
Hilary W. Heuer, Stefanie Tokiyama, and Stephen G. Lisberger

Howard Hughes Medical Institute, W. M. Keck Foundation Center for Integrative Neuroscience, Department of Physiology, University of California, San Francisco, California

Submitted 21 April 2008; accepted in final form 20 June 2008

Heuer HW, Tokiyama S, Lisberger SG. Doing without learning: stimulation of the frontal eye fields and floccular complex does not instruct motor learning in smooth pursuit eye movements. J Neurophysiol 100: 1320–1331, 2008. First published June 25, 2008; doi:10.1152/jn.90492.2008. Under natural conditions, motor learning is instructed by sensory feedback. We have asked whether sensory signals that indicate motor errors are necessary to instruct learning or if the motor signals related to movements normally driven by sensory error signals would be sufficient. We measured eye movements in trained rhesus monkeys while employing electrical microstimulation of the floccular complex of the cerebellum and the smooth eye movement region of the frontal eye fields to alter ongoing pursuit eye movements. Repeated electrical stimulation at fixed times after the onset of target motion and pursuit failed to cause any learning that was retained beyond the time period used to instruct learning. Learning was not uncovered when the target was stabilized with respect to the moving eye to prevent competition between instructive signals created by electrical stimulation and visual image motion signals evoked when stimulation drove the eye away from the tracking target. We suggest that signals emanating from motor-related structures in the pursuit circuit do not instruct learning. Instead, instructive sensory error signals seem to be necessary.

INTRODUCTION

In learning motor skills, we think of the old adage that “practice makes perfect.” Movements are improved through repetition under conditions where errors are signalled by sensory input and corrected in two senses. In one sense, the sensory-motor system responds immediately to the sensory error with a corrective movement that leads to an improved outcome. In the other sense, the sensory-motor system treats the sensory errors as “instructions” for learning and undergoes long-term changes so that errors are eliminated. After successful instruction and learning, the first attempt at making the movement is perfect and immediate sensory-motor corrections no longer are needed. An important element of the long-term changes is that they are remembered so learning is expressed even in the absence of the instructive stimuli. This typical sequence of motor learning raises the question of whether the mechanisms of motor learning are instructed by sensory signals that indicate errors in movement or by the actual motor commands for immediate corrective movements.

Smooth pursuit eye movements are subject to learning when an ongoing target motion changes either speed (Kahlon and Lisberger 2000) or direction (Boman and Hotson 1992; Medina et al. 2005). Under typical pursuit learning conditions, a change in target motion provides a large visual motion signal because the tracked target suddenly moves at a different speed or in a different direction relative to the eye. Approximately 100 ms later, the image motion drives a rapid change in eye velocity. If the same target motion, and therefore the same instructive signal, is provided repeatedly, then a learned response gradually appears at a time that anticipates changes in target motion. In a recent paper, our laboratory showed that learning was instructed when sensory stimulation was replaced by microstimulation in the sensory input to the pursuit circuit from extrastriate visual area MT, indicating that sensory signals were sufficient to instruct learning (Carey et al. 2005). To examine the necessity of sensory errors for instructing pursuit learning, we now ask whether learning occurs when we attempt to instruct learning with electrical stimulation in motor parts of the pursuit circuit.

There are likely to be multiple sites of learning in the pursuit system, and the floccular complex of the cerebellum has been implicated as one of them (Kahlon and Lisberger 2000). Purkinje cells in the floccular complex show strong modulation of simple spike firing rate during pursuit, and inputs to the floccular complex converge (via the pontine nuclei) from both the visual motion pathways of MT and MST and the motor-related outputs from the smooth eye movement region of the frontal eye fields (FEFSEM) (Leichnetz 1989; Robinson and Fuchs 2001). Available evidence does not point toward MT or the FEFSEM as likely loci of learning. However, stimulation of MT instructs learning (Carey et al. 2005), and learning is expressed in the eye movements that result from stimulation of the FEFSEM (Chou and Lisberger 2004). These data imply that outputs from both MT and the FEFSEM are transmitted through a locus of learning. Thus the motor signals that arise from the FEFSEM could instruct learning, as do the sensory signals that emanate from MT (Carey et al. 2005).

We tested the roles of sensory and motor error signals in pursuit learning by replacing the usual instructive change in target motion with electrical stimulation in either the cerebellum or the FEFSEM. Although no sensory error signal was provided, stimulation in the floccular complex (Belknap and Noda 1987; Lisberger et al. 1994a; Ron and Robinson 1973) and the FEFSEM (Gottlieb et al. 1993, 1994; Tanaka and Lisberger 2001) produced a change in eye velocity at a consistent time. Even when we prevented conflicting sensory signals during electrical stimulation by stabilizing the target with respect to the moving eye, stimulation in either the
floccular complex or the FEF<sub>SEM</sub> failed to instruct learning. Our data suggest, with caveats related to the uncertainties of interpreting negative results from electrical stimulation experiments, that sensory error signals are necessary to instruct learning in pursuit eye movements.

**METH ODS**

Eye movements were recorded from four male rhesus monkeys (Macaca mulatta) during smooth pursuit eye movements. Each monkey had been outfitted surgically with a stainless steel or titanium socket for head restraint and a scleral search coil for monitoring eye position using methods described elsewhere (Ramachandran and Lisberger 2005). Two monkeys had recording chambers placed stereotaxically over the right frontal eye fields; the other two monkeys had stimulating electrodes implanted chronically in the right floccular complex of the cerebellum. The methods for implanting floccular electrodes were the same as used by Lisberger et al. (1994b), and the monkeys also were used for brain stem recording experiments that are currently under preparation for publication. In one monkey (W), the stimulating electrode had been stable for 2 yr before our pursuit learning experiments were performed; in the other monkey (H), pursuit learning experiments were performed in the first 2 mo after stimulating electrode implantation. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Tracking stimuli and behavioral methods**

Monkeys had been trained to sit in a primate chair with their head restrained while they fixated and tracked spots of light projected onto a screen in front of them in exchange for droplets of fluid. We used different experimental rigs and different visual displays for experiments performed in the FEF<sub>SEM</sub> versus in the floccular complex of the cerebellum. For the FEF<sub>SEM</sub>, targets were presented on an analog oscilloscope (Hewlett-Packard HP1304A) with a refresh rate of 250 Hz. The visual target was a small (0.3°) bright square. The display subtended 49° horizontally by 38° vertically at a viewing distance of 30 cm. Nominal spatial resolution of 216 pixels across the screen was achieved by driving the oscilloscope with 16-bit digital-to-analog converters on a digital signal processing board in our experimental control computers. For the cerebellum, the target was a bright circle of diameter ~0.5°, created by focusing the light beam from a fiber optic light source onto a pair of moveable mirrors and projecting the beam onto the back of a large tangent screen. The screen subtended ~50 × 40° at a distance of 114 cm from the monkeys’ eyes. The positions of the mirror galvanometers were controlled by the D/A converters in our experimental control computer. Both the fiber optic and oscilloscope target types have been used previously in studies of pursuit learning in this laboratory (Carey et al. 2005; Chou and Lisberger 2002), and no difference was noted with target type.

In some experiments, we applied an image stabilization technique (Morris and Lisberger 1987) in an attempt to eliminate retinal image motion resulting from small tracking errors or from driving the eye off the target with electrical stimulation in the brain. Stabilization was enabled selectively on stimulation trials in the learning block with the goal of exploring whether learning could be unmasked by eliminating visual instructive signals for pursuit learning at times when they might be competing with electrically induced signals. Our experimental control program allowed us to enable target stabilization selectively during electrical stimulation. To stabilize the target with respect to the moving eye, we drove target position with electronic feedback of eye position on a millisecond time scale. Because stabilization is only as good as the calibration of the eye coil, we endeavored to obtain excellent calibration in every experiment. In addition, we imposed stabilization in a few trials without electrical stimulation and used the absence of consistent eye accelerations or staircases of saccadic eye movements (Morris and Lisberger 1987) as independent verification of the accuracy of the calibration. Stabilization is never perfect, but we think that any stabilization errors were small and therefore were not likely to have any impact on the analyses presented here.

Experiments were divided into a series of trials, where each trial began with an interval of randomized duration from 600 to 1,800 ms when the monkey was required to fixate within 1–2° of a target at the center of the screen. The initial target motion then was provided by a standard “step-ramp” pursuit task (Rashbass 1961). The stationary target was displaced in one direction by 1–3° and then moved back toward the position of fixation at 10°/s (for floccular stimulation experiments) or 20°/s (for experiments in FEF<sub>SEM</sub>). Target velocity was chosen to optimize the initiation of pursuit and to ensure excellent tracking at the time the potentially instructive stimulus was delivered in each monkey. Target step size was adjusted for each monkey to minimize early catch-up saccades. During pursuit, the monkey was required to keep its eye within ~5° of the target, or the trial was aborted and was not subjected to further analysis.

We used an electrical-stimulation version of the directional learning paradigm described in detail in prior papers (Carey et al. 2005; Medina et al. 2005). In each experiment, the monkey first completed a “preinstruction” block of ~100 trials to establish a control level of performance. In this block, ~80% of trials provided target motion in the direction of target motion that would be used for subsequent instruction blocks; 5–20% of trials provided target motion in the opposite direction. In addition, 5–10% of the trials in this preinstruction block sometimes delivered electrical microstimulation to document the evoked eye movements before stimulation was delivered repeatedly in attempts to instruct learning.

Next, we ran an “instruction” block of 300–1,000 trials to assess whether electrical stimulation in the cerebellum or the FEF<sub>SEM</sub> could instruct learning. Now 80% of the trials were “instruction trials” that delivered electrical stimulation during step-ramp target motion in the direction chosen for the particular experiment, ~10% of the trials were “probe” trials comprising the same target motion without electrical stimulation, and ~2–10% of trials were catch trials in the opposite direction. Probe trials were identical to the trials presented in the preinstruction block. The potential instructive signal for learning was provided by microstimulation that began 200–300 ms after the onset of target motion and lasted for 75–300 ms, depending on animal and stimulus conditions. Fixation requirements were relaxed during the stimulation period for the instruction trials and during the corresponding epoch of the probe trials. Thus the monkey had an equal likelihood of obtaining a reward in all trials and was not punished for the imposition of electrical stimulation that drove his eyes off target albeit only by a small amount. In a few of the experiments where the target was stabilized during stimulation, we also included a small percentage of trials that delivered electrical stimulation during pursuit in the probe direction, but without target stabilization. We ran the instruction block for ~800–1,000 trials as long as the monkey continued to perform well and the stimulation evoked eye movements remained consistent. If the monkey completed 800–1,000 trials, then we transitioned to an “extinction” block consisting of 100–200 probe trials; otherwise, the extinction block was performed prior to the next experimental session. Extinction blocks were a luxury in the sense that we didn’t observe any learning that needed to be extinguished, but we ran them at convenient times as a precaution. Within each block, all trial types were interleaved in pseudorandom order by our experimental control program. For all experiments, ~80 probe trials were used in calculating control performance; a minimum of 200 instruction and 20 postinstruction probe trials were averaged for evaluating learning.

For experiments with microstimulation in the FEF<sub>SEM</sub>, electrodes were introduced daily, and we chose the direction of target motion for instruction and probe trials on the basis of the eye movements evoked
at the stimulation site. The goal was to select the direction of pursuit so that microstimulation caused eye movement orthogonal to the ongoing pursuit eye velocity. Due to the variable nature of the sites, some experiments were performed using stimulation evoked eye movements along the same axis as the ongoing pursuit. For experiments with stimulation in the floccular complex, the electrodes were implanted surgically so that the evoked eye movements were very similar from day to day. Again, the direction of target motion was chosen so that stimulation caused an orthogonal smooth eye movement. In addition, a few experiments used target motions along a cardinal axis not necessarily orthogonal to the eye movements evoked by floccular stimulation.

Electrical stimulation

All electrical stimulation was performed using a Grass S-88 stimulator controlling two constant-current SIUs configured to provide biphasic current pulses where each pulse was 0.2 ms in duration and the frequency of the stimulus train was 200 Hz. For floccular stimulation, we implanted a bipolar stimulating electrode (Peter Rhodes Medical Instruments, Woodland Hills, CA) at a location chosen on the basis of stereotaxic coordinates and refined by evaluating the horizontal eye movements evoked by stimulation as we drove the electrode through the cerebellum. The stimulus train duration was 300 ms and current amplitude was 30 µA. For monkey H, the stimulation was initiated 300 ms after target motion onset for both normal and target-stabilized conditions. For monkey W, stimulation occurred at 200 and 250 ms for normal and target-stabilized conditions. Because of the size of the stimulating electrode, we do not consider this to represent “microstimulation.” Indeed the eye movements evoked by stimulation in the floccular complex with bipolar stimulating electrodes have proven to be remarkably consistent from monkey to monkey, whereas true microstimulation through a recording microelectrode activates Purkinje cell axons strongly, whereas stimulation through the same electrodes with smaller impendence: 800 –1,500 kΩ) and a tungsten microelectrode (Frederick Haer, Cape Medical Instruments, Woodland Hills, CA) at a location chosen on the basis of stereotaxic coordinates and refined by evaluating the horizontal eye movements evoked by stimulation as we drove the electrode through the cerebellum. The stimulus train duration was 300 ms and current amplitude was 30 µA. For monkey H, the stimulation was initiated 300 ms after target motion onset for both normal and target-stabilized conditions. For monkey W, stimulation occurred at 200 and 250 ms for normal and target-stabilized conditions. Because of the size of the stimulating electrode, we do not consider this to represent “microstimulation.” Indeed the eye movements evoked by stimulation in the floccular complex with bipolar stimulating electrodes have proven to be remarkably consistent from monkey to monkey, whereas true microstimulation through a recording microelectrode activates Purkinje cell axons strongly, whereas stimulation through the recording microelectrode may be activating local elements in the cerebellar cortex, many of which inhibit Purkinje cells.

For microstimulation in the FEF, the electrodes were introduced daily. A transdural guide tube was inserted at known locations within a plastic coordinate grid system in the frontal recording chamber (Crist et al. 1988), and a tungsten microelectrode (Frederick Haer, impedance: 800–1,500 kΩ) was advanced through the guide tube and into the arcuate sulcus using a hydraulic microdrive (Kopf Instruments). Because of the challenge of finding good smooth eye movement sites, we explored the relevant area of the arcuate sulcus quite thoroughly, and we are confident that our results reflect the organization of sites throughout the FEF. Sites were selected for learning experiments when electrical microstimulation elicited a smooth pursuit eye movement of a consistent direction and speed. For both monkeys, stimulation occurred 250 ms after target motion onset and the stimulus current was set at 50 µA. For monkey L, train duration was 200 ms for both normal and target-stabilized conditions; for monkey D, trains longer than 75 caused a characteristic saccade back toward the target under normal tracking conditions. Therefore train duration was set to 75 and 150 ms for normal and target-stabilized pursuit in this animal. The times of electrical stimulation were chosen to maximize the chance of instructing learning: with natural stimuli, a change in target direction 200–300 ms after the onset of target motion is optimal for inducing directional learning in pursuit eye movements (Medina et al. 2005).

Data analysis

Eye position signals from the scleral search coil were differentia ted by an analog circuit to obtain signals proportional to horizontal and vertical eye velocity. The circuit also served as a filter, differentiating frequencies <25 Hz and rejecting higher frequencies with a roll-off of ~20 db/decade. Eye velocity and position signals were digitized at 1 kHz and stored for later analysis. For each completed trial, eye position and velocity traces were displayed on a computer screen. The start and ending times of each saccade were marked using a combination of automated detection and visual inspection. The automated detection algorithm determined when a saccadic deflection of radial eye velocity rose above and fell below 50°/s and then defined saccade onset and offset as 15 ms before the first crossing and 15 ms after the second crossing. After automated detection, each trace was checked visually and saccade detection was corrected if necessary. Trials were discarded from further analysis if a saccade occurred either during the initiation of pursuit or in the first 100 ms after the onset of electrical stimulation, if there were excessive saccades (generally ≥3/ trial) or if a saccade occurred during the analysis window for responses to electrical stimulation. Remaining saccades, which were rare, were replaced by smooth segments of eye velocity that had been interpolated linearly between saccade onset and offset separately for horizontal and vertical velocities. Generally, <5% of trials were discarded for any experiment.

RESULTS

Stimulation of the cerebellar flocculus

As previously demonstrated (Lisberger et al. 1994a); Ron and Robinson 1973, microstimulation in the right floccular complex of the cerebellum induced eye velocity to the right, even during fixation of a stationary target. In the examples in Fig. 1, a 300-ms train of 30 µA pulses at 200 Hz produced ~3°/s of horizontal eye velocity (top) toward the side of recording in both monkeys. Floccular stimulation also evoked upward eye velocity in both monkeys, although the vertical response was more pronounced for monkey H and smaller in monkey W. Because the electrodes were cemented in place for floccular stimulation, the responses to a given current remained consistent within each monkey over a period of months or even years. In both monkeys, we also knew from prior or subsequent recordings that stimulation through the same electrodes with single shocks caused monosynaptic inhibition of a group of neurons in the vestibular nucleus known as “floccular target neurons” (Lisberger et al. 1994a).

FIG. 1. Eye movements evoked by stimulation of the cerebellar flocculus during fixation. Left and right: data from monkeys H and W, respectively. The bold horizontal bar in each panel indicates the time of stimulation. Records were obtained by averaging across multiple repetitions of the same stimulation train in a single experimental session. Top panels: horizontal eye velocity; bottom panels: vertical eye velocity. Upward deflections of the traces indicate eye movement toward the side of stimulation (ipsiversive) or upward. The width of the ribbon around each trace indicates ±1 SE.
Figure 2 shows an example of a typical instruction trial in which we attempted to induce learning by pairing electrical stimulation in the floccular complex with responses to step-ramp target motion. In each experiment, we used a single direction of target motion contrived so that the eye velocity evoked by floccular stimulation was orthogonal to ongoing target motion and eye velocity. Here the step-ramp target motion took the target and eye to the left and up, and floccular stimulation (bold red horizontal line) caused the eye velocity in the instruction trial (red traces) to divert to the right and slightly up relative to those in a preinstruction trial (black traces).

Figure 3 shows the full set of data for an example experiment and explains how we analyze and present the data throughout the paper. For both the horizontal (A) and vertical (B) components of eye movement, eye position records were not particularly informative because stimulation was too brief to cause large deviations of eye position from the preinstruction controls. The effects are clear, however, in eye velocity traces. Here stimulation in the instruction trials (red traces) caused both horizontal and vertical eye velocity to deviate from that in the preinstruction trials (black traces), which used the same target motion but did not include electrical stimulation. Further, the postinstruction probe trials (blue traces) produced eye velocity essentially identical to that in preinstruction probe trials, indicating that instruction by stimulation in the floccular complex did not induce learning that persisted beyond the instruction trials. In contrast, changes in the direction of target motion (Medina et al. 2005) and stimulation in area MT (Carey...
et al. 2005) both cause changes in eye velocity that persist in postinstruction probe trials.

To evaluate the immediate effect of stimulation in the flocculus and its longer term effects on smooth eye movements in isolation from the preinstruction response to the target motion, we computed the “difference eye velocity” defined as the average eye velocity evoked in instruction or probe trials during the instruction block minus the average eye velocity in target motions during the preinstruction block. Comparison of the full eye velocity records with the difference eye velocities in Fig. 3 shows that this presentation focuses attention on the immediate and long-term effects of instructive electrical stimulation without distorting the data in any other way. The difference eye velocity shows the evoked changes in eye velocity during stimulation in instruction trials (red traces) as well as the absence of any effects that might be related to learning in the postinstruction probe trials (blue traces): the difference eye velocity on postinstruction probe trials remained near zero during the epoch corresponding to the stimulation.

To present the data in a way that we find intuitive and that unifies the separate horizontal and vertical eye velocity traces, we also created two-dimensional plots that show vertical versus horizontal eye position or velocity. For eye position, Fig. 4 shows that the eye started at straight ahead gaze (0, 0) and moved upward and to the left under all conditions. However, because of the small effects of stimulation on eye position, there are only tiny differences among preinstruction (black), instruction (red), and postinstruction (blue) trials. For eye velocity, Fig. 4D shows that the initial trajectory was upward and to the left and was the same for preinstruction, instruction, and postinstruction trials. At the point indicated by the arrow, the eye velocity in the instruction trials (red trace) deviated substantially from the other two traces. However, the postinstruction trials (blue trace) and preinstruction trials (black trace) were the same throughout the response. If the instructional stimulus had been a change in target direction, then the postinstruction trace (blue) would have deviated from the preinstruction trace (black) even sooner than did the instruction trace (red) because the learned eye velocity anticipates the time of the stimulus that instructs learning (Medina et al. 2005). The two-dimensional plots have the advantage of showing the results of our experiments in a single view, along with the disadvantage that time is not represented explicitly. Temporal information can be gleaned from the eye velocity records in Fig. 3, A and B.

Figure 4 shows the course of pursuit responses over a full instruction block in one example experiment. Each line in a graph shows the response for an individual trial and uses a color scale to plot the residual eye velocity defined as the eye velocity in that trial minus the mean for the same target motion in the preinstruction block of trials. The instruction and probe trials were interleaved, but Fig. 4 plots them separately for both horizontal (left) and vertical (right) eye velocity to show the absence of any systematic changes in pursuit responses as the experiment proceeded from the top to the bottom of each graph.

Inspection of Fig. 4 reveals two small changes in pursuit that developed over the course of most experiments, independent of any expressions of learning. First, a small positive residual develops over the first 50 test trials during the initiation of pursuit, just over 100 ms after the onset of target motion. This residual is $<0.5°/s$ on a baseline of $10°/s$ and resulted from a small decrease in the strength of initiation of leftward pursuit. We saw similar slight decreases in the strength of pursuit initiation in almost all experiments, including many that did not involve learning, and we think that this small shift is a consequence of fatigue, not a result of learning induced by instruction with electrical stimulation. Second, in this experiment and most others using floccular stimulation on monkey H, a negative residual developed in the instruction trials, in the middle of the stimulation interval from 300 to 600 ms after the onset of target motion (top left panel). The negative residual reflects a tendency for the eye velocity evoked by electrical stimulation of the floccular complex to return toward zero in the middle of the stimulation period. The same effect can be seen in the average horizontal eye velocity for instruction trials in Fig. 3A (red traces). We attribute this deflection to the monkey’s improved use of visual feedback during the experiment, in an attempt to overcome the retinal image motion that occurs when floccular stimulation drives the eye off the target. There is, however, no evidence that this response was expressed in the interleaved probe trials as any learned effect of
the electrical stimulus should be. Also note that learning should be expressed in postinstruction probe trials before the time of the instructional stimulation in the floccular complex (Carey et al. 2005; Medina et al. 2005) and should appear as white and yellow in the residuals of eye velocity in postinstruction probe trials. Inspection of the plots of horizontal and vertical eye velocity for postinstruction probe trials reveals some variation in responses around the blue, vertical line drawn at 300 ms, but no sign of a consistent learned change.

To evaluate the effects of instruction on pursuit, we performed quantitative analysis only for postinstruction probe trials that occurred ≥100 trials into the instruction block (below the bold, blue, horizontal lines in each graph). Previous work has shown that pursuit learning emerges within the first 30–50 trials and stabilizes (Carey et al. 2005); limiting our analyses to later trials reduces the possibility that any small learning effects were being obscured by including early, and potentially unaffacted, probe trials in the average. We inspected early probe trials as well and saw no indication that learning occurred in the trials prior to our analysis.

The apparent failure of electrical stimulation in the floccular complex to instruct learning in pursuit eye movements could reflect a competition between instructional signals in one direction from the electrical stimulation and instructional signals in the opposite direction from the visual image motion induced when stimulation dragged the eyes along at a velocity different from that of the target. In Figs. 3 and 4, the dip in middle of the eye velocity evoked by stimulation is presumably due to visual feedback, and these sensory signals could be in conflict with any instructive signals provide by electrical stimulation. We tested this possibility by stabilizing the target with respect to the moving eye during the period of electrical stimulation (see Carey et al. 2005; Morris and Lisberger 1987). In general, stimulation of the floccular complex induced somewhat larger eye movements during target stabilization (Fig. 5A) than during normal tracking, presumably because stabilization prevented visual feedback from reducing the stimulation effect. Further, the dip in eye velocity in the middle of the stimulation interval is much less pronounced, supporting our suggestion that the dip results from the use of visual feedback to overcome the retinal image motion created when stimulation drives the eye off target. Even under conditions of target stabilization, however, and even without the dip in eye velocity in the middle of the stimulation period, floccular stimulation still did not instruct learning. As shown in the two-dimensional plot of eye velocity in Fig. 5B, the average eye velocities evoked in postinstruction probe trials (blue) did not differ from those in preinstruction trials (black), even though electrical stimulation of the floccular complex caused a large deviation of eye velocity in instruction trials (red). Although time is not explicitly represented in the two-dimensional plot of Fig. 5B, the large symbols provide some temporal reference points by indicating eye velocity responses on the separate traces at the same times.

The lack of stimulation-induced learning held true across multiple experiments in both monkeys tested with floccular stimulation (13 experiments without target stabilization, 8 from monkey H, 5 from monkey W; 16 experiments with target stabilization, 10 from monkey H, 6 from monkey W). We have summarized all the data from stimulation of the floccular complex in Fig. 6 by plotting the mean horizontal (A–C) and vertical (D–F) eye velocities for instruction trials and postinstruction probe trials versus the mean velocity in preinstruction trials at three different times within the trial. In these plots, any effect of stimulation will cause points to plot above the diagonal line. The main finding is that all the responses during postinstruction probe trials (open symbols) plotted on the diagonal line, meaning that electrical stimulation did not cause any learning. As expected, the responses during instruction trials (filled symbols) plotted above the diagonal line 50 ms (B and E) after the onset of stimulation but not at the onset of stimulation (A and D). They also plotted above the diagonal line at 150 ms after the onset of stimulation for instruction trials that included target stabilization (filled red symbols, C and F) but not for trials that did not include target stabilization (filled black symbols, C and F). We think that a visually guided attempt to overcome the initial eye movements evoked by stimulation explain the absence of an increase in eye velocity 150 ms after the onset of stimulation in the instruction trials without target stabilization. The similarity of the data for stabilized (red symbols) and non-stabilized (black symbols) targets demonstrates that the absence of learning in the normal, non-stabilized target, experiments was not due solely to visual signals inhibiting instruction. We conclude that simply altering the motor commands for eye movement by stimulating the floccular complex during pursuit is not sufficient to instruct learning in pursuit. Learning does not result simply from cerebellar commands to execute a given movement over and over.

Stimulation of the smooth eye movement region of the FEFs

Previous work from our laboratory showed that stimulation in area MT, within the sensory domain of the pursuit pathways, is sufficient to instruct learning in pursuit eye movements
(Carey et al. 2005), suggesting that instructive signals may originate early in the pursuit circuit. Therefore we tested whether pursuit learning can be instructed by stimulation of the smooth eye movement region of the frontal eye fields (FEFSEM), which is a motor-related area earlier in the pursuit circuit than the cerebellum. Stimulation in FEFSEM induces smooth eye movements, and those eye movements are modified in parallel with visually instructed learning in pursuit (Chou and Lisberger 2004). Perhaps execution of eye movements induced by microstimulation in the FEFSEM provides sufficient instructive signals to downstream sites of learning.

We began each experiment by searching for a site in the FEFSEM where microstimulation through the electrode evoked consistent smooth eye movements. Sites were excluded if stimulation caused reliable or stereotyped saccades even if smooth pursuit was also evoked or if stimulation evoked eye movement along a direction that the animal pursued poorly (upwards for monkey L); this occurred infrequently. Sites also were discarded if the evoked eye movement drifted substantially in amplitude or direction over the course of the experimental session. To characterize stimulation sites, we used the approach explored thoroughly by Tanaka and Lisberger (2002) and stimulated during ongoing pursuit in different directions. As they reported, different sites had somewhat different patterns of effects, depending on the direction of the pursuit at the time of stimulation. At one extreme, stimulation evoked an increase in the amplitude of the eye velocity in the ongoing direction of pursuit (Fig. 7A); this site was in monkey D, for which we had to limit the duration of stimulation to 75 ms to avoid frequent saccades in the opposite direction from ongoing pursuit. The short pulse train generally evoked a distinct, biphasic effect on eye velocity; the initial increase was followed by a short period of slowing. At the other extreme, stimulation in the FEFSEM evoked smooth eye movement toward the side of stimulation (Fig. 7B) without regard for the direction of ongoing pursuit; this site was in monkey L, for
whom we used longer pulse trains without eliciting a consistent saccade.

Microstimulation in the FEFSEM reliably evoked changes in eye velocity in instruction trials both with (Fig. 8A) and without (C) target stabilization but did not instruct any learned changes in eye velocity that persisted on postinstruction probe trials. In both sets of example traces, the difference between the eye velocity in instruction trials and that in preinstruction trials (red traces) was reliable during the period of stimulation. However, the difference was not evident on probe trials that occurred after the first 100 trials in the instruction block (blue traces). The absence of any instructive effect can be seen clearly in the two-dimensional eye velocity plots of Fig. 8, B and D. Here the eye velocity traces for the preinstruction (black) and postinstruction probe (blue) trials superimposed, while those for the instruction trials themselves (red) had an extra loop that was an immediate consequence of the electrical stimulation in the FEFSEM. As before, the large symbols in Fig. 8, B and D, were plotted at the same two times on all three traces and should help to align the different traces in time.

Altogether, we conducted microstimulation without target stabilization at 19 sites in the FEFSEM (9 in monkey D, 10 in monkey L) and with stabilization at 15 sites (8 in monkey D and 7 in monkey L). Figure 9 summarizes the results of these experiments by analyzing eye velocity at three times in the pursuit response. As in Fig. 6, we have plotted average eye velocity for both instruction trials (solid symbols) and postinstruction probe trials (open symbols) against the mean preinstruction eye velocity for both horizontal (A–C) and vertical (D and E) components. For graphical clarity, we have assigned positive values to eye velocity in the direction of the target motion used for each experiment. At the time corresponding to stimulation onset (Fig. 6, A and D), both postinstruction probe and instruction eye velocities lay on or slightly below the diagonal. At later times (Fig. 6, B, C, E, and F), eye velocity on instruction trials mostly plotted above the diagonal, whereas that in postinstruction trials continued to plot on or below the diagonal indicating no change from preinstruction trials. Had learning occurred and been retained beyond the instruction trials, we would have expected the open symbols to plot as far above the diagonal line as do the filled symbols. Further, because directional learning in pursuit is expressed at the time of the instructive stimulus (Medina et al. 2005), the absence of any change in the eye velocity at the time of microstimulation in the instructional trials (Fig. 9, A and D, filled symbols) provides additional evidence that learning did not occur. We also did not observe any change in eye velocity at the time of microstimulation in comparisons of the first and last 200 instruction trials except for some generalized slowing of pursuit responses. We conclude that stimulation of the FEFSEM did not instruct learning in pursuit eye movements.

Figure 9, A and D, reveals modest but equal decreases in eye velocity in both instruction trials and postinstruction probe trials at the time corresponding to stimulation onset. We have two reasons for thinking that these decreases represent drift in the baseline pursuit relative to preinstruction trials, and not learning. First, we do not see the same decreases either 50 or 150 ms after the onset of stimulation as we should if the early effects were due to learning. Second, if the decreases are related to learning, then the direction of the change in eye velocity at the onset of microstimulation in the FEFSEM should be related to the direction of the eye velocity caused by stimulation. It was not as shown in the example in Fig. 10A. Here the initiation of pursuit was weaker in the instruction trials and the postinstruction probe trials than in the preinstruction control trials, so that eye velocity was smaller than control at the start of the microstimulation. However, microstimulation

---

**FIG. 8.** Example data from 1 experiment with the target stabilized (A and B) or not stabilized (C and D) with respect to the moving eye during stimulation of the FEFSEM. A and C: averages of the difference between eye velocity in the instruction block and in preinstruction control trials, plotted vs. time. Red and blue traces show averages for instruction trials and probe trials that were delivered after the 1st 100 trials in the instruction block. The 2 pairs of traces show horizontal and vertical eye velocity. B and D: average vertical eye velocity is plotted vs. average horizontal eye velocity to illustrate eye velocity trajectories in 2 dimensions. Black, red, and blue traces show responses in preinstruction trials, instruction trials that included electrical stimulation of the FEFSEM, and probe trials that occurred after the 1st 100 trials in the instruction block. Circles and squares indicate the value of eye velocity at the start and end of the interval that delivered electrical stimulation in instruction trials.
caused an increase in leftward horizontal eye velocity and, if learning had occurred, should have caused an increase we did not observe in leftward eye velocity in the postinstruction probe trials. Figure 10, B and C, summarize the results at all of our stimulation sites and show that there was a complete lack of relationship between the direction of the difference eye velocity at the onset of microstimulation in postinstruction probe trials (x axis) and that 150 ms after the onset of stimulation in instruction trials (y axis). We conclude that execution of eye movements induced through stimulation of the FEFSEM is not sufficient to instruct directional learning in pursuit.

Normal pursuit learning for visual instructive signals

The four monkeys used in these experiments were veterans of experiments on pursuit learning and all showed strong learning in conditions where changes in the speed or direction of target motion were used as instructive sensory signals. Importantly, learning tests with natural stimuli employed changes in target motion at the same times as the application of electrical stimulation in the floccular complex and the FEFSEM; 200–300 ms after the onset of target motion, ensuring that the time of potentially instructive electrical stimulation was in a window where learning would normally occur. In addition, we verified in monkey D that stimulation in area MT instructed learning, as our laboratory had reported previously (Carey et al. 2005). The success of these control experiments indicates that our inability to induce learning through electrical stimulation of the cerebellar flocculus or the FEFSEM represents a genuine feature of the organization of the pursuit circuit and is not a consequence of a general failure of any of our monkey subjects to undergo pursuit learning or of an incorrect choice of the time of electrical stimulation.

**DISCUSSION**

One important step in determining how and where motor learning occurs in the brain is to evaluate the identity of the neural pathways that deliver instructive signals to the site(s) of learning. Our prior work had shown that extrastriate visual area MT delivers signals to the instructive pathways for learning in pursuit (Carey et al. 2005). Our goal in the present paper was to evaluate other potential areas and signals that might also have access to the instructive pathways for learning in pursuit.

**FIG. 9.** Quantitative summary of absence of instructive effects of electrical stimulation in the FEFSEM. A–C: horizontal eye velocity. From left to right, graphs show eye velocity measured at the time of onset of FEFSEM stimulation and 50 and 150 ms later. Red and black symbols show data for trials with vs. without target stabilization during the time of electrical stimulation. Triangles and diamonds show data from monkeys L and D. Note that open symbols have been drawn on top of the filled symbols, so that many of the latter are invisible in A and D, where many of the points lay on the unity line. For experiments where the eye velocity was negative on preinstruction trials (i.e., left- or downward target motions), eye velocities were inverted for graphical clarity.

**FIG. 10.** Detailed analysis of changes in eye velocity at the onset of microstimulation. A: averages from an example experiment. Black, red, and blue traces show responses in preinstruction trials, instruction trials, and probe trials. The 2 sets of traces show vertical and horizontal eye velocity. The bold, horizontal, red line shows the time of microstimulation in the FEFSEM. The up and down arrows indicate the measurement times used to create the 2 graphs (B and C) at the onset of microstimulation and 150 ms later. B and C: each point shows data from 1 experiment and plots horizontal (B) and vertical (C) difference eye velocity in instruction trials 150 ms after the onset of microstimulation as a function of difference eye velocity in probe trials at the time when microstimulation would have occurred. Difference values were obtained by subtracting the mean eye velocity in preinstruction trials from the average eye velocities in probe and instruction trials.
We have found that learning is not instructed by electrical activation of the outputs from either the floccular complex of the cerebellum or the FEFSEM.

Our criteria for results that would constitute learning instructed by electrical stimulation in the floccular complex or FEFSEM are based on the features of learning instructed by changes in the direction of target motion (e.g., Medina et al. 2005). When a target changes direction 200–300 ms after the onset of motion, learning is expressed during postinstruction probe trials in which the target simply moves continuously in its initial direction. The expression of learning starts before and reaches a peak near the time when the target would have changed direction. In the present experiments, we never found any evidence that instruction by electrical stimulation commencing 200–300 ms after the onset of target motion caused changes that persisted in postinstruction probe trials. Thus we conclude that electrical stimulation did not instruct the form of learning that occurs with natural stimuli.

We did see minor changes in eye velocity evoked by floccular stimulation as the monkey went through a sequence of instruction trials, but these changes did not persist in postinstruction probe trials and occurred too late relative to the onset of the instructive stimulus to be classified as the form or learning we have investigated. Thus while electrical stimulation might have been associated with some modest modifications in behavior, these did not fit the previously established criteria for behavioral learning in smooth pursuit eye movements.

Necessity of sensory errors

Previous work on motor learning in the pursuit system has used paradigms in which it was not possible to segregate the sensory and motor signals that might be used to instruct learning. When the learning paradigm involves an instructive change in the direction or speed of target motion, the initial learning stimulus provides sensory signals related to the imposed error as well as evoking an immediate motor response to the error, along with potentially instructive motor signals inside the brain. Motor instructive signals were not excluded even in a related study from our laboratory that used microstimulation in area MT to instruct learning, because activation of MT also caused a small smooth eye movement (Carey et al. 2005) and, again, the associated motor signals inside the brain. Still, we think that microstimulation in MT generates a primarily sensory signal, because it both exerts a directional effect on pursuit eye movements (Groh et al. 1997; Komatsu and Wurtz 1989) and biases perceptual judgments on a motion discrimination task (Salzman et al. 1992). In contrast, stimulation in the FEFSEM and the floccular complex produces motor signals that drive eye motion with quite short latencies of 25 and 10 ms, respectively. The successful instruction of pursuit learning from stimulation in area MT, combined with our failure to instruct learning with stimulation in the FEFSEM and the floccular complex, suggests that sensory signals are necessary, and possibly also sufficient, to instruct learning in pursuit.

We are suggesting that the failure of stimulation in the floccular complex or the FEFSEM to instruct learning reflects the organization of the pursuit pathways that instruct learning. Importantly, the stimulation was successful in evoking movement, showing that the stimulation effectively activated the floccular complex or the FEFSEM. However, negative results from electrical stimulation could have many causes, making it important to consider alternative interpretations. We do not think that the absence of an explicit reward for responding to the electrical stimulation can account for the absence of learning. First, the reward structure of our trials is the same as those that used a change in target direction (Medina et al. 2005) or electrical microstimulation in MT (Carey et al. 2005) to successfully instruct learning. The fixation requirements were suspended whenever the target changed direction or electrical stimulation was delivered, so that the monkeys were never punished for events that were outside their control, and they received rewards at the end of each trial for having eye position close to target position. Second, our attempts to induce learning by providing extra rewards only for larger or smaller eye velocities at a given time have not been very successful in modulating pursuit behavior (K. Bouchard and S. G. Lisberger, unpublished observations). Thus it is hard to imagine how reward contingencies could account for the failure of stimulation in the floccular complex or the FEFSEM to instruct learning.

If sensory signals instruct learning while motor signals do not, then one of the counterintuitive aspects of our results is the absence of learning in the direction opposite the eye movements induced by electrical stimulation. For example, a rightward smooth eye movement evoked by electrical stimulation causes leftward visual motion of the target. We might anticipate that learning would cause a leftward shift in eye velocity on probe trials. For stimulation in area MT, it was possible to segregate the learned component of eye velocity into two temporally distinct components. One component was in the direction of the eye movement instructed by stimulation in MT, and the other was in the opposite direction, instructed by the visual motion signals created when the electrical stimulation drove the eye away from the moving target.

The absence of sensory-instructed learning in the direction opposite to the eye movements evoked by stimulation in the present experiments cannot be attributed to competition with motor-instructed learning because the latter did not emerge when we eliminated sensory signals by stabilizing the target with respect to the moving eye during stimulation. It cannot be attributed to the small size of some of our stimulation effects because learning was instructed successfully in experiments where target speed or direction was altered with similarly small magnitudes either by target manipulation or by microstimulation in area MT (Carey et al. 2005). It also cannot be explained by a complete failure of sensory signals to alter the smooth eye movements evoked by electrical stimulation: at least for stimulation of the floccular complex, sensory signals are able to modulate eye velocity under non-stabilized target conditions, as seen in Figs. 3 and 4, even though the sensory signals do not instruct pursuit learning. Instead we suggest that stimulation of the floccular complex or the FEFSEM drives the system in a way that trumps conflicting sensory error signals, preventing learning from occurring even when appropriate sensory instruction signals are present.

In the floccular complex, electrical stimulation could be trumping sensory error signals by interfering with signal processing in the cerebellar cortex or one synapse downstream in the vestibular nucleus. Prior recording experiments have argued that the locus of learning may be in the floccular cortex (Kahlon and Lisberger 2000). Thus electrical activation of the floccular complex might be preventing any learning that nor-
mally occurs in the cerebellar cortex. This explanation fits with our conclusion that cerebellar output signals are not suitable for instructing pursuit learning. By analogy to eyelid conditioning (Mauk 1997) and adaptation of the vestibuloocular reflex (Lisberger 1994), another likely site of pursuit learning would be at the floccular target neurons in the vestibular nucleus. We might expect electrical stimulation in the cerebellar cortex to drive learning at the next synapse, but it might instead prevent learning by causing highly abnormal signals to be delivered to the floccular target neurons. Evidence that pursuit learning occurs at the floccular target neurons would turn this explanation into a plausible explanation for our finding that electrical stimulation of the floccular complex does not instruct pursuit learning.

In the FEFSEM, it seems unlikely that electrical stimulation trumps visual instructions for learning by disrupting neural signals immediately within the FEFSEM; some, and possibly all, pursuit learning occurs downstream from the FEFSEM, and microstimulation of the FEFSEM transmits some signals through a site of learning (Chou and Lisberger 2004). Thus microstimulation in the FEFSEM fails to instruct learning even though a site of learning receives the signals that arise from stimulation. One concern is that the FEFSEM has been implicated in multiple components of smooth pursuit and its output may not be a true motor command signal. For example, microstimulation in FEFSEM enhances the gain of pursuit eye movements and of the pursuit response to a given visual motion input (Tanaka and Lisberger 2001, 2002). A second concern is that electrical stimulation of the FEFSEM may not provide signals in a natural enough form to instruct pursuit learning. We have some hints that this may be a genuine problem because stimulation of the FEFSEM evokes responses in floccular Purkinje cells that seem to be unnatural in the sense that they cannot be explained simply by the tuning of Purkinje cells for smooth eye velocity (Rottman and Lisberger 2007). Either of these explanations is compatible with our suggestion that the failure of stimulation in the FEFSEM reflects the organization of the pursuit circuit and, in particular, of the pathways that instruct pursuit learning. We think that feedback signals related to eye movement impact the learning system differently than do feedback signals related to image motion.

Doing without learning

From introspection, it seems reasonable to think that we learn a motor skill by doing it and that we perfect it through doing it repeatedly. Although “practice makes perfect” may be true at the level of behavioral performance, our data imply that simply executing a pursuit eye movement is not sufficient to learn it, at least based on motor signals evoked by stimulation of the floccular complex of the cerebellum and the FEFSEM. Sensory error signals seem to be necessary, although concurrent motor activity also may be required. We performed the experiments reported here to better understand learning in pursuit eye movements and not to test any specific hypothesis of learning. Still, it is worth noting that our conclusions are entirely consistent with the cerebellar learning theory, in which signals arising over the climbing fiber system are instructive and cause learning through depression of parallel fiber inputs. Visual error signals are reported to the floccular complex through the climbing fiber system (Alley et al. 1975; Maekawa and Simpson 1972; Stone and Lisberger 1990), while signals related to ongoing motor activity are prominent in mossy fiber inputs (Lisberger and Fuchs 1978; Miles and Lisberger 1981). Climbing fiber inputs seem to be indicators of sensory events and to be unrelated to the motor commands. Thus the necessity of a sensory error signal to instruct learning, and the failure of signals that emanate from motor structures, could reflect the critical importance of the climbing fiber pathways or other purely sensory inputs to the pursuit circuit, in instructing motor learning.

It is worth noting that other forms of motor learning rely predominantly on sensory error signals rather than signals related to motor adjustments. For example, saccadic gain adaptation can be elicited in the absence of corrective saccades (Noto and Robinson 2001; Wallman and Fuchs 1998). Similarly, sensory errors seem to be sufficient to instruct adaptation of visually guided reaching (Tseng et al. 2007); adding corrective motor adjustments in conjunction with visual error during a reaching task did not influence the amount of adaptation. Because of the ease of studying the neural basis for eye movements, pursuit learning seems like an excellent system for demonstrating the neural mechanisms of the more general phenomenon of motor learning induced by sensory instruction signals.

Acknowledgments

We thank K. MacLeod, E. Montgomery, S. Ruffner, L. Bosckai, D. Frank, K. McGary, D. Wolfgang-Kimball, and D. Kleinhessselink for technical assistance.

Grants

This research was supported by the Howard Hughes Medical Institute.

References


