Title: Smooth Pursuit Preparation Modulates Neuronal Responses in Visual Areas MT and MST

Abbreviated Title: Activity in MT and MST during smooth pursuit initiation.

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Abstract

Primates are able to track small moving visual targets using smooth pursuit eye movements. Target motion for smooth pursuit is signaled by neurons in visual cortical areas MT and MST. In this study, we trained monkeys to either initiate or withhold smooth pursuit in the presence of a moving target to test whether this decision was reflected in the relative strength of “go” and “no-go” processes. We found that the gain of the motor response depended strongly on whether monkeys were instructed to initiate or withhold pursuit, thus demonstrating voluntary control of pursuit initiation. We found that the amplitude of the neuronal response to moving targets in areas MT and MST was also significantly reduced (by 2.1 spikes/sec on average). The magnitude of the neural response reduction was small compared to the behavioral gain reduction. There were no significant differences in neuronal direction selectivity, spatial selectivity, or response reliability related to pursuit initiation or the absence thereof. Variability in eye speed was negatively correlated with firing rate variability after target motion onset during go trials, but not during no-go trials, suggesting that MT and MST activity represents an error signal for a negative feedback controller. We speculate that modulation of the visual motion signals in areas MT and MST may be one of the first visual cortical events in the initiation of smooth pursuit and that the small early response modulation may be amplified to produce an all-or-none motor response by downstream areas.
The primate oculomotor system incorporates a number of strategies to detect and respond to retinal image motion. When gaze is fixed on a small moving object of interest, a voluntary eye movement called smooth pursuit rotates the eye to match angular target velocity. Smooth pursuit typically requires a visual stimulus, however, even in the presence of a moving target, voluntary effort or attention is required to fully initiate the response (Wyatt and Pola 1987; Pola and Wyatt 1993; Burke and Barnes 2011; Mulligan et al 2013). Thus, the sensory-motor transformation for pursuit might be characterized as a “switch” that gates activation of the motor system by appropriate sensory inputs. The switch could be implemented as a gain element that controls the ratio of eye speed to target speed and that changes from a small value during fixation to a larger value during pursuit. The change in gain could be stepwise or graded. Although much is known about the neural pathways for generating smooth pursuit, the mechanisms underlying the decision to initiate pursuit have not been fully determined. Elucidation of these mechanisms may reveal principles that apply to voluntary movement initiation in general.

The computation of target motion for smooth pursuit involves visual cortical areas MT and MST. These areas contain a high proportion of neurons that have direction and speed selective responses to moving visual targets prior to and during smooth pursuit. Some MST neurons also carry an extraretinal signal proportional to eye velocity (Newsome and Wurtz, 1988). Lesions of these areas impair smooth pursuit (Dursteler et al, 1987), while electrical stimulation alters pursuit but does not evoke smooth eye movements during fixation (Komatsu and Wurtz, 1989; Groh et al 1997).

MT and MST project directly to frontal cortex, including the saccade and smooth pursuit regions of the frontal eye field (FEFsac and FEFsem). These areas also have cells that respond to visual motion in a direction and speed selective manner (Gottlieb et al., 1994; Xiao et al
Stimulation of FEFsem does evoke smooth eye movements (Gottlieb et al., 1993, 1994; MacAvoy et al., 1991), while stimulation of the saccade region of the FEF can suppress pursuit (Izawa et al. 2011.) MT and MST also project to the basilar pontine nuclei (DLPN: dorsolateral pontine nuclei, NRTP: nucleus reticularis tegmenti pontis), which provide input to the cerebellum, as well as to the nucleus of the optic tract (Mustari et al. 2009).

In this study, we trained monkeys to either initiate or withhold smooth pursuit in the presence of a moving target. In go/no-go paradigms, monkeys decide whether to initiate pursuit or continue to fixate and this decision may reflect the relative strength of "go" and "no-go" processes (Heinen et al. 2006, 2011; Schichinohe et al., 2009; Yang et a. 2010; Fukushima et al., 2011; Fukushima et al. 2013; Kurkin et al. 2011.) We found that the gain of the motor response was reduced almost to zero when monkeys were instructed to withhold pursuit, thus demonstrating voluntary control. We found that the gain of neuronal responses to the moving target in MT and MST was also significantly reduced. The magnitude of the neural response modulation was small compared to the behavioral gain reduction. The enhanced response to target motion on go trials was associated with a negative correlation between eye speed fluctuations and neuronal activity. This correlation might reflect the computation of an error signal used in a negative feedback loop that drives smooth pursuit initiation.

We speculate that modulation of the gain of direction selective responses in visual cortex is among the earliest cortical events leading to the initiation of smooth pursuit, and that it provides a biasing input to downstream areas that transform the initial bias into a full motor response. The initiation of smooth pursuit might be compared to the watershed of a river; small, early signals in sensory cortex being the rivulets whose contributions channel together to drive the motor output. Confirmation of this idea could help to resolve the issue of whether voluntary movement commands arise fully formed in the motor circuitry or are foreshadowed by weaker signals in sensory cortex.
Materials and Methods

Experiments were performed on two adult male rhesus monkeys (Macaca mulatta). All methods were approved by the Institutional Animal Care and Use Committee at Columbia University, the New York State Psychiatric Institute, and the University of California San Francisco. Monkeys were prepared for experiments by surgical implantation of a post used for head restraint, a recording chamber for positioning microelectrodes within the neocortex, and a monocular scleral search coil for recording eye position (Robinson 1963). Monkeys were trained to sit in a primate chair for the duration of the experiment with their heads restrained and to perform visual fixation and tracking tasks for liquid reward.

Visual stimulation. Visual stimuli were generated and controlled by a CRS VSG2/3F video frame buffer. The output from the video board was displayed on a calibrated color monitor with a 60 Hz non-interlaced refresh rate. The spatial resolution of the display was 1280 pixels by 1024 lines. The central fixation target was a 0.5 deg white or yellow square, luminance 15 cd/m², presented on a uniform dark background. Moving targets were 1.0 deg white squares, luminance 15 cd/m². The frame buffer was programmed to send out digital pulses (frame sync) for timing purposes at the beginning of each video frame in which a target was turned on or off. These pulses were recorded by the computer using a hardware timer and stored together with the eye movement data.

Neuronal recording. Recording chambers were placed at stereotaxic coordinates 5P, 15L. Neural activity was recorded extracellularly using parylene insulated or epoxy-coated tungsten microelectrodes (impedance 0.15-2.0 Mohms). Electrode penetrations were oriented in the coronal and sagittal planes such that the electrode passed first through the dorsal and then the ventral bank of the superior temporal sulcus. Areas MT and MST were identified based on depth relative to the pial surface, and on physiological characteristics. Upon completion of the experiments, both animals were euthanized and perfused with 4% paraformaldehyde. The brains were sectioned at 40 micron
thickness on a freezing microtome. The sections were treated with a silver stain for myelin (Gallyas, 1971) and examined microscopically for patterns of myelination associated with areas MT and MST (Van Essen et al., 1981; Maunsell & Van Essen, 1983). Two parasagittal sections from monkey F are shown in Fig. 1. The sections are spaced 960 microns apart. Three electrode tracks (which were oriented close to the plane of sectioning) were reconstructed and can be seen to pass through MST on the dorsal/anterior bank of the STS, and then into MT on the ventral/posterior bank. These results were typical and demonstrate that the orientation of electrode penetrations made it relatively straightforward to assign neurons to either MST or MT. Moreover, all neurons recorded on the dorsal/anterior bank of the STS were the dorsal subdivision of MST (MSTd).

Both MT and MST have a high proportion of direction selective neurons, but have strikingly different receptive field sizes (Van Essen et al., 1981; Albright and Desimone, 1987; Komatsu and Wurtz, 1988; Raiguel et al., 1997). Receptive fields were mapped using a hand-held light projector while monkeys kept their eye fixed on a small LED to receive a reward. MST neurons had large receptive fields that often included the ipsilateral visual field. MT neurons had smaller receptive fields and were restricted to the contralateral visual field. Receptive fields did not overlap with the fixation target. The relationship between RF size and eccentricity had a slope of 0.69 for MT, and 1.16 for MST (Ferrera & Lisberger, 1997a).

Action potentials were detected using a time-amplitude window, and converted to digital pulses with 0.1 msec precision. Spike trains were converted to spike density functions by convolving with a causal filter. The filter was an alpha function defined as $a(t) = k * t * \exp[(1 - t) / \tau]$, with $\tau = 8$ msec. The constant, $k$, was set to a value such that the time integral of $a(t)$ was equal to 1.0.

To compute average firing rate for a given set of trials relative to a given event, spike times relative to event time were binned with 1 msec resolution to create a histogram of spike frequency (mean spike count) across trials. The spike probability histogram was smoothed by convolving with the alpha function described in the previous paragraph. The result was multiplied by 1000.0 to convert to spikes/sec. Variance was computed similarly except that mean spike count was replaced...
by the variance of the spike count in each histogram bin. To compute population averages, the mean firing rate and variance were averaged across cells.

Eye movement recording and analysis. Eye position was monitored using a monocular scleral search coil system (CNC Engineering, Seattle, WA). The horizontal and vertical eye position signals were digitally sampled by computer at 1 kHz/channel and stored on disk for offline analysis. Eye velocity was computed by convolving eye position with a digital filter. The filter was constructed by taking the first derivative of a temporal Gaussian, $G(t)$, such that $dG/dt = -k^* t \exp(-t^2/\tau^2)$, $\tau = 8$ msec, $k$ is a constant that sets the overall filter gain to 1.0. This filter does not introduce any time delay between the position input and velocity output, but adds temporal uncertainty to the velocity estimates. Horizontal $[h'(t)]$ and vertical $[v'(t)]$ eye velocities were combined to estimate radial eye speed $[r'(t)]$, where speed is the magnitude of the velocity vector using the formula: $r'(t) = [h'(t)^2 + v'(t)^2]^{1/2}$.

Saccades that achieved radial eye speed greater than a criterion (30 or 10 deg/sec, depending on the analysis) were detected by an automated procedure. For some analyses, saccades were deleted (the entire saccade was deleted, not just the portion above the threshold) and the eye velocity samples were replaced by interpolating between the eye velocity samples immediately before and after the saccade.

Behavioral task. Monkeys were trained to initiate or withhold visual tracking of a small moving target presented in or near their central visual field (Fig. 2). At the start of each trial, a small square appeared in the center of the video display and the monkey had 600 msec to achieve fixation. After holding fixation for a random amount of time (200 – 1800 msec), a moving target appeared a few degrees perifoveally and moved either toward or away from the center of the screen. The axis of target motion was not always along a radius relative to the fixation point. Rather the axis was adjusted so that at least one direction of target motion was close to the cell’s optimal direction.
The initial position of the moving target was within the receptive field of the cell being recorded on half the trials, and at the opposite location on the other trials. "Target location" refers to the location at the onset of motion, unless otherwise indicated. MT and MST receptive fields were often large enough to have some response at both locations, hence we refer to "preferred" and "non-preferred" locations, rather than "inside" and "outside." Thus, there were two target locations (preferred/non-preferred) and two directions of motion (toward/away). By randomizing target location and direction, this design minimizes anticipatory pursuit. The average initial target eccentricity was 4.0 deg (range: 1.0 to 10.8 deg). The speed of the moving target was chosen to evoke a strong response from the cell, but no attempt was made to optimize target speed. The average target speed was 10.3 deg/sec (range: 3.8 to 19.2 deg/sec).

Go and no-go trials were distinguished by the color of the initial fixation target, which was white on go trials and yellow on no-go trials. The luminance of both colors was the same (15 cd/m2). The fixation target color remained constant throughout the trial. For half the trials, the fixation target remained on for the entire trial ("fixon" trials). For the other half ("fixoff" trials), the fixation target was extinguished when the moving target appeared. The fixation target remained off for 200 msec on no-go trials and was then re-illuminated. On go trials, the fixation target stayed off for the remainder of the trial. This manipulation was done to test whether leaving the fixation target on would bias the animal to maintain fixation, whereas turning it off would bias the animal to initiate pursuit. The total number of trial conditions was 16 (2 initial target locations x 2 directions x go/no-go x fixon/fixoff).

The monkey was required to keep his eye position within ±2 deg of the fixation target. When the moving target appeared, the fixation requirement was turned off for 200-300 msec. After this grace period, eye position was again required to be within ±2 deg of the fixation target on no-go trials, or within the same proximity to the moving target on go trials. These windows were large relative to the monkeys' typical fixation accuracy. The average fixation error (absolute value of eye position - target position) during the first 100 msec after the moving target appeared was 0.23 deg on no-go trials (0.13 s.d., n = 7491) and 0.27 on go trials (0.18 s.d., n = 7565).
On a small proportion of trials, monkeys failed to keep their eye position within these limits and the trial was terminated without reward. However, the behavioral constraints on eye position were lenient enough that unrewarded trials were extremely rare and generally occurred when the monkey failed to initiate a trial, looked away from the display in the middle of a trial, or otherwise disengaged from the task. We made no attempt to quantify the proportion of unrewarded trials or to analyze behavior or neural activity on such trials. All of the analyses below are based on correctly completed and rewarded trials. All correct trials were rewarded with an equal quantity of juice.

Results

Behavior

In this study, two male rhesus monkeys performed an eye tracking task in which they either initiated or withheld smooth pursuit in the presence of a moving target. Eye movements were analyzed to determine how well monkeys withheld pursuit when instructed to do so (“no-go” trials), and to determine an appropriate time window for comparing neural activity when monkeys initiated or withheld pursuit. The data were collected during 68 recording sessions (43 from monkey B, 25 from monkey F), one to three sessions per animal per day. The mean number of trials per session was 220 (14 repetitions of each trial condition, on average).

To estimate the latency and gain of smooth pursuit, we computed radial eye speed (see Methods) for each trial. We then normalized by dividing eye speed by target speed, and then averaged over all trials in each session, keeping go and no-go trials separate. The normalized eye speeds for all sessions are plotted in Fig. 3A. On go trials, pursuit was initiated between 100 and 200 msec after the appearance of the moving target. On no-go trials, there was a slight increase in radial eye speed, peaking around 200 msec after target onset, but the maximum eye speed was much less than on go trials, indicating that monkeys were successful in withholding smooth pursuit.
The gain of pursuit (eye speed / target speed) on go trials was generally less than 1.0 and was somewhat variable, with several sessions having gains below 0.5. Some of this variability is likely due to differences in initial target location; more eccentric targets generally elicit weaker pursuit (Lisberger and Westbrook 1985). It is also possible that the gain of pursuit on go trials was reduced by the fact that go and no-go trials were randomly interleaved, requiring the animal to change its preparatory set each time the trial condition switched from go to no-go or vice-versa, i.e. on about half of the trials.

We quantified the gain of pursuit by averaging normalized eye speed during two task epochs: 0 to 100 msec and 250 to 300 msec after target onset (Fig. 3B). During the earlier epoch, there was very little eye movement for either go or no-go trials (average normalized eye speed go-trials = 0.051 ± 0.002 s.e., no-go trials = 0.052 ± 0.002 s.e.), and the difference between go and no-go trials was not significant (p = 0.77, t-test paired by session). During the later epoch, the gain was nearly zero when monkeys were instructed to withhold pursuit (avg. gain no-go trials = 0.06 ± 0.003) as compared to trials when monkeys were instructed to initiate pursuit (avg. gain go trials = 0.77 ± 0.024). This difference was significant (p < 10⁻³⁰, t-test paired by session). On no-go trials, the mean change in gain between the earlier and later epoch was 0.01 (p = 0.03, paired t-test), while for go trials, the change in gain was 0.72 (p<0.0001, paired t-test).

In the go/nogo paradigm, monkeys decide whether to initiate pursuit or continue to fixate. This decision may reflect the relative strength of “go” and “no-go” processes. To further manipulate this balance, we conducted 39 sessions in which the fixation target was either extinguished for 200 msec (fix off) or remained illuminated (fix on) after the moving target appeared. Fig. 4 shows eye speed (with saccades removed) averaged over the 39 sessions in which the fixon/fixoff manipulation was used. On go trials, pursuit was initiated an average of 18 msec later when the fixation target remained illuminated. Once initiated, pursuit eye speed followed a parallel timecourse on both fixon and fixoff trials, although after 400 msec pursuit
gain remained 4.4% lower on fixon trials. The presence or absence of the fixation target had no
significant effect on no-go trials. These results show that the net effect of the fixation target was
to delay the onset of pursuit and reduce the gain, suggesting that the fixation ("no-go") signal
may not be fully extinguished after the onset of pursuit.

Visual tracking of moving targets comprises smooth eye movement as well as corrective
saccades. One might expect that the preparation to initiate or withhold pursuit would also affect
the saccadic component. This expectation was confirmed. The frequency of saccades with
peak velocities $\geq 30$ deg/sec that occurred in a time window $\pm 100$ msec around target onset
was approximately 1 per 20 trials and did not differ between "go" and “nogo” conditions (go:
mean number of saccades = 0.053, no-go: mean = 0.055, paired t-test $p = 0.69$, $n = 68$.)
However, if the eye velocity threshold was lowered to 10 deg/sec to include smaller saccades,
then the frequency of saccades was significantly higher on no-go trials (go mean = 0.21, no-go
mean = 0.29, paired t-test $p < 0.0001$, $n = 68$.) Thus, prior to pursuit onset, saccades with peak
velocities of at least 10 deg/sec were more prevalent on no-go trials, however, their amplitude
was significantly smaller (go mean saccade amplitude = 0.60 deg, no-go mean = 0.41 deg, t-
test $p<0.0001$).

To examine saccades during pursuit initiation, we detected rapid eye movements with
minimum velocity of 30 deg/sec (this threshold was used to avoid including smooth eye velocity)
in a window 100 to 200 msec after target onset. The frequency of saccades was many times
greater on go trials (go mean number of saccades per trial: 0.62, no-go mean: 0.04, paired t-test
$p < 0.0001$, df = 67). The amplitude of each saccade was computed by integrating eye velocity
during the saccade. To estimate the change in position due to pursuit, the saccade was deleted
before integrating eye velocity. The net saccade amplitude was then calculated as the integral of
the total eye velocity minus pursuit eye velocity. Saccades that occurred 100 to 200 msec after
target onset were significantly larger on go trials (go mean amplitude = 2.52 deg, no-go mean =
0.62 deg, t-test $p < 0.0001$). Thus, saccade frequency and amplitude varied significantly with
go/no-go condition prior to and during pursuit initiation. This raises two questions: 1) Did saccades occurring around the time of target onset affect neuronal activity, and 2) did neuronal activity predict saccade characteristics during pursuit initiation. These issues are addressed in the next section.

Neuronal responses

To identify activity potentially related to the decision to initiate smooth pursuit, we recorded well-isolated action potentials from 68 neurons in MT and MST of two monkeys (MT: n = 30 cells, MST: n = 38 cells). The population response in MT began to deviate from baseline firing 47 msec after the appearance of the target in the receptive field. The population response in MST started 53 msec after target motion onset (Fig. 5). By comparison, smooth pursuit latencies were typically in the range of 100-150 msec (Fig. 3). Sixty cells (88%) had a significant change in response 50 to 150 msec after target onset compared to a baseline period 50 msec before to 50 msec after target onset (t-test, p<0.05)

To examine the time course of activity in MT and MST, we computed spike density functions by convolving the spike train on every trial with a causal temporal filter (alpha function, see Methods). We then averaged the spike density functions for each cell, using only trials where the target initially appeared at the preferred location (inside the receptive field). We separated go and no-go trials, but combined over the remaining conditions (target direction and fixation target on/off). The average population spike density functions were then computed by taking the mean spike density across all cells. This process estimates the effect of go and no-go conditions in the face of other sources of neuronal response variability. Fig. 5 shows that the initial responses in both MT and MST were slightly stronger for go trials than for no-go trials.

To define an appropriate time epoch in which to analyze neural responses uncontaminated by image motion due to pursuit, we considered the behavioral results in Fig. 3. These show that pursuit was not initiated until at least 100 msec after target onset and that
there was no significant difference in eye speed between go and no-go trials during the interval 0 to 100 msec after target onset. If we use conservative estimates of 80 msec after target motion onset as the time of the earliest pursuit eye movement, and 40 msec as the minimum visual response latency of MT and MST neurons, then there is a time window of 120 msec after target onset within which retinal image motion due to pursuit cannot influence neuronal firing in MT and MST. In subsequent analyses, we consider only neural activity within this time window.

To quantify the response difference between go and no-go conditions, we computed the average firing rate on each trial (without smoothing or normalizing) within a window 50 to 120 msec after target motion onset. We also computed firing rate during a background period -70 to 0 msec relative to target onset. We then took the difference between the later and earlier firing rates as the evoked response for each trial. This response was averaged over all trials for each cell, keeping go and no-go trials separate. The mean response evoked on go trials vs. no-go trials is plotted for each cell in Fig. 6A. The distribution of differences (go minus no-go) is plotted in Fig. 6B (black bars). The average difference was 2.1 spikes per second and was statistically significant (p = 0.0002, paired t-test) for the entire population (n = 68). For MT, the average difference was 2.0 ± 0.71 sp/sec (p = 0.0045, paired t-test, n = 30). For MST, the average difference was 2.2 ± 0.78 sp/sec (p = 0.0049, paired t-test, n = 38). The average difference during a background period (-100 to 0 msec relative to target motion onset) was not significantly different from zero (Fig. 6B, grey bars). On a cell-by-cell basis, the percentage reduction \((\text{go firing rate} – \text{no-go firing rate})/(\text{go firing rate})\) averaged 23%.

The average population response 50-120 msec after target onset was highly selective for initial target location and direction (Fig. 7A). For each target location and direction, there was a stronger response on go trials compared to no-go trials. A five-way ANOVA (dependent variables = firing rate, explanatory variables = cell, go/no-go condition, target initial location, target direction, and fixon/fixoff) showed significant effects (p < 0.005) for all factors except fixon/fixoff (Table 1). Location and direction selectivity, measured as the difference in firing rate
between preferred and non-preferred locations or directions, did not vary significantly between
go and no-go trials. Direction and location selectivity were further examined by constructing
ROC curves to estimate the discriminability of preferred and non-preferred locations and
directions based on evoked firing rates. Area under the ROC curves did not depend
significantly on go/no-go condition for target direction or initial location.

Analysis of eye movements revealed that, prior to pursuit initiation, saccades were more
prevalent on no-go trials but were of smaller amplitude compared to go trials. To determine if
saccade amplitude affected firing rate, we detected saccades with minimum eye velocity of 10
deg/sec in a window 50 msec before to 50 msec after target onset (this time window excludes
the response to the moving target). Saccade amplitude was coded as 0 on trials in which no
saccade was detected in this window. We ran a 5-way ANOVA (Table 1, dependent variables =
firing rate 50 to 120 msec after target onset, explanatory variables = cell, go/no-go condition,
target location, direction, saccade presence). The effect of saccade presence on firing rate was
not significant (p >= 0.05). Furthermore, there was no significant correlation between firing rate
and saccade amplitude (Pearson's r = 0.005, p = 0.53, n = 14,947; note: the overall mean firing
rate for each cell was subtracted from the firing rate on each trial prior to calculating the
correlation).

Smooth pursuit was delayed on trials in which the fixation target remained on when the
moving target appeared (fix on trials) as compared to trials in which the fixation target was
extinguished (fix off trials). Neuronal responses 50 to 120 msec after moving target onset were
reduced during fix on trials by an average of 1.5 sp/sec compared to fix off trials, but the
reduction was not statistically significant (paired t-test p = 0.0564, n = 39).

Previous studies have suggested that attention modulates the variability of neural firing
in some visual areas (Mitchell et al 2007). This was examined here by computing the Fano
factor (variance divided by mean, Fano 1947) of spike counts during the initial target
presentation (50 to 120 msec after target onset) and background (70 to 0 msec before target
onset). For each cell, trials were sorted by go/no-go condition, target direction and initial location. Conditions where the mean spike count was zero were excluded, as this results in an infinite Fano factor.

In several conditions (5/8), the stimulus driven response had a significantly lower Fano factor than the background activity (Fig. 7B, p-values are the result of two-sided Wilcoxon rank sum test). Stimulus presence reduced the Fano factor even for conditions in which there was no change in the mean firing rate (i.e., null direction, non-preferred location). These observations are consistent with a previous report (Churchland et al. 2010). Within each condition, we compared the Fano factors for go and no-go trials. There was no significant difference for any of the 8 comparisons (p > 0.25, Wilcoxon rank sum test). For targets at the preferred location, the target-onset associated reduction in Fano factor was larger on go trials (average Fano factor difference, stimulus-background = -0.79) than no-go trials (average difference = -0.66). This difference in FF reduction between conditions was not significant (paired t-test, p = 0.11, n = 68).

One explanation for the reduction in Fano factor after stimulus onset is that spike count regularity might depend on firing rate. To explore this, we plotted Fano factor as a function of spike count (Fig. 8). During the background period (Fig. 8A), the average Fano factor was 1.47, and had a broad distribution (std dev = 0.76) with a long right tail. During the stimulus period, the Fano factor depended strongly on mean spike count (Fig. 8B), and was typically below 0.5 for spike counts above 3.5 per 70msec (50 spikes/sec). Fano factors below 1.0 indicate that the trial-to-trial spike count variability is less than expected from a Poisson process.

In neither the background nor stimulus period was there a significant difference in Fano factor between go and no-go conditions (t-test, paired by cell, background period p=0.12, stimulus period p=0.76), consistent with the results in the previous paragraph and Fig. 7.

To form an expectation about how ideal, randomly firing neurons might behave, given the parameters of our data and analysis, we ran simulations that generated random spike trains
with the same range of average spike counts (5 - 300 spikes/sec), duration (70 msec) and number of trials (14) as in our experiments. The simulated spike counts were Poisson distributed with mean Fano factor = 1.0. The Fano factor distributions for the simulated spike trains were broad (std dev = 0.37) and had long right tails. Neither the mean Fano factor nor the shape of the Fano factor distribution depended on mean firing rate. We added an absolute refractory period to the spike train generator and found that refractoriness always decreased the mean Fano factor below 1.0.

The simulations suggest that some of the scatter in measured Fano factors seen in Fig. 8 is an expected consequence of sampling a random spike generation process in short (70 msec) time intervals. However, the mean Fano factor of 1.47 and standard deviation of 0.76 found in the background period data are both significantly higher than the values of 1.0 and 0.37, respectively, in the simulations. The spike-count dependent reduction of Fano factor below 1.0 that was observed in the data was also observed in simulations when a refractory period was included in the algorithm that generated the spike trains. In Fig. 8B, the gray circles represent Fano factor vs. spike count for simulated spike trains with a refractory period of 5 msec.

Previous studies have explored the relationship between fluctuations in MT activity and smooth pursuit velocity (Osborne et al. 2005). Hohl and Lisberger (2011) showed that small eye movements can drive activity in MT, while Lee and Lisberger (2013) reported that random variability of neuronal activity in area MT is correlated with variability in eye velocity during smooth pursuit. Schoppik et al. (2008) showed a similar relationship for frontal pursuit area activity and pursuit. Such correlations suggest a direct coupling between neurons and behavior. We examined this relationship in the current data set, restricting the analysis to trials where the moving target appeared at the preferred location. We calculated the mean firing rate for each neuron and direction of target motion and then subtracted this mean from the firing rate on each
trial, separating preferred and null directions. Horizontal and vertical eye velocity were combined to yield radial eye speed. Saccades were removed with a threshold of 30 deg/sec.

The p-values for the correlations, each weighted by the sign of the corresponding correlation coefficient, were log transformed [-ln(p)] and plotted in Fig. 9. For targets moving in the preferred direction (Fig. 9, top row), there was a significant negative correlation between firing rate and eye speed fluctuations for go trials. For no-go trials, there are hints of a correlation for the preferred direction, but nothing was significant at the corrected p < 0.05 level. There were no significant (p > 0.05, Bonferroni corrected) correlations for the null direction (Fig. 9, bottom row). These results show that both target direction and go/no-go condition modulate the noise correlations between neural responses in MT/MST and eye speed.

If neural activity fluctuations drove eye movement variability, then the strongest correlations should be found for eye movement fluctuations following neural activity by about 50 msec. In fact, the opposite was true. The time lag that produced the strongest negative correlations was when eye movement fluctuations occurred about 50 msec prior to neural activity. This suggests that eye movements suppressed the neural response and is opposite to the effect found in the frontal pursuit area (Schoppik et al. 2008).

One way that eye movements can drive neural activity in MT and MST is by producing retinal image motion. If the noise correlations were the result of neuronal sensitivity to retinal image motion of the background scene, then they should be the same for a given time lag, i.e. the pattern of correlations should show up as a diagonal band spanning the entire time range in Fig. 9. This was not the case. Rather, the region of significant correlations starts about 50 msec after target onset and tapers after pursuit onset. The correlations appear to be gated by the presence of the moving target.

To account for the lack of significant correlations on no-go trials, the retinal slip hypothesis would require that there be more variability in eye speed on go trials compared to no-go trials. In fact, the variance in eye speed, pooled over all sessions, was almost always
greater on no-go trials. For example, in the time bin representing 50-100 msec after target
onset for the neuronal response and 0-50 msec for the eye movement, the variance in eye
speed was 4.89 \((\text{deg/sec})^2\) on go trials, compared to 5.62 \((\text{deg/sec})^2\) on no-go trials \((F\text{-test } p =
0.031, \text{ df(go) } = 728, \text{ df(no-go)= 729})\). The appearance of negative correlations after target
motion onset is consistent with the computation of an error signal in a negative feedback control
system (Robinson et al., 1986) that computes the difference between target velocity and eye
velocity. A simple model will be presented in the Discussion to explain the timing and sign of
these correlations.

As mentioned above, corrective saccades were more frequent on go than no-go trials.
To address the possibility that enhanced neuronal responses on go trials might be related to the
production of such saccades, we did the following analysis. First, we removed the smooth
component of eye velocity, leaving only the saccades (the velocity threshold was 30 deg/sec).
Then we performed the correlation analysis described above using saccadic eye velocity rather
than smooth eye velocity. We found no significant correlations at the \(p < 0.05\) level (Bonferroni
corrected.) It is therefore unlikely that firing rate was either driving or driven by saccades.

Discussion

How the brain initiates voluntary movements is still a profound mystery. Smooth pursuit eye
movements present an excellent opportunity to shed light on this process because the behavior
is easily quantified and the neural pathways are well-defined (Ilg and Thier, 2008). At what level
of the sensory-motor circuit for pursuit are sensory signals converted to motor commands?
Does this happen suddenly or gradually (either in terms of time or processing stages)?
Movement initiation also provides an opportunity to study the neural mechanisms of simple
decisions. Such decisions could be implemented by a modulation of mean firing rate, a
reduction of variability, changes in selectivity or sensitivity, or a combination of these and other
mechanisms.
The current results provide evidence for a large behavioral gain control and a small but reliable modulation of stimulus evoked activity in visual areas MT and MST. The behavioral gain control is not like an on/off switch, but appears to allow a continuum of gain values, like a dimmer switch. Smooth pursuit onset was delayed when the stationary fixation target remained on after the appearance of the moving target. Hence, fixating a visual target delays pursuit more than fixating without a target, as if there is an interaction between the visual fixation and pursuit targets, in addition to or instead of an interaction between fixation and pursuit responses. Modulation of neuronal activity (go vs. no-go) was present from the beginning of the response to the visual target, which occurred approximately 50 msec before the onset of the motor response. Thus, there is sufficient time for a small modulation of direction selective responses in visual cortex to be amplified into an all-or-none motor response by downstream structures in frontal cortex, cerebellum, or brainstem that are involved in generating smooth pursuit (Ilg and Thier, 2008, Mustari et al. 2009). Target motion onset was also associated with a reduction in neural firing variability, and this reduction was slightly (but not significantly) greater preceding pursuit initiation. Negative correlations between eye speed and firing rate were found on go trials when the target moved in the preferred direction, supporting the idea that MT and MST are involved in computing an error signal for smooth pursuit initiation.

Previous studies. There are only a few prior studies that have examined smooth pursuit in the context of a go/no-go task. Kurkin et al. (2011) recorded from MSTd during a go/no-go pursuit task and reported that there was no modulation of activity related to the go/no-go instruction, in agreement with the current finding that instruction has no effect on baseline activity. However, Kurkin et al. also found that nearly all MSTd cells in their sample responded after the onset of smooth pursuit and hence were not able to assess the modulation of response to a moving target prior to the initiation of pursuit. Yang et al. trained monkeys to make go/no-go pursuit decisions based on a visual cue (Yang et al., 2010; Heinen et al 2011) and found that the
decision altered activity in the supplemental eye field. Burke and Barnes (2011) used a go/no-go pursuit task in human subjects who underwent fMRI. They found that this task evoked differential activity in a number of areas involved in oculomotor control. However, they did not report modulation of the BOLD signal in human MT+. If the magnitude and duration of neural modulation in humans is comparable to that in monkeys, it may be too small and too brief to evoke measurable changes in cerebral blood flow or oxygenation.

Role of attention and preparatory set. Smooth pursuit engages several cognitive processes including attention, decision-making, and prediction or preparation (Barnes 2008, Ferrera and Lisberger, 1995, 1997b, Knox and Bekkour, 2002). However, there are few reports on the role of voluntary attention specifically in smooth pursuit initiation. Wyatt and Pola (1987) distinguished between active and passive pursuit and noted that attention modulated the gain of the oculomotor response to moving targets. We have shown here that monkeys can voluntarily withhold smooth pursuit in the presence of a moving target. Because initial target position and direction of motion were randomized, it is unlikely that monkeys were able to anticipate either property of the target. Therefore, they could not systematically deploy spatial attention or prepare a specific motor response before the target appeared. However, the moving target likely draws attention when it appears, and the strength of this orienting response may be modulated by the intent to initiate pursuit.

Attention modulates the activity of MT neurons during covert or overt tracking (Treue and Maunsell, 1996; Siedemann and Newsome, 1999; Ferrera and Lisberger 1997a, Recanzone and Wurtz, 2000.) However, the effect of attention on firing rate is often strongest during the later component of the response. The effect of attention on the initial visual response is typically very weak. Considering both the task design and the nature of attentional modulation in MT, it is more likely that the neural response modulation reported here is due to a general preparatory
set for initiating or withholding pursuit, but may also reflect covert spatial attention or movement planning.

**Firing rate variability.** Trial-to-trial variability in firing rate can be quantified by the Fano factor (spike count variance divided by mean, Fano 1947). Several studies have reported that stimulus onset reduces Fano factor from a baseline level of about 1.3 or 1.4 to a value closer to 1.0, which is typical of a Poisson process (Mitchell et al., 2009; Churchland et al., 2010). Here, we found that stimulus onset reliably reduced variability across most conditions, even when there was no increase in mean firing rate, as reported by Churchland et al. (2010). We also found that stimulus-driven activity could be associated with Fano factors well below 1.0. This indicates that trial-to-trial firing was less variable than a Poisson process, an effect that was not reported by Mitchell et al. (2009) or Churchland et al. (2010).

Maimon and Assad (2009) also reported that firing rate variability in areas MT and MST was Poisson-like, whereas LIP and area 5 neurons were more regular (Fano factor < 1.0) as measured by interspike intervals and trial-to-trial spike count. The current study joins several others in reporting sub-Poisson variability in MT and MST (Bair and Koch, 1996; Osborne et al., 2004; Huang and Lisberger 2013). In our hands, regularity in MT and MST increased with increasing spike count, an effect that is expected for spike trains with a finite refractory period. It should be noted that the mean firing rates evoked by preferred stimuli averaged over 50 spikes/sec in the current study, while the mean firing rates for MT in Churchland et al. (2010) and Maimon and Assad (2009) appear to be much lower (roughly 30 spikes/sec). Hence, the stimulus or behavioral conditions in previous studies may not have driven the cells as strongly as in the current study.

Mitchell et al. (2009) reported that the reduction in firing variability associated with stimulus onset was enhanced by spatial attention. In the current study, we found that variability reduction occurred for both go and no-go trials and there was no significant difference between
the two conditions. If changes in mean firing and variability are both characteristic of spatial
attention, then the lack of a difference in variability argues against the notion that the neural
modulation seen in this study is due to attention.

Noise Correlations: Fluctuations in neural activity in area MT and in the frontal pursuit area
precede and may give rise to fluctuations in smooth pursuit velocity (Osborne et al. 2005, Hohl
and Lisberger 2011, Lee and Lisberger, 2013, Schoppik et al., 2008). It has also been reported
that image motion due to small eye movements may drive responses in MT (Hohl and Lisberger,
2011.) Here, we found evidence for coupling between MT/MST activity and pursuit eye speed
when the target was moving in the preferred direction of the neurons. Noise correlations
between eye speed and firing rate were found only on go trials. Temporally, the effect arose
only after target motion onset. Others have reported positive correlations such that increases in
eye speed drive increases in firing rate, or vice-versa. Here, we found that the correlations were
negative.

The eye speed fluctuations were not produced by variability in firing rate. Rather, the
timing of the effect shows that eye speed fluctuations were correlated with neural activity that
occurred 50 msec later, approximately the visual latency of MT and MST neurons. The timing of
the correlations was therefore consistent with a neuronal response to retinal image motion
induced by eye movements during fixation. However, the fact that the correlations appeared
only after the onset of the moving target suggests that they represent a response to target
motion and not motion of the background image.

The sign and approximate timing of the noise correlations is consistent with the
operation of a negative feedback control system believed to underlie the generation of smooth
pursuit eye movements (Robinson et al. 1986). The error signal in this negative feedback
controller is related to the difference between target velocity and eye velocity, and could be
represented by the firing of MT and MST neurons. Under certain conditions, the output of the
controller (a command for eye velocity, \(y\)) will be negatively correlated with the error. To show this, we simulated a simple PID (proportional, integral, derivative) controller. The equations for the PID controller were:

\[
e(t) = T'(t) - E'(t)
\]

where \(e(t)\) is the error signal (image velocity), \(T'\) is target velocity and \(E'\) is eye velocity. The control signal, \(y(t)\), is given by:

\[
y(t) = k \ [e(t) + k_i \int e(t) dt + k_D \frac{de(t)}{dt}]
\]

Where \(k\) is the overall feedback gain. The controller takes target velocity as input and produces eye velocity as output. The internal dynamics are governed by the time constants of the controller’s integrator and differentiator (\(k_i\), \(k_D\)), and also include sensory and motor time delays. This simulation is not intended as a realistic model of smooth pursuit. However, it suggests how the error signal and eye velocity can become negatively correlated.

Simulations were run under two conditions: high gain (\(k = 0.1\), “go” trials, Fig. 10A) and low gain (\(k = 0.001\), “no-go” trials, Fig 10B, \(k_i\) and \(k_D\) did not vary between simulations.) Independent Gaussian noise was added to the target velocity input and to the controller output (\(y\)), to represent sensory and motor variability. The two noise sources were uncorrelated. The correlation between the error and control signals was computed as a function of time relative to target motion onset. To simulate target motion input, the target velocity stepped from zero to 1.0, creating an error signal, which the controller attempted to eliminate by increasing eye velocity. The presence of the error signal during pursuit initiation coupled with the high gain of the controller resulted in a negative correlation between the error and control signals as the increase in controller output worked to reduce the error (Fig. 10C).

When the gain was low (Fig. 10B), the error signal was present but eye velocity did not increase, probably because the controller output had little effect on the error. The result was that the error was uncorrelated with eye velocity (Fig. 10D). The sign and strength of the
correlation between the error signal and eye velocity appear to be determined by the gain of the negative feedback. The negative correlation only appears during pursuit initiation when there is a sufficiently large error signal. Thus, the correlations between eye velocity and firing rate can be explained if firing rate in MT and MST represents an error signal for smooth pursuit initiation, and the gain of the controller using that error signal is greater on “go” than “no-go” trials. Interpreted in the light of these simulations, the experimental results (Fig. 9) suggest that the gain of the controller depends on behavioral state (go/no-go) as well as target direction relative to the cells’ preferred direction.

Conclusion: The decision to initiate or withhold smooth pursuit in the presence of a moving target has a powerful effect on the gain of the behavioral response and a weaker, but reliable effect on the response of neurons in visual areas MT and MST. The decision does not affect the selectivity or reliability of the neurons. The neural modulation may be due to attention or the preparation of a specific motor response, but may also reflect a non-specific preparation to initiate or withhold pursuit. Activity in MT and MST may represent an error signal that is correlated with fluctuations in eye speed when the gain of the system that converts target velocity to eye velocity is large.

Figure Legends

Figure 1. Histology. Myelin-stained sections from one monkey showing electrode tracks (red dashed lines labeled a, b, c) in relation to cortical areas MSTd and MT. Blue lines show approximate borders of MSTd, while green lines show MT borders. MSTd = dorsal Medial Superior Temporal, MT = Middle Temporal, STS = Superior Temporal Sulcus.
Figure 2. Task design. A) Schematic of video display with targets for initial fixation and smooth pursuit. The gray circle suggests the size and location of a receptive field of a typical MT cell during initial fixation. B,C) Target and eye position as a function of time for nogo trials. Yellow line is fixation target position (gap in C indicates when target is blanked for fixoff trials), black line is moving target position, red line is eye position. D,E) Target and eye position for go trials. Black lines are fixation and moving target position, green line is eye position.

Figure 3. Eye movement behavior. A) Normalized target speed (black line) and eye speed for go (green) and no-go (red) trials. B) Average gain before (0 to 100 msec after target onset) and after (250 to 350 msec after target onset) pursuit initiation.

Figure 4. Comparison of normalized eye speed (eye speed / target speed) for all sessions with fix on and fix off conditions.

Figure 5. Time course of population response for all neurons. A) MT neurons. Green solid trace is activity (smoothed mean firing rate) during go trials, red is during no-go trials. Dashed lines are smoothed spike count variance. Black tick marks indicate individual time bins in which the firing rates were significantly different (t-test, p<0.05). B) MST neurons, same conventions as A.

Figure 6. Average firing rate 50-100 msec after moving target onset. A) Go vs. nogo trials. Each point is one cell. Black dots are MT cells, grey are MST cells. B) Histograms of firing rate differences (go – nogo) during background (-100 to 0 msec relative to target motion onset) and stimulus (50-100 msec after target motion onset.)
Figure 7. Mean firing rate and reliability. A) Firing rate for go (black circles) vs. no-go (white circles) trials sorted by target location and direction. Error bars are ±1 s.e.m. B) Fano factor

Figure 8. Fano factor as a function of spike count. A) Background period (50 to 0 msec before target onset). B) Stimulus period (50 to 100 msec after target onset).

Figure 9. Significance of correlations between residual eye speed and firing rate. Color code represents –In(p), yellow for positive correlations, blue for negative, Bonferroni corrected. The color bar indicates p values. A) Go trials with target moving in the preferred direction. Vertical and horizontal lines represent time of target motion onset. Diagonal dashed line separates eye leading neuronal firing (above diagonal) from eye lagging firing (below diagonal). B) No-go trials, preferred direction. C) Go trials, null direction. D) No-go trials, null direction.

Figure 10. Simulations of a negative feedback PID controller. A,B) Target and eye velocity on high and low gain trials, respectively. C,D) Correlations between controller error and eye velocity as a function of time (target motion onset at t = 50, horizontal and vertical solid lines). The color bar indicates correlation strength (r); yellow for positive, blue for negative.

References


Table 1. Five-way ANOVA. *Two different 5-way models were used: one that included fixon/fixoff, and one that included saccade presence/absence, all other explanatory variables (factors) were the same in both models. Firing rate was the dependent variable.

<table>
<thead>
<tr>
<th>Factor</th>
<th>F</th>
<th>P</th>
<th>d.f.</th>
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<tbody>
<tr>
<td>Cell</td>
<td>56.5</td>
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<tr>
<td>Go vs. no-go</td>
<td>8.3</td>
<td>0.004</td>
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<tr>
<td>Initial target location</td>
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<td>3</td>
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<tr>
<td>Target direction</td>
<td>262.7</td>
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<td>1</td>
</tr>
<tr>
<td>*Fixon vs. fixoff</td>
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<td>0.064</td>
<td>1</td>
</tr>
<tr>
<td>*Saccade presence</td>
<td>0.07</td>
<td>0.79</td>
<td>1</td>
</tr>
</tbody>
</table>
go, fix off
go, fix on
nogo, fix off
nogo, fix on

Normalized speed

Time re: stimulus onset (msec)
A: MT

- Mean firing rate (sp/sec)
- Time re: stimulus onset (msec)

B: MST

- Mean firing rate (sp/sec)
- Time re: stimulus onset (msec)
A

NOGO firing rate (sp/sec)

MT cells
MST cells

GO firing rate (sp/sec)

B

Number of cells

mean = 2.1 sp/sec
p = 0.0002 (paired t)

mean = −0.5 sp/sec
p = 0.1896 (paired t)

FR difference (GO–NOGO)
Firing rate (sp/sec)

pref location

null direction

pref location

null direction

Median Fano factor

p < 0.001

p < 0.001

p < 0.001

p < 0.001

p = 0.091

p = 0.243

p = 0.031

p = 0.076

B

Median Fano factor

pref location

null direction

pref location

null direction

go, background

go, stimulus

nogo, background

nogo, stimulus
A: Go, preferred

B: No-go, preferred

C: Go, null

D: No-go, null