbody>
</table>

Number of neurons that were recorded when the fixation time was fixed (1200 ms or 3000 ms) or when it was sampled from a rectangular distribution (1000 – 1200 ms, 1200 – 1500 ms, or 1200 – 3000 ms).
A. FEF

Correlation vs. Time from stimulus (ms)

Separation time:
- 100 ms
- 200 ms
- 300 ms
- 400 ms
- 500 ms

n = 33

B. V4

Correlation vs. Time from stimulus (ms)

Separation time:
- 100 ms
- 200 ms
- 300 ms
- 400 ms
- 500 ms

n = 40
Correlation within baseline activity

Correlation between baseline activity and stimulus-driven activity

*FEF*

n = 33

*V4*

n = 40