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The role of the FEFsem in smooth pursuit: Insights from varying retinal input

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Abstract

The role of the FEFsem in smooth pursuit:
Insights from varying retinal input

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Primates are exceptionally adept at tracking moving objects with their eyes, providing high-acuity vision throughout the duration of tracking. This behavior, called smooth pursuit, relies on incoming visual signals being successfully transformed into eye movement commands. Though a seemingly simple process, in practice this requires the processing of retinal signals, integrating them with extraretinal signals like memory, prior experience, prediction, motivation, and attention, among others, and weighting these various factors appropriately. One area known to be vital to the execution of volitional smooth pursuit is the smooth eye movement subregion of the Frontal Eye Field (FEFsem). I sought to advance our understanding of the role of the FEFsem in

smooth pursuit by varying the retinal stimuli during pursuit, and estimating the relative contributions of retinal and extraretinal signals to neuronal activity.

In chapter 1, I review what is known about smooth pursuit behavior with an emphasis on the way different signals impact the behavior. I also review the signal transformations at different nodes in the pursuit pathway, focusing on those areas known to be most closely linked to the FEFsem. In chapter 2, I manipulate the retinal input during pursuit by temporarily extinguishing the target. This affords me the opportunity to assess the relative contributions of retinal and extraretinal signals to both the behavioral and neuronal response. I found that both the smooth pursuit system as a whole, as well as individual FEFsem neurons exhibit changing reliance on retinal input throughout pursuit. The degree to which the weights of these components change and the type of response observed allows me to discuss their possible functions.

In chapter 3, I use a large-field, textured background moving concurrently with a small target spot to investigate the behavioral and FEFsem neuronal response to a combined volitional and reflexive pursuit task. I found that the addition of the large-field motion increased the gain of pursuit eye movements, and decreased the neuronal activity level in the FEFsem, on average, consistent with the hypothesis that a different cortico-ponto-cerebellar pathway underlies reflexive ocular following compared to volitional pursuit. Additionally, I found that the response of individual FEFsem neurons to the addition of the background motion was positively correlated with the response to the target blink, suggesting that these neurons may respond to "disruptions" in pursuit similarly, irrespective of whether the disruption consists of the addition or subtraction of retinal input. Finally, in chapter 4, I summarize the findings, discuss their relevance to current models of pursuit, and suggest future directions to address remaining questions as to the mechanisms underlying volitional smooth pursuit eye movements.

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DEDICATION

For Barry, Feige, David, and Paula, who,

with their remarkable resilience, ceaseless optimism,

extraordinary bravery, and love of learning,

surely provided the foundation for this work.

Chapter 1. INTRODUCTION

Our behavior and survival in the world relies upon our ability to process incoming information and take action. As highly visual creatures, one important way we do this is by transforming incoming visual signals into commands for eye movements. The ability to follow a moving object with our eyes is called smooth pursuit, and our brain's ability to turn incoming visual signals into motor commands for smooth pursuit is one example of a sensorimotor (specifically visuomotor) transformation. However, as vital as this kind of visuomotor transformation is to our survival and behavior, it is still relatively poorly understood.

The majority of this review is dedicated to defining neural circuits related to visual-oculomotor behavior as exemplified in the smooth pursuit eye movement system. I will begin with an overview of the behavior of smooth pursuit, from the initiation of the eye movement to its offset, with a discussion of the way that different components (e.g. retinal and extraretinal signals) and higher-level functions (e.g. target selection and gain) influence the basic behavior.

I will then review the signal transformations at different nodes in the pursuit pathways within the brain, demonstrating the relative specialization of circuit components and loops. The signal progresses from purely visual to purely motor as it travels through a circuit beginning in the retina, moving through the dorsal stream, which carries motion and depth information for perception and action, and ending with oculomotor neurons in the brainstem.

1.1 BEHAVIOR

Smooth pursuit eye movements allow the continual tracking of moving objects, providing high-acuity vision throughout the eye movement (for review, see Krauzlis 2004). The pursuit system is a controlled feedback system -- the eye movement output affects the retinal input. On

average, it takes about 100 ms to initiate smooth pursuit, and, consequently, about 100 ms to see the effects of visual input on the motor output (Lisberger et al. 1987). Smooth pursuit is typically broken down into two phases: initiation (open-loop) and maintenance (closed-loop).

The initiation phase consists of the first approximately 100 ms of the pursuit eye movement, and is largely made up of the eye accelerating to the target. These first 100 ms are open-loop because there is no visual feedback in the system. This phase is notable for the presence of large amounts of retinal image motion (Robinson 1965, Lisberger et al. 1987).

The maintenance phase, also known as steady-state pursuit, follows initiation, and continues until the eyes decelerate at the end of the behavior. It is considered closed-loop because visual feedback has already made its way through the system. This phase is marked by low amounts of retinal image motion, and, ergo, the need for some sort of extraretinal signal to drive the continual movement of the eye (Lisberger et al. 1987).

1.1.1 *Initiation*

The transition from fixation to active smooth pursuit is referred to as pursuit initiation. The initiation of pursuit can include both saccades and smooth components, which are coordinated but have independent latencies (Rashbass 1961). Moving stimuli, which produce retinal image motion (also termed velocity errors), are effective stimuli for evoking pursuit. Even small velocity errors, when consistent, can elicit pursuit from fixation. Position errors, though, are generally ineffective stimuli for eliciting pursuit eye movements from fixation (Morris and Lisberger 1987). When a visual target is moving at speeds greater than 5°/s, the latency of smooth pursuit onset is highly consistent. For targets that move more slowly, the latency of movement onset increases (Carl and Gellman 1987). Generally, pursuit initiation is thought to be divided into two phases. Depending on the characteristics of the target motion, these phases may

be separated by a saccade that moves the eyes to the target. However, even in the absence of a saccade, two initial phases of pursuit are observed.

The first approximately 30 ms of pursuit, called the early phase of initiation, features eye acceleration that matches the direction of target motion, but the acceleration is only sensitive to target velocity up to 10°/s. Beyond 10°/s, the eye acceleration saturates. In this early phase, the dynamics of the eye movement are independent of the presence or absence of a background. If a saccade is evoked, the early phase is generally the presaccadic portion of pursuit (Lisberger and Westbrook 1985; Carl and Gellman 1987; Krauzlis and Lisberger 1994).

The second phase of initiation, called the late phase, is 40-100 ms after pursuit onset. The dynamics of this late phase show the effects of various target characteristics including target velocity, target position, and the presence or absence of a background. Eye acceleration in the late phase of initiation has a nearly linear relationship to target velocity, increasing up to 150°/s. When the background is diffusely illuminated, the eye acceleration peaks for target velocities between 30 and 60°/s. The eye acceleration is greatest for targets close to the fovea, and decreases with increasing target eccentricity (Lisberger and Westbrook 1985; Krauzlis and Lisberger 1994). These differing sensitivities in the early and late phases of initiation suggest that there is likely a transient target motion onset component that acts to initiate the movement, and then a later image velocity component that more precisely determines the characteristics of pursuit.

In an experiment where participants are told to either actively track a moving target or passively view the visual display, there are minimal differences in the initial eye movements seen following target motion onset. Many subjects show substantial smooth eye movements during the passive condition, which suggests that the initial portion of active pursuit is likely

reflexive (Wyatt and Pola 1987). This is in accord with what has been described for the early phase of active pursuit: initial eye movements match the direction of the target motion, but reflect few other characteristics. This early reflexive response rapidly moves the eyes in the appropriate direction, and then the pursuit system subsequently provides a stronger and more accurate driving signal for volitional pursuit.

1.1.2 *Anticipation*

Although pursuit onset is usually triggered by image motion on the retina, anticipatory pursuit can be observed before the target begins to move in predictable contexts. When target motion is predictable, both humans and monkeys can initiate smooth eye movements prior to target motion onset. These eye movements are scaled to the expected velocity of the target (Freyberg and Ilg 2008). Cues that indicate the direction and speed of subsequent target motion can also be used by subjects to scale their anticipatory pursuit (Jarrett and Barnes 2002). Additionally, anticipatory pursuit shows the effect of previous experiences: the eye velocity of anticipatory pursuit depends on the velocity of prior trials, with the heaviest weights on the most recent trials (Kowler et al. 1984).

Interestingly, though the addition of a textured background only minimally affects visually-guided pursuit, it strongly impedes anticipatory pursuit (Freyberg and Ilg 2008). Thus, extraretinal signals related to the expectation of target motion are strong enough to initiate pursuit on their own, but not strong enough to override retinal signals from a visible background.

1.1.3 *Retinal vs. extraretinal components*

The existence of anticipatory pursuit indicates that retinal signals are not strictly required for the initiation of pursuit eye movements. Beyond this, explicit tests of the role of retinal and

extraretinal signals in pursuit eye movements have provided a more complete picture of the importance of these different components. First, temporarily extinguishing the target during pursuit, also called blinking the target, removes retinal input to the pursuit system. This removal causes small reductions in eye velocity during both pursuit initiation and maintenance (see chapter 2). Interestingly, if the target moves behind an occluder instead of merely disappearing during pursuit maintenance, the reduction in eye velocity is nearly abolished. The use of an occluder does not cause the same recovery of eye velocity during pursuit initiation. Anticipatory eye acceleration is observed just prior to target reappearance in these cases as well (Churchland et al. 2003).

These results suggest a number of things. First, though there are reductions in eye velocity when the target is blinked, the eyes do not entirely stop moving. Thus, there are extraretinal components that support pursuit during both initiation and maintenance. Second, when an occluder is used, it diminishes the velocity reductions observed during pursuit maintenance, suggesting that extraretinal signals are strengthened by the presence of the occluder to the extent that they can nearly overcome the removal of retinal input. Third, this strengthening of extraretinal signals does not occur equally throughout pursuit. Thus, the reliance on extraretinal signals or the ability to engage them is very likely changing throughout pursuit. Lastly, the presence of anticipatory eye acceleration indicates the ability of extraretinal signals to drive pursuit when there is an expectation of target motion returning, much like the anticipatory pursuit initiation discussed above.

These results are corroborated by other findings that used target stabilization to remove retinal image motion and position error signals specifically, instead of all retinal signals entirely. In this case, there are minimal decrements in eye velocity during pursuit maintenance, which

suggests that some sort of velocity memory or other extraretinal component supports steady-state pursuit (Morris and Lisberger 1987).

Tasks that involve memory-based pursuit also indicate the role of both retinal and extraretinal components. In memory-based pursuit tasks, there are few errors in pursuit direction or go/no-go choice, but there are longer latencies for pursuit onset and corrective saccades, lower initial pursuit velocity and acceleration, as well as a lower peak velocity and longer time to peak, as compared to traditional, visually-guided pursuit (Ito et al. 2013). This again demonstrates that pursuit is possible in the absence of retinal signals, but at a lower gain; the extraretinal signals are insufficient to generate pursuit that is as fast and accurate. However, the lack of retinal signals does not impede decision processes.

Additionally, pursuit can be evoked without a foveal stimulus. Whether two parafoveal stimuli are used to create an "invisible" foveal stimulus for pursuit (Ilg and Thier 1999), or a perceptually-completed contour or a rotating wheel (Steinbach 1976) are used as the targets of pursuit, all of these instances show that accurate pursuit is possible without a foveal stimulus. This is likely related to the fact that perceived rather than actual motion is the stimulus used for pursuit (Yasui and Young 1975).

Another way that retinal signals come into play during pursuit is from visible backgrounds over which pursuit can occur. With such a static, textured background, the gain of steady-state pursuit is reduced only by about 15%, but initial eye acceleration is much more affected, while latency remains unchanged (Collewijn and Tamminga 1984; Keller and Khan 1986; Kimmig et al. 1992). Interestingly, even if a substantial amount of the background is removed such that the target of pursuit is not moving over visible background, the eye acceleration seen at the onset of pursuit is still much reduced (Kimmig et al. 1992). Thus, the effects of a visible background on

pursuit initiation are not due to local differences in contrast, but instead relate to the integration of motion over large areas. Additionally, the absence of notable deficits in pursuit suggests that the retinal signals from the background are not a hindrance during steady-state, while they perhaps require additional processing during initiation. This might mean that the system relies more on retinal signals during pursuit initiation as compared to maintenance, making the background easier to ignore later on.

This idea of the pursuit system integrating information over large visual areas can be confirmed by comparing the open-loop phase of pursuit for both a small, single target and for a large patch of random dot motion. In both cases, there are similar movement dynamics during pursuit initiation (Watamaniuk and Heinen 1999), which suggests that motion information used by the pursuit system is being integrated over large areas for use as the basis of eye movement execution.

1.1.4 *Motion processing for movement vs. perception*

The fact that motion information can be integrated over large swaths of visual area for pursuit eye movements as well as perception suggests that motion processing might be common for both processes. In fact, the directional precision of pursuit tracking of random dot motion with various amounts of coherence mimics the psychophysical performance on a direction discrimination task using the same stimuli (Watamaniuk and Heinen 1999). This is also observed on a trial-by-trial level, in that perceptual decisions and tracking behavior covary in addition to being similar on average (Stone and Krauzlis 2003).

When perceptual and pursuit tasks are combined such that the target for the perceptual task is not necessarily the target of pursuit, and pursuit and perception are given different priorities, pursuit gain follows the task priorities. Thus, the gain of pursuit is lower when the perceptual

task is given the priority and is higher when pursuit is given priority. However, the perceptual performance does not diminish when the priority is given to the pursuit task (Kerzel et al. 2009). In another version of the combined perception-pursuit task, it was found that pursuit *did*, in fact, enhance the perceptual task performance (Khurana and Kowler 1987), suggesting that perceptual performance may also follow task priorities. Therefore, it is likely that many resources for motion processing are shared between perception and movement, but they are not necessarily entirely overlapping.

1.1.5 *Pursuit offset*

Unlike pursuit initiation, in which the eyes smoothly accelerate with limited overshoot, pursuit offset can have quite different dynamics. If target motion offset is unpredictable, the eyes overshoot the target and actually reverse direction before ceasing to move. However, if target motion offset is predictable, the eye velocity gradually decreases to zero with limited overshoot, much like the dynamics seen during initiation (Krauzlis and Miles 1996).

Additionally, if the target decelerates but does not stop moving, the eyes' deceleration ringing dynamics are highly similar to acceleration dynamics. The ringing dynamics differ, though, if the target decelerates to zero (Luebke and Robinson 1988). This likely implies that the system governing pursuit, including eye acceleration and deceleration, is different than the system that regulates fixation. When the eyes have to stop pursuit to maintain a static position, the pursuit system might switch off while a fixation system comes online.

Other work has also indicated that the oculomotor system is in a different state during pursuit offset as compared to initiation or maintenance. When inserting a 1° target step during either pursuit initiation, maintenance, or offset, different eye movement responses can be observed. During initiation and maintenance, the eye speed change is greatest the earlier the perturbation is

inserted. During pursuit offset, if target motion offset is predictable then the perturbation has limited effect on eye velocity. However, if target motion offset is unpredictable, target perturbations inserted during pursuit offset caused marked increases in eye velocity when the steps are in the direction of target motion (Krauzlis and Miles 1996). This suggests that visuomotor processing during pursuit offset can be similar or highly different than the processing that occurs during pursuit onset. In toto, these results imply that the transition from pursuit to fixation likely involves separate systems and the context of the task can modulate the smoothness of the transition.

1.1.6 *Gain*

As evinced by the variability of responses to target steps during pursuit offset, the pursuit system does not always react identically to the same input. Thus, it is clear that some mechanism must be regulating the gain of the visuomotor transformation. One common way to probe the gain state of the pursuit system is to use a single cycle of high-frequency, sinusoidal target motion to evoke a reflexive ocular following response. The size of the ocular following response can serve as a readout of the gain state of the system: high gain states would result in large eye movement responses to the target motion whereas low gain states would show only small responses, even though the visual input is identical.

One of the first findings using this kind of gain probe paradigm was that the gain states during fixation and pursuit are not the same. The reflexive eye movement evoked during fixation is much smaller than that evoked during pursuit (Schwartz and Lisberger 1993). Additionally, the eye movement response increases with higher pursuit velocity (either eye or gaze velocity; Schwartz and Lisberger 1993; Churchland and Lisberger 2002; Carey and Lisberger 2004). The direction of the target motion also has an effect: the largest movements are evoked by motion in

the direction of ongoing pursuit, while the smallest are evoked by orthogonal directions (Schwartz and Lisberger 1993).

The same gain probe can be used in paradigms that vary the probability of requiring pursuit: the higher the probability that pursuit will be required, the larger the reflexive response (Tabata et al. 2005). Similarly, the expectation of pursuit generated by the experience in previous trials of either performing pursuit or fixation can affect the gain state. When previous trials require pursuit, the gain state is higher than if fixation was previously required (Tabata et al. 2008).

These results are also recapitulated using a task in which target motion for pursuit has a high-frequency sinusoidal component superimposed (multiple cycles instead of one). The evoked eye movements are dependent on eye velocity, but not image velocity. They also depend on the direction of the sinusoidal motion, and are modulated by the expectation of future pursuit (Keating and Pierre 1996).

Overall, these results cumulatively show that the gain of the visuomotor transformation for smooth eye movements is dynamically regulated. The presence or absence of ongoing eye movements, combined with prior experiences and the expectation of future pursuit (or lack thereof) all have a hand in regulating the transmission of visual information to be used by the oculomotor system. Though there have been multiple clear demonstrations of this dynamic gain control in the smooth pursuit system, the locus of this control in the brain remains an open question, though there are hints that the smooth eye movement subregion of the Frontal Eye Field (FEFsem) is involved (discussed below; Tanaka and Lisberger 2001, 2002).

1.1.7 *Position input to the pursuit system*

Although it has been shown that image velocity is the main driver of pursuit initiation, there is also evidence for a position input to the pursuit system. In pursuit maintenance, the insertion of

position errors (the difference between eye and target) can induce changes in pursuit velocity that are dependent on the direction of the position error (Morris and Lisberger 1987; Barnes and Asselman 1992). Additionally, if a visual stimulus is flashed during ongoing pursuit, a smooth eye movement is evoked towards the flash with a velocity proportional to the position error. The evoked smooth eye movement is not influenced by the ongoing pursuit velocity or the presence of a subsequent saccade to the flashed target (Blohm et al. 2005).

As discussed before, position errors imposed during fixation are not adequate stimuli for pursuit (Morris and Lisberger 1987). However, when a square wave stimulus is provided, smooth eye movements can be evoked, even though there is no actual retinal image motion (Pola and Wyatt 1980). Other kinds of position inputs can also evoke pursuit by mimicking retinal image motion as well. Apparent motion, created by a sequence of flashes, can elicit pursuit. As flash separation increases, pursuit latency also increases, but, paradoxically, initial eye acceleration also increases. With further flash separation, though, the eye acceleration eventually decreases. The spatial limit of the flashes increases with eccentricity (Churchland and Lisberger 2000). Thus, though simple position errors do not evoke pursuit outright, position signals can be drivers of pursuit in certain conditions during both initiation and maintenance. This suggests that though position error signals on their own are unlikely to be sufficient drivers of pursuit, information about position error plays a powerful role as a modifier of ongoing pursuit and is likely essential for coordinating pursuit and saccadic eye movements.

1.1.8 *Target selection*

Although many laboratory tasks use a single visual target to examine oculomotor behavior, in the real world, individuals must select targets for saccades and pursuit amongst many options. To begin to tease apart the way the pursuit and saccadic systems handle target selection, two or

more moving targets can be used. When two visual stimuli are used and without any cues as to which is the intended target, pursuit begins as a vector-average of the eye movements seen in response to each stimulus independently (Lisberger and Ferrera 1997; Ferrera 2000; Garbutt and Lisberger 2006). This vector-average is entirely determined by the directions of motion of the targets and not by the speed of the motion (Ferrera and Lisberger 1997).

However, the use of cues can bias such pursuit towards the target and away from the would-be distractor. The cues can take the form of the color or shape of the intended target, the direction in which the target will be moving, or the initial location of the target. Generally, these cues serve to bias the vector-average towards the target's direction of motion, but there is usually still substantial influence of the distractor motion. Color/shape cues tend to be the least effective in biasing pursuit, while motion and location cues are more effective (Ferrera 2000; Garbutt and Lisberger 2006). These vector-average biases can persist through delays of up to 1 second (Garbutt and Lisberger 2006).

Similar effects are seen for pursuit latency: color/shape cues are less effective at reducing pursuit latencies than location cues (Krauzlis et al. 1999; Adler et al. 2002). Motion cues are also less effective than location cues at reducing pursuit latency (Adler et al. 2002). Shape/color cues are also less effective at initiating accurate pursuit. Following shape/color cues, pursuit is often first directed at the distractor (Krauzlis et al. 1999). In sum, location cues seem to be the most effective for target selection, allowing for the fast and accurate initiation of pursuit, while color and shape provide the least reliable information.

Similar results are seen in tasks that require both pursuit and saccades. Color/shape cues are the least effective at decreasing latency and increasing initial accuracy, whereas location cues are much more effective (Krauzlis et al. 1999). Without cues, pursuit generally has a shorter latency

than saccades and is initially a vector-average of the movements evoked by each target alone. If the eventual saccade is oriented towards one of the targets, postsaccadic pursuit is biased towards the motion of that target. However, if the saccade endpoint is between the two targets, pursuit remains vector-averaging. This scheme holds true even for saccades with latencies longer than 300 ms (Gardner and Lisberger 2001). Thus, pursuit and saccades are ordinarily directed towards the same target. When mistakes are made and pursuit is directed towards the distractor, saccades are often misdirected as well (Krauzlis et al. 1999).

Occasionally, though, pursuit and saccades are not directed at the same target. In one set of experiments, target selection differed between pursuit and saccades in between 1 and 13% of trials (Liston and Krauzlis 2003). In another estimate, the selected target differed in up to approximately 20% of trials (Case and Ferrera 2007). In these instances, pursuit is directed to one target, and then often reverses its direction before the saccade is executed (Liston and Krauzlis 2003). This suggests that the machinery for target selection and eye movement execution are closely coordinated between the pursuit and saccadic systems, but are not necessarily the same.

To further investigate the extent to which target selection mechanisms for pursuit and saccades are yoked, saccades to targets can be evoked by electrically stimulating the superior colliculus (SC) or the frontal eye field (FEF). If a saccade is evoked to one of two targets moving in orthogonal directions, the same target is then pursued (Gardner and Lisberger 2002). However, if a saccade is evoked during pursuit such that the eyes are shifted to a distractor moving in the opposite direction, the eye velocity is only minimally changed in the 50 ms following the saccade. In fact, when there is a moving distractor present at the endpoint of the saccade, the eye velocity is identical to the eye velocity seen when there is no visual stimulus at

the saccade endpoint (Krauzlis et al. 2012). Thus, the execution of a saccade is not sufficient to select a target for pursuit, though there is clearly close coordination of target selection for both saccades and pursuit.

1.1.9 *Coordination of saccades and pursuit*

Beyond coordinating saccades and pursuit in the domain of target selection, the oculomotor system must regulate the execution of these eye movements more generally to maintain accurate, high-acuity vision of desired targets. The oculomotor system seems to use an estimate of when the eyes would intersect the target during pursuit to decide whether or not to trigger a catch-up saccade. If the intersection would occur in 40 to 180 ms, no saccade is triggered, whereas if the intersection falls outside of that range a catch-up saccade is executed (de Brouwer et al. 2002). Ergo, there must be some central mechanism that computes a representation of target and eye trajectory, likely utilizing both position and velocity error, and uses that to coordinate oculomotor behavior.

Additionally, the execution of a saccade can impact pursuit in domains beyond target selection. If a catch-up saccade is generated during pursuit, the eye velocity in the 10 ms following the saccade is significantly higher than in the 10 ms prior to the saccade. This is true for saccades in all directions, including those orthogonal to the direction of ongoing pursuit. Initial target position and whether target motion is towards or away from the position of fixation do not strongly affect this postsaccadic velocity enhancement. Moving a textured background to simulate a saccade during pursuit does not induce similar eye velocity enhancement (Lisberger 1998). This suggests that it is the execution of the movement itself that changes the gain of the eye movement and not the visual input.

Interestingly, similar postsaccadic enhancements can also be observed during ocular following. The magnitude of the enhancement depends on the amount of retinal stimulation during the saccade, and moving the visual scene to simulate a saccade causes a similar enhancement unlike during voluntary smooth pursuit. Thus, in the case of reflexive ocular following eye movements, it is the visual input that causes the gain change of the movement and not the execution of a saccade (Kawano and Miles 1986).

1.2 Pursuit Pathways

Although signal processing for pursuit begins at the retina and ends with oculomotor neurons in the brainstem, this dissertation is focused on the role of the smooth eye movement subregion of the Frontal Eye Field (FEFSem) in smooth pursuit. Therefore, this review of pursuit pathways focuses on those regions most closely linked to the FEFsem: MT, MST, FEFsem, FEFsac, SEF, SC, NRTP, and DLPN. The information presented for each region is broken down into five categories: anatomy, lesions, inactivation, stimulation, and recording.

The anatomy section examines the afferent and efferent connections for each area as discovered using neuroanatomical tract-tracing methods. This section is not meant to be exhaustive, but rather to provide a sense of the connectivity of the pursuit system, with an emphasis on the direction of information flow. The lesions section presents what is often the earliest evidence of involvement in pursuit behavior. Following up on this, the inactivation section provides a more acute account of the role of the area. In contrast, the stimulation section discusses the effects of injecting electrical current into the region, which are often opposite that of inactivation. Lastly, the recording section discusses the results of neurophysiology studies. Though not exhaustive, this section attempts to give the reader a sense of the possible role(s) of the area in pursuit eye movements. Finally, a summary is provided that discusses all of the

evidence presented in concert. The role of the area in pursuit eye movements, as well as in associated higher-level functions like attention and target selection discussed.

1.2.1 *Middle Temporal area (MT)*

Anatomy: MT is located in the region just posterior to the superior temporal sulcus (STS). It receives input from striate and extrastriate cortices V1, V2, V3, and V4 (Maunsell and Essen 1983; Boussaoud et al. 1990). MT projects to cortical areas that include: MST (medial superior temporal area), FST (fundus of the STS), inferotemporal area TEO, PP (posterior parietal area), LIP (lateral intraparietal area), and the FEF (Ungerleider and Desimone 1986; Tusa and Ungerleider 1988; Boussaoud et al. 1990). MT also has numerous subcortical projections including the claustrum, caudate, putamen, pulvinar, DLPN (dorsolateral pontine nucleus), thalamic nuclei, and the SC (superior colliculus) (Spatz and Tigges 1973; Maunsell and Essen 1983; Ungerleider et al. 1984). Within MT, there are regions with sensitivity to foveal vision (MTf), and extrafoveal vision. Additionally, there are bands of neurons with reinforcing surrounds and interbands containing neurons that possess suppressive surrounds (Born and Tootell 1992). These pathways provide visual information that could play a role in sensory-motor behaviors.

Lesions: Unilateral MT lesions cause two clear movement-related deficiencies: first, deficits are observed in matching eye speed to target speed. Second, lesions also cause errors in saccades to moving targets. Both effects are strongest when the target is in the hemifield contralateral to the lesion. No deficits are seen for saccades to stationary targets in either hemifield. These deficits appear to be permanent, as no recovery is observed (Newsome et al. 1985). MT lesions also cause perceptual problems. Specifically, a functional scotoma for visual motion is produced rendering the individual unable to perceive motion in the retinotopic location represented by the

damaged cortex. Additionally, the threshold intensity at which a monkey can reliably perform a motion direction discrimination task is dramatically elevated following MT lesions. However, contrast thresholds remain unchanged (Newsome and Paré 1988).

Stimulation: There are two main categories of effects of stimulation of MT. The first category of effects is on overt movement. When MT is stimulated during ongoing pursuit towards the stimulated side, the eyes accelerate. When the stimulation occurs during contralateral pursuit, the eyes decelerate. These effects are larger with increased pursuit velocity. Additionally, catch-up saccades occur normally, which suggests that the stimulation acts on estimations of image velocity and not position. Interestingly, when the target is stabilized on the retina during stimulation, there is no difference in the amount of eye acceleration. This means that the effect of stimulation is likely affecting perception itself, and not movement directly. These effects are primarily seen after stimulation of MTf. Stimulation has limited effects during fixation (Komatsu and Wurtz 1989).

The second category of effects is perceptual. In this case, if stimulation is delivered while monkeys perform a motion direction discrimination task, perceptual decisions are biased towards the direction of motion encoded by the stimulated neurons (Salzman et al. 1990). This kind of effect could underlie a top-down attention mechanism in which projections from higher-order areas alter tonic levels of activity in MT to allow the system to be more sensitive to particular directions of motion.

Recording: MT cells are known for their visual motion sensitivity. They are often active during visual motion as well as during smooth pursuit. The cells are direction-selective, as well as speed-sensitive (Maunsell and Essen 1983). MTf cells, in particular, prefer small target spots as opposed to large moving stimuli, and have small receptive fields (RFs) (Komatsu and Wurtz

1988a, 1988b). Throughout MT, there are bands that have reinforcing surrounds, which likely help encode global motion, and interband regions that have suppressive surrounds, which likely emphasize local motion (Born and Tootell 1992). During pursuit, the average latency of MT neuronal response to target motion is 90 ms, but shorter latencies are found for large-field motion (Lisberger and Movshon 1999).

MT's response to motion does not seem to include an explicit representation of acceleration itself. Although there are cells that increase their activity in response to steps in target speed and speed ramps, this likely reflects underlying velocity sensitivity and not acceleration sensitivity itself (Lisberger and Movshon 1999; Price et al. 2005).

Other research has demonstrated that MT's visual motion sensitivity is strongly dependent on retinal input. If the visual target is briefly extinguished during pursuit, MT neuronal responses drop off sharply (Newsome et al. 1988). Additionally, in a task involving pursuit of an "invisible" target, in which the visual stimulus falls only on the periphery, no MTf cells respond during tracking (Ilg and Thier 2003). This suggests that the MT sensitivities described above are entirely visual and not reliant on the eye movement itself.

In pursuit tasks involving two targets, the neuronal response seems to be best represented by a vector average that shifts to a winner-take-all pattern, which is also true of the behavior itself (Recanzone and Wurtz 1999). When this paradigm includes a color cue indicating the target to pursue, only 14% of MT neurons show any predictive component based on either color or direction of motion. Thus, the majority of cells responds only to the visual stimulus itself, and do not participate directly in a target selection process (Ferrera and Lisberger 1997).

Although MT neurons do not seem to underlie target selection, their responses do indicate attentional effects. In 40% of MT neurons, the neuronal response is modulated in excess of 50%

by attentional cues. This attentional modulation takes several hundred milliseconds to reach its peak, demonstrating a clear, albeit slow, attentional effect (Recanzone and Wurtz 2000).

Summary: MT is a brain area critical for motion processing for perception and for visually-driven eye movements. MT neurons have relatively small visual receptive fields located in the contralateral hemi-field (Dubner and Zeki 1971). MT participates in visual motion processing through its strong afferents from striate and extrastriate areas and its projections to a variety of eye movement-associated areas throughout the cortex and brainstem. Although attentional effects have been found in MT, high-level signals directly involved in processes like target selection and decision-making have not been observed. MT is likely a key component of multiple cortico-ponto-cerebellar loops underlying both reflexive and voluntary smooth eye movements.

1.2.2 *Medial Superior Temporal area (MST)*

Anatomy: MST is adjacent to MT along the STS. It is made up of two subregions: the dorsal-medial region (MSTd) on the anterior lip of the STS, and the lateral-anterior region (MSTl) on the posterior lip of the STS (Komatsu and Wurtz 1988a). MST receives input from MT, and also has reciprocal connections with PP and FEF (Tusa and Ungerleider 1988; Tian and Lynch 1996; Boussaoud et al. 1990). MST also receives input from earlier visual areas including V1, V2, and V3. It has connections to VIP (ventral intraparietal area), LIP, the inferior parietal gyrus, and TEO. MST also projects to the claustrum, caudate, putamen, pulvinar, and pontine nuclei including DLPN (Boussaoud et al. 1992). The functional role of most of these connections has not been explored.

Lesions: There are two types of deficits observed following a lesion of MST: retinotopic and directional. Retinotopic deficits affect all directions of motion that occur in the hemifield

opposite the lesioned MST. These deficits render the animal unable to match eye speed to target speed, or to make an accurate saccade to a moving target. The directional deficits occur once the eye is on or near the target, and only involves directions of motion towards the side of the lesion, irrespective of the initial location of the target. Additionally, optokinetic nystagmus is also affected: decreased gain is seen in both the slow and fast phases of nystagmus, and the greatest effects are observed for directions towards the lesion. There are no deficits for saccades to stationary targets. The directional deficits are mostly seen following lesions of MSTl, while retinotopic deficits are observed following lesions to both MSTl and MSTd (Dürsteler and Wurtz 1988).

Inactivation: Reversible chemical inactivation of MSTd increases behavioral thresholds in a heading direction discrimination task. In this task animals must report their perceived direction of movement by executing a saccade toward the perceived directin of motion. The effect is strongest when cues are only visual and weakest when cues are only vestibular. However, though there is a change in threshold, animals are still able to behave near optimally despite large, bilateral MSTd inactivation (Gu et al. 2012). This suggests that MSTd is participating in encoding heading direction but is likely one of multiple areas involved in the process.

Stimulation: Stimulating MST enhances ongoing pursuit rather than evoking smooth eye movements outright. Stimulation has limited effects during fixation, but it increases smooth pursuit eye velocity towards the stimulated side. The effects intensify with increasing pursuit speed. Interestingly, if the target is stabilized on the retina during the stimulation, the dynamics of the evoked eye movement do not change. This suggests that the effects of stimulation are perceptual and not movement-related. Catch-up saccades remain accurate throughout

stimulation, which suggests that the stimulation solely affects velocity perception and not position. These effects were most common in MSTl and not MSTd (Komatsu and Wurtz 1989).

Stimulation can also affect perceptual decisions. In a heading direction discrimination task, monkeys' perceptions of optic flow stimuli are biased following electrical stimulation. However, the stimulation does not bias discrimination based on vestibular inputs (Gu et al. 2012).

Recording: MST contains pursuit cells that are direction-selective and include the fovea in their RF. MSTd neurons prefer large, moving visual stimuli, whereas MSTl has a mixture of preferences for large or small visual stimuli (Komatsu and Wurtz 1988a). Cells that respond to large-field stimuli also often have some response to small stimuli moving in the opposite direction. Thus, at some size and speed, there is a reversal of direction preferences for these cells. These preferences render the response to a small spot moving over a visible background synergistic: the small spot and visible background elicit a larger response than to the small spot or background alone. However, the MSTl cells that prefer small spots show no reversal of preference and have weaker responses when the spot moves over a visible background (Komatsu and Wurtz 1988b).

In addition to being direction-selective, some MSTd cells (22%) are modulated by target position in addition to their response to target motion. An additional 43% are insensitive to target position, and 18% are modulated solely by target position and not, in fact, by pursuit (Squatrito and Maioli 1997). These characteristics render the neurons equally sensitive as the animal as a whole during psychophysical motion discrimination tasks. Indeed, MST activity is well correlated with the behavior in such a task (Celebrini and Newsome 1994).

To discern whether pursuit responses are primarily visual or movement-related in origin, the target can be briefly extinguished during ongoing pursuit. In MSTd, neurons show little

decrement in activity in response to the removal of retinal input, indicating the presence of extraretinal signals. Some MSTl cells respond similarly, while others show a great deal of reliance on retinal input. Of the cells that have strong extraretinal signals, most prefer large-field stimuli to small targets. However, 92% of cells begin their pursuit response after eye movement onset (Newsome et al. 1988). This means that they are not responding early enough to play a causal role in the initiation of pursuit.

These results are recapitulated when an "invisible" target is used in which the visual stimuli fall on the periphery and no visual stimuli are present on the fovea. Nearly all MST neurons respond identically to such an invisible target and traditional pursuit, indicating that the neurons are clearly not highly dependent on foveal input (Ilg and Thier 2003). To further differentiate responses to *volitional* pursuit from other types of smooth eye movements, MSTd cells can be tested during vestibulo-ocular reflex-induced (VOR) eye movements. MSTd neurons show responses during traditional pursuit as well as during cancelation of the VOR, but do not respond to any VOR-induced movements in dark or light conditions (Ono and Mustari 2006). Therefore, the activity in MSTd is likely related only to *volitional* smooth eye movements.

MST activity is also related to other functions. MSTd cells seem to be involved in determining heading direction, as they show strong responses to both optic flow and pursuit. The cells also compensate for changes in the apparent heading direction when pursuit occurs during self-movement. Although the neurons do not show perfect compensation, that some compensation is present suggests that MSTd is likely involved in encoding heading direction for self-motion (Shenoy et al. 2002; Page and Duffy 2003).

Investigations of the role of MST in other higher-level functions show limited evidence of MST's direct influence. In a dual-target task, MST activity seems to be best described by a

vector average that shifts to a winner-take-all pattern, much like behavior (Recanzone and Wurtz 1999). Only a minority (~25%) of MST cells shows a predictive component, some based on the color of the cue, some based on direction (Ferrera and Lisberger 1997). These signals seem unlikely to underlie target selection behavior as they are present in only few cells and the cells have long latencies. In a memory-based pursuit task, 96% of MSTd neurons show no evidence of visual motion memory or pursuit preparation signals (Kurkin et al. 2011). It is therefore unlikely that MSTd is playing a role in these functions.

MSTd also seems uninvolved in dynamic gain control. It had previously been shown that the gain of a reflexive eye movement response to a small target perturbation increases with pursuit velocity. Only 36% of MSTd neurons show any kind of response to this target perturbation, and the response is delayed 102 ms from the behavioral response, on average (Ono et al. 2010). Thus, it is unlikely that MSTd is playing a role in dynamic gain control.

There does, however, seem to be some effect of attention in MST. Although the effect takes several hundred milliseconds to peak, about 25% of MST neurons show attentional modulations in excess of 50% (Recanzone and Wurtz 2000).

Lastly, some preliminary investigations into the properties of specific projections do not reveal major differences between that subpopulation and the overall MST population as a whole. When using antidromic activation to identify neurons projecting from MST to the FEF, no significant differences were found for speed, direction, and optic flow tuning (Churchland and Lisberger 2005).

Summary: MST is found in the parietal lobe along the STS. It is made up of two subregions: MSTd and MSTl, which have some similarities, but are likely playing distinct roles in visuomotor behavior. Both areas play a likely role in the perception of visual motion in the

contralateral hemifield, and MSTl in particular seems likely to provide directional signals for pursuit eye movements. Although both play a role in visual motion perception, it is MSTd that evinces the greatest contribution from extraretinal signals, while MSTl has mixed reliance on retinal signaling. It seems unlikely that MSTd or MSTl are involved in higher-level cognitive processes like decision-making, target selection, or gain control.

As MSTd preferentially responds to large-field visual motion, and, in fact, often has opposite directional preferences for small target motion, it is likely involved in encoding heading direction and compensation for self-motion. Unlike MSTl, which has limited responses to large-field motion, MSTd seems uniquely well suited to detect eye or gaze movements with respect to background motion.

MSTl, however, remains a bit of a mystery. With its shorter latencies, small-target visual motion responses, and demonstrated effects on pursuit eye movements, it seems reasonable to hypothesize a causal role in pursuit generation. However, its dependence on retinal signals belies this supposition. Whether MSTl plays a role in ocular following eye movements through its projections to the pons, and/or a role in volitional pursuit through projections to the FEFsem, despite its seeming contradictions, requires further investigation.

1.2.3 *Smooth Eye Movement subregion of the Frontal Eye Field (FEFsem)*

Anatomy: The FEFsem is located in the fundus and deep posterior bank of the arcuate sulcus in the macaque frontal lobe. It is adjacent to FEFsac (saccadic subregion of the FEF) and somatic premotor regions (Gottlieb et al. 1993). High-resolution fMRI (functional magnetic resonance imaging) studies have shown a great deal of homology between the findings in macaques and humans, with the human FEF lying in and around the precentral sulcus (Rosano et al. 2002). Although early studies focused on the FEF as a whole, instead of its constituent saccadic and

pursuit subregions, multiple groups have found FEFsem-specific projections to MT, MST, VIP (ventral intraparietal area), and NRTP (nucleus reticularis tegmenti pontis) (Leichnetz 1989; Tian and Lynch 1996). Afferent connections have also been found from DLPFC (dorsolateral prefrontal cortex), SEF (supplementary eye field), MST, MT, VIP, and thalamic nuclei including: VA (ventral anterior), MD (medial dorsal), VL (ventral lateral) (Selemon and Goldman-Rakic 1988; Tian and Lynch 1996; 1997; Stanton et al. 2005). More recently, the FEFsem was found to project to the caudate nucleus, as well as the SC (Cui et al. 2003).

Lesions: Ablation of FEFsem causes marked deficits in voluntary smooth pursuit eye movements in both humans and non-human primates (Lynch 1987; Morrow and Sharpe 1995). Unilateral lesions tend to show stronger effects on pursuit towards the lesioned side (Morrow and Sharpe 1995). The effects include both decreased eye acceleration at the initiation of pursuit, and also decreased eye velocity during maintenance (MacAvoy et al. 1991). There are conflicting reports of deficits in anticipatory initiation, but the prediction necessary for accurate sinusoidal pursuit was abolished in the face of a lesioned FEFsem (Keating 1993). Although voluntary pursuit is significantly impaired after FEFsem lesion, optokinetic eye movements are unaltered (Keating et al. 1996). This may reflect differential projections of FEF and MST to pontine nuclei including NRTP and DLPN. Additionally, saccadic eye movements are unaffected, although saccades to targets moving toward the side opposite the lesion are often inaccurate (Morrow and Sharpe 1995). These effects are most prominent in the first 1-3 weeks following the lesion, as substantial recovery is seen after several additional weeks (Keating 1991).

Inactivation: Injection of the GABA agonist muscimol into the FEFsem causes large reductions in eye acceleration and velocity by about 70-90% of preinjection levels. The effects are strongest when pursuit is in the direction preferred by the neurons in the affected area. Saccades are

unaffected (Shi et al. 1998). Additionally, when tested during a go/no-go task with multiple targets, FEFsem inactivation does not affect the number of directional or no-go errors (Fukushima et al. 2011). It also does not alter choice behavior in a target selection task (Mahaffy and Krauzlis 2011).

Beyond muscimol, transcranial magnetic stimulation (TMS) has also been used to disrupt brain activity with more temporal precision. When TMS is applied over the FEFsem during pursuit of a target spot moving with superimposed sinusoidal motion, both the overall eye velocity as well as the reflexive eye movement in response to the superimposed motion are decreased. This implies that the overall gain *and* the dynamic gain control of the system are both affected (Nuding et al. 2009).

Stimulation: When electrical stimulation is applied to the FEFsem, low velocity, smooth eye movements are evoked, even during attempted fixation. The average eye velocity evoked is 11°/s with a latency of 39 ms. Increasing the intensity of the stimulation causes concurrent increases in eye acceleration and velocity, but no change in the direction of evoked pursuit, which seems skewed ipsilaterally (MacAvoy et al. 1991; Gottlieb et al. 1993).

Beyond evoking smooth eye movements outright, subthreshold stimulation of FEFsem also has interesting effects. Using a small sinusoidal perturbation of a target spot, the internal gain state of the oculomotor system can be tested. Without electrical stimulation, the gain state is low during fixation, and increases with higher pursuit speeds. When stimulation is applied, the gain state during fixation is higher, and in fact equivalent to that seen during active pursuit. This suggests that there are two effects of stimulation on FEFsem: (1) a directional signal that drives eye velocity and (2) a signal that increases gain omnidirectionally (Tanaka and Lisberger 2001, 2002).

Subthreshold stimulation does not alter choice behavior in a target selection task (Mahaffy and Krauzlis 2011). Interestingly, subthreshold stimulation of FEF*sac* decreases eye acceleration during pursuit initiation as well as eye velocity during pursuit maintenance (Izawa et al. 2011).

TMS has also been used as a means of stimulating brain regions of interest. When the FEFsem is stimulated during sinusoidal tracking, it enhances the gain of the coming velocity change: when stimulation occurs just before the target reverses direction, eye velocity is enhanced in the new direction, when it occurs mid-cycle before the deceleration, eye velocity decreases in the same direction (Gagnon et al. 2006).

Recording: Neurons in the FEFsem respond to voluntary smooth pursuit eye movements and visual motion, and are directionally tuned (Bizzi 1968; Bizzi and Schiller 1970; MacAvoy et al. 1991). Tuning is relatively broad with a full-width at half maximum of about 117 degrees on average. Preferred directions are uniformly distributed. About 60% of neurons exhibit directional selectivity before pursuit onset. FEFsem neurons have a median latency of about 100 ms, which leads pursuit onset by about 15 ms (Tanaka and Lisberger 2001, 2002a). Blink testing of neurons reveals the presence of extraretinal signals (Tanaka and Fukushima 1998).

Beyond simple direction-selectivity, more complex signals have also been reported. Neurons in the FEFsem have been shown to be eye velocity sensitive, as well as modulate their activity in response to retinal motion (Barborica and Ferrera 2002; Fukushima et al. 2002; Tanaka and Lisberger 2002c). Neurons that project from the FEFsem to NRTP have also been shown to be preferentially sensitive to eye acceleration (Ono and Mustari 2009). FEFsem neurons also show predictive responses during sinusoidal tracking, even in the presence of a target blink (Fukushima et al. 2002).

Additionally, when monkeys are given a double-target task, the monkey initially performs vector-averaging pursuit before target selection occurs. During this behavior, about half of the neurons mimic this vector-averaging pursuit showing an intermediate response. A third of the neurons respond as if to one of the targets alone. The remaining neurons have a stronger response to the presence of two targets than either alone (Tanaka and Lisberger 2002b). Interestingly, though, FEFsem neurons do not discriminate between target and distractor early enough to play a causal role in target selection (Mahaffy and Krauzlis 2011b).

Signals related to visual motion memory and a no-go cue are also found in the FEFsem, but not in particularly high numbers (Fukushima et al. 2011). Additionally, according to some reports, when the fluctuations in individual neurons' activity are compared to fluctuations in eye velocity, there are short periods of time in which the neuronal activity is predictive of eye velocity. Across the FEFsem population, these periods of prediction tile the entire duration of pursuit (Schoppik et al. 2008).

Summary: Given the volitional pursuit responses, short latencies, and sensitivity to eye velocity and acceleration, it is highly likely that the FEFsem plays a causal role in smooth pursuit. This capacity is likely subserved by afferents from MST (likely MSTl) and MT, and efferents to the brainstem and superior colliculus. As the FEFsem also has strong inputs from other frontal lobe structures like the DLPFC and SEF, it seems probable that it is also involved in higher-level functions. The FEFsem likely plays a role in gain control, which might be akin to the attentional signals demonstrated in the FEFsac (see below). However, the FEFsem does not seem to be involved in target selection or the decision of whether or not to pursue. More work needs to be done to establish the role of the diverse FEFsem projections, identifying whether distinct signals

are transmitted to different brain regions and underlie various aspects of volitional smooth pursuit.

1.2.4 *Saccadic subregion of the Frontal Eye Field (FEFsac)*

Anatomy: The FEFsac is located in the anterior portion of the arcuate sulcus in macaques, with its lateral component representing small saccade vectors and its medial portion consisting of larger vectors. It has cortical connections that include LIP, VIP, V2, V3, V4, IT (inferotemporal cortex), and TEO (Stanton et al. 1995). It also has subcortical projections to the caudate, putamen, claustrum, thalamic nuclei (VA, MD, CL [central lateral]), SC, subthalamic nucleus, midbrain tegmentum, and NRTP (Astruc 1971; Künzle et al. 1976; Künzle and Akert 1977; Stanton et al. 1988). These projections often maintain their topographical organization. One example of this organization is the projection to the SC: the lateral portion of the FEFsac projects to the anterolateral region of the SC, while the medial portion of the FEFsac projects to the posteromedial SC (Astruc 1971; Künzle et al. 1976; Künzle and Akert 1977; Stanton et al. 1988a). The FEFsac also receives projections from cortical retinotopically-organized visual areas like V4 and LIP, as well as cortical visual areas without such organization like IT (Schall et al. 1995). The FEFsac also receives subcortical projections from the thalamus, SC, dentate nucleus in the cerebellum, and the substantia nigra pars reticulata (Lynch et al. 1994).

Lesions: Long-lasting deficits are seen in visually-guided saccades in tasks that require a sequence of saccades following FEFsac lesion. Both visual processing speed and temporal ordering are disrupted (Schiller and Chou 1998). However, for simple visually-guided saccade tasks, only minor deficits are seen following chronic lesions (Dias and Segraves 1999).

Inactivation: Marked deficits in both visually- and memory-guided saccades are seen following inactivation with muscimol (Dias et al. 1995). These deficits include longer latencies, slower

velocities, and larger errors. Premature saccades and impaired fixation accuracy are also observed, primarily towards the direction of the inactivation, likely due to a disruption of the balance between the two FEFsacs (Sommer and Tehovnik 1997; Dias and Segraves 1999).

TMS can also be used to disrupt FEFsac function. When applied during a visual search task in which it is neither necessary nor required to make a saccade, simple search is unaffected, but search is impaired in more complex tasks. This is primarily evident in an increase in false alarms during the complex search (Muggleton et al. 2003). These findings suggest that the FEFsac may be involved in visual search, and not just the eye movements subsequent to the search.

Stimulation: Electrical stimulation of the FEFsac evokes conjugate saccades from fixation (Robinson and Fuchs 1969). In regions of the FEFsac with presaccadic activity and cells classified as visuomovement or movement-related, only a low level of current is required to evoke saccades. In areas with visual neurons and a lack of presaccadic activity, the threshold required is much higher. There is a notable correspondence between the vector evoked by electrical stimulation and the vector of saccade to which the neurons in the area respond (Bruce et al. 1985). Application of bicuculline also evokes saccades in a staircase fashion (Dias et al. 1995).

Subthreshold stimulation also has the potential to *suppress* saccades. If the animal attempts to make a saccade that is quite different than the vector evoked from the stimulated location, it is often highly delayed. This effect is strongest for memory-guided saccades, and less so for visually-guided saccades. There are some sites that suppress saccades with low-level current and evoke saccades at higher current levels. Stimulation at other sites, primarily located in the fundus of the arcuate sulcus, does not evoke saccades at any current level and only induces the suppressive effect (Burman and Bruce 1997).

Beyond evoking and suppressing saccades, subthreshold stimulation of the FEFsac also has other effects. It modulates the gain of V4 visual responses such that stimulation of retinotopically-corresponding locations enhances visual responses, and non-corresponding locations suppress visual responses (Moore and Armstrong 2003). These results suggest a role for the FEFsac in modulating the gain of the visuomotor transformation.

Recording: The FEFsac exhibits both visual and eye movement-related activity. FEFsac neuronal RFs are quite large at over 50° x 50° (Mohler et al. 1973). FEFsac neurons are divided into three main types: visual, movement, and visuomovement. Neurons classified as visual respond to visual stimuli regardless of the execution of an eye movement. Visual cells tend to be the most sharply tuned. Visuomovement cells respond to both the presence of visual stimuli as well as the execution of saccades, while movement cells respond only to the execution of a saccade, regardless of the presence of visual stimuli. Movement cells are the most broadly tuned. Visual responses tend to be seen about 90 ms following the onset of the stimulus, on average, whereas movement responses following a cue to execute a saccade have a latency of about 150 ms, on average. Some cells also show anticipatory activity (Bruce and Goldberg 1985). Fixation-related neurons have also been identified in the FEFsac (Hanes et al. 1998).

In a countermanding saccade task, some classes of FEFsac cells show signals sufficient to control gaze shifts. Movement, visuomovement, and fixation classes all contain neurons that change their activity in response to the stop signal within a window that would allow them to causally affect movement. Visual neurons, however, do not respond to the stop signal (Hanes et al. 1998).

However, in a visual search task, FEFsac cells do not discriminate between target and distractor early enough to be playing a causal role in target selection. The activity of movement

cells does ultimately reflect the presence of target or distractor, but not such that it could be causally involved in the decision itself (Schall and Hanes 1993).

In a task involving speed categorization, FEFsac neurons are found that reflect the boundary between categories, the speed of the stimulus itself, or both (Ferrera et al. 2009). Additionally, as FEFsac neurons encode position and their activity is modulated by target velocity, the population readout can include both target position and velocity, likely facilitating saccades to moving targets (Cassanello et al. 2008).

The specific function of FEFsac neurons that project to the SC has also been investigated using antidromic activation. The projection contains movement cells (53%), visuomovement cells (6%), visual cells (22%), and other types as well (20%). The lowest threshold required for antidromic activation occurs when the vectors in the SC and FEFsac are in register (Segraves and Goldberg 1987). Accordingly, this projection is highly topographically organized. Additionally, fixation-related signals are present in the projection and cognitive signals are also sent to the SC in the form of delay period activity (Sommer and Wurtz 2000). This delay period activity is present in 77% of FEFsac cells found to project to the SC. Using a go/no-go task to further investigate this activity, some neurons show delay period activity that relates to the go/no-go decision and are varyingly dependent on visual stimuli and movement. This seems to indicate the presence of many types of signals in this projection: visual, movement, visual attention, and working memory, among others (Sommer and Wurtz 2001).

Experiments done to assess the characteristics of FEFsac neurons *orthodromically* activated from the SC found that they all had visual responses and about half had movement responses as well (Sommer and Wurtz 1998).

Summary: The FEFsac is known for its involvement in saccadic eye movements. Its retinotopic organization, inputs from visual areas, and outputs to midbrain and brainstem oculomotor structures support this function. The FEFsac, like the FEFsem, does not seem to be involved in high-level functions like visual search or target selection, but is likely involved in modulating the gain of the visuomotor transformation. This gain modulation can also be thought of as a top-down attentional mechanism, affecting the tonic activity levels of earlier visual areas like V4. Interestingly, some FEFsac neurons are modulated by visual motion velocity, which is perhaps due to the interconnected nature of FEFsac and FEFsem. The coordination of saccades and pursuit to moving objects indicates close communication between saccade- and pursuit-related areas.

1.2.5 *Supplementary Eye Field (SEF)*

Anatomy: The SEF is located in the frontal lobe, just lateral to the midline and medial to the superior limb of the arcuate sulcus (Heinen 1995). The SEF has reciprocal connections with many areas including the claustrum, thalamic nuclei, FEF, supplementary motor area (SMA), premotor cortex, and the PFC. It receives additional input from areas around the STS, and also projects to the caudate, putamen, subthalamic nucleus, intermediate/deep layers of the SC, pons, and NRTP (Huerta and Kaas 1990).

Lesions: To what extent lesions of the SEF cause deficits for overt pursuit behavior remains up for debate. Some reports suggest that SEF lesion may cause lowered ipsilesional pursuit gain (Lekwuwa and Barnes 1996; Morrow and Sharpe 1995), but others only observe a longer latency to reverse pursuit direction in periodic pursuit tasks (Heide et al. 1996). It is possible that more complex tasks, like those used following inactivation (see below), would show more robust deficits following SEF lesion, but there seem to be only mild effects on basic smooth pursuit.

Inactivation: Muscimol injections during a memory-based pursuit task cause direction errors in pursuit, errors in whether or not to pursue (the go/no-go decision), and also inaccurate pursuit initiation. These are higher-level deficits than those seen following FEFsem inactivation, which mostly involve pursuit metrics (Shichinohe et al. 2009; Fukushima et al. 2011).

TMS has also been used to disrupt activity in the SEF. During pursuit of predictably- and unpredictably-moving targets, TMS over the SEF has somewhat similar effects to FEF in that it delays contraversive pursuit more than ipsiversive. However, the effects are stronger following TMS of the FEF. Additionally, TMS of the SEF shows no difference between predictable and unpredictable conditions, while the effects are more severe for unpredictable targets following TMS over FEF (Drew and van Donkelaar 2007).

Stimulation: Electrically stimulating the SEF has been shown to evoke both saccadic and smooth eye movements, much like the FEF (Tian and Lynch 1995). However, there are some differences between the two areas. For saccades, prolonged stimulation of the FEF generates a staircase of sequential saccades, whereas it evokes one saccade followed by fixation in the SEF. Additionally, while the saccade vector evoked in the FEF is independent of initial orbital position, there are effects of initial position on the saccades evoked from the SEF (Schall 1991).

Smooth eye movements are also affected by stimulation of the SEF, but they are not evoked from fixation. Stimulation increases the eye acceleration and velocity in the direction of target motion, specifically during pursuit initiation. These effects are not seen during pursuit maintenance. The effects are strongest when the stimulation occurs just prior to eye movement onset and serves to enhance eye velocity by 20%, on average. The sites that have these effects on pursuit do not evoke saccades, and stimulation has no effect on saccades to stationary targets (Missal and Heinen 2001).

The facilitation seen during pursuit initiation can also be observed during anticipatory pursuit of highly predictable targets. The latency of pursuit onset is decreased and the eye velocity is increased following stimulation just prior to the end of fixation. Without the expectation of movement, however, no pursuit (anticipatory or otherwise) can be evoked (Missal and Heinen 2004).

Recording: Neurons in the SEF show direction-selective responses during voluntary pursuit. These responses typically peak during pursuit initiation, with latencies of 97 ms, on average. SEF neurons tend to show some visual motion sensitivity, but this sensitivity does not fully account for observed pursuit activity (Heinen 1995). When a predictable change in target motion is introduced, neural activity peaks at the time of the change. Altering the timing of this predictable change causes the peak activity to systematically shift (Heinen and Liu 1997). These results indicate that the response of SEF neurons to pursuit might be due to some higher-level aspects of pursuit and/or prediction.

One of the ways that higher-level functions of the SEF can be tested is to use go/no-go tasks. In a saccadic version of the task, many types of neuronal responses are found. Visual cells are identified, and though largely similar to visual cells in FEF, the SEF cells have longer latencies and are less phasic. Cells that discharge after the targets appear but before the saccade is executed are also found. These cells are called preparatory set cells and are found in higher proportions in the SEF than FEF. The SEF preparatory set neurons also respond to auditory stimuli. Visuomovement neurons are also identified, and these neurons have lower firing rates, slightly longer latencies, and reach peak activation later in the SEF than the FEF. Presaccadic movement neurons have similar properties and are present in similar proportions in both areas, but there are more postsaccadic movement neurons in the FEF than SEF (Schall 1991).

Another version of this task reveals that there are distinct groups of cells that respond to error in the go/no-go decision, successful cancelation of saccades, and reinforcement (Stuphorn et al. 2000). Some of these neurons might fall into the "preparatory set" category from the previous task, but together, these results indicate the range of response types and possible signals present in the SEF.

These high-level signals can also be demonstrated in pursuit tasks. In a memory-based pursuit task that involves a go/no-go component, SEF neurons respond to retinal image motion, motion memory, motion direction, the decision of whether or not to pursue (go/no-go), and pursuit preparation (Shichinohe et al. 2009). The visual motion memory and no-go signals are present in higher numbers in the SEF than the FEF, reinforcing its likely role in higher-level cognitive function (Fukushima et al. 2011). This is also demonstrated in a two-target, free choice task, which allows the identification of neurons that predict the future choice of the animal. The SEF has a much higher proportion of these neurons than FEF or LIP, and its neurons show this activity earlier than the other areas (Coe et al. 2002).

To try to establish whether this activity is associated with the oculomotor decision or the rule itself, more complex tasks can be used, like the oculomotor "baseball" task from Heinen and colleagues (Kim et al. 2005). In one version of the task where the boundary between go and no-go changes on a trial-by-trial basis, some SEF neurons encode the oculomotor choice independent of the direction of motion or other low-level characteristics (Heinen et al. 2011). Additionally, in a saccade-based paradigm, the SEF neurons did not seem to encode the rule *per se* but encoded where the target was within an array (Tremblay et al. 2002).

Another attempt to dissociate the oculomotor decision from the rule used a task sufficiently difficult that many errors were made. In this case, the neurons take longer to develop their

response, but the activity is indeed related to the rule rather than the oculomotor decision. This is true in both directional and non-directional neurons, in both delay and movements periods (Yang et al. 2010). It may be that only particular types of rules are encoded by the SEF, or there might be separate groups of neurons that encode the decision and the rule. Either way, it is clear that high-level signals are robustly represented in the SEF, and this is a departure from what is observed in the FEF.

Summary: The SEF is likely involved in voluntary eye movement behavior, but is farther removed from regulating pursuit and saccade metrics than areas like the FEF. The SEF is most likely participating in higher-level aspects of eye movement behaviors, like the decision of whether or not to move, visual motion memory, and target selection. To what extent the SEF encodes rules or categories for oculomotor/visual perception tasks remains contested. However, it is clear that the SEF is unlikely to be involved in generating eye movements directly, and instead likely plays a role in the planning of such movements, possibly relaying the plans to the FEF so they can be acted upon.

1.2.6 *Superior Colliculus (SC)*

Anatomy: The SC is a midbrain structure with two main groups of layers: superficial and intermediate/deep. Both sets of layers possess a retinotopic organization representing the contralateral visual hemifield. There is a direct retinal projection to the SC, with the superior hemifield represented medially and central vision represented rostrally (Cynader and Berman 1972). The SC receives input from a number of cortical visual areas including V1, V2, V3, MT, FST, FEF, and SEF, as well as non-visual areas like motor cortex, premotor cortex, PP, IT, and auditory cortex. The strongest projections are from V1, V2, and MT, which tend to project to the superficial layers of the SC, while other cortical areas like PP, IT, auditory and motor cortices

project to the intermediate and deep layers (Fries 1984; Collins et al. 2005). The FEF has a topographically organized projection to the intermediate layers as well (Leichnetz et al. 1981). The SC, in turn, projects to DLPN, NRTP, the reticular formation, substantia nigra, FEF, and contralateral SC (Harting 1977; Lynch et al. 1994).

Lesions: Ablation of the SC causes acute sensory deficits in the contralateral visual hemifield. However, there is a recovery of sensory function over the subsequent two weeks. Long-term, mild motor deficits are observed: fewer saccades are seen on the contralateral side towards both targets and distractors, and saccade frequency and velocity are reduced (Wurtz and Goldberg 1972; Schiller et al. 1980; Albano et al. 1982). There is also more time spent with the eyes directed towards the ipsilateral hemifield (Albano et al. 1982).

However, though the long-term effects of SC lesion are mild on their own, they become much more severe when combined with a lesion of the FEF. Following the ablation of both areas, fixation is generally inaccurate, and the range of eye movements, as well as the frequency and velocity of saccades, are greatly reduced. There is limited recovery over time, unlike the substantial recovery seen when each region is damaged individually (Schiller et al. 1979, 1980). Interestingly, if the SC alone is ablated, stimulation of the FEF results in normal saccades, while stimulation of primary visual cortex (V1) evokes no saccades. This suggests parallel oculomotor streams for FEF and SC, while the normal ability of V1 to evoke saccades is clearly dependent on SC function (Schiller 1977).

Inactivation: Muscimol injection into the SC affects pursuit, saccades, and fixation. More saccades to distractor stimuli are seen when the target is in the contralateral visual hemifield, and this is not due to low-level motor impairments (McPeek and Keller 2004). Additionally, muscimol injected into the rostral SC (rSC) causes shorter-latency saccades and an inability to

suppress unwanted saccades. It also causes difficulty in maintaining fixation (Munoz and Wurtz 1993). Inactivation of the rSC also suppresses contraversive pursuit initiation, while enhancing ipsiversive pursuit (Basso et al. 2000). Given the variety of effects on multiple types of oculomotor behavior, the likely role for the SC is to provide some sort of position error/desired eye position signal that can be used by multiple systems and is not strictly related to the generation of any particular eye movement.

Stimulation: The SC is perhaps best known for being an area in which electrical stimulation evokes saccades. The location of the stimulation within the SC determines the vector of the saccade. Immediately after evoking a saccade there is a refractory period in which is it much more difficult to evoke another saccade (Robinson 1972). Interestingly, though stimulation of the SC is known for its ability to evoke saccades, it can also, in fact, increase saccade latency as well. Bilateral rSC stimulation completely suppresses saccades, and saccades will be executed only following the cessation of stimulation. Pulses of stimulation to the rSC can even interrupt ongoing saccades "mid-flight." This can also be observed under the influence of bicuculline for both remembered and visual targets (Munoz and Wurtz 1993).

Stimulation, much like inactivation, also affects pursuit eye movements. When the deep layers of the rSC are stimulated during pursuit initiation or maintenance, suppressive effects that are strongest for ipsiversive pursuit are seen. The effects are greatest for the most rostral sites and the highest underlying pursuit speeds. No smooth eye movements can be evoked from fixation by stimulating the SC and there are virtually no effects on the VOR, suggesting that the rSC is not providing some sort of general inhibitory signal (Basso et al. 2000).

Additionally, subthreshold stimulation has effects beyond those on overt eye movements, as well. In a target selection task, stimulation increases the proportion of contralateral targets

selected for both pursuit and saccades. For pursuit, the direction of motion is irrelevant; only the initial position matters (Carello and Krauzlis 2004). The SC, then, is likely also playing a role in target selection.

In one interesting experiment, stimulation was applied to both the SC and FEF simultaneously. This resulted in a weighted average of the two saccade vectors, where the weight is the intensity of the stimulation. This is likely facilitated by a downstream integrator, as paired stimulation within either the FEF or SC alone showed similar vector-averaging effects. It is unlikely to be due to local neural integration (Schiller et al. 1979a, 1980).

Recording: The SC is typically broken down into two groups of layers with different functional properties: superficial and intermediate/deep. The superficial layers contain neurons with strong visual responses that do not depend on eye movement execution, while neurons in the deep layers respond to eye movements regardless of retinal stimulation (Mays and Sparks 1980).

The superficial visual neurons respond to binocular input and tend to have small circular or elliptical RFs with suppressive surrounds (Cynader and Berman 1972). The RFs are less than 1° in diameter at the fovea and 10-30° in the periphery, and the foveal representation is highly expanded taking up over one-third of the whole SC (Cynader and Berman 1972; Goldberg and Wurtz 1972). Most of the superficial cells respond to stationary spots of light in their RFs, but about 10% of neurons respond to moving stimuli and are direction-selective. Little summation is observed over the RF, and the size, shape, and orientation of the visual target does not influence activity (Goldberg and Wurtz 1972). However, half the cells do have enhanced responses when the visual stimulus is ultimately the target of a saccade (Cynader and Berman 1972; Goldberg and Wurtz 1972a). The RFs represent the contralateral visual hemifield and are arranged topographically such that the superior hemifield is represented medially, and the inferior

hemifield is represented laterally. The rostral pole of the SC represents central vision, while the caudal SC contains information related to peripheral vision (Cynader and Berman 1972).

In contrast, neurons in the intermediate/deep layers are active for saccades of a particular vector. They have movement fields (MFs) that are in register with the RFs in the superficial layers above, and are active for saccades to visual and remembered targets, as well as during the fast phase of nystagmus (Wurtz and Goldberg 1972a; Schiller et al. 1980; Albano et al. 1982). They also maintain their firing rate in the presence of brief target blinks, and only respond to visual targets moving across the retina as opposed to the eye moving across a target (Robinson and Wurtz 1976; Krauzlis 2001). This provides further evidence for the presence of extraretinal signals in the SC.

These intermediate/deep-layer neurons can be subdivided into two categories: burst and buildup. Burst neurons have high-frequency activity that begins just prior to saccade onset, whereas buildup neurons have low-frequency activity long before saccade onset and continue to fire through the execution of the saccade. This buildup activity can occur even in the absence of a saccade, whereas burst neurons require a saccade to become active. The burst neurons are also anatomically segregated from buildup neurons, forming a layer that is more superficial than the buildup neurons (Munoz and Wurtz 1995).

Buildup neurons begin to fire at least 100 ms prior to the saccade. About 25 ms before saccade onset, the burst neurons begin to respond. During the saccade, all of the buildup neurons rostral to the original locus of activity begin to fire: the more rostral the location of the neurons, the later their peak activity. The burst neurons do not show this shift in activity and instead, their activity rapidly diminishes following saccade onset (Munoz and Wurtz 1995a).

The buildup neurons that lie in the rSC respond to stationary spots, much like their more caudal counterparts, but they also respond to other types of stimuli including moving targets as well as during fixation. In fact, these neurons were originally known as "fixation cells" because of this increase in tonic discharge during fixation. However, this increase in tonic discharge is also seen during pursuit eye movements (Munoz and Wurtz 1993a). Interestingly, their MF centers are slightly different for moving and stationary stimuli. For moving stimuli, MFs tend to be pushed farther from the fovea, and saccades to these targets tend to be slower and have secondary peaks and longer decelerating phases. These neurons also have a 14% lower firing rate for moving stimuli, on average (Keller et al. 1996), and do not exhibit much direction or speed sensitivity (Krauzlis 2004). They also pause during saccades and tend to show a postsaccadic increase in activity (Munoz and Wurtz 1993b).

Because they are sensitive to parafoveal targets on the contralateral side, they tend to be most active during both fixation and pursuit, when there are small position errors. This is true even during saccade-free pursuit, and thus, this activity is not obligatorily related to saccade generation itself (Krauzlis et al. 2000). Ergo, these rSC neurons are likely providing a position error signal that can be used in multiple types of oculomotor behavior and is an extension of the caudal SC neurons that signal larger position errors, rather than a separate class of cells (Krauzlis 1997).

Beyond initiating oculomotor behavior, this kind of position error signal can be useful for higher-level functions like target selection. When multiple visual targets are present in a display, the probability of a saccade to any given target is low. This introduces uncertainty into the system. Neuronal activity in the SC reflects this uncertainty: the more targets in the display, the lower the activity preceding target selection. This is most true for buildup neurons, and is not as

prominent in burst neurons (Basso and Wurtz 1997, 1998). In a cued target selection task, buildup neurons start firing shortly after the cue and the activity is predictive of saccade choice (Glimcher and Sparks 1992). Purely visual neurons do not show predictive activity when tested similarly (McPeek and Keller 2002).

Choice behavior can also be observed in motion direction discrimination tasks. A small subset of intermediate/deep-layer neurons show choice-predicting responses in such a task (Horwitz and Newsome 2001; Krauzlis and Dill 2002). Some of these responses seem to relate to the choice itself, while others are more associated with post-selection movement preparation (Horwitz and Newsome 1999). Interestingly, choice-related activity was always found in neurons whose MFs were in the region of space towards which the motion flowed (Horwitz et al. 2004). This provides strong evidence that the SC is involved in complex behavior beyond the execution of saccades; it is likely providing a position error signal to multiple oculomotor systems and may even participate in high-level perceptual decision-making.

Summary: Although originally thought only to be involved in saccade-generation, the role of the SC in oculomotor behavior is now much expanded to include a role in pursuit, fixation, and even potentially target selection and perceptual decision-making. The superficial layers of the SC contain neurons that are primarily sensory, responding to visual stimuli. As such, these neurons are unlikely to underlie high-level function. However, the intermediate/deep-layer neurons show a close relationship to eye movements, from fixation and pursuit at the rostral end to saccades more caudally, and are modulated by complex tasks like motion direction discrimination and visual search. These neurons are likely providing a position error signal that can be used to generate multiple types of oculomotor behavior, and may even possess decision or planning signals that are reliant on spatial aspects of vision. These lower- and higher-level signals are

likely subserved by projections from occipital, parietal, and frontal visual areas. The FEF in particular could shape some of this behavior through its direct projections to the SC. The oculomotor behavior and cognitive-level effects of the SC are likely mediated by brainstem and cortical targets, respectively.

1.2.7 *Nucleus Reticularis Tegmenti Pontis (NRTP)*

Anatomy: NRTP is a retinotopically-organized brainstem structure thought to be a vital part of a cortico-ponto-cerebellar pathway that plays a role in eye movement generation (Crandall and Keller 1985). It projects to the cerebellum, specifically to the vermal visual area, the flocculus, and less so to the anterior lobe and paramedian lobule (Brodal 1979, 1980, 1982). This cerebellar projection maintains the topographic organization seen within the structures themselves (Brodal 1980). Inputs to NRTP include motor, premotor, somatosensory, cingulate, and frontal cortices, as well as the subthalamic and red nuclei. These projections often also maintain a topographic organization (Brodal 1979, 1980b, 1982). Although NRTP is important for eye movement generation, is does not receive input from MT, MST, or LIP, which project to other pontine areas instead (Giolli et al. 2001).

Lesions: Chemical lesions of NRTP cause deficits in both smooth pursuit initiation and maintenance. The gain of pursuit during initiation is decreased by 48%, and the gain during maintenance is similarly decreased by 44%. The impairments seen are directional, with upward pursuit being most affected, downward most preserved, and both ipsi- and contraversive pursuit equally affected. However, these effects are acute and significant recovery is seen by three days post-lesion (Suzuki et al. 1999).

Inactivation: Muscimol injections into unilateral NRTP have profound effects on horizontal saccades. The gain of ipsiversive saccades decreases, with both amplitudes and peak velocities

showing significant reduction. Latencies are lengthened for ipsiversive saccades, as well. Contraversive saccades are largely unaffected (Kaneko and Fuchs 2006).

Stimulation: Electrical stimulation of NRTP evokes smooth eye movements that accelerate to a constant velocity that is then maintained for the duration of stimulation. This constant eye velocity ranges from 3.7 to 23°/s and averaged 11°/s. The eye velocity increases with stimulation intensity, but the direction does not change. The vast majority of stimulation sites (89%) evoke upward eye movements. The presence of retinal image motion in the direction opposite the evoked eye movement causes eye velocity to decrease (Yamada et al. 1996).

Recording: NRTP has neuronal responses to both smooth and saccadic eye movements. The neurons that respond to saccades do not respond to large-field optokinetic stimulation. Saccade-related neurons have high-frequency bursts in conjunction with saccade execution, but the bursts do not predict saccade metrics. The MFs of the neurons vary between small and circumscribed to quite large, up to 180°. These responses are similar for saccades in total darkness and those to visual targets. About half of the neurons have visual responses, most of which are enhanced by a saccade made to the target. These visual responses had latencies of approximately 60-70 ms, on average (Crandall and Keller 1985).

Rostral NRTP (rNRTP) neurons also exhibit pursuit sensitivity. They are direction-sensitive, with an even split between ipsilateral and contralateral preferences, and a bias towards upwards pursuit (60% of neurons) (Suzuki et al. 2003). The direction tuning is rather broad, and the neurons are mostly classified as gaze-velocity or gaze-acceleration related. Interestingly, some reports indicate a preference for velocity (Suzuki et al. 2003), while others describe a bias towards acceleration (Ono et al. 2005).

Given the preferential projection of eye acceleration related signals from the FEF to NRTP (Ono and Mustari 2009), it is clear that there is a strong acceleration component to the activity in NRTP, whether or not it is true for the majority of NRTP neurons. Many neurons in NRTP also respond to VOR in darkness, and some are additionally modulated by large-field motion stimuli (Ono et al. 2004).

Summary: NRTP is closely linked to the execution of saccadic and smooth eye movements, although its activity does not predict the metrics of these behaviors. Its input from FEF and outputs to cerebellar visual areas support such a causal role in eye movements. NRTP neuronal activity is most closely associated with movement, often either gaze/eye velocity or acceleration, although some minimal visual motion sensitivity is observed. NRTP is most likely involved in the drive for pursuit and perhaps saccadic eye movements, and not in higher-level processes.

1.2.8 *Dosolateral Pontine Nucleus (DLPN)*

Anatomy: DLPN is located in the brainstem within the base of the pons. It has projections to the cerebellum: to the vermal visual area, uvula, and the contralateral flocculus (Brodal 1979, 1982). DLPN receives input from cortical visual areas like MT, MST, FST, and minimal input from other visual association areas and primary visual cortex (Glickstein et al. 1980, 1985; Distler et al. 2002).

Lesions: When DLPN is unilaterally lesioned with ibotenic acid, there are notable deficits in smooth pursuit initiation and maintenance, primarily for directions towards the lesioned side. Deficits are also seen for vertical pursuit. Eye acceleration during pursuit initiation is less than half of pre-lesion levels. There are also deficits in initial eye acceleration for ocular following towards the side of the lesion. The visual hemifield in which the target first appears does not affect the deficits. Saccades to stationary targets are unaffected, but saccades to targets moving

towards the lesioned side are hypometric. Remarkably, rapid recovery is observed in the 3-7 days post-lesion, despite extensive cell loss (May et al. 1988).

Inactivation: The effects of inactivation are quite similar to the acute effects observed following lesions of DLPN. Unilateral lidocaine injections into DLPN cause reductions in eye acceleration and velocity primarily for ipsilesional pursuit. Initial eye acceleration is also affected for ocular following eye movements. Deficits are independent of the initial location of the visual target, and are seen for vertical pursuit, as well. Saccades to stationary targets are unaffected, while saccades to ipsilesionally-moving targets are hypometric (May et al. 1988).

These results are corroborated by experiments using muscimol to inactivate DLPN. Initial eye acceleration and steady-state eye velocity are reduced by about 50-75% of control values for ipsilesional pursuit only. In an adaptation paradigm in which the target either steps up or down in velocity, deficits are seen in the step-up paradigm for ipsilesional directions, while the step-down paradigm is only affected for contralesional directions (Ono and Mustari 2007). These findings suggest that DLPN is likely sending vital eye and/or visual motion information to the cerebellum for smooth pursuit execution as well as more complex processes like adaptation.

Recording: DLPN neurons tend to fall into one of three groups: visual, eye movement, and visuomovement. Visual neurons tend to respond to visual motion, and not during smooth pursuit. Eye movement neurons do not respond to visual motion, but, instead, are active during smooth pursuit. Visuomovement neurons have responses to both visual motion and smooth pursuit (Suzuki and Keller 1984; Mustari et al. 1988; Thier et al. 1988). All types of neurons are direction-selective, with a full-width at half-maximum of 65-180° (Mustari et al. 1988; Suzuki et al. 1990). All directions are represented, but there is a bias towards vertical and near-vertical directions (Mustari et al. 1988). Some of the neurons with visual motion sensitivity are

additionally modulated by the speed of the motion (Suzuki and Keller 1984; Suzuki et al. 1990). Neurons with visual sensitivity tend to begin their response prior to pursuit onset, while those most associated with the eye movement respond after pursuit onset (Mustari et al. 1988).

Visual neurons cease firing during target blinks, while eye movement neurons continue discharging, indicating the reliance on retinal and extraretinal signals, respectively. Visuomovement neurons have a mix of responses, from those that show significant reductions in activity to those that continue firing in response to target blinks (Mustari et al. 1988). Visual RFs for DLPN neurons include the fovea and range from as small as 2° x 2° to as large as 70° x 50° (Mustari et al. 1988; Suzuki et al. 1990).

Many DLPN neurons also have responses during ocular following eye movements. The responses contain two components: an initial transient and a sustained later component. Higher stimulus speeds tend to increase both components and decrease the latency of the response. Like the visual motion responses, the ocular following responses are direction-selective. These visual/ocular following responses tend to prefer either the same or opposite direction as the preferred direction for pursuit, split approximately evenly (Mustari et al. 1988; Thier et al. 1988; Kawano et al. 1992).

Beyond basic responses to pursuit and/or visual motion, more recent work has established that DLPN neurons tend to show sensitivity to eye position, eye velocity, or a combination of the two. There is limited sensitivity to eye acceleration (Dicke et al. 2004; Ono et al. 2005). DLPN also shows gaze velocity sensitivity when the head is permitted to move, but only a very small number of neurons respond to eye movements generated by the VOR in the dark (Ono et al. 2004). Some neurons also show limited saccade responsivity, with saccade-related bursts and memory-period activity (Dicke et al. 2004).

Summary: DLPN is likely involved in the execution of smooth eye movements, including both reflexive ocular following and volitional smooth pursuit. Its inputs from parietal areas like MT and MST and its outputs to cerebellar visual areas support this role. The observed sensitivity to eye/gaze velocity and position, as opposed to acceleration, indicate that DLPN might be more involved in pursuit maintenance than initiation, but DLPN visual neurons with short latencies could be playing a role in the initiation of ocular following eye movements.

Chapter 2. TEMORAL DYNAMICS OF RETINAL AND EXTRARETINAL SIGNALS IN THE FEFSEM DURING SMOOTH PURSUIT EYE MOVEMENTS

2.1 ABSTRACT

Neurons in the smooth eye movement subregion of the Frontal Eye Field (FEFsem) are known to play an important role in voluntary smooth pursuit eye movements. Underlying this function are a number of projections to parietal visual association areas, other frontal cortical areas, brainstem nuclei, and midbrain structures, all known to play vital, but differing roles in the execution of smooth pursuit. Additionally, the FEFsem has been shown to carry a diverse array of signals including eye velocity and acceleration, gain control, visual motion memory, and prediction, among others. We hypothesized that distinct subpopulations of FEFsem neurons subserve these diverse functions and projections, and that the relative weights of retinal and extraretinal signals could form the basis for such a categorization. To investigate this, we used a step-ramp tracking task with a target blink to determine the relative contributions of retinal and extraretinal signals in individual FEFsem neurons throughout pursuit. We found that the contributions of retinal and extraretinal signals to the activity of individual neurons in FEFsem, and to smooth pursuit behavior as a whole, change throughout the timecourse of pursuit. Additionally, we used these relative weights to group neurons into distinct clusters and discuss their possible functions for pursuit. A comparison with a sample of medial superior temporal (MST) neurons has also been performed to assess similarities and differences between the two areas. The results indicate the utility of simple tests like the target blink for parsing the complex and multifaceted roles of cortical areas in behavior.

2.2 INTRODUCTION

Primates are exceptionally good at tracking moving objects with their eyes using smooth pursuit eye movements. This continual tracking supports high-acuity vision throughout the duration of pursuit. These eye movements are the result of complex processes, including prediction and anticipation, attention, memory, motivation, gain control, and ultimately, the initiation and maintenance of the eye movements themselves.

The initial sensory processing of the incoming visual signals necessary for smooth pursuit is primarily completed in the early visual areas of the occipital and parietal lobes, while the motor commands are mainly generated in the brainstem and cerebellum. However, other cortical areas, including the smooth eye movement subregion of the frontal eye field (FEFsem) and the medial superior temporal area (MST) are also critical for accurate voluntary smooth pursuit eye movements (see Lisberger et al. 1987 and Krauzlis 2004 for reviews).

Early studies showed that lesions of the FEFsem cause a clear decrement in eye velocity and acceleration during smooth pursuit (Lynch 1987; Morrow and Sharpe 1995), while electrical stimulation of the FEFsem evokes smooth eye movements even from fixation (Gottlieb et al. 1993). Recent work has also indicated a plethora of signals in the FEFsem including: eye velocity (Gottlieb et al. 1994; Fukushima et al. 2000), eye acceleration (Tanaka and Fukushima 1998; Ono and Mustari 2009), anticipation (MacAvoy et al. 1991), prediction (Fukushima et al. 2002), visual motion memory (Fukushima et al. 2011), retinal image motion (Fukushima et al. 2000), gain control (Tanaka and Lisberger 2001, 2002), vestibular input (Fukushima et al. 2000), and potentially even signals that peak at different times during the course of pursuit (Schoppik et al. 2008; Li and Lisberger 2011).

Beyond the diversity of signals found within the FEFsem, there are also a variety of anatomical connections. These include projections to the nucleus reticularis tegmenti pontis (NRTP) in the brainstem (Künzle and Akert 1977; Brodal 1980; Giolli et al. 2001), the basal ganglia (Cui et al. 2003; Hikosaka and Wurtz 1989), the superior colliculus (Komatsu and Suzuki 1985), MST and the middle temporal area (MT) (Lynch and Tian 2006), among others. In this study, we hypothesized that the diversity of signals and projections found in the FEFsem are comprised of subpopulations of neurons with distinct functional and anatomical properties.

In order to investigate the existence of such subpopulations, we sought to evaluate the relative contributions of retinal and extraretinal signals in the activity of FEFsem neurons. These relative contributions could be informative as to the function of individual neurons, as the importance of retinal input shifts over the course of pursuit. Initially, the retinal image motion occurring before the onset of pursuit is the major drive for the pursuit system (Lisberger et al. 1981; Lisberger and Westbrook 1985; Tychsen and Lisberger 1986; Krauzlis and Lisberger 1994). However, once the eyes have reached steady-state pursuit, approaching unity gain, there is limited retinal motion and the main drive for pursuit is unlikely to be retinal signals (Lisberger et al. 1987; Nuding et al. 2008). The drive for ongoing pursuit will instead be some combination of extraretinal signals (e.g. visual motion memory or efference copy).

Because of the changing role of retinal signals during pursuit initiation and maintenance, we hypothesized that these signals would be informative as to the likely function of individual neurons. As an example, neurons involved in the command to initiate pursuit would likely be more sensitive to retinal input, whereas neurons involved in pursuit maintenance would likely show a smaller contribution of retinal signals.

We used a simple target blink paradigm to measure the relative contributions of retinal and extraretinal signals throughout pursuit and a clustering algorithm to test for the presence of distinct subpopulations of cells in FEFsem. Our study demonstrates that the relative retinal and extraretinal contributions to a given neuron's activity are not necessarily static. Indeed, many neurons show changing reliance on retinal input throughout pursuit. Furthermore, the reliance of neuronal activity on retinal signals over time, combined with other easily-measurable neuronal characteristics, can provide a simple and useful tool for neuronal classification. We also examined a sample of MST neurons to assess similarities and differences between these two interconnected cortical areas.

2.3 Materials & Methods

2.3.1 *Surgical Procedures*

Behavioral and neuronal data were collected from three normal rhesus monkeys (*Macaca mulatta*) weighing 5.5 - 14.0 kg. Detailed descriptions of most surgical procedures can be found in prior publications (Ono and Mustari 2010, 2012). Surgery was performed under aseptic conditions using isoflurane anesthesia (1.25 - 2.5%) to stereotaxically implant a titanium head stabilization post and titanium recording chambers (Crist Instruments, Hagerstown, MD). In a subsequent surgery, scleral search coils were implanted underneath the conjunctiva of both eyes using the methodology of Judge et al. (1980). All surgical procedures were performed in strict compliance with National Institutes of Health guidelines, and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Washington.

2.3.2 *Data Collection*

During all experiments, monkeys were seated in a primate chair (Crist Instruments) with their head stabilized in the horizontal stereotaxic plane. Eye movements were detected and calibrated using standard electromagnetic methods (Fuchs and Robinson 1966) and precision hardware (CNC Electronics, Seattle, WA). Eye and target position feedback signals were processed with anti-aliasing, six-pole Bessel filters (200 Hz) prior to digitization at 1 kHz with 16-bit precision using CED-Power1401 hardware (Cambridge Electronic Designs, Cambridge, England). Velocity and acceleration data were filtered using an 80-point finite impulse response (FIR) digital filter with a passband of 50 Hz. Saccades were removed from smooth pursuit traces using a manually-overseen custom detection algorithm in MatLab (MathWorks, Natick, MA). The missing eye data was replaced with a linear interpolation between the pre- and post-saccadic regions of data.

Single-unit activity was recorded using modified commercial glass- or epoxy-coated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA; Frederick-Haer Corporation, Brunswick, ME) with the impedance ranging from 0.5 to 5 MΩ. Spike2 software was used for data acquisition and preliminary offline analyses, including 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 et al. 1987).

2.3.3 *Localization of FEFsem and MST*

We verified the location of our neurons using functional criteria (e.g. response directionally tuned during voluntary smooth pursuit eye movements) and stereotaxic location. Prior to surgery, magnetic resonance imaging (T-1 weighted, fast spin-echo; Siemens 3T magnet) was used to localize the FEF and MST. Recording chambers were then stereotaxically implanted with centers

at anterior 22, lateral 14 (at a 10° angle) for FEF, and posterior 5, lateral 15 (at a 0° angle) for MST. The locations were also verified using depth measurements taken from microdrive readings while FEFsem or MST neurons were being recorded. These corresponded to depths expected from each animal's MRI.

2.3.4 *Behavioral Paradigms*

All visual stimuli were rear projected on a tangent screen 57 cm away from the monkey, and were delivered using computer-controlled two-axis mirror galvanometers (General Scanning, Watertown, MA) and appropriate optic bench hardware. Monkeys were trained to track a small-diameter target spot (0.2°; produced by a red laser light emitting diode), moving in sinusoidal or step-ramp trajectories (Rashbass 1961). Monkeys were also trained to perform fixation and visually-guided saccade tasks.

Neurons were first tested for responsivity to smooth pursuit eye movements or saccades. Neurons classified as saccade-related were not included in this study. Neurons that responded during pursuit of the target spot moving at low frequency (0.15 – 0.35 Hz) were included. The neuronal activity was recorded while the target moved in one of the eight cardinal directions, and the neurons were ultimately tested using step-ramp motion in the preferred and anti-preferred directions for the given neuron. The target moved at 7, 15 and/or 30°/s, with an excursion of 15°.

Neurons were also tested with a target blink task (Newsome et al. 1988; Tanaka and Fukushima 1998; Ono and Mustari 2006). In this task, the target spot was extinguished for 150 ms during step-ramp tracking, starting at various times after target motion onset (50, 100, 200, 300, 400, or 500 ms). Trials with and without blinks were randomly interleaved. Well-trained monkeys show limited decrement in eye velocity following target blink, and decrements were consistent across blink times (**Figure 2.2**). Due to losing some cells early, we were not always

able to collect the same number of trials for each blink. To ensure we collected data from both the early and late periods, we prioritized collecting data from the 100, 300, and 500 ms blink times.

2.3.5 *Data Analysis*

To quantify the behavioral blink responses, we used parameters similar to Bogadhi and colleagues (2013). Briefly, we found the maximum eye velocity after the target blink, in a window from 50 to 150 ms after the target blink. This was designated V_0. We then found the minimum eye velocity (V_{min}) in the 150 ms after V_0, which is equal to the length of the target blink. The difference between V_0 and V_{min} was V_{drop}. We also calculated the ratio between blink and control eye velocities at the time of V_{min} to assess not just the absolute drop but the relative drop in eye velocity. We also identified the eye velocity 150 ms after V_0 (V_{150}) to look for any changes in anticipatory eye velocity. The difference between V_{min} and V_{150} was designated V_{ant}. As for V_{min}, we also calculated the ratio of blink to control at V_{150} to be able to assess the relative differences in eye velocity.

Averaged data, taken from at least 10 trials, was used to calculate neuronal and eye movement latencies. The times at which the neuronal response and eye velocity exceeded three standard deviations above baseline (the 100 ms prior to target motion onset) were designated the neuronal and eye movement latencies, respectively. The overall latency was then expressed as the difference between eye and neuron, such that negative values represent neurons that began responding before eye movement onset.

We also attempted to reconstruct the response profiles of neurons using a combination of eye position (E), velocity (E’), and acceleration (E’’), as well as retinal position error (R) and velocity error (R’) (Ono and Mustari, 2009). Retinal error parameters were calculated as the

difference between target and eye motion. The impulse in target velocity due to differentiation of the step in target position at the beginning of the trial was removed prior to its use in the model. Additionally, target acceleration was set to $0°/s^{2,}$ as the step in target velocity results in no steady-state target acceleration. Data averaged from at least 10 trials was used to calculate coefficients in the following model:

$$FR(t) = A + BE(t+\tau_1) + CE'(t+\tau_1) + DE''(t+\tau_1) + GR(t+\tau_2) + HR'(t+\tau_2)$$

in which FR(t) represents the spike density function at time t, E(t) denotes the eye position at time t, and coefficients are defined by terms A-D, G, and H. Latency of unit activity is represented by both τ_1, which is the unit latency with respect to pursuit onset, and τ_2, which is the latency with respect to target motion onset. Thus, the model attempts to account for the unit activity using a combination of eye and retinal motion parameters. The coefficient of determination (CD) was used to determine goodness of fit, and a series of τ_1 and τ_2 values were used to find the maximum CD (Ono et al. 2005).

Partial correlation (r) values were also calculated for each component to estimate its relative contribution, while controlling for the other parameters. All statistical tests used a significance value of 0.05 unless otherwise specified.

To quantify the relative contributions of retinal and extraretinal components of unit activity, the blink task was utilized. 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 visual processing delays (Newsome et al. 1988; Tanaka and Fukushima 1998). This blink average was compared to averages over the same interval for control trials, and the difference was expressed as percent change. Negative changes indicate that the unit was less active during the blink than during

control trials, whereas positive values indicate that the cell increased its activity during the blink as compared to control.

To assess the existence of subpopulations in our sample of neurons, a k-means clustering algorithm was used. The following variables were included as input for the algorithm: neuronal latency with respect to eye movement onset, partial correlations from the multiple linear regression, percent change due to early blink (50-100 ms after target motion onset), and percent change due to late blink (300-500 ms). All input variables were normalized on a scale of 0 to 1. The gap statistic (Tibshirani et al. 2001) was used to identify the appropriate number of clusters. The lowest number of clusters with a positive gap statistic is used as k; in our case $k = 3$.

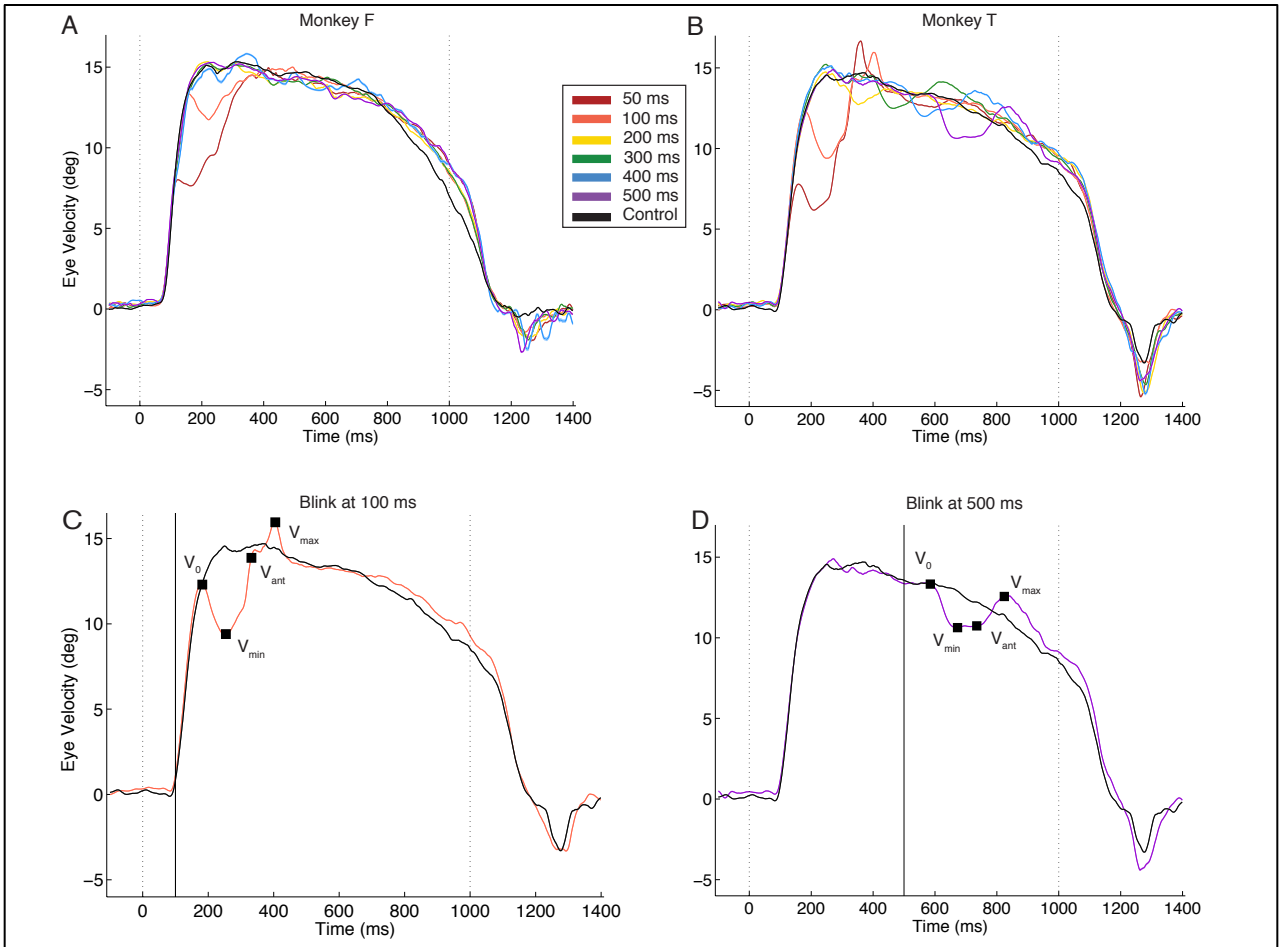


Figure 2.1. Behavioral response to target blink. A: Average eye movement traces for Monkey F. The black trace represents control trials (N=752) with no target blink, and color traces represent different blink timings (N=564, 924, 706, 820, 155, and 563, for 50, 100, 200, 300, 400, and 500 ms, respectively). Target motion onset at 0 ms, and offset at 1000 ms, as indicated by dotted lines. B: Average eye movement traces for Monkey T, same conventions as in A. Control trials N = 921, Blink trials N = 603, 884, 591, 810, 363, 641. C: Detailed view of the 100 ms average trace from B (red) alongside the control trace (black). Points used for quantification of blink effects indicated by black squares. Target motion onset and offset indicated by dotted lines at 0 and 1000 ms, blink onset at the solid vertical line. D: Same conventions as in C, but for trials with blink at 500 ms.

2.4 RESULTS

2.4.1 *Behavioral response to target blinks throughout step-ramp tracking*

We first assessed the behavioral response to target blinks at different timings throughout step-ramp tracking. The eye movements associated with the blinks differed slightly between the three monkeys (B, F, and T), as shown in Figure 1A-B for monkeys F and T. The preferred direction of the neuron is plotted as positive. All animals had persistent, small decrements in eye velocity following target blinks. In order to quantify these decrements, four parameters were used along with their timings: V_0, V_{min}, V_{ant}, and V_{max} (**Figure 2.1**C-D). The average eye velocity in blink trials when it begins to deviate from control trials is V_0. V_{min} is the minimum eye velocity occurring after V_0. The difference between V_{min} and V_0 represents the magnitude of the behavioral effect of the target blink (V_{drop}). V_{ant} is the eye velocity 150 ms after V_0, which is the length of the target blink, and the difference between V_{min} and V_{ant} indicates whether there was any anticipatory eye acceleration. V_{max} is the maximum eye velocity after V_{min}.

Although there was some variability within individual monkeys' behavior, the quantification revealed similar magnitudes for the drop in eye velocity following the target blink (V_{drop}, **Figure 2.2**A). Notably, V_{drop} was relatively stable across all blink timings, with the possible exception of a smaller velocity decrement for early blinks beginning at 50 ms for monkey F, and 100 ms for monkey B (**Figure 2.2**A). For monkeys F and T, the largest values of V_{drop} were seen for blinks at 100 ms, while for monkey B the largest value was at 200 ms. This finding is especially important for the assessment of the neuronal response to the target blinks. If there was a larger drop in eye velocity for certain blink timings, it would be difficult to differentiate the effects of the lack of visual input from those due to decreased eye velocity.

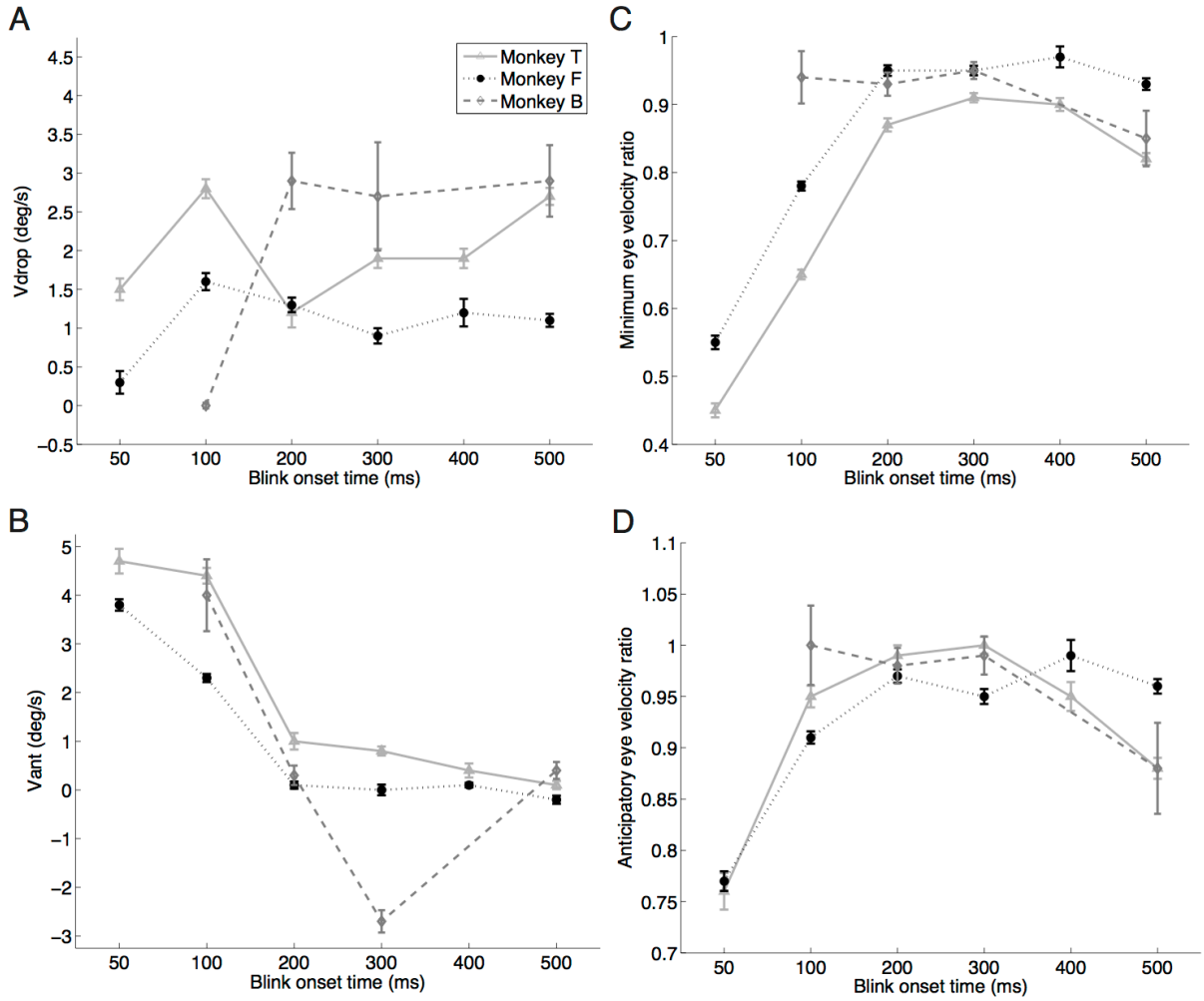


Figure 2.2. **Quantification of Behavior.** A: Magnitude of eye velocity drop following target blink (V_{drop}), according to time of blink onset. Each line represents behavior from an individual monkey. Monkey B had 0, 364, 211, 345, 0, and 107 trials for the 50, 100, 200, 300, 400, and 500 ms blinks, respectively. Monkey F had 564, 918, 708, 823, 155, and 565 trials. Monkey T had 603, 884, 591, 810, 363, and 641 trials. Error bars are SEM. B: Same conventions as in A, but for magnitude of anticipatory eye velocity increase (V_{ant}). C: Ratio of minimum eye velocity following target blink to control (blink/control). D: Ratio of anticipatory eye velocity (blink/control).

Interestingly, the anticipatory eye acceleration (V_{ant}) did show a more consistent difference between earlier and later blink timings across the three monkeys. V_{ant} was greater for target blinks at 50 and 100ms than for those at 200-500 ms, and the magnitude of this anticipatory behavior was remarkably consistent across animals (**Figure 2.2**B).

In addition to assessing the change decrement and anticipatory changes in eye velocity, we also calculated the ratio of eye velocity between the blink and control trials at the minimum and anticipatory eye velocity (**Figure 2.2**C-D). For both, we found that the ratio of blink to control was much lower for early blinks than later blinks. For the minimum eye velocity (**Figure 2.2**C), the ratio was smallest for the 50 ms blink, slightly greater for the 100 ms blink, and then achieves a plateau level between 0.82 and 0.97. For anticipatory eye velocity (**Figure 2.2**D), again the ratio is smallest for the 50 ms blink, and has nearly reached plateau levels (0.88 to 1.00) by the 100 ms blink.

2.4.2 *Response of individual FEFsem neurons to target blinks throughout step-ramp tracking*

We included a total of 137 neurons recorded from the FEFsem of three monkeys for this study (monkey B, N=49; monkey F, N=46; monkey T, N=42). Only neurons that responded to tracking of a small-diameter (0.2°) target spot during the step-ramp task were included. **Figure 2.3** illustrates the response of an example FEFsem neuron during step-ramp tracking in its preferred direction. Smooth pursuit begins 111 ms after target motion onset, while the neuronal response begins 8 ms later, on average (N=28 trials, Monkey T). During the acceleration phase of the eye movement, the firing rate increases continuously until it reaches a plateau, which corresponds to a period of constant eye velocity. To assess the relative contributions of retinal and extraretinal components of activity in individual FEFsem neurons, target blinks were delivered and the colored bars indicate the timing of these target blinks used across portions of

pursuit behavior that have different amounts of retinal image motion (**Figure 2.3**, grey shaded region).

These blinks consisted of the extinction of the target for 150 ms and occurred at different times after target motion onset (50, 100, 200, 300, 400, or 500 ms). Example target and eye position traces during trials when the target was extinguished at 100 ms can be seen in **Figure 2.4**A (N=21 trials). The missing region of the dashed line shows the time at which the target was blinked. Four representative FEFsem neurons are also shown to illustrate the range of blink responses (**Figure 2.4**B-E). Both of the neurons in **Figure 2.4**B and 4C (Monkey T and F, respectively) show less neuronal activity during blink trials (thick black line; N=21 and 17, respectively) than control trials (grey shaded region; N=28 and 52, $p = 0.001$ and $p = 0.003$, respectively). However, the neuron shown in **Figure 2.4**D (Monkey F) shows minimal difference between blink (N=32) and control (N=25, $p = 0.44$) trials, while the neuron in **Figure 2.4**E (Monkey F) shows a marked increase in firing rate in response to the target blink (N=27) compared to control (N=26, $p < 0.001$).

To quantify these responses, the average firing rate over an interval equal to the length of the target blink, but delayed 60 ms to allow for visual processing delays, was used (indicated by the vertical dashed lines in **Figure 2.4**B-E). The blink response is given as a percent change in neuronal activity during blink trials compared to control, with negative values indicating less activity in blink trials than control. The four neurons had blink responses of -20%, -40%, -4%, and +30%, respectively (**Figure 2.4**F).

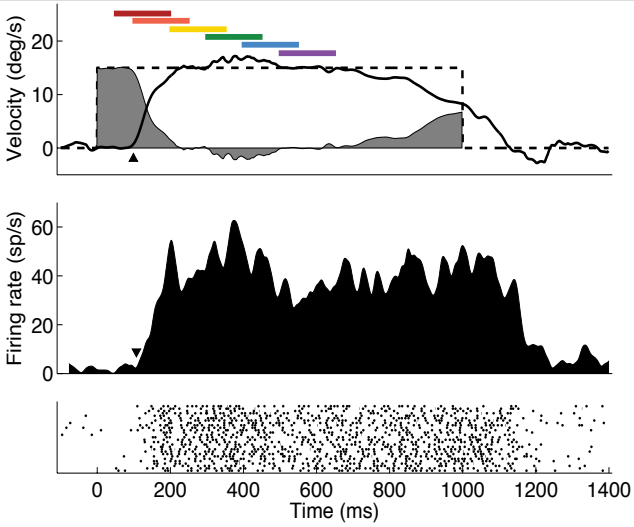
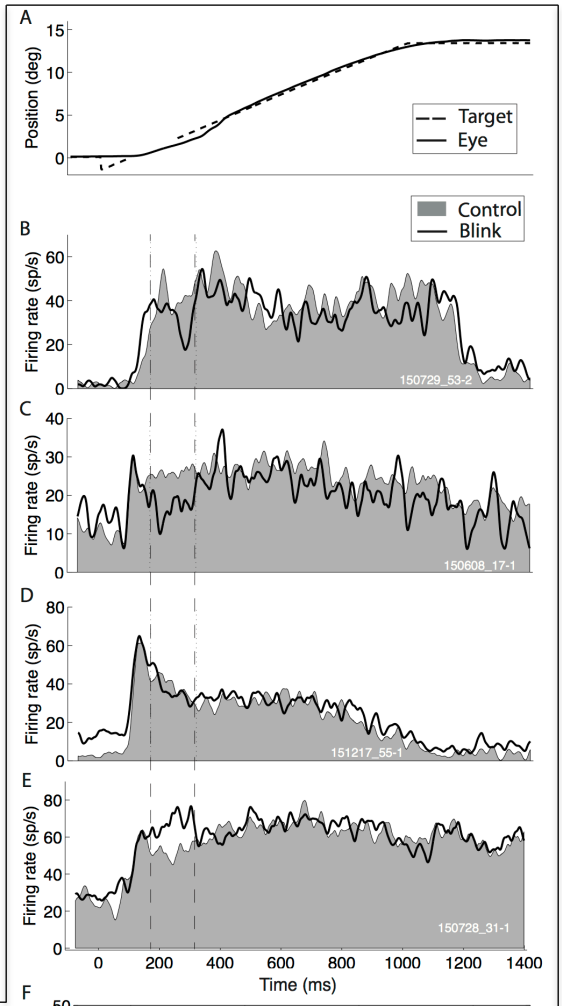


Figure 2.3. Response of an example FEFsem neuron. Top panel shows the target velocity (dashed line), average eye velocity (solid line), and average retinal image motion (shaded region) during step-ramp tracking at 15°/s (N=28; Monkey T). Colored bars represent blink timings: 50, 100, 200, 300, 400, 500 ms. Arrowhead indicates the eye movement latency (110 ms after target motion onset). Middle panel shows the average spike density function. Arrowhead indicates the neuronal latency (119 ms after target motion onset). Bottom panel shows the raster plot.



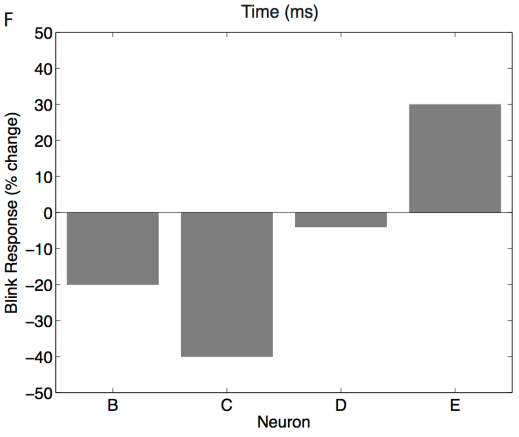


Figure 2.4. Representative examples of blink responses in FEFsem neurons. A: Target position (dashed line) and average eye position (solid line; N=21) during the step-ramp task with target blink. Target was extinguished between 100 and 250 ms after target motion onset, as indicated by the gap in the target trace. B-E: Each panel shows the average spike density function for control (shaded region) and blink trials (solid line) for example FEFsem neurons. Vertical dashed lines show the time range used for neuronal response quantification. Panel B is same neuron as in Figure 2. B: Control trials N=28, blink trials N=21, Monkey T. C: Control trials N=52, blink trials N=17, Monkey F. D: Control trials N=25, blink trials N=32, Monkey F. E: Control trials N=26, blink trials N=27, Monkey F. F: Quantification of blink response for neurons in B-E. Difference in firing rate within the interval is expressed as percent change. Negative numbers indicate firing rates that are lower in blink trials than control.

In addition to the variation in blink responses seen across neurons, there are also individual neurons that have changing blink responses across time. One representative FEFsem neuron is shown in **Figure 2.5**. The eye position traces (colored lines in **Figure 2.5**A, top panel; Monkey F) indicate the behavioral response to target blinks at different times throughout the trial, which are largely overlapping and accurate. The average neuronal activity for this unit during blink trials (colored lines in **Figure 2.5**A, bottom panel) is shown compared to control activity (grey shaded region; **Figure 2.5**A, bottom panel). The red and orange lines, which represent activity during trials with early target blinks (50 and 100 ms, respectively), show the only notable deviation from control levels during their respective target blinks (colored horizontal bars).

Using the same quantification procedure as in **Figure 2.4**F, blink responses were calculated for this neuron for each blink timing, as shown in **Figure 2.5**B. To compare this neuron's blink responses during periods of pursuit with high and low retinal image motion, the blink times were grouped into "early" (50 and 100 ms target blinks) and "late" (300, 400, and 500 ms target blinks) categories. The 200 ms timepoint was excluded as it often fell during the transitional period of high to low retinal image motion and during the transition from the initiation to the maintenance phase of behavior (**Figure 2.3**). The blink responses during early and late timings were significantly different (Student's t-test $p < 0.001$; N=26 early trials, N=42 late trials).

The change in blink response over time is also visible across trials (**Figure 2.5**C). Here, blink timing with respect to movement onset is compared to the firing rate following the blink as normalized by control levels of activity. This comparison shows increased neuronal activity as the blink is delivered later in the trial, as fit by the line: normalized FR=0.0011(blink time) + 0.7436.

Again, collapsing the trials into early and late categories (**Figure 2.5**D) shows that this neuron's blink responses change over time, and therefore, the contributions of retinal and extraretinal input to its firing rate are not static.

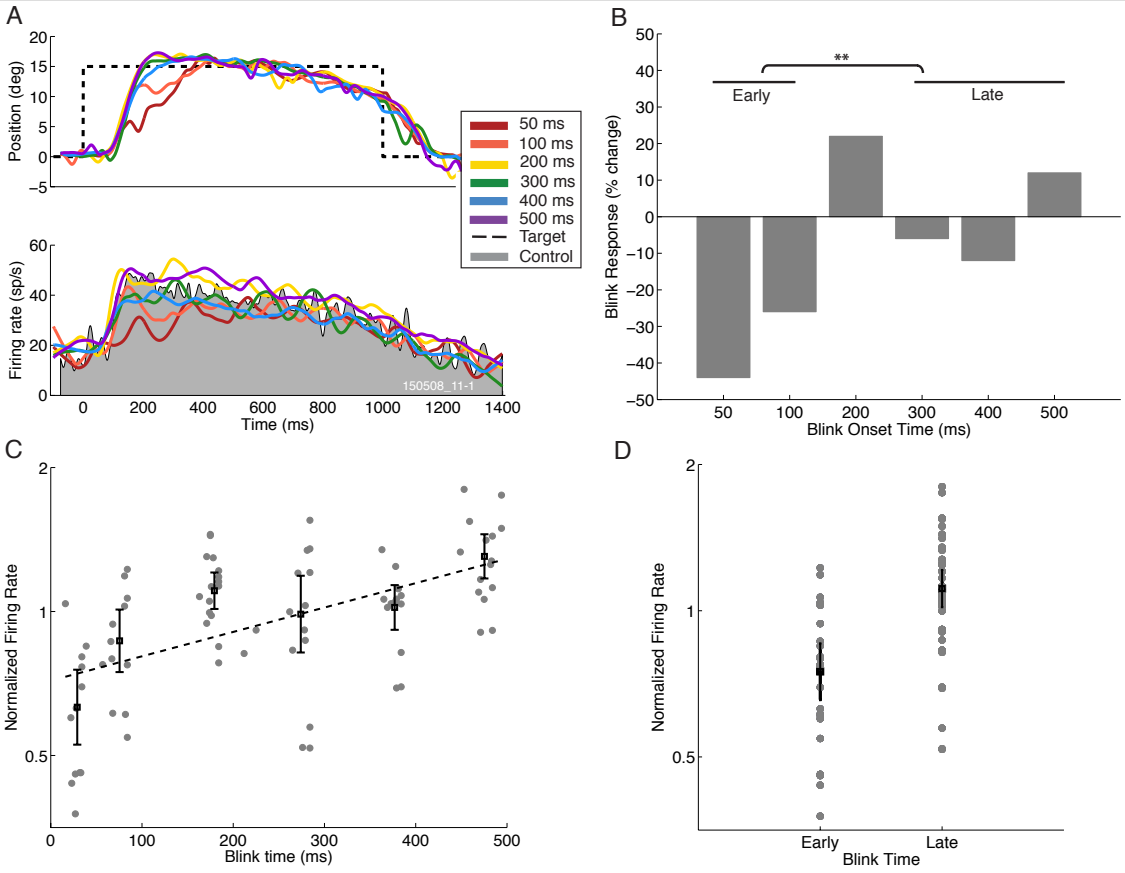


Figure 2.5. Example FEFsem neuronal blink response over time. A: Top panel shows behavior during step-ramp trials with blinks at different times. Target position during control trials (dashed line) shown alongside eye position traces (solid lines) for the various blink times (N = 14, 12, 17, 14, 13, 15, for 50, 100, 200, 300, 400, and 500 ms, respectively). Bottom panel shows average firing rate in control trials (shaded region), as compared to the average firing rate during blink trials (colored traces). Quantification intervals shown as horizontal colored lines. B: Quantified blink response over time. Difference in firing rate within the interval between blink and control is expressed as percent change. Early (50-100 ms) blink responses significantly different than late (300-500 ms) blink responses ($p < 0.001$). C: Change in blink response over time shown across individual trials. Blink time is with respect to eye movement onset. Firing rate plotted on a log2 scale. Each grey point is the blink response in one trial compared to average of all control trials. Means and 95% CI shown superimposed in black, along with best fit line with the equation: normalized FR = 0.0011(blink time) + 0.7436. D: Individual trial blink responses (grey points) shown condensed across the early and late categories (N = 26 and 42, respectively), with means and 95% CI shown atop in black.

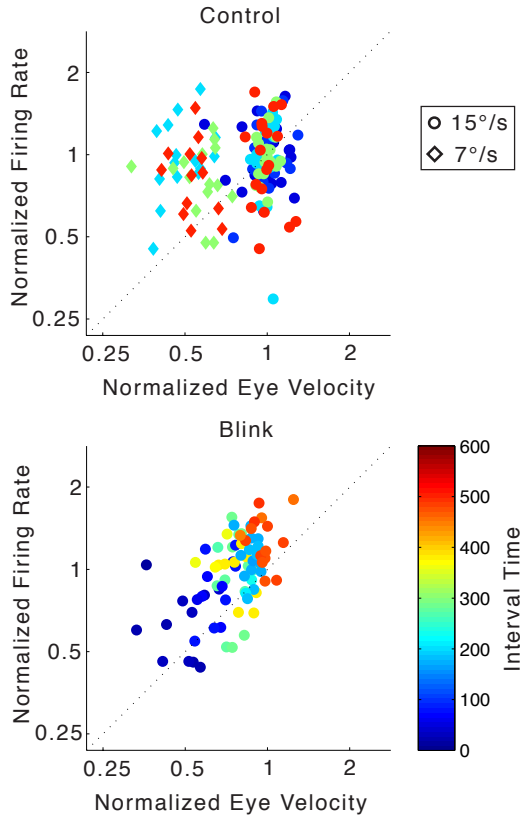


Figure 2.6. Eye velocity and firing rate comparison for an example FEFsem neuron. Data from the same neuron as in **Figure 2.5**. Top panel shows data from control trials. Average of firing rate and eye velocity were taken over intervals of 50 ms on individual trials and normalized by the average during that interval for all control trials. Circles represent data from trials at 15°/s (N=18), diamonds are data from trials at 7°/s (N=15), both are compared to the average at 15°/s. The color of the points indicates the time during the step-ramp trial the interval began, as indicated by the color bar. Dotted line represents unity. Both firing rate and eye velocity are plotted on a log2 scale. Bottom panel shows data from blink trials (N=85). Averages of firing rate and eye velocity over intervals starting 60 ms after blink onset and lasting 150 ms in individual trials were normalized by the average during that interval for all control trials. Color represents timing of the start of the interval.

2.4.3 *The influence of eye velocity*

Although we did not find a greater decrement in eye velocity following early blinks as compared to late blinks in our behavioral analyses, we noticed that many neurons showed a change in blink response over time (**Figure 2.5**). To be sure that this was not due to greater deviations in eye velocity during early blinks on some trials, we compared the normalized firing rate to the normalized eye velocity in both control and blink trials. An example is shown in **Figure 2.6** using data from the same neuron as shown in **Figure 2.5**. For control trials, we calculated the average firing rate and eye velocity over 50 ms intervals on individual trials, and normalized them by the average for all control trials (N=33; Figure 6, top panel). The color of the data points and the accompanying color bar represent the timing of the interval. To get a full picture of the neuron's baseline sensitivity to eye velocity, we used control trials in which the

target moved at both 15°/s (N=18; circles) and 7°/s (N=15; diamonds), and compared them to the average at 15°/s. It is clear that although there is variability in both firing rate and eye velocity, the two variables are unrelated.

In comparison, the bottom panel shows all blink trials (N=85). For these trials, we calculated the average firing rate and eye velocity on individual trials over the 150 ms interval delayed 60 ms from the blink onset, and normalized it by the average during that interval for all control trials. The timing of the blink is represented by the color of the data points and the color bar at right. Initially, it seems as though the increase in blink response is due to differences in eye velocity, but the control data shows that this neuron is not, in fact, sensitive to eye velocity.

We tested 137 neurons for eye velocity sensitivity in control trials at one pursuit speed and found that 122 (91%) showed the same profile as the neuron in **Figure 2.6**, while only 13 (9%) exhibited eye velocity sensitivity. Only 7 neurons were tested with two speeds, but 6 (87%) of the neurons showed similar profiles to Figure 6, and 1 neuron (14%) evinced eye velocity sensitivity. This supports the hypothesis that the differences in blink response seen through time are not artifacts of deviations in eye velocity, but are the result of the removal of retinal input itself.

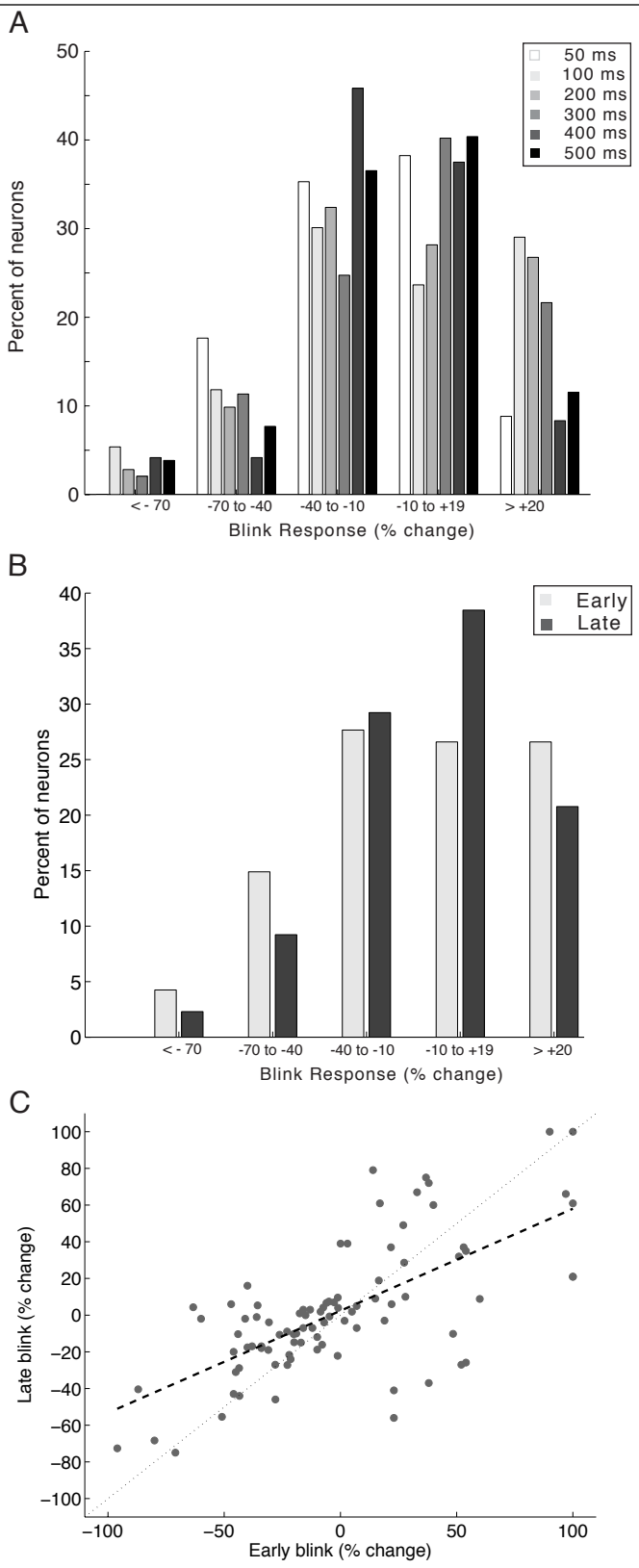


Figure 2.7. Blink responses over time across the FEFsem population. A: Distribution of responses from neurons tested at each blink time (colors represent different timings; N = 34, 93, 71, 97, 24, and 52 neurons for 50, 100, 200, 300, 400, and 500 ms, respectively). Blink responses are grouped into 30%-wide bins along the abscissa, and the percentage of neurons in each bin is shown on the ordinate. B: Same conventions as in A, but blink times are grouped into early (50-100; N=93) and late (300-500; N=130) responses. C: Early and late blink responses for individual neurons. The dotted line represents unity, and the best linear fit (dashed line) is represented by equation: Late Blink Response = 0.56(Early Blink Response) + 2.37.

2.4.4 *FEFsem population response to target blinks throughout step-ramp tracking*

To assess the distribution of blink responses over time in a manner similar to prior studies (Newsome et al. 1988; Tanaka and Fukushima 1998), we divided the possible blink responses into 30%-wide bins and calculated the percentage of neurons falling into each bin (**Figure 2.7**A). Each shade represents the findings from blink testing at a particular time during step-ramp tracking. Consistent with previous work, the majority of neurons, on average 68.8% (95% CI: 60.0 – 77.7), fell into the -40 to -10 and -10 to +19 bins at all blink times. This indicates the strong influence of extraretinal signals in the FEFsem overall. To probe whether there were any population-level differences in the distributions of early and late blinks, we grouped the data into early (50 and 100 ms) and late (300-500 ms) categories (**Figure 2.7**B), and visualized them as in **Figure 2.7**A. Although it appears that more neurons fall into the lowest two bins (< -70 and -70 to -40) when tested early on, there was no significant difference between the distributions of early and late blink responses (Student's t-test $p = 0.71$; N=93 neurons tested with early blinks, N=130 neurons tested with late blinks).

Beyond the basic distribution of blink responses, we were also interested in the predictive value of one blink timing for another. In **Figure 2.7**C, early and late blink responses are compared. Although there is a positive relationship – indicating that there is indeed some predictive value – the line of best fit (dashed) has a slope of 0.56 (R=0.67), which is less than the unity line (dotted). Additionally, there are many neurons whose blink responses place them at quite a distance from the unity line, revealing more of what was seen in **Figure 2.5**: the contributions from retinal and extraretinal signals are not consistent throughout step-ramp tracking for some neurons.

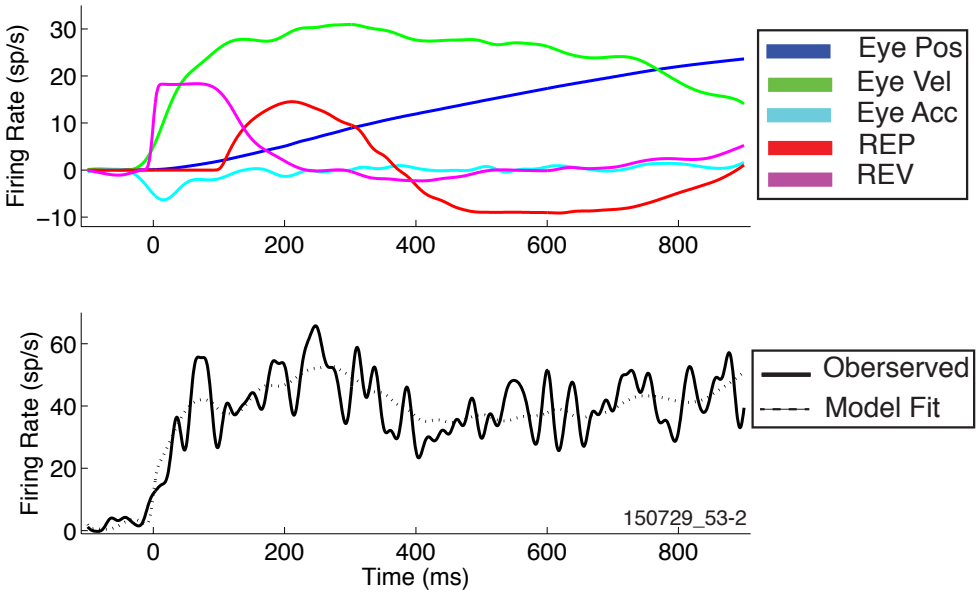


Figure 2.8. Multiple linear regression modeling for an example FEFsem neuron (same neuron as in **Figure 2.3**). Top panel shows the eye and retinal motion component contributions as determined by the model. The bottom panel shows the observed spike density function (solid line; N=28) compared to the best fit from the model (dotted line). The model fit had a CD of 0.77. The equation for the model is: FR(t) = 2.23 + 1.82E(t+8) + 1.83E'(t+8) – 0.03E''(t+8) + 12.60R(t+119) + 1.22R'(t+119).

2.4.5 *Do FEFsem neurons fall into distinct subgroups?*

In previous work, neurons have been categorized into subgroups on the basis of their activity's relationship to various eye and visual motion components (e.g., Ono et al. 2005). Most commonly, this allows the description of FEFsem neurons as primarily eye acceleration or eye velocity sensitive, or not particularly sensitive to either. However, it is evident from other work that FEFsem activity includes signals beyond just eye acceleration and velocity (for some examples see Tanaka and Lisberger 2001; Schoppik et al. 2008; Fukushima et al. 2011). To expand on this, we first used multiple linear regression modeling to describe the sensitivity of the neurons to eye position, velocity, and acceleration, as well as retinal position error and retinal image motion. One representative example is shown in **Figure 2.8**. For each neuron, we estimated the contribution of each component (colored lines in the top panel), and used them to

generate a model fit for the data (dotted line in bottom panel). We used the coefficient of determination (CD) to determine goodness of fit (CD = 0.77 for this example). In this way we were able to derive an estimate of the neuronal sensitivity to each component, represented by the partial correlation (*r*).

We used these partial correlation results, combined with neuronal latency, and the blink responses at early and late timepoints as inputs to a k-means clustering algorithm to elucidate whether distinct clusters were present within our sample. The results of the k-means clustering can be seen in **Figure 2.9**. In order to determine the appropriate number of clusters for the algorithm to use (the *k* in k-means), we used the gap statistic (**Figure 2.9**J-K; Tibshirani et al. 2001). The lowest value of *k* for which the gap statistic was positive was 3, indicating that we should initialize the *k*-means algorithm with *k*=3.

As the input to the k-means algorithm is essentially made up of neurons represented as points in 8-dimensional space, it is challenging to visualize the results. To that end, we have plotted the cluster assignments for each variable separately: eye position sensitivity, eye velocity sensitivity, eye acceleration sensitivity, retinal position error sensitivity, and retinal velocity error sensitivity, neuronal latency with respect to movement onset, early blink response, late blink response (**Figure 2.9**A-H, respectively). Because the k-means algorithm does not accept inputs with missing data, some neurons were excluded from the analysis (N=47), but they are present in the "All Data" plots (**Figure 2.9**A-H).

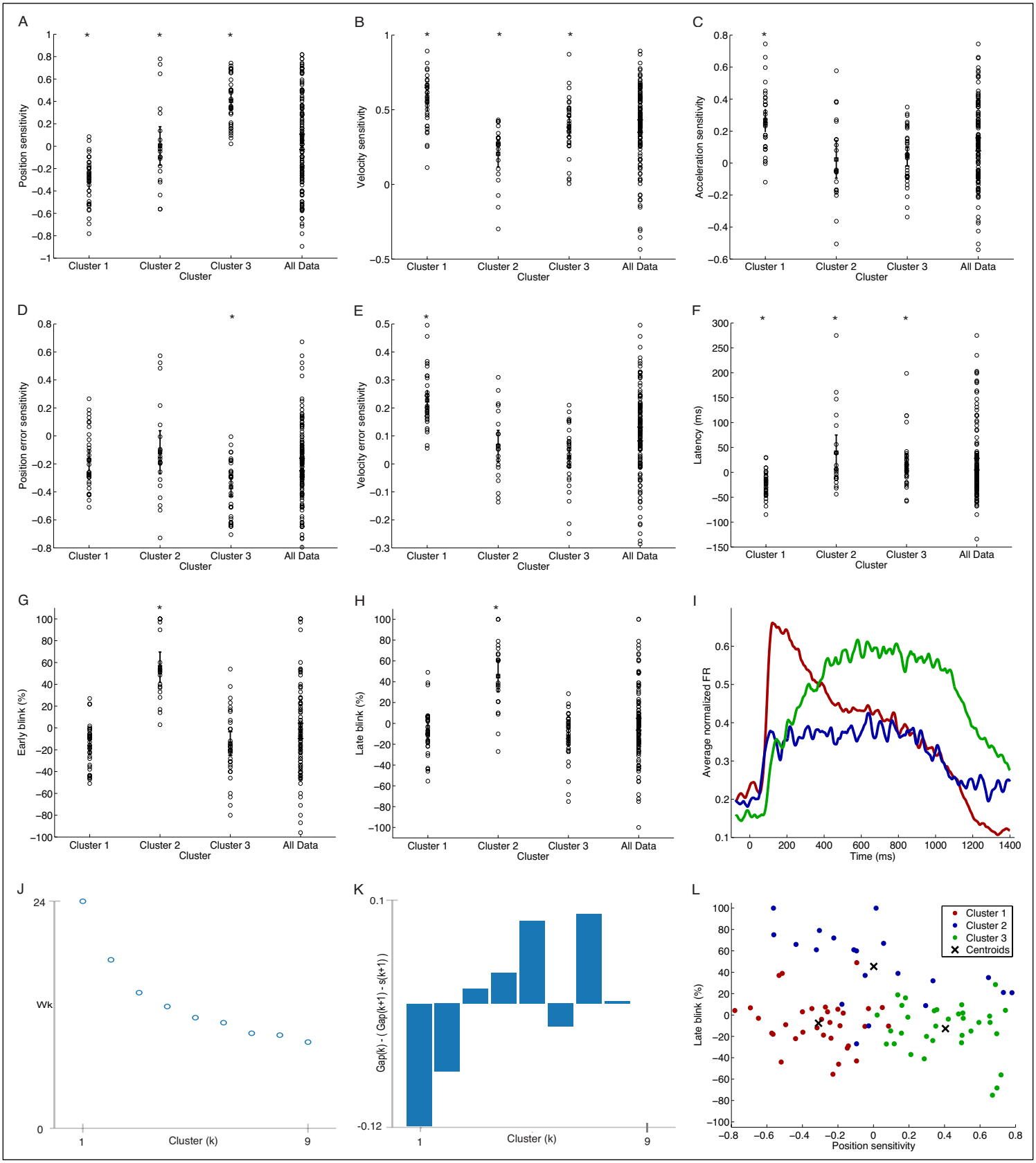

A
Position sensitivity
Cluster 1
Cluster 2
Cluster 3
All Data
Cluster
B
Velocity sensitivity
C
Acceleration sensitivity
D
Position error sensitivity
E
Velocity error sensitivity
F
Latency (ms)
G
Early blink (%)
H
Late blink (%)
I
Average normalized FR
Time (ms)
J
Wk
Cluster (k)
K
Gap(k) - (Gap(k+1) - s(k+1))
L
Late blink (%)
Position sensitivity
Cluster 1
Cluster 2
Cluster 3
Centroids

Figure 2.9. K-means clustering results for FEFsem neurons. A-H: Cluster assignments for each variable: (A) eye position sensitivity, (B) eye velocity sensitivity, (C) eye acceleration sensitivity, (D) position error sensitivity, (E) velocity error sensitivity, (F) neuronal latency with respect to eye movement onset, (G) early and (H) late blink response. Open circles represent each neuron; black squares indicate means and 95% CI for each cluster. Asterisks indicate clusters that are significantly different. Cluster 1 N=33; Cluster 2 N=20; Cluster 3 N=33. I: Average normalized firing rate for each cluster. J: Within-cluster variance (Wk) by number of clusters. K: Gap statistic for each number of clusters. The appropriate number of clusters is the smallest number with a positive gap statistic. L: Comparison of eye position sensitivity and late blink to show the three distinct clusters. Colors indicate cluster assignment, and black x's represent means.

For the majority of the variables, the data appear to form a continuum rather than separate clusters when inspected visually. However, there are a number of significant differences between the clusters. In terms of eye position sensitivity, all clusters have significantly different sensitivities (**Figure 2.9**A; $p < 0.01$ for all comparisons; mean (SEM): C1 -0.31 (0.04), C2 0.002 (0.09), C3 0.42 (0.04)). All clusters also have significantly different velocity sensitivities (**Figure 2.9**B; $p < 0.01$; C1 0.55 (0.03), C2 0.20 (0.05), C3 0.39 (0.03)). C1 has significantly higher acceleration sensitivity than C1 or C2 (**Figure 2.9**C; $p < 0.001$ for C1 comparisons, $p = 0.76$ for C2 compared to C3; C1 0.266 (0.03), C2 0.023 (0.06), C3 0.04 (0.03)). For position error sensitivity, C3 is significantly less sensitive than C1 or C2 (**Figure 2.9**D; $p < 0.01$ for C3 comparison, $p = 0.47$ for C1 and C2; C1 -0.173 (0.04), C2 -0.117 (0.09), C3 -0.366 (0.03)). In terms of velocity error sensitivity, C1 has a higher sensitivity than C2 or C3 (**Figure 2.9**E; $p < 0.001$ for C1 comparisons, $p = 0.23$ for C2 and C3; C1 0.227 (0.02), C2 0.064 (0.03), C3 0.024 (0.02)). In the case of neuronal latency, all clusters are significantly different (**Figure 2.9**F; $p < 0.01$; C1 -22.6 (4.5), C2 39.6 (18.1), C3 17.4 (8.9)). For the early blink, C2 has a significantly greater response than the other two clusters (**Figure 2.9**G; $p < 0.001$ for C2 comparisons, $p = 0.61$ for C1 and C3; C1 -17.7 (3.6), C2 55.8 (7.1), C3 -14.3 (5.6)). As with the early blink, C2

had a significantly larger late blink response (**Figure 2.9**H; $p < 0.001$ for C2 comparisons, $p = 0.38$ for C1 and C3; C1 -7.7 (4.0), C2 -45.4 (7.7), C3 -12.8 (4.1)).

To show how these clusters are distinct from one another, we have plotted the average normalized firing rate for each cluster (**Figure 2.9**I). Here, it is visible that C1 has a strong, short-latency response that peaks that decreases to a plateau until pursuit ceases. C2 has a response that is a stable plateau throughout the pursuit interval, although its lower response level suggests that this group may have more variable firing dynamics. In contrast, C3 has a longer-latency response that increases gradually to a much higher plateau. The firing rate begins to return to baseline much later in C3 than in either of the other clusters.

Additionally, to illustrate the separation of the clusters, we compared position sensitivity and late blink response in **Figure 2.9**L. Here, it is clear that C1 and C3 have blink responses that are largely less than or unchanged from control, whereas C2 neurons show a positive blink response. C1 and C3 are differentiated by their position sensitivity, which is positive and negative, respectively. Although not all dimensions show this degree of separation of the clusters, this example gives a sense of the spread of the data and the separation of the clusters in multiple dimensions.

2.4.6 *Comparison with MST: Distribution of population response*

As FEFsem and MST are two of the major cortical contributors to voluntary smooth pursuit eye movements, and their strong reciprocal connections underlie a number of similarities between the areas, we wanted to test whether recapitulating these analyses on data from MST neurons would yield the same results. We included 51 neurons from MST in monkey F. All included neurons responded to tracking of the target spot during the step-ramp task in their preferred direction. To evaluate the distribution of blink responses over time, we divided the

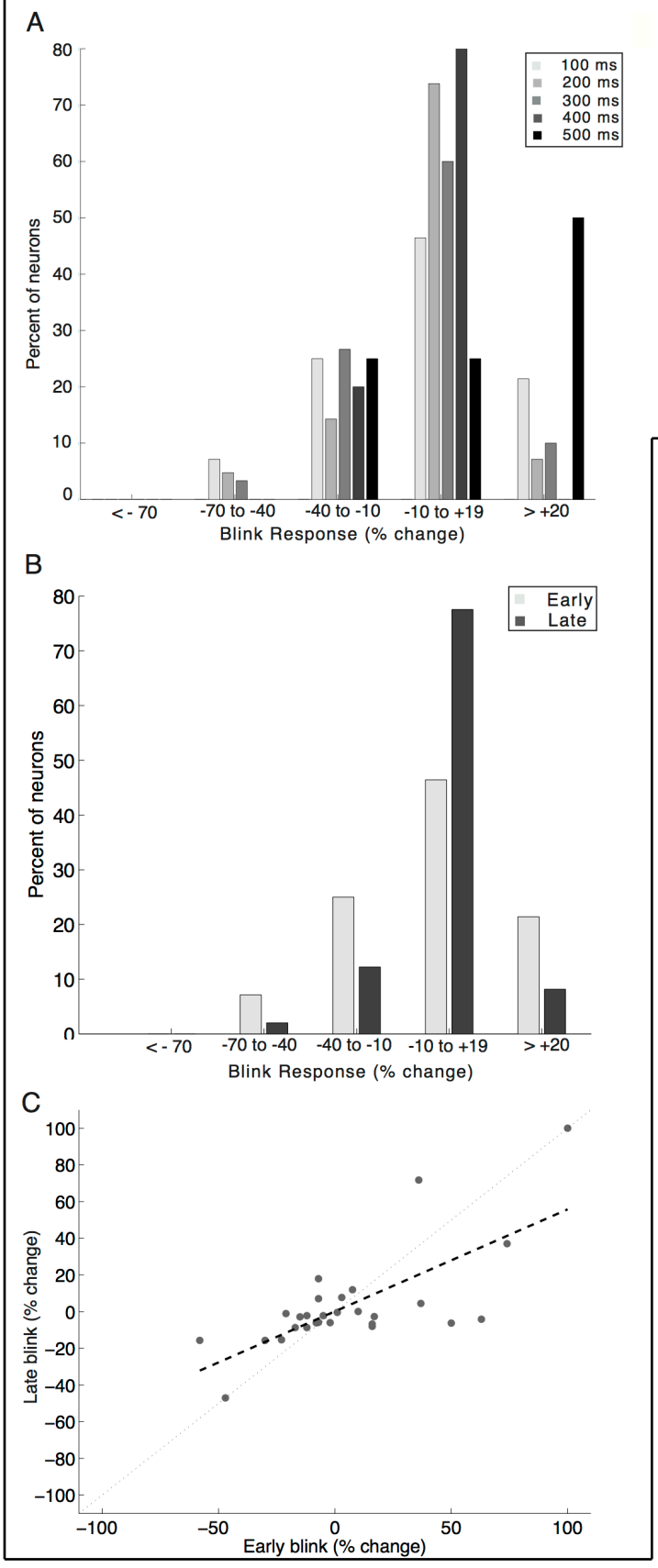


Figure 2.10. Blink responses over time across the MST population. Same conventions as **Figure 2.7**. A: Distribution of responses from neurons tested at each blink time (N=28, 42, 30, 5, and 4 for 100, 200, 300, 400, and 500 ms, respectively). B: Blink times are grouped into early (50-100 ms; N=28) and late (300-500 ms; N=49) responses. C: Early and late blink responses for individual neurons. The best linear fit (dashed line) is represented by equation: Late Blink Response = 0.56(Early Blink Response) + 0.13.

blink responses into 30%-wide bins, as we did for the FEFsem (**Figure 2.10**A). Although there were fewer neurons and only one monkey tested (N=51), the distribution is similar to that found in the FEFsem: most responses fall into the -10 to +19% bin. This indicates the strong presence of extraretinal input in MST, as has been reported previously (Newsome et al. 1988; Ono et al. 2010).

To probe population-level differences in early and late categories of blinks, we again grouped the data into early (100 ms) and late (300-500) categories (**Figure 2.10**B). Although there appears to be a larger proportion of neurons falling in the lower bins when tested with early blinks, there was no

significant difference between the two distributions (p = 0.53, N=28 neurons tested with early blinks, N=49 neurons tested with late blinks). To assess the relationship between early and late blinks, data from neurons tested at both times were compared (**Figure 2.10**C; N=28). Like in the FEFsem, there is a positive relationship between the blink responses, although some neurons show notable differences between their early and late blink responses. Interestingly, the slope of the line that best describes the population relationship between early and late blinks in MST is nearly identical to that from FEFsem: slope = 0.56, R = 0.71.

2.4.7 *Comparison with MST: Distinct subgroups*

To facilitate comparison with the FEFsem results, we also used a k-means clustering algorithm on the MST neurons. Because we only had 28 neurons tested for early blink response, we excluded that variable from the analysis. We used neuronal latency, partial correlation results (eye position, velocity, and acceleration sensitivity, and retinal position and velocity error sensitivity), as well as the late blink responses as input variables. The results of the analysis are shown in **Figure 2.11**. We imposed three clusters on the algorithm (k=3) to allow for direct comparisons between the two brain regions. The comparison of number of clusters and within-cluster variance is shown in **Figure 2.11**I.

As in the FEFsem, the clusters seem to form more of a continuum than discrete clusters, but significant differences were found. For neuronal latency, there was a significant difference between C1 and C2 ($p < 0.01$; mean (SEM): C1 110 (16.2), C2 53.6 (10.1), C3 106.3 (62.1)). All clusters showed significant differences in their position sensitivity ($p < 0.001$; C1 0.39 (0.05), C2 0.11 (0.03), C3 -0.28 (0.13)). Significant differences were also found for velocity sensitivity ($p < 0.01$; C1 0.23 (0.04), C2 0.50 (0.02), C3 -0.09 (0.09)) as well as acceleration sensitivity ($p < 0.01$; C1 -0.07 (0.03), C2 -0.16 (0.01), C3 0.11 (0.04)). No differences were

found for position error sensitivity ($p > 0.05$; C1 -0.42 (0.03), C2 -0.38 (0.02), C3 -0.37 (0.04)). For velocity error sensitivity, only C2 was found to be different than the others ($p < 0.05$; C1 -0.05 (0.02), C2 0.11 (0.02), C3 0.02 (0.03)). For the late blink response, C3 was found to be significantly different ($p < 0.01$; C1 -3.9, (3.4), C2 -1.0 (2.2), C3 26.9 (16.3)).

To show how the separation between the clusters, we have plotted the average normalized firing rate for each cluster (**Figure 2.11**H). Here, it is visible that C1 has a response that increases gradually to a plateau that lasts the duration of pursuit. Much like C2 for the FEFsem, the lower response level suggests variability in the response dynamics. C2 has a response similar to C1 in the FEFsem that increases rapidly, peaks, and then decreases to a slightly lower level for the rest of pursuit. In contrast, C3 seems to be somewhat of a combination of the other two clusters. It has a smaller early peak, followed by a plateau at a higher level than C1.

To further illustrate the separation of the clusters, eye velocity and acceleration sensitivity are compared in **Figure 2.11**J. There is a clear negative correlation between the two variables, and the three clusters represent progressively lower levels of acceleration sensitivity and greater levels of velocity sensitivity. These clusters show some separation, but together they seem to form more of a continuum than distinct subgroups.

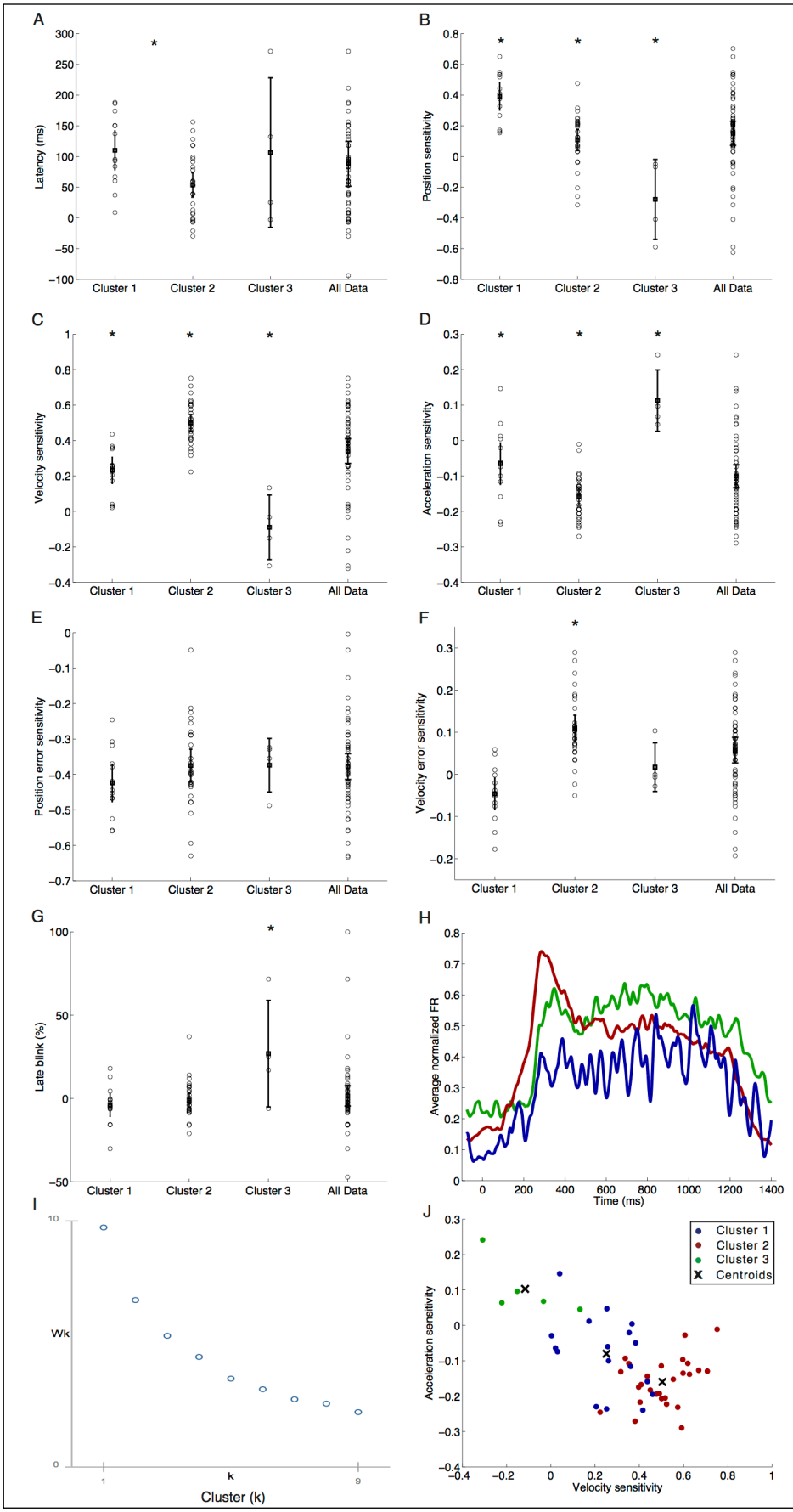


Figure 2.11. K-means clustering results for MST neurons. A-G: Cluster assignments for each variable: neuronal latency with respect to eye movement onset (A), eye position sensitivity (B), eye velocity sensitivity (C), eye acceleration sensitivity (D), position error sensitivity (E), velocity error sensitivity (F), and late blink response (G). Same conventions as Figure 9. Cluster 1 N=27; Cluster 2 N=17; Cluster 3 N=5. H: Average normalized firing rate for each cluster. I: Within-cluster variance (Wk) by number of clusters. J: Comparison of eye velocity sensitivity and eye acceleration sensitivity to show the three distinct clusters. Colors indicate cluster assignment, and black x's represent means.

2.4.8 *Comparison with MST: Direct comparison of variables*

As a final comparison between FEFsem and MST, we looked at the distribution of values for each dimension used in the k-means clustering algorithm (**Figure 2.12**). The FEFsem had a significantly lower neuronal latency than MST (**Figure 2.12**A; $p < 0.001$; mean ± SEM, FEFsem 16.4 ± 5.9, N=137; MST 87.9 ± 18.6, N=51). Although the distributions of eye position sensitivity appear visually different in **Figure 2.12**B, there was no significant difference between FEFsem and MST ($p = 0.08$; FEFsem 0.038 ± 0.036; MST 0.151 ± 0.040). There was also no significant difference between FEFsem and MST in terms of eye velocity sensitivity (**Figure 2.12**C; $p = 0.23$; FEFsem 0.391 ± 0.022; MST 0.340 ± 0.036). For eye acceleration sensitivity, the FEFsem had significantly higher values than MST (**Figure 2.12**D; $p < 0.001$; FEFsem 0.116 ± 0.021; MST -0.101 ± 0.017). The same was true for position error sensitivity (**Figure 2.12**E; $p < 0.001$; FEF -0.204 ± 0.023; MST -0.378 ± 0.019). There was also a significant difference between the velocity error sensitivity in FEFsem and MST (**Figure 2.12**F; $p = 0.03$; FEFsem 0.107 ± 0.013; MST 0.057 ± 0.015). For both early and late blink responses there were no significant differences between FEFsem and MST (early blink: **Figure 2.12**G, $p = 0.40$, FEFsem -2.7 ± 4.5 N=90, MST 5.7 ± 6.6; late blink: **Figure 2.12**H, $p = 0.79$, FEFsem -0.8 ± 3.0 N=130, MST 1.6 ± 3.2). In the discussion, we consider the relationship of retinal and extraretinal signals to neuronal activity in both the FEFsem and MST, and what it reveals about possible subgroups within these neuronal populations.

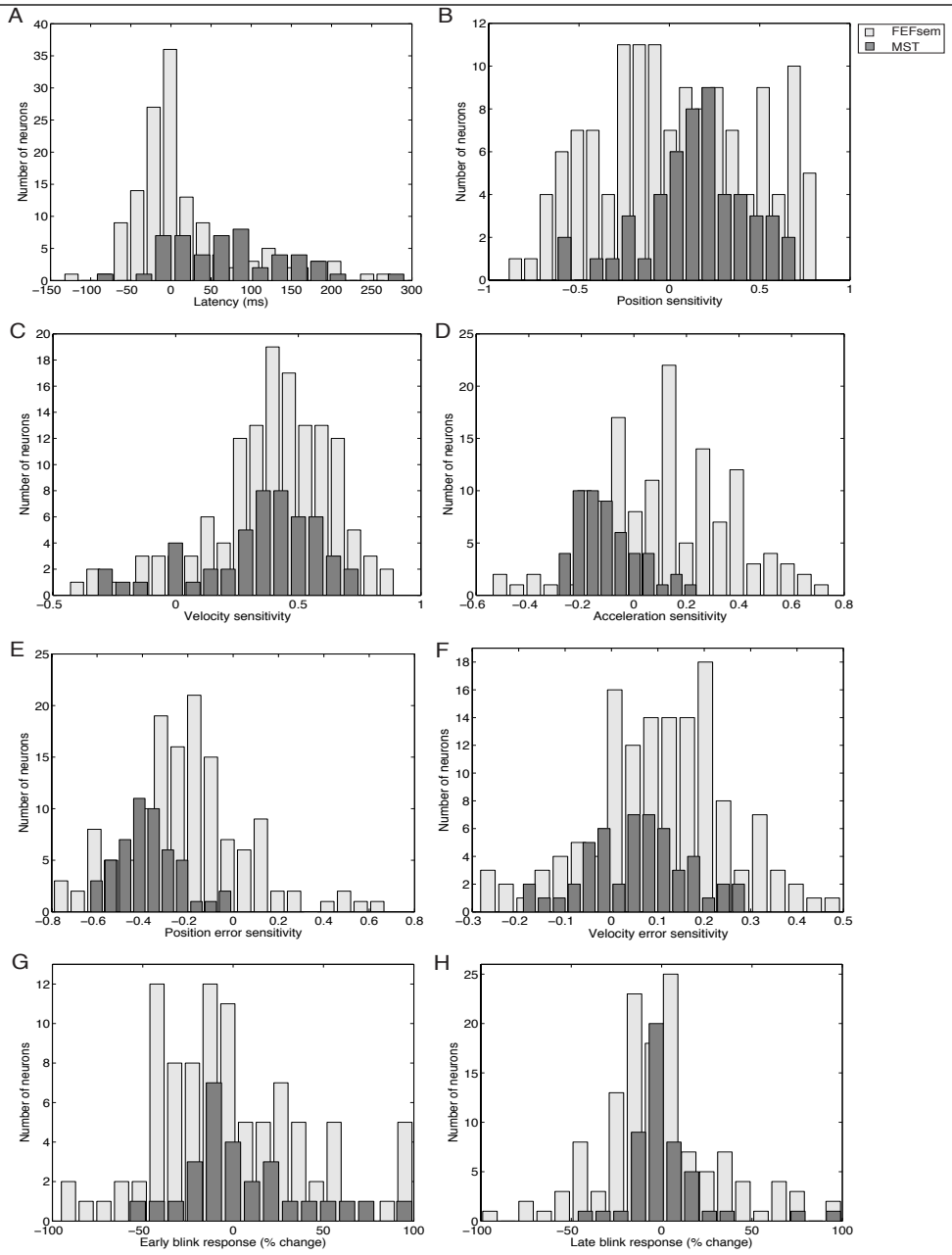


Figure 2.12. Comparison of FEFsem and MST. A-H: Histograms of data from FEFsem (light grey) and MST (dark grey). A: Neuronal latency with respect to eye movement onset. FEFsem (N=137) and MST (N=51) are significantly different ($p < 0.001$). B: Position sensitivity from partial correlation. No significant difference between FEFsem (N=137) and MST (N=51; $p = 0.08$). C: Velocity sensitivity. No significant difference between FEFsem (N=137) and MST (N=51; $p = 0.23$). D: Acceleration sensitivity. FEFsem (N=137) and MST (N=51) are significantly different ($p < 0.001$). E: Position error sensitivity. FEFsem (N=137) and MST (N=51) are significantly different ($p < 0.001$). F: Velocity error sensitivity. FEFsem (N=137) and MST (N=51) are significantly different ($p = 0.03$) G: Early blink response (percent change from control). No significant difference between FEFsem (N=94) and MST (N=28; $p = 0.40$). H: Late blink response. No significant difference between FEFsem (N=130) and MST (N= 49; $p = 0.79$).

2.5 DISCUSSION

The target blink has been previously used to test for the presence of extraretinal components within neural activity. However, it has not been established whether these relative contributions of extraretinal and retinal signals are static over time within the neuronal responses. Others, like Bogadhi and colleagues (2013), have indicated that the relative contributions of retinal and extraretinal signals to pursuit behavior are likely dynamically weighted over time, and there is also evidence of cognitive influence on FEFsem neuronal responses (e.g. Fukushima et al. 2011). Thus, we sought to establish whether we could find evidence of dynamic reweighting of these signals in FEFsem activity. Moreover, the idea that these relative contributions could be informative as to the function of individual neurons in complex areas like the FEFsem has not been tested. In this study, we sought to characterize the relative weights of retinal and extraretinal signals over time in a population of FEFsem neurons. We then investigated whether these relative weights, combined with other basic neuronal characteristics, could provide a basis for the functional categorization of neurons.

2.5.1 *Behavioral response to target blinks*

In earlier work, the eye velocity of highly trained monkeys has been presumed to be sufficiently accurate following target blinks to guarantee that any deviations in neuronal activity are due to the lack of retinal input as opposed to changes in eye velocity (Newsome et al. 1988; Heinen 1995; Tanaka and Fukushima 1998). This has also been explored using “imaginary” targets and parafoveal pursuit (Ilg and Thier 2003). However, the magnitude of the effect has not been directly investigated. In a study in which human participants tracked a tilted bar that translated horizontally, eye velocity *did* reliably change following the target blink. When the blink occurred late in the trial, the drop in eye velocity was around 4°/s, on average. However,

when the blink occurred early in the trial, the drop in eye velocity was only around 1.5°/s (Bogadhi et al. 2013). This would suggest that the pursuit system as a whole has a changing reliance on retinal input throughout the trial, with less reliance on retinal input early on. Based on their results and previous work on motion integration (Bogadhi et al. 2011; Montagnini et al. 2007), the authors proposed a new closed-loop two-stage recurrent Bayesian model in which retinal and extraretinal signals are dynamically weighted. A similar model has also been proposed by Orban de Xivry and others (2013) to explain how sensory and internal memory signals are combined to produce both predictive and sensory-driven pursuit characteristics.

Our results perhaps show a different finding than Bogadhi et al. (2013). The absolute eye velocity drop for the earliest blink times seems to be similar to the drop at other times, and the ratio of blink to control eye velocity is much lower early on (**Figure 2.2**A and 2C). This indicates that the retinal input may be playing a greater role at the beginning of pursuit.

Interestingly, we also found a difference between early and late blinks in terms of anticipatory eye acceleration. As we used the same blink duration (150 ms) throughout all experiments, the reappearance of the target was always equally predictable irrespective of the time of blink onset. However, we saw greater anticipatory eye acceleration in response to the early blinks than the later blinks. This has also been shown in humans, even in cases where the duration of the blink was not as predictable (Bogadhi et al. 2013). These findings suggest that the contributions of extraretinal signals is stronger or more effective for anticipatory eye acceleration during pursuit initiation as compared to maintenance. Where this is borne out in neural activity has yet to be identified.

These results suggesting the importance of retinal signals early on are are in keeping with the results of control systems models (Robinson et al. 1986; Lisberger et al. 1987; Nuding et al.

2008) and other experimental work (Lisberger et al. 1981; Lisberger and Westbrook 1985; Tychsen and Lisberger 1986; Krauzlis and Lisberger 1994) that show the central importance of retinal image motion in the initiation of smooth pursuit eye movements. Though these models differ in their reliance on retinal parameters, both the Robinson (1986) model that uses extraretinal signals and the Lisberger (1987) model that relies exclusively on retinal signals both demonstrate the priority of retinal image motion to the initiation of pursuit. In part, the explanation could lie in the rapid conversion of the initial pulse of visual motion into a movement command that includes an anticipatory component.

Though our results also suggest a stronger role for extraretinal signals during initiation for functions like anticipation, it is worth noting that the context in which this occurs is one with a high degree of predictability. In circumstances with more complex scenes and motion, the utility of extraretinal signals might be less than in this laboratory setting. Thus, though the import of extraretinal signals in these behaviors is evident, it is critical to acknowledge the context in which they are generated. One could easily imagine changing weights for retinal and extraretinal signals depending on the context of predictability, as suggested before (Orban de Xivry et al. 2013).

Beyond a more detailed characterization of the behavioral response to blinks, one important reason to carefully assess the behavioral effects of target blinks is to ensure that changes in neuronal activity are not due to larger deviations in eye velocity at certain timepoints. We found that early blinks do not induce larger absolute deviations in eye velocity than later blinks, and the maximum difference between eye velocity deviations across timepoints is only about 3°/s. Ergo, evidence of changes in neuronal activity in response to target blinks are likely not attributable to a substantial difference in eye velocity.

2.5.2 *Single neuron responses to target blinks*

As has been shown previously, neurons in the FEFsem are not entirely reliant on retinal input, which indicates the presence of extraretinal signals in their activity (Tanaka and Fukushima 1998). However, not all neurons in the FEFsem show the same relative contributions from retinal and extraretinal input (**Figure 2.4**). While some neurons show large contributions from retinal input, and thus their firing rates decrease in response to a target blink, others show almost no influence of retinal signals, barely changing their firing rates at all. Indeed, some cells even show an increase in activity in response to the target blink, indicating reliance on retinal signals but in a way that generates a higher level of activity with the removal of retinal input.

This diversity of responses likely reflects the variety of signals and functions present in the FEFsem. Evidence of signals related to eye velocity (Gottlieb et al. 1994; Fukushima et al. 2000; Ilg and Thier 2008) and eye acceleration (Tanaka and Fukushima 1998; Ono and Mustari 2009) are common, as are those relating to the gain of the visuomotor transformation (Tanaka and Lisberger 2001, 2002). Beyond this, there have been reports indicating the presence of visual motion memory and go/no-go cues (Fukushima et al. 2011), anticipation and prediction (MacAvoy et al. 1991; Fukushima et al., 2002; Ilg and Thier 2008), as well as vestibular and image motion signals (Fukushima et al. 2000), likely among others.

The variety of blink responses found in the FEFsem exists not just across neurons, but also within individual neurons over time. We showed that the response to a target blink early on in the step-ramp trial was significantly different than the response later in the trial in some neurons (Figure 5). In the representative case illustrated here, the neuron exhibits a greater reliance on retinal input during pursuit initiation than during maintenance. This is interesting in view of the fact that though the absolute deviation in eye velocity following target blinks is relatively

consistent across time, the discrepancy between blink and control is greater earlier on. It is possible that neurons like these are playing a role in this aspect of behavior.

In keeping with this, neurons like these could be involved in the initiation of pursuit, perhaps sending a driving signal related to eye acceleration to the brainstem (Ono and Mustari 2009). As eye acceleration neurons typically have neuronal activity that includes a sharp, transient peak that begins before the onset of pursuit, and the remainder of this neuron's response profile is more of a flat plateau, it is also possible that this neuron participates in eye acceleration and some other function. Perhaps this neuron participates in gain control (Tanaka and Lisberger 2001, 2002), which would likely be dependent on incoming visual signals early on and then dependent on ongoing eye movement signals later. Although we cannot conclusively state the function(s) in which the neuron plays a role, the combination of blink response and other neuronal characteristics provides a useful basis for making predictions about its role.

2.5.3 *Distribution of blink responses and emergent clusters in FEFsem*

Our results show that the overall distribution of blink responses in the FEFsem is similar to what has been described previously: most neurons show little to no decrement in activity in response to target blinks (Tanaka and Fukushima 1998). Although a greater percentage of neurons fell into the smallest bins when tested early, there was no significant difference in the overall distribution of blink responses between early and late (**Figure 2.7**). This is notable for two reasons. First, given what we found at the individual neuron level, there might be some expectation that testing early and late would provide different distributions of responses. Second, given that the behavioral results suggest that the system as a whole is relying on changing weights of retinal and extraretinal signals, we expected to see a difference between the early and late blink response distributions.

While it is clear that some neurons have changing contributions of retinal and extraretinal signals (**Figure 2.7**C), overall, the FEFsem maintains only a minimal reliance on retinal input during both pursuit initiation and maintenance. However, this does not necessarily mean that neurons in the FEFsem are not underlying the differences in the behavior described above. It only means that the population level might not be fine-grained enough to identify all of the relevant changes in the FEFsem. This is unsurprising given the wide variety of signals found in the area. The challenge, then, is how to comb through and group the neurons to ascertain meaningful results.

In an attempt to address exactly this problem, we used a k-means clustering algorithm to group the FEFsem neurons and assess whether these groups relate to previously identified functions (**Figure 2.9**). We had the algorithm separate the neurons into three clusters based on the gap statistic (Tibshirani et al. 2001). The clusters separate as follows: the first cluster has significantly shorter latencies, and is the most sensitive to eye velocity, eye acceleration, and retinal image motion. Cluster 1 also shows blink responses that range from no change to negative. The second cluster has the longest latencies on average, and the lowest eye velocity and acceleration sensitivities. Additionally, the second cluster has blink responses that were almost entirely positive for both early and late timepoints. The third cluster has somewhat long latencies as well, is quite sensitive to eye position, and has the most negative sensitivities to position error. Cluster 3 also shows minimal change in response to target blinks, on average.

These clusters have remarkably different characteristics, and although the functions that they serve require further study, we can begin to generate hypotheses about the likely roles these clusters are playing. For instance, the second cluster's blink response is telling: its constituent neurons all increase their firing rates following the blink. Given that the eye velocity is largely

maintained and anticipatory eye acceleration is also seen following target blinks, the blink responses seen in cluster 2 are likely compensating for the decrease in activity found in other neurons. These neurons could be part of a network involving multiple areas carrying extraretinal signals.

In contrast, the third cluster seems to possess signals related to eye position and/or target offset. Its long latencies and lack of change in activity in response to target blinks are in line with this hypothesis. The first cluster seems to be involved in other types of processing entirely. Its eye acceleration and velocity sensitivities, combined with some reliance on retinal input as evinced by the partial correlation and blink tests indicate that it likely contains neurons that are involved in eye acceleration during initiation and/or eye velocity during maintenance. These neurons could be seen as part of a network integrating retinal signals to produce adequate motor commands.

2.5.4 *Comparison with MST*

The distribution of blink responses in MST closely resemble those found in the FEFsem (**Figure 2.10**). Remarkably, the slope of the relationship between early and late blink responses in MST (**Figure 2.10**C) is nearly identical to that found in the FEFsem (**Figure 2.7**C), likely subserved by their strong reciprocal connections (Tian and Lynch 1996; Churchland and Lisberger 2005; Stanton et al. 2005). These findings are also in agreement with what has been shown previously for MST, in that there is clear evidence of the presence of extraretinal signals in the activity of most neurons in the area (Newsome et al. 1988; Ono and Mustari 2006).

The results of the k-means clustering algorithm for MST neurons are also revealing. The first cluster has the longest latencies, greatest position sensitivity, and no effect of target blink. The second cluster shows shorter latencies (although longer than in the FEFsem), higher eye velocity

and image velocity sensitivity, low eye acceleration sensitivity, and no effect of target blink. The third cluster has only five constituent neurons and therefore, only limited interpretation is possible. It has the lowest eye position and velocity sensitivity, highest eye acceleration sensitivity, and seems to have positive blink responses.

The first cluster in MST is similar to the third cluster in FEFsem, and is likely carrying some sort of eye position signal. The second cluster could potentially be involved in a driving signal for eye velocity, as it is insensitive to target blinks and highly correlated with eye velocity. The third cluster is small but is quite distinct from the other MST neurons in a few dimensions. The acceleration sensitivity is fairly rare for MST, as are the large blink responses. These cells may be involved in some kind of anticipation or compensation function during the target blinks, as was more commonly observed in the FEFsem.

In addition to the results of the clustering algorithm, we also compared the distributions of the various variables directly. As expected, the FEFsem has significantly shorter latencies, and greater eye acceleration sensitivities than MST. Somewhat unexpectedly, the FEFsem was also found to have greater position and velocity error sensitivities. This might underlie the broader distribution of blink responses seen in the FEFsem.

2.5.5 *Conclusions*

This study describes in detail the behavioral effects of target blinks throughout pursuit eye movements in non-human primates, corroborating results as yet only reported in humans. There appears to be a greater system-wide reliance on extraretinal signals early on, as evinced by the larger anticipatory eye acceleration. However, there may also be enhanced retinal sensitivity given the greater discrepancy between eye velocity in blink and control trials during the initiation of pursuit.

Additionally, this study goes beyond simply verifying the presence of extraretinal signals in a given neuronal population and uses the relative retinal and extraretinal contributions to assert the likely functions of individual neurons. For the first time, we have shown evidence of changing contributions of retinal and extraretinal signals in the FEFsem and, to a lesser extent, MST, and these results inform our ability to begin to functionally classify these pursuit neurons.

Further work that functionally characterizes the diverse projections from the FEFsem will be vital in continuing this exploration into the likely roles of the FEFsem and identifying a simple series of tests to characterize individual neurons in the awake, behaving animal.

Chapter 3. BEHAVIOR AND FEFSEM RESPONSE DURING COMBINED VOLITIONAL AND REFLEXIVE PURSUIT

3.1 ABSTRACT

Although much is known about volitional and reflexive smooth eye movements individually, not much is known about how they are jointly 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, while the FEF-NRTP pathway is understood to be vital for volitional smooth pursuit eye movements. However, the role that these loops play in a combined volitional and reflexive behavior is unknown. We used a large, textured background moving in conjunction with a small, rewarded 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) in this combined task. Given that the pursuit system likely has a decreased reliance on volitional drive in this task, we hypothesized less involvement of the FEFsem in the behavior, and, accordingly, less FEFsem activity. We found that, on average, FEFsem neurons were 11% less active in a combined volitional and reflexive pursuit task than in a purely volitional pursuit task. We also found that 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 neurons may respond similarly to any kind of "disruption" to pursuit, whether it is the addition or subtraction of retinal input. These results begin to clarify the

role(s) of the FEFsem in more complex behaviors, and characterize the behavior of the smooth pursuit system as a whole in a combined reflexive and volitional task.

3.2 INTRODUCTION

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

This behavior, though it feels effortless, 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 world passing by (Gellman et al., 1990). Additionally, primates are known to be highly skilled at tracking a moving target with their eyes, a process called smooth pursuit (for review, see Krauzlis, 2004). Both reflexive and volitional smooth eye movements rely on largely overlapping brain regions.

The visual motion input from such background movement travels through retinal-geniculo-striate pathways to dorsal stream areas including the middle temporal area (MT) and the medial superior temporal area (MST) (Maunsell and Essen, 1983; Ungerleider and Desimone, 1986; Tusa and Ungerleider, 1988; Boussaoud et al., 1990). MT, and to a lesser extent the lateral subdivision of MST (MSTl), are known for being highly retinal-dependent, while the dorsal subdivision of MST (MSTd) evinces the strong influence of extraretinal signals (Newsome et al., 1988). MST has dense reciprocal connections with the smooth eye movement subregion of the Frontal Eye Field (FEFsem) (Tian and Lynch, 1996; Stanton et al., 2005), and both MST and FEFsem project to the brainstem (Leichnetz, 1989; Boussaoud et al., 1992; Tian and Lynch,

1996; Ono and Mustari, 2009). MST projects to the dorsolateral pontine nucleus (DLPN), and the FEFsem projects to the nucleus reticularis tegmenti pontis (NRTP) (Leichnetz, 1989; Boussaoud et al., 1992; Tian and Lynch, 1996; Ono and Mustari, 2009). Both DLPN and NRTP play a role in volitional smooth pursuit and optokinetic eye movements, as shown by inactivation or lesion of DLPN is known to cause deficits in reflexive ocular following movements as well (May et al., 1988; Yamada et al., 1996; Suzuki et al., 1999). Both DLPN and NRTP then project to the floccular complex and vermal visual areas, respectively, which ultimately connect to brainstem oculomotor areas (Voogd and Barmack, 2006).

These distinct cortico-ponto-cerebellar pathways (FEF-NRTP-cerebellum; MST-DLPN-cerebellum) could be serving different roles in volitional and reflexive smooth eye movements. The reliance on retinal signals and the effects of lesions and inactivations support a role for the MST-DLPN-cerebellar loop in reflexive eye movements, while the FEF-NRTP-cerebellar loop is likely involved in the initiation of volitional smooth pursuit (Nuding et al., 2008; Mustari et al., 2009).

In this study, we sought to examine the role of the FEFsem in a combined reflexive-volitional pursuit task. Based on the postulated role for these cortico-ponto-cerebellar pathways, we hypothesized that the FEFsem would be less active for a combined reflexive-volitional task than for volitional pursuit of a small target alone. We found that this was indeed the case for 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 smooth pursuit.

3.3 Materials and Methods

3.3.1 *Surgical procedures*

Behavioral and neuronal data were collected from three normal rhesus monkeys (*Macaca mulatta*) weighing 5.5-14 kg. Detailed descriptions of most surgical procedures can be found in prior publications (Ono and Mustari 2010, 2012). Surgery was performed under aseptic conditions using isoflurane anesthesia (1.25-2.5%) to stereotaxically implant a titanium head stabilization post and titanium recording chambers (Crist Instruments, Hagerstown, MD). In a subsequent surgery, scleral search coils were implanted underneath the conjunctiva of both eyes using the methodology of Judge et al. (1980). All surgical procedures were performed in strict compliance with National Institutes of Health guidelines, and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Washington.

3.3.2 *Data collection*

During all experiments, monkeys were seated in a primate chair (Crist Instruments) with their head stabilized in the horizontal stereotaxic plane. Eye movements were detected and calibrated using standard electromagnetic methods (Fuchs and Robinson 1966) and precision hardware (CNC Electronics, Seattle, WA). Eye and target position feedback signals were processed with anti-aliasing, six-pole Bessel filters (200 Hz) prior to digitization at 1 kHz with 16-bit precision using CED-Power1401 hardware (Cambridge Electronic Designs, Cambridge, England). Velocity and acceleration data were filtered using an 80-point finite impulse response (FIR) digital filter with a passband of 50 Hz. Saccades were removed from smooth pursuit traces using a manually-overseen custom detection algorithm in MatLab (MathWorks, Natick, MA). The

missing eye data was replaced with a linear interpolation between the pre- and post-saccadic regions of data.

Single-unit activity was recorded using modified commercial glass- or epoxy-coated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA; Frederick-Haer Corporation, Brunswick, ME) with the impedance ranging from 0.5 to 5 MΩ. Spike2 software was used for data acquisition and preliminary offline analyses, including 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 et al. 1987).

3.3.3 *Localization of FEFsem*

We verified the location of our neurons using functional criteria (e.g. response directionally tuned during voluntary smooth pursuit eye movements) and stereotaxic location. Prior to surgery, magnetic resonance imaging (T-1 weighted, fast spin-echo; Siemens 3T magnet) was used to localize the FEF and MST. Recording chambers were then stereotaxically implanted with centers at anterior 22, lateral 14 (at a 10° angle) for FEF. The locations were also verified using depth measurements taken from microdrive readings while FEFsem neurons were being recorded. These corresponded to depths expected from each animal's MRI.

3.3.4 *Behavioral paradigms*

All visual stimuli were rear projected on a tangent screen 57 cm away from the monkey, and were delivered using computer-controlled two-axis mirror galvanometers (General Scanning, Watertown, MA) and appropriate optic bench hardware. Monkeys were trained to track a small-diameter target spot (0.2°; produced by a red laser light emitting diode), moving in sinusoidal or

step-ramp trajectories (Rashbass 1961). Monkeys were also trained to perform fixation and visually-guided saccade tasks.

Neurons were first tested for responsivity to smooth pursuit eye movements or saccades. Neurons classified as saccade-related were not included in this study. Neurons that responded during pursuit of the target spot moving at low frequency (0.15 – 0.35 Hz) were included. The neuronal activity was recorded while the target moved in one of the eight cardinal directions, and the neurons were ultimately tested using step-ramp motion in the preferred and anti-preferred directions for the given neuron. The target moved at 7, 15 and/or 30°/s, with an excursion of 15°.

Neurons were then tested with a large-field (LF) motion task. The target spot continued to move in a step-ramp trajectory, but in this case was accompanied by a large, textured background (over 50°x50°) moving with the same trajectory. The step-ramp motion was towards the preferred and anti-preferred directions of the neuron. Due to technical limitations, the LF trials were not interleaved, and were instead delivered in one block.

Neurons were also tested with a target blink task (Newsome et al. 1988; Tanaka and Fukushima 1998; Ono and Mustari 2006). In this task, the target spot was extinguished for 150 ms during step-ramp tracking, starting at various times after target motion onset (50, 100, 200, 300, 400, or 500 ms). Trials with and without blinks were randomly interleaved.

3.3.5 *Data analysis*

Averaged data, taken from at least 10 trials, was used to calculate neuronal and eye movement latencies. The times at which the neuronal response and eye velocity exceeded three standard deviations above baseline (the 100 ms prior to target motion onset) were designated the neuronal and eye movement latencies, respectively. The overall latency was then expressed as

the difference between eye and neuron, such that negative values represent neurons that began responding before eye movement onset.

We also used multiple linear regression modeling to reconstruct the response profiles of neurons using a combination of eye position (E), velocity (E'), and acceleration (E''), as well as retinal position error (R) and velocity error (R') (Ono and Mustari, 2009). Retinal error parameters were calculated as the difference between target and eye motion. The impulse in target velocity due to differentiation of the step in target position at the beginning of the trial was removed prior to its use in the model. Additionally, target acceleration was set to $0°/s^{2,}$ as the step in target velocity results in no steady-state target acceleration. Data averaged from at least 10 trials was used to calculate coefficients in the following model:

$$FR(t) = A + BE(t+\tau_1) + CE'(t+\tau_1) + DE''(t+\tau_1) + GR(t+\tau_2) + HR'(t+\tau_2)$$

in which FR(t) represents the spike density function at time t, E(t) denotes the eye position at time t, and coefficients are defined by terms A-D, G, and H. Latency of unit activity is represented by both τ_1, which is the unit response time with respect to pursuit onset, and τ_2, which is the response with respect to target motion onset. Thus, the model attempts to account for the unit activity using a combination of eye and retinal motion parameters. The coefficient of determination (CD) was used to determine goodness of fit, and a series of τ_1 and τ_2 values were used to find the maximum CD (Ono et al. 2005).

Partial correlation (r) values were also calculated for each component to estimate its relative contribution, while controlling for the other parameters. All statistical tests used a significance value of 0.05 unless otherwise specified.

To quantify the response to the blink task, 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 visual processing delays

(Newsome et al. 1988; Tanaka and Fukushima 1998). This blink average was compared to averages over the same interval for control trials, and the difference was expressed as percent change. Negative changes indicate that the unit was less active during the blink than during control trials, whereas positive values indicate that the cell increased its activity during the blink as compared to control. Blink timings were grouped into early (50 and 100 ms) and late (300-600 ms), to assess whether blink responses changed between the initiation and maintenance phases of pursuit.

Similarly, to quantify the response to the addition of LF motion during step-ramp tracking, the percent 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. These timeframes allow for easy comparison to the results of the blink task, and again allow for the assessment of any change between the initiation and maintenance phases of pursuit.

Neurons whose responses began after the early blink interval or the LF initiation interval were not included in analyses using those values. Thus, there are fewer neurons included in analyses involving the early part of the trial as compared to later on.

3.4 RESULTS

3.4.1 *Smooth pursuit with large-field motion*

We assessed the effects of adding concurrent large-field (LF) motion to a step-ramp tracking task in three monkeys (**Figure 3.1**). 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 to control, however, monkey F actually showed the opposite effect (**Table 3.1**). The gain in LF trials as compared to control also showed similar results: monkeys T and B had gains greater than 1.0 during the initiation interval (50-200 ms after target motion onset, first grey shaded region **Figure 3.1**), monkey F had a gain of 0.9 (**Table 3.1**).

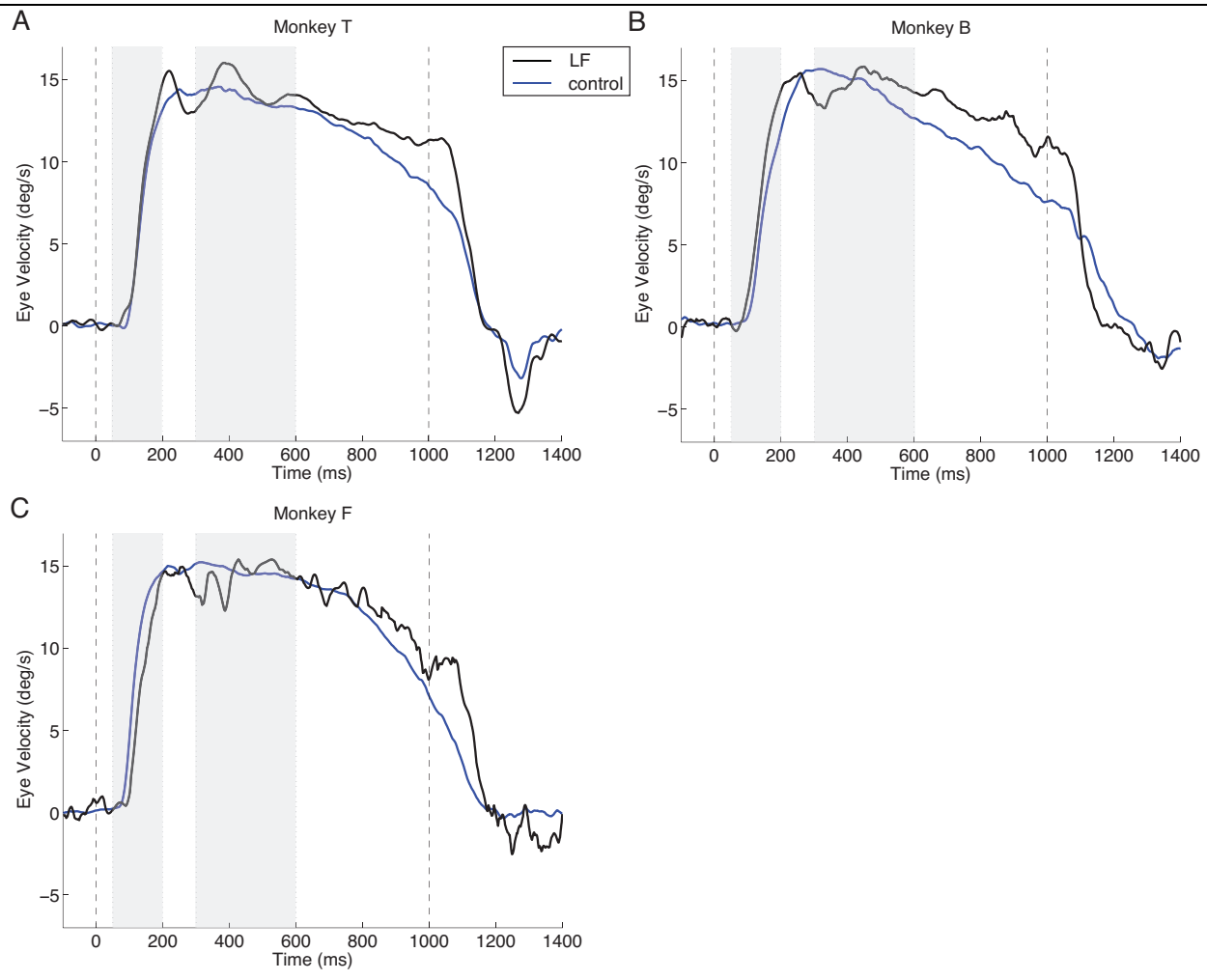


Figure 3.1. Smooth pursuit in combined volitional and reflexive task. A: Average eye movement traces for Monkey T. The blue trace represents control trials (N=921) with no LF motion, and the black trace represents trials with concurrent LF motion (N=532). Target motion onset and offset at 0 and 1000 ms, respectively, indicated by the dashed vertical lines. The grey 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.

Table 3.1. Gain and latency for trials with LF motion

	Monkey T	Monkey B	Monkey F
Initiation (gain)	1.1	1.3	0.9
Maintenance (gain)	1.0	1.0	1.0
Whole Interval (gain)	1.1	1.1	1.0
Control latency (ms)	86	93	69
LF latency (ms)	67	66	89
N (trials)	532	202	90

Table 3.1 Gain is the ratio of the eye velocity for trials with LF motion compared to 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). Behavioral latency for control trials and LF trials, given as time following target motion onset. Number of trials included for each animal.

Following the initiation interval, the eye velocity in LF trials briefly decreased before rebounding to control levels again, though only monkey T exhibited an eye velocity that overshot control levels (**Figure 3.1**A). Monkey B had only one such decrease (**Figure 3.1**B), while monkeys T and F arguably have two separate cycles of this behavior (**Figure 3.1**A and C). 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 3.2**A), and calculated the differences between the peak and subsequent trough (**Figure 3.2**B). The peak-trough differences were all around 2.0-2.5°/s, and similar across animals and cycles of peak-trough behavior. However, monkey B only exhibited one cycle of peak-trough behavior.

Though this peak-trough behavior is present at the very beginning of the maintenance interval (300-600 ms after target motion onset, second grey shaded region **Figure 3.1**), the gain during that interval is equal to control levels due to the subsequent increase in eye velocity for all monkeys (**Table 3.1**). This comparatively greater eye velocity continues throughout the duration of pursuit, becoming even more noticeable towards the end of target motion and pursuit offset (**Figure 3.1**). This is also represented in the greater-than-1.0 gain observed for the whole pursuit interval for monkeys T and B (**Table 3.1**).

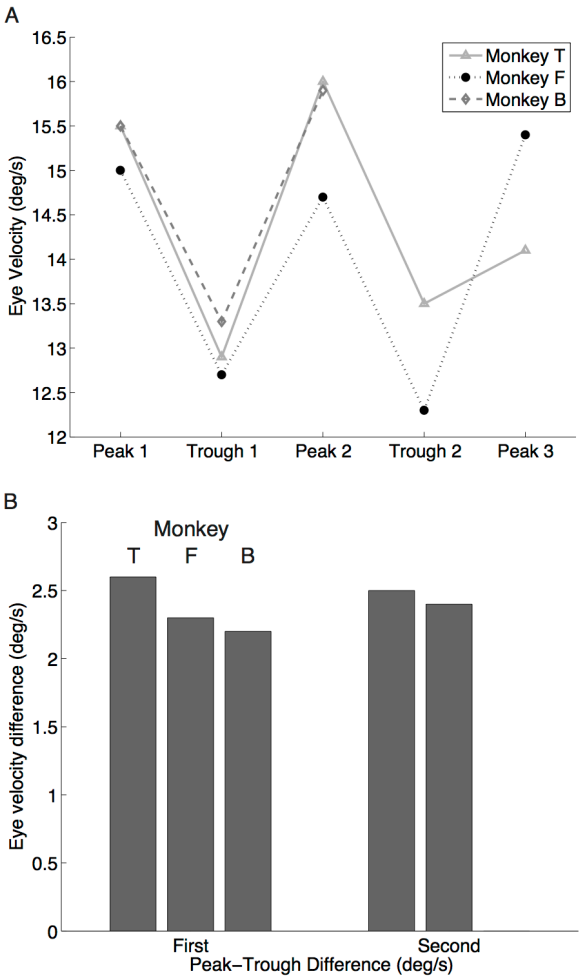


Figure 3.2. Quantification of behavior. A: Eye velocity at peaks and troughs during pursuit, as seen in **Figure 3.1**. Each line represents behavior from an individual monkey. Monkeys T and F both had two quantifiable peaks and troughs, whereas monkey B only exhibited one. B: Peak-trough differences in eye velocity for each monkey.

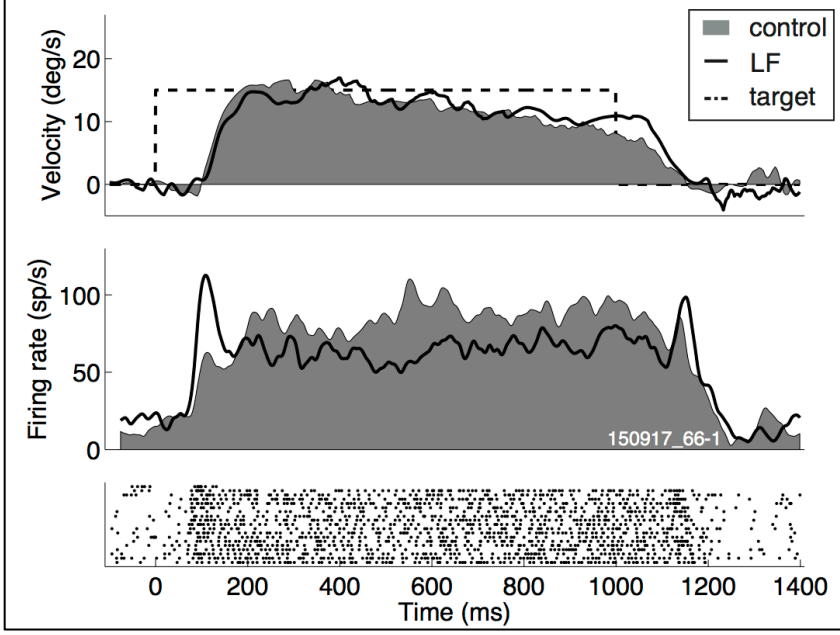


Figure 3.3. Example FEFsem neuronal activity during combined volitional and reflexive pursuit. Top panel shows the target velocity (dashed line), average eye velocity in control trials (grey shaded region, N=26), and LF trials (black line, N=19). Middle panel shows the average spike density function for control and LF trials; conventions as in the top panel. The bottom panel shows the raster plot for the LF trials. Data from Monkey T.

3.4.2 *Response of individual FEFsem neurons during pursuit with large-field motion*

We included a total of 43 FEFsem neurons recorded from three monkeys for 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. An example FEFsem neuronal response to step-ramp tracking in both control and LF trials is shown in **Figure 3.3**. The eye velocity and firing rate traces are broadly similar between control (N=26, grey shaded region) and LF trials (N=19, black line). The latencies are also similar 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°/s in LF trials compared to 11.1°/s in control trials), though they exhibit similar dynamics overall (**Figure 3.3**, top panel).

Interestingly, there are some notable differences in the neuronal activity between control and LF trials (**Figure 3.3**, middle panel). In the control trials (grey shaded region), there is a fairly 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, transient peak that coincides with the time the eyes stop moving, following which the neuronal activity returns to baseline levels. Though there are some similarities between the control and LF neuronal responses, the transient peaks featuring higher activity than control and the sustained activity at lower levels than control are conspicuous differences.

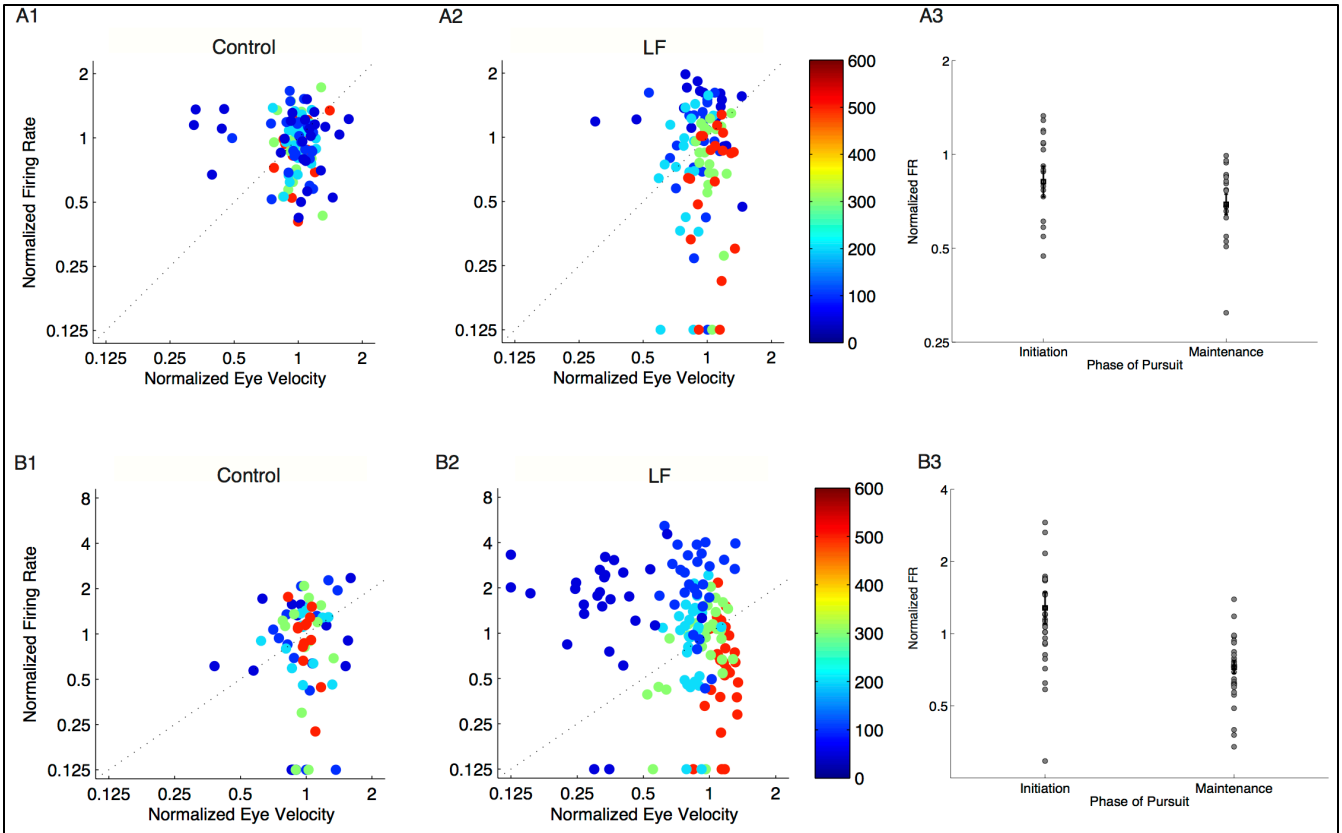


Figure 3.4. Relationship of firing rate to eye velocity in two representative FEFsem neurons. The neuron in A is the same as from **Figure 3.3**. A1: Data from control trials. Average of firing rate and eye velocity were taken over intervals of 50 ms on individual trials, normalized by the average for all control trials during that interval (N=26). The color of the points indicates the time during the step-ramp trial that the interval began, as shown by the color bar. Dotted line represents unity. Both firing rate and eye velocity are plotted on a log2 scale. A2: Data from LF trials (N=19). Same quantification procedure as in A1, but individual LF trial interval averages were normalized by the average from all control trials. A3: Comparison of normalized firing rate during initiation (50-200 ms after target motion onset) and maintenance (300-600 ms) during LF trials. Grey points are the normalized firing rate in individual trials, normalized by the average during the same interval for all control trials. Mean and 95% confidence intervals shown atop in black. There is no significant difference between initiation and maintenance ($p = 0.1$) B1-3: Same conventions as in A1-3. Control trials (N=13), LF trials (N=28). Data from Monkey T. B3: Normalized firing rate during initiation is significantly greater than during maintenance ($p < 0.01$)

To investigate whether the observed differences in neuronal activity (**Figure 3.3**) could be related to differences in eye velocity (**Figure 3.1** and **Figure 3.3**), we directly assessed the relationship between firing rate and eye velocity (**Figure 3.4**). Here, we show two representative FEFsem neurons and their sensitivity to eye velocity. In **Figure 3.4**A1-2, we compare

normalized eye velocity to normalized firing rate throughout the pursuit interval for both control and LF trials. This is the same neuron whose activity was shown in **Figure 3.3**. Each point represents the average firing rate and eye velocity over a 50 ms interval on an individual trial, compared to the average for all control trials in that interval. The color of the data point and accompanying color bar indicate the timing of the interval with respect to target motion onset.

For both control and LF trials (**Figure 3.4**A1-2), though there is variability in both normalized eye velocity and firing rate, there does not appear to be a relationship between them. However, it does seem evident that the points representing the earliest intervals in LF trials exhibit a higher normalized firing rate than those intervals later in the trial, though this is not due to differences in eye velocity (**Figure 3.4**A2). This is in accordance with the transient peak observed at the onset of the neuronal response in **Figure 3.3**. Additionally, this difference in activity between initiation and maintenance is visible in a direct comparison between these two intervals, seen in **Figure 3.4**A3. In this case, the average firing rate was calculated over the initiation (50-200 ms) and maintenance (300-600 ms) intervals in LF trials and normalized by the average from the same intervals in all control trials. Though they look visually distinct, the normalized activity in the initiation and maintenance intervals are not significantly different for this neuron (Student's t-test, $p = 0.1$).

The second example FEFsem neuron shows similar results (**Figure 3.4**B). Again, there is no clear relationship between neuronal activity and eye velocity in control trials (**Figure 3.4**B1). Though it looks as though there could possibly be some relationship between eye velocity and firing rate in LF trials, with the dark blue points having the highest activity and lowest velocity and the red points having the lowest activity and highest velocity (**Figure 3.4**B2), it is clear that this is not the case given the lack of relationship between the two variables in control trials.

Therefore, there is some effect of the addition of the LF motion over time that changes the activity of the neuron, independent of the effect on eye velocity. This is borne out in the direct comparison of the normalized firing rate during the initiation and maintenance intervals in **Figure 3.4**B3. The normalized firing rate in the initiation interval is significantly greater than that in the maintenance interval (Student's t-test, $p < 0.01$).

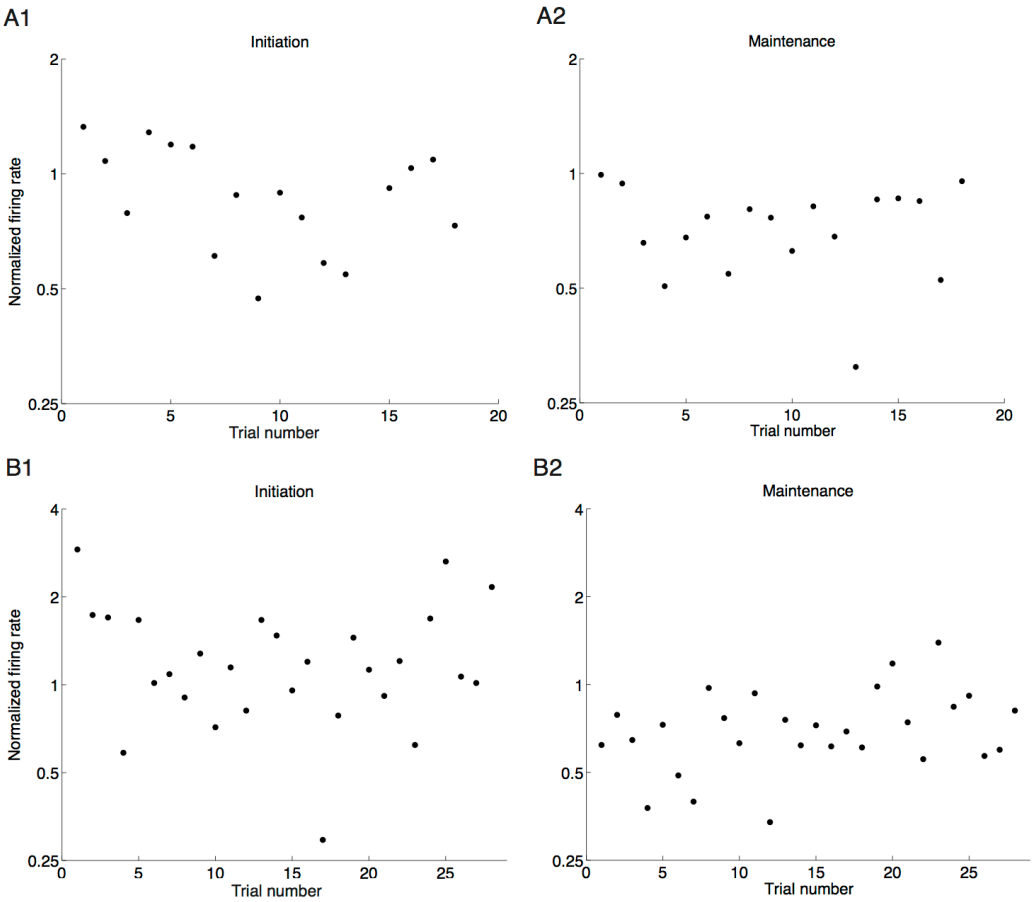


Figure 3.5. Neuronal response in two example FEFsem neurons across trials. A: Neuron from **Figure 3.3** and 3.4A. A1: Normalized firing rate during the initiation interval (50-200 ms after target motion onset) across trials within LF block. Each black point represents the average firing rate in one LF trial normalized by the average from all control trials. A2: Same conventions as A1, but for the maintenance interval (300-600 ms). B1-2: Same conventions as A1-2, but for the neuron from **Figure 3.4**B.

Because the conditions were blocked, we also wanted to assess the firing rate across trials within an LF block. To do so, we used the same quantification procedure as in **Figure 3.4**A3, and compared the normalized firing rate across trials within the LF block (**Figure 3.5**). **Figure 3.5**A consists of data from the same neuron as in **Figure 3.4**A, and **Figure 3.5**B is from the same neuron as in **Figure 3.4**B.

In **Figure 3.5**A1, it seems as though the activity in the initiation interval in LF trials is greater than control levels for the first several trials, and then begins to decrease approximately through trial 15. The normalized firing rate is certainly variable across trials, making it difficult to definitively conclude that there is a systematic change in the neuronal activity as the LF block progresses, but it is possible that there is some sort of habituation or other change going on. This is quite different than what is observed in the maintenance interval (**Figure 3.5**A2), which has a relatively flat level of activity across all trials.

In our second example neuron (**Figure 3.5**B), though the normalized activity level is variable throughout the LF block, there is no evidence of a systematic shift in the amount of activity for either the initiation (**Figure 3.5**B1) or the maintenance intervals (**Figure 3.5**B2). It is clear, though, that there is a comparatively higher level of activity in initiation than maintenance, as was shown in **Figure 3.4**B3.

Using this kind of cross-trial assessment, we found that 27 neurons (66%) did not show any evidence of a systematic shift in activity across trials, much like the example neuron shown in **Figure 3.5**B. The remaining 13 neurons (33%) could not be definitively classified as showing or not showing evidence of a systematic shift, like the neuron in **Figure 3.5**A. This analysis suggests that the responses of our population would not be different with interleaved conditions. The implications of this are discussed below.

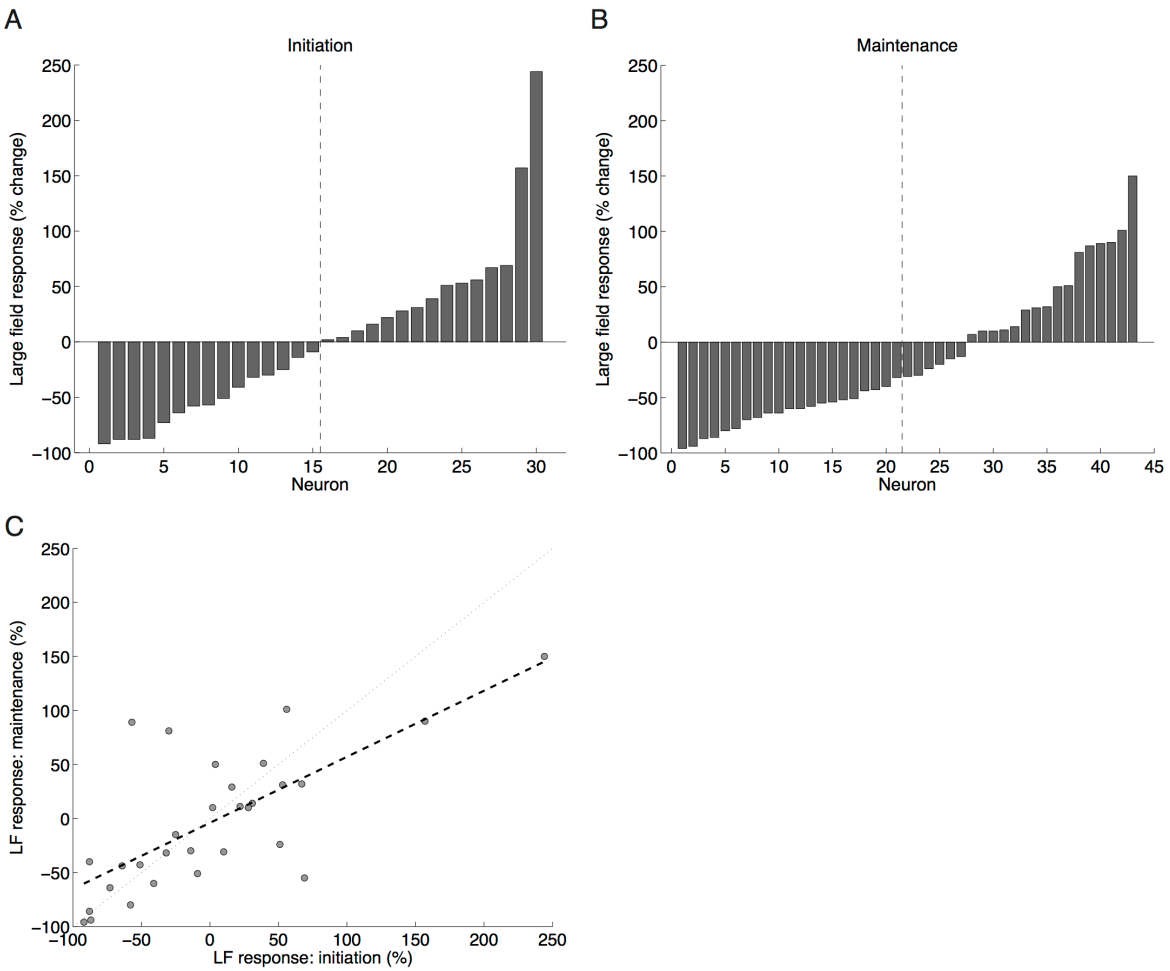


Figure 3.6. Distribution of neuronal responses across the FEFsem population. A: Distribution of responses for the initiation interval; each bar represents an individual neuron's response (N=30). Difference in firing rate between control and LF trials expressed as percent change. Negative numbers indicate firing rates that are lower in LF trials than control. Median of responses shown by dashed vertical line (-3.5%). B: Same conventions as in A, but for the maintenance interval (N=43). Median = -31%. C: Comparison of LF responses during initiation (LF_{init}) and maintenance (LF_{maint}). Each grey point represents data from one neuron. Dotted line represents unity, and the dashed line is the line of best fit (r^2=0.511, $p < 0.01$), with the equation: $LF_{maint} = 0.61(LF_{init}) - 4.02$.

3.4.3 *FEFsem population response during pursuit with large-field motion*

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 3.6**). We expressed the difference as a percent change from control levels. In this scheme, negative differences represent neurons whose activity levels in LF trials were less than

control levels. We assessed this LF response in both the initiation interval (LF_{init}, **Figure 3.6**A; N=30) and the maintenance interval (LF_{maint}, **Figure 3.6**B; N=43). For the initiation interval, we excluded any neuron whose latency was sufficiently long so as to have little or no response within the interval. In both cases there is a broad range of responses from highly negative to positive. The median value for initiation is -3.5% (dashed line, **Figure 3.6**A), which is the point in the distribution at which the responses go from negative to positive. The median value for maintenance is -31% (dashed line, **Figure 3.6**B), which is solidly in the negative portion of the distribution. This indicates that a greater proportion of neurons exhibit lower activity levels than control during the maintenance interval as compared to initiation.

To determine whether the LF response at one time bares any predictive value for the LF response at another time, we compared the LF_{init} and LF_{maint} responses (**Figure 3.6**C). There is a strong, positive relationship between the two LF responses, indicated by the best-fit line (dashed; r^2=0.511, $p < 0.01$). This indicates that the LF response for the majority of neurons is relatively reliable over time.

Given that the differences between LF and control smooth pursuit are generated by characteristics of retinal image motion, we wanted to compare the effects of LF motion during pursuit to another manipulation of retinal input: the target blink. This comparison can be seen in **Figure 3.7**. 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 percent change following the target blink compared to control levels in the same interval. Again, negative responses represent neurons that were less active following the blink as compared to the same interval in control trials.

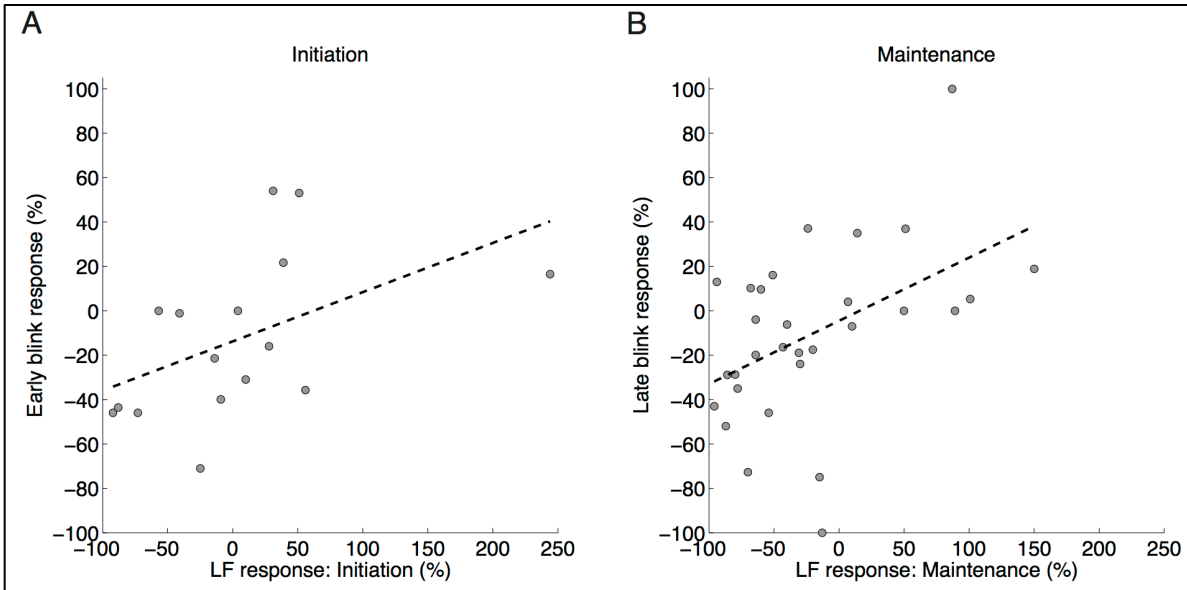


Figure 3.7. Comparison of LF and blink responses in the FEFsem. A: Comparison of the LF response during initiation (50-200 ms after target motion onset) and the early blink response (110-310 ms). Each grey point represents data from one neuron (N=16). Dashed line is the line of best fit (r^2=0.241, p = 0.05), with the equation: Early Blink Response = 0.22(LF_{init}) – 13.80. B: Same conventions as in A, but for the LF response in the maintenance interval (300-600 ms), and late blink response (360-710 ms; N=30). The line of best fit (r^2=0.22, $p < 0.01$) has the equation: Late Blink Response = 0.28(LF_{maint}) – 4.53.

We compared the LF_{init} response to the early blink response (**Figure 3.7**A, N=16), and did not find a significant relationship, though there seems to be a trend towards a positive relationship (dashed line, r^2=0.241, p = 0.05). This relationship was even stronger for the maintenance interval (**Figure 3.7**B, N=30), with those neurons exhibiting the most negative LF responses also exhibiting 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=0.22, $p < 0.01$). In the discussion, we consider the role of the FEFsem in combined volitional and reflexive pursuit, as well as the relationship of retinal and extraretinal signals to these processes.

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

3.5.1 *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 and Miles, 1986; Gellman et al., 1990). Additionally, it has been shown that the gain of reflexive ocular following smooth eye movements 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 8 of 9 intervals analyzed (3 intervals for 3 monkeys). The average gain in initiation is 1.10, while in maintenance it is 1.07, indicating that the eyes begin moving sooner and move faster in the presence of LF motion.

Interestingly, there is less stability in the initiation of pursuit in LF trials than control (**Figure 3.1** and 2). 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. For two of the animals, there are two cycles of this peak-trough behavior, while the other animal exhibits only one cycle. Compared to the control trials, it takes

longer for the eye velocity to settle and reach steady-state. This suggests that though the LF motion causes the eyes to start moving faster and earlier than control, it induces more uncertainty as to the actual movement of the target. This uncertainty results in increased image velocity on the retina, and the animal seems to be less able to utilize extraretinal signals to override these changing retinal errors.

Following this ringing, 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, continuing through another 100 ms after target motion ceases. This suggests that though 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 in a reflexive task.

3.5.2 *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 and Lisberger, 2001, 2002; Ono and Mustari, 2009). However, these neurons are not thought to play these roles for reflexive eye movements, which are thought to be subserved by the MT-(MST)-DLPN pathway. Thus, one reasonable hypothesis is that the neurons in the FEFsem will be less active for a task that combines reflexive and volitional eye movements.

Though 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. While eye velocity was at or above control levels, the firing rate was often

below control levels, with a median response that was 11% less than control activity over the whole pursuit interval.

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. When comparing normalized firing rate to normalized eye velocity in control trials, no neurons had a linear fit that accounted for more than 10% of the variance, suggesting that very few of the tested neurons are strongly sensitive to eye velocity. In contrast, when performing the same comparison for LF trials, the number of neurons with linear fits accounting for more than 10% of the variance is 8 out of 41 (20%). This means that the majority of FEFsem neurons tested exhibit limited eye velocity sensitivity in both control and LF tasks, suggesting that differences in firing rates between LF and control tasks are not due to increased eye velocity.

For the small minority of cells that exhibited more eye velocity sensitivity during LF trials, it is still unlikely that the differences in activity are due to augmented eye velocity, given the lack of sensitivity during control trials. The presence of the LF motion must cause differential effects during the initiation and maintenance intervals (an example of which can be seen in **Figure 3.4**A3). Similar to what was shown in the previous chapter (Bakst et al. 2016 *Submitted*), this could be due to changing weights of retinal and extraretinal signals throughout the timecourse of pursuit.

In addition to the influence of eye velocity, we also wanted to assess the effects of LF motion on neuronal activity across trials. As technical limitations rendered us unable to interleave LF and control trials, we frequently presented these tasks in separate blocks, with the preferred and anti-preferred directions randomly interleaved. Most neurons (66%) show no evidence of systematic changes in activity throughout the block, but the other 33% could not have such an

effect ruled out. These possible systematic changes generally took the form of gradual decreases in activity over the course of the block within a given analysis interval (e.g. initiation). The data in **Figure 3.5**A1 provide an example.

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 and Lynch, 1996; Stanton et al., 2005), there may be some form of habituation or a reweighting of signals to lessen the impact of this continuously-present LF background as the block progresses. Whether such habituation, if it is indeed taking place, follows from similar habituation in earlier structures like 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.

3.5.3 *FEFsem population response and comparison to other neuronal sensitivities*

Overall, the FEFsem neuronal response is less in LF trials than control. This is observed to a limited extent in the initiation interval, with a median neuronal response that is 3.5% less than control, and to an even greater extent during the maintenance interval with a median response that is 31% lower than control levels. These differential effects could be due to the fact that, though the combined pursuit relies more on pathways for reflexive pursuit, the large pulse of image motion at the beginning of each trial is sufficient to replace the lower activity levels in the FEFsem. Thus, we see activity in the initiation interval that, on average, is similar to control levels, while later on in the trial, when there is less image motion, there is a larger decrement in neuronal activity.

This would also explain the tight correlation between the LF responses during initiation and maintenance. The overall contribution of the FEFsem neurons to the behavior could be

unchanging throughout pursuit interval, but the strength and importance of the image motion does change between initiation and maintenance. Thus, the increased contribution from image motion early on in the trial masks the decreased involvement of the FEFsem in the combined reflexive-volitional behavior.

The LF response is also moderately correlated with the blink response. Though the results from the initiation interval are not quite 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 and r^2 values. Interestingly, this means that taking away the visual target induces a similar response to the addition of retinal input with the LF background. This could potentially be due to neurons being sensitive to anything that "disrupts" the expected step-ramp pursuit task, like the removal of the target or the addition of other visual stimuli.

For neurons that increase their activity in response to both "disruptions," 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. In the case of the LF motion, the textured background is composed of many spots, dispersed randomly across the screen. These spots could ostensibly be targets of pursuit, though only the red laser spot is rewarded. It is therefore possible that this compensatory activity seen in the blink trials also provides an increased attention or gain signal for the retinal input from the rewarded target spot. In order to out-compete all of the other possible visual targets, the system may increase the gain or weight of the signals coming from the target spot.

In contrast, neurons may decrease their activity in response to both types of "disruption," possibly due to the lack of signals propagating from earlier visual areas like MT and MST. In the case of the target blink, the retinal signals are no longer present in areas dependent on retinal input, like MT, and this in turn decreases the input to MST and FEFsem. In the case of the additional LF motion, though there is a great deal of retinal input, it may be flowing through pathways designed to handle full-field stimuli rather than small spots. These possibilities could explain the seemingly paradoxical findings that both subtracting and adding retinal stimuli have the same effects in individual FEFsem neurons.

3.5.4 *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 initial pursuit less stable, possibly due to a diminished ability to utilize extraretinal components to override early retinal image motion.

Chapter 4. CONCLUSIONS

4.1 SUMMARY

In this thesis, I have explored the role of the FEFsem in the generation of smooth pursuit eye movements, primarily by manipulating the retinal information available to the pursuit system. These experiments have revealed several insights about the pursuit system as a whole, and the FEFsem in particular.

In chapter two, I used a target blink task to test the relative contributions of retinal and extraretinal components in the activity of FEFsem neurons, as well as in pursuit behavior itself. Confirming prior results in humans (Bogadhi et al., 2013), I established that the pursuit system as a whole exhibits changing reliance on retinal signals throughout the duration of pursuit. Additionally, beyond confirming the presence of extraretinal signals in FEFsem (Tanaka and Fukushima, 1998), I assessed the relative contribution of these retinal and extraretinal signals to the activity of individual neurons. For many neurons, these contributions are not fixed. Building on this, I used multiple linear regression modeling (e.g., Ono et al. 2009) to assess the sensitivity of each neuron to eye movement and visual motion parameters. Combining these findings, I used a k-means clustering analysis to assess whether distinct subgroups of neurons exist in the FEFsem. Though there appears to be a continuum of responses rather than discrete groups, the relative contributions of retinal and extraretinal signals combined with eye and visual motion parameters provides clues as to the likely function of individual neurons in pursuit behavior.

In chapter three, I used a large, textured background moving in conjunction with a small target spot to investigate the coordination of volitional and reflexive behavior. As it is thought

that the MT-MST-DLPN pathway underlies reflexive ocular following behaviors and the FEFsem-NRTP pathway is likely vital for volitional pursuit, I hypothesized that a task requiring less volitional drive would result in lower levels of activity in the FEFsem. Indeed, I found that FEFsem neurons are less active for combined volitional and reflexive pursuit than for purely volitional pursuit on average. Additionally, by comparing the neuronal responses to LF motion and target blinks, I found that FEFsem neurons might respond to pursuit "disruptions" similarly, regardless of whether the disruption comprises the addition or subtraction of retinal input. Some neurons seem to decrease their activity during such disruptions, while others increase their activity, seemingly compensating for the difference in pursuit drive coming from the retinal input.

Together, these findings support a conception of the FEFsem as being involved in the generation of the eye movement – providing a driving signal for pursuit initiation – as well as in more complex functions like gain control and anticipation. Below, I discuss the implications of these results in terms of proposed models of pursuit. Additionally, many questions remain unanswered in the quest to determine the likely role(s) of the FEFsem in the generation of pursuit. A discussion of these open questions and potential avenues for future work follows.

4.2 Models of Pursuit

Generating a successful model of a behavior is a meaningful step in understanding the mechanisms underlying that behavior. Specification of the input and output, as well as various parameters affecting the process, requires a high level of understanding and can generate testable predictions for future research. For pursuit, control systems models have been popular tools to describe likely components of the smooth pursuit system.

At the most basic level, models of pursuit include image velocity – the comparison between target motion and eye movement – as the initial input to the pursuit system (**Figure 4.1**). Appropriately, the output of these models is a command for eye velocity and the subsequent movement of the eye. Unsurprisingly, these features are common across many iterations of pursuit models. In contrast, it is the question of which internal computations are occurring and where that is controversial.

One of the main challenges to modeling smooth pursuit is the combination of the inherent delay in the system, and the limited image velocity present during accurate pursuit. These two aspects can result in highly unstable pursuit. The three most common models of pursuit each deal with this problem differently: the efference copy model (Robinson et al. 1986), the image motion model (Krauzlis and Lisberger 1989, 1994), and the tachometer feedback model (Ringach 1995). In the efference copy model, a copy of the eye velocity command is provided as feedback and this allows for the reconstruction of target velocity. This target velocity signal can then be used to drive the eye even in the absence of image velocity. In contrast, the image motion model suggests that instead of relying on an efference copy, the system uses a representation of the image acceleration, which helps to dampen oscillations that can occur due to instability. Lastly, the tachometer feedback model posits an eye acceleration feedback signal (as opposed to the eye velocity signal in the efference copy model).

Critically, for any model to successfully represent what is actually carried out in the brain, it must not only account for the behavior observed but there must also be signals in the brain matching the signals represented in the model. Signals related to target velocity and eye velocity have been reported in multiple areas (Their and Ilg 2005, Barborica and Ferrera 2002, Mustari et al. 2009), as have signals related to eye acceleration (Mustari et al. 2009, Ono and Mustari 2009,

Ono et al. 2005). However, there are limited reports of sensitivity to image acceleration (Price et al 2005, Ono et al. 2005).

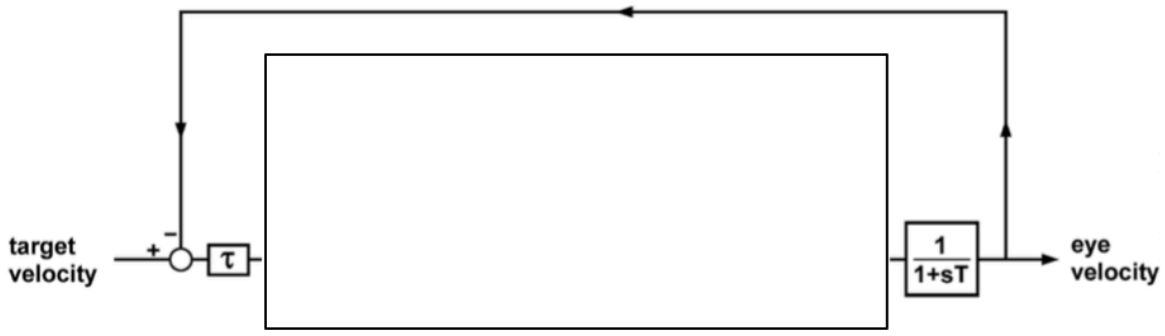


Figure 4.1. Basic features of control systems model of pursuit. Target velocity and eye velocity are compared at a summing junction to generate an image motion signal for input to the system. Eye velocity command and subsequent eye movement comprise the output of the model. The blank rectangle in the middle represents the inner computations that transform the input into the output. Other common elements are in Laplace notation. Adapted from *Lencer and Trillenberg 2008*.

More recently, these basic models have been modified to include other features of pursuit, like dynamic gain control and the effects of expectations, attention, and experience (Nuding et al. 2008, Bennett and Barnes 2004, Barnes and Collins 2008). Nuding and colleagues (2008) postulate a dynamic gain controller arising in either the FEFsem or MST, and add a multiplicative junction of image velocity and a function of eye velocity to represent this (**Figure 4.2**). The addition of online gain control was prompted by a number of studies showing that the gain of visuomotor transmission varies and is dependent on factors like internal expectations and the speed of ongoing pursuit (Schwartz and Lisberger 1993, Tabata et al. 2005, 2008, Tanaka and Lisberger 2001, 2002).

Barnes and colleagues (2004, 2008) do not put forth specific anatomical locations for their additional functions, but their model, which also builds on the efference copy model, includes a direct loop that allows for the influence of expectations and experience and an indirect loop that

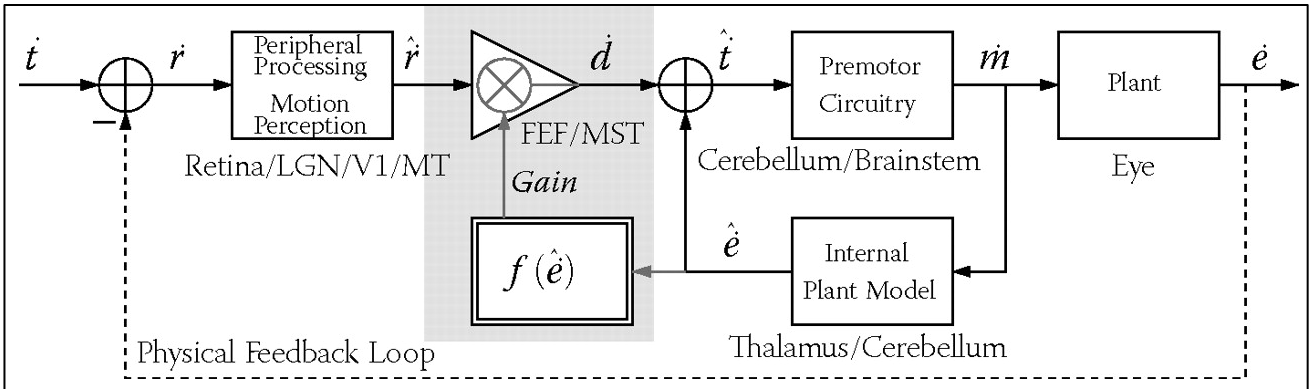


Figure 4.2. Control systems model of smooth pursuit with dynamic gain control. Expanded version of the Robinson et al. (1986) model, with putative locations in the brain. Dynamic gain control, postulated to take place in FEF and/or MST, involves a multiplicative combination of image velocity and a function of eye velocity. From *Nuding et al. 2008*.

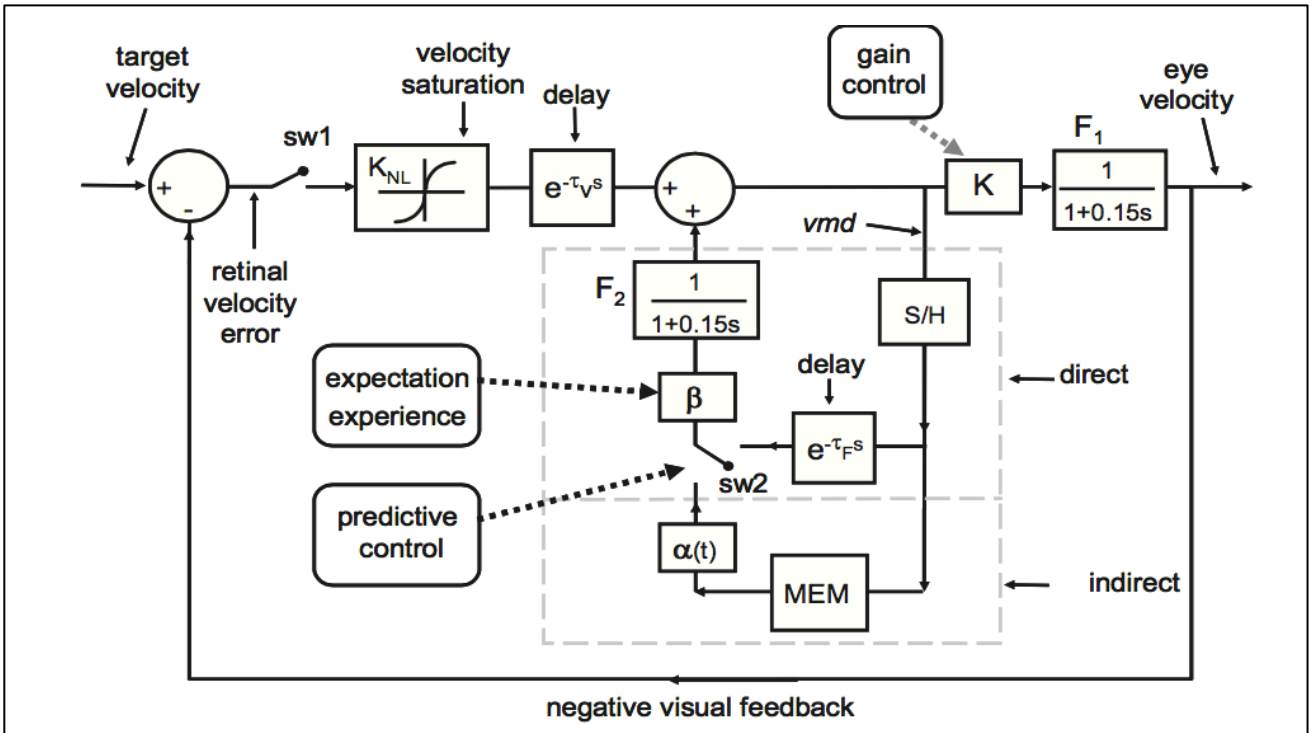


Figure 4.3. Control systems model of smooth pursuit with the added influence of expectation, experience, and prediction. Expanded version of the basic Robinson et al. (1986) model with direct and indirect loops facilitating the influence of internal expectations, prior experience, and predictions based on a short-term velocity memory store. From *Barnes and Collins 2008*.

facilitates predictive control via a short-term velocity memory store (**Figure 4.3**). It is reasonable to hypothesize that the FEFsem plays an important role in facilitating functions like these for smooth pursuit, given prior findings (Tanaka and Lisberger 2001, 2002, Fukushima et al. 2011).

My results also provide additional support for a role for the FEFsem in these functions. In the absence of retinal input, as in the target blink task, the pursuit system must have some kind of compensatory signal to support ongoing pursuit. This signal could result from expectations or memory stores, for instance, and could facilitate anticipatory pursuit and increase the gain of visuomotor transmission to promote accurate tracking once the target has returned. I have shown that a subset of neurons in the FEFsem increase their activity in response to target blinks, and this is precisely the type of response one would expect from an area involved in compensating for missing retinal input. Additionally, many of these same neurons also increased their activity during pursuit in the presence of a concurrently moving background. This could again represent some sort of attention or gain signal meant to emphasize the retinal input from the rewarded target, thereby deemphasizing all of the additional retinal input.

Beyond the addition of ancillary signals like gain control and prediction, more recent models have also moved to incorporate uncertainty through the use of Bayesian inference (Bogadhi et al. 2013, Orban de Xivry et al. 2013). In these models, retinal and extraretinal signals (including memory, expectations, etc.) are weighted according to the amount of associated uncertainty (**Figure 4.4**).

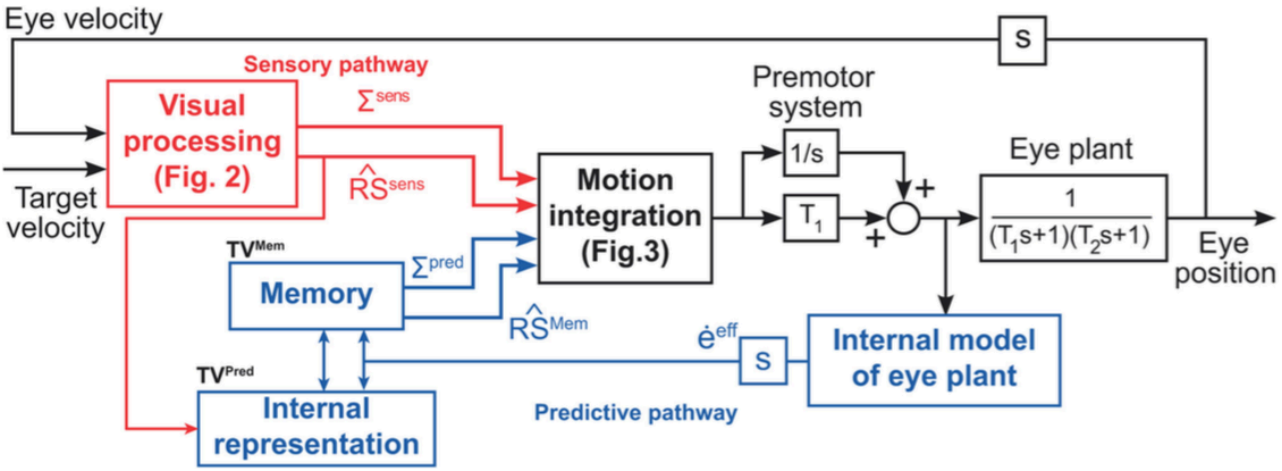


Figure 4.4. Model of smooth pursuit with Bayesian inference. Although notated slightly differently, this version of the model includes the traditional visual processing of retinal signals as well as extraretinal signals like internal expectations and memory. These retinal and extraretinal signals are then integrated using their associated uncertainties as weights. From *Orban de Xivry et al. 2013*.

These changing weights of retinal and extraretinal signals have been demonstrated at the level of behavior in both humans (Bogadhi et al. 2013) and now in non-human primates. Additionally, I have shown that these changing weights are observable even at the level of individual neurons in the FEFsem, and to some extent in MST. Although it remains unknown how this reweighting occurs, and its relationship to the uncertainty of the signals, we have shown clear evidence of the dynamic reweighting of retinal and extraretinal components, in support of models like those proposed by Bogadhi and Orban de Xivry and colleagues (2013). More work must be done to elucidate how these models might be instantiated in the brain, and the contribution of each area to these various pursuit modules.

4.3 Future directions

Over the last 40 years, much has been learned about the smooth pursuit system. By systematically describing pursuit behavior and characterizing the many brain regions likely playing a role in pursuit eye movements, scientists have established a rich foundational

understanding of the mechanisms underlying basic smooth pursuit. However, these advancements have mostly been within the realm of pursuit metrics. Much remains to be done to develop a full understanding of volitional pursuit in complex, natural conditions.

Some of the more recent pursuit models provide suggestions and testable predictions as to the integration of a plethora of extraretinal signals including anticipation/prediction, gain control, memory, reward/motivation, expectations, and prior experience, among others. Additionally, the role of uncertainty in the execution of pursuit eye movements, as well as its effects on the weighting of various signals is ripe for future investigation. Tasks designed to specifically manipulate these factors (e.g. the amount of uncertainty, prior experience, reward, etc.) would allow for a more precise description of their effects on the behavior as well as their representation in neuronal activity in areas like the FEF, SEF, and beyond. Even more specifically, the data presented in this thesis point to the existence of a subpopulation of FEFsem neurons that seem to be involved in some form of compensation for disruptions of smooth pursuit. These neurons are likely involved in some of these extraretinal functions like anticipation and/or gain control, and specific tasks designed to probe this functionality would be greatly informative. Though some research has already begun to use more complex tasks to identify more "cognitive" roles for areas like the FEF and SEF (e.g. Fukushima et al. 2011, Shichinohe et al. 2009), this is only a small fraction of what needs to be done to thoroughly elucidate the ways extraretinal signals influence pursuit behavior.

In addition to the development of tasks tailored to particular types of extraretinal signals, a complete description of how the brain generates smooth pursuit eye movements requires the characterization of the information flow between various nodes in the pursuit circuitry. Using antidromic activation, scientists can discern both anatomical and functional information about a

given neuron. This has been done to some extent, and a preliminary characterization of the information passed between several brain regions exists (e.g. Churchland and Lisberger 2005, Ono and Mustari 2009). However, there are many projections that have not been functionally characterized, and the ones that have largely relied on tasks that only gave us information about basic parameters like direction/speed tuning, latency, and eye and retinal motion sensitivity. Though technically difficult, these kind of anatomical and functional characterizations of information flow within the pursuit system would help take the extraretinal signals in control systems models out of the conceptual realm and establish how they are instantiated in the brain.

It is an exciting time for smooth pursuit research. Current experiments are moving towards a more integrated perspective, assessing the coordination of smooth pursuit and saccadic eye movements, the mechanisms of target selection, and the way attention and reward affect eye movement behaviors. Ultimately, a complete account of volitional smooth pursuit will require an understanding of the many "cognitive," sensory, and motor factors that influence behavior, and where and how they are represented in the brain. Though we have come a long way, there is still much to learn.

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APPENDIX A

List of Abbreviations

CL = central lateral nucleus of the thalamus
DLPFC = dorsolateral prefrontal cortex
DLPN = dorsolateral pontinue nucleus
FEF = frontal eye field
FEFsac = saccadic subregion of the frontal eye field
FEFsem = smooth eye movement subregion of the frontal eye field
fMRI = functional magnetic resonance imaging
FST = fundus of the superior temporal sulcus
IT = inferotemporal cortex
LIP = lateral intraparietal area
MD = medial dorsal nucleus of the thalamus
MF = movement field
MST = medial superior temporal area
MSTd = dorsal subregion of the medial superior temporal area
MSTl = lateral subregion of the medial superior temporal area
MT = middle temporal area
MTf = foveal subregion of the middle temporal area
PP = posterior parietal area
NRTP = nucleus reticularis tegmenti pontis
RF = receptive field
rNRTP = rostreal nucleus reticularis tegmenti pontis
rSC = rostral superior colliculus
SC = superior colliculus
SEF = supplementary eye field
SMA = supplementary motor area
STS = superior temporal sulcus
TEO = inferotemporal area TEO
TMS = transcranial magnetic stimulation
V1 = primary visual cortex/striate cortex
V2 = visual area V2/extrastriate cortex
V3 = visual area V3/extrastriate cortex
V4 = visual area V4/extrastriate cortex
VA = ventral anterior nucleus of the thalamus
VIP = ventral intraparietal area
VL = ventral lateral nucleus of the thalamus
VOR = vestibulo-ocular reflex