Exp Brain Res (2007) 181:477–492
DOI 10.1007/s00221-007-0947-7

RESEARCH ARTICLE

Subthreshold microstimulation in frontal eye fields updates spatial memories

Robert L. White III · Lawrence H. Snyder

Received: 28 March 2006 / Accepted: 28 March 2007 / Published online: 8 May 2007

Abstract The brain's sensitivity to self-generated movements is critical for behavior, and relies on accurate internal representations of movements that have been made. In the present study, we stimulated neurons below saccade threshold in the frontal eye fields of monkeys performing an oculomotor delayed response task. Stimulation during, but not before, the memory period caused small but consistent displacements of memory-guided saccade endpoints. This displacement was in the opposite direction of the saccade that was evoked by stronger stimulation at the same site, suggesting that weak stimulation induced an internal saccade signal without evoking an actual movement. Consistent with this idea, the stimulation effect was nearly absent on a task where an animal was trained to ignore self-generated eye movements. These findings support a role for the frontal eye fields in accounting for self-generated movements, and indicate that corollary discharge signals can be manipulated independent of motor output.

Keywords Eye movements · Cortex · Population code · Feedback

This work was supported by the EJLB Foundation, the Washington University Silvio Conte Center, and the National Eye Institute. R.W. was supported by grants from the National Institute of Health's Medical Scientist Training Program (GM07200) and the National Eye Institute (EY13360).

R. L. White III · L. H. Snyder (✉)
Department of Anatomy and Neurobiology,
Box 8108, Washington University School of Medicine,
660 S. Euclid Ave, St. Louis, MO 63110, USA
e-mail: larry@eye-hand.wustl.edu

Introduction

Neurons in the frontal eye fields (FEF) are believed to play a role in the planning and execution of saccades (Bruce and Goldberg 1985; Schall et al. 1995). FEF has also been implicated in the maintenance of spatial working memory (Deng et al. 1986; Funahashi et al. 1989, 1993; Sommer and Tehovnik 1997; Chafee and Goldman-Rakic 1998). It has been proposed that neurons in this area store a short-term representation for the spatial location of an upcoming eye movement.

Short-term spatial memories are not static. When an observer shifts their gaze, the locations of remembered objects shift relative to the center of gaze. Many visuospatial brain areas have gaze-centered representations (e.g., Colby et al. 1995). In order for a gaze-centered representation to encode accurate information about a remembered spatial location that is fixed in the world (world-fixed), the representation must be updated each time gaze shifts. For example, if an intervening saccade is made to a new fixation point while a subject remembers the location of a target, neurons in FEF (Goldberg and Bruce 1990; Tian et al. 2000) and other brain areas (Goldberg and Bruce 1990; Duhamel et al. 1992; Walker et al. 1995; Batista and Andersen 2001; Nakamura and Colby 2002) change their activity to represent the gaze-centered coordinates of the remembered location relative to the new eye position. As a result of this computation, these neurons encode remembered world-fixed spatial information that is accurate despite shifts in gaze. Similar results have been found for other types of self-movement, not just saccades (McKenzie and Lisberger 1986; Schlag et al. 1990; Powell and Goldberg 1997; Baker et al. 2002). Updating of gaze-centered representations appears to be important in guiding oculomotor behavior (Baker et al. 2003).

Spatial updating relies on corollary discharge, or efference copy, signals to compensate for self-generated movements (Guthrie et al. 1983; Bridgeman 1995; Lewis et al. 2001). For neurons to update accurately for saccades, they must receive input signals that convey the direction and magnitude of the intervening saccade. A number of models have shown that this computation can be performed in a simple network of neurons receiving a gaze position, gaze velocity or gaze displacement signal (Droulez and Berthoz 1991; Xing and Andersen 2000; White and Snyder 2004a). However, the source and nature of the specific signals used for spatial updating in the brain are yet to be fully determined. Neurons that project to FEF from the mediodorsal nucleus of the thalamus (MD) appear to convey corollary discharge signals for saccades (Sommer and Wurtz 2004a). Thus, FEF could be an important locus for combining stored spatial information with corollary discharge signals.

At the behavioral level, spatial updating is not obligatory. Many objects move, and in particular many objects move with the gaze of the observer. For example, when a moving object is tracked, all features of the tracked object maintain their locations on the retina. In the laboratory, animals can be trained to suppress updating, keeping memories fixed with respect to the center of gaze (gaze-fixed) (Baker et al. 2003). In this case, information about where the eyes have moved must be ignored to maintain a spatial memory in a gaze-fixed frame. One might expect to see a neural correlate of this at the level of FEF: updating of neuronal representations should occur for world-fixed but not gaze-fixed targets, so corollary discharge signals should be effective in modifying world-fixed representations but not gaze-fixed representations.

We examined the role of FEF neurons during a memory task by using electrical microstimulation. Microstimulation of sites within FEF elicits saccades of fixed direction and amplitude in both monkeys and humans (Bruce et al. 1985; Blanke et al. 2000). Saccades of smaller amplitude are elicited from the ventrolateral FEF and those of larger amplitude from progressively more dorsomedial locations (Robinson and Fuchs 1969; Bruce et al. 1985). Stimulation can also affect spatial processing without evoking saccades (Burman and Bruce 1997; Schiller and Tehovnik 2001). Recent studies have shown that subthreshold stimulation at sites within FEF improves contrast threshold for detection (Moore and Fallah 2004) and enhances V4 visual responses (Moore and Armstrong 2003) at the retinal location corresponding to the response field of the FEF site and not at locations outside of this field, consistent with a role of FEF in directing covert spatial attention.

We hypothesized that if FEF were involved in maintaining a memory trace for a saccade target, then subthreshold stimulation might enhance, degrade or otherwise systematically bias the memory trace, resulting in a systematic bias of memory-guided saccades. If FEF were instead involved in updating saccade plans for changes in gaze direction, then subthreshold stimulation might bias memory-guided behavior via a corollary discharge pathway, and mimic an eye movement that was not actually performed. We found that memory-guided saccades were biased by stimulation, and that the effect was more consistent with spatial updating in response to an corollary discharge signal than with a direct perturbation of the memory trace itself.

To confirm this result, we performed two additional experiments to verify that stimulation in FEF specifically affects corollary discharge signals. In the first, stimulation was applied either during or before a memory period, and resulted in changes in memory-guided saccade endpoints only when applied during the memory period. The results of this experiment indicated that subthreshold microstimulation specifically modified stored spatial information, and did not produce a more generalized effect on the motor processes involved in saccade execution.

In the second control experiment, we stimulated in either a world-fixed or gaze-fixed context, and found that stimulation affected memory-guided endpoints when world-fixed targets were remembered, but not when gaze-fixed targets were remembered. We found evidence suggestive of contextual control over the effects of FEF microstimulation. This finding demonstrates that the effects introduced by microstimulation may not be fixed, but can be influenced by the cognitive state of the subject.

A preliminary version of these results has been presented in abstract form (White and Snyder 2004b).

Methods

Subjects

Two male *Macaca mulatta* (M1, 7.5 kg; M3, 9.4 kg) and one male *Macaca fascicularis* (M2; 4.4 kg) were used as subjects. Monkeys were fitted with a prosthetic device to stabilize the head, a single scleral search coil for eye movement recording (Robinson 1963; Judge et al. 1980), and recording chamber over either the left or right arcuate sulcus. Sterile surgery was performed under inhalation anesthesia (isoflurane, 0.5–2.0%). Post-operative analgesics were provided as necessary. All surgical and behavioral procedures conformed to National Institutes of Health guidelines and were approved by the Washington University Institutional Animal Care and Use Committee.

Recording and stimulation procedures

During experiments, the monkey was seated in a Lexan box (Crist Instruments). Eye movements were monitored using

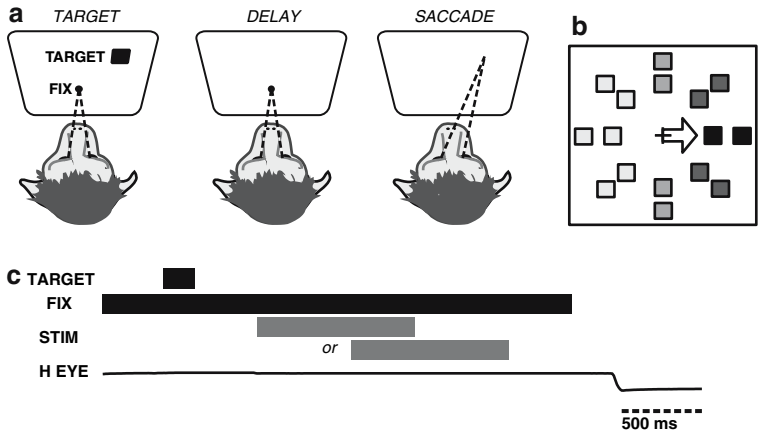

Fig. 1 **a** Schematic of delayed saccade task. The monkey fixated a central fixation point (FIX). A peripheral target (TARGET) was flashed for 200 ms. The animal withheld a response during the delay. When the fixation target was turned off, the monkey made a saccade to the location of the previously flashed target. **b** Target locations were grouped by proximity to the MF, shown for an example MF at 0° (*arrowhead*). Shading indicates category (*black*, towards; *dark gray*, adjacent; *gray*, orthogonal; *light gray*, distal). **c** Timing of events in the delayed saccade task. Subthreshold stimulation (*gray bar*) was applied at one of two time points during the delay for 1 s

earth-mounted 4′ rectangular field coils (CNC Engineering). Visual stimuli were projected (Electrohome, Model ECP 4100) onto a 100 × 80 cm screen placed 58 cm from the animal. The room was otherwise completely dark, as confirmed by a dark-adapted human observer. All aspects of the experiment were computer-controlled (custom software). Eye position was logged every 2 ms. Visual stimulus presentation times were accurate to within one video refresh (17 ms).

Electrophysiological recording and stimulation were performed with tungsten microelectrodes (FHC; 0.2–2.0 MΩ). Extracellular potentials were amplified (FHC) and filtered (band pass 400–5,000 Hz; Krohn-Hite). Single units were isolated with a dual time-amplitude window discriminator (BAK Electronics). Bipolar stimulation pulses (negative leading, 250 μs/phase) were applied with a stimulus isolator (FHC). Prior to electrical microstimulation at a cortical site, we recorded the responses of single and/or multiple units from the microelectrode.

Frontal eye fields sites were defined as those at which electrical microstimulation with current less than 50 μA evoked consistent saccadic eye movements (333 Hz, 70 ms duration; Bruce et al. 1985), to facilitate comparison with previous studies. In a screening task, animals began by fixating a central target for 400 ms. The target was extinguished, and in half of trials, stimulation began 100 ms later. The fixation point reappeared 300 ms after the initial offset. The animal was rewarded on all stimulation trials and on control trials in which the eyes remained at the fixation target.

Subthreshold microstimulation was delivered at 93 μA and 1 s duration. For five sites in M1, stimulation was delivered at 50 μA. The threshold for evoking saccades as a function of stimulation frequency was measured at each site while the animal fixated. The frequency of stimulation was then adjusted to below this threshold. Although manipulation of stimulation current has been typically used to achieve subthreshold stimulation in FEF (Bruce et al. 1985; Moore and Fallah 2004), manipulation of frequency has been used to bias saccade metrics in the superior colliculus (Glimcher and Sparks 1993). The rationale for stimulation at a constant current with variable frequency was to activate a constant pool of neurons at a variable rate. With increases in stimulation current, current density around the microelectrode increases, resulting in a greater pool of stimulated neurons over a larger cortical region (Tehovnik 1996). In theory, stimulation at constant current would be more likely to activate a fixed number of neurons at a rate determined by the stimulation frequency.

Stimulation frequency was initially set to the highest level at which no saccades were evoked in a series of four trials. However, we found that the threshold could change over the course of a block of trials, indicated by breakthrough saccades at the onset of stimulation during the delayed saccade task (see below). We adjusted the stimulation frequency stepwise in 5, 10, or 20 Hz increments until breakthrough saccades were no longer evident. The average stimulation frequency across all experiments was 77 Hz (range: 10–333 Hz). Stimulation frequency decreased by an average of 16 ± 2.5 Hz over each session. Stimulation frequency decreased in 63% of sessions, remained constant in 34%, and increased in 2.4%.

Delayed saccade task

A schematic of the delayed saccade paradigm is illustrated in Fig. 1a. Monkeys were trained to fixate a central target to within 2°. Once the animal had maintained fixation for

600 ms, a peripheral target appeared for 200 ms in one of eight directions at either 10° or 20°eccentricity (*target*). After the target was extinguished, the animal was required to continue fixation of the central target for 2.8 s (*delay*). The fixation target was then extinguished, cuing the animal to make a saccade to within 3.5–6.8° of the target's location. During the delay, subthreshold microstimulation (*stimulate*) lasting 1 s was applied at one of two time points: either early (400 ms after target offset; 1,400 ms before the fixation offset) or late (1,400 ms after target offset; 400 ms before the fixation offset). On control trials, no stimulation was applied. The 400 ms offset was used to avoid memory-nonspecific effects either on the initial encoding of the target's location or on the preparation of a saccade before/during the cue to move. The three types of trials (early, late, control) were fully interleaved. The peripheral target never reappeared on stimulation trials to prevent the animals from receiving feedback on any stimulation-induced inaccuracies. The target reappeared on 50% of unstimulated trials. Feedback was used in this case to motivate animals to maintain a high level of accuracy throughout the experiment. We found that behavior did not differ significantly between the feedback and no-feedback conditions on unstimulated trials, and these data were combined for analysis.

The data revealed an equivocal difference between the early and late stimulation conditions. Where significant displacement vectors were found, there was no difference between the mean size of the vectors in the early and late conditions (2.55° vs. 2.30°, $P > 0.2$); however, there were more significant vectors found in the late condition (42/354 vs. 64/354, $P < 0.05$, χ^2 test). We combined data from early and late stimulation trials for analysis.

Data analyses

Saccade endpoints were measured as the average eye position in the interval 100–300 ms following the end of the saccade to the memorized target location. Presaccadic eye positions were measured in the 200 ms interval preceding the offset of the fixation point. Saccades on error trials were excluded. To measure displacements between stimulated and unstimulated endpoints, we projected the two-dimensional saccade endpoints onto an axis that connects the mean stimulated and unstimulated endpoints for each target location. To determine if stimulation produced an effect on saccade endpoints in each experiment, differences between the two distributions of endpoints along this axis were assessed with the *t* test, corrected for multiple comparisons across target locations (Bonferroni method).

Displacements were not artifacts of eye movements during the delay. Effects were similar when either absolute saccade endpoints or endpoints relative to presaccadic eye positions were analyzed. Furthermore, when we eliminated trials post hoc in which small, detectable saccades occurred during the stimulation interval, similar results were obtained.

To pool data across the population, we defined the mean endpoint of saccades evoked by microstimulation as the movement field (MF) of at a site and the vector between fixation and the MF as the evoked saccade (Fig. 1b, arrow). Data from each stimulated site were aligned to the target closest to the MF measured at the site. Occurrence and magnitude of stimulation effects were then grouped by target location: "towards" targets closest to the MF, "adjacent" for targets ±45°, "orthogonal" for targets ±90°, and "distal" for targets ≥135° (Fig. 1b).

Our preliminary observations indicated that extensive stimulation quieted neurons surrounding the microelectrode (Tehovnik 1996). Therefore, in most experiments we balanced the number of targets presented and trials collected per target to avoid stimulation for more than 200 trials. Experiments were performed with 3–8 target directions possible and with 8–20 trials per direction. In 50% of sessions (29/58), targets were presented uniformly throughout the visual field. In the remainder of sessions, we either tested targets in a quadrant centered on the evoked vector direction plus a null direction target (20%, 11/58), or we tested two targets at the "adjacent" target locations and one target in the null direction (31%, 18/58). In total, 52 "towards", 122 "adjacent", 70 "orthogonal", and 110 "distal" target locations were sampled (see Results).

We classified FEF neurons recorded at each stimulation site based on their visual and motor responses in a delayed-saccade task. For each response (visual, motor), the spike rate in each interval was subtracted from the spike rate in the preceding baseline interval. The visual response was measured in the interval 50–300 ms after target appearance, relative to baseline activity (200–400 ms before target appearance). The motor response in the perisaccadic interval (100 ms interval prior to the time of peak saccade velocity) was measured relative to late delay period activity (200–300 ms prior to peak saccade velocity). A *visuomotor index* was constructed for each neuron by calculating the contrast ratio between visual and motor responses ([motor − visual]/[motor + visual]). Small manipulations (≤200 ms) in the length and location of the intervals used to calculate the visual and motor responses did not qualitatively alter any of the results.

Memory-related effects of stimulation: hypothesis testing

One possible outcome of subthreshold microstimulation would be to bias saccades towards the MF, by preferentially stimulating neurons that encode the MF. If saccade direction were decoded from FEF activity as a population vector aver-

age (Georgopoulos et al. 1986), microstimulation of a subset of similarly tuned neurons near the electrode tip might be expected to bias the population vector towards the MF of stimulated neurons. In fact, this phenomenon was very clearly not observed (see Results). A second possible outcome would be for microstimulation to shift saccades away from the MF. Transient microstimulation in FEF can be followed by a prolonged hyperpolarization of cortex (Seidemann et al. 2002). This phenomenon might effectively silence neurons during and after stimulation in our experiment. In this case, we might expect saccades to deviate away from the MF (Fig. 2a, "REPULSE"). Such repulsion is evident when small regions of the superior colliculus (SC) are inactivated, consistent with the idea that these neurons contribute to a "population average" of activity that determines the endpoint of a saccade (Lee et al. 1988). If subthreshold microstimulation results in a subsequent transient reduction in neuronal excitability, then saccades to targets adjacent to the MF should be deviated roughly orthogonal to the saccade vector. Saccades to targets beyond the MF should be deviated even more eccentrically.

A third possible outcome is that stimulation might shift saccade endpoints in a direction anti-parallel to the direction of the evoked saccade. This phenomenon would result if subthreshold stimulation mimics a signal indicating that a small saccade has occurred (Fig. 2b, "UPDATE"), and could occur if stimulation introduced a corollary discharge signal. In this case, stimulation should deviate saccades in the opposite direction of the evoked saccade at all target locations.

To test predicted outcomes two and three (above), we compared two different rotations of the data. For each stimulation site and target location, we rotated the observed displacement vector such that, if the predicted outcome were correct, the vector would point directly to the left (polar angle of 180°). For the REPULSE hypothesis, the 0° polar axis was oriented along the line connecting each mean unstimulated endpoint and the MF. For example, for the target and MF illustrated in Fig. 2a, the observed displacement vector would be rotated 30° counterclockwise, pointing the MF directly to the right and the predicted REPULSE displacement vector directly to the left. In order to test the UPDATE prediction, the 0° polar axis was oriented in the same direction as the evoked saccade (Fig. 2b). For the example shown, no rotation of the data would be necessary, since the evoked saccade direction points directly to the right. For each possible outcome, the rotated displacement vectors were averaged (Fig. 6a, b). The magnitude and direction of the resultant vector indicates the extent to which the data conform to the predicted outcome (see Results).

Motor-related effects of stimulation: hypothesis testing

In addition to, or instead of, biasing saccades by altering spatial memory, subthreshold microstimulation could directly bias the motor processes involved in saccade execution. In order to distinguish a motor effect from an effect on memory, we applied microstimulation either during or prior to the memory period. The memory-based hypotheses described in the previous section predict that saccades will be altered only when stimulation occurs during a memory period. In contrast, a motor-based effect of stimulation would occur regardless of whether memory is engaged at the time of stimulation.

We constructed a control experiment to test the motor and memory hypotheses. With two monkeys, we interleaved two variants of the delayed saccade task (Fig. 3a). The goal of this experiment was to hold constant the time between stimulation offset and the 'go' cue, while applying microstimulation either during the memory period (in which case the target was flashed before stimulation onset), or before the memory period (in which case the animal fixated while stimulation was applied, and the target was presented afterwards).

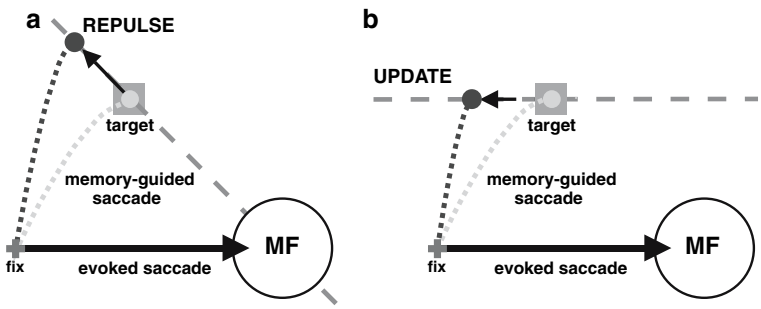

Fig. 2 Possible effects of stimulation for a hypothetical FEF site with a movement field (*MF*) at 0°. The memory-guided saccade endpoint without stimulation (*light gray circle*) is accurately directed from the fixation point (*gray cross*) towards the target (*gray square*). Stimulation could possibly bias saccades away from MF (**a**, *black circle*), indicated by the endpoint shifted in the direction away from the MF. Stimulation could also shift saccades anti-parallel to evoked saccade (**b** *black circle*), indicated by the endpoint shifted antiparallel to the evoked saccade vector (*large arrow*). *Coarse dashed lines* indicate the axes of alignment used to examine the REPULSE and UPDATE hypotheses and correspond to the 0° polar axis in Fig. 6a and b, respectively

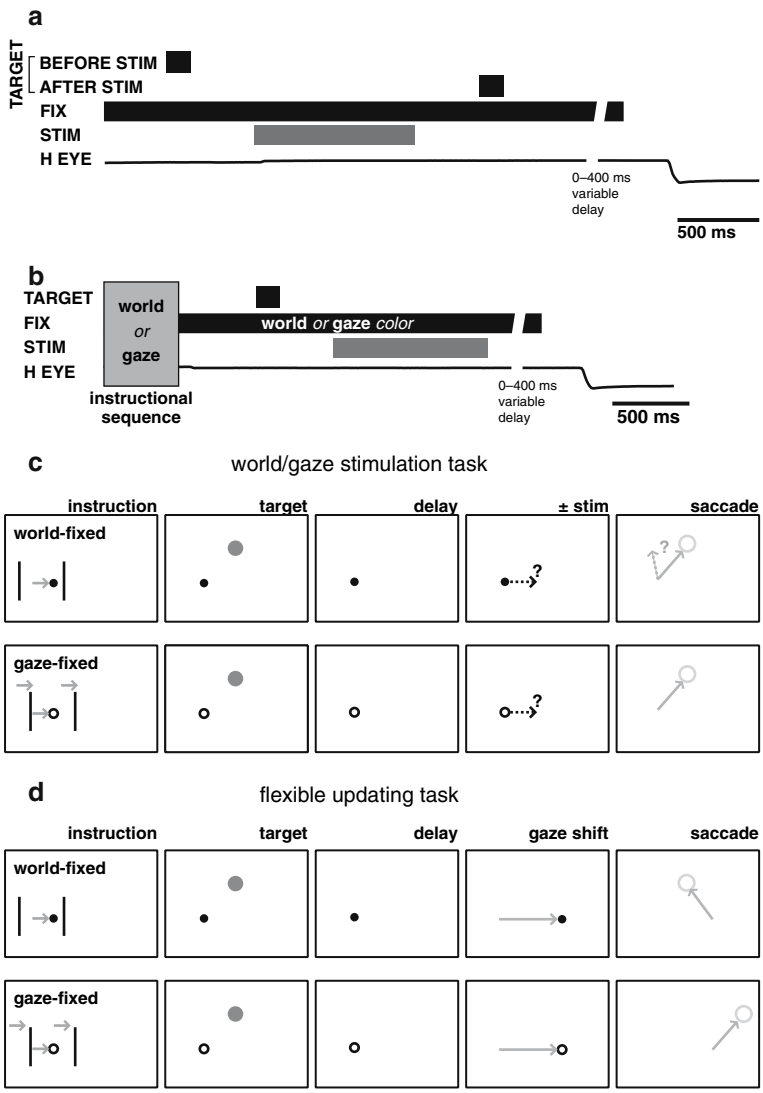

Fig. 3 **a** Timing of events in the stimulate during/before memory task. The spatial target was flashed either before (stimulation during the memory period) or after (stimulation before the memory period) the onset of stimulation. **b** Timing of events in the world-fixed/gaze-fixed stimulation task. After an initial task instruction (either world-fixed or gaze-fixed), the target was briefly flashed, and a 1,700–2,100 ms delay period followed. **c** Schematic of the world-fixed/gaze stimulation task. The animal fixated a leftward or rightward fixation point (*leftward shown here*). The motion of the initially presented flanking bars and the color of the fixation point (*solid points*, *yellow*; *open circles*, *purple*) indicated whether the target would be world-fixed or gaze-fixed. A peripheral target was then flashed, and the animal withheld a response during the delay. In half the trials, stimulation was applied during a segment of the delay, which was could have introduced a signal that the eyes had moved (*dashed arrow*). Offset of the fixation point cued the animal to make a memory-guided saccade to either the world-fixed or gaze-fixed location of the target (*solid arrow*), based on the instructional sequence and color of the fixation point. If stimulation mimicked a corollary discharge, a shift in endpoints would be expected only for the world-fixed condition (*grey dashed arrow*). **d** Schematic of the flexible updating task. The animal fixated a leftward or rightward fixation point (*leftward shown here*). A peripheral target was flashed, followed by a delay. The animal then made a horizontal smooth pursuit or saccadic eye movement towards the center of the screen. A second delay occurred, followed by offset of the fixation point, cuing the animal to saccade to the appropriate world-fixed or gaze-fixed target location

In the "during memory" condition, the peripheral target was flashed for 150 ms and extinguished 400 ms before the onset of stimulation. A 2.5–2.9 s delay period followed. This is analogous to the early condition of the original experiment. In the "before memory" condition, the target was flashed 400 ms after stimulation offset. A 550–950 ms delay followed. Both conditions included stimulation and control trials (4 trial types total), and were fully interleaved. In all stimulation trials, electrical microstimulation was applied 1.1–1.5 s before fixation point offset.

Context-specific effects of stimulation: hypothesis testing

Data from the experiments described above indicated that delay period microstimulation produced effects most consistent with an updating effect (see Results). This result

predicts that microstimulation could act as an artificial eye movement signal. If so, then the effect of the stimulation signal might be influenced by top-down contextual control signals. For example, work from our laboratory has shown that monkeys can remember target locations that are either fixed in the world (world-fixed) or fixed with respect to the center of gaze (gaze-fixed) (Baker et al. 2003; White and Snyder 2004a). Animals compensate for eye movements in remembering a world-fixed target, but ignore eye movements to keep a remembered target gaze-fixed. We therefore asked whether the effect of stimulation was modulated by context (either world-fixed or gaze-fixed).

One monkey had been previously trained to perform a flexible updating task, in which the animal makes a saccade or pursuit eye movement during a delay while remembering the location of either a world-fixed or gaze-fixed target (see Flexible Updating Task, below). This animal was very proficient at the task (typical performance >90% correct). Unfortunately, this was the only trained animal available for use in these experiments; training a second animal on this complex task would have required 1–2 years of training.

We decided to test whether the animal's treatment of a target as either world-fixed or gaze-fixed would influence the effect of microstimulation. We constructed a task in which a world-fixed or gaze-fixed cue was given, but no eye movements were performed during the delay (Fig. 3b, c). Instead, subthreshold microstimulation was applied. We predicted that stimulation during the delay would have little or no effect when a gaze-fixed target was being remembered, but would update spatial memories when a world-fixed target was being remembered.

In this task, the monkey fixated a point either 10° to the left or right of the center of the workspace. The task instruction (either world-fixed or gaze-fixed) was indicated by both the color of the fixation target, as well as by an instructional sequence presented at the beginning of the trial, as previously described (Baker et al. 2003). A purple fixation point indicated that the upcoming target would be gaze-fixed, while a yellow fixation point indicated the target would be world-fixed. The instructional sequence consisted of the following events. After initial fixation, two vertically oriented flanking bars appeared 7° to either side of the fixation point. After 250 ms, the fixation spot slid to the right or left (10°/s for 300 ms). If the flanking bars moved with the fixation spot, then the target in the upcoming trial would be gaze-fixed. If the flanking bars remained fixed in the world, then the target in the upcoming trial would also be world-fixed. After the instructional sequence was complete, the flanking bars disappeared.

After a short delay, a peripheral target was flashed for 150 ms at one of eight locations with horizontal displacement relative to the fixation target of 0°, +20°, or −20° and a vertical displacement of 2.5°, +18°, or −18° (approximating the vertices and midpoints of a rectangle). A variable (1,700–2,100 ms) delay period followed the disappearance of the target. On half the trials, microstimulation lasting 1 s was applied 350 ms after the offset of the target (stimulation trials). On the other half of trials, no stimulation was applied (control trials). At the end of the delay, the fixation point disappeared, and the animal made a saccade to the remembered location of the target. To expedite data collection, unique target locations were presented for left and right fixation locations and the data subsequently pooled over both locations. All other experimental parameters were identical to those in the delayed saccade task.

Flexible updating task

The flexible updating task was interleaved with the world-fixed/gaze-fixed task described in the previous section. Its purpose was simply to reinforce the behavioral relevance of the world-fixed/gaze-fixed contextual cue. Importantly, no data were collected during these trials. A brief description of the task follows; for more details on task design and training, please refer to Baker et al. (2003).

In this task (Fig. 3d), the monkey fixated a point either 10° to the left or right of the center of the workspace. The task instruction (either world-fixed or gaze-fixed) was indicated by both the color of the fixation target (yellow or purple) as well as by an instructional sequence presented at the beginning of the trial. A peripheral target was flashed for 150 ms at one of eight locations with horizontal displacement relative to the fixation target of 0°, +20°, or −20° and a vertical displacement of 2.5°, +18°, or −18°. These conditions were identical to previously described world-fixed/gaze-fixed stimulation task.

After a 250 ms delay, a horizontal smooth pursuit eye movement or visually guided saccade followed. On pursuit trials, the fixation point moved smoothly 10°/s either to the left or right for 1,000 ms. On saccade trials, the fixation point jumped either 10° to the left or right and subjects were required to re-acquire the fixation point within 700 ms. The fixation point was extinguished 400−1,200 ms following the end of the pursuit or saccade, cuing the subject to make a memory-guided saccade to the appropriate target location based on the world-fixed/gaze-fixed cue.

Results

Subthreshold stimulation during the memory period of a delayed saccade task was applied at 58 FEF sites in three monkeys (42 in M1; 12 in M2; 4 in M3). Stimulation lasting 1 s was applied either early or late in the delay (Fig. 1). On control trials, no stimulation was applied. All three

conditions (early stimulation, late stimulation, no stimulation) were fully interleaved.

During initial tests, we discovered that microstimulation did not severely disrupt the monkey's ability to make a memory-guided saccade. Normal saccades demonstrate a linear relationship between amplitude and peak velocity, termed the main sequence (Bahill et al. 1975). The main sequence relationship was no different between stimulated and unstimulated trials (Bonferroni method, $P > 0.10$), indicating that stimulation did not disrupt the animals' ability to generate saccades of normal character. Mean reaction time for memory-guided saccades into the contralateral field was faster on stimulated trials than control trials (238 vs. 247 ms, t test, $P < 10^{-14}$), and was slower for saccades into the ipsilateral field (256 vs. 248 ms, t test, $P < 10^{-12}$). For all data collected, monkeys were rewarded for making a saccade to within ~7° of the target location. Small on-line adjustments were made in window position and size to keep performance levels roughly the same on stimulation and control trials (95.4 vs. 97.0% success rates on stimulated versus control trials, $P = 0.08$). The endpoints of these saccades are the subject of our subsequent analyses.

Figure 4 shows the results from a typical experiment. Saccades evoked from fixation by 333 Hz, 50 μA microstimulation were directed into the upper left quadrant. The receptive field of a single neuron recorded at this site was also in the upper left quadrant. We defined the mean endpoint of saccades evoked by microstimulation as the movement field (MF) of the site and the vector between fixation and the MF as the evoked saccade (yellow arrow). In this experiment, the animal made memory-guided saccades to eight locations at 20° eccentricity. During experimental trials, stimulation was applied for 1 s, using stimulation frequency and current levels below the threshold for evoking a saccade (42–71 Hz, 93 μA in this example; see Methods). Although subthreshold stimulation was applied at 93 μA, all stimulation sites had a current threshold less than 50 μA at 333 Hz, which matches the criterion for FEF from many previous studies (see Methods). For targets close to the MF, saccade endpoints on stimulation trials (filled red circles) were deviated down and to the right of saccade endpoints on control trials (filled green circles). We measured the scalar deviation between control and stimulation endpoints along the axis of the displacement vector to assess the significance of displacement at each target location (Bonferroni method, $P < 0.05$, see Methods). In this example, deviations were statistically significant at four of the eight targets ($P < 0.05$; significant deviations indicated by heavy black lines). In contrast, saccades to targets far from the MF were unaffected by stimulation (open red and green circles; thin lines).

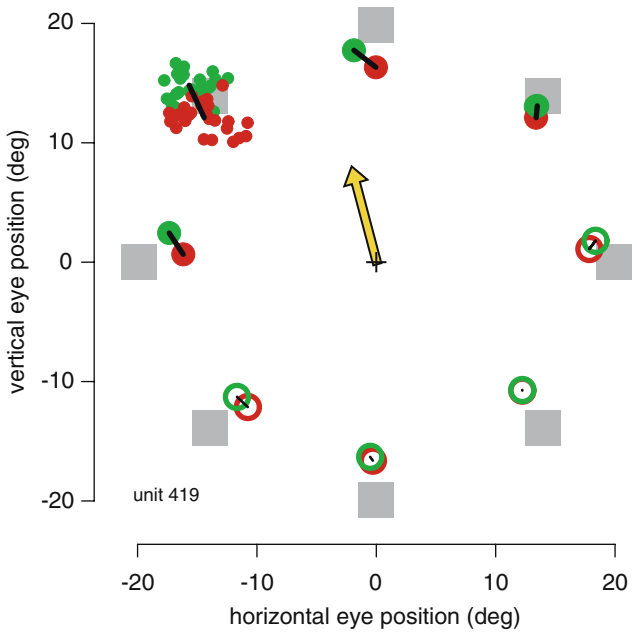

Fig. 4 Results from a single FEF site. The yellow arrowhead indicates the mean endpoint of microstimulation-evoked saccades. The monkey performed delayed saccades to eight target locations (*gray squares*). Displacement vectors (*line segments*) are between the mean stimulated memory-guided saccade endpoints (*red circles*) and mean unstimulated endpoints (*green circles*) for each target location. Thicker vectors with filled circles indicate displacement vectors with a significant difference in the distance between mean stimulated and unstimulated endpoints ($P < 0.05$, Bonferroni method). Individual trial endpoints are shown for the upper left target location only

Of the 58 sites lying within low-threshold FEF, 39 (67%) showed a significant difference, as described above, between stimulation and control endpoints for at least one target location (30/42 in M1; 5/12 in M2; 4/4 in M3), and 17 of those showed a difference at two or more target directions (11/42 in M1; 2/12 in M2; 4/4 in M3).

To analyze data from multiple sites, we rotated the saccade endpoints such that the target closest to the MF was oriented at 0°, that is, to the right of fixation (Fig. 5). As a result, the evoked saccades were brought into approximate alignment with one another (yellow lines). Next, we defined a displacement vector as the difference between the mean saccadic endpoint on stimulated versus control trials. We computed a displacement vector for each target in each experiment. Each vector originates from the location of the associated saccade target (green circles). Red vectors indicate significant differences between stimulation and control, whereas black vectors are not significant. On average, we used 6 target locations in each of the 58 experiments, for a total of 354 displacement vectors. Of these, 62 (18%) demonstrated a significant displacement, that is, subthreshold delay period microstimulation resulted in a significant difference in the endpoint of the memory-guided saccade. Displacement vectors were non-uniformly distributed in a direction away from the MF (Rayleigh test, $P < 10^{-12}$; mean direction = 185.1° ± 2.73°). Significant displacements were most common for sites that were close to the MF (Fig. 5d).

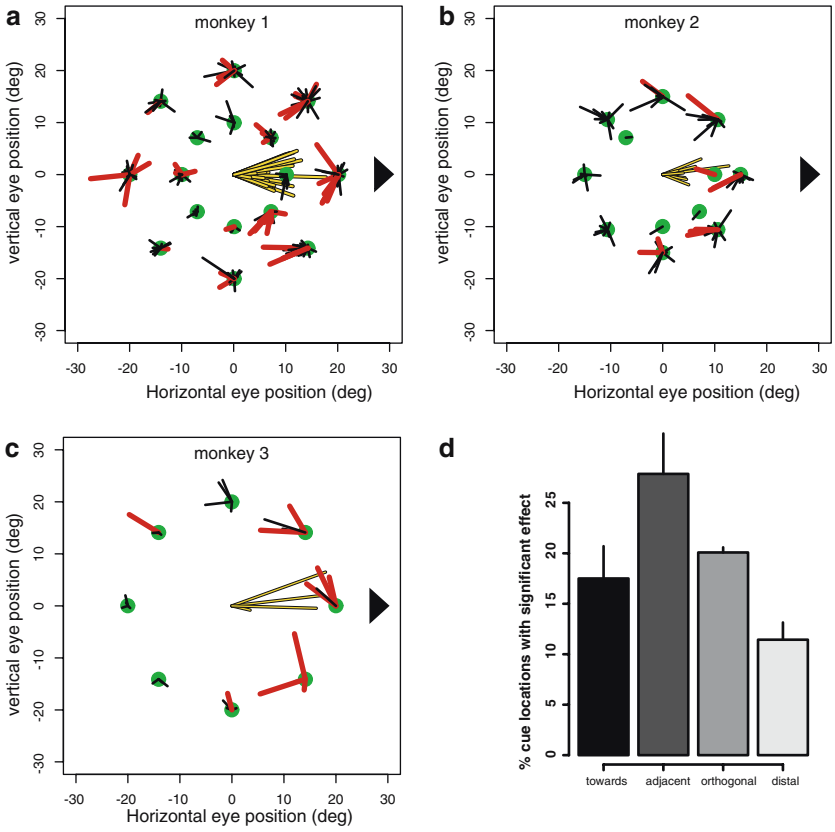

Fig. 5 Effects of stimulation for all FEF sites in each monkey. **a–c** Control endpoints have been drawn at the corresponding target location (*green circles*), and all endpoints have been rotated such that the target closest to the MF is at 0° (*arrowhead*). Line segments indicate the displacement vector between stimulation and control endpoints and are shown at 2×. Individually significant difference vectors are red ($P < 0.05$, Bonferroni method). Yellow lines indicate the mean endpoints of microstimulation-evoked saccades. **d** Effect prevalence. The frequency of significant deviation is shown by target distance from the MF. *Error bars* indicate SEM for 3 monkeys. *Shading* corresponds to target location, as shown in Fig. 1b

Although the scatter of displacement vectors appeared to be large, this was likely a result of the inherent variability of memory-guided saccades combined with the relatively small magnitude stimulation effect. We compared the within- and between-site variability of significant displacement vectors. We did not find a significant effect of recording site on either displacement magnitude (ANOVA, $F = 1.15$, $P = 0.37$, $n = 60$) or direction (circular ANOVA, $F = 1.56$, $P = 0.13$, $n = 60$).

Subthreshold stimulation mimics a corollary discharge signal

Why are saccades on stimulated trials displaced? Saccades tended to deviate away from the direction of the MF (Fig. 5), so it is unlikely that microstimulation biased spatial memories towards the MF. Stimulation might shift saccades away from the MF (Fig. 2, "REPULSE"), or it might shift saccades in a direction anti-parallel to the evoked saccade (Fig. 2, "UPDATE").

We compared these two hypotheses quantitatively (see Methods). To test the first possibility, we rotated the data from each stimulation site and target location such that displacement vectors directed away from the MF would lie directly to the left (polar angle of 180°). To test the second possibility, we rotated the data instead such that displacement vectors oriented anti-parallel to the evoked saccade would lie to the left.

The data shown in Fig. 6a, b reveal that the effect of delay period stimulation is best described as a shift of saccades anti-parallel to the evoked saccade (UPDATE), rather than as a repulsion away from the MF (REPULSE). For saccades to target locations adjacent to the MF (where the largest proportion of effects were observed, Fig. 5d), vectors aligned parallel to the evoked vector (Fig. 6b) had a significantly larger mean component (1.5° vs. 0.02°, $P < 10^{-3}$, $n = 33$ significant vectors) and were more tightly clustered (mean direction ± SEM: 189° ± 6.5° vs. 90° ± 13°) than those aligned on the axis oriented towards the MF (Fig. 6a). A similar result was obtained when both significant and nonsignificant vectors were analyzed (mean component 0.74° vs. 0.08°, $P < 10^{-5}$; mean direction 198° ± 6.6° vs. 117 ± 9.5°; $n = 122$). These data are more consistent with the idea that stimulation counterfeits an eye movement signal than with the idea that stimulation has a direct, repulsive effect on spatial memory.

To compare the effect of stimulation predicted by the two hypotheses, we calculated the projection of each displacement vector in the direction predicted by the repulsion or updating hypothesis (the negative horizontal component in Fig 6a or b, respectively). A visual comparison of the target-by-target effect magnitude for all adjacent target

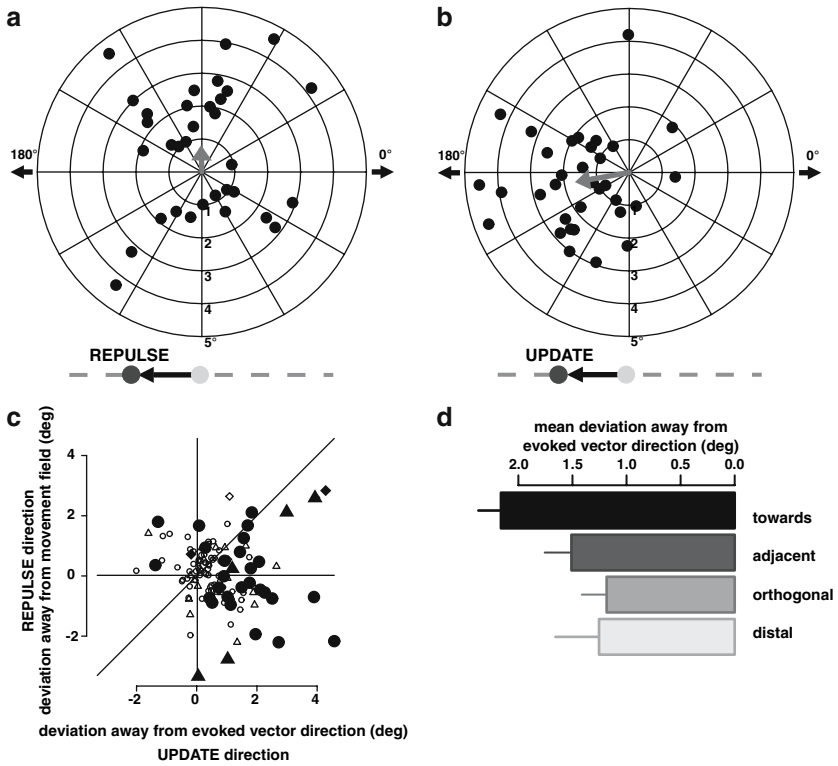

Fig. 6 **a** Points indicate the magnitude and direction of each significant displacement vector for adjacent target locations, aligned towards the MF (repulsion hypothesis). The *gray arrow* indicates the mean displacement. Direction, in degrees of polar angle, is oriented such that 0° is directed towards the MF. Magnitude is measured in degrees of visual angle. **b** Displacement vectors for all FEF sites aligned on the evoked direction (updating hypothesis). Direction in this case is oriented such that 0° is directed parallel to the evoked vector. Vectors in *b* are more tightly clustered and have a larger mean effect. **c** Comparison of effect magnitude in the direction of *repulsion* (ordinate) versus the direction of *updating* (abscissa) for adjacent target locations. Effects are larger in the updating direction. *Solid data points* indicate individually significant vectors. *Circles* monkey 1; *triangles* monkey 2; *diamonds* monkey 3. **d** Stimulation effect magnitude in the direction of updating, grouped by target location for all significant FEF displacement vectors. All effects are significantly greater than zero, and are strongest at the 'towards' target location

locations is shown in Fig. 6c. Effects are larger when aligned towards the axis predicted by the updating hypothesis, as seen by points that fall below the unity line.

To determine the average effect of stimulation, we calculated the mean projection of the significant displacement vectors in the direction of updating. This value is plotted as a function of target location in Fig. 6d. A significant displacement in the direction of updating was observed for all target locations (t test vs. 0, $P < 0.05$). Somewhat larger effects were observed for target locations near the MF ('towards') compared to other directions ($P = 0.01$). We examined whether sites with larger evoked saccades correlated with a larger updating effect. There was a weak positive trend between displacement vector magnitude and evoked saccade, but this relationship was not statistically significant (slope = 0.038°/°, $r = 0.15$, $P = 0.10$).

Before stimulating in each experiment, we recorded from isolated units while the animal performed a delayed saccade task. Data from one monkey (M1) contained a suitable range of single unit responses to warrant further inspection. We quantified the relative strength of each unit's movement-related activity to visually related activity by calculating a visuomotor index (see Methods). In 42 experiments, we found a correlation between stronger movement responses (larger visuomotor index values) and larger spatial updating effects induced by microstimulation ($r = 0.20$, $P < 0.002$). Furthermore, a greater proportion of significant effects was found where movement-related activity was greater than visual activity (index > 0) as compared to the converse (30/131 vs. 12/98 displacement vectors, $P < 0.001$). Thus, it appears that subthreshold microstimulation was more effective at introducing a corollary discharge signal where movement-related cells were present.

Subthreshold stimulation perturbs memory, not saccades

The data presented thus far support the hypothesis that subthreshold microstimulation counterfeits a signal indicating that the eyes have moved, thereby resulting in the adjustment, or updating, of spatial locations stored in memory ("updating" hypothesis; Fig. 6). A possible alternative explanation, however, is that subthreshold microstimulation

merely disrupts the motor processes involved in saccade execution without any direct effect on memory ("motor" hypothesis). This scenario might occur if stimulation had a long-lasting disruptive effect on neuronal function that persisted into the saccade phase of each trial. To minimize this possibility, stimulation on "late stimulation" trials concluded at least 400 ms before the cue to initiate a saccade (fixation point offset). Nonetheless, an abnormal saccade might result if some of the neurons that would normally be recruited were still disrupted at the time of the saccade. In this case, it is reasonable to suppose that the disrupted neurons might code saccades towards the MF, and therefore the absence of their contribution might bias the saccade endpoint in a direction opposite that of the MF.

We designed a task to test whether subthreshold microstimulation had a non-specific effect on subsequent saccades, or had a specific effect only when applied during a memory period (see Methods). It consisted of two conditions, where stimulation was applied either during the memory period or before the memory period. If stimulation exerted its effect on the saccade-generating machinery, then we would expect displaced saccade endpoints in both conditions. If stimulation exerted a memory-specific effect, displacements would be observed only in the "during memory" condition.

The results of a representative experiment are shown in Fig. 7a, b. Saccade endpoints were significantly deviated from control when stimulation was applied during the memory period (Fig. 7a: filled circles to either side of the MF), but not when stimulation was applied prior to the memory period (Fig. 7b: hollow circles).

This experiment was performed at 11 FEF sites in two monkeys (5 in M2; 6 in M3). When stimulation was applied *during* the memory period, 16 out of 80 displacement vectors (20%) showed significant effects. Overall, at least one significant displacement vector occurred for 9 of the 11 simulation sites (82%). In comparison, when stimulation was applied *before* the memory period, we found no significant displacements (0/80, 0%).

The population data are summarized in Fig. 7c, which compares the component of each displacement vector antiparallel to the evoked saccade for stimulation during the memory period (abscissa) or prior to target presentation (ordinate). Most data points fall well below the unity line (62/80, 78%), and the effects are significantly larger for stimulation during the memory period ($1.37 \pm 0.19°$ vs. $0.20 \pm 0.11°$, t test, $P < 1 \times 10^{-6}$). Stimulation prior to target presentation produced an effect that was not significantly different from zero (t test, $P = 0.082$). The results of this experiment indicate that subthreshold microstimulation must occur within the memory period to produce its effects.

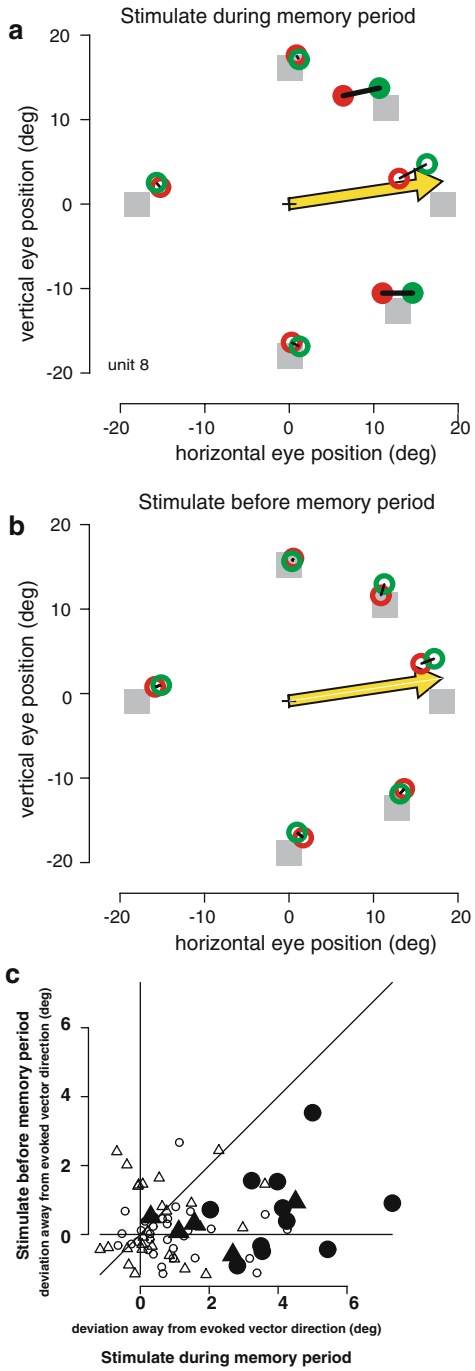

Fig. 7 **a–b** Results from an example FEF site. Symbols are the same as in Fig. 4. Microstimulation evoked large saccades to the right (*arrow*). Stimulation caused significant displacements of memory-guided saccades to adjacent target locations when stimulation was applied during the memory period, but not before. **c** Stimulation effect magnitude in the direction of updating when stimulation was applied *during memory* (ordinate) versus *before memory* (abscissa) for all 11 recording sites. Effects are large and significant only when stimulation occurs during a memory delay. *Solid data points* indicate individually significant vectors in this condition; there were no significant vectors when stimulation was applied before the memory period. *Triangles* monkey 2; *circles* monkey 3

This supports the interpretation that stimulation has a specific effect on a corollary discharge pathway and not on saccade execution.

Use of the stimulated signal is under cognitive control

The gaze-fixed/world-fixed stimulation task was designed to test whether a contextual cue (whether a target was world-fixed or gaze-fixed) was sufficient to modulate the effect of subthreshold microstimulation (see Methods). The "update" hypothesis predicted that stimulation would produce an effect only when the target was world-fixed.

One subject performed both stimulation and control trials in which either a world-fixed or gaze-fixed cue was given (4 trial types total, see Methods). The cue in this task was identical to the cue provided during the flexible updating task. Unlike the flexible updating task, stimulation experiments did not involve any memory period gaze shifts. Thus, world-fixed and gaze-fixed trials of the stimulation experiment differed only in the instructional sequence presented at the beginning of the trial and the color of the central fixation spot. To encourage the animal to utilize the task cue information, we interleaved the flexible updating task with the world-fixed/gaze-fixed stimulation task in an equal ratio.

The results of one experiment are shown in Fig. 8a, b. When stimulation was applied in combination with a world-fixed cue, there was a significant displacement of saccade endpoints in a direction anti-parallel to the evoked saccade vector at the two adjacent target locations. By contrast, when stimulation was applied with the gaze-fixed cue, it produced no effects. Thus, the task cue (world-fixed or gaze-fixed) profoundly altered the effect of delay period microstimulation in this experiment.

This finding was true across stimulation sites ($n = 12$). Saccades were significantly deviated at 7 sites (58%) in the world-fixed context and at only 2 sites (17%) in the gaze-fixed context (of the 2 sites with gaze-fixed effects, one showed an effect that was much larger in the world-fixed case, while the other showed similar effects in both conditions). Figure 8c compares the effects of stimulation under world-fixed versus gaze-fixed conditions. As in the previous analyses, we examined the projection of the displacement vector onto the evoked saccade vector. For displacement vectors where significant effects were observed ($n = 12$ displacement vectors), the mean component was larger in the world-fixed condition (t test, $P = 0.02$), indicated by points that fall below the unity line in Fig. 8c. By least-squares regression, we found this relationship to be linear ($R^2 = 0.730$, $P < 0.001$; intercept $P > 0.10$) and the regression slope significantly less than 1 (slope = 0.325 gaze vs. world, $P < 10^{-6}$). Thus, it appears that the animal's cognitive

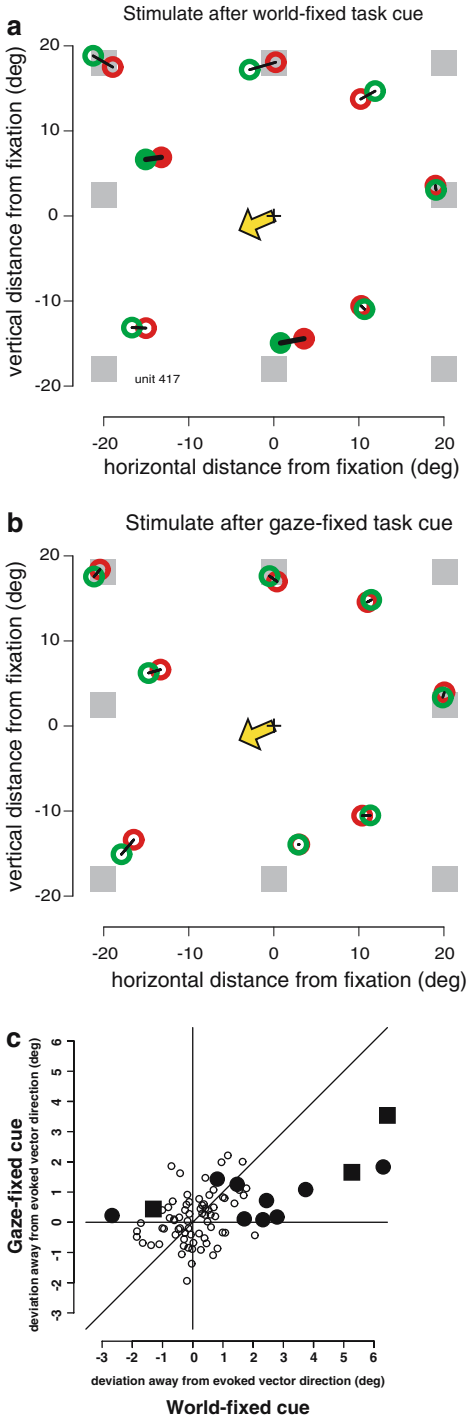

Fig. 8 **a–b**: Results from an example FEF site. *Symbols* are the same as in Fig. 4. Microstimulation evoked small saccades down and to the left (*arrow*). The monkey performed delayed saccades from either left or right fixation positions. Eye positions are shown relative to the fixation point for clarity. **c** Stimulation effect magnitude in the direction of updating in the gaze-fixed (ordinate) versus world-fixed (abscissa) conditions for all 12 recording sites. Effects are larger in the world-fixed case, where the monkey is instructed to compensate for eye movements during the memory period. *Filled circles* indicate individually significant vectors in the world-fixed task; *filled squares* indicate significance in both tasks ($n = 3$); there were no vectors significant in only the gaze-fixed task

representation of the task was capable of partially suppressing the use of the stimulated signal for updating.

Discussion

Many experiments point to FEF as a locus of spatial working memory in monkeys (Deng et al. 1986; Funahashi et al. 1989, 1993; Sommer and Tehovnik 1997; Dias and Segraves 1999) and humans (Ungerleider et al. 1998; Ploner et al. 1999; Corbetta et al. 2002). We hypothesized that subthreshold microstimulation of neurons in FEF should bias or disrupt memory-related activity, thereby altering behavior in a memory-guided saccade task.

We found that memory-guided saccades following subthreshold stimulation were largely normal, but endpoints were systematically displaced away from the direction of saccades evoked with stimulation above threshold (Fig. 5). This effect of stimulation was most consistent with the introduction of an artificial corollary discharge signal (Fig. 6). We propose that the target's location in memory was updated in response to this fictive eye movement. In a control experiment, stimulation resulted in updating only when it was applied during a memory period (Fig. 7), indicating that stimulation did not simply disrupt the read-out of the saccade plan or the ability to generate a saccade command. In a third experiment, we found that world-fixed targets were updated to a greater degree than gaze-fixed target in response to microstimulation (Fig. 8).

Corollary discharge signals in FEF

The displacement of memory-guided saccades by microstimulation is consistent with updating in response to a fictive eye movement (Fig. 6). Updating in response to saccades relies on corollary discharge from neural command signals (Guthrie et al. 1983; Bridgeman 1995; Lewis et al. 2001). FEF gives rise to saccadic motor commands that could be the source of such corollary discharges (Bruce and Goldberg 1985), either locally or through downstream pathways. FEF neurons project to subcortical structures involved in oculomotor control: FEF neurons that project to SC carry presaccadic signals (Segraves and Goldberg 1987; Sommer and Wurtz 2000), and FEF neurons also project directly to brainstem oculomotor nuclei (Huerta et al. 1986; Stanton et al. 1988). A pathway exists that conveys saccadic signals from SC to FEF via the mediodorsal nucleus of the thalamus (MD) (Sommer and Wurtz 2004a) and this pathway appears to contribute to spatial updating: when MD is pharmacologically inactivated with muscimol, subjects fail to fully compensate for the intervening eye movement in a sequential saccade (double-step) task (Sommer and Wurtz 2002, 2004b). Thus, subthreshold signals introduced by microstimulation in FEF could be insufficient to excite oculomotor structures below the level of the SC, but could contribute to spatial updating as a corollary discharge via MD.

There is also evidence to suggest that cortico-cortical pathways are important for spatial updating (Berman et al. 2003, 2004). Split-brain monkeys lacking the forebrain commisures have impaired behavioral performance for updating locations across visual hemifields, but not for updating within a single hemifield in a double-step saccade task. In our experiments, we note that fewer effects of microstimulation were observed for distal targets (which were most often in the opposite hemifield), suggesting perhaps that the stimulated signal did not transfer as well across hemispheres as within hemispheres.

Some FEF neurons update remembered locations in response to saccades, while others signal saccade amplitude and direction; therefore, the transformation of a corollary discharge signal into an accurately updated spatial memory could conceivably occur entirely within FEF.

There is good evidence, described above, that the FEF contains corollary discharge signals for saccades. However, FEF may well contain other signals that encode changes in gaze direction, for example, corollary discharge signals for pursuit eye movements, or vestibular signals (Fukushima et al. 2000). It is therefore also possible that FEF stimulation counterfeits a more general gaze shift signal, rather than a signal that is specific to saccadic eye movements.

Comparison with previous results

Electrical microstimulation can have effects on the generation of saccades. For example, low-intensity stimulation delivered shortly before or during a saccade can suppress saccades altogether (Burman and Bruce 1997) or modify their latency (Izawa et al. 2004).

Recently, Opris and colleagues (Opris et al. 2005) performed a similar experiment, in which subthreshold stimulation was applied to FEF during a delay period. Following Groh et al. (1997), Opris et al. asked whether the interaction between the planned memory saccade and the microstimulation was best described by vector addition, vector subtraction or vector averaging. They concluded that FEF microstimulation results in both vector subtraction and vector averaging, but did not quantify the relative contribution of each effect. The vector subtraction effect is identical in principle to our "update" effect (Fig. 4). Vector averaging can be described as an "attraction" of memory-guided saccades towards the MF.

In contrast to these results, the current study indicates that only vector subtraction (spatial updating) takes place, and not vector averaging. This is an important difference, because it confirms that the principle finding in both studies is vector subtraction, an effect that has not been reported as

a consequence of previous stimulation experiments. The current study also tests the mechanism by which vector subtraction occurs, distinguishing between a direct modification of a stored memory versus an effect on saccade generation. We tested these hypotheses explicitly in a separate experiment and found an effect of stimulation on stored memory, but a negligible effect on saccade execution (Fig. 7). The experiment was designed to hold constant the time between stimulation and the cue to saccade. However, this necessitated comparing the effects of stimulation in trials of different delay lengths. Stimulation altered saccade endpoints when the memory delay was long (stim during memory), but did not cause a detectable effect when the delay was short (stim before memory). Thus, if microstimulation exerted a weak effect on spatial memory, larger, i.e., more difficult, delays might be expected to magnify its effect. This alternative explanation still refutes the null hypothesis, which argued that stimulation would exert a memory-nonspecific effect on saccade generation. Although we cannot rule out an interaction between stimulation and delay length/difficulty, this still implies that stimulation exerted its effect on a component of spatial memory. In addition, this explanation does not explain the direction of displacement in the main experiment or the effects of world/gaze context of the effect of microstimulation.

We believe that the precise timing of the stimulation in the two studies may account for the finding of vector averaging in the Opris et al. study but not in the current study. Electrical stimulation in the SC preceding a visually guided saccade is known to produce vector averaging (Schiller and Sandell 1983; Glimcher and Sparks 1993). Since both studies used a fixed delay period, subjects likely anticipated the end of the delay, and saccade generation was likely initiated prior to fixation point offset (Findlay 1981; Bruce and Goldberg 1985; Kowler 1990; Dorris and Munoz 1998). In the current study, electrical stimulation ceased either 1,400 ms (early trials) or 400 ms (late trials) prior to fixation point offset. In the study of Opris and colleagues, stimulation ceased coincident with fixation point offset. As a result, it is possible that, while stimulation early in the delay period produced a spatial updating response, stimulation occurring immediately prior to the movement in the Opris et al. study had a direct effect on saccade generation, analogous to that seen with stimulation of the SC around the time of a saccade (Schiller and Sandell 1983; Glimcher and Sparks 1993).

Cognitive control of the stimulated signal

Animals can update spatial memories in response to pursuit eye movements, saccadic gaze perturbations, or whole body rotations but can also be trained to suppress updating, keeping memories fixed with respect to the center of gaze (Baker et al. 2003). In the latter case, shifts in gaze direction must be ignored to maintain a spatial memory in a gaze-fixed frame (White and Snyder 2004a). Thus, high level cognitive instructions regarding whether a target is world-fixed or gaze-fixed can be used to "gate" whether or not gaze shifts are allowed to alter the oculocentric (i.e., gaze-centered) representation of a remembered location.

In this report, we describe how FEF microstimulation appears to counterfeit a signal representing a gaze shift. Whether or not this counterfeit signal is allowed to alter a remembered location depends on how and where the cognitive gating of signals representing gaze shifts occurs. Our results indicate that the effects of FEF stimulation in an animal instructed to treat targets as gaze-fixed were almost completely suppressed (Fig. 8). Signals introduced by microstimulation could be gated by the animal in much the same manner as signals produced from an overt saccade (Baker et al. 2003). This suggests that microstimulation adequately mimics the physiologically normal discharge that occurs with a true gaze shift, and furthermore, that the locus of cognitive control lies within or downstream of FEF.

These conclusions on cognitive control, unlike the other conclusions in this report, are drawn from experiments in only one animal. Although in the past we trained three animals to memorize targets as either world- or gaze-fixed on otherwise identical interleaved trials, only one was still available for our use at the time of this study. Training a second animal for this report would have taken 1–2 years, and we therefore chose to publish these results with only a single subject.

Spatial memory in FEF

An unexpected finding of this study was the apparent lack of a direct effect of microstimulation on working memory. Rather than disrupting or altering working memory directly, microstimulation instead appears to introduce an eye movement signal that is subsequently incorporated into memory via spatial updating. Why might spatial memories be largely unaffected by FEF stimulation? Perhaps FEF does not itself store signals over time, but instead merely reflects the continuous output of some other brain area, which does provide such a function. There is some evidence to support this claim (Balan and Ferrera 2003). Neural correlates of spatial working memory have been observed in the lateral intraparietal area (LIP) (Gnadt and Andersen 1988) and the dorsolateral prefrontal cortex (DLPFC) (Funahashi et al. 1989). Both LIP and DLPFC are strongly and reciprocally interconnected with FEF (Huerta et al. 1987; Andersen et al. 1990; Stanton et al. 1993, 1995; Schall et al. 1995; Tian and Lynch 1996; Lewis and Van Essen 2000). Given the rich connectivity of these areas, we find it difficult to believe that microstimulation in FEF could not affect neural activity in LIP or DLPFC. However, it

remains to be seen whether subthreshold microstimulation in these areas directly perturbs working memory storage.

An alternative explanation is that FEF does have a direct role in spatial memory storage, but that the parameters of this experiment did not reveal it. First, the memory activity introduced by microstimulation might be infinitesimally small compared to the sustained neural activity representing the remembered target location. This possibility is analogous to the use of unambiguous motion stimuli used in many studies of MT. Microstimulation in MT during presentation of these stimuli produces little or no effect on perception (Salzman et al. 1992; DeAngelis et al. 1998, but see Nichols and Newsome 2002), likely because the neural activity in response to the visual stimulus far outweighs the activity induced by stimulating a small number of neurons. Similarly, in the experiments we describe, the animal's memory of the target might swamp any effect on memory introduced by microstimulation.

Second, the parameters of microstimulation used in this report might preferentially excite a pool of movement-related neurons, which might play a minor role in memory storage. Movement-related activity is correlated with lower thresholds for evoked saccades (Bruce et al. 1985), suggesting that the production of saccade commands is tied to movement-related activity in single cells. We found that effects of stimulation were more frequent and larger when they occurred where movement-related activity was recorded. Thus, it appears that the updating effect we observed was more pronounced when movement-related neurons were stimulated. However, the effect observed from excitation of movement-related cells does not preclude (perhaps more subtle) effects of microstimulation on other types of cells in FEF. It is possible that different stimulation parameters could preferentially excite a different population of FEF neurons, which might include those neurons involved in buffering spatial memory.

Acknowledgments We thank Dora Angelaki, Justin Baker, Steve Chang, Brian Coe, Bonnie Lawrence, and Doug Munoz for insightful discussions and comments on the manuscript. We thank Justin Baker for the monkey illustration.

References

Andersen RA, Asanuma C, Essick G, Siegel RM (1990) Corticocortical connections of anatomically and physiologically defined subdivisions within the inferior parietal lobule. J Comp Neurol 296:65–113

Bahill AT, Clark MR, Stark L (1975) The main sequence: a tool for studying human eye movements. Math Biosci 24:191–204

Baker JT, White RL, Snyder LH (2002) Reference frames and spatial memory operations: area LIP and saccade behavior. In: Society for Neuroscience, Program No. 57.16

Baker JT, Harper TM, Snyder LH (2003) Spatial memory following shifts of gaze. I. Saccades to memorized world-fixed and gaze-fixed targets. J Neurophysiol 89:2564–2576

Balan PF, Ferrera VP (2003) Effects of gaze shifts on maintenance of spatial memory in macaque frontal eye field. J Neurosci 23:5446–5454

Batista AP, Andersen RA (2001) The parietal reach region codes the next planned movement in a sequential reach task. J Neurophysiol 85:539–544

Berman RA, Heiser LM, Saunders RC, Colby CL (2003) Remapping of visual signals depends on the forebrain commissures; transfer of motor signals does not. In: Society for Neuroscience, Program No. 14.14

Berman RA, Heiser LM, Colby CL (2004) Does neural activity in macaque lateral intraparietal cortex predict spatial behavior? In: Society for Neuroscience, Program No. 649.644

Blanke O, Spinelli L, Thut G, Michel CM, Perrig S, Landis T, Seeck M (2000) Location of the human frontal eye field as defined by electrical cortical stimulation: anatomical, functional and electrophysiological characteristics. Neuroreport 11:1907–1913

Bridgeman B (1995) A review of the role of efference copy in sensory and oculomotor control systems. Ann Biomed Eng 23:409–422

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

Burman DD, Bruce CJ (1997) Suppression of task-related saccades by electrical stimulation in the primate's frontal eye field. J Neurophysiol 77:2252–2267

Chafee MV, Goldman-Rakic PS (1998) Matching patterns of activity in primate prefrontal area 8a and parietal area 7ip neurons during a spatial working memory task. J Neurophysiol 79:2919–2940

Colby CL, Duhamel JR, Goldberg ME (1995) Oculocentric spatial representation in parietal cortex. Cereb Cortex 5:470–481

Corbetta M, Kincade JM, Shulman GL (2002) Neural systems for visual orienting and their relationships to spatial working memory. J Cogn Neurosci 14:508–523

DeAngelis GC, Cumming BG, Newsome WT (1998) Cortical area MT and the perception of stereoscopic depth. Nature 394:677–680

Deng SY, Goldberg ME, Segraves MA, Ungerleider LG, Mishkin M (1986) The effect of unilateral ablation of the frontal eye fields on saccadic performance in the monkey. In: Keller EL, Zee DS (eds) Adaptive processes in visual and oculomotor systems. Pergamon Press, Oxford, pp 201–208

Dias EC, Segraves MA (1999) Muscimol-induced inactivation of monkey frontal eye field: effects on visually and memory-guided saccades. J Neurophysiol 81:2191–2214

Dorris MC, Munoz DP (1998) Saccadic probability influences motor preparation signals and time to saccadic initiation. J Neurosci 18:7015–7026

Droulez J, Berthoz A (1991) A neural network model of sensoritopic maps with predictive short-term memory properties. Proc Natl Acad Sci USA 88:9653–9657

Duhamel JR, Colby CL, Goldberg ME (1992) The updating of the representation of visual space in parietal cortex by intended eye movements. Science 255:90–92

Findlay JM (1981) Spatial and temporal factors in the predictive generation of saccadic eye movements. Vis Res 21:347–354

Fukushima K, Sato T, Fukushima J, Shinmei Y, Kaneko CR (2000) Activity of smooth pursuit-related neurons in the monkey periarcuate cortex during pursuit and passive whole-body rotation. J Neurophysiol 83:563–587

Funahashi S, Bruce CJ, Goldman-Rakic PS (1989) Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61:331–349

Funahashi S, Bruce CJ, Goldman-Rakic PS (1993) Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic "scotomas". J Neurosci 13:1479–1497

Georgopoulos AP, Schwartz AB, Kettner RE (1986) Neuronal population coding of movement direction. Science 233:1416–1419

Glimcher PW, Sparks DL (1993) Effects of low-frequency stimulation of the superior colliculus on spontaneous and visually guided saccades. J Neurophysiol 69:953–964
Gnadt JW, Andersen RA (1988) Memory related motor planning activity in posterior parietal cortex of macaque. Exp Brain Res 70:216–220
Goldberg ME, Bruce CJ (1990) Primate frontal eye fields. III. Maintenance of a spatially accurate saccade signal. J Neurophysiol 64:489–508
Groh JM, Born RT, Newsome WT (1997) How is a sensory map read Out? Effects of microstimulation in visual area MT on saccades and smooth pursuit eye movements. J Neurosci 17:4312–4330
Guthrie BL, Porter JD, Sparks DL (1983) Corollary discharge provides accurate eye position information to the oculomotor system. Science 221:1193–1195
Huerta MF, Krubitzer LA, Kaas JH (1986) Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys: I. Subcortical connections. J Comp Neurol 253:415–439
Huerta MF, Krubitzer LA, Kaas JH (1987) Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys. II. Cortical connections. J Comp Neurol 265:332–361
Izawa Y, Suzuki H, Shinoda Y (2004) Suppression of visually and memory-guided saccades induced by electrical stimulation of the monkey frontal eye field. I. Suppression of ipsilateral saccades. J Neurophysiol 92:2248–2260
Judge SJ, Richmond BJ, Chu FC (1980) Implantation of magnetic search coils for measurement of eye position: an improved method. Vis Res 20:535–538
Kowler E (1990) The role of visual and cognitive processes in the control of eye movement. Rev Oculomotor Res 4:1
Lee C, Rohrer WH, Sparks DL (1988) Population coding of saccadic eye movements by neurons in the superior colliculus. Nature 332:357–360
Lewis JW, Van Essen DC (2000) Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey. J Comp Neurol 428:112–137
Lewis RF, Zee DS, Hayman MR, Tamargo RJ (2001) Oculomotor function in the rhesus monkey after deafferentation of the extraocular muscles. Exp Brain Res 141:349–358
McKenzie A, Lisberger SG (1986) Properties of signals that determine the amplitude and direction of saccadic eye movements in monkeys. J Neurophysiol 56:196–207
Moore T, Armstrong KM (2003) Selective gating of visual signals by microstimulation of frontal cortex. Nature 421:370–373
Moore T, Fallah M (2004) Microstimulation of the frontal eye field and its effects on covert spatial attention. J Neurophysiol 91:152–162
Nakamura K, Colby CL (2002) Updating of the visual representation in monkey striate and extrastriate cortex during saccades. Proc Natl Acad Sci USA 99:4026–4031
Nichols MJ, Newsome WT (2002) Middle temporal visual area microstimulation influences veridical judgments of motion direction. J Neurosci 22:9530–9540
Opris I, Barborica A, Ferrera VP (2005) Effects of electrical microstimulation in monkey frontal eye field on saccades to remembered targets. Vis Res 45:3414–3429
Ploner CJ, Rivaud-Pechoux S, Gaymard BM, Agid Y, Pierrot-Deseilligny C (1999) Errors of memory-guided saccades in humans with lesions of the frontal eye field and the dorsolateral prefrontal cortex. J Neurophysiol 82:1086–1090
Powell KD, Goldberg ME (1997) Remapping of visual responses in primate parietal cortex during smooth changes in gaze. Soc Neurosci Abstr 23:17
Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Eng 10:137–145
Robinson DA, Fuchs AF (1969) Eye movements evoked by stimulation of frontal eye fields. J Neurophysiol 32:637–648
Salzman CD, Murasugi CM, Britten KH, Newsome WT (1992) Microstimulation in visual area MT: effects on direction discrimination performance. J Neurosci 12:2331–2355
Schall JD, Hanes DP, Thompson KG, King DJ (1995) Saccade target selection in frontal eye field of macaque. I. Visual and premovement activation. J Neurosci 15:6905–6918
Schiller PH, Sandell JH (1983) Interactions between visually and electrically elicited saccades before and after superior colliculus and frontal eye field ablations in the rhesus monkey. Exp Brain Res 49:381–392
Schiller PH, Tehovnik EJ (2001) Look and see: how the brain moves your eyes about. Prog Brain Res 134:127–142
Schlag J, Schlag-Rey M, Dassonville P (1990) Saccades can be aimed at the spatial location of targets flashed during pursuit. J Neurophysiol 64:575–581
Segraves MA, Goldberg ME (1987) Functional properties of corticotectal neurons in the monkey's frontal eye field. J Neurophysiol 58:1387–1419
Seidemann E, Arieli A, Grinvald A, Slovin H (2002) Dynamics of depolarization and hyperpolarization in the frontal cortex and saccade goal. Science 295:862–865
Sommer MA, Tehovnik EJ (1997) Reversible inactivation of macaque frontal eye field. Exp Brain Res 116:229–249
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 (2002) A pathway in primate brain for internal monitoring of movements. Science 296:1480–1482
Sommer MA, Wurtz RH (2004a) What the brain stem tells the frontal cortex. I. Oculomotor signals sent from superior colliculus to frontal eye field via mediodorsal thalamus. J Neurophysiol 91:1381–1402
Sommer MA, Wurtz RH (2004b) What the brain stem tells the frontal cortex. II. Role of the SC-MD-FEF pathway in corollary discharge. J Neurophysiol 91:1403–1423
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
Stanton GB, Bruce CJ, Goldberg ME (1993) Topography of projections to the frontal lobe from the macaque frontal eye fields. J Comp Neurol 330:286–301
Stanton GB, Bruce CJ, Goldberg ME (1995) Topography of projections to posterior cortical areas from the macaque frontal eye fields. J Comp Neurol 353:291–305
Tehovnik EJ (1996) Electrical stimulation of neural tissue to evoke behavioral responses. J Neurosci Methods 65:1–17
Tian JR, Lynch JC (1996) Corticocortical input to the smooth and saccadic eye movement subregions of the frontal eye field in Cebus monkeys. J Neurophysiol 76:2754–2771
Tian J, Schlag J, Schlag-Rey M (2000) Testing quasi-visual neurons in the monkey's frontal eye field with the triple-step paradigm. Exp Brain Res 130:433–440
Ungerleider LG, Courtney SM, Haxby JV (1998) A neural system for human visual working memory. Proc Natl Acad Sci USA 95:883–890
Walker MF, Fitzgibbon EJ, Goldberg ME (1995) Neurons in the monkey superior colliculus predict the visual result of impending saccadic eye movements. J Neurophysiol 73:1988–2003
White RL III, Snyder LH (2004a) A neural network model of flexible spatial updating. J Neurophysiol 91:1608–1619
White RL, Snyder LH (2004b) Delay Period Microstimulation in the Frontal Eye Fields Updates Spatial Memories. In: Society for Neuroscience, Program No. 527.529
Xing J, Andersen RA (2000) Memory activity of LIP neurons for sequential eye movements simulated with neural networks. J Neurophysiol 84:651–665