Changes in the Response Rate and Response Variability of Area V4 Neurons During the Preparation of Saccadic Eye Movements.

Abbreviated title: Signatures of Saccade Preparation in Area V4.

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Abstract

The visually driven responses of macaque area V4 neurons are modulated during the preparation of saccadic eye movements, but the relationship between presaccadic modulation in area V4 and saccade preparation is poorly understood. Recent neurophysiological studies suggest that the variability across trials of spiking responses provides a more reliable signature of motor preparation than mean firing rate across trials. We compared the dynamics of the response rate and the variability in the rate across trials for area V4 neurons during the preparation of visually guided saccades. As in previous reports, we found that the mean firing rate of V4 neurons was enhanced when saccades were prepared to stimuli within a neuron’s receptive field (RF) in comparison with saccades to a non-RF location. Further, we found robust decreases in response variability prior to saccades and found that these decreases predicted saccadic reaction times for saccades both to RF and non-RF stimuli. Importantly, response variability predicted reaction time whether or not there were any accompanying changes in mean firing rate. In addition to predicting saccade direction, the mean firing rate could also predict reaction time, but only for saccades directed to the RF stimuli. These results demonstrate that response variability of area V4 neurons, like mean response rate, provides a signature of saccade preparation. However, the two signatures reflect complementary aspects of that preparation.
Introduction

Visual perception and oculomotor control are known to interact. In one direction, the features of a visual scene influence the patterns of saccadic eye movements (Yarbus, 1967; Vishwanath and Kowler, 2003). Underlying this influence is presumably the projection of visual cortical representations onto oculomotor structures (Keller and Edelman, 1994; Edelman and Keller, 1996; Moore, 1999). Conversely, psychophysical evidence demonstrates that the preparation of saccadic eye movements informs perception of visual targets, enhancing visual sensitivity at the intended saccade location (Hoffman and Subramaniam, 1995; Deubel and Schneider, 1996). Correspondingly, the mean firing rates of single neurons in some areas of visual cortex have been shown to be modulated during the preparation of saccades to receptive field stimuli, suggesting a direct influence of saccade preparation on these neurons (Fischer and Boch, 1981; Chelazzi et al., 1993; Moore et al., 1998; Sheinberg and Logothetis, 2001; Tolias et al., 2001; Nakamura and Colby, 2002; Mazer and Gallant, 2003). Mimicking endogenous saccade signals by electrically stimulating sites within the Frontal Eye Field (FEF) yields similar modulation of visually driven responses in visual area V4 (Moore and Armstrong, 2003; Armstrong and Moore, 2007), suggesting that the perisaccadic modulation observed during voluntary saccades originates from oculomotor structures (Moore et al., 2003). In spite of the above evidence, our understanding of the nature of the oculomotor influence on visual cortex and the contribution of extrastriate areas to saccade preparation remains incomplete.

Thus far, evidence of an influence of saccade preparation on extrastriate neurons has been exclusively examined in terms of perisaccadic modulations in mean firing rate
(Fischer and Boch, 1981; Tolias et al., 2001; Moore and Chang, 2009). However, a recent study suggests that the across-trial variability of neuronal firing rate provides a more robust signature of motor preparation (Churchland et al., 2006). This study examined the relationship between the activity of neurons in dorsal premotor cortex and the reaction time of monkeys performing a delayed reach task. Although the mean firing rate of premotor neurons did not predict reaction time, changes in the across-trial variability of firing rate did. This observation suggests that firing rate variability may be a more sensitive measure of behavioral state than mean firing rate and thus may be a more robust signature of motor preparation. A recent study of extrastriate area V4 observed attention-dependent changes in across-trial variability of neuronal response rates (Mitchell et al., 2007). Given the well-established relationship between attention and saccade preparation (Moore, 2006), across-trial variability of response rates of V4 neurons may also provide an index of motor preparation.

To assess the interaction between saccade preparation and visual cortical representations, we measured the mean firing rate and variability across trials of spike trains recorded from area V4 neurons in monkeys trained to make saccades to visual targets. Response variability was measured by the Fano factor (FF), which was computed by dividing the across-trial variance in spike counts within a small window by the mean count. As expected, the mean firing rate of V4 neurons was enhanced when saccades were prepared to stimuli within a neuron’s receptive field (RF) in comparison with saccades to a non-RF location. In contrast, we found robust decreases in FF prior to saccades both to RF and non-RF stimuli, and these decreases predicted saccadic reaction times for saccades to all stimuli. Mean firing rate also predicted reaction time, but only
for saccades directed to the RF stimuli. For saccades directed away from the RF, no mean firing rate change was observed, yet FF still predicted saccadic reaction time. These results demonstrate that response variability of area V4 neurons, like mean response rate, provides a signature of saccade preparation. However, the two signatures reflect complementary aspects of that preparation.
Methods

Subjects

Two male monkeys (*Macaca mulatta*, 8-12 kg) were used in these experiments. All experimental procedures were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals, the Society for Neuroscience Guidelines and Policies. General surgical procedures have been described previously (Graziano et al., 1997).

Behavioral Task

Monkeys performed a visually-guided, delayed saccade task which was initiated by fixation to within 1.0° of the central fixation spot (Figure 1). Immediately following fixation, an oriented bar stimulus appeared in the RF of the neuron under study and remained there until the end of the trial. Following the onset of the RF stimulus, the monkey was required to maintain fixation for a fixed delay (0.5 to 1 seconds, for a given experiment), while it waited for the appearance of a saccade target (0.25° diameter) at one of two locations distant from the RF. In two-thirds of the trials (away conditions), the target appeared, the fixation spot was extinguished and the monkey was rewarded for making a saccade to the target. In these away conditions, the saccade target could appear either directly upward from the fixation spot (“up” condition) or in the opposite visual hemifield to the RF stimulus (“opposite” condition). In the remaining one-third of trials (“toward” condition), the saccade target did not appear. Instead, when the fixation spot was extinguished the monkey was rewarded for saccades to the RF stimulus. All conditions were identical until the cue to saccade (disappearance of the fixation spot) and
were randomly interleaved. During all behavioral trials, eye position was measured via the scleral search coil method, and digitized at 200 Hz for offline analysis. Trials in which the monkey broke fixation prematurely or made a saccade to an incorrect target were discarded.

**Recording**

The activity of single V4 neurons was recorded via glass-coated platinum-iridium electrodes lowered into the dorsal surface of the prelunate gyrus. Neural activity was sampled at 32 kHz, digitized and stored. The waveforms of single neurons were isolated by offline clustering (DataWave Technologies).

**Receptive Field Stimuli**

RF stimuli were displayed on a 34 x 27 cm Sony video monitor that was driven by a Number Nine graphics board (640 x 480) at a 60 Hz, non-interlaced, refresh rate. The video display was positioned 57 cm in front of the monkey. Visual stimuli consisted of gray, red, green or blue colored bars appearing at one of four orientations (0°, 45°, 90°, or 135° θ), presented at the center of a V4 neuron’s RF. The contrast of the oriented bars varied between 5% and 80%, and the sizes varied between 1.0° x 0.1° and 8.0° x 0.8°. In a single block of trials, the RF stimulus varied along only one of the four stimulus dimensions (color, orientation, contrast, or size). The fixation spot was a small (0.25° diameter) circle displayed at the center of the video display. The non-RF saccade target stimulus used in some behavioral conditions was identical to the fixation spot but located peripherally (>5.0°).
Data Analyses

We distinguished between tuned and untuned neurons by performing an unpaired t-test between firing rates for trials of each stimulus identity (i.e. “red” or “green”) and trials of each other stimulus along the same dimension (size, contrast, color, or orientation). If any comparison was significant (p<0.05) then the neuron was defined as tuned and the maximal stimulus was taken as “preferred” while the minimal was “non-preferred”. For trials corresponding to each neuron, each stimulus identity, and each saccade direction (10-20 trials (mean 15.6), hereby defined as a “neuron-condition”), we used the median RT for that neuron-condition to determine the faster (“short RT”) and slower (“long RT”) trials. Thus the “long RT” and “short RT” trials are exactly controlled for the effects of stimulus identity, neuron identity, stimulus preference, and saccade direction. Trials with RTs equal to the median of the neuron-condition were randomly assigned to the short or long RT groups.

Fano factor (FF) was computed by calculating the variance divided by mean of the spike counts across trials for an 80-ms window centered on successive 1-ms time bins, for each neuron-condition, i.e. those trials with the same recording site, visual stimulus, and saccade direction. For example, for a time bin centered at -45 ms relative to saccade onset, counts were made within the 80 ms window around that time point (-85 to -5 ms) on each of the 10-20 trials of the neuron-condition, and both the mean and the variance were computed on the resulting set of 10-20 numbers. Finally the variance was divided by the mean to yield the FF, and the population estimate was simply the average of the FF values from all neuron-conditions. Note that the FF measures across-trial variability (Churchland et al., 2006) as opposed to within-trial variability of spike times.
or inter-spike intervals (de Ruyter van Steveninck et al., 1997), or variability across neurons (Cohen et al., 2007). Windows with no spikes on any of the trials were excluded from FF calculations. 80 ms was chosen as a window size, prior to computing any statistics, after trying values between 5 and 150 ms and selecting visually for a window that yielded traces retaining salient features of those generated with shorter windows while smoothing the noise effectively. Mean firing rates were likewise computed with the same 80 ms window.

To determine whether mean firing rate or FF traces at a particular point deviated significantly from a baseline period, we performed Wilcoxon ranked sum tests on the difference between data at the point of interest and data from a set of baseline period time points chosen to include the entire delay period without overlap, since each point contains data from an 80ms window. For saccade aligned data, the delay period was -640 to -320 ms relative to saccade onset, so the selected data points composing the delay period were -600, -520, -440, and -360 ms.

To control for a possible effect of variable firing rates on FF, we employed a “mean-matching” procedure in which the population distribution of mean spike counts was equalized across time (See Figure 4, (Churchland et al., 2007)). The algorithm computed the mean spike counts for all neuron-conditions, where each “neuron-condition” consists of a complete set of trials, 10-20 total, from a particular neuron, visual stimulus, and saccade direction. Each plotted dot in Fig 2G represents the mean and variance across the trials of one neuron-condition. The algorithm determined a common distribution of these mean spike counts that can be found at all time points. It then randomly eliminated neuron-conditions until this common distribution was achieved.
at each time point. Since individual trials were never deleted from within neuron-
conditions, the relationship between the mean and variance of spike counts for any
neuron-condition was never altered by this procedure; rather, a different selection of the
neuron-conditions (i.e. variance/mean pairs) is taken at each time point to meet the
common distribution. The elimination was independent at each time point. The algorithm
discarded a minority of the data in each case, keeping 69% for the upward saccade
condition, 63% for opposite saccades, and 53% for saccades toward the RF. The FF was
then computed only on these remaining data. The process was repeated 10 times and the
results averaged to control for variation due to the randomness of the procedure. We
performed this analysis using the “Variance Toolbox” for MATLAB provided by M.M.
Churchland.

In order to assess the possible influence of microsaccades on the mean rate and
variability of V4 responses, we performed control analyses in which trials containing
microsaccades within relevant time windows were eliminated. Thus for analyses of
presaccadic firing rates and FFs, we excluded trials with microsaccades occurring within
200 ms of saccade onset (0.6% of trials). Likewise, for analysis of RT effects around the
time of cue onset, we excluded trials with microsaccades occurring within 200 ms of cue
onset (2.4% of trials). Microsaccade detection was performed as in (Armstrong et al.,
2006). Microsaccades were defined as eye movements which exceeded 0.1° amplitude
and had maximum velocity greater than 10°/sec for at least 10ms.

For comparison of two conditions (for example, “long reaction time trials” versus
“short reaction time trials”) we computed a Wilcoxon signed rank test on the mean firing
rate or FF values for all neurons under the first condition versus those for the second
condition at a certain time point. For stimulus aligned responses, we used $t=100\text{ms}$ post-stimulus onset, approximately at the peak of responsiveness. For cue aligned, we used $t=0\text{ms}$ (exactly at cue onset) and for saccade aligned we used $t=-45\text{ms}$ (just prior to saccade onset without including any post-saccadic visual responses). Since a window of 80ms was used for the computation of both mean firing rate and FF, the values at these points include spikes from 40ms on either side of the point. For comparisons in which many time points were examined to determine the time course of an event, the Simes procedure was used to control the false discovery rate (Benjamini and Hochberg, 1995).

To analyze differences in the magnitude of the presaccadic decline in FF between saccade directions, we performed an analysis of covariance (ANCOVA) on the change in FF over the final 80ms of saccade preparation, with the change in mean firing rate over the same time period as a covariate and the saccade direction as a factor. Thus for each neuron-condition, without mean-matching, we subtracted the FF and mean rate values at -80ms relative to saccade from the values at the time of saccade onset. These two sets of numbers, $\Delta FF$ (dependant variable) and $\Delta$ mean firing rate (independent variable), were grouped according to saccade direction and analyzed with the ANCOVA. The $y$-intercept of the $\Delta FF$ versus $\Delta$ mean firing rate measures the component of the changes in FF that is independent of changes in mean firing rate.

**Results**

We computed the Fano factor (FF) and the mean firing rate for 102 single neurons recorded in area V4 of two macaque monkeys ($n=28$ neurons from one and $n=74$ from the other) during the visually guided saccade task. Neurons were visually stimulated with
single oriented bars that varied in orientation, color, size or contrast. Figure 2 shows both
the mean firing rate and mean FF changes in the population following onset of the RF
stimulus, around the time of cue onset, and at the time of saccades to the RF stimulus or
to non-RF targets. Stimulus-onset-aligned data from the most effective (‘preferred’) and
least effective (‘non-preferred’) RF stimuli are plotted separately; cue- and saccade-
aligned data are divided according to the direction of the saccade. Overall, the sample of
V4 neurons was highly selective for the RF stimuli employed, shown by the substantial
difference in mean firing rate following stimulus onset between the preferred and non-
preferred stimuli (Figure 2A). In contrast, the FF exhibited a marked decrement following
stimulus onset (Figure 2B), and there was no apparent difference in that decrement
between the preferred and non-preferred responses (Wilcoxon signed rank test, p=0.234;
see Methods for further details on statistical procedures). Instead, the dynamics of the
stimulus-driven FF changes were similar for the two stimulus divisions during both the
initial onset transient and the sustained response in the delay period. The overall
decrement in the FF following stimulus presentation is consistent with the stimulus-
driven changes in variability reported across many other cortical areas (Churchland M et
al., 2009). Cue-aligned firing rate and FF are shown only to emphasize that at the time of
cue onset the rewarded direction of saccade was unknown to the monkey, and therefore
the overall mean firing rate and FF did not differ between the three saccade direction
conditions (Figure 2C and D).

The mean firing rate changes we observed prior to saccade onset (Figure 2E)
confirmed previous findings. Specifically, there was a significant increase in mean firing
rate for saccades to the RF stimulus (“toward” condition; Wilcoxon rank sum test,
However, there was no change in mean firing rate for saccades to the opposite hemifield or upward saccade target locations (“opposite” and “up” conditions; Wilcoxon rank sum test, p=0.155 and p=0.069 respectively) (Fischer and Boch, 1981; Moore et al., 1998; Moore and Chang, 2009). In contrast to the mean firing rate effects, the FF decreased significantly for all saccade directions when compared to its value during the delay period (Wilcoxon rank sum test, p<0.001 for each direction) (Figure 2F). The decrement in the FF was present within the final 100 ms of saccade preparation for each of the three saccade directions, shown by the decreased slope in the presaccadic variance/mean relationship relative to baseline (Figure 2G). Saccades to the RF stimulus generally had much longer reaction times than saccades to non-RF targets (reaction times, mean±standard deviation: toward = 224±50ms; up = 115±28ms; opposite = 115±24ms; toward vs. up, p<0.001; toward vs. opposite, p<0.001). The larger reaction times of the saccades to the RF stimuli is presumably due to the lack of an abrupt onset of the target (i.e. the RF stimulus) in this condition, in contrast to the other two conditions (Yantis and Jonides, 1984). Nonetheless, the pattern of presaccadic FF decline was largely similar to the other saccade conditions.

“Mean-matched” control for presaccadic firing rate changes.

Neural firing patterns are commonly approximated as Poisson processes, for which the variance of spike counts across trials is equal to the mean and thus FF is unity. However, this assumption may be violated and FF may decrease for extraneous reasons, for example due to an increasing influence of the refractory period at high firing rates. Although average firing rates were low (<~40Hz), and thus the refractory period is
unlikely to have a large impact on spike train variability (Mitchell et al., 2007), we nonetheless performed an analysis to control for the influence of firing rate dynamics on the FF (Figure 3A, B). In this analysis, “neuron-conditions” (sets of trials corresponding to each neuron and stimulus condition; see Methods) were discarded randomly at each time point to equalize the distribution of mean firing rates across the entire presaccadic period. This was done separately using data for each saccade condition (toward, up, and opposite), and the FF was computed on the remaining data (see Methods). Thus this procedure eliminated changes in mean firing rate preceding saccades. Nevertheless, the significant decline in FF prior to saccade onset persisted for all three saccade conditions (Wilcoxon signed rank, p<0.001 for toward condition; p<0.05 for up and opposite) even in this “mean-matched” data set. Thus, the observed presaccadic decreases in FF were not due to the rising mean firing rate.

Dependence of FF changes on saccade direction

We compared the magnitude of the presaccadic decline in FF between the three saccade directions during the final 80 ms period prior to the saccade onset. We used an ANCOVA to factor out the effect of presaccadic changes in mean firing rate. We found main effects of saccade direction (p<0.016) and mean firing rate (p<10^{-4}) on the magnitude of FF decline (Figure 3C). The latter effect demonstrates that firing rate indeed influences the presaccadic change in FF. The main effect of saccade direction, however, demonstrates that the FF declines with different magnitude for the different saccade directions, and that this difference is independent of changes in mean firing rate. The overall decline for all saccade directions (p<0.01 for all directions) corroborates the
results of the mean-matching analysis in that it confirms a presaccadic decline in FF that
is independent of changing firing rates. However, the FF decline was greatest for
saccades directed toward the RF compared to the up and opposite conditions. Thus in
addition to a robust overall decline in FF for all saccade directions, we observed a
component of that decline that depended on saccade direction.

Predicting saccadic reaction time.

A recent study found that across trial firing rate variability provides a better
predictor of motor preparation than does the mean firing rate (Churchland et al., 2006).
We sought to determine whether the variability of V4 responses, measured by Fano
factor, might reflect the state of saccade preparation. To do this, we examined the extent
to which the FF was predictive of saccadic reaction time. We divided the trials obtained
from all saccade directions and all RF stimuli into two subsets, long and short RT trials,
with equal numbers of all conditions in each subset. We then recomputed mean firing rate
and FF on these new trial divisions. We reasoned that if either FF or mean firing rate
reflects the state of saccade preparation then we should observe differences in these
measures between short and long RT saccades at the time of the movement cue. Since the
analysis window was 80 ms in duration, it included spikes occurring from 40 ms prior to
the movement cue onset up to 40 ms after. V4 neurons have visual onset latencies of
approximately 50 ms (Maunsell, 1987), and in our data closer to 70 ms (Figure 2A). Thus
the analysis window includes only the activity of neurons prior to any measurable
responses to the movement cue (fixation offset) or target onset.
Despite the lack of differential visual stimulation at the time of cue onset, the FF of V4 neurons was significantly different between long and short RT trials, though mean firing rate was not (Figure 4). We computed the mean firing rate and FF around the time of movement cue onset separately for trials corresponding to each RT group and saccade direction, and depict these data as percent changes from short to long RT trials, plotted for each saccade direction separately (Figure 4A). A two-way repeated measures ANOVA revealed no main effect of either RT or direction on mean firing rate at exactly the time of cue onset, though there was an interaction between the two (Figure 4B, p<0.001). Considering only those saccades directed toward the RF, there was a difference in mean firing rate between short and long RT trials at the time of cue onset, with 8.6% larger mean firing rate for short RT trials (p<0.001). Mean firing rate did not differ significantly between RT groups for saccades to other locations, though the trend was towards a suppression of mean firing rate for saccades to the opposite hemifield on short RT trials relative to long (2.4% lower mean firing rate for short RT trials; p=0.16).

We observed a main effect of RT on FF, with short RT saccades having lower FF across directions (Figure 4A, right column; p=0.003). However, there was no main effect of saccade direction (p=0.66) and no interaction between RT and saccade direction (p=0.59). Thus for saccades toward the RF, both mean firing rate and FF predicted RT. However, for saccades directed to the upwards and opposite saccade targets, the FF predicted saccadic RT even though there was no change in mean firing rate. Due to the interaction between saccade direction and RT for mean firing rate, collapsing the data across saccade direction largely eliminated the difference between short and long RT trials (p=0.15). In contrast, collapsing the FF data across saccade directions yielded a
robust difference between short and long RT trials (p = 0.006). FF was significantly lower for short RT trials than long from -35 to 148 ms relative to cue onset (p<0.014; Figure 4C). The difference between FF of short and long RT trials (4.5%) is similar in magnitude to the effects reported in the study of premotor cortical neurons in a reaching task (5%; (Churchland et al., 2006)). Our results demonstrate that, in contrast to the presaccadic decline in FF, a component of which depended on saccade direction, the relationship between FF and RT at the time of cue onset was independent of saccade direction.

Possible influence of microsaccades

Since it is known that fixational saccades (i.e. microsaccades) can affect the firing rates of V4 neurons (Leopold and Logothetis, 1998), we considered their possible influence on the rate and variability of V4 activity in this study. For example, since the rate of microsaccades necessarily (and empirically) decreases in the time leading up to a saccade, this decline in the incidences of microsaccades might have contributed to the decline in FF (Figure 2D). To control for any influence of microsaccades, we discarded all of the trials in which a microsaccade occurred within the time window of interest and re-performed the analyses described above. There were no differences in the primary effects in this reduced data set compared to the data set in which all trials with microsaccades were included. In particular, the presaccadic decline in FF remained significant for all saccade directions (Wilcoxon rank sum test, p<0.001). The magnitude of the presaccadic decline still depended on saccade direction (p<0.018). The mean firing
rate still predicted saccadic RTs for saccades toward the RF but not for other directions (p<0.001), and the FF predicted saccadic RTs for all directions (p=0.05).

Discussion

We measured the mean and variability of firing rates across trials of spike trains recorded from area V4 neurons during visually guided saccades. As expected, the mean firing rate of V4 neurons was enhanced when saccades were prepared to stimuli within a neuron’s receptive field (RF) in comparison with saccades to a non-RF location. In contrast, we found robust decreases in Fano factor (FF) prior to saccades both to RF and non-RF stimuli, with only a small influence of saccade direction on the magnitude of the FF decrease. These FF decreases predicted saccadic reaction times for all saccade directions. Although mean firing rate also predicted reaction time, this effect depended on saccades being directed to the RF stimuli. These results demonstrate that mean firing rate and FF exhibit different and complementary signatures of saccade preparation in area V4: while mean firing rate conveys more information about the direction of an imminent saccade, FF primarily reflects the progress of saccade preparation.

The way in which saccades toward the RF were cued differed from that of saccades directed to up or opposite targets. Specifically, in the latter case the appearance of a saccade target indicated the location of the rewarded saccade, whereas in the former case, the absence of such a target indicated that the rewarded saccade was to the RF stimulus. It could be argued that this unbalanced task design could confound the interpretation. For example, the presaccadic enhancement of mean firing rate for toward saccades might be explained by a difference in the cueing method or by increased RTs in
that condition. A previous report has shown that the presaccadic enhancement of mean firing rate is independent of any cue (Moore and Chang, 2009) and thus these mean firing rate changes are not due to the differences in cueing method. The novel changes in FF that we report were also independent of cueing method. Specifically, the ability of FF to predict reaction time and the presaccadic decline in FF were largely independent of saccade direction and thus cannot be explained by the task design.

FF is a measure of variability normalized to the mean, so it reflects changes in variance relative to concurrent changes in mean firing rate. Nevertheless, the FF may vary due to indirect effects caused by changes in mean rate that do not reflect true changes in variability. For example, spike trains may be regularized by an increasing influence of the refractory period at high mean firing rates, such as those observed in our task prior to saccades toward RF stimuli. In some conditions, such indirect effects could not possibly account for the dynamics in the FF. For example, although saccades in different directions were preceded by either enhanced or unchanged mean firing rates, the FF decreased for all directions uniformly (Figure 2C, D). Thus, differences in the dynamics of mean firing rate across conditions do not necessarily result in the same direction of differences in the dynamics of FF. In addition, we controlled for the effect of changes in mean rate on FF by matching the mean across-trial firing rate distributions across time. The effect of this manipulation is to produce a subset of the data that has stable mean firing rate across time. We found that the FF decrease during saccade preparation was still present in the mean-matched data, indicating that the dynamics of the FF response were independent of changes in mean firing rate. The dissociation of
responses of these two measures of neural activity demonstrates that they represent
different information about the state of the visuosaccadic network.

Firing rates of single neurons predict behavioral reaction times in many frontal
and parietal cortical regions, such as motor and premotor cortex (Riehle and Requin,
1993), the parietal reach region (Snyder et al., 2006), the frontal eye fields (Hanes and
Schall, 1996), and the lateral intraparietal area (Ipatas et al., 2006). These correlations may
reflect the role of these individual neurons in generating motor behaviors such as arm and
eye movements. Recent studies have also shown that several measures of visual cortical
activity predict reaction time, such as LFP in striate and extrastriate cortex (Zhang et al.,
2008), spike-field coherence in area V4 (Womelsdorf et al., 2006), and multiunit activity
in area V1 (Super and Lamme, 2007) as well as single unit activity in areas MT and VIP
(Cook and Maunsell, 2002). Our results are thus consistent with a growing body of
evidence that neural activity in visual cortex can predict reaction time. Our results also
demonstrate that the FF of V4 responses provides a reliable prediction of RT in that it
predicted RTs of saccades in all tested directions, rather than simply those which target
the neuron’s RF. Taken together, these findings argue for a more integrated view of the
role of visual cortical areas in visually guided behavior, a view that could take advantage
of the myriad signatures that predict that behavior.

Fano factor has been interpreted as reflecting the true underlying variability of
neuronal firing rate across trials (Churchland et al., 2006). In this view, every spike train
recorded from a neuron is a noisy instantiation of some “true” firing rate for that trial.
This true firing rate may itself be variable across trials, so that the recorded spike trains
are in fact noisy realizations of a different true firing rate on each trial. While averaging
the firing rate eliminates both sources of variability, FF instead estimates the extent of the underlying “true” variability with the assumption that spiking noise is invariant. With this context, we can interpret our data in much the same way as did Churchland et al. (2006). The decreased FF for short RT trials relative to long at the time of the cue to move reflects less variability in underlying firing rate, i.e. that more of the trials had the same “true” firing rate at cue onset for the short RT condition than the long. The precise value of this “true” firing rate may depend on the particular task, as in this experiment neurons had higher firing rates for saccades toward the RF and were on average unresponsive to saccades away from the RF. Nevertheless, variability decreased in all three conditions, so the FF provides an index of the state of saccade preparation.

Importantly, FF revealed a signature of saccade preparation in the responses of area V4 neurons even when there was no change in mean firing rate. Traditionally, a neuron without changes in mean firing rate would be viewed as non-modulated and its activity as uninformative during these conditions. Our results indicate that such a view is inaccurate. Even though a neuron may not be modulated in terms of its mean firing rate, a measure of the firing rate distribution may reveal that the activity of the neurons is indeed modulated. Our results show that such modulation present during saccade preparation occurs to such a degree that the activity predicts saccadic RTs. FF therefore provides a sensitive measure of the influence of saccade preparation on V4 activity that is complimentary with mean firing rate, revealing that neuronal responses are influenced by saccade preparation even when mean firing rate is neither enhanced nor suppressed.

Nonetheless, our results do not in any way undermine the important role that firing rate likely plays in determining how neurons drive behavior. On the contrary, likely it is
not the variability *per se*, but rather the particular firing rates on individual trials, as indexed by the FF, which relates to the state of saccade preparation. We assume that the proximity of the firing rate on individual trials to some ‘optimal’ mean firing rate relates directly to motor preparation (Churchland et al., 2006), an assumption consistent with our result that groups of trials with shorter RTs tend to have firing rates closer to the mean (i.e. lower FF). This view is depicted schematically in Figure S1, where the difference between baseline and pre-saccadic periods, as well as the difference between short and long RT trials, can be understood as a narrowing of the width of the firing rate distribution across trials. Due to a relatively small number of trials for each neuron-condition, and the noisiness of the firing rate measure, these firing rate distributions cannot be visualized directly, but are instead estimated from both the mean and variance of the measured single trial spike counts.

There may be some relationship between the presaccadic modulation of variability reported here and modulation of the gamma frequency (30-70Hz) spectral power of visual cortical responses. Gamma modulation has been demonstrated to occur presaccadically at least for microsaccades (Bosman et al., 2009) and coherence effects in the gamma range are predictive of behavioral reaction times (Womelsdorf et al., 2006) on some tasks, as is the FF reported here. Despite these similarities, it should be re-emphasized that we have measured variability across trials rather than variability in the spike times within trials, which would be most directly related to oscillatory processes. Future studies might address the potential relationship between observed decline in across-trial variability and frequency domain properties of neural activity.
The predictive activity of the mean firing rate for some, and FF for all, saccade directions can also be interpreted in the context of the influence of attention on saccadic reaction time (Kustov and Robinson, 1996). Because the interval between fixation onset and cue onset during a particular experiment was fixed, monkeys might have anticipated the impending saccade and directed spatial attention accordingly prior to the cue to move. In fact, increased anticipation of a behaviorally relevant stimulus does increase the magnitude of attentional modulation of the firing of area V4 neurons (Ghose and Maunsell, 2002). Thus, for example, on some trials the monkey may have anticipated the cue and attended to the RF stimulus, which could have resulted both in reduced FF (Mitchell et al., 2007) and “short” RTs on those trials for which that stimulus became the saccade target (Posner et al., 1980). Likewise, higher FFs and “long” RTs may have resulted from allocation of attention to incorrect target locations or lack of attentional allocation altogether.

Our results do not allow us to determine whether the two measures (mean firing rate and FF) are signatures solely of attentional deployment or saccade preparation. However, given the preponderance of evidence that the effects of attention and saccade preparation on V4 neurons are very similar, if not identical, (Moore et al., 2003), it is unclear to what extent such a distinction is possible in this area. However, our results cannot be explained solely by the known influences of covert spatial attention on variability (Mitchell et al., 2007). Since we observed a robust decline in FF even when the monkey directed saccades, and thus spatial attention (Hoffman and Subramaniam, 1995; Deubel and Schneider, 1996), away from the neuron’s RF, any known influence of covert spatial attention on FF must have been combined with some other influence that is independent
of the saccade direction. For example, there may be a saccade-direction independent influence of attention, perhaps merely related to the disengagement of fixation prior to saccades of any direction. On the other hand, such a non-spatial influence need not be directly related to the preparation of the eye movement *per se*. A number of studies have observed neural correlates of other spatially non-selective factors such as stimulus and reward expectation as well as elapsed time (e.g. (Ghose and Maunsell, 2002; Janssen and Shadlen, 2005)). Moreover, although the magnitude of presaccadic decline in FF depended on saccade direction, there was a substantial decline for all saccade directions. Thus a more global influence, for example arousal or reward anticipation, could be considered to explain the non-spatial component of the effects. Indeed, like attention, these other influences may be associated with saccade preparation, but may not require an actual movement to produce the dynamics we observe. Nonetheless, the FF predicts saccadic reaction times for saccades in all tested directions and thus provides a reliable signature of saccade preparation.
Figure Captions

Figure 1. The visually guided delayed saccade task. In the task, the monkey fixates a central dot while an oriented bar is displayed in the receptive field (RF; dashed circle) of a single V4 neuron. After a delay, the monkey is cued (by fixation spot offset) to make a saccade in one of three directions. On two thirds of the trials, a target dot appears in one of two locations, conditions “up” (left panel) and “opposite” (center), and the monkey is rewarded for making a saccade to that dot. If no target appears, the monkey is rewarded for executing a saccade to the RF stimulus (right).

Figure 2. Effects of RF stimulation and saccade preparation on the mean firing rate and response variability for the population of area V4 neurons. The left column shows mean firing rate (A) and Fano factor (B) aligned to the time of RF stimulus onset and divided into responses to preferred versus non-preferred visual stimuli. These traces, as well as those in C-F, are all smoothed with an 80-ms box filter (see Methods). C and D, Mean firing rate and FF aligned to cue onset (i.e. fixation offset) and split by direction of saccade. E and F, the same but aligned to saccade onset. In all traces, means (dark lines) and SEMs (shading) are shown. In E and F, horizontal bars indicate significant difference from baseline. In E, translucent plots above traces show distributions of cue onset times relative to saccade onset. G, Data from individual neuron-conditions for two time points: baseline and immediately prior to saccade onset. Each dot represents the mean and variance of spike counts within an 80ms window for just one neuron-condition (those trials corresponding to a particular neuron, stimulus, and saccade direction). Black dots represent variance/mean pairs taken from windows during the baseline period of each
saccade condition (first arrow in F). Colored dots represent variance/mean pairs taken from windows just prior to saccade onset (second arrow in F). Thick lines are linear regressions on the data.

Figure 3. Presaccadic changes in FF for “mean-matched” conditions. The mean-matching algorithm was applied to presaccadic spike trains from the population of recorded V4 neurons to equalize firing rate distributions across time for each of the saccade directions. A, Mean-matched firing rates for each of the saccade directions (toward, left; up, center; opposite, right), which no longer vary over time. B, FF of the mean-matched data, which still declines presaccadically despite removing variation in firing rate. C, The magnitude of FF decline in the final 80 ms period before the saccade for each of the three saccade directions. The FF decline plotted corresponds to the component of the FF decline independent of the presaccadic change in mean firing rate, computed in an ANCOVA. Error bars represent 95% confidence intervals from ANCOVA.

Figure 4. Relationship of presaccadic mean firing rate and FF to saccadic reaction time for the population of V4 neurons. In A, left column traces show percent difference in mean firing rate between short and long RT trials for each saccade condition. Right column shows percent differences in FF. B, differences in mean firing rate and FF for short and long RT trials in each saccade condition at the time of the movement cue (t = 0). C, same data as in A, but collapsed across the three saccade conditions. Horizontal bar indicates a significant difference between long and short RT traces.
Figure S1. Cartoon model of changes in firing rate distributions during saccade preparation. Each firing rate distribution depicted corresponds to the hypothetical probability distribution of firing rates for a given neuron and stimulus. The distributions depicted, which cannot be directly visualized from the data are instead estimates based on the measured means and FFs, with the assumption of a Poisson distribution. A, The baseline firing rate distribution has a comparatively wide spread, reflected in its higher FF. B, Distributions during the presaccadic period move to separate means depending on saccade direction but become narrower, and thus have lower FFs, for all directions. C, At the time of cue onset, firing rates may be within any of the three presaccadic distributions due to advance planning or directed spatial attention (see Discussion). On those trials with the correct plan, firing rates are already in the correct presaccadic distribution and reaction times tend to be short (green). For trials with either of the incorrect plans, firing rates are in either of the other two presaccadic distributions (summed and divided by two) and RTs tend to be longer (red). “Optimal” firing rates are shown as dotted lines corresponding to the presaccadic mean firing rate appropriate for a given condition (note alignment with distribution means in B). Short RT distributions in each case have smaller standard deviations ($\sigma$) than long RT distributions while the differences between the means ($\mu$) of the short and long RT distributions in this model correspond qualitatively to our observed results (Figure 4A).
References


Figure 1

1.5 columns
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Figure 2
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Figure 3
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Figure 4

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