Learned timing of motor behavior in the smooth eye movement region of the frontal eye fields

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Summary

Proper timing is a critical aspect of motor learning. We report a relationship between a representation of time and an expression of learned timing in neurons in the smooth eye movement region of the frontal eye fields (FEFSEM). During pre-learning pursuit of target motion at a constant velocity, each FEFSEM neuron is most active at a distinct time relative to the onset of pursuit tracking. In response to an instructive change in target direction, a neuron expresses the most learning when the instruction occurs near the time of its maximal participation in pre-learning pursuit. Different neurons are most active, and undergo the most learning, at distinct times during pursuit. We suggest that the representation of time in the FEFSEM drives learning that is temporally linked to an instructive change in target motion, and that this may be a general function of motor areas of the cortex.

Keywords

smooth pursuit; motor learning; frontal pursuit area; temporal coding; representation of time; neuron-behavior correlations

Introduction

Young children jumping rope soon learn the importance of timing: jumping too early or too late can be as bad as failing to jump at all. Precise timing is critical to all aspects of motor control, at levels ranging from the coordination of joints and muscles during simple reflexive movements to the acquisition of complex skills such as playing a musical instrument. Indeed, timing is so important for motor control that it can be learned. There now are multiple demonstrations that the motor system can learn not just what to do, but also when to do it (Mauk and Ruiz, 1992; Medina et al., 2005; de Hemptinne et al., 2007; Doyon et al., 2009). In the smooth pursuit system, repeated presentations of a precisely-
timed instructive change in the direction of a moving target elicits a learned smooth pursuit eye movement that peaks near the time when the instructive motion is expected to occur (Medina et al., 2005; Carey et al., 2005).

The ability to learn timing in motor control requires a representation of time during movements. The most relevant temporal signals for motor control are typically on the order of tens to hundreds of milliseconds (Buonomano and Karmarkar, 2002; Mauk and Buonomano, 2004). In eyelid conditioning and smooth pursuit eye movements, learning is largest for an instructive signal that occurs in the range from 200-400 ms after the onset of a conditioned stimulus that references time (Mauk and Ruiz, 1992; Medina et al., 2005). Possible timing signals have been observed via imaging or electrophysiological studies throughout the brain, for example in the basal ganglia (Rao et al., 2001; Chiba et al., 2008; Jin and Graybiel, 2009), the cerebellum (Lewis and Miall, 2003; Smith et al., 2003), the prefrontal cortex (Sakurai et al., 2004; Oshio et al., 2006; Jin and Graybiel, 2009), the supplementary motor cortex (Shih et al., 2009; Onoe et al., 2001), and the parietal cortex (Leon and Shadlen, 2003). The next step is to establish a link between a representation of time and a neural expression of learning.

A prior paper from our laboratory reported a representation of time in the smooth eye movement region of the frontal eye fields (FEFSEM) (Schoppik et al., 2008). Each neuron in the FEFSEM reaches its maximal firing rate at a particular time during pursuit, and the peak responses of the full population tile the entire duration of pursuit. Thus, the representation of smooth pursuit in the FEFSEM is such that each neuron primarily contributes to a particular moment in the eye movement. In contrast, most of the brain regions in the pursuit circuit have stereotyped responses as a function of time during pursuit. Neurons in extrastriate visual area MT tend to have transient responses that are driven by, and time-locked to, the visual motion signals caused by the initial target motion (Newsome et al., 1988). Similarly, Purkinje cells in the cerebellar flocculus show transient responses that are well timed to the onset of target motion, followed by sustained responses that are monotonically related to the smooth eye velocity (Stone and Lisberger, 1990; Krauzlis and Lisberger, 1994).

The unique, temporally-selective representation of pursuit in the FEFSEM raises the possibility we tested here, that this cortical area plays a temporally-specific role in the modulation of pursuit through learning. We recorded changes in the responses of FEFSEM neurons during pursuit learning induced by a precisely-timed instructive change in target direction, to ask whether the learned eye movement would be driven selectively by neurons that contribute to pursuit around the time of the instruction. In agreement with this prediction, we found that the magnitude of learning in any given neuron is correlated with how strongly the same neuron would have responded (during pre-learning pursuit) at the time of the instructive change in target trajectory. We suggest that the representation of time within the FEFSEM may be harnessed to guide the temporal specificity of pursuit learning, and that temporally-specific modulation of motor behavior could be a general function of the motor regions of the cerebral cortex.

**Results**

We recorded from 100 FEFSEM neurons in two monkeys during directional smooth pursuit learning. The neurons we selected for investigation responded vigorously during pursuit prior to learning, and were tuned for the direction of pursuit. In the pre-learning behavioral block, we characterized the direction tuning of each FEFSEM neuron by measuring its mean firing rate during pursuit in each of eight directions spaced 45° apart. The neuron in Figure 1A responded most strongly for pursuit that was upward or obliquely up and left, and
therefore had a preferred direction between 90° and 135°. The neuron was only weakly active for purely horizontal pursuit to the right or left.

**Behavioral learning**

The tuning of the neuron under study specified the direction parameters of the learning experiment (see schematic in Figure 1B). We chose the learning direction to be the cardinal direction closest to the neuron’s preferred direction: 90° in Figure 1. The cardinal axis orthogonal to the learning direction defined the probe and control directions: 360° and 180° in Figure 1.

Each learning experiment began with a baseline block of trials that used step-ramp target motions in the probe and the control direction to establish the baseline pursuit response prior to learning. After the monkey fixated a stationary central target, the target stepped 2 or 3° in one direction and ramped immediately in the opposite direction at 20°/s (Figure 1H). For the probe trials in Figure 1F and H, the mean horizontal eye velocity was zero for almost 100 ms after target motion onset, accelerated to the right for 100 to 200 ms, and then approximated the target speed of 20°/s for the remainder of the 750 ms target motion. Vertical target velocity was zero throughout the trial, as was the mean vertical eye velocity prior to learning.

The subsequent learning block introduced learning trials that started like probe trials with a step-ramp of target motion in the probe direction, but underwent a predictable change in target direction at a fixed time. In Figure 1E and G, the initial 20°/s ramp motion took the target to the right. After 250 ms, an upward motion at 30°/s began so that the target moved up and to the right for 500 ms. The direction of the added component of target motion defines the learning direction; the 250 ms delay between the onset of target motion and the change in target direction defines the instruction time. Both the learning direction and instruction time were fixed for a given learning experiment. Learning trials comprised 45% of the trials in a learning block. The remaining 55% consisted of control trials (45%) and probe trials (10%), which were identical to the control and probe trials in the baseline block.

The average vertical eye velocity from the learning trials (Figure 1E, lower panel, red traces) shows a small upward deflection that starts before the instructive change in target direction and represents the learned response. The initial, early response is followed by a later, more abrupt, “visually-driven” change in eye velocity that is the immediate consequence of the instructive upward target motion. The learned response is not present in the first few learning trials, but grows rapidly and asymptotes after about 20-40 learning trials. This early, upward response reflects behavioral learning because it 1) precedes the onset of the instructive stimulus, and 2) occurs in the infrequent probe trials interspersed in the learning block, even though they lack an instructive change in target motion (Figure 1F, lower panel, blue trace). As reported before, the peak of the learned vertical eye velocity deflection in the probe trials coincides with the instruction time (Medina et al., 2005).

Our learning paradigm elicits robust, but short-term behavioral changes. For any given learning experiment, behavioral learning was quantified as the difference in mean eye velocity between the learning trials and the baseline probe trials, integrated across 100 to 320 ms (Figure 1E, grey shaded region). Integrating eye velocity yields the change in eye position. Behavioral learning averaged 0.8° in Monkey G (SD: 0.2°, range: 0.4° to 1.2°) and 2.1° in Monkey S (SD: 0.7°, range: 0.7° to 4.5°), and was significantly different from zero in all experiments (Mann-Whitney U test: p<0.001). Residual behavioral learning did not persist across learning experiments; the mean eye velocity measured in the sessions following training on a particular learning direction was not significantly different from the mean eye velocity in the sessions following learning in the opposite direction (Monkey G:

*Neuron. Author manuscript; available in PMC 2011 July 13.*
The rate of behavioral learning also did not vary as the study progressed. Behavioral changes continued to reach a plateau after about 20 to 40 learning trials. We conclude that learning proceeded anew for each experiment, so that we could pool neural data across recording sessions to assess the effect of directional pursuit learning on the activity of the population of neurons in the FEFSEM.

Neural correlates of learning

The example neuron in Figure 1 produced only a few spikes during the baseline block probe trials (Figure 1D, black raster), because the probe direction was orthogonal to the neuron’s preferred direction. During learning trials, the neuron produced the expected vigorous response to the visually-driven eye movement in the learning direction, and also acquired a small learned response that appeared before the instructive change in target direction (Figure 1C, red raster). The learned neural response also appeared in probe trials during the later part of the learning block (Figure 1D, blue raster) and, like the learned eye velocity, began before the time when the instructive change in target direction would have occurred in learning trials.

Different neurons expressed varying degrees of learning. The two neurons whose responses appear in Figure 2 were recorded on different days with strong behavioral learning that reached almost 4°/s by the time of the instructive change in target direction in both experiments (Figure 2 C, D). However, neuron #1 exhibited a large learned change in mean firing rate, while neuron #2 did not. Neuron #2 did respond strongly to the instructive change in target direction, but only after the visual latency of 70 ms typically found in the FEFSEM (Figure 2B, red trace; Gottlieb et al., 1994).

The learned change in firing rate, when present, had several important features. First, it appeared in temporal register with the learned change in eye velocity, in the interval preceding the visual input caused by the instructive target motion. Second, it was present in the probe trials in the learning block (Figure 2A, blue trace), and had a transient time course that peaked near the instruction time. Third, it appeared during target motion in a direction that did not evoke much neural activity before learning, as seen by comparison of the blue and black traces in Figure 2A. Therefore, the learned firing rate is related to the acquisition of a vertical response to the horizontal target motion and not to the horizontal eye movement itself, which changed very little as a consequence of learning (Figure 1F, top panel).

Figure 2 shows an important feature of the data that motivated our analysis procedures. The averages of both eye velocity and firing rate followed the same trajectory during learning trials and the interleaved probe trials, up to about 70 ms after the instruction time (Figure 2). Thereafter, the mean eye velocity and firing rate in the learning trials, but not the probe trials, showed large visually-driven reactions to the instructive change in target direction. The sequence of identical responses followed by divergence due to the visual stimulus is expected because the learning and probe trials were interleaved randomly. It allowed us to assess neural changes related purely to learning from the more frequent learning trials in the 220 ms interval from 100 ms after the onset of target motion to 70 ms after the instruction time.

We showed in Figure 2 that the size of the learned response could be very different across FEFSEM neurons even when the concomitant behavioral changes were similar. Only 35% of neurons (15/55 in Monkey G, 20/45 in Monkey S) exhibited a significant learned change in firing rate (Mann-Whitney U test: p<0.001). All neurons with statistically significant changes in firing rate showed increases in activity as a result of learning. Because the firing rate in the preceding fixation period almost always remained stable in spite of learning, we argue that the neural changes in the analysis interval probably are due to learning and not to
Neural preference for the instruction time predicts the magnitude of neural learning

Each neuron's response during pursuit of a ramp target motion at constant velocity showed a distinct and repeatable trajectory as a function of time (e.g. Figure 3A). The smoothed firing rate for this FEFSEM neuron increased rapidly after the onset of pursuit, peaked approximately 340 ms after the onset of target motion, and declined gradually thereafter. We defined the neural preference for a particular time during the pursuit trial as the firing rate at that time normalized for the peak firing rate. At 250 ms after the onset of target motion (intersection of dashed lines), this particular neuron had a neural preference of 0.7, indicating that it fired at 70% of its maximum. The neuron's preferred time was 340 ms after the onset of target motion. We measured neural preference from data acquired in the pre-learning pursuit block, using step-ramp target motion in the direction subsequently chosen to be the learning direction.

The preferred time varied widely across the full sample of FEFSEM neurons. In Figure 3B, each row uses color to depict the neural preference for a single FEFSEM neuron as a function of time. Neurons are ordered by the latency to 95% of their peak response. The narrowness of the red diagonal band indicates that the time of maximal neural activity is well defined, and its distribution across the full duration of the pursuit movement indicates that the population of FEFSEM neurons shows a wide range of preferred times. Thus, individual neurons are most active during limited distinct temporal chunks of the eye movement, only a fraction of the population is close to maximal response at any given time, and the population of FEFSEM neurons encodes all times throughout the entire movement. In our sample, preferred times were fairly evenly distributed across the full pursuit movement duration, with some preponderance of neurons that preferred the initiation of pursuit, from 100 to 200 ms after the onset of target motion (Figure 3C).

Much of the variation in the magnitude of learning across neurons was related to the wide range of neural preferences at the time of the instructive change in target direction. When we plotted the size of the mean learned response in each neuron as a function of its neural preference for the instruction time of 250 ms (Figure 3E), we obtained positive correlations that were statistically significant in both monkeys (Monkey G: r = 0.50, p<0.0001; Monkey S: r = 0.58, p<0.0001). Figure 3E uses the mean response averaged across all learning trials as an index of the magnitude of learning, but we obtained similar correlations when we estimated the magnitude of learning from the first or last 40 learning trials within each learning block.

Figure 3E shows the relationship between the neural preference at the single time of 250 ms during pre-learning pursuit and the magnitude of neural learning. For this one time point, the correlation coefficients were quite high. To judge the importance of neural preference at the time of the instructive change in target direction in determining its susceptibility to learning, we performed the same analysis shown in Figure 3E, except that we varied systematically the time used to obtain neural preference from 0 to 750 ms relative to the onset of target motion, and computed the correlation between neural preference at each time and the magnitude of neural learning for instructions delivered at 250 ms. For each monkey (Figure 3F), the size of learning across our sample of FEFSEM neurons showed the highest correlation with the neural preference near 250 ms, the time of instruction, and lower correlations with neural preference at earlier or later times. Thus, learning with an
instruction time of 250 ms engages neurons that specifically prefer 250 ms. The temporally-selective relationship between neural preference and the magnitude of neural learning in Figure 3F provides evidence that the distributed representation of time within the FEFSEM may be used to regulate the temporal specificity of pursuit learning.

As an alternate way to examine the relationship between the amount of neural learning in an FEFSEM neuron and its temporal preference during pursuit, we plotted the magnitude of neural learning as a function of the difference between the neuron’s preferred time and 250 ms (Figure 3D). There is considerable scatter in the plot, but for the population as a whole learning is largest in neurons with preferred times close to 250 ms, and is smaller in neurons with earlier or later preferred times. A small subpopulation of neurons exhibited negative learned responses, but the preferred times of these neurons were evenly distributed before and after the instruction time.

The size of neural learning also was positively correlated with the size of the learned eye velocity and the opponent response of the neuron, defined as the difference in mean firing rate between pre-learning pursuit in the probe direction versus in the learning direction, measured in the interval from 100 to 320 ms after the onset of target motion. Partial correlation analysis (Table 1) revealed that a strong correlation between the magnitude of neural learning and the neural preference for 250 ms persisted even when the correlations with the other variables were taken into account. The size of the opponent response during pre-learning pursuit was not a statistically significant predictor of the magnitude of learning. Not surprisingly, the magnitude of the learned eye velocity was a strong predictor of the magnitude of neural learning in monkey S, who had wider variation in the size of his behavioral learning.

We now ask whether the magnitude of neural learning varies systematically within an individual neuron when we alter the instruction time. The same neuron was exposed to two learning experiments featuring different instruction times associated with disparate neural preferences. The results in Figure 3 predict that the example neuron in Figure 4A should show larger learning for an instruction time of 150 ms, when its neural preference was 1.0, versus an instruction time of 250 ms, when its neural preference was 0.6. The prediction was borne out by performing two different learning experiments with instruction times of 250 and 150 ms, respectively. The amount of neural learning was greater when the instruction time was 150 ms (Figure 4B, top panel), even though the learned change in eye velocity was somewhat larger when the instruction time was 250 ms (Figure 4B, bottom panel).

We studied the activity of 31 neurons (11 in Monkey G, 20 in Monkey S) during two sequential learning experiments that were identical in all respects except the instruction time. The instruction time for one experiment was always 250 ms; the instruction time for the other experiment was chosen amongst 150 ms, 350 ms, or 450 ms. We sorted the 31 neurons into two groups based on whether their neural preference for 250 ms was larger or smaller than for the other instruction time. Then, we computed the size of learning for a 250 ms instruction time minus that for the other instruction time. These values would be positive or negative depending on whether neural learning was larger or smaller when the instruction occurred at 250 ms.

Neurons with larger preferences for 250 ms showed more learning for an instruction time of 250 ms than for the other instruction time, while neurons with larger preferences for the other instruction time showed less learning for an instruction time of 250 ms, results that were confirmed statistically (Figure 4C, Monkey G: p=0.01; Monkey S: p=0.01; Mann-Whitney U test). The magnitude of neural learning did not depend significantly on alternative explanatory variables, such as the disparity in the sizes of the mean learned
behavior elicited by the two instruction times (Monkey G: p=0.76; Monkey S: p=0.88), or the order of presentation of the two instruction times (Monkey G: p=0.24; Monkey S: p=0.28). Finally, the magnitude of neural learning produced with the most frequently used other instruction time, 150 ms, was correlated much better with neural preference for 150 ms (Monkey G: r=0.61, p=0.11, 8 neurons; Monkey S: r=0.75, p=0.001, 15 neurons), than with neural preference for 250 ms (Monkey G: r=0.075; Monkey S: r=0.31).

In conclusion, we have demonstrated that pursuit learning with specific timing requirements selectively engages FEF\textsubscript{SEM} neurons that encode the relevant time.

Changes in firing related to learning versus eye movement performance

Do learned changes occur in FEF\textsubscript{SEM} neurons because the FEF\textsubscript{SEM} plays a direct role in behavioral learning, or simply because learning causes changes in eye velocity to which the FEF\textsubscript{SEM} responds? To distinguish between the two scenarios, we presented “mimic” trials in which target motion presented in the absence of learning created an eye movement similar to that produced by learning with an instruction time of 250 ms. During a mimic trial (Figure 5A), a target moving at 20°/s in the probe direction underwent a brief motion in the learning direction. The target motion evoked a mean eye velocity that closely resembled the mean learned eye velocity expressed in the probe trials from the learning block (Figure 5B, top panel). We verified the excellence of the mimicry across neurons using a millisecond by millisecond regression analysis of the mimic versus the learned mean eye velocities in the interval from 100 to 320 ms after the onset of target motion. Regression slopes averaged 1.00 across neurons (range: 0.88 to 1.19), and correlation coefficients averaged 0.95 (range: 0.83 to 0.99).

The example neuron in Figure 5 exhibited notably different changes in firing rate as a result of learning versus during the mimic trials (Figure 5B, middle panel), even though the changes in eye velocity were nearly identical. For the 21 neurons from Monkey S that were studied during both learning and the mimic experiment, we quantified the size of the evoked firing rate in the mimic trials as we had for the learning data, in a comparable interval of duration 220 ms (shaded grey region, Figure 5B). We did not find any correlation between the size of the neural responses to the mimic target motion and the learned change in firing rate in the corresponding learning block (Figure 5C, filled circles, r=0.05, p=0.83). Some neurons had similar responses in the learning and mimic conditions, while many others had quite different responses. Measuring the sensitivity to eye velocity as the mimic and learned neural responses divided by the magnitude of the corresponding changes in mean eye velocity also failed to reveal a significant correlation (r=-0.06; p=0.78), reaffirming that minor behavioral differences are unlikely to account for the disparate neural responses.

To control for recording instabilities, we also compared the firing rate during probe trials in the two baseline blocks that preceded the learning and mimic blocks. Most neurons showed very similar responses during the two sets of baseline trials (Figure 5C, open symbols), and plotted along the line of slope one. Finally, to ascertain whether the mismatch between the learned response and the response to mimic target motion originates from the differing visual inputs under the two conditions, we measured the activity of individual neurons during passive, coherent motion of a 5°×5° patch of dots while the monkey fixated a stationary target at the center of the patch. We found no relationship between the size of the disparity between the mimic and learned responses and the neuron’s visual sensitivity, computed as the difference in mean firing rate produced by passive dot motion in the learning direction versus in the opposite direction (21 neurons; r=-0.12, p=0.66).

In contrast to what we found in individual neurons, averaging the responses across the 21 neurons we studied revealed very similar population responses for the mimic and learning
conditions (Figure 5B, bottom panel). We conclude that the learned responses of individual neurons in the FEFSEM cannot be thought of solely as secondary consequences of learned changes in smooth eye movement. At least in some neurons, the changes in firing rate are related selectively to an eye movement produced in the context of learning. However, the response of the population is balanced across different behavioral conditions so that the FEFSEM as a whole is always making the same contribution to the smooth eye movement. A similar conclusion has been reached for the cerebellar floccular complex (Kahlon and Lisberger, 2000; Medina and Lisberger, 2009).

Finally, we characterized differences in the temporal preferences of neurons activated by learning versus by the mimic stimulus. For our dataset of 21 neurons, the correlation between neural preference and the size of the learned neural response reached a peak when the neural preference was taken at 250 ms (Figure 6, gray trace), as expected. In contrast, the correlation between neural preference and the size of the mimic response reached a peak for neural preference earlier in the trial (Figure 6, black trace), suggesting that the mimic target motion was most effective for neurons that preferred times during the initiation of pursuit.

Effects of the prior trial on neural and behavioral learning

Previous studies have suggested that motor learning occurs on multiple time scales (Lee and Schweighofer, 2009; Ethier et al., 2008; Smith et al., 2006), including situations where the behavior on a given trial reflects the instruction provided on the previous trial (Yang and Lisberger, 2010). To measure the relative contributions of single-trial versus longer-term learning processes to the behavioral and neural changes reported here, we sorted learning trials based on the identity of the immediately preceding trial. The size of the learned eye velocity was smaller if it had been preceded by a control trial versus by another learning trial. The effect averaged 7.1% and 21.5% in Monkeys S and G and was statistically significant in 15.6% (7/45, Monkey S) and 61.8% (34/55, Monkey G) of the learning experiments in the two monkeys (Mann-Whitney U test, p<0.05).

The small trial-over-trial changes in the size of behavioral learning frequently were not present in a similar analysis of the size of neural learning (for example, Figure 7A). In the 35 neurons that showed a significant change in mean firing rate as a result of learning, the trial-over-trial changes in neural learning were distributed fairly evenly above and below zero, and were unrelated to the trial-over-trial learning of eye velocity (Figure 7B). The neural response on learning trials preceded by a control trial were on average 2.1% larger (Monkey S) and 4.4% smaller (Monkey G) than those preceded by another learning trial. Neural response differences were statistically significant in 15% (3/20, Monkey S) and 6.7% (1/15, Monkey G) of the neurons (Mann-Whitney U test, p<0.05). We conclude that the neural learning in the FEFSEM results from a longer-term process that does not contribute to trial-over-trial changes in the learned behavior.

Nature of temporal information in the FEFSEM

To explore the basis of the temporal responses in the FEFSEM, we used pursuit trials featuring target motion at 5, 10, or 20°/s in the future learning direction to ask whether the activity of individual FEFSEM neurons was most consistent with a representation of speed, elapsed time, or of traversed distance. We collapsed the data across the three speeds and performed regression of firing rate (or normalized firing rate) versus each variable. A regression slope and correlation coefficient of one for any particular variable would indicate that the neuron encodes the value of that variable unambiguously. For the variable of elapsed time, we obtained an average regression slope of 0.90 and correlation coefficient of 0.79 in both monkeys. Slopes and correlations were somewhat smaller for distance (slope=0.62 and 0.61; r=0.75 and 0.71) and for speed (slope=0.67 and 0.66; r=0.65 and
Discussion

For a learned movement to be effective, it not only needs to have the correct trajectory, but must also be produced at the desired time. We have provided evidence that the FEFSEM is involved in regulating the timing of learned pursuit eye movements. We show that when driven by a temporally precise instructive stimulus, learned changes in firing rate are preferentially expressed in neurons that respond best at the time of the instructive stimulus during pre-learning step-ramp pursuit. Our results suggest that the FEFSEM may be a site where the timing of sensory errors is processed during learning and integrated into appropriate, learned motor commands.

We provide several lines of evidence that the learned responses of neurons in the FEFSEM are related selectively to learning, and are not secondary to the altered eye movement. Comparing the changes in firing rate resulting from two different instruction times showed that the magnitude of the learned neural response depended more on the temporal properties of the instructive stimulus than on the size of the learned eye movement. Our analysis of the learned changes in eye velocity and firing rate across single trials revealed a dissociation between the magnitudes of the behavioral and neural responses. Finally, for the same neuron, the change in firing rate associated with a visually-driven eye velocity was often quite different from the change in firing rate produced by learning, even though the visually-evoked eye velocity mimicked the learned eye velocity closely.

Neural mechanisms for temporally selective motor learning

Why should a precisely-timed instructive signal induce learned responses selectively in certain FEFSEM neurons, and how might these neural changes give rise to an appropriately timed eye movement? Based on the knowledge that depolarization of the post-synaptic neuron is a key regulator of synaptic plasticity (Malenka and Bear, 2004), we suggest that neurons with a high preference for the instruction time are more susceptible to plasticity than other neurons in the same population because they have higher membrane potentials around the time of the instructive signal. Further, the same neurons presumably receive inputs that are maximally active around the onset of the instruction. The convergence of elevated pre- and post- synaptic activity should favor plasticity in these neurons around the time of the instruction, which in turn will alter the eye movement selectively around the time of the instructive change in target direction.

We cannot answer definitively the question of whether the learned timing of pursuit or neural responses in the FEFSEM results from the timing contingencies of the cellular mechanisms of plasticity that are involved, or from timing that emerges out of neural circuit properties. We think it is important to remember that timing is inherent in the responses of neurons in the FEFSEM before learning, and that the FEFSEM is suited for processing the 250 ms intervals utilized in our learning paradigm because FEFSEM neurons track time on the order of hundreds of milliseconds. In contrast, cellular mechanisms such as spike timing-dependent plasticity, in isolation, process intervals on the order of tens of milliseconds (Bi and Poo, 1998). Modeling results indicate that the temporal specificity of order 100 ms in FEFSEM responses could emerge and be maintained via network properties (Buonomano, 2005). Thus, we suggest that temporal selectivity in pursuit learning could be the consequence of associative forms of synaptic plasticity acting upon the time-varying pattern of activity created by the properties of the circuit through the FEFSEM.
Factors contributing to timing signals in the FEFSEM

A temporally specific encoding of smooth pursuit is unique to the FEFSEM and has not been reported in any other locus within the pursuit circuit, including MST (Newsome et al., 1988; Squatrito and Maioli, 1997; Ono and Mustari, 2006), the dorso-lateral pontine nucleus (Ono et al., 2005), and the floccular complex in the cerebellum (Krauzlis and Lisberger, 1994; Lisberger, 2010). Further, the representation of time during smooth pursuit appears to be an inherent feature of the population response in the FEFSEM and is present in animals that had never been exposed to a task that requires learned timing (Schoppik et al., 2008).

The motor system has access to both implicit and explicit information about the passage of time (Mauk and Ruiz, 1992; Ivry, 1996; Buonomano and Karmarkar, 2002; Regan and Gray, 2000; Sherk and Fowler, 2001; Caijou et al., 2004; Medina et al., 2005), and is able to rapidly assimilate temporal information to modify behavior. Here, we are using the terms “explicit” and “implicit” to refer to the nature of the signals the brain uses to estimate the duration of a time interval. “Explicit” timing mechanisms would function like a stopwatch, creating a neural state that depends entirely on the number of elapsed milliseconds. “Implicit” mechanisms, on the other hand, would estimate time from less direct cues generated by one’s self or the environment. In our learning paradigm, either elapsed time or the distance traveled by the target/eye can be used to cue an upcoming change in target direction (Medina et al., 2005). The FEFSEM appears to be an explicit source of temporal information because neural responses during pursuit at three speeds were well correlated with elapsed time, and less so with an implicit measurement such as distance traveled by the eye. Other potential sources of temporal information, such as image motion and eye velocity/acceleration, fail to account for the timed pursuit responses because all are fairly constant during steady state pursuit when the temporal selectivity of FEFSEM responses is still clearly present.

Relationship to other sites of pursuit learning

The FEFSEM occupies a prime position within the pursuit circuit for mediating motor learning. It receives information that reports discrepancies between the eye and the target via visual motion sensory areas MT and MST (Leichnetz, 1989; Stanton et al., 2005). Lesion and micro-stimulation studies have pinpointed the FEFSEM as a major player in regulating the sensory-motor gain for pursuit (Lynch, 1987; MacAvoy et al., 1991; Tanaka and Lisberger, 2001), a mechanism that could determine what gets learned, and how well. Finally, the FEFSEM is strongly connected to the caudate nucleus (Cui et al., 2003), an area involved in assessing reward contingencies, which could be used to guide motor learning.

A previous study in the FEFSEM failed to uncover a consistent expression of neural learning using a training procedure that provided a change in target speed 150 ms after the onset of target motion in the learning direction (Chou and Lisberger, 2004). There are two possible reasons for the discrepancy between this earlier finding and our present results. First, behavioral learning is larger and more consistent for changes in target direction than target speed (compare results presented here with Kahlon and Lisberger, 1996). Thus, the direction-learning paradigm may induce more persuasive neural changes than the speed learning paradigm, as has been found in the cerebellar flocculus (compare Medina and Lisberger, 2008, 2009 with Kahlon and Lisberger, 2000). Second, the recordings during speed learning did not examine how learned FEFSEM responses varied as a function of neural preference for the time of the instructive stimulus. The instructive change in target speed occurred 150 ms after the onset of target motion, implying that learning should be expressed mainly in neurons that respond most strongly at the initiation of pursuit. Averaging across neurons having a range of temporal preferences would dilute any learning-
related effects. Consistent with this explanation, a subpopulation of FEF\textsubscript{SEM} neurons did exhibit significant changes in firing rate during speed learning (Chou and Lisberger, 2004).

The cerebellar flocculus, several synapses downstream of the FEF\textsubscript{SEM}, also may play a causal role in temporally specific pursuit learning. Purkinje cells show changes in simple spike activity around the time of the instructive stimulus during learning in both the OFF and ON directions of the Purkinje cell under study (Medina and Lisberger, 2008, 2009). In one model of the cerebellar microcircuit, a sparse representation of time in the granule cell population provides the excitatory drive for Purkinje cells. Different granule cells would provide inputs to Purkinje cells at different times during a movement so that visually-driven climbing fiber inputs could potentiate or depress the granule-Purkinje synapses that were active 100 ms prior to the arrival of the climbing fiber signal (Buonomano and Mauk, 1994). Thus, the cerebellum could act independently in learning motor timing, or inputs from the FEF\textsubscript{SEM} could contribute to the temporal sparseness of the granule cell population in a way that is enhanced by learning in the FEF\textsubscript{SEM}. Recent work also has highlighted the possibility that learning occurs on different time scales (Lee and Schweighofer, 2009; Ethier et al., 2008; Smith et al., 2006; Yang and Lisberger, 2010) with the possibility of very rapid short term learning in the cerebellar cortex as a prelude to slower, longer-term changes in the FEF\textsubscript{SEM}.

Utility of a high-dimensional motor code

Neurophysiological studies of motor and perceptual learning reveal a common theme: changes are localized to neurons whose properties best capture the features of the training stimulus (Arce et al., 2010; Paz et al., 2003; Recanzone et al., 1993; Schoups et al., 2001; Yang and Maunsell, 2004). In real life, the learning rule can be very complex. Thus, the dimensionality of the neural representation of movements limits the flexibility of the motor system in terms of what can be learned quickly. For many years, it was commonly believed that the responses of motor cortex neurons could be modeled by a time-invariant combination of limb kinematics and dynamics (Evarts, 1968; Georgopoulos et al., 1982; Moran and Schwartz, 1999). Recently, examination of a broader population of neurons in M1, PMd, and the FEF\textsubscript{SEM} has revealed considerable heterogeneity in movement-related neural responses (Hatsopoulos et al., 2007; Churchland and Shenoy, 2007). Many response patterns are explained poorly using standard movement parameters such as acceleration, speed, and direction. We propose that the FEF\textsubscript{SEM} and other motor cortices are important for facilitating action selection. The FEF\textsubscript{SEM} encodes smooth pursuit movements flexibly along seemingly baroque but perhaps behaviorally relevant dimensions, such as time, so that error and reward signals can act selectively on a subregion within the movement space to drive rapid, precise motor learning.

Experimental Procedures

Two male rhesus monkeys (Macaca mulatta) aged 6 and 8 years, tracked smoothly moving targets in exchange for a water reward. Both monkeys had prior experience in experiments on pursuit, but neither had participated in learning studies. Throughout each experiment, head position was fixed, and eye position in the orbit was monitored using a scleral search coil system. The recording chamber and eye coil were attached during surgery with sterile procedure using approaches described before (Ramachandran and Lisberger, 2005) with the monkey under anesthesia with isofluorane. After surgery, monkeys received analgesics for several days and careful monitoring by veterinary staff. All experimental procedures and protocols used were approved by the Institutional Animal Care and Use Committee of UCSF, and are in accordance with use and care guidelines established by the NIH Guide for the Care and Use of Laboratory Animals.
Data acquisition

Horizontal and vertical eye positions were sampled at 1 kHz and passed through an analog differentiator with a cutoff of 25 Hz to produce the corresponding eye velocity traces. Quartz shielded tungsten electrodes (Thomas Inc) were lowered anew each day into the frontal eye fields. FEFSEM neurons were identified by direction-tuned activity during smooth pursuit, and weak or non-existent responses to saccades or changes in eye position. Spike waveforms were retained using a threshold crossing criterion, and were sorted into single units based on waveform shape and the absence of refractory period violations defined as two waveforms occurring within 1 ms. For a typical recording session, the waveforms from recorded neurons were 3 to 10 times the amplitude of the background noise. Sorted waveforms were converted into spike trains with a temporal precision of 1 ms.

Presentation of visual stimuli

All behavioral experiments took place in a dimly lit room. Visual stimuli were displayed on a BARCO monitor (model number: CCID 7651 MkII) that was placed 40 cm from the eye and subtended 61°×42° of the visual field. Targets were white squares measuring 0.5° along each side. Target motions were presented in discrete trials. Each trial started with a stationary fixation target at the center of the screen for an interval that was randomized between 500 and 1000 ms. Targets then underwent standard step-ramp motion in an unpredictable direction for 750 ms, and then stopped for 500 ms in a second fixation period. For step-ramp motion, the step size was chosen to minimize saccades during pursuit onset and typically ranged between 2° to 3°, depending on the initial direction of target motion. To successfully complete a trial and receive a water reward, monkeys were required to keep their eyes within a window centered on the target. The window was 1.5°×1.5° during fixation, 3°×3° during smooth target motion, and 5°×5° for 300 ms after an instructive change in target direction. For tests of neural responses to passive visual stimuli, monkeys fixated a small square target centered in an invisible square aperture that was 5° long on each side. The aperture contained 10 dots that moved with 100% coherence at 5°/s in one of the four cardinal directions.

Behavioral paradigms

The direction and temporal tuning of each neuron were characterized in a pre-learning block of pursuit trials where the pursuit target moved at 20°/s in one of 8 possible directions, including all four horizontal and vertical directions and the 45° oblique directions. In some experiments, targets moved in the four cardinal directions with speeds of 5, 10, or 20°/s in different trials.

Each learning experiment consisted of a baseline block and a learning block. During the baseline block (80 to 100 trials), the target moved at 20°/s in one of two opposing cardinal directions, designated the probe (55% of the trials) and control directions (45%). In the learning block, (250 to 300 trials) the pursuit target also initially moved in either the probe (55%) or control (45%) directions; however, targets moving in the probe direction had an 82% chance of adopting a 30°/s orthogonal velocity component at a fixed time after the onset of target motion. The direction and timing of the instructive stimulus was fixed for a given learning block. In some recording sessions, we performed an additional learning experiment after residual behavioral learning had been extinguished with a second baseline block (100-150 trials), or a two-block sequence of learning in the opposite direction (50-100 trials) followed by a baseline block (50 trials). The residual eye velocity measured after the two reversal procedures averaged 27.7% (SD: 30.8%, range: 61.7% to -34.1%) of the original learned response after a baseline block and -1.3% (SD: 16.3%, range: 47.9% to -33.1%) after a learning block in the opposite direction and another baseline block. For 21 neurons, we followed the reversal procedure with a mimic experiment, which consisted of a
baseline block followed by a mimic block. The mimic block featured “mimic” trials
designed to evoke an eye velocity with the same time course and trajectory as the learned
component of eye movement, but without any learning. To prevent learning during the
mimic block, mimic trials in the learning direction were counterbalanced by trials that
contained the same target perturbation in the opposite direction.

Data analysis
Trials were examined individually by eye to identify the onset and offset times of any
saccades; we replaced the intervening eye velocity with a linear interpolation whose
endpoints were the eye velocity values at the onset and offset of the saccade. We quantified
the magnitude of neural learning in the interval from 100 ms after the onset of target motion
to 70 ms after the instruction time, as the difference in mean spike count between the
learning trials in the learning block and the probe trials from the baseline block. Neural
responses are reported as firing rates, obtained by dividing the spike counts by the duration
of the analysis intervals. We verified that all analyses produced similar results if the firing
rate changes were converted to Z-scores. Firing rates were smoothed with a 50 ms duration
rectangular filter for our figures, but unsmoothed traces were used for quantitative analysis.
In the bottom panel of Figure 5B, we took advantage of the larger number of traces to
smooth the data with a narrower, 15 ms rectangular filter.

Acknowledgments
Research supported by the Howard Hughes Medical Institute, NIMH grant MH077970, and predoctoral fellowships
from the National Science Foundation and the National Institutes of Health (NS655982). We thank Karen
MacLeod, Elizabeth Montgomery, Stefanie Tokiyama, Lazslo Bocskai, Darrell Floyd, Dirk Kleinhesselink, Ken
McGary, and Scott Raffner for technical assistance. Finally, we thank colleagues for helpful comments and
discussions.

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Neuron. Author manuscript; available in PMC 2011 July 13.


Figure 1. Expression of learning in the FEFSEM

Figure 2. Expression of pursuit learning in two example FEFSEM neurons
Mean firing rates (A,B) and eye velocity (C,D) as a function of time from the onset of target motion. The vertical dashed lines show the time of the instructive change in target direction. Black, blue, and red traces show data obtained respectively during probe trials in the baseline block, probe trials in the learning block, and learning trials. The grey shading indicates the analysis interval from 150 ms before to 70 ms after the time of the change in target direction.
Figure 3. Relationship between the size of neural learning and neural preference for the instruction time of 250 ms

A: Example of neural preference as a function of time from target motion onset. The neuron’s preference for 250 ms is specified by the intersection of the vertical and horizontal dashed lines. B: Summary of neural preference during pre-learning pursuit for all 100 FEFSEM neurons in our sample, sorted by the time when neural preference first reaches 0.95. Each horizontal line of the color map shows the preference of one neuron as a function of time; neural preference is quantified by the pixel color. C: Distribution of times of maximal neural preference. D: The size of neural learning is plotted as a function of the neuron’s preferred time minus 250 ms. E: The size of neural learning is plotted as a function of the neural preference for 250 ms. The lines show linear regression fits to the data from each of the two monkeys. In D and E, each symbol shows data for one neuron, and filled versus open symbols show data from the two monkeys. F: Correlations across neurons between the size of neural learning and the neural preference as a function of the time used to assess neural preference. The two traces show results from the two monkeys. The vertical dashed line indicates the time of the instructive change in target direction, 250 ms after the onset of target motion.
Figure 4. Effect of the instruction time on the magnitude of the learned neural response
A: Neural preference as a function of time for an example neuron. B: Baseline-subtracted mean firing rate (top panel) and eye velocity (bottom panel) when the same neuron underwent learning using an instruction time of 250 ms (grey trace) or 150 ms (black trace). C: Each symbol plots data from a single neuron (n=31). Each neuron is sorted into one of two x-axis groups according to whether its neural preference was greater for 250 ms or for the other instruction time used in the experiment. The y-axis plots the size of the neural learning for an instruction time of 250 ms minus the size of the neural learning for the other instruction time. The horizontal lines indicate the group means. Filled versus open symbols and different line styles indicate data for the two monkeys.
Figure 5. Assessment of whether learned changes in neural firing can be attributed solely to changes in eye movement performance

A: The dashed and continuous traces show target and eye velocity during a tracking that mimicked the trajectory of the learned eye velocity measured from probe trials in the learning block. B: Black traces show data from the mimic trials and grey traces show the learned responses. Top panel: averages of eye velocity for an example neuron. Middle panel: average firing rates for the example neuron. Bottom panel: the population responses, averaged across all neurons. C: Filled symbols compare learned firing rates with mimic responses for the full sample of neurons. Open circles compare the responses from the probe trials in the baseline blocks preceding the learning or the mimic experiments. The dashed line has a slope of one.
Figure 6. Time course of correlation between neural preference and size of learned and mimic responses
Correlations between neural preferences at the times on the x-axis and the learned response with an instruction time of 250 ms (grey trace), or the mimic response (black trace). Results reflect all 21 FEF_{SEM} neurons studied in the mimic experiments.
Figure 7. Trial-over-trial effects on learned eye velocity and firing rate
A: Example from a single experiment of baseline-subtracted mean firing rate (top panel) and eye velocity (bottom panel) from learning trials preceded by a control trial versus by another learning trial. B: Population analysis: each symbol shows data from an individual neuron and open versus filled symbols show data from the two monkeys.
Table 1
Partial correlation coefficients between the magnitude of neural learning and other neural or behavioral parameters. For assistance in interpreting the correlations, the table also shows the mean and range of each variable in the two monkeys.

<table>
<thead>
<tr>
<th></th>
<th>Monkey G</th>
<th>Monkey S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation, significance</td>
<td>Mean [range]</td>
</tr>
<tr>
<td>Neural preference, for 250 ms</td>
<td>0.43 p=0.001</td>
<td>0.63 [0.06 to 0.98]</td>
</tr>
<tr>
<td>Opponent firing rate</td>
<td>0.22 p=0.11</td>
<td>19.2 spikes/s [0.6 to 88.7]</td>
</tr>
<tr>
<td>Behavioral learning</td>
<td>0.18 p=0.19</td>
<td>0.8 degrees [0.4 to 1.2]</td>
</tr>
</tbody>
</table>

*Neuron.* Author manuscript; available in PMC 2011 July 13.