# Manipulation of object choice by electrical microstimulation in macaque frontal eye fields

Ayano Matsushima & Masaki Tanaka

*Department of Physiology, Hokkaido University School of Medicine, Sapporo 060-8638, Japan.*

*Running head*: A role of FEF in object choice for covert tracking

*Keywords*: eye movement; object selection; primate ; single unit recording; target selection

*Abstract*: 200 words
*Text pages*: 30 (including References and Figure legends)

*Figures*: 6
*Table*: 0

*Supplementary Figure*: 1

*Correspondence to*:
Dr. Masaki Tanaka
Department of Physiology
Hokkaido University School of Medicine
North 15, West 7, Sapporo 060-8638, Japan
Tel: +81 11-706-5039, FAX: +81 11-706-5041
E-mail: masaki@med.hokudai.ac.jp

## Abstract

For each saccade, we select an object to direct gaze and specify the direction and amplitude of eye movement. Although these two processes are inevitably interdependent when visual stimuli are held stationary, several lines of evidence suggest that the neuronal signals in the frontal eye fields (FEF) that underlie the selection of visual objects are distinct from those underlying the selection of saccades. In the present study, we overtly dissociated these two processes spatially and temporally using the covert object tracking paradigm, in which four identical objects moved randomly for 3 seconds before monkeys made a saccade to a previously selected target. To assess the causal role of the FEF in the two selection processes, we applied electrical microstimulation to the FEF at various times during the motion period. When stimulation was delivered at the motion onset, animals tended to choose an object that was initially presented at a particular location depending on the stimulation site. In contrast, the same stimulation delivered at the motion end failed to alter saccade endpoints. These results indicate that manipulation of FEF activity can change the selection of a visual object without affecting saccade goals, suggesting the existence of neurons solely regulating visual selection.

## Introduction

The frontal eye fields (FEF) plays an essential role in target selection (Schall 2002). Previous studies have shown that visual responses to the target for impending saccades are enhanced (Wurtz and Goldberg 1972; Schall and Hanes 1993), and that electrical stimulation to the FEF alters the choice of saccade target (Opris et al. 2005; Schiller and Tehovnik 2005). Because the locations of visual stimuli and saccade endpoints were identical in previous studies, "target selection" could either mean the selection of a visual object (visual selection), or the specification of a movement goal (motor selection). Although these processes are inevitably interdependent in many natural situations, results from recent studies suggest that the neural basis of visual selection might differ from that of motor selection. For example, the enhanced visual response to the target in the FEF has been found in the absence of eye movements toward the target (Sato and Schall 2003; Thompson et al. 2005). In addition, the trajectories of saccades evoked by FEF stimulation have been found to deviate to the goal of planned saccade but not to the location of the attended object (Juan et al. 2004). The separation of sensory selection from motor selection has also been demonstrated by recent experiments in the superior colliculus (SC, Carello and Krauzlis 2004; Nummela and Krauzlis 2010; Song et al. 2011), which mutually communicates with the FEF both directly and indirectly via the thalamus (Hanes and Wurtz 2001; Sommer and Wurtz 2001). However, it remains unclear whether the separation of visual selection from saccade specification also holds true for the FEF because visual stimuli were held stationary in space in the previous experiments.

To dissociate the two selection processes spatially and temporally, we used the

covert object tracking task, in which a selected object moved randomly with identical distractors before the execution of targeting saccades (Matsushima and Tanaka 2012). By applying subthreshold electrical stimulation to the FEF at various times during the 3-s motion period, we directly assessed the causal role of the FEF in visual and motor selection. Stimulation at sites with visually responsive neurons was found to affect animals' choices when delivered at motion onset, while the same stimulation delivered at motion end did not alter saccade goals. Our data show that, in addition to a role in the generation of saccades to specific locations, neuronal signals in the FEF can also regulate object selection without affecting saccade goals. These findings appear to be compatible with the previous studies suggesting that visually responsive neurons and purely movement-related neurons in the FEF might play different functional roles (Bruce et al. 1985; Thompson et al. 2005).

## Materials and Methods

### *Animal preparation*

Experiments were conducted on two female Japanese macaques (*Macaca fuscata*, 6–7 kg, monkeys J and L). All experimental protocols were approved in advance by the Animal Care and Use Committee of Hokkaido University, and were in accord with the Guide for the Care and Use of Laboratory Animals. The animal preparation procedure is described elsewhere in detail (Tanaka 2005; Matsushima and Tanaka 2012) and will be summarized only briefly. After animals were trained to sit in a primate chair, a pair of head holders for running through stainless steel bars horizontally was implanted to the skull using titanium screws and dental acrylic under general isoflurane anesthesia, using sterile procedures. A

few weeks after the first surgery, a coil of stainless steel wire was implanted under the conjunctiva to record eye movements, under the same isoflurane anesthesia and sterile conditions. During subsequent training and experimental sessions, the monkey's head was secured to the primate chair, and horizontal and vertical eye positions were continuously recorded using the search coil technique (MEL-25; Enzanshi Kogyo, Chiba, Japan). The signals proportional to eye position were calibrated before each experimental or training session by having monkeys fixate on stationary target spots at known visual angles. After training on behavioral tasks for approximately one year, a recording cylinder was installed over a small craniotomy under the same surgical conditions. Animals received analgesia with intramuscular injection of either pentazocine or ketoprofen after each surgery. Topical antibiotics were administered around the implant and in the cylinder as necessary. The monkeys' water intake was controlled daily so that they were motivated to perform the tasks.

***Visual stimuli and behavioral paradigms***

Experiments were controlled by a Windows-based real-time data acquisition system (TEMPO; Reflective Computing, St. Louis, MO) running on laboratory PCs. Visual stimuli were presented on a 24-inch cathode-ray tube monitor (GDM-FW900, refresh rate 60 Hz; Sony, Tokyo, Japan) positioned 38 cm from the eyes, subtending 64 × 44° of visual angle. All visual stimuli were presented within a 40° square contour (background luminance, 9.6 $cd/m^2$) that was presented on the center of the screen and was visible throughout the

experiments. Experiments were carried out in a darkened booth.

In the covert tracking paradigm (Fig. 1), four visual stimuli (2° white circles, 25.6 cd/m$^2$ for monkey J, 71.3 cd/m$^2$ for monkey L) were presented during central fixation. The initial stimulus locations were defined in polar coordinates. In all experiments, the initial polar angle of one object was chosen randomly from 0–350° (10° increments, measured from rightward), and those for the other three objects were 90°, 180°, and 270° from the first angle. In the first behavioral experiment (Fig. 2), the initial eccentricity of each object was selected from one of four different groups (5–7°, 9–11°, 13–15°, or 17°), and the initial motion direction was chosen randomly from 0–350° (10° increments) for each object. In the second behavioral experiment (Fig. 3), all objects appeared at the same eccentricity (5–17°), but the initial motion direction was chosen randomly for each object. In the third experiment that included trials with electrical stimulation or a flashing distractor (see below), all objects again appeared at the same eccentricity but moved symmetrically about the fixation point (FP). This stimulus configuration allowed us to examine the effects of electrical stimulation without the interplay with the intrinsic selection bias depending on the object eccentricity and motion direction found in the behavioral experiments (Figs. 2 and 3).

Prior to the motion period, the color of one object changed briefly (red, 300 ms, 34.4 cd/m$^2$) in approximately 40% of trials ("cued" condition, Fig. 1A) and was designated as a target. In the other trials ("free-choice" condition, Fig. 1B), all objects remained white. The four objects moved along straight paths at 20°/s in different directions and bounced against the sides of a 40° visible square (motion period, 3000 ms). Monkeys were required

to keep their eyes within 4° (monkey L) or 6° (monkey J) of the FP throughout the motion and the following delay (500 ms) periods, and to make a saccade to one of the objects (5° window) within 400 ms after the FP offset. We used a relatively large fixation window because monkeys sometimes generated small vertical saccades associated with eye blinks during the motion period. However, the fixation during the motion period was quite accurate for both monkeys; the distance of eye position from the center of the FP averaged 0.52° ± 0.06° (SD) and 0.70°± 0.10° for monkeys L and J, respectively. When the object eccentricities at the end of the motion period were < 5° from the FP, the motion interval was extended until all object eccentricities became > 5° from the FP. In the cued trials, the animals obtained a drop of liquid reward only when they chose the target. In the free-choice trials, they obtained reward in half of trials selected randomly.

To test whether electrical stimulation had its effect indirectly by producing a sensation of a flash of light (phosphene), we substituted electrical stimulation with a brief flash of one object. In this experiment, one object changed its color to yellow (125 cd/$m^2$) for 100 ms either at 0, 1000, 2000 ms after motion onset, or at motion end (usually 3000 ms after motion onset, see above). In the remaining trials, all objects remained white (25.6 cd/$m^2$) throughout the trial, serving as a control. The same object trajectories were presented five times for different conditions (non-flash control and four different flash timings). The flashed object was chosen randomly in each trial.

***Physiological procedures***

Neuronal activity was recorded using tungsten electrodes (FHC Inc., Bowdoin, ME) that

were advanced with a micromanipulator (MO-97S; Narishige, Tokyo, Japan). The location of electrode penetration was adjusted using the x-y stage attached on top of the cylinder that was located over the arcuate sulcus. Signals obtained from the electrodes were amplified (Model 1800; A-M Systems, Sequim, WA), filtered (Model 3625; NF Co., Tokyo, Japan), and monitored online using oscilloscopes and an audio device.

A recent study in our laboratory reported that many neurons in and around the FEF elevated their activity for the target presented in the receptive fields, while other neurons responded to the distractor during a covert tracking task similar to the one used in the present study (Matsushima and Tanaka 2012). The distractor-selective neurons were located within a small area anterior to the FEF and were often found near the target-selective neurons without any topographic arrangement of the receptive fields. The distribution of distractor-selective neurons seem to overlap with neurons that elevated the activity during the suppression of specific saccades (Hasegawa et al., 2004), suggesting the close functional linkage between the two neuronal signals. In contrast, target-selective neurons were recorded from a wider area including the FEF, and their receptive fields were topographically organized (Matsushima and Tanaka 2012). We therefore applied electrical stimulation to the FEF in an attempt to manipulate the activity of target-selective neurons with similar response properties.

In advance of the experiments, we systematically applied electrical stimulation during spontaneous eye movements so as to locate the FEF, defined as the region where low-current microstimulation ($\leq$ 50 μA, 0.2 ms biphasic 34 pulses at 333 Hz) evokes contraversive saccades (Bruce et al. 1985). We then determined the sites of stimulation in

the following way. In each experimental session, we initially searched for sites where multi- or single-unit activity exhibited presaccadic bursts, and confirmed that applying microstimulation at these sites evoked saccades of ~10° in amplitude. We then moved the electrode along the same penetration to search for the site where neuronal activity was elevated during the motion period in the covert tracking task. Although we did not attempt to isolate single neurons during the stimulation experiments, background multi-unit activity often exhibited the target-selective visual response. Such sites were usually found within a few millimeters from the saccade-evoking sites (Fig. 6), and no saccades were evoked by stimulation there (100 ms at 333 Hz, 80 μA). Around these sites, we could not find presaccadic neurons responding exclusively to contraversive saccades without any visual response (i.e., movement neurons; Bruce and Goldberg 1985). The effects of stimulation on object selection were systematically tested at those locations (see below).

During the stimulation experiments, cued trials (40%, Fig. 1A) were randomly interleaved with the free-choice trials (60%, Fig. 1B) in a block, in order to assure that monkeys selected an object at the beginning of the trial and tracked it throughout the motion period; otherwise, they might passively viewed the moving objects. In 80% of the free-choice trials, we delivered electrical stimulation (100 ms at 333 Hz, 70–80 μA) at either 0, 1000 or 2000 ms after motion onset, or at motion end (usually 3000 ms after motion onset). The remaining non-stimulation trials (20%) served as a control. The same object trajectories were presented five times for different stimulation conditions (non-stimulation and four different stimulation timings). The sites of electrical stimulation were verified based on coordinates of electrode penetrations and the MR images taken after

the experiments.

### *Data analysis*

The effects of stimulation at four different times were statistically verified for each site. Initially, the probability of selecting an object presented within each 6° square (1° steps, 1600 squares) was calculated for each of the five conditions (non-stimulation and four different stimulation timings). The changes in probability caused by stimulation were then examined statistically for each square (both-side *z* test, $P < 0.05$, Figs. 4E and F). Since the *z* test applied to each square was not corrected for the multiple comparisons, we next tested whether the number of squares exhibiting significant changes was greater than that expected by chance using the permutation methods. For this analysis, the data from trials with and without electrical stimulation were shuffled 1000 times for every object trajectory, and the number of squares with significant changes in choice probability was counted repeatedly to obtain the baseline distribution of the null hypothesis. When the value computed from the actual data at any of the four times (0, 1000, 2000 ms after motion onset, and at motion end) in any stimulation condition was statistically greater than the baseline distribution ($P < 0.05$), stimulation was considered to affect object selection. The details of other statistical tests are described in the relevant text and figure legends.

## Results

Data were obtained from two monkeys that were well-trained in the covert tracking task with the cue (Fig. 1A). In all experimental sessions, both animals chose the target correctly

in almost all of the cued trials (monkey J, 98 ± 1.4%; monkey L, 97 ± 0.9%) that were randomly interleaved with the free-choice trials (Fig. 1B). In all subsequent analyses, we will consider the data in the free-choice trials (approximately 60% of trials in each block, Methods) to examine the intrinsic bias of target choice and the effects of FEF stimulation.

***Endogenous bias of object selection in free-choice trials***

Prior to the stimulation experiments, we performed two behavioral experiments in order to understand the intrinsic properties of target selection in free-choice trials. In the first behavioral experiment, four visual stimuli were initially presented at different eccentricities. Figure 2A plots the initial (left panel) and the final (right panel) locations of all objects (gray symbols) and the selected objects (red or blue symbols) in 624 free-choice trials. The orientation of each bar indicates the direction of object motion. Although the four objects always appeared at different eccentricities, most of the selected objects were located close to the FP at motion onset (Fig. 2A, red symbols). In contrast, the selected objects were distributed uniformly at motion end (blue symbols), indicating that there was no spatial preference in saccade endpoints. To quantify the strength of selection bias, Figure 2B shows the choice probability plotted as a function of object eccentricity at the two task intervals. The data revealed that both monkeys often chose objects presented within 8° from the FP at the motion onset (Fig. 2B, red lines), while the choice probabilities were comparable across different eccentricities at motion end (blue lines). These results indicate that both monkeys chose an object at the very beginning of object motion and exhibited a preference for the one presented close to the FP.

In the second behavioral experiment, all visual stimuli were initially presented at the same eccentricity. Figure 3A shows the distributions of the initial (left panel) and final (right panel) locations of all objects (gray symbols), and the selected objects (colored symbols). Animals tended to select objects presented in the upper visual field at the motion onset (Fig. 3A, red symbols), but exhibited no clear bias at the motion end (blue symbols). To assess the selection bias on object location, the choice probability was computed for every 30° sector at different times (Fig. 3B). When the strength of selection bias was quantified by computing the mean vectors (Fig. 3B, central arrows), the magnitude of selection bias was significantly greater at motion onset compared with motion end (*t* test for 1000 bootstrap samples, $P < 0.0001$). A similar result was obtained for the other monkey (monkey J), although the animal exhibited a preference for objects in the lower-right visual field.

In addition to the positional bias described above, the animals also exhibited a preference in motion direction. Figure 3C plots the choice probability for each motion direction relative to the FP at different task intervals. Both monkeys tended to select objects moving toward the FP at motion onset (red data points). When the data were sorted according to the relative direction of object motion and the strength of selection bias was quantified by computing the mean vectors as in Figure 3B, the magnitude of selection bias was significantly greater at the motion onset than the motion end (*t* test for 1000 bootstrap samples, $P < 0.0001$). A similar preference in motion direction was also found for the data in the first behavioral experiment shown in Figure 2 ($P < 0.0001$). Thus, when the initial eccentricities of all objects were the same, our monkeys selected an object based on its

initial location and motion direction relative to the FP. Taken together, the data in both behavioral experiments indicated that monkeys chose a target at an early stage of object motion, even in the free-choice trials.

***Effects of FEF stimulation on object selection***

Our behavioral experiments demonstrated that, even in the free-choice trials, monkeys were likely to initially select an object at the motion onset and track it throughout the motion period, but unlikely to ignore the moving objects until the motion end. This might be due to the extensive training on the cued trials that were also interleaved in the block of trials in the current study (Methods). The results in the behavioral experiments ensured that the selection of a visual object and the selection of a saccade goal were spatially and temporally dissociated in free-choice trials. Taking advantage of this dissociation, we attempted to manipulate visual and/or motor selection by applying electrical stimulation to the FEF.

In stimulation experiments, the trajectories of four objects in individual trials were symmetrical about the FP to eliminate the interplay with the endogenous selection bias depending on object eccentricity and motion direction. We initially attempted to manipulate object choice by applying subthreshold stimulation (either 30 μA at 100 Hz, 40 μA at 200 Hz, or 70 μA at 50 Hz) to the saccade-evoking sites in the FEF ($n$ = 9 sites, amplitude of evoked saccades, 15.1 ± 5.7°). However, stimulation at any time during the motion period in the free-choice trials failed to alter object choice (four stimulation timings were tested for seven sites, and three were tested for two sites, Supplementary Fig. 1). Because neurons at

the saccade-evoking sites exhibited transient activity associated with saccades but often exhibited decreased activity during the motion period in the covert tracking task, we next examined the effects of electrical stimulation at the sites in the same penetration where neurons increased their activity during the motion period (Methods).

Stimulation at the FEF sites containing visually responsive neurons exhibited significant effects on object selection. Figure 4 shows the data from a representative experiment in monkey J. Figures 4A and B plot the locations of all objects (A) and the selected objects (B) at different timings in the non-stimulation controls, showing that the monkey tended to select objects initially presented in the lower-right visual field (Fig. 4B, left panel), while visual stimuli in each quadrant were presented at equal probabilities (Fig. 4A, Methods). As stimulation was delivered at motion onset (80 μA, 100 ms at 333 Hz), the animal often chose objects initially presented in the lower-left visual field (Fig. 4C, left panel), but the locations of the selected objects at motion end, which were identical to the goals of saccades, were not altered (Fig. 4C, right panel). Although no saccade was evoked from this stimulation site for the current intensity up to 80 μA, the left-downward shift of object choice was in good agreement with the direction of saccades evoked from the adjacent stimulation sites along the same penetration; saccades evoked from the sites 3900 and 2900 μm above the Fig. 4 site were in the polar direction of 259° (measured from rightward, amplitude, 5.1°; 48 μA, 100 ms, 333 Hz) and 252° (amplitude, 3.2°; 65 μA, 100 ms, 333 Hz), respectively. In contrast, stimulation delivered at motion end exerted no effect throughout the trial (Fig. 4D). The effects of FEF stimulation could also be appreciated by comparing the mean locations of selected objects in each panel (green arrows in Figs. 4B–

D).

To assess the stimulation effect statistically, we compared the choice probability between trials with and without stimulation for every 6° square. Figure 4E plots the center of squares where FEF stimulation at motion onset affected the choice probability (both-side z test, $P < 0.05$). The numbers of effective locations gradually decreased and were statistically greater than the chance level only at motion onset (permutation test for the correction of multiple comparisons, 1000 repeats, $P < 0.05$). These results demonstrated that electrical stimulation at motion onset altered which object to track, without affecting the selection of saccade goals. The lack of a stimulation effect on saccade selection was also evident for trials with stimulation at motion end (Fig. 4F, right panel).

Stimulation experiments were conducted at a total of 73 sites (32 and 41 sites for monkeys J and L, respectively) where the preceding multiunit recordings verified elevated activity during the motion period (Methods). As an example shown in Figures 4E and F, the effects of stimulation in each site were quantified by counting the number of 6° squares exhibiting significant changes in choice probability. Among 73 sites tested, stimulation at 39 sites (53%, 15 and 24 sites in monkeys J and L, respectively) exhibited significant effects, showing a greater number of effective locations than expected by chance (null distribution was generated using the permutation method, $P < 0.05$) for at least one of 16 conditions (four task intervals × four stimulation timings). For the majority of these sites (69%, 27/39), stimulation was effective when delivered at motion onset, while stimulation at other times also exhibited a weak but significant effect at some sites (31%, 12/39). To compare the magnitude of stimulation effects at different timings, Figure 5A plots the

number of locations exhibiting significant changes in choice probability at four task intervals for trials with different stimulation timings (0, 1000, 2000 ms after motion onset, and at motion end). For comparison, baseline was computed using the permutation method (1000 repeats) for each stimulation timing, and the mean values for all stimulation timings are shown in Figure 5A (dotted lines). The data show that FEF stimulation could alter object choice only when delivered at motion onset, although stimulation at the motion end was the closest in time to the execution of saccades. On the other hand, the effects of subthreshold stimulation applied to the saccade-evoking sites on object choice were not statistically significant at any stimulation timings for both monkeys (the means of the number of significant locations according to the z test ranged from 2.8 to 12.0, permutation test, $P > 0.05$).

In addition, we confirmed the results shown in Figure 5A using a different measure. As an example shown in Figures 4B–D, we quantified the effects of stimulation by comparing the mean locations of the selected objects in trials with and without stimulation. Figure 5B plots the sizes of difference vectors normalized for the object eccentricity at the corresponding times, exhibiting greater stimulation effects as delivered at the motion onset. Two-way factorial ANOVA (four task intervals × four stimulation timings) applied to the data in Figures 5A and B revealed the significant main effects and interaction between them ($P < 0.05$). Post hoc multiple comparisons confirmed that the values measured at motion onset were greatest when stimulation was applied at the motion onset. On the other hand, the values measured at motion end were comparable between four stimulation timings. When we performed the same analysis on individual animals, similar results were obtained

from each monkey except that the interaction effect was not statistically significant in difference vectors for monkey L. The shifts of selected object locations were in the direction contralateral to the stimulation site (Fig. 5B, inset) and correlated with the direction of saccades evoked from nearby low-threshold ($\leq$ 50 μA) sites in the same penetrations (circular-circular correlation, $r = 0.43$, $P < 0.05$, $n = 39$), indicating that the animals tended to select an object in the contralateral visual field during stimulation. The contralateral bias was unlikely resulting from the occurrence of microsaccades, because the number of microsaccades *decreased* during electrical stimulation (Fig. 5C). These results demonstrated that FEF stimulation at motion onset could alter visual object selection without affecting the choice of saccade goal.

Figure 6 illustrates the stimulation sites reconstructed from MR images. Electrode penetrations were located near the genu of the arcuate sulcus and were mostly confined within its anterior bank just posterior to the caudal tip of the principal sulcus. The stimulation sites with significant effects on object choice (red circles) were found within the area containing saccade-evoking sites (i.e., physiologically defined FEF, blue circles), indicating that sites with stimulation effects on object selection resided in the FEF.

***Ineffectiveness of task-irrelevant object flash***

Our data suggest that FEF stimulation might alter the salience, or significance, of an object located at a specific location in the visual field, resulting in the change in the object locations that were ultimately selected. Alternatively, FEF stimulation might alter object choice indirectly through the enhancement of visual perception, for example, by generating

a sensation of a flash of light (phosphene). Recent stimulation experiments exploring the roles of the FEF and SC in visuospatial attention were confronted with a similar problem, and assessed this alternative by substituting electrical stimulation with irrelevant target flashes (Muller et al. 2005; Schafer and Moore 2007). To address this issue, we introduced object flashes in the free-choice trials. In the flash experiments, a randomly selected object was flashed for 100 ms at one of four different times (0, 1000, 2000 ms after motion onset or at motion end). As in the stimulation experiments, the same object trajectories were repeatedly presented five times for different flash conditions (non-flash and flash at four different timings). Unlike electrical stimulation, object flash did not alter object choice in either monkey. The probability of choosing the object flashed at motion onset was not significantly different from the probability of choosing the corresponding object in non-flash trials (monkey J, 28% versus 26%, $n = 1090$; monkey L, 28% versus 24%, $n = 836$; both-side $z$ test, $P > 0.05$). Similarly, choice probability for objects flashed at motion end was comparable to that for non-flashed objects in trials with the same object trajectories (monkey J, 26% versus 26%, $n = 1088$; monkey L, 28% versus 24%, $n = 832$; both-side $z$ test, $P > 0.05$). We therefore conclude that electrical stimulation directly manipulated the selection bias for each object rather than injecting sensory signals that enhanced visual perception.

## Discussion

The present study provides direct evidence for the causal role of the FEF in visual object selection. We applied electrical stimulation to the FEF while monkeys performed a covert

object tracking task, in which visual and motor selection were spatially and temporally dissociated. Even without stimulation, object selection strongly depended on the initial, but not the final, location, indicating that monkeys selected an object at the very beginning of their motions. When stimulation was delivered at the motion onset, the animals tended to choose an object that was initially presented at a particular location, depending on the site of stimulation. In contrast, the same stimulation delivered at motion end exerted no effect. Because the task-irrelevant object flash failed to alter object choice, the stimulation effects might not result from the perceptual enhancement of visual stimuli. These results suggest that, in addition to the well-established role in saccade selection and generation (Schall 2002; Opris *et al.* 2005; Schiller and Tehovnik 2005), signals in the FEF could also prioritize an object at a particular location in the process of visual selection.

***Intrinsic bias of object selection in the absence of electrical stimulation***

Prior to the stimulation experiments, we examined behavioral data in free-choice trials to understand the properties of intrinsic selection bias. When the locations of objects selected at the end of trials were examined retrospectively, their initial locations were strongly dependent on particular visual attributes while their final locations were not, indicating that monkeys selected an object at motion onset and covertly tracked it throughout the motion period. The selection bias observed at motion onset was likely due to overtraining of the monkeys in the covert tracking task with an initial explicit cue, which was interleaved with the non-cue, free-choice trials.

By systematically varying object locations and motion directions, the results

revealed several visual properties that guided object choice. Both monkeys often chose objects presented close to (Fig. 2B) or moving toward the FP (Fig. 3C). These results might be attributed to the central magnification of the visual system (Dow et al. 1981), and could be related to the gain enhancement of smooth pursuit eye movements for targets moving toward the FP (Lisberger and Westbrook 1985). Moreover, both animals exhibited a strong preference for objects presented at a particular location. Interestingly, these preferred locations were stable for several months. Although the origin of this spatial bias remains unknown, it might result from the imbalance of population activities in the FEF induced by long-term experience (Bichot et al. 1996), or from activities in the other oculomotor areas, such as the supplementary eye fields (Coe et al. 2002), the lateral intraparietal area (LIP; Platt and Glimcher 1999; Shadlen and Newsome 2001) and the SC (Horwitz and Newsome 1999).

***Effects of FEF stimulation on object selection***

Our behavioral results demonstrated that object selection took place at motion onset in the free-choice trials, and was separable from the specification of saccade goal that occurred after the object motion. Taking advantage of this dissociation, we used microstimulation to address whether the FEF controls the selection of visual objects and/or the selection of saccade goals. If the FEF regulates visual selection, stimulation would be expected to shift the initial locations of selected objects. On the other hand, if the FEF is involved in the selection of saccade goals, stimulation would be expected to shift saccade endpoints that are manifested as the locations of selected objects at motion end.

Our data clearly showed that stimulation altered the selection of visual objects (Fig. 4). Stimulation delivered at motion onset biased the initial locations of selected objects toward a particular location without affecting saccade endpoints. On the other hand, the same stimulation delivered at motion end exerted no effect, indicating that neuronal signals at our stimulation sites played a causal role in the selection of visual stimuli but not in the specification of saccade goals. Interestingly, we found no significant effect of stimulation delivered in the middle of the motion period (Fig. 5). These results seem to contradict our previous findings that neurons in the FEF continuously represent object locations during covert tracking (Matsushima and Tanaka 2012). However, considering that electrical microstimulation modulates the activity of only a fraction of FEF neurons, it is likely that a small bias introduced by stimulation could not counteract the robust representation of a selected object in the population of neurons during covert object tracking. A previous study also reported similar results that neuronal activity in the LIP that accumulate evidence for one direction of motion could be altered by a brief motion perturbation applied soon after motion onset, but not by a perturbation applied later in the trial (Huk and Shadlen 2005). Thus, once a perceptual decision has been made, a robust representation of subjective choice in a neuronal population might be immune to brief perturbations.

Since we previously showed that a subset of neurons in the pre-FEF modulated their activity depending on the distractor locations while many others responded to the target (Matsushima and Tanaka 2012), one might argue that the stimulation effects reported here could be confounded by the activation of different populations of neurons. However, because the distractor-selective neurons were found in the penetrations anterior to the FEF

and only a few were located within the FEF, they were unlikely to contribute to the current results of FEF stimulation. The stimulation of distractor-selective neurons would be an issue of future study.

Although previous studies demonstrated that saccade target selection could be altered by subthreshold microstimulation at saccade-evoking sites (Opris *et al.* 2005; Schiller and Tehovnik 2005), we failed to obtain any significant effect at saccade-evoking sites. The lack of stimulation effect at the time of saccade selection might be due to the difference in experimental conditions or the use of lower stimulation parameters. Practically, we were unable to use greater stimulation current at saccade-evoking sites in this study because suprathreshold stimulation directly triggered contraversive saccades, which inevitably specifies saccade goals irrespective of behavioral context and might mask the possible stimulation effects on visual selection. In contrast, stimulation at different depth in the same penetrations neither evoked saccades nor biased saccade endpoint, but altered visual object selection at motion onset in the covert tracking trials. These contrasting stimulation effects in terms of visual selection and saccade specification might indicate the existence of different functional groups of neurons in the FEF.

Indeed, several lines of evidence support the hypothesis that visually-responsive and purely motor-related neurons in the FEF subserve different functional roles. Originally, Bruce et al. (1985) demonstrated that the sites containing visually-responsive neurons in the FEF had higher threshold for evoking saccades, while those with burst of activity prior to saccades had lower threshold. Moreover, Thompson et al. (2005) showed that visually-sensitive neurons in the FEF responded to the target during pop-out visual search,

while neurons with exclusively movement-related activity ceased firing. Anatomically, the superficial layers in the FEF interconnect with V4 (Pouget et al. 2009; Ninomiya et al. 2012), while the deep layers send projections to the SC and lower brainstem (Segraves and Goldberg 1987; Stanton et al. 1988; Segraves 1992; Sommer and Wurtz 2000). These distinct projection patterns might be associated with the different functional roles exerted by the two layers, which are thought to be related to selective attention (Noudoost and Moore 2011) and the generation of saccades (Hanes and Wurtz 2001), respectively. Our data in the current study might further corroborate these findings and reinforce the concept that neurons with visual activity at the higher-threshold sites are more involved in visual selection while those solely exhibiting saccade-related activity are more involved in saccade generation. However, considering the current spread and the stimulation effects on passing fibers, the interpretation of our results should be limited. To prove the functional duality within the FEF depending on cortical layers or anatomical connections, further research using a proper technique such as optogenetics must be needed (Cavanaugh et al. 2012).

## Acknowledgements

We are grateful to T. Shiraishi and T. Mori for the animal care and training, J. Kunimatsu for participating in the surgery, A. Yoshida for taking MRI, M. Suzuki for administrative help. This work was supported in part by the grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the Takeda foundation; the Uehara Memorial Foundation; the Brain Science Foundation; the Smoking Research Foundation; the Akiyama Life Science Foundation; and the Suhara Memorial Foundation. A.M. was supported by the Graduate Research Fellowship from the Japan Society for the Promotion of Science. Animals were provided by the National Bio-Resource Project.

## References


Bichot NP, Schall JD, Thompson KG.. 1996. Visual feature selectivity in frontal eye fields induced by experience in mature macaques. Nature. 381:697-699.

Bruce CJ, Goldberg ME. 1985. Primate frontal eye fields. I. Single neurons discharging before saccades. J Neurophysiol. 53:603-635.

Bruce CJ, Goldberg ME, Bushnell MC, Stanton GB. 1985. Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. J Neurophysiol. 54:714-734.

Carello CD, Krauzlis RJ. 2004. Manipulating intent: evidence for a causal role of the superior colliculus in target selection. Neuron. 43:575-583.

Cavanaugh J, Monosov IE, McAlonan K, Berman R, Smith MK, Cao V, Wang KH, Boyden ES, Wurtz RH. 2012. Optogenetic inactivation modifies monkey visuomotor behavior. Neuron. 76:901-907.

Coe B, Tomihara K, Matsuzawa M, Hikosaka O. 2002. Visual and anticipatory bias in three cortical eye fields of the monkey during an adaptive decision-making task. J Neurosci. 22:5081-5090.

Dow BM, Snyder AZ, Vautin RG, Bauer R. 1981. Magnification factor and receptive-field size in foveal striate cortex of the monkey. Exp Brain Res. 44:213-228.

Hanes DP, Wurtz RH. 2001. Interaction of the frontal eye field and superior colliculus for saccade generation. J Neurophysiol. 85:804-815.

Hasegawa RP, Peterson BW, Goldberg ME. 2004. Prefrontal neurons coding suppression of specific saccades. Neuron. 43:415-425.

Horwitz GD, Newsome WT. 1999. Separate signals for target selection and movement specification in the superior colliculus. Science. 284:1158-1161.

Huk AC, Shadlen MN. 2005. Neural activity in macaque parietal cortex reflects temporal integration of visual motion signals during perceptual decision making. J Neurosci. 25:10420-10436.

Juan CH, Shorter-Jacobi SM, Schall JD. 2004. Dissociation of spatial attention and saccade preparation. Proc Natl Acad Sci U S A. 101:15541-15544.

Lisberger SG, Westbrook LE. 1985. Properties of visual inputs that initiate horizontal smooth pursuit eye movements in monkeys. J Neurosci. 5:1662-1673.

Matsushima A, Tanaka M. 2012. Neuronal Correlates of Multiple Top-Down Signals during Covert Tracking of Moving Objects in Macaque Prefrontal Cortex. J Cogn Neurosci. 24:2043-2056.

Muller JR, Philiastides MG, Newsome WT. 2005. Microstimulation of the superior colliculus focuses attention without moving the eyes. Proc Natl Acad Sci U S A. 102:524-529.

Ninomiya T, Sawamura H, Inoue K-i, Takada M. 2012. Segregated Pathways Carrying Frontally Derived Top-Down Signals to Visual Areas MT and V4 in Macaques. J Neurosci. 32:6851-6858.

Noudoost B, Moore TC. 2011. Control of visual cortical signals by prefrontal dopamine. Nature. 474:372-375.

Nummela SU, Krauzlis RJ. 2010. Inactivation of primate superior colliculus biases target choice for smooth pursuit, saccades, and button press responses. J Neurophysiol. 104:1538-1548.

Opris I, Barborica A, Ferrera VP. 2005. Microstimulation of the dorsolateral prefrontal cortex biases saccade target selection. J Cogn Neurosci. 17:893-904.

Platt ML, Glimcher PW. 1999. Neural correlates of decision variables in parietal cortex. Nature. 400:233-238.

Pouget P, Stepniewska I, Crowder EA, Leslie MW, Emeric EE, Nelson MJ, Schall JD. 2009. Visual and motor connectivity and the distribution of calcium-binding proteins in macaque frontal eye field: implications for saccade target selection. Front Neuroanat. 3:2.

Sato TR, Schall JD. 2003. Effects of stimulus-response compatibility on neural selection in frontal eye field. Neuron. 38:637-648.

Schafer RJ, Moore T. 2007. Attention governs action in the primate frontal eye field. Neuron. 56:541-551.

Schall JD. 2002. The neural selection and control of saccades by the frontal eye field. Philos Trans R Soc Lond B Biol Sci. 357:1073-1082.

Schall JD, Hanes DP. 1993. Neural basis of saccade target selection in frontal eye field during visual search. Nature. 366:467-469.

Schiller PH, Tehovnik EJ. 2005. Neural mechanisms underlying target selection with saccadic eye movements. Prog Brain Res. 149:157-171.

Segraves MA. 1992. Activity of monkey frontal eye field neurons projecting to oculomotor regions of the pons. J Neurophysiol. 68:1967-1985.

Segraves MA, Goldberg ME. 1987. Functional properties of corticotectal neurons in the monkey's frontal eye field. J Neurophysiol. 58:1387-1419.

Shadlen MN, Newsome WT. 2001. Neural basis of a perceptual decision in the parietal cortex (area LIP) of the rhesus monkey. J Neurophysiol. 86:1916-1936.

Sommer MA, Wurtz RH. 2000. Composition and topographic organization of signals sent from the frontal eye field to the superior colliculus. J Neurophysiol. 83:1979-2001.

Sommer MA, Wurtz RH. 2001. Frontal eye field sends delay activity related to movement, memory, and vision to the superior colliculus. J Neurophysiol. 85:1673-1685.

Song JH, Rafal RD, McPeek RM. 2011. Deficits in reach target selection during inactivation of the midbrain superior colliculus. Proc Natl Acad Sci U S A. 108:E1433-1440.

Stanton GB, Goldberg ME, Bruce CJ. 1988. Frontal eye field efferents in the macaque monkey: II. Topography of terminal fields in midbrain and pons. J Comp Neurol. 271:493-506.

Tanaka M. 2005. Involvement of the central thalamus in the control of smooth pursuit eye movements. J Neurosci. 25:5866-5876.

Thompson KG, Biscoe KL, Sato TR. 2005. Neuronal basis of covert spatial attention in the frontal eye field. J Neurosci. 25:9479-9487.

Wurtz RH, Goldberg ME. 1972. Activity of superior colliculus in behaving monkey. 3. Cells discharging before eye movements. J Neurophysiol. 35:575-586.

## Figure Legends

**Figure 1.** Covert tracking paradigm. (A) Sequence of events in a cued trial. (B) Sequence of events in a free-choice trial. In both conditions, monkeys were required to maintain fixation while the central fixation point was visible. Although these trials were presented randomly in a block, only the data in the free-choice trials were considered in the following figures. In 80% of free-choice trials, electrical stimulation was delivered during the motion period. The color of one object was changed immediately after saccades to indicate the target. Note that monkeys were rewarded only for correct choices in the cued trials, but in half of the free-choice trials irrespective of their choice.

**Figure 2.** Preference for objects presented closer to the fixation point at motion onset. (A) Data in non-stimulation trials with different initial eccentricities of objects for monkey J. Each oriented gray bar indicates the location and motion direction of all visual stimuli presented in 624 free-choice trials (three daily sessions). Different panels show the data from the same trials but at different timings during the motion period. Each object was initially presented at a different eccentricity chosen from the four levels indicated by broken circles. Each colored bar indicates the location and motion direction of the object that was selected by saccades at the end of trials. (B) Probability of object choice as a function of object eccentricity at motion onset (red circles) and motion end (blue triangles). Data from different monkeys are plotted with different fillings and connected lines.

**Figure 3.** Preference for the specific location and motion direction relative to the fixation

point. (A) Data in non-stimulation trials with the same initial eccentricity of objects for monkey L. Each gray bar indicates the location and motion direction of all visual stimuli presented in 1656 free-choice trials (two daily sessions). Colored symbols show the locations of selected objects at the time of motion onset (left panel) or motion end (right panel). In each trial, four objects were initially presented at the same eccentricity but with different polar angles chosen from 36 directions (10° steps). (B) Probability of object choice dependent on the polar angle of object location (30° bins) at different motion intervals. Red and blue arrows indicate the mean vectors for the data at motion onset and motion end, respectively. (C) Probability of object choice as a function of motion direction relative to the fixation point. Note that both monkeys tended to choose the object that initially moved toward the fixation point.

**Figure 4.** Shift of selection bias caused by stimulation at a representative FEF site. (A) Locations of all objects presented during a single experiment. Object locations at different intervals in the free-choice trials are shown in separate panels. The orientation of each line indicates motion direction. Trials with the same object trajectories were presented repeatedly for five different stimulation conditions (non-stimulation control and four different stimulation timings). In each trial, all object eccentricities and motion directions were symmetrical about the fixation point. (B) Locations of selected objects in non-stimulation control trials. Green arrow at the origin indicates the mean of selected object locations. (C and D) Locations of selected objects in trials with stimulation at motion onset (C) or motion end (D). Note that when stimulation was applied at motion onset, the

monkey tended to select objects that were initially presented in the lower-left visual field (C, left panel), while stimulation at motion end had no effect (D). (E and F) Object locations exhibiting significant changes in choice probability by electrical stimulation delivered at motion onset (E) and motion end (F). The changes in choice probability were assessed for every 6° square area (1° step, both-side z test, $P < 0.05$). Data were obtained from monkey J.

**Figure 5.** Summary of the stimulation effects. (A) Total counts of locations showing significant changes in choice probability as a function of time. Different colors and symbols indicate the data for trials with different stimulation timings. Black dotted lines indicate the baseline derived from the permutation test (see Results for detail). (B) Normalized length of the difference vector between the mean locations of selected objects in trials with and without stimulation. Conventions are the same as in A. Inset illustrates the difference vectors during stimulation at motion onset for all sites. In both panels, the error bar indicates 95% confidence interval. The error bars for the baseline are less than 1.0 (A) and 0.005 (B) for all data points, and have been omitted. Data were obtained from 39 stimulation sites in two monkeys (15 and 24 sites for monkeys J and L, respectively) where no saccades were evoked by stimulation at 80 μA. For both measures, subthreshold stimulation applied to the saccade-evoking sites ($n = 9$) at any timing did not alter object choice (permutation test, $P > 0.05$). (C) Transient suppression of microsaccades during electrical stimulation. The frequency of microsaccades was plotted as a function of time in trials with (red trace) and without electrical stimulation (blue trace). Black bars on the

abscissa indicate stimulation timing. Vertical broken line indicates the onset of visual stimuli. Shaded area corresponds to 95% confidence interval. The size of microsaccades averaged 0.97 ± 0.94° (SD) and 1.81 ± 2.1° for monkeys L and J, respectively.

**Figure 6.** Locations of stimulation sites. (A) MRI surface image perpendicular to the electrode penetrations for monkey J. Red lines indicate the locations of coronal planes shown in (B). (B and C) The locations of stimulation sites were reconstructed from MR images for each monkey (slice thickness, 2.0 mm). Sites with significant stimulation effects on object selection (red dots) were located along the same penetrations as saccade-evoking sites (blue circles). The size of the blue circles indicates the amplitude of evoked saccades. Antero-posterior coordinates were determined relative to the center of the recording cylinder (A28 for monkey L, A 29 for monkey J). AS, arcuate sulcus; PS, principal sulcus; CS, central sulcus; IPS, intraparietal sulcus; LS, lateral sulcus; STS, superior temporal sulcus.

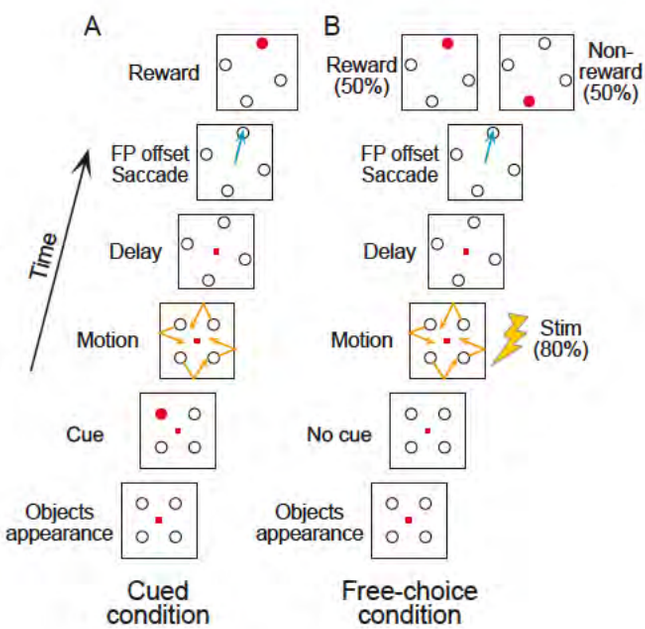



*Figure 1, Matsushima & Tanaka*

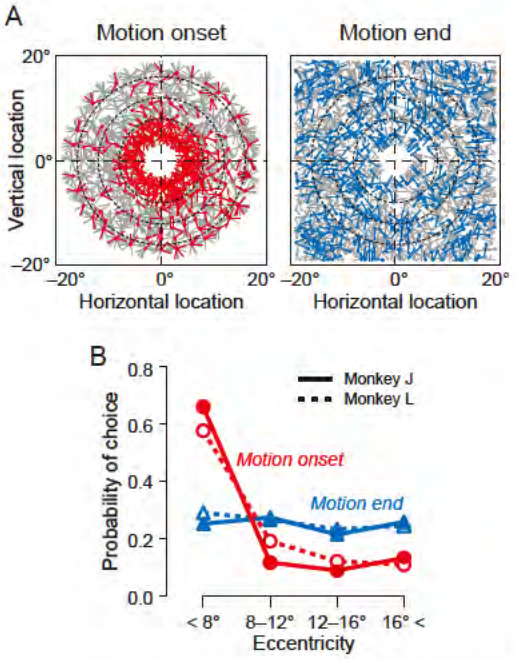



*Figure 2, Matsushima & Tanaka*

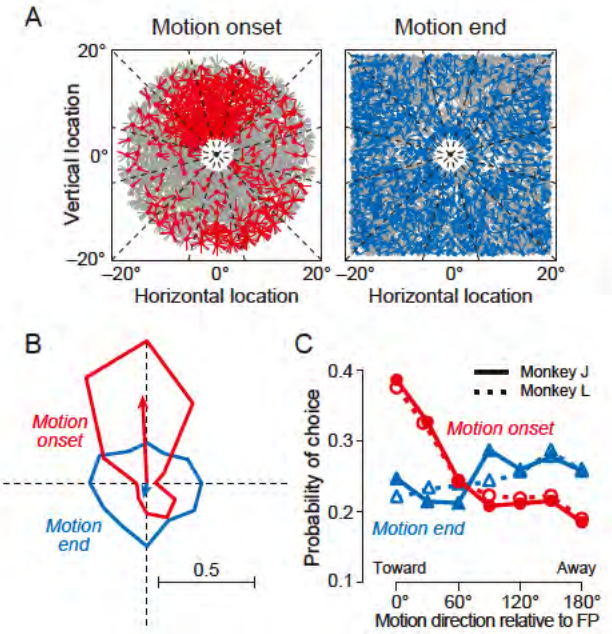



*Figure 3, Matsushima & Tanaka*

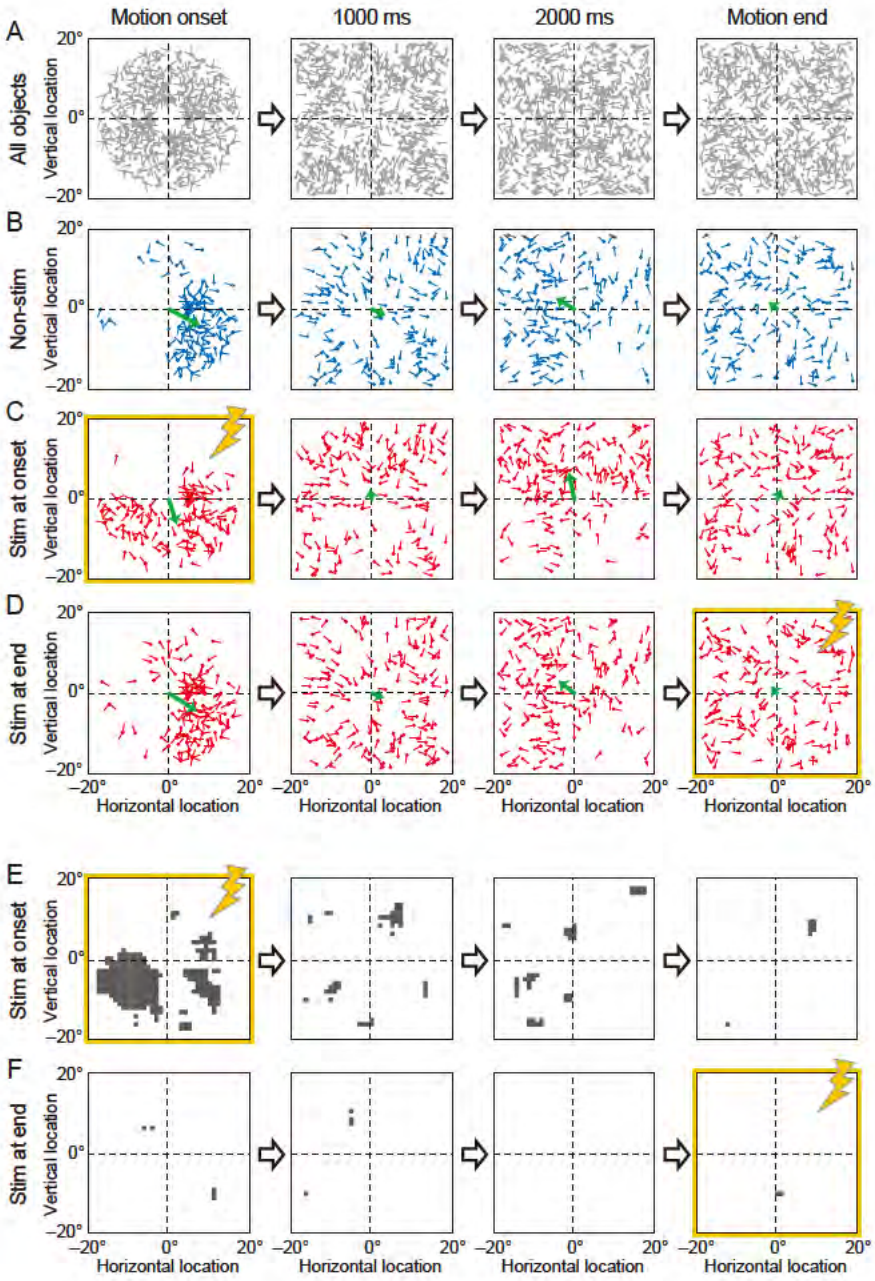



*Figure 4, Matsushima & Tanaka*

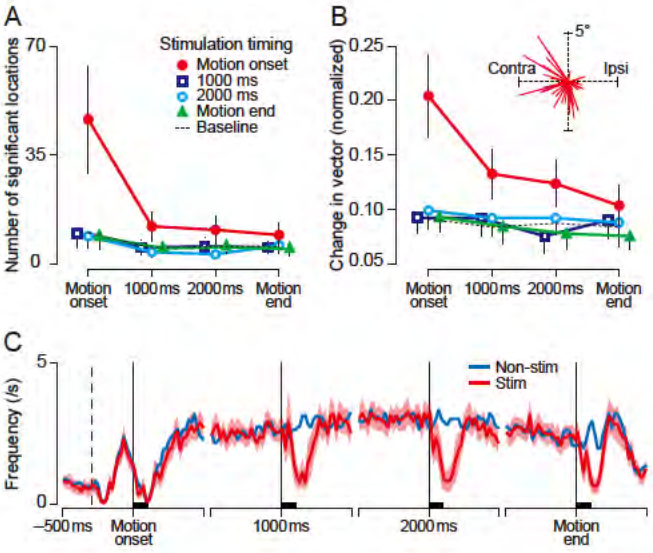



*Figure 5, Matsushima & Tanaka*

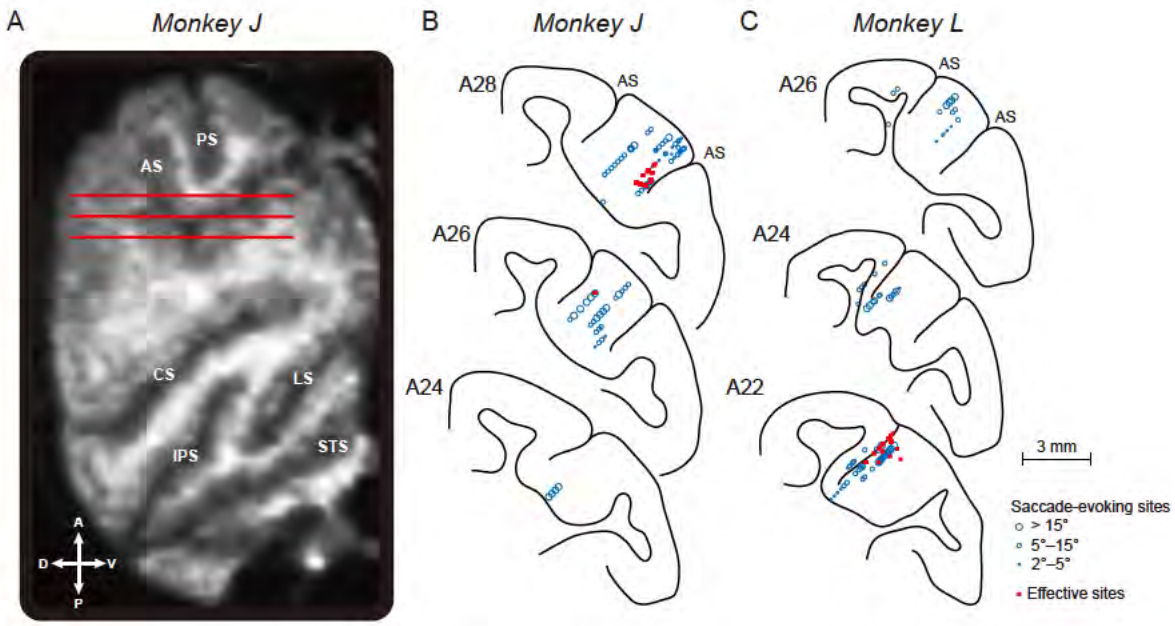
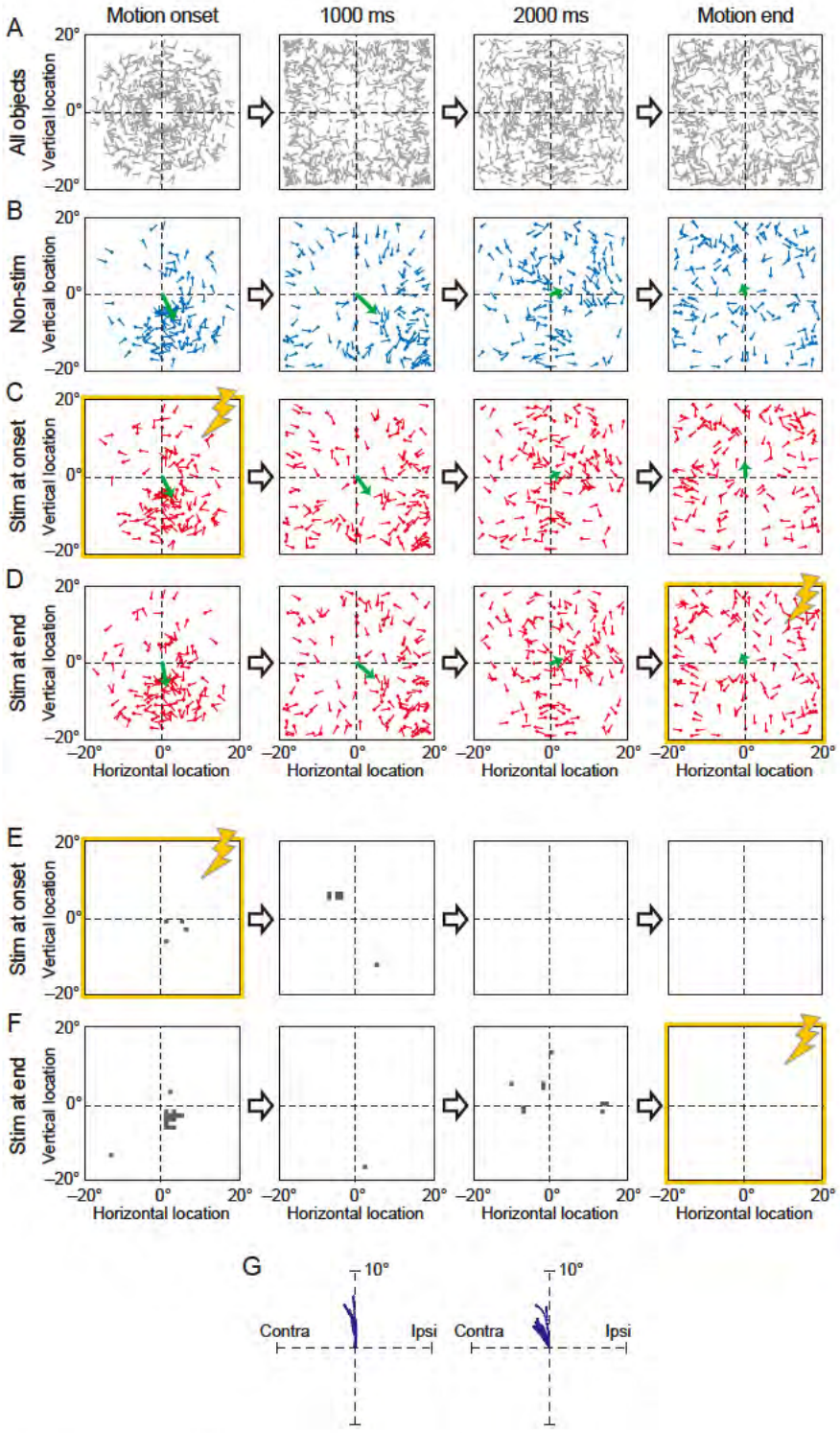



Figure 6, Matsushima & Tanaka




**Supplementary Figure 1. The absence of stimulation effect at a saccade-evoking FEF site.** (A) Locations of all objects presented during a single experiment. The conventions are the same as those in Figure 4. (B) Locations of selected objects in non-stimulation control trials. (C and D) Locations of selected objects in trials with stimulation at motion onset (C) or motion end (D). Note that stimulation (30 μA, 100 ms, 100 Hz) had no effect on object choice, irrespective of the times of delivery. (E and F) Object locations exhibiting significant changes in choice probability by electrical stimulation delivered at motion onset (E) and motion end (F). (G) Saccades evoked by suprathreshold stimulation (50 μA, 100 ms, 333 Hz) before (left) and after (right) the experiment. Data were obtained from monkey J.