Supplementary Eye Fields Stimulation Facilitates Anticipatory Pursuit

M. Missal¹,² and S. J. Heinen¹
¹The Smith-Kettlewell Eye Research Institute, San Francisco, California 94115; and ²Laboratoire de Neuropsychologie, Université Catholique de Louvain, 1200 Brussels, Belgium

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INTRODUCTION

A candidate structure to search for a neural correlate of anticipatory pursuit could be the frontal lobe. Indeed, the frontal pursuit area (FPA) of the frontal eye fields (FEFs) seems to be involved in the determination of the smooth pursuit response to a given moving stimulus, thereby participating in target selection for pursuit (Tanaka and Lisberger 2001).Lesions of the FEF decrease the performance of pursuit as a whole and could cause a deficit of anticipatory or predictive responses (MacAvoy et al. 1991; but see Keating 1993). In this study, we focused on the role of the supplementary eye fields (SEFs) of the dorsomedial frontal cortex (Schlag and Schlag-Rey 1987) because it has been shown that neurons in the SEF are active during smooth pursuit in the absence of saccades (Heinen 1995), especially when predictable changes in target motion occur (Heinen and Liu 1997). Activation of the SEF during smooth pursuit has been confirmed using imaging techniques in humans (O’Driscoll et al. 2000), but the precise role of this structure in the pursuit pathway is unknown. In the behaving monkey, it has been shown that electrical microstimulation in the SEF can facilitate smooth pursuit initiation toward a moving target, suggesting that activation of the SEF might change the gain of smooth pursuit (Missal and Heinen 2001). In this study, we report a set of microstimulation experiments that show that the SEF could play a role in anticipatory smooth pursuit, before the appearance of an expected moving target.

METHODS

Two male rhesus monkeys (Macaca mulatta; referred to as GU and SA) were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee and were in compliance with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals. A stainless steel recording chamber (Crist Instruments) was positioned at 24 mm anterior in Horsley-Clark stereotaxic coordinates and centered on the midline of the brain in monkey GU and 5 mm right to the midline in monkey SA. Eye movements were recorded with the scleral search-coil method.

Monkeys had their heads immobilized and were trained to pursue a 1° target spot back-projected on a tangent screen. Each trial started with a 500-ms fixation period. At the end of the fixation period, the target disappeared for 200 ms. This 200-ms “gap” of the fixation target was used to favor the anticipatory response, as has been shown previously (Boman and Hotson 1988). After the reappearance of the target, it immediately stepped to a position 2–4° eccentric and then started to move at a constant velocity toward the fovea (usually at 40 or 60°/s).

Stimulation trains consisted of bipolar pulses (0.25 ms for each phase; frequency, 300 Hz). Train duration was usually 200 ms (except at 9/38 sites, 400 ms). Most results were obtained with a 75-μA current intensity (20/38 sites). The current intensity statistics were as follows: mean, 107.2 ± 58 μA (n = 38); median, 75 μA; min, 50 μA; max, 200 μA.

Eye velocity was obtained by computing the difference in eye position between two successive digital samples and by dividing this difference by the time elapsed between two samples (1 ms). We define anticipatory pursuit as a nonzero eye velocity at the time of target motion onset. To reduce spurious effects due to noise, eye velocity was averaged during a 20-ms window period centered on the time of target motion onset. By definition, an anticipatory pursuit movement occurred if this average eye velocity exceeded the average velocity of the eye during fixation. Theoretically, eye velocity during fixation should be close to zero on average, but it showed variations due to noise, making the detection and the determination of the latency of anticipatory pursuit difficult. Indeed, during anticipatory pursuit the eye had a very low initial acceleration, and anticipatory eye velocity progressively emerged from the eye velocity signal observed during fixation. To overcome this problem, the following procedure was adopted to estimate the value of the eye velocity signal during fixation. 1) Trials containing a prolonged period of steady fixation
(≥400 ms) were selected. 2) For these trials, average eye velocity during a period of 100-ms fixation before gap onset was computed. Other trials were discarded for the estimation of eye velocity during fixation. 3) The grand average was computed from all the selected fixation periods in a block of trials (n ~ 10–20). This average eye velocity signal during fixation usually varied between 0.1 and 0.3°/s. 4) For all trials and on a trial-by-trial basis, eye velocity at the time of target motion onset (that was averaged in a 20-ms bin) was compared with this estimation of the eye velocity signal during fixation for a particular block of trials. To be considered as anticipatory pursuit, eye velocity at the time of target motion onset had to exceed the limits of 2 SD of the fixational eye velocity signal for ≥50 ms. Anticipatory pursuit latency was defined as the time when eye velocity exited the 2 SD limit. The duration criterion of 50 ms was used to avoid that an accidental crossing of the threshold could be considered as an anticipatory pursuit response.

RESULTS

Stimulation was delivered at 38 stimulation sites in the region of the SEF of two monkeys. Robust anticipatory pursuit movements were obtained by having monkeys pursue a target that moved always in the same direction in a particular block of trials, and stimulation increased the velocity of anticipatory pursuit. Typical anticipatory pursuit responses are shown on Fig. 1 for the control (blue traces) and SEF stimulation conditions (red traces; all traces aligned on target motion onset). In the control condition, eye velocity reached 7.2°/s at the time of target motion onset (Fig. 1, filled vertical arrow) during this particular trial. On average, leftward eye velocity reached 7.6 ± 0.6°/s (SE) in controls (n = 21; Fig. 1C, blue traces). In the stimulation condition (60 μA at 300 Hz), eye velocity was larger than in the control situation and reached 16.9°/s (Fig. 1B, red trace). Average eye velocity during stimulation trials was 14.2 ± 1.2°/s (n = 14; Fig. 1C, red traces). The main effect of electrical stimulation was to increase anticipatory eye velocity (P < 0.01) in the direction of the expected future target motion. This effect will be referred to as anticipatory pursuit facilitation. As a consequence of facilitation, the eye reached the target 87 ms after target motion onset during stimulation and only after 223 ms in the control trial, after a catch-up saccade. Anticipatory pursuit started 141.8 ± 11.5 ms (n = 21) before target motion onset in controls and 157.9 ± 12.2 ms (n = 14) before this event in stimulation trials with this particular set of stimulation train parameters (t-test; P = 0.3582; not significant). With the stimulation train starting at the end of the fixation period or during the gap, a significant increase of anticipatory eye velocity was observed at 25/38 sites tested (66%). At six additional sites, the number of anticipatory pursuit movements in blocks of control trials was too small (n < 5) to allow a reliable statistical analysis. On average across all sites, the proportion of anticipatory smooth movements was significantly larger in stimulation blocks of trials (0.83 ± 0.20; n = 38) than in controls (0.65 ± 0.30; n = 38; paired t-test; P < 0.0001; n = 38).

At the beginning of each stimulation experiment at a particular site in the SEF, we first rapidly determined the direction in which facilitation of anticipatory pursuit was the largest by comparing eye velocity traces in control and stimulation trials obtained by analog on-line differentiation. We found that at 17 stimulation sites, ipsilaterally directed facilitation was observed (17/25). Subsequent experiments were carried on in the preferred direction. However, at the site presented on Fig. 1 (site SA43, right hemisphere), eye velocity was also increased by electrical stimulation with the same current parameters during rightward anticipatory pursuit obtained after training to pursue a rightward moving target (3.7 ± 1.6, n = 18 vs. 8.8 ± 3.4°/s, n = 20; P = 5.2 × 10⁻⁷). This result shows that stimulation at the same site could facilitate anticipatory pursuit in both horizontal directions after the animal became accus-
tom ed to the target moving in one direction (usually 10–20 trials in the same direction). The possibility to facilitate anticipatory pursuit in both horizontal directions was investigated at 13 stimulation sites. At nine sites, anticipatory pursuit could be facilitated in both horizontal directions (9/13; 69%). At all sites where a significant facilitation was observed (n = 25), we found that it depended strongly on the onset time of the stimulation train with respect to target motion onset. At 13 sites, we further investigated the influence of stimulation timing by changing the onset time of the stimulation train with respect to target motion onset. The statistics of the different onset times were as follows: mean, 4.1 different onset times; median, 4 different onset times; minimum, 2 different onset times; maximum, 6 different onset times. Figure 2A shows a schematic of the experimental design for a particular site tested. Different onset times of the stimulation train were tested in different blocks of trials. The order of the blocks of 20–30 trials was randomized. The earliest onset time of the 200-ms stimulation train was 600 ms before target motion onset. At this stimulation site, six different onset times were tested, regularly separated by steps of 100 ms (600–100 ms before target motion onset). Figure 2B shows the anticipatory eye velocity in controls (blue symbols) and stimulation trials (red symbols) for the different timings of the stimulation trains. It can be seen that stimulation onset time played a dominant role in the process of anticipatory pursuit facilitation. The largest increase in eye velocity was observed when the train started 300 ms before target motion onset. The electrically induced increase in eye velocity was less for earlier or later onset times. Timing of the stimulation train had a significant effect on eye velocity at all 13 sites where it was systematically investigated (2-way ANOVA, interaction stimulation × timing; P < 0.05). The largest effect on eye velocity most often occurred when stimulation began either 300 (5/13) or 200 ms before target motion onset (4/13 sites). This period corresponded to the end of the fixation period and the beginning of the 200-ms gap.

As represented on Fig. 2C, stimulation also significantly altered the latency of anticipatory pursuit with respect to target motion onset, although more variability was observed. At this site, the largest effect on anticipatory pursuit latency was observed for a stimulation train that started 500 ms before pursuit onset at this site. With this onset time, the eye started to move 108.8 ± 14.5 ms (n = 19) before target motion onset in controls and 188.3 ± 46.0 ms (n = 12) in stimulation trials. Timing of stimulation trains had also a significant effect on anticipatory pursuit latency at 10 of 13 stimulation sites where a significant effect on eye velocity was found. The largest effect on latency most often occurred when stimulation began either 300 (6/10) or 200 ms before target motion onset (4/10 sites). On average, the peak effect of the influence of stimulation train timing on latency occurred 60 ms before the peak of the effect on eye velocity. These results show that the optimal

Fig. 2. Influence of stimulation train timing. A: schematic representation of the experimental procedure. Filled arrow shows average anticipatory pursuit latency (approximately −120 ms) with respect to target motion onset. B: relationship between stimulation train onset and latency of the 2000-ms gap. C: relationship between stimulation train onset and anticipatory pursuit latency at a single site. Reference time is the beginning of target motion period (time 0). Negative values indicate that the 200-ms stimulation train started before target motion onset. Same color conventions as Fig. 1. Current intensity: 60 μA at 300 Hz. C: relationship between stimulation train onset and anticipatory pursuit latency at the same site. D: relationship between anticipatory pursuit latency and anticipatory eye velocity for a site where both variables were significantly altered by microstimulation. Current intensity: 60 μA at 300 Hz. In controls (open symbols, interrupted line), equation of regression line is Y = 1.0 – 44.8X (r = −0.82, P < 0.001, n = 18, intercept not significantly different from 0). In stimulation trials (filled symbols, continuous line), equation of regression line is Y = 0.8 – 66.2X (r = −0.82, P < 0.001, n = 22, intercept not significantly different from 0). E: relationship between anticipatory pursuit latency and anticipatory eye velocity for a site where only eye velocity was significantly altered by microstimulation. In controls, equation of regression line is Y = 2.3 – 50.2X (r = −0.43, n = 23, P = 0.04, intercept not significantly different from 0). In stimulation trials, equation of regression line is Y = −2.7 – 139.5X (r = −0.50, n = 22, P = 0.018, intercept not significantly different from 0). F: example of anticipatory pursuit facilitation when the stimulation train was delivered during the fixation period and ended before the beginning of the earliest anticipatory response. The eye was immobile during the stimulation period.
timing to alter latency and velocity were close. The effect of stimulation on eye velocity could simply be caused by an earlier onset of the movement. Indeed, if anticipatory pursuit started earlier, eye velocity could reach a higher level at the time of target motion onset because of the prolonged acceleration period. Therefore we systematically measured the correlation between latency (independent variable) and eye velocity (dependent variable). We found a significant correlation between latency and eye velocity at all sites in controls as well as in stimulation trials ($P < 0.05$). Two different typical cases can summarize the results. In the first case, both eye velocity and latency were significantly altered and co-varied (see Fig. 2D). Anticipatory eye velocity was higher, and movements started earlier during stimulation trials (Fig. 2D, filled symbols and continuous line) than in controls (open symbols and interrupted line). However, experimental data points were clustered around a different regression line in stimulation trials compared with controls. The slope of the regression line was higher in stimulation trials than in controls.

This result shows that eye velocity in stimulation trials was not simply caused by an earlier movement onset. Indeed, in the latter case, stimulation data points would be in the continuation of the regression line for controls. At the population level (all sites with significant facilitation, $n = 25$), a significant effect of stimulation on eye velocity and on latency together was observed at 12 sites. In the second case, only eye velocity was significantly altered by stimulation but not latency (see Fig. 2E). Stimulation data points were in the same range of latencies but a different range of eye velocities. Regression lines were also different (see Fig. 2). At the population level (all sites with significant facilitation, $n = 25$), a significant effect of stimulation on eye velocity in the absence of an effect on latency was observed at 13 sites. We experimentally manipulated the onset of the stimulation train and found that it was indeed possible to affect anticipatory eye velocity independently of latency. For the example site presented on Fig. 2, B and C, it can be observed that, although an optimal effect was observed at ~300 ms on eye velocity, the effect on latency was not significant for the same onset time. An increase in eye velocity could occur independently of a reduction in movement latency. We conclude that, although eye velocity and latency were correlated, the increase in anticipatory pursuit velocity was not simply due to an earlier onset of the response in stimulation trials compared with controls.

We found that electrical stimulation as early as 500 ms before target motion onset could alter anticipatory pursuit velocity even if stimulation ended before the onset of the anticipatory response (observed at all 4 sites tested with early stimulation). Figure 2F shows control and stimulation traces (site SA24, not the same site as on Fig. 2, A–C) when electrical stimulation started 500 ms before target motion onset. Control and stimulations trials were randomly interleaved. It can be seen that, during electrical stimulation, the velocity of the eye did not increase as the animal was involved in the attentive fixation of the central target. Toward the end of the fixation period, eye velocity started to increase in stimulation trials. Anticipatory pursuit latency was significantly altered (controls: $-48 \pm 5$ ms, $n = 20$; stimulation trials: $-156 \pm 5$ ms; $P = 0.001$). At the time of target motion onset, the difference between eye velocity in controls ($4.5 \pm 0.7^\circ$/s, $n = 20$) and stimulation trials ($11.0 \pm 1.0^\circ$/s, $n = 18$) was statistically significant ($P = 1.9 \times 10^{-6}$). This example shows that stimulation in the SEF did not directly trigger a motor response during the stimulation period, although it significantly changed the latency and velocity of the subsequent smooth movement.

Figure 3A shows the distribution of the percentage increase in eye velocity for all sites tested in this study. The average percentage increase in anticipatory eye velocity due to stimulation was $35.5 \pm 2.8\%$ ($n = 38$) and was significantly different from zero ($t$-test; $P < 1 \times 10^{-6}$). Stimulation increased anticipatory eye velocity at 37/38 sites (note that at 6 sites, the number of anticipatory movements in controls was extremely low, $n < 5$). On a site-by-site analysis, a statistically significant increase in eye velocity was observed at 25 sites. At
these 25 sites, stimulation was repeated in several blocks of trials, yielding a total of 70 experiments where facilitation was observed. Figure 3B shows a distribution of the difference in anticipatory pursuit latency between control and stimulation trials. On average, the latency of anticipatory pursuit decreased by 24.5 ± 3.8 ms (n = 38; t-test; P < 1 × 10⁻⁶). A statistically significant reduction of anticipatory pursuit latency was observed in 37% of all stimulation sites (14/38). The data presented on Fig. 3 was obtained with the best timing of the stimulation train to increase anticipatory eye velocity. Figure 3C shows that eye velocity during stimulation trials increased linearly as a function of eye velocity in controls, and a significant correlation between these quantities was found (Y = 0.67 + 1.42X, r = 0.95, n = 38, P < 0.0001; intercept not significantly different from 0, P = 0.09). This result suggests that facilitation was caused by an electrically evoked increase of the gain of the anticipatory smooth movement.

In the experiments described above, target motion direction was always the same, and the future direction of the target was entirely predictable. This was the most efficient method to show a potential effect of microstimulation on anticipatory responses. However, it has been shown that altering the predictability of target motion direction can alter anticipatory pursuit in humans (Kowler et al. 1984). If the SEF play a role in anticipatory pursuit, altering the predictability of target motion direction could also lead to significant differences in the facilitatory effect. Therefore we compared anticipatory pursuit responses when there was only one target motion direction in a given block of trials (left only or right only) or when two different target motion directions were equally likely to occur (left or right). Figure 4A shows that, in controls, anticipatory pursuit in a given direction is stronger when only one target motion direction was presented to the subject (compare Fig. 4A, left open symbols). During stimulation trials, a significant facilitation was obtained only when target motion direction was predictable to the right (facilitatory effect indicated by Fig. 4A, 2-headed arrow) and did not occur when target motion direction was randomized (Fig. 4A, right symbols). At this particular site, facilitation occurred only during rightward anticipatory pursuit when target motion direction was entirely predictable. We repeated this experiment at seven additional stimulation sites. The result was that the effect of electrical microstimulation in the SEF was always stronger when the direction of future target motion was entirely predictable. At most sites (6/8), facilitation was significant only when there was only one target motion direction, justifying our choice to keep target motion direction entirely predictable during most experiments.

When two different target motion directions were randomly interleaved, it happened that particular sequences of target motion direction could occur. For instance, two trials to the right or to the left could occur in a row (with P = 0.5²). It has been shown previously that such sequences are used to guide anticipatory pursuit (Kowler et al. 1984). Therefore we analyzed the effect of SEF microstimulation, taking into account the number of trials in a particular direction. Figure 4B shows the result of this analysis. On this figure, 0 labels average eye velocity before classification of trials according to the sequence of previous target motion directions; 1 labels the category of anticipatory movements preceded by one trial to the right or to the left; 2 labels the category of movements where the two previous target motion directions were in the same direction; and >2 shows anticipatory eye velocity in independent blocks of trials with only one direction of target motion. Figure 4B shows that anticipatory pursuit velocity increased as the number of trials in a particular direction increased. Anticipatory
pursuit during stimulation trials was also larger as the number of trials in a given direction increased. The direction of the facilitatory effect varied with the direction the trials in a sequence, to the left (Fig. 4B, negative values) or to the right (positive values). A total of 100 controls and 100 stimulation trials at the same site were collected to establish this result.

Figure 4C shows that electrical stimulation did not evoke an anticipatory pursuit response when the monkey was not expecting an upcoming target motion. In this control experiment, the target reappeared at the end of the gap but remained stationary instead of starting to move at a constant velocity. The monkey did not anticipate the movement of the target, there was no anticipatory pursuit in controls, and the effect of microstimulation was not significant at target motion onset (Fig. 4C, vertical arrow; t-test, \( P = 0.78 \)). At the same site, anticipatory pursuit was strongly facilitated when the gap was followed by target motion (controls: 2.7°/s; stimulation trials: 6.6°/s; t-test, \( P < 0.05 \)). This result suggests that electrical stimulation in the SEF during fixation periods at sites where anticipatory pursuit was facilitated did not evoke anticipatory smooth eye movements in the absence of an expectation of target motion. At sites with significant facilitation (\( n = 25 \)), stimulation during a fixation period when the animal was not involved in an experimental task and was freely moving its gaze to different positions on the screen had no effect.

**DISCUSSION**

In this study, we showed that electrical stimulation in the SEF can alter the latency and velocity of anticipatory pursuit movements. Early electrical stimulation during the fixation period increases the velocity of anticipatory movements, when no sensory information about the upcoming pursuit target could be available. The effect of the stimulation was purely multiplicative; eye velocity during stimulation was 1.4 times eye velocity in controls. It was not possible to trigger movement initiation from the SEF in the behaving macaque monkey if the subject was not expecting an upcoming target motion. The effect of electrical stimulation increased when target motion direction was predictable and with sequences of trials in the same direction. We did not systematically test the influence of electrical stimulation in the SEF during sustained pursuit. The absence of a thorough study of the effect of stimulation during sustained pursuit does not preclude the main result of the paper that anticipatory smooth eye movements can be facilitated when target motion is predictable.

The inability to directly trigger a motor response from the SEF differentiates this structure from the frontal pursuit area (FPA), where smooth eye movements can be evoked with low currents during fixation (Gottlieb et al. 1993; Tanaka and Lisberger 2001). The relative role in smooth pursuit of the two frontal oculomotor regions, the SEF and the FPA, is unknown. Indeed, electrical stimulation in both the FPA (Tanaka and Lisberger 2001) and the SEF (Missal and Heinen 2001; this study) can alter the response of the smooth pursuit system to a moving target (“internal” gain of smooth pursuit), and both areas are interconnected and share afferent and efferent projections (Huerta and Kaas 1990). However, the findings reported here and previously by Missal and Heinen (2001) suggest that the SEF pursuit area could be involved in using past experience to guide the upcoming movement.

Anticipatory responses based on endogenous cues are also generated by the saccadic system. Previous studies have shown an involvement of the SEF in sequences of saccades (Grosbras et al. 2001). Sequences of saccades can be based on an endogenous source of information, and a neural correlate of sequences of saccades has been found in the saccadic part of SEF (Lu et al. 2002). During sequences of saccades, anticipatory activity is more pronounced in the SEF than in the FEF or in area LIP (Coe et al. 2002). These results suggest that the SEF could be involved in the process of planning a movement based on memorized information about past stimuli or previously executed movements and could be involved in distinctly cognitive functions in both the saccadic and smooth pursuit domains.

**REFERENCES**


