

FEFsem neuronal response during combined volitional and reflexive pursuit

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Although much is known about volitional and reflexive smooth eye movements individually, much less is known about how they are coordinated. It is hypothesized that separate cortico-ponto-cerebellar loops subserve these different types of smooth eye movements. Specifically, the MT-MST-DLPN pathway is thought to be critical for ocular following eye movements, whereas the FEF-NRTP pathway is understood to be vital for volitional smooth pursuit. However, the role that these loops play in combined volitional and reflexive behavior is unknown. We used a large, textured background moving in conjunction with a small target spot to investigate the eye movements evoked by a combined volitional and reflexive pursuit task. We also assessed the activity of neurons in the smooth eye movement subregion of the frontal eye field (FEFsem). We hypothesized that the pursuit system would show less contribution from the volitional pathway in this task, owing to the increased involvement of the reflexive pathway. In accordance with this hypothesis, a majority of FEFsem neurons (63%) were less active during pursuit maintenance in a combined volitional and reflexive pursuit task than during purely volitional pursuit. Interestingly and surprisingly, the neuronal response to the addition of the large-field motion was highly correlated with the neuronal response to a target blink. This suggests that

FEFsem neuronal responses to these different perturbations—whether the addition or subtraction of retinal input—may be related. We conjecture that these findings are due to changing weights of both the volitional and reflexive pathways, as well as retinal and extraretinal signals.

Introduction

Most people have had the experience of sitting on a train with the landscape sweeping by. You notice something of interest that you watch for a few seconds until it is out of view. This probably happens many times over the course of the ride, all seemingly effortlessly.

This behavior, although it feels simple, actually requires the coordination of both reflexive and voluntary eye movements. The movement of a large, textured background is known to evoke reflexive ocular following eye movements, whether in a lab using a digital display or on a train with the visual world passing by (Miles, Kawano, & Optican, 1986; Gellman, Carl, & Miles, 1990). In addition, primates are known to be highly skilled at volitionally tracking a moving

Citation: Bakst, L., Fleuriet, J., & Mustari, M. J. (2017). FEFsem neuronal response during combined volitional and reflexive pursuit. *Journal of Vision*, 17(5):13, 1–14, doi:10.1167/17.5.13.

doi: 10.1167/17.5.13

Received January 6, 2017; published May 24, 2017

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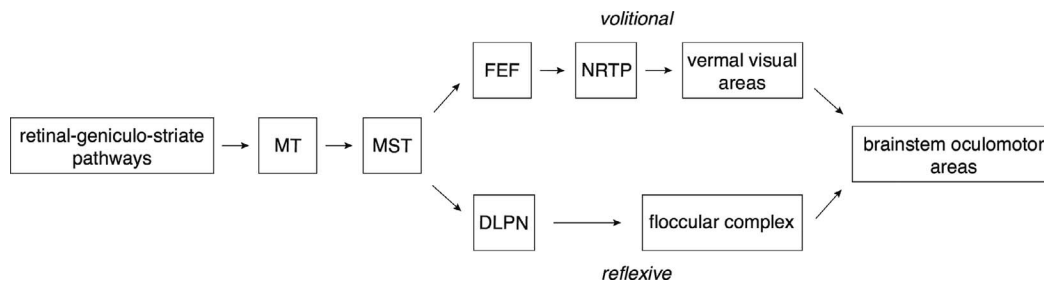


Figure 1. Schematic representation of pathways for volitional and reflexive pursuit. The two pathways have common beginnings and ends but discrete cortico-ponto-cerebellar portions. The upper part of the schematic represents the likely neural pathways subserving volitional pursuit, whereas the bottom portion represents the likely pathways underlying reflexive ocular following. MT = middle temporal area; MST = medial superior temporal area; FEF = smooth eye movement subregion of the frontal eye field; NRTP = nucleus reticularis tegmenti pontis; DLPN = dorsolateral pontine nucleus.

target with their eyes, a process called *smooth pursuit* (for review, see Krauzlis, 2004). Reflexive and volitional smooth eye movements likely rely on both common and distinct brain regions (Figure 1).

The visual motion input for both types of eye movements first travels through common retinal-geniculo-striate pathways to dorsal stream areas including the middle temporal area (MT) and the medial superior temporal area (MST; Maunsell & Van Essen, 1983; Ungerleider & Desimone, 1986; Tusa & Ungerleider, 1988; Boussaoud, Ungerleider, & Desimone, 1990). It is at this point that the pathways are thought to diverge to form two distinct cortico-ponto-cerebellar pathways (Nuding, Ono, Mustari, Büttner, & Glasauer, 2008; Mustari, Ono, & Das, 2009). Visual motion information for *volitional* pursuit is likely sent from MST to the smooth eye movement subregion of the frontal eye field (FEFsem) via dense reciprocal connections (Tian & Lynch, 1996; Stanton, Friedman, Dias, & Bruce, 2005). The FEFsem then projects to the nucleus reticularis tegmenti pontis (NRTP) in the brainstem (Leichnetz, 1989; Boussaoud, Desimone, & Ungerleider, 1992; Tian & Lynch, 1996; Ono & Mustari, 2009), which in turn sends information to cerebellar vermal visual areas and, ultimately, brainstem oculomotor areas (Voogd & Barmack, 2006). These pathways comprise the volitional cortico-ponto-cerebellar pathway, which is thought to be involved in volitional pursuit most often of smaller targets. However, the pursuit system is capable of tracking larger objects as well, which likely activates both the volitional and reflexive pathways to varying extents (Heinen & Watamaniuk, 1998).

MST is also the likely origin of the *reflexive* cortico-ponto-cerebellar pathway. In addition to sending visual motion information to the FEFsem, it also projects to the dorsolateral pontine nucleus (DLPN) in the brainstem (Leichnetz, 1989; Boussaoud et al., 1992; Tian & Lynch, 1996; Ono & Mustari, 2009), which then projects to the floccular complex in the cerebellum (Voogd & Barmack, 2006). The floccular complex

projects to the final common pathway within the brainstem oculomotor nuclei (Voogd & Barmack, 2006). The reflexive pathway is thought to be more involved in ocular following movements, which are most robustly elicited by large or full-field visual stimuli. In these ways, some of the machinery that underlies volitional and reflexive smooth eye movements is common, and some distinct.

In this study, we used coplanar small target and large-field (LF) motion to create a tractable version of a combined reflexive-volitional pursuit task, as can often happen in real-world pursuit of a target in a complex, featured environment. Given the known importance of the FEFsem in volitional smooth pursuit (Lynch, 1987; MacAvoy, Gottlieb, & Bruce, 1991; Gottlieb, MacAvoy, & Bruce, 1994; Morrow & Sharpe, 1995) and the lack of involvement in pure optokinetic nystagmus (Keating, Pierre, & Chopra, 1996), we sought to investigate the response of the FEFsem in combined reflexive-volitional eye movements.

Pursuit of a small target spot with concurrent LF motion engages brain regions in the reflexive pathway in addition to those involved in pursuit of a small spot alone, as discussed above; we therefore predicted two main findings. First, we predicted that the added drive provided by the LF motion to the reflexive pathway (including areas MT, MST, and DLPN) would serve to increase the gain of the pursuit eye movements. We found this to be true, which is in keeping with the higher gain observed during pursuit of large patches of random dot motion (Heinen & Watamaniuk, 1998). Second, we further predicted that this added drive in the reflexive cortico-ponto-cerebellar pathway would be complemented by a decrease in the drive provided by the volitional pathway, thereby maintaining accurate tracking behavior. Specifically, we predicted that this decreased drive would be evident in the lowered activity level in the FEFsem for combined volitional-reflexive pursuit, as compared with volitional pursuit of a small target alone. This compensatory decrease in activity could result from feedback connections that travel from

the cerebellum to thalamic nuclei (e.g., the medial dorsal nucleus and the most caudal portion of the ventral lateral nucleus; Stanton, 1980; Asanuma, Thach, & Jones, 1983a, 1983b), which, in turn, project to the FEFsem (Tian & Lynch, 1997). This feedback information from the reflexive pathway could dampen the drive provided by the volitional pathway. We did find such a decrease in many FEFsem neurons, but not all. We contrast these results with findings from a target blink task used to assess the relative contributions of retinal and extraretinal components to FEFsem neuronal activity during volitional pursuit. Some of the neurons in our study were also part of the sample in prior work (Bakst, Fleuriot, & Mustari, 2017; see the Materials and methods section).

Materials and methods

Surgical procedures

Behavioral and neuronal data were collected from three normal rhesus monkeys (*Macaca mulatta*, 5.5–14.0 kg). Detailed descriptions of surgical procedures can be found in earlier publications (e.g., Ono & Mustari, 2010, 2012). Surgery was performed under aseptic conditions using isoflurane anesthesia (1.25%–2.5%) to stereotactically implant a head stabilization post and recording chambers (titanium; Crist Instruments, Hagerstown, MD). In a second surgery, scleral search coils were implanted underneath the conjunctiva of both eyes (Judge, Richmond, & Chu, 1980). The protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Washington, and all surgical procedures were performed in strict compliance with the National Institutes of Health Guide.

Data collection

During recording sessions, monkeys were seated in a primate chair (Crist Instruments) with their heads stabilized in the horizontal stereotaxic plane during all experiments. All experiments were performed in a lightproof, sound-attenuated room. Precision hardware (CNC Electronics, Seattle, WA) and standard electromagnetic methods (Fuchs & Robinson, 1966) were used to detect and calibrate eye movements. Prior to digitization at 1 kHz with 16-bit precision using CED-Power1401 hardware (Cambridge Electronic Designs, Cambridge, UK), eye and target position feedback signals were processed with anti-aliasing, six-pole Bessel filters (200 Hz). Velocity and acceleration data were filtered using an 80-point finite impulse response

digital filter with a bandpass of 50 Hz. Saccades were removed from smooth pursuit traces using a custom detection algorithm in MatLab (MathWorks, Natick, MA). The removed saccades were replaced with a linear interpolation.

Single-unit activity was recorded using modified commercial glass- or epoxy-coated tungsten micro-electrodes (Alpha-Omega, Alpharetta, GA; Frederick-Haer Corporation, Brunswick, ME; impedance = 0.5 to 5 M Ω). Spike2 software was used for data acquisition and initial offline analyses such as spike sorting (Cambridge Electronic Designs). The neuronal response was represented as a spike density function generated by convolving spike times with a 5-ms Gaussian function (Richmond, Optican, Podell, & Spitzer, 1987).

Localization of FEFsem

We verified the location of our neurons using stereotaxic location and functional criteria (including directionally-tuned responses during volitional smooth pursuit eye movements). Prior to surgery, the FEFsem was localized using magnetic resonance imaging (T1 weighted, fast spin echo; Siemens 3T magnet). Recording chambers were then stereotactically implanted over the FEFsem region. The location of the FEFsem was also verified using depth measurements taken from microdrive readings while neurons were recorded. The depths corresponded to those expected from each animal's magnetic resonance image.

Behavioral paradigms

All visual stimuli were rear projected on a tangent screen (91.4 cm \times 91.4 cm) that was 57 cm in front of the monkey and delivered using appropriate optic bench hardware and computer-controlled two-axis mirror galvanometers (General Scanning, Watertown, MA). Monkeys were trained to track a small-diameter target spot (0.2°; produced by a red laser light-emitting diode) that moved in sinusoidal or step-ramp trajectories (Rashbass, 1961). The sinusoid task was used to ascertain whether a neuron was sensitive to smooth pursuit and determine its preferred direction, whereas the step-ramp task was used for more in-depth analyses (see below). Monkeys were also trained to perform fixation and saccade tasks.

Neurons were first tested for responses during smooth pursuit or saccadic eye movements using the sinusoid and saccade tasks. Only neurons that responded during pursuit of the target spot moving in a sinusoidal pattern at low frequency (0.15–0.35 Hz) were included. While the target moved in the eight cardinal

directions, neuronal activity was recorded, and the neurons were subsequently tested using step-ramp motion in the preferred and antipreferred directions.

In the step-ramp task, the target was first stationary at the center of the screen for 1000 ms. The target then moved at a velocity of $15^\circ/\text{s}$ to an excursion of 15° , at which point it stopped and remained static and illuminated for another 1000 ms. The preferred and antipreferred directions for each neuron were randomly interleaved. These step-ramp trials with the target spot alone are referred to as “control” trials for all subsequent tasks.

Neurons were also tested with an LF motion task. The small target spot moved in the same step-ramp trajectory as described above and was accompanied by a large, textured, black-and-white background moving with the same trajectory ($>50^\circ \times 50^\circ$; Supplementary Figure S1). Motion was made coherent by projecting the image of a random-dot pattern and the target spot off the same galvanometers. Preferred and antipreferred directions were randomly interleaved, but the LF trials were not interleaved with control trials and were instead delivered in blocks. Monkeys were rewarded only for accurate tracking of the small target spot; they were not rewarded for tracking other features of the LF stimulus or other types of eye movements.

Neurons were additionally tested with a target blink task (Newsome, Wurtz, & Komatsu, 1988; Tanaka & Fukushima, 1998; Ono & Mustari, 2006). In this task, the target spot alone moved in the same step-ramp trajectory but was extinguished for 150 ms, starting 50, 100, 200, 300, 400, or 500 ms after target motion onset. Blink trials were randomly interleaved with control trials, and preferred and antipreferred directions were interleaved as well. Blink trials comprised between 50% and 70% of the trials during the blink task. These neurons represent 20% (28/137) of the population in the Bakst et al. (2017) article and 65% (28/43) of the population in this article.

Most neurons were also tested for visual motion sensitivity. Monkeys were required to fixate the small target spot while it remained stationary at the center of the screen and the LF stimulus moved in a sinusoidal trajectory. This allowed us to assess whether neurons respond to visual motion independent of pursuit and determine if such sensitivity was in the same or opposite direction of the preferred direction for pursuit.

Data analysis

Data taken from at least 10 trials were averaged and used to calculate neuronal and behavioral latencies. The times at which the neuronal response and eye velocity exceeded three standard deviations above baseline were designated the neuronal and eye move-

ment latencies, respectively. Baseline was defined as the 100 ms prior to target motion onset. The overall neuronal latency was then expressed as the difference between eye and neuron, where negative values represent the latencies of neurons that began responding before eye movement onset.

To quantify the response to the addition of LF motion during step-ramp tracking, the percentage change from control was calculated. Negative changes indicate less activity during the LF trials than control. Two intervals were used for this calculation: initiation (50–200 ms after target motion onset) and maintenance (300–600 ms after target motion onset). The neuronal responses to the LF motion during these intervals are referred to as LF_{init} and LF_{maint} , respectively. Neurons whose responses began after the initiation interval were not included in these particular analyses. Thus, there are fewer neurons included in analyses involving the early part of the trial.

To quantify the response to the target blink, the eye velocity and firing rate were averaged over a 150-ms interval, delayed 60 ms from the onset of the blink to allow for delays in visual processing (Newsome et al., 1988; Tanaka & Fukushima, 1998). This average was compared with averages over the same interval for control trials, and the difference was expressed as percentage change and called the *blink response*. Negative blink responses indicate that the unit was less active during the blink than during control trials, whereas positive values indicate that the cell increased its activity compared with control. Blink timings were divided into early (50 and 100 ms) and late (300–600 ms) groups, to assess whether blink responses changed throughout the time course of pursuit. Neurons whose responses began after the early blink interval were not included in this particular analysis.

Results

Smooth pursuit with LF motion

We assessed the effects of adding concurrent LF motion to a step-ramp tracking task in three monkeys (Figure 2). The onset dynamics were largely similar between control trials without LF motion and LF trials for all three animals. For Monkeys T and B, the behavioral pursuit latency was slightly shorter for LF trials as compared with control; however, Monkey F actually showed the opposite effect ($p < 0.001$ for all animals; Table 1). The gain in LF trials as compared with control also showed similar results: Monkeys T and B had gains greater than 1.0 during the initiation interval along with significantly greater eye velocity (50–200 ms after target motion onset, first gray shaded

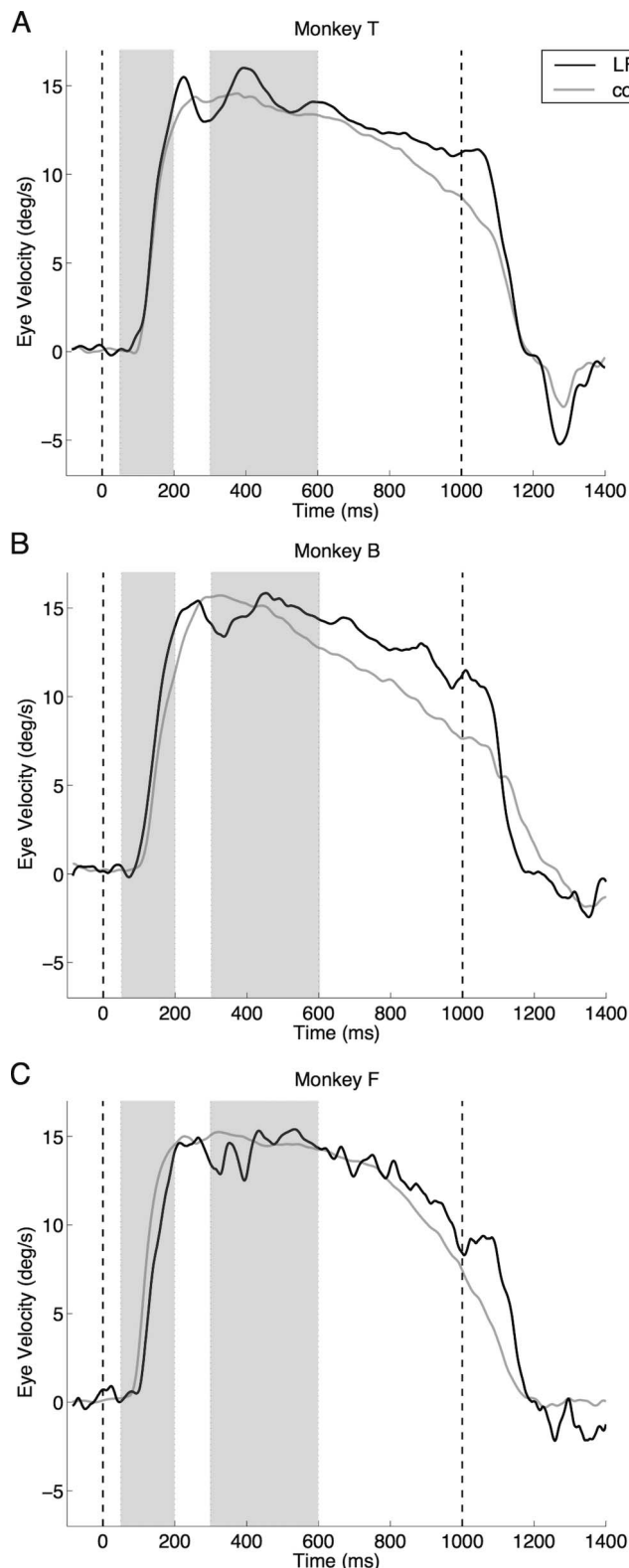


Figure 2. Smooth eye movements in a combined volitional and reflexive tracking task. (A) Average eye movement traces for Monkey T. The gray trace represents control trials ($n = 921$) with no LF motion, and the black trace represents trials with concurrent LF motion ($n = 532$). Target motion onset and offset

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at 0 and 1000 ms, respectively, indicated by the dashed vertical lines. The gray shaded regions indicate the quantification intervals used for initiation (50–200 ms after target motion onset) and maintenance (300–600 ms). (B) Average eye movement traces for Monkey B, same conventions as in (A). Control trials $n = 752$, LF trials $n = 202$. (C) Average eye movement traces for Monkey F, same conventions as in (A). Control trials $n = 978$, LF trials $n = 90$.

region Figure 2), and Monkey F had a gain of 0.8 (Table 1).

Following the initiation interval, the eye velocity in LF trials briefly decreased before rebounding to control levels again (Figure 2). Monkey B had only one such decrease (Figure 2B), whereas monkeys T and F arguably have two separate cycles of this behavior (Figure 2A, C). For all monkeys, the eye velocity overshoot control levels at around 400–450 ms after target motion onset (Figure 2). To quantify these early decreases in eye velocity unique to LF trials, we found the local maxima and minima for each peak-trough cycle (Figure 3A) and calculated the differences between the peak and subsequent trough (Figure 3B). The peak-trough differences were all about 2.0–2.5°/s, and similar across animals and cycles of peak-trough behavior.

Although this peak-trough behavior is present at the very beginning of the maintenance interval (300–600 ms after target motion onset, second gray shaded region Figure 2), the gain during that interval is equal to control levels because of the subsequent increase in eye velocity for all monkeys (Table 1). This comparatively greater eye velocity continues throughout the duration of pursuit, becoming even more noticeable toward the end of target motion and pursuit offset (Figure 2). This is also demonstrated in the greater-than-1.0 gain and a significantly greater eye velocity for LF trials compared with controls for the whole pursuit interval for monkeys T and B ($p < 0.001$ for both).

Response of individual FEFsem neurons during pursuit with LF motion

We included a total of 43 FEFsem neurons recorded from three monkeys in this study (Monkey B, $n = 15$; Monkey F, $n = 6$; Monkey T, $n = 22$). Only neurons that responded to tracking of a small-diameter (0.2°) target spot during step-ramp tracking were included. When tested for visual motion sensitivity (response to LF motion during fixation), 20 neurons (47%) exhibited a visual motion response, 19 (44%) did not, and 4 (9%) were not sufficiently tested. Of those that were sensitive to visual motion, 60% had the same preferred direction

	Monkey T	Monkey B	Monkey F
Gain (LF/control)			
Initiation	1.1 (0.4)	1.3 (0.7)	0.8 (0.3)
Maintenance	1.0 (0.2)	1.0 (0.3)	1.0 (0.2)
Whole interval	1.1 (0.2)	1.1 (0.3)	1.0 (0.2)
Latency (ms)			
LF	95 (21)	94 (27)	92 (13)
Control	101 (20)	122 (40)	84 (16)
<i>n</i> (trials)			
LF	532	202	90
Control	921	752	992

Table 1. Gain and latency for trials with LF motion. *Notes:* Gain is the ratio of the eye velocity for trials with LF motion compared with the eye velocity in control trials, for three intervals: initiation (50–200 ms after target motion onset), maintenance (300–600 ms), and the whole interval (50–1100 ms). Standard deviations in parentheses. Average behavioral latency for control trials and LF trials given as time following target motion onset. *n* = number of trials included for each animal.

for visual motion and pursuit, whereas 35% exhibited the opposite relationship. One neuron (5%) exhibited visual motion sensitivity in both the preferred and antipreferred direction of pursuit. Visual motion sensitivity was not associated with differences in any other tested variables, aside from neuronal latency in control trials. Cells with visual motion sensitivity had significantly shorter neuronal latencies (99.4 ± 9.4 ms [$M \pm SEM$]) compared with neurons without visual motion sensitivity (160.5 ± 22.6 ms, $p = 0.01$; data not shown).

An example FEFsem neuronal response to step-ramp tracking in both control and LF trials is shown in Figure 4. The eye velocity and firing rate traces are broadly similar between control ($n = 26$, gray shaded region) and LF trials ($n = 19$, black line). The latencies are also similar for control and LF trials for both the behavior and neuronal responses, at about 100 ms and 70 ms after target motion onset, respectively. The eye velocity in LF trials is slightly higher than in control trials, on average ($11.5^\circ/\text{s}$ in LF trials compared with $11.1^\circ/\text{s}$ in control trials), although they exhibit similar dynamics overall (Figure 4, top panel).

Interestingly, there are some notable differences in the neuronal activity between control and LF trials (Figure 4, middle panel). In the control trials (gray shaded region), there is a gradual increase in firing rate during the initiation phase of pursuit, followed by a plateau that persists throughout the duration of pursuit. In contrast, during LF trials (black line), there is a sharp, transient peak in activity that coincides with pursuit onset. The activity then drops to a level that is just below the plateau seen in control trials, and this level of activity is maintained throughout the duration of pursuit. At pursuit offset, there is a similar sharp,

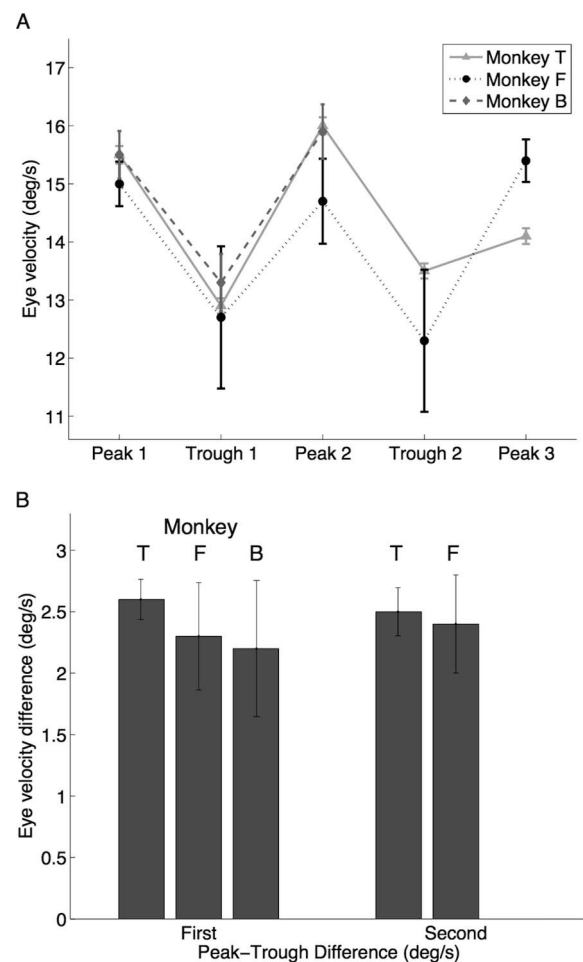


Figure 3. Quantification of behavior. (A) Eye velocity at peaks and troughs during pursuit, as seen in Figure 2. Each line represents data from an individual monkey. Both Monkeys T and F had two quantifiable peaks and troughs, whereas Monkey B exhibited only one. (B) Peak-trough differences in eye velocity for each monkey.

transient peak that coincides with the time the eyes stop moving, following which the neuronal activity returns to baseline levels. Although there are some similarities between the control and LF neuronal responses, the transient peaks featuring higher activity than control and the sustained activity at levels lower than control are conspicuous differences.

To investigate whether the observed differences in neuronal activity (Figure 4) could be related to differences in eye velocity (as seen in Figures 2 and 4), we directly assessed the relationship between firing rate and eye velocity in the initiation (Figure 5A1, B1) and maintenance (Figure 5A2, B2) intervals. The neuron in Figure 5A is the same one whose activity was shown in Figure 4. Each point represents the average firing rate and eye velocity over the interval (initiation: 50–200 ms, maintenance: 300–600 ms) on an individual trial. Significant differences in eye velocity and firing rate

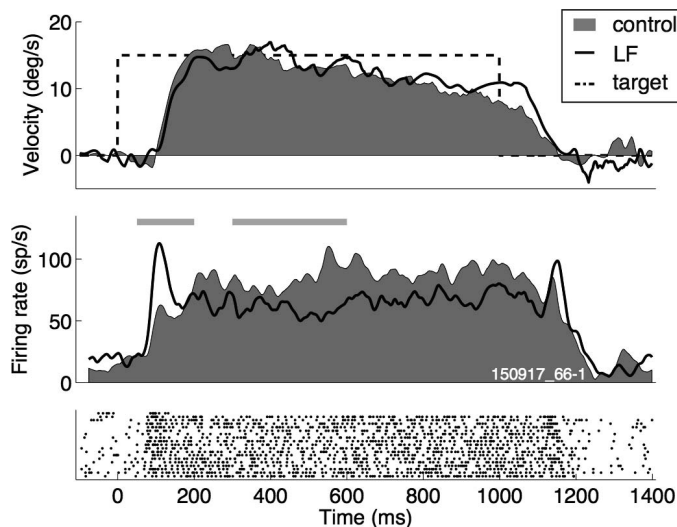


Figure 4. Example FEFsem neuronal activity during combined volitional and reflexive pursuit. Top panel shows the target velocity (dashed line), average eye velocity in control trials (gray shaded region, $n = 26$), and LF trials (black line, $n = 19$). Middle panel shows the average spike density function for control and LF trials; conventions as in the top panel. Gray horizontal lines represent the intervals used for initiation and maintenance. Bottom panel shows a raster plot for the LF trials. Data from Monkey T.

between LF and control trials for both intervals are observed ($p < 0.05$), but there is no significant relationship between eye velocity and firing rate for LF or control trials, for either interval (control: $R^2 = 0.056$, $p = 0.25$ for initiation and $R^2 = 0.109$, $p = 0.10$ for maintenance; LF: $R^2 = 0.003$, $p = 0.82$ for initiation and $R^2 = 0.148$, $p = 0.11$ for maintenance). This suggests that the observed differences in firing rate between control and LF trials are unlikely to have resulted from differences in eye velocity.

In contrast, the neuron in Figure 5B does have a significant relationship between eye velocity and firing rate in control trials ($R^2 = 0.243$, $p = 0.02$ for initiation; $R^2 = 0.200$, $p = 0.04$ for maintenance) with an increase in firing rate with higher eye velocity. However, the same relationship was not observed in LF trials ($R^2 = 0.002$, $p = 0.85$ for initiation; $R^2 = 0.138$, $p = 0.14$ for maintenance). It is clear from this comparison that differences in eye velocity do not underlie the significant differences in firing rate between LF and control trials ($p < 0.001$).

In addition, the effect of the added LF motion changed throughout the time course of pursuit for both neurons (Figure 5A3, B3). LF response was calculated for both initiation (LF_{init}) and maintenance (LF_{maint}) intervals. Both neurons showed a significant difference between LF_{init} and LF_{maint} (Student's t test, $p < 0.001$ for both neurons).

Because LF and control trials were not interleaved, we also wanted to assess the firing rate across trials within an LF block (Supplemental Figure S2). We found that in the initiation interval, five of 39 neurons (13%) had a significant relationship between trial number and firing rate (e.g., Supplemental Figure S2B), whereas in the maintenance interval, nine of 43 neurons (21%) had a significant relationship (e.g., Supplemental Figure S2A). Notably, of these neurons with significant relationships, only one showed such a relationship within both intervals. This analysis suggests that the responses of our population of neurons would not likely be different with interleaved conditions.

FEFsem population response during pursuit with LF motion

Over the whole population of neurons, 10 of 39 (26%) had significant differences between LF and control trials in both firing rate and eye velocity during the initiation interval. Of those 10 neurons, only one (Figure 5B) showed a significant correlation between eye velocity and firing rate in control trials, and even this correlation could not explain the observed differences in firing rate between LF and control trials. For the initiation interval, we excluded any neuron whose latency was sufficiently long so as to have little or no response within the interval (four of 43 neurons).

In the maintenance interval, 15 of 43 neurons (35%) had significant differences in both firing rate and eye velocity. None of these neurons had significant correlations between eye velocity and firing rate in control trials, again suggesting that eye velocity alone is not sufficient to explain the significant differences in firing rate seen between LF and control trials.

To assess the distribution of FEFsem responses to combined volitional and reflexive pursuit, we compared the average activity for each neuron in LF trials to the average activity in control trials (Figure 6). We expressed the difference as a percentage change from control levels. In this scheme, negative differences represent neurons whose activity levels in LF trials were less than in control trials. We assessed this LF response in both the initiation interval (LF_{init}, Figure 6A; $n = 39$) and the maintenance interval (LF_{maint}, Figure 6B; $n = 43$). In both cases, there is a broad range of responses from highly negative to positive. The mean value for initiation is 37% (dashed line, Figure 6A), whereas the mean value for maintenance is -23% (dashed line, Figure 6B). However, there is no significant difference between these two distributions (Student's t test, $p = 0.85$).

To determine whether the LF response is consistent over time, we compared the LF_{init} and LF_{maint} responses (Figure 6C). There is a clear, positive

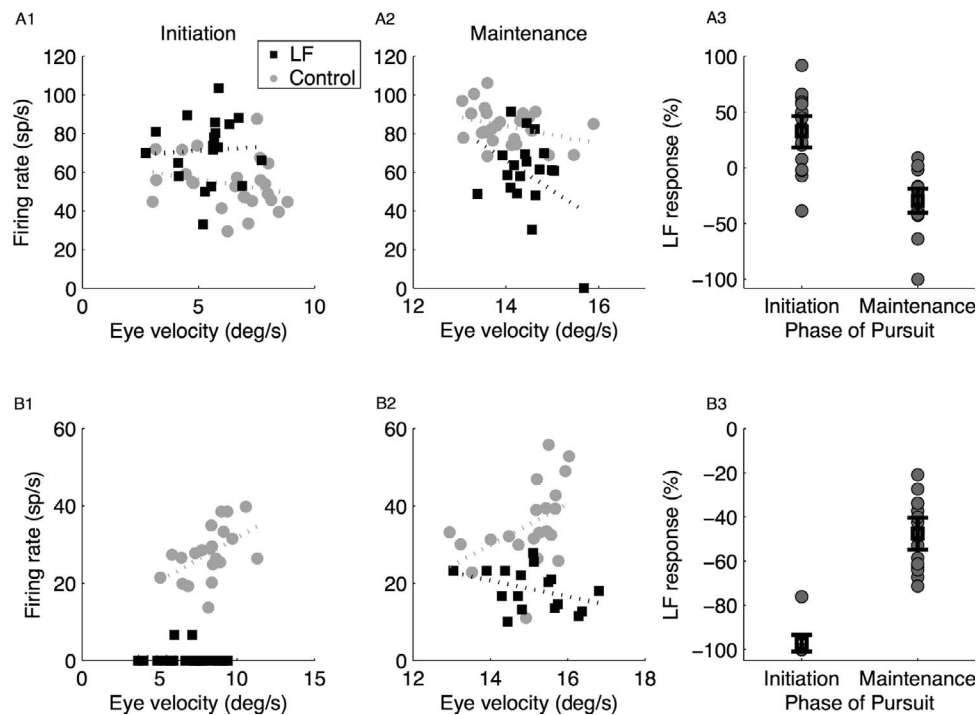


Figure 5. Relationship of firing rate to eye velocity in two representative FEFsem neurons. The neuron in (A) is the same as from Figure 4. (A1) Data from initiation interval. Average of firing rate and eye velocity from the initiation interval for control (gray, $n = 26$) and LF (black, $n = 19$) trials. Dotted lines represent best linear fits. (A2) Data from maintenance interval, same conventions as in A1. (A3) LF response for both intervals for each LF trial. Mean and 95% confidence intervals shown atop in black. Significant difference between initiation and maintenance ($p < 0.001$). (B1–3) Same conventions as in (A1–3). Control trials ($n = 21$), LF trials ($n = 17$). Data from Monkey T. Significant difference between LF_{init} and LF_{maint} , $p < 0.001$.

relationship between the two LF responses, indicated by the best-fit line (dashed; $R^2 = 0.392$, $p < 0.001$). This indicates that the LF response for the majority of neurons is relatively reliable over time despite the fact that some individual neurons show significantly different activity during initiation and maintenance (e.g., Figure 5A3, B3).

Comparison of FEFsem response during pursuit with LF motion and pursuit with target blink

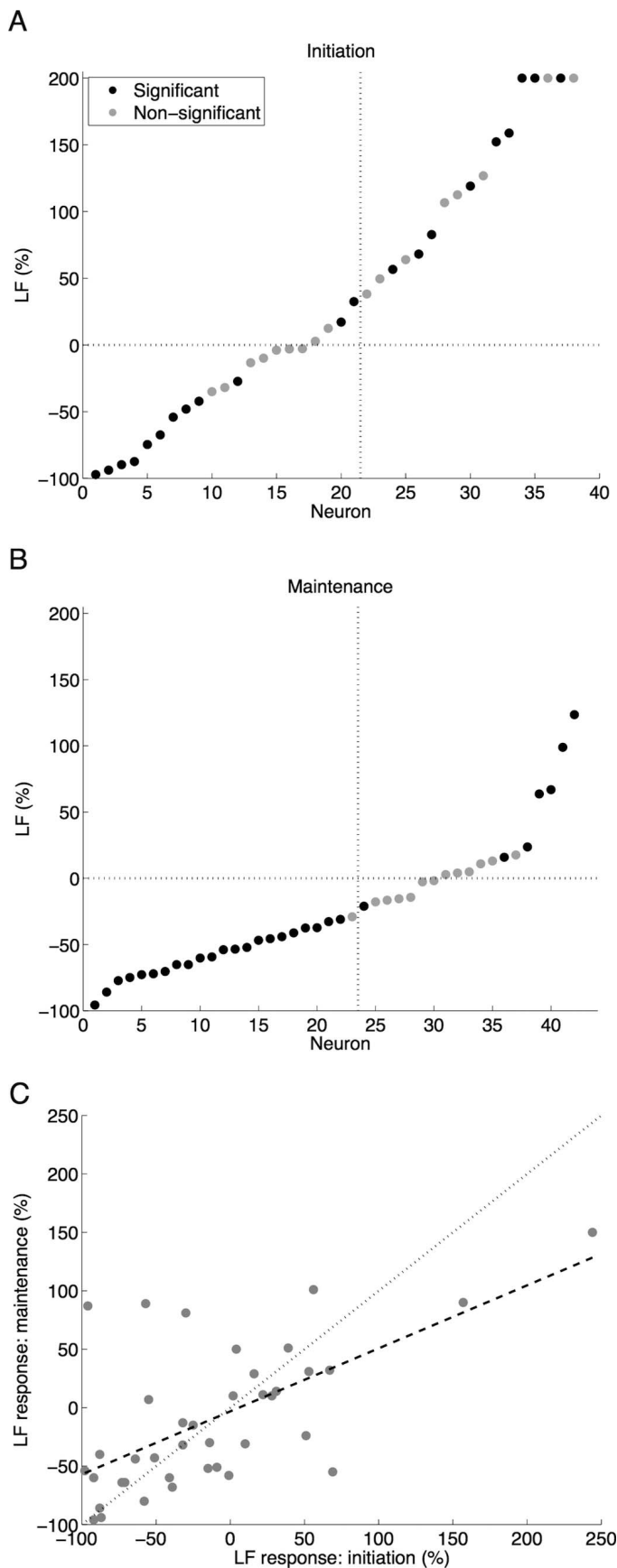
Given that the differences in tracking behavior between LF and control trials are generated by characteristics of retinal image motion, we wanted to compare the effects of LF motion to another manipulation of retinal input: the target blink. This comparison can be seen in Figure 7. Target blinks that began at either 50 or 100 ms after target motion onset were considered to be “early” blinks, whereas target blinks beginning at 300–500 ms after target motion onset were considered “late” blinks. The blink response, similar to the LF response, was expressed as the percentage change in firing rate following the target blink compared with control levels in the same interval. Again, negative responses represent neurons that were

less active following the blink as compared with the same interval in control trials.

We compared the LF_{init} response to the early blink response (Figure 7A, $n = 20$) and did not find a significant relationship (dashed line, $R^2 = 0.049$, $p = 0.35$). However, a relationship was evident for the maintenance interval (Figure 7B, $n = 30$), with those neurons exhibiting the most negative LF responses also showing the most negative blink responses. In this case, there is a significant, positive relationship between the LF_{maint} response and the late blink response (dashed line, $R^2 = 0.220$, $p < 0.01$). Somewhat surprisingly, this suggests that neuronal responses to the removal of retinal input are related to the responses to the addition of retinal input.

Discussion

The generation of smooth eye movements is generally studied using either volitional smooth pursuit or reflexive ocular following behaviors. In this study, we sought to describe whether and how the behavior for combined volitional and reflexive pursuit differs from that of pursuit of a small spot alone. We also



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Figure 6. Distribution of neuronal responses across the FEFsem population. (A) LF_{init} : each point represents an individual neuron's response ($n = 39$). Difference in firing rate between control and LF trials expressed as percentage change; significant differences between LF and control shown by black points. Mean of responses shown by dashed vertical line (37%). (B) Same conventions as in (A) but for the LF_{maint} ($n = 43$). Mean = -23%. (C) Comparison of LF_{init} and LF_{maint} . Each point represents data from one neuron. Dotted line represents unity, and the dashed line is the line of best fit ($R^2 = 0.392$, $p < 0.001$), with the equation: $LF_{maint} = 0.54(LF_{init}) - 3.17$.

investigated the response of FEFsem neurons to such combined pursuit. We then compared the responses of FEFsem neurons in the LF task to neuronal retinal and extraretinal sensitivities.

Behavior in a combined volitional and reflexive pursuit task

LF motion is known to evoke neuronal and behavioral responses at shorter latencies than those associated with motion of small visual targets alone (Kawano & Miles, 1986; Miles et al., 1986; Gellman et al., 1990). In addition, it has been shown that the gain of reflexive ocular following is greater than in volitional smooth pursuit eye movements (Kawano, 1999). We found that combined volitional and reflexive pursuit also induced shorter behavioral latencies than purely volitional pursuit for two of the three animals, with the mean latency decrease for the three monkeys being 9 ms. Beyond this, the eye velocity in LF trials is greater than or equal to control eye velocity in eight of nine intervals analyzed (three intervals for three monkeys).

Interestingly, there is less stability at the end of the initiation phase in LF trials than control (Figures 2 and 3). There appears to be some sort of ringing dynamic (e.g., underdamped oscillations) in the eye velocity traces of all three animals as the eye velocity approaches its peak, around 200–300 ms after target motion onset. Compared with the control trials, it takes longer for the eye velocity to settle and reach steady state. This suggests that although the LF motion causes the eyes to start moving faster and earlier than in control trials, it induces more uncertainty as to the actual movement of the target. This uncertainty could result from the higher open-loop accelerations that are seen during pursuit of large patches of random-dot motion (Heinen & Watamaniuk, 1998), which is somewhat similar to our combined volitional and reflexive task. Increased open-loop acceleration could contribute to the initial overshoot and subsequent retinal velocity errors that the animal needs to override

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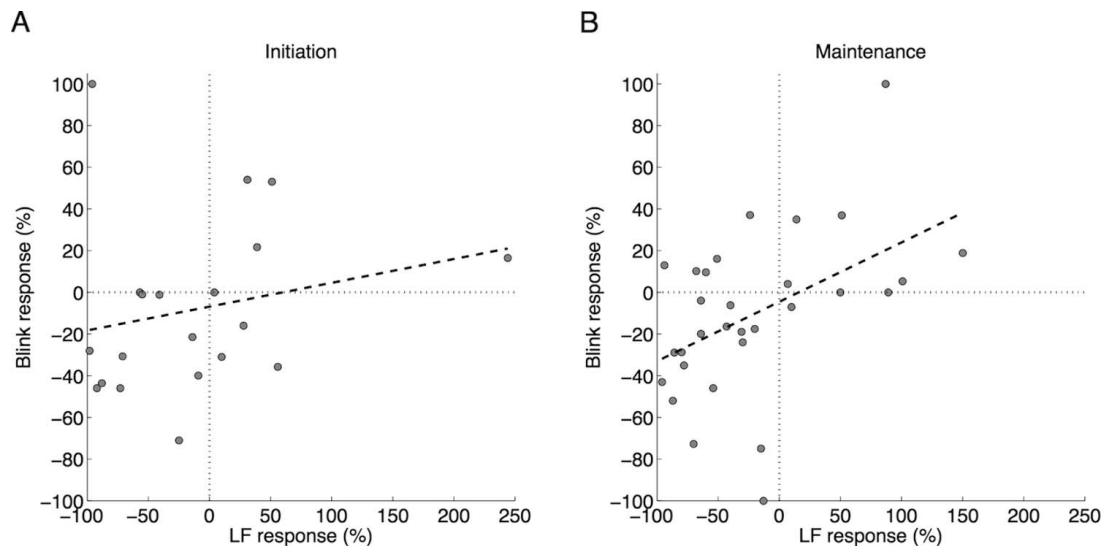


Figure 7. Comparison of LF and blink responses in FEFsem neurons. (A) Comparison of LF_{init} (50–200 ms after target motion onset) and the early blink response (110–310 ms). Each gray point represents data from one neuron ($n = 20$). Dashed line is the line of best fit ($R^2 = 0.049$, $p = 0.35$), with the equation: Early Blink Response = $0.11(LF_{init}) - 6.86$. (B) Same conventions as in (A) but for LF_{maint} (300–600 ms) and late blink response (360–710 ms; $n = 30$). The line of best fit ($R^2 = 0.220$, $p < 0.01$) has the equation: Late Blink Response = $0.28(LF_{maint}) - 4.54$.

through the use of extraretinal signals to achieve accurate pursuit.

Following this instability, the animal maintains a high steady-state eye velocity at levels greater than control throughout the remainder of the pursuit interval. In fact, some of the greatest differences in eye velocity between LF and control trials are in the last 200–400 ms of target motion. This suggests that although the animal is exerting volitional control to keep the eyes on target, the added LF motion is quite effective in increasing the eye velocity above control levels, as would be expected of reflexive eye movements.

Single neuron response to a combined volitional and reflexive pursuit task

Neurons in the FEFsem are theorized to be involved in the initiation of volitional smooth pursuit eye movements as well as dynamic gain control for pursuit (Tanaka & Lisberger, 2001, 2002; Ono & Mustari, 2009). However, FEFsem neurons are not understood to play these roles for reflexive eye movements, which are thought to be subserved by the MT-MST-DLPN pathway. Thus, we hypothesized that the neurons in the FEFsem would be less active for a task that combines reflexive and volitional eye movements because of the increased drive provided by the MT-MST-DLPN pathway.

Although there were many overall similarities in the firing rate dynamics for FEFsem neurons when comparing volitional (control) and reflexive + volitional

(LF) pursuit, there were also notable differences.

Although eye velocity was at or above control levels, the firing rate was often below control levels during steady-state pursuit, with a mean response that was 23% less than control activity.

However, given the differences in eye velocity between control and LF trials, we wanted to ensure that the neuronal effects were not due to these increases in eye velocity. Of the neurons that had significantly different firing rates and eye velocities when comparing control and LF trials, only one had a significant correlation between eye velocity and firing rate within control trials. This means that the vast majority of FEFsem neurons exhibit limited eye velocity sensitivity within the range of observed eye velocities, suggesting that differences in eye velocity are not sufficient to explain changes in firing rates between LF and control tasks.

In addition to the influence of eye velocity, we also wanted to assess the effects of LF motion on neuronal activity across trials. Only one neuron showed a significant relationship between firing rate and the number of trials in both initiation and maintenance intervals, whereas 13 other neurons (30%) had a significant relationship in only one interval. These relationships generally took the form of gradual decreases in activity over the course of the block within a given analysis interval (e.g., initiation).

In cases like these, it may be that the sudden presence of LF motion provides a strong retinal signal to the FEFsem in the beginning of the block, likely via projections from MST (Tian & Lynch, 1996; Stanton et

al., 2005). Then, some habituation or reweighting of signals could occur to lessen the impact of this continuously present LF background as the block progresses, although this possible habituation does not seem to correlate with visual motion sensitivity. Whether such habituation, if it is indeed taking place, follows from similar habituation in earlier structures such as MST and MT or appears *de novo* in the FEFsem is an open question. However, it is clear that the majority of FEFsem neurons do not seem to be affected by the block design of our study.

In addition, for some neurons, the presence of the LF motion causes differential effects during the initiation and maintenance intervals (two examples of which can be seen in Figure 5A3, 5B3). According to our central hypothesis, these changes in activity could be due to changing weights of the drives from the MT-MST-DLPN and FEF-NRTP pathways. Similarly, it has been recently shown that the weights of retinal and extraretinal signals in FEFsem activity are not static throughout the time course of pursuit (Bakst et al., 2017), as has been demonstrated previously for behavior (Bogadhi, Montagnini, & Masson, 2013). This could also contribute to the differential effects of the LF motion during initiation and maintenance. Below, we discuss the possible explanations for these results in more detail.

FEFsem population response and comparison to other neuronal sensitivities

Overall, the FEFsem neuronal response is greater in LF trials than control during the initiation interval and less than control during the maintenance interval, on average. According to our main hypothesis, this result would suggest that the voluntary drive from the FEFsem is stronger during initiation than during maintenance. These differential effects could be due to the fact that, although the combined pursuit relies more on reflexive pursuit pathways, the large pulse of image motion during initiation provides a strong, transient increase in activity to many neurons in the FEFsem. Thus, the increased contribution from image motion early in the trial masks the decreased involvement of the FEFsem in the combined reflexive-volitional behavior.

Surprisingly, the LF response is also moderately correlated with the blink response. Although the results from the initiation interval are not significant, they are similar to the significant findings from the maintenance interval. Both intervals show positive relationships between the blink response and LF response with similar slopes. Interestingly, this means that the addition of retinal input from the LF motion induces a similar response to taking away the visual target within

the same neurons. This could potentially be due to neurons being sensitive to anything that perturbs the expected step-ramp pursuit task, such as the removal of the target or the addition of other visual stimuli.

In both tasks, the changes in activity could reflect the weights of the signal from the rewarded target spot. For neurons that increase their activity in response to both perturbations, this could be a form of attention or compensation. When the target is blinked, the retinal signal, and therefore some of the drive for pursuit, is removed. Thus, in order for the eyes to continue moving in the absence of a visual target, some other neurons must increase their activity to compensate, as has been reported before (Tanaka & Fukushima, 1998; Bakst et al., 2017). In the case of the LF motion, the textured background is composed of many spots, dispersed across the screen. These spots could ostensibly be targets of pursuit, although only the red laser spot is rewarded. It is therefore possible that some neurons increase their activity to provide a stronger attention or gain signal for the retinal input from the rewarded target spot. To outcompete all of the other possible visual targets, the system may increase the weight of the signals coming from the target spot. Thus, the same neurons could increase their activity in response to both the addition and subtraction of retinal stimuli.

In contrast, neurons may decrease their activity in response to both types of perturbations, possibly because of the lack of signals propagating from earlier visual areas such as MT and MST or because of a compensatory decrease in drive from the FEF-NRTP pathway effected by cerebellar-thalamocortical feedback signals. In the case of the target blink, the retinal signals are no longer present in areas dependent on retinal input, such as MT, and this in turn decreases the input to MST and FEFsem. In the case of the additional LF motion, although there is a great deal of retinal input, it may be flowing through reflexive pathways that handle full-field stimuli rather than small spots. In response to this increased reflexive drive, the volitional pathway might decrease its drive to maintain accurate eye movements in response to feedback signals traveling from the cerebellum to the thalamus and back to the FEFsem (Stanton, 1980; Asanuma et al., 1983a, 1983b; Tian & Lynch, 1997). These possibilities could explain the seemingly paradoxical findings that both subtracting and adding retinal stimuli have similar effects in individual FEFsem neurons. These results are also in keeping with prior findings suggesting that there are multiple subgroups of neurons within the FEFsem that likely play different functional roles in volitional smooth pursuit eye movements (Bakst et al., 2017).

Conclusions

This study assesses the role of the FEFsem in combined volitional and reflexive pursuit. As expected, the behavior during such a combined task begins sooner and is executed at a higher gain than purely volitional pursuit. Somewhat surprisingly, we also found evidence that this addition of the LF motion makes the transition to steady-state pursuit less stable, possibly because of a diminished ability to use extraretinal components to override early retinal image motion or the difficulty in determining the appropriate balance between volitional and reflexive drives.

We also found that the FEFsem as a whole participates less in the combined behavior than in purely volitional pursuit, again confirming our expectations. We also show that these results are in accordance with the findings from a target blink task, indicating that there may be a subset of neurons that compensate in the presence of perturbations such as the removal of the target spot or the appearance of LF motion.

Further work will be necessary to verify that the introduction of LF motion into a volitional smooth pursuit task lessens the information flow between MST and FEF and increases the flow through the MT-MST-DLPN pathway. Testing the response of MST and MT neurons to this combined volitional and reflexive pursuit task will likely help clarify the role(s) of these cortical nodes of pursuit.

Keywords: smooth pursuit, FEF, ocular following, retinal input

Acknowledgments

The authors thank R. Koepke and B. Congdon for technical support, as well as S. Ono for mentorship and guidance. This work was supported by the National Institutes of Health (NEI EY07031, EY013308, EY06069, ORIP ODO10425) and Research to Prevent Blindness.

Author contributions: LB, JF, and MJM contributed to experimental design. LB and JF collected the data. LB performed the data analysis. LB prepared the manuscript with assistance from JF and MJM. This work comprised part of LB's PhD thesis.

Commercial relationships: none.

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