Multiple spatial memories in the brain: decoding and modification using microstimulation

by

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Abstract

Sequential processing --- using multiple sensory stimuli to plan and control a set of ordered movements --- is a central aspect of human behavior. Because previous and future movements must be stored during the execution of any movement in a sequence, memory is an indispensable aspect of sequential behavior. To study how memory is used to link sensory inputs to sequential motor outputs, we have used the oculomotor system as a model. We trained monkeys to remember the location of two spatial cues over a brief delay, and then make two eye movements to the remembered locations in the order that they appeared. We explored the role of two different frontal eye movement areas, the frontal and supplementary eye fields (FEF and SEF) during this memory delay.

While both the FEF and SEF have shown to be important for sequential behavior, their individual roles are unknown. Here, using physiology, we show that the FEF is important for storing the location of multiple cues and their order in memory. In the SEF, we show that memory period stimulation can affect the order of a sequence, changing the goal of the entire sequence but not the individual movement components. Thus, both areas appear to play complementary roles in sequential planning: the FEF stores target locations, while the SEF appears to control the order of a response sequence, coding entire sequences without affecting the locations of the intermediate targets.

This work bears on several outstanding questions in the field. It clarifies the individual roles of the FEF and SEF during sequencing: the FEF may serve as a buffer for multiple memories while the SEF plays a role in organizing movement sequences. It relates several prior SEF results, suggesting that a primary role of SEF may be to specify movements by their goal. Finally, we suggest that this goal-centered scheme may be a fundamental way that many different types of movements are encoded.

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Chapter 1: Introduction

Background: sequential order

How sequentially ordered information is processed is one of the most important 20th century questions in the study of brain and mind. This question motivated some of the first experimental psychologists studying the behavior of humans and animals. Some of these researchers, including Pavlov and James, put forth the theory of "response chaining" in the late 19th century to explain how sequential behaviors could be controlled (James, 1890; reviewed by Adams, 1984). This theory of response chaining was adopted and extended by the behaviorists to explain sequential behavior; they attempted to express each behavior in a sequence as a simple function of the elements prior to it (e.g. Skinner, 1935, 1959). Eventually, it became apparent that this theory --- and the behaviorist approach itself --- was insufficient to explain the complex repertoire of human behavior (Lashley, 1951; Chomsky, 1959). While Chomsky used the example of

sequences of words in language to make his argument, Lashley pointed out that the same large, flexible repertoire of behaviors is seen also in the realm of movement control. More recently, specific models for movement control have been proposed that incorporate a hierarchy of representations (which include the internal states anathema to the behaviorists) to describe how sequential movements might be controlled by the brain. (Hollerbach, 1982; Soechting and Flanders, 1991) In this work, we explore how these sorts of movement sequences are flexibly planned, using spatial cues from vision and memory to maintain internal state.

There are several issues of sequential encoding that we distinguish in this work. First, the ability to flexibly order components into a sequence based on short-term, working memory is the type of complex behavior mentioned above. This type of sequencing, in the form of sequences of movements based on spatial working memories, is the focus of our work. A parallel line of investigation has focused on skilled motor behavior, the ability to temporally coordinate a large set of muscle activations to effect a complex motor output. Although there are many more detailed taxonomies, these two broad types of sequential planning are distinguished in large part by their different time courses of learning and retention. (Squire et al., 1993; Mishkin et al., 1988). For example, if you learn to ride a bike at a young age, an activity that takes hours to weeks, it is likely that you could execute this particular skilled motor program today even if you have not practiced it in the intervening years. In contrast, the sequential effect we examine is seen when you remember over a period of seconds a phone number that is read to you, and after dialing it, forget the number. This short-term, working memory of the digits is used

to plan the motor sequence required to dial the number, and many different digit sequences can be remembered over this brief period with equal facility (Baddeley, 1992; Milner, 1962). Although it is possible that the control of skilled motor coordination shares underlying neural circuitry with sequences planned from working memory, we will not address this issue here, focusing instead on motor sequences planned using working memory.

A second distinction that we draw is between sequential planning and execution. Most neurophysiological studies have focused on the latter effect, using repeated movements in a skilled sequence and tracking neural responses only during the performance of the sequence. In contrast, we have used a task where the sequence to be performed changes on every trial, and we focus on how several actions can be planned in advance. As will be later described, it is likely that these different types of tasks have different neural mechanisms, as in the former case other investigators have seen little activity during the planning period, where we observe robust effects. The work reported here centers on this planning period.

The oculomotor system and persistent activity

To examine how these sequential movements are organized, we have chosen to use the oculomotor system as a model. Because of its simplicity, this system has a long history in neuroscience as a way to study the neural basis of motor control. For example, much of what is known of the motor function of the basal ganglia and cerebellum, structures that play a large role in skeletal as well as eye movements, comes from the study of their

oculomotor function. We have chosen to study sequential control of one particular kind of eye movements: saccades. This is both because saccades are very common --- occurring once every few hundred milliseconds throughout our waking hours --- and because they are subject to volition, being the only eye movements which can be made voluntarily without specialized training (Schiller and Tehovnik, 2001; Carpenter, 1988).

The eyes are moved by a set of six extraocular muscles, controlled by a set of brainstem nuclei which innervate these muscles (Robinson, 1973, see e.g. Sparks, 2002, for a review). This is in contrast to the dozens of muscles which are activated when the arm is moved. Beyond the relatively straightforward muscular structure, the physical nature of the eye itself lends simplicity to its motor control. The eyeball is light and thus responds quickly to force, and the system is highly damped --- that is, little force from the muscles is needed to decelerate the eye to end a movement.

One fruitful line of research that has used as a model the voluntary saccadic eye movement system is the study of persistent activity underlying very short-term or working memories. In the prototypical paradigm, monkeys are asked to remember a cued spatial location over a brief delay period and respond after that delay by making an eye movement to the remembered location of the cue. In many visual areas of the brain, extracellularly-recorded neurons show phasic (quickly-adapting) visual responses that are selective for the location; that is, the magnitude of the response varies based on the location. In some brain areas, neurons show persistent activity ("delay activity") that shows similar tuning to the visual phasic activity and is sustained throughout the time the

animal must remember the cued location. It is thought that this persistent activity carries mnemonic information of the location through the delay period until the animal can use that information to guide its response (Goldman-Rakic, 1995; Fuster, 1995). Although the connection to memory has been most widely studied in the monkey, this has inspired a wave of recent work on persistent activity as a common mechanism for memory across many systems and organisms (Major and Tank, 2004).

Persistent activity has been seen for spatial locations in the frontal areas that we studied: the frontal eye fields (FEF), and supplementary eye fields (SEF), as well as parietal areas involved in eye movements such as the lateral intraparietal area (LIP) (Bruce and Goldberg, 1985; Schall 1991a, 1991b; Schlag and Schlag-Rey, 1987; Gnadt and Andersen, 1988). Similar delay activity related to memory for objects has been observed in the dorsolateral prefrontal cortex (which we define as distinct from the FEF) and inferotemporal cortex, a temporal lobe area involved in object recognition (Fuster, 1973; Mikama and Kubota, 1980; Fuster and Jervey, 1981). Thus, delay activity has been observed in many disparate brain areas, but the ways in which areas differ is not clear. Below, we describe our results showing one way that delay activity in FEF can be differentiated from that seen in other areas; it can encode more than one spatial location.

The FEF and SEF: history and anatomy

The earliest work identifying areas in the frontal lobe of monkeys that were involved in eye movements was that of Ferrier (1875). Using techniques that were cutting-edge for the time, he stimulated the brains of monkeys using small platinum disc electrodes placed

on the surface of the brain. He found that a strip of frontal cortex from the midline to the ventral region of the arcuate sulcus caused movements of the eyes when stimulated. Our knowledge of the system under study has increased greatly, but it is remarkable that after more than 100 years, we report here on an experiment which employs a stimulation technique different from Ferrier's mainly in that our electrodes now penetrate into the cortex.

Beginning in the 1950s and 60s, neuroscience research focused on what is now called the FEF, found in the anterior bank of the arcuate sulcus. Bizzi (1967) found that cells there fired before saccades, and Robinson and Fuchs (1969) demonstrated that intra-cortical stimulation of this area evoked saccades. Since that time, a large number of studies have been done on the FEF exploring its role in controlling eye movements. We know that it has a direct output path to the brainstem (Schiller et al., 1987), connects strongly to other areas that play a role in eye movements like the colliculus, parietal cortex (LIP), oculomotor thalamus, basal ganglia, and cerebellum (Leichnetz, 1981; Giguere and Goldman-Rakic 1988; Stanton et al. 1988), shows persistent delay activity related to memory, and may play a role in high level functions like attention and visual target selection (Moore and Armstrong; 2003, Schall and Hanes, 1993).

The FEF, located in the ventral part of the oculomotor strip identified by Ferrier, has been the focus of many studies since the late 1960s. In contrast, modern interest in the medial part of Ferrier's oculomotor region, what we now call the SEF (also MEF or DMFC, see Tehovnik et al 2000; Schiller and Chou 1998), was ignited by the work of Schlag and

Schlag-Rey in the 1980s. (Schlag and Schlag-Rey, 1987). Since that time, several distinctions between the FEF and SEF have been observed. Saccades elicited from the FEF are usually vectorial: the eyes move the same distance in the same direction regardless of the initial eye position (except when restricted by the physical maximum deviation of the eye). In contrast, saccades elicited from the SEF tend to be goal-directed or convergent: from many different initial eye positions, stimulation drives the eyes to a single final location. The current threshold for eliciting a saccade from the FEF (sometimes $\leq 5\mu A$) is in general lower than the SEF. And animals appear to have more control over the effects of stimulation in the SEF. When animals are fixating, stimulation thresholds rise by a factor of 15-20 in the SEF compared to when they are not voluntarily attempting to hold their eyes fixed. In the FEF, this factor ranges from 3 to 5. (Tehovnik et al. 1999). Finally, the SEF appears to be more involved in learned eye movements, while a wider variety of visual effects have been seen the FEF (Chen and Wise, 1995; Schall 2002).

The FEF and SEF are highly interconnected, both within a cortical hemisphere and across hemispheres. The SEF is strongly connected to other oculomotor areas, similar to the connections of the FEF described above, a marked difference from the surrounding dorsomedial cortex (supplementary and pre-supplementary motor cortex, Brodmann's area 6; see Shook et al. 1991). Oculomotor areas to which it projects include the parietal cortex, colliculus, basal ganglia, and cerebellum. In particular, it is reciprocally connected to "almost every known pre- and paraoculomotor structure of the brainstem" (Shook et al, 1990). The SEF also is interconnected with prefrontal and skeletal

premotor structures including post-arcuate premotor cortex, cingulate areas, and dorsolateral prefrontal cortex (Huerta and Kaas, 1990). In conclusion, while the SEF and FEF have similar connections, it appears that the FEF is more strongly connected with vision-related structures and the SEF more connected with motor and prefrontal areas (Huerta and Kaas 1990). The possible involvement of the FEF with vision and the SEF with motor control is a point which we will later explore.

The FEF and SEF: sequence involvement

Prior studies have examined the role that the FEF and SEF play in sequencing, with somewhat contradictory results. When the FEF is lesioned, major deficits result in double-step saccade tasks using no memory delay between the appearance of the cues and the animal's saccade. In contrast, lesions of the SEF have only minor effects (Schiller and Chou, 1998, 2000). In these lesion studies, the time between lesion and first behavioral test is on the order of a day. Over the course of weeks and months, improvement is seen, suggesting that cortical reorganization can restore function over this time period. In summary, lesion studies find larger sequence deficits in the FEF than SEF.

Reversible inactivation through infusion of pharmacological agents has been performed in both areas. When this is done, weak sequence deficits are seen in the SEF, with little effect on other sorts of saccadic behavior. In the FEF, reversible inactivation causes a major deficit in memory-guided saccades to contralateral targets, while visually-guided saccades are relatively unaffected (Sommer and Tehovnik, 1997, 1999). These reversible inactivation experiments are done on a timescale of minutes to hours: the time elapsed

from inactivation and behavioral testing is typically on the order of a few minutes and persists for a few hours. There is also an anecdotal report of a similar effect obtained in humans using transcranial magnetic stimulation (TMS): when TMS was applied over the FEF, memory-guided saccades were affected, but when TMS was applied over the SEF, interference with sequential saccades was observed in a small number of cases. (Tobler and Muri, 2002). In summary, inactivation studies find larger sequence effects in FEF than SEF.

Finally, there have been several recent physiological studies on the activity of neurons in the FEF and SEF during sequential tasks. Lu et al. (2002) was the first to demonstrate that during sequential saccade execution, cells in the SEF show sequence selectivity: that is, cells' firing rate depends not only on the parameters of the saccade being executed, but also its position in the sequence. Isoda and Tanji (2003) compared cellular activity in the FEF and SEF in a similar repeated skilled motor saccade task. They found that while sequence selectivity was observed in the activity of many SEF cells, very few FEF cells were sequence tuned. In contrast, Tian et al. (2003) did find some sequence effects in the FEF during the execution of saccade sequences, but their task had more memory requirements than the task used by Isoda and Tanji. In summary, physiological studies find larger sequence effects in the SEF than FEF, but we suggest that this may depend on the type of task used.

One more study which compares SEF and FEF relates to our work. Subthreshold microstimulation (below the current level needed to evoke a saccade) of both the SEF and

FEF has been performed while animals make visually-guided saccades to one of two peripheral targets depending on which appeared first, a task related to sequential behavior. (Schiller and Tehovnik, 2001). In these studies, it has been found that in both SEF and FEF, stimulation biases the animal to choose the target in the motor field of the stimulated site. This study provides some evidence that the role of the FEF and SEF may be similar when saccades to one of a set of targets are made without memory requirements.

In summary, lesion, reversible inactivation, and physiology experiments have come to conflicting conclusions on the relative importance of the FEF and SEF in sequencing of saccades. While lesion work has implicated the FEF in sequential control, data from inactivation work suggests that the SEF may play a more important role. Physiology studies also have come to conflicting results, in some cases arguing that the SEF is more important than the FEF in the execution of sequences of saccades, and in some cases that FEF does play a large role. Our results bear on this question, and using our results, we propose a hypothesis to explain the observed differences. When working memory is required, it appears that the SEF may play a major role in control of movement sequences but not in location memory, while the FEF may be involved in sequencing *through* its role in maintaining locations over a brief delay. In skilled motor tasks, on the other hand, neither area is responsive during a memory period, while during execution only the SEF seems to be involved. After a discussion of the results obtained in microstimulation and physiology experiments in the FEF and SEF (Chapters 2-3), we will return to this point in the discussion (Chapter 4).

Chapter 2:

Microstimulation of the supplementary eye field reorders sequential movements to specify a goal

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Abstract

Sequential processing – the brain's ability to serially order memories and assemble compound, temporally coordinated, movements – is a fundamental aspect of intelligent behavior. To study how sequential actions are planned, we trained monkeys to remember and execute two successive saccadic eye movements to remembered locations. Microstimulation of the supplementary eye fields (SEF) during the memory delay changed the order of the movements, but not the memory for which locations were cued, by biasing the final endpoint of the sequence. A key aspect of motor planning may be to code complex movements in terms of their goals.

Understanding how the brain represents sequence information is key to understanding how temporally-extended, goal-directed behaviors are planned and executed. Neurophysiological and neuropsychological experiments in monkeys have identified cortical areas involved in the execution of learned sequences (Tanji, 2001), but important questions remain, including the nature of their coding. Many computational neural network models of sequential behavior use a form of associative chaining: using persistent activity or feedback, neurons representing a given movement trigger neurons representing the next movement (Berns and Sejnowski, 1998; Nakahara et al., 2001; Abeles, 1991). This is likely to be the case at some level because the movements must be "read out" in order during behavior. Indeed, neurophysiological studies have demonstrated neurons with order-dependent selectivity for movements during performance of a learned sequence, as predicted by associative chaining (Tanji and Shima, 1994; Kermadi and Joseph, 1995; Mushiake and Strick, 1995). But what about planning and remembering a behavioral sequence? Some models of motor control suggest that this involves higher-order representations of whole sequences that are separate from the details of individual movements (Hollerbach, 1982; Tanji, 2001), but it is possible for motor sequences to be represented using associative chaining alone. We sought to address these issues in a frontal cortical area thought to be involved in the planning and execution of volitional sequences of eye movements, the supplementary eye fields (SEF). (Isoda and Tanji 2002; Lu et al., 2002; Sommer and Tehovnik, 1999; Tobler and Muri, 2002; Pierrot-Deseilligny et al., 2004). We applied microstimulation to the SEF of monkeys while they held information about a saccade sequence in memory before its execution.

Monkeys were rewarded for remembering the location and order of two briefly presented spatial cues over a short delay and then saccading to them in the order in which they appeared (Fig. 1). We changed the interval between the cues' onsets (the stimulus onset asynchrony, or SOA) to vary the difficulty of recognizing the correct order and thus test the effects of stimulation on the resulting psychophysical curve. We applied microstimulation (below threshold for eliciting eye movements) during the memory delay (see Methods and Fig. 1).

If SEF delay activity played a major role in representing the memory related to the individual cued locations, we would expect that microstimulation of the SEF might sometimes produce *target errors* (a saccade to a "wrong" location, i.e., one that was not cued). If it also played a role in representing sequence information, we might sometimes see *sequence errors* (saccades to the cued locations but in the wrong order). If the SEF represented sequences by associative chaining alone, we would expect to sometimes produce errors in making the first saccade and sometimes in making the second saccade, as if we were randomly selecting different elements of the sequence chain. We found a consistent pattern of results: SEF stimulation produced *sequence* errors, not *target* errors. Furthermore, it affected the endpoint of the sequence, not the first saccade --- stimulation biased the animal such that sequence endpoints converged toward a zone in the contralateral hemifield.

The effect of 50μA stimulation at three example SEF sites is shown in Figure 2. The lower panels show the psychophysical functions, with the unstimulated trials plotted in

blue and the stimulated trials in red. Positive SOAs indicate that the more ipsilateral target appeared first, and negative SOAs, the more contralateral target first. At zero SOA, the targets appeared simultaneously (thus no order information), and the reward for a correct trial was given randomly (P=0.5) after either response order. At all three sites (Fig 2A-C), stimulation during the memory delay biased the monkeys to choose the ipsilateral target first and the contralateral second; there was a significant upward shift in the psychophysical curve on stimulated trials, shown in red. ($P < 10^{-4}$, via logistic regression (Agresti, 2002; Salzman et al., 1992; see Methods). This is also apparent in the eye traces for the three smallest SOAs (-60, 0, +60 ms, upper panels); in Fig. 2A, for example, the animal *always* chose the ipsilateral target first following memory delay microstimulation. Surprisingly, while stimulation above threshold evokes saccades that are contraversive (directed contralateral to the stimulated SEF), the monkeys' first saccade was *ipsiversive* on most stimulated trials when one cue was contra- and the other ipsilateral (e.g. Fig. 2A and 2C) resulting in a sequence whose endpoint was contraversive. Across the two animals, stimulation at 31 of 55 sites (56%) produced a significant bias (at $P<0.05$, corrected for multiple comparisons). Importantly, stimulation did not disrupt the monkeys' ability to saccade to *which* locations were cued, only their correct *order*; monkeys made target selection errors on fewer than 2% of trials, unaffected by stimulation. Furthermore, saccade metrics and dynamics were not affected by stimulation (see Supplementary Figs. 2-4).

The pattern of effects across all six cue locations for three different SEF sites is seen in Figure 3, which shows which sequences were significantly biased by stimulation during

the memory delay ($P<0.05$, logistic regression, corrected for multiple comparisons). For example, in Fig. 3A, the arrow showing a first saccade up and to the right and a second saccade to the left (i.e., first to near one o'clock and then to near 11 o'clock) indicates that stimulation resulted in a significant bias of that sequence over the alternative from that target pair (to 11 o'clock, then to one o'clock). The magnitude of the bias is expressed as the shift in milliseconds of SOA of the psychometric function, i.e., the change in SOA needed to produce the same behavioral effect as microstimulation (biases with absolute value >= 200ms were set to 200ms, see Methods). When one cue is contralateral and one ipsilateral (e.g., the two pairs directly above and directly below the fixation point), stimulation causes a bias towards the contralateral endpoint, as shown in Fig 2A-B and summarized in Fig 4A. But when both cues appear on one side, the biases are not explained by a final saccade to the most contralateral point *per se*. Sometimes, the bias could be for a final saccade directed in the ipsilateral direction (e.g., in Fig 3A, the second saccade from 9 o'clock to 11 o'clock). The best explanation of the effect of SEF stimulation was not simply a bias of the final saccade to be contraversive, but instead a bias of the endpoints of the sequences to converge to a zone in the contralateral hemifield. For example, the sequences in Fig 3A seem to converge on a zone in the upper contralateral field, while in Fig 3B, they seem to converge to a zone in the lower contralateral field.

The convergent nature of the biases is summarized across all experiments in Figures 4A and 4B. For each site with two or more target pairs that showed a significant effect of stimulation, we calculated the vector difference between the biased and non-biased

endpoint. The mean difference vector for each SEF site gives an approximate final direction or zone to which stimulation biases the endpoint of the sequences. Difference vectors in this direction (i.e. within a 180° interval centered on the vector) indicate the bias was to the endpoint nearer this zone. If an individual difference vector pointed away from the mean, the bias for that pair was away from the zone. Figure 4A plots all the difference vectors as a function of distance from their mean vector. Almost all of the difference vectors are *towards* the mean vector for each site (in the range between 90 and 270 degrees), indicating that endpoints converge to a similar final direction. In fact, only one difference vector was in a different direction than the mean (compared to 23 that would be expected by chance; $p<10^{-10}$, χ^2 test; see Methods). The difference vector could also represent the second saccade vector, but it is unlikely that stimulation affected the second saccade vector only. If this were the case, we would expect to see target errors, which we did not observe, as the animals made the first saccade correctly but the second saccade was influenced by stimulation. Because the direction of each individual pair affects the direction of the mean vector, we confirmed the convergence using a Monte Carlo simulation. We calculated the null distribution of the absolute difference in angle between each difference vector and its mean, assuming each vector's direction was independent. (Fig. 4B, see Methods). The distributions were significantly different ($p < 10^{-6}$, KS test), confirming that endpoints selected by stimulation were clustered around the region given by the mean vector.

The magnitude of the biases caused by stimulation is illustrated in histograms in Figure 4C and 4D. Statistically significant shifts ($p<0.05$, corrected for multiple comparisons)

are purple and non-significant shifts are gray. Fig. 4C shows all cue pairs where one cue was ipsilateral to the fixation point and the other cue contralateral. The distribution shows a strong leftward shift, indicating a bias towards sequences with contralateral endpoints. As noted above, stimulation causes a bias away from contraversive *first* saccades. Fig. 4D shows the same plot, incorporating every cue pair where a mean difference vector could be calculated, not just the pairs where one cue is ipsi- and the other contralateral. This distribution is similarly strongly biased to the left; stimulation causes convergence around the difference vector mean.

These results demonstrate that microstimulation of the SEF during a memory delay affects the *order* of saccades to two remembered locations but neither the monkeys' ability to saccade to *which* locations were cued nor the metrics of the saccades. Further, stimulation biases sequence endpoints to converge toward a zone in contralateral space, a finding that parallels observations that stimulation of the SEF using currents above oculomotor threshold elicits single contraversive, convergent saccades (Schlag and Schlag-Rey, 1987; reviewed by Tehovnik et al., 2000). These results support hierarchical models of motor sequence control, suggesting a role for the SEF in "higher-order" representation of entire oculomotor sequences coded in terms of their endpoints.

We observed that stimulation of SEF can bias the endpoint of a sequential movement. This goal-directed encoding could reflect a general principle of the organization of motor sequencing in the premotor regions of both the oculomotor and skeletal systems. There are many parallels between the SEF and the premotor cortical areas that control skeletal

movements (e.g., dorsal and ventral premotor cortex, PMd and PMv, and the supplementary motor areas, SMA and pre-SMA). SMA and pre-SMA contain neurons selective for the order of an arm movement during performance of a sequence (Shima and Tanji, 2000), while PMd neurons encode movement goals (Hatsopoulos et al., 2004) and neurons in PMv encode movements in extrinsic, world-centered coordinates (Kakei et al., 2003). Furthermore, stimulation of PMv can produce single goal-directed arm movements (Graziano et al., 2002). It may be that planning of behavioral sequences depends, in general, on organizational schemes in which whole sequences are encoded in terms of their goals.

Figures

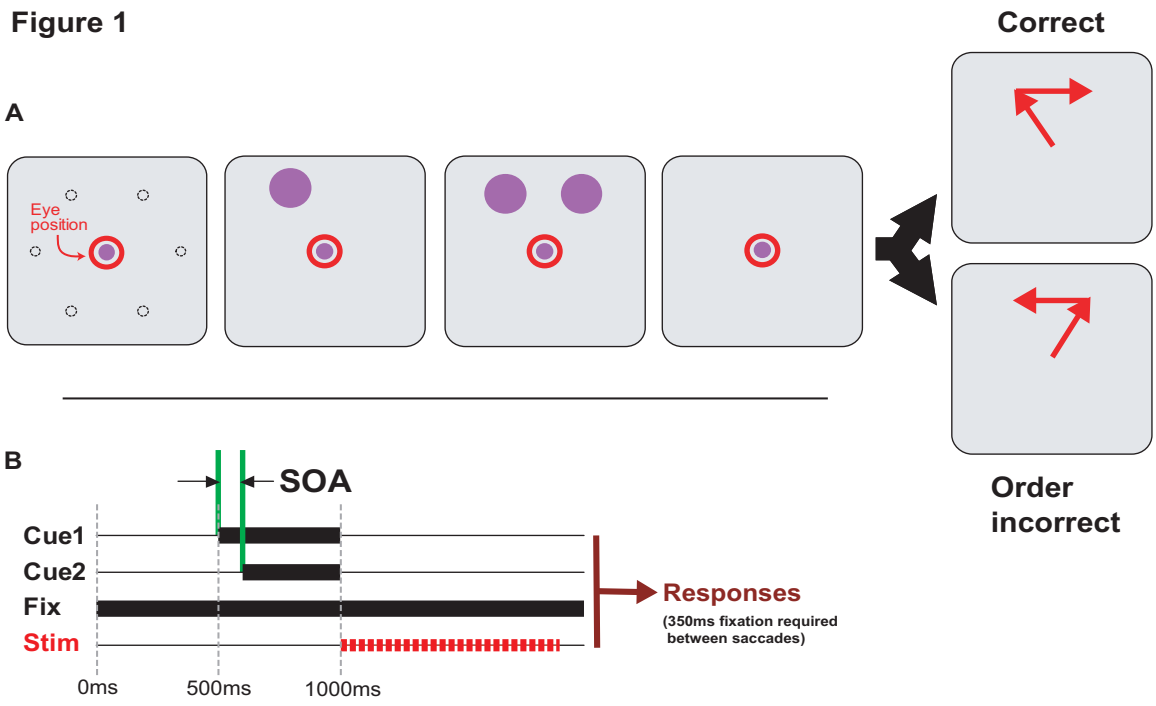


Figure 1: Task

A task schematic is shown in **(A)**. Each trial began with the illumination of a central fixation point. This was followed by 500ms of fixation and then by the onset of the two peripheral cues, separated by a short interval (the SOA). The cues were extinguished together and animals maintained fixation over a one second memory delay which ended with the offset of the fixation point. Animals then made saccades to the remembered locations of the cues in the order in which they appeared. To ensure that they were two separate movements, 350 ms of fixation was required after the first saccade. The two cues were always presented at adjacent positions, and the pair could occur at any of six positions (as shown). **(B)** Timing of trial events. Intracortical microstimulation was applied for the first 900ms of the 1000ms delay period.

Figure 2

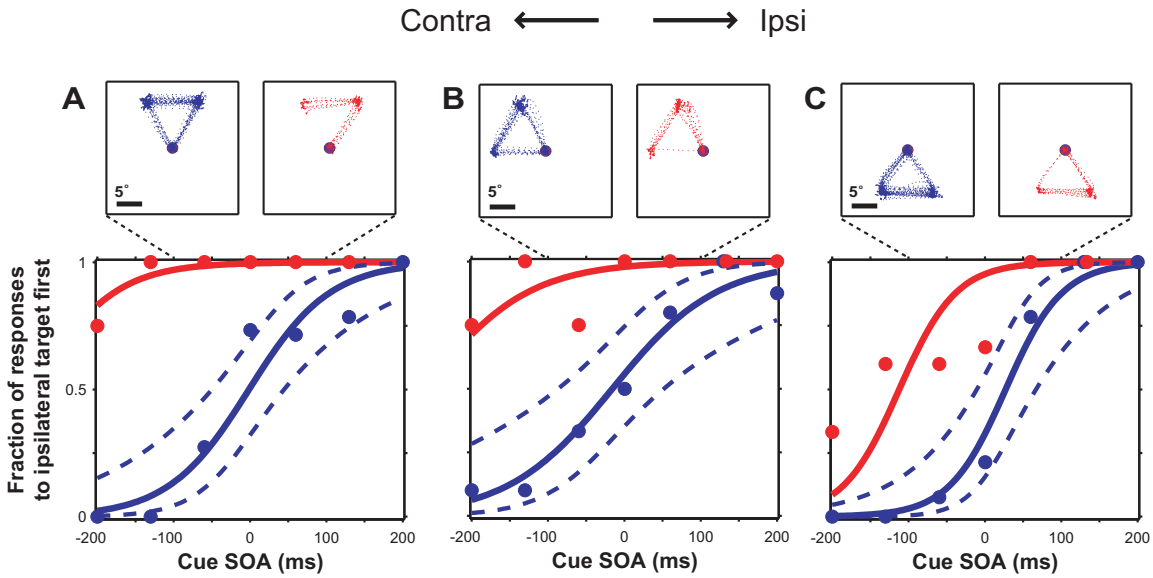


Figure 2: Examples of stimulation effects at three SEF sites

Each column (A-C) shows the effect of microstimulation three different SEF sites, each using one of three different pairs of targets. The lower panels show the psychometric behavioral curves. Horizontal axis is the SOA, vertical axis is the fraction of trials on which there was a saccade to the more ipsilateral target first. Data from unstimulated trials are plotted in blue, stimulated trials are plotted in red. Solid lines show the best-fit logistic curve. Dotted blue lines show a simultaneous 95% confidence interval around the unstimulated (blue) curve. The upper panels contain the eye traces from every trial at the three lowest SOAs: 0, -60 and +60. Blue traces (left) are from unstimulated trials. Red traces (right) are from stimulated trials. Fixspots not drawn to scale. In each case, stimulation strongly biased the animal to choose the more ipsilateral target first (and thus the more contralateral second).

Figure 3

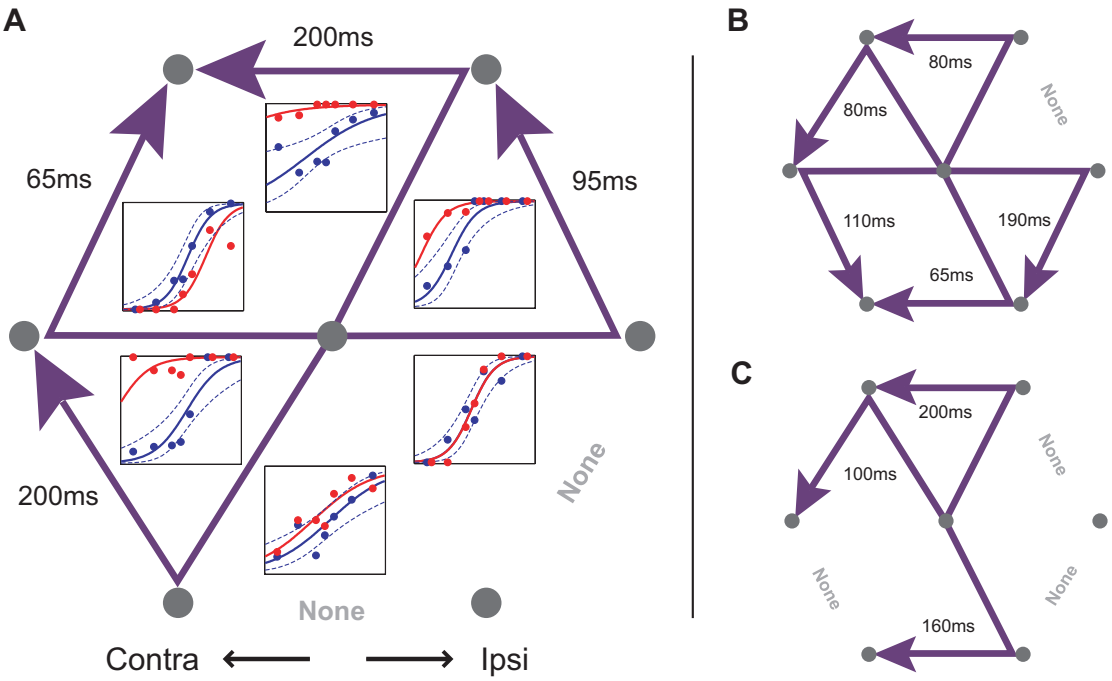


Figure 3: Stimulation biases animals to choose the contralateral target last

The pattern of stimulation-induced bias at all pair locations from three different SEF sites **(A-C)**. Purple arrows indicate the sequence of two saccades that was preferred on stimulation trials. Numbers outside each pair give the magnitude of the shifts in milliseconds. In **(A)**, psychometric curves are shown for each pair; conventions are the same as in **Fig. 2**.

Figure 4

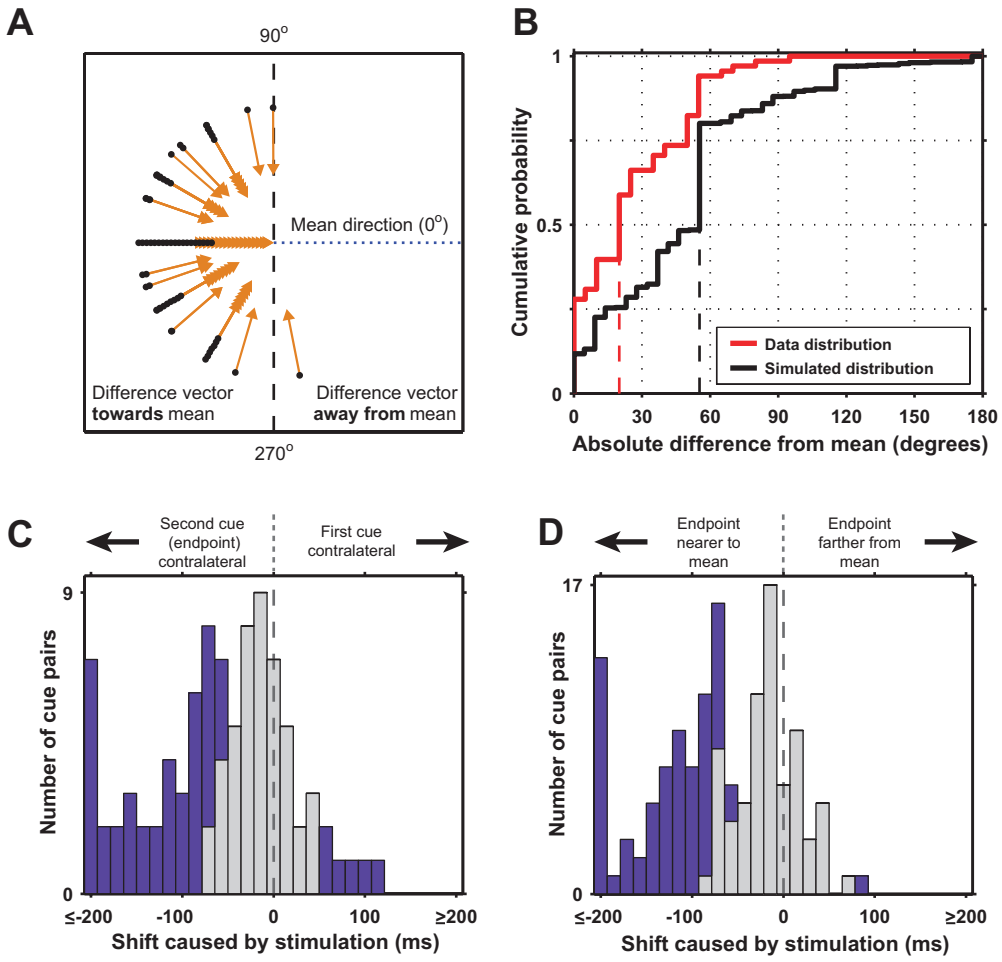


Figure 4: Bias directions are coordinated for each site

(**A** and **B**) Convergence of bias directions. (**A**) The difference vectors for each site around the mean difference vector at that site; the mean vector points to the right (dotted horizontal line). Black dots indicate origin of each difference vector; they are offset in the radial direction for display purposes only. Each vector's endpoint is at the center of the plot, and vectors beyond the 90-270° dashed vertical line indicate that the shift direction at that pair was *away* from the mean direction. Only 1/59 shifts were away from the mean, while 23 would be expected by chance (see Methods). (**B**) The cumulative

distribution of absolute differences between each difference vector and the mean at that site. Red line shows the true distribution, black line the simulated null distribution; the medians are shown by dashed vertical lines. The distributions are significantly different ($p < 10^{-6}$; KS statistic = 0.36). **(C-D**) Magnitude of sequence effects. (**C**) Histogram of bias directions for each pair with one target ipsilateral and the other contralateral to fixation. The horizontal axis shows the size of the shift in estimated SOA. Vertical axis is the number of shifts of a given size. Statistically significant shifts (logistic regression, $p<0.05$) are plotted in purple, non-significant shifts are plotted in gray. Dashed vertical line represents a shift of zero. Positive shifts mean that the contralateral target was chosen *first*, negative shifts that the contralateral target was chosen *second*. The histogram median is shifted significantly to the left. ($P<10^{-6}$, Wilcoxon test). **(D)** Histogram of bias directions for all pairs from sites that elicited more than one target pair with a significant shift; conventions are the same as in **(C)**.

Materials and Methods

We performed stimulation experiments in two rhesus monkeys (*Macaca mulatta*): monkey A (15kg, male) and monkey B (5kg, female). Animals were surgically implanted with a titanium head-holding device and a recording chamber was placed stereotaxically over the medial frontal lobe and secured to the skull with cortical bone screws (Synthes, Inc.). Tungsten epoxy-coated electrodes (FHC, Bowdoinham, ME) were inserted transdurally for stimulation (electrode impedance post-stimulation >= 100kOhm at 1kHz). Surgeries were conducted under isoflurane anesthesia and animals were given analgesics as part of post-operative care. All animal procedures conformed to NIH guidelines and those of the MIT Institutional Animal Care and Use Committee.

Site localization

We placed the recording chambers stereotaxically over the frontal cortex between the superior branch of the arcuate sulcus and the midline. These brain landmarks were identified by structural MRI; a reconstruction of the gray or white matter surface was computed from the MRI images and used to find the location of electrode penetrations, the recording chambers, and cortical sulci and gyri (Supplementary Fig. 1). For animal A, the highest-contrast boundary in the MRI images was between the gray matter and CSF, so we plotted (Supp. Fig. 1) the gray matter surface. For animal B, the highest-contrast boundary was between the gray and white matter, and we plotted the surface of the white matter.

To functionally localize the SEF, we mapped the cortex beneath the recording chamber by stimulating to elicit saccades. For this suprathreshold stimulation, we used biphasic, cathodal-first pulses, each phase 0.2ms, in trains 200-400ms long at 250-333Hz. Initial stimulation was at 100-120μA; once saccades were elicited, currents were reduced to determine thresholds. In addition, we recorded signals from each electrode while it was being lowered into the brain, to determine the electrode location relative to the cell layer in cortex. Most sites at which we stimulated during the task were in the superficial layers.

Experimental setup

Animals sat in a primate chair facing a flat computer monitor, 34cm away, on which stimuli were presented. An LCD monitor (NEC LCD1850E) was used to avoid flicker associated with CRTs. Pixel rise time to half-maximum intensity was measured as 6.1±0.4ms, mean±standard deviation. All visual stimuli (fixation points and cue stimuli) were round white spots. The fixation spot was presented at a central position and cue stimuli were displayed in the periphery; each was the same distance from the fixation point. Two cue stimuli, always adjacent to one another, were presented on any given trial.

Stimuli were presented and animals' responses monitored with Cortex (NIH, Salk Institute: http://www.cortex.salk.edu). Eye position was monitored with the Eyelink II system (SR Research Inc., Mississauga, Ontario, Canada). The position of the left eye was sampled digitally by the eye tracker at 500Hz, recorded as an analog signal at 2kHz,

smoothed with a 2.5ms moving-average window, and downsampled to 500Hz for off-line analysis. The time of occurrence of visual stimuli was recorded with a photodiode and amplifier. All analog signals were recorded using a Plexon system (Dallas, TX, USA).

Behavioral task

Fixation was required for 500ms before the first cue onset, during the cue presentations, and during the memory delay and stimulation. Both cues were always offset at the same time, and the offset of the fixation point served as the signal to begin the first saccade. Animals were required to hold their gaze within 1.0-1.75 degrees of the fixation point, or the trial was aborted. The animals fixated within 0.5 degrees of the central spot for the entire period on >90% of trials.

If the animal's first saccade was to any of the six possible target locations, whether or not it was the correct location, the fixspot was moved to the acquired location and animals fixated it for 350ms. Then, this fixspot was extinguished and animals made a saccade to one of the remaining possible target locations, where a fixspot appeared for 350 ms. Only at the end of the set of saccades was behavioral feedback given (unless the animal made no saccade or failed to fixate; in these cases an error was signaled immediately). If the animal had chosen the correct two cues in the correct order, it was rewarded and the next trial began. If the correct two cues were chosen, but in the *incorrect* order, a smaller reward was delivered (1/6 of the reward used on correct trials), and a short (3 sec) timeout ensued while a negative feedback stimulus was displayed. If the animal chose an incorrect location, did not stay within the fixation window, or did not wait for the

required amount of time before beginning the second saccade, no reward was delivered and a long timeout with a different negative feedback stimulus was interposed before the next trial. Saccades were made to incorrect locations on fewer than 2% of trials.

For each trial, the position of the pair, SOA value, and whether stimulation was applied was pseudorandomly chosen by drawing with uniform probability without replacement from a set of all combinations of pair, SOA, and stimulation state. All three parameters were thus randomly intermixed from trial to trial. These sets of trials were repeated so that animals performed 600-1000 correct trials per experiment.

For monkey B (19 sites) the possible cue locations were always indicated during the trial by a small (0.1°) dim spot at each location. For monkey A (36 sites), these were not present. Cues were 8° away from the fixation point for animal A and in animal B the distance varied from 5-13°. No difference in behavior was observed. Seven SOAs were used, 3 on either side of the 0ms point. We used six possible pair locations arranged in a hexagon in 39 of 55 total sites (36 of 36, animal A, 3 of 19, animal B). At other sites, a subset of the six was used to improve statistical power; once we determined that strong effects could be obtained using all six pairs at 50μA or below, we used these values exclusively.

Stimulation

On stimulation trials, stimulation was applied for the first 900ms of the 1000ms delay period. These stimulation trains were sets of 0.2ms per phase, biphasic, cathodal-first

square constant-current pulses delivered at 250Hz. We often used two stimulation electrodes (spaced at least 1mm apart) for a single experiment; stimulation was delivered on separate trials to each electrode so that there could be no interaction between electrodes, and no correlation between effects of stimulation at the two electrodes was observed. If one stimulation electrode was used, stimulation was delivered on 33% of trials (67% unstimulated); if two were used, stimulation was delivered to each on 25% of trials (50% unstimulated). For animal A, 50μA of current was used for each pulse phase unless that current elicited saccades during fixation, in which case the current was set below the threshold for eliciting saccades. For animal B, either 50μA, 75μA, or 120μA of current was used unless similarly reduced due to elicited saccades. 42 of 55 sites were stimulated with <= 50μA of current, of which 23 (42%) showed a significant effect.

The electrical stimulation we used passively spreads through the area at the tip of the electrode, and likely activates a large number of neurons; at 50μA high and low-threshold units are activated over an estimated radius of, respectively, 0.1-0.5mm (Stoney et al. 1968; reviewed by Tehovnik, 1996). However, it is probable that there is additional active propagation due to activation of local circuits through lateral interconnections. (Tolias et al., submitted) Other functions have been proposed for SEF in e.g. object-centered coding, reward monitoring, and associative learning, which our results do not exclude. Rather, our stimulation may selectively trigger a subset of local circuits (possibly those relying on lateral connections), which when activated specify the endpoint of a planned movement.

Data analysis

We used logistic regression (Agresti 2002) to determine the size and significance of stimulation effects. This is a special case of a generalized linear model (GLM) (McCullagh and Nelder, 1989) where the data are transformed by a logit link function (Equation 1) and then a linear model is fit using maximum-likelihood. Using a probit link function produced qualitatively identical results. The model we fit was:

(1) $$\ln \frac{\pi}{(1-\pi)} = \beta_0 + \beta_1 T_{SOA} + \beta_2 D_{stim}$$

Here, $ß_0$ specifies the intercept (threshold), $ß_1$ specifies the slope of the psychometric function, and $ß_2$ gives the change in threshold (shift) due to stimulation. D_{stim} is a dummy variable indicating the presence (1) or absence (0) of stimulation, and T_{SOA} is the SOA time. Fitting the model specified the parameters $ß_0$, $ß_1$, and $ß_2$, and these were used to compute the best-fit curves, plotted as the solid lines in Figure 2. We also calculated 99% Wald confidence intervals around these best-fit curves (Agresti, 2002, p172). Threshold shift magnitude, in milliseconds, was computed as the difference between the medians (i.e. 50% points or LD_{50}'s) of the stimulated and unstimulated curves. Analysis was done with custom programs in MATLAB (The Mathworks, Natick, MA) and R (http://www.r-project.org).

We report how stimulation consistently biased animals to more often saccade to the targets in a particular order. Another possible outcome was that stimulation merely increased the animals' error rate without causing a consistent direction bias (i.e. “injected

noise" into the order representation). This would be seen as a flattening in the psychometric curve around the 50% point (chance performance) in the stimulation case relative to the unstimulated case. To rule out this possibility, for every site and pair of cues, we fit a logistic regression model which included an interaction term between the stimulation dummy variable and the SOA value. Only 6% of site and cue pair combinations (19/286) showed a significant effect of slope change in the absence of threshold shift at $p<0.05$, almost exactly what would be expected by chance. Therefore, we rejected the hypothesis that stimulation flattened the curves around 50% and used the model shown above. For a more detailed discussion of this method see Salzman et al. (1992).

To determine the number of stimulation sites which showed a significant effect, we tested if any of the pairs used at that site showed a significant threshold shift at $p<0.05$, using the Bonferroni correction for multiple comparisons.

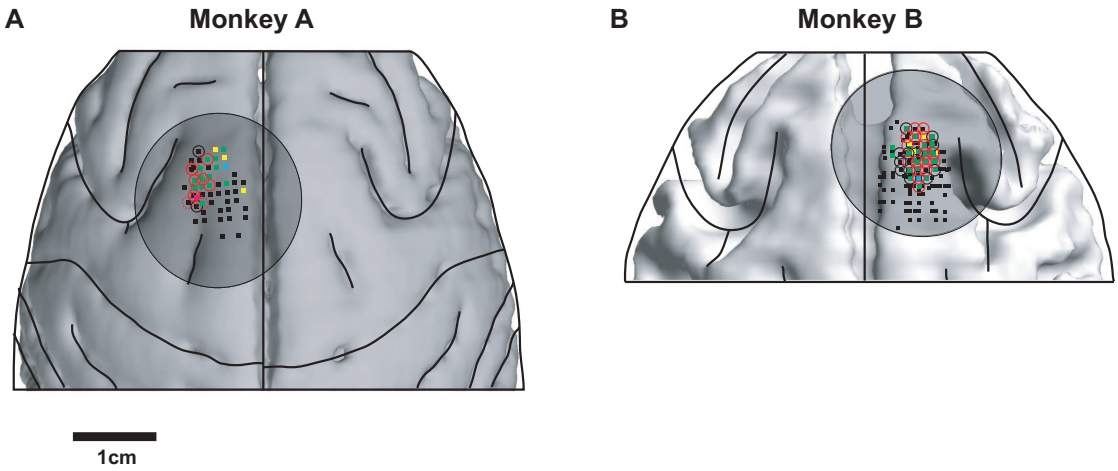
Figures 4A and 4B are calculated using endpoint difference vectors. These were found for each significant ($p<0.05$) cue pair by subtracting the vector from the fixation point to the endpoint selected by stimulation and the vector of the non-selected endpoint. We considered only vectors at sites with more than one significant pairs for all analyses on difference vectors, as a mean could only be calculated with two or more difference vectors. For each site, we calculated the mean of the difference vectors for those pairs (the "convergence zone") and rotated the vectors such that the mean vector pointed in the 0° direction. To determine whether the observed concentration about the mean was due

to chance, we calculated the null distribution of a set of endpoint vectors about their mean, matched to our data set. First, we simulated the null distribution of difference vector directions, for that number of pairs, using the same target locations used in the experiment. The simulation produced the null probability distribution of the difference vectors when the identity of the significant pairs and their shift directions were chosen randomly. We then averaged these null distributions, matched to each site, over all sites. We plotted all vectors by aligning all of their endpoints at the origin in Fig. 4A; to evaluate the significance of the effect shown there, we computed the expected number (by chance) of vectors whose angles were more than 90° away from the site's mean, using the null distribution described above.

In order to plot the observed and simulated distributions with respect to a scalar quantity, we calculated the absolute difference, in degrees, of each difference vector and the mean for that site. The corresponding observed and null distributions are shown in Fig. 4B. A two-sample Kolmogorov-Smirnov test was used to determine the significance of the difference between the two distributions.

Supplementary Figures

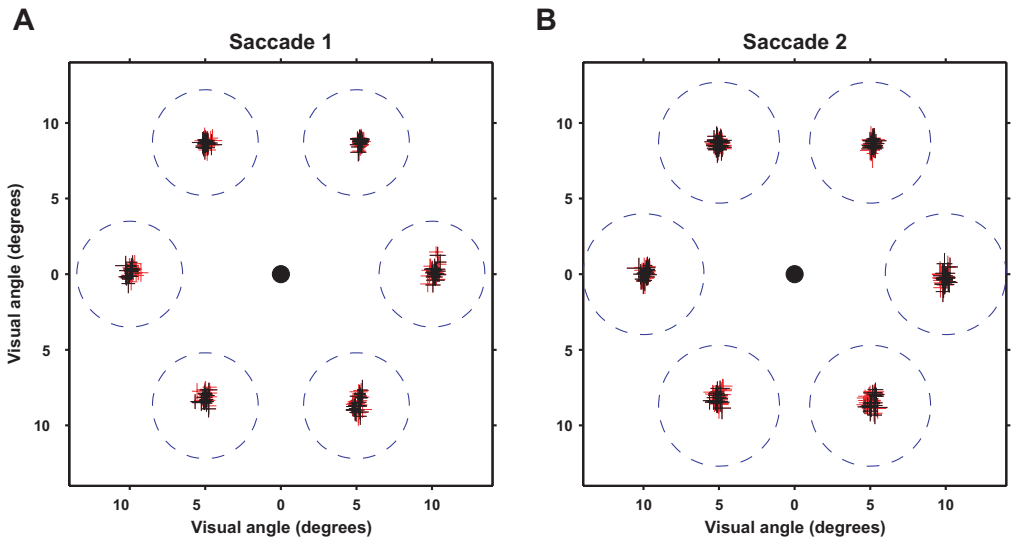
Supplementary Figure 1



Supplementary Figure 1: Stimulation locations

Shown are brain images constructed from MRI images. *Left,* locations of electrode penetrations in Monkey A; MRI image shows the surface of the **gray** matter**.** *Right*, electrode penetrations in Monkey B; MRI image shows the outer surface of the **white** matter, i.e. the border between the gray and white matter, as this was the most salient boundary in these MRI images (see Methods). Black lines show locations of sulci, circles are locations of recording chambers. *Squares*: result of suprathreshold mapping stimulation (all thresholds <= 50μA). *Green*, convergent saccades elicited; *yellow*, vector saccades; *cyan*, stimulation produced fixation; *magenta*, pursuit movements elicited; *black,* no effect. *Circles:* result of stimulation during the memory delay period. *Solid red circles:* stimulation caused response order bias for at least one cue pair, threshold <= 50μA. *Dashed red circles*: stimulation caused response order bias, threshold between 50μA and 120μA. (N=9, monkey A only). *Black circles:* no significant effect.

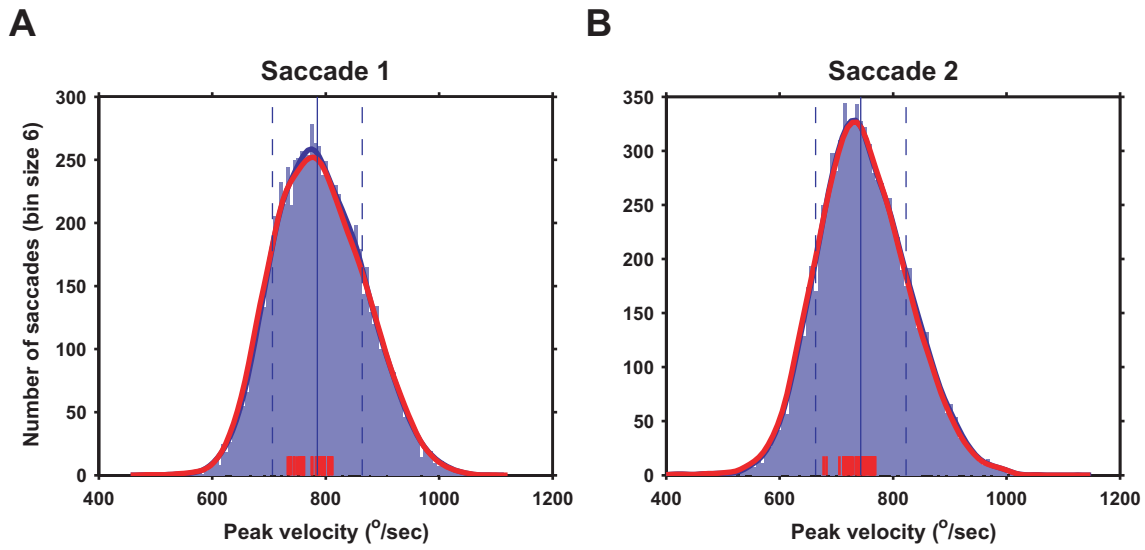
Supplementary Figure 2



Supplementary Figure 2: endpoint locations are unchanged by stimulation

(**A**) Endpoints of first saccade. (**B**) Endpoints of second saccade. (Data from animal B only; no visual cues were available to guide saccades.) Plus signs indicate mean (intersection point) and standard deviations in *x* and *y* directions (length of horizontal and vertical bar in plus symbol) of saccade endpoints over an experimental session. Stimulated endpoints are plotted in red, unstimulated endpoints in black. Blue dashed circles indicate the size of the window outside which a saccade endpoint would be considered incorrect.

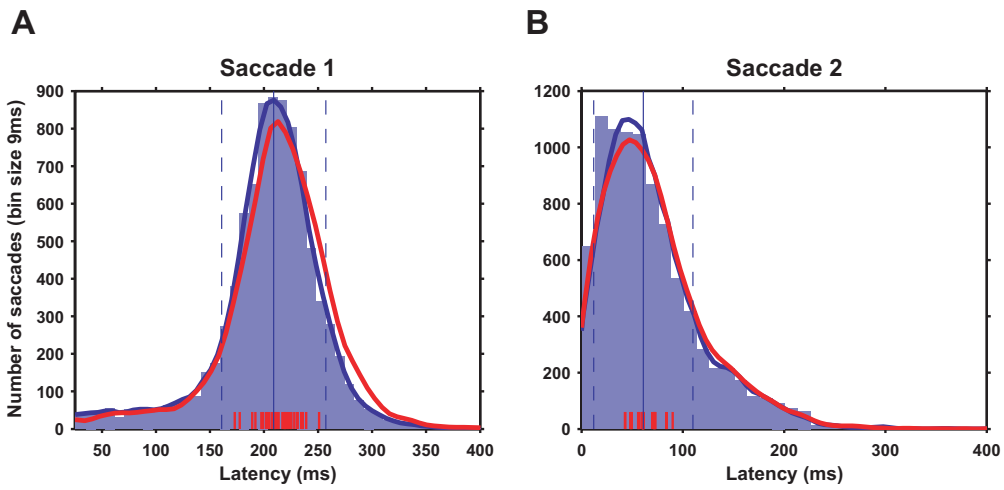
Supplementary Figure 3



Supplementary Figure 3: Peak saccade velocities are unchanged by stimulation

(**A**) Velocities of first saccade (**B**) Velocities of second saccade The blue region is a histogram of peak saccade velocities on unstimulated trials. Thick blue lines (largely hidden by red lines) are a kernel density estimate of the distribution of unstimulated peak velocities. Thick red lines indicate an estimate of the distribution of *stimulated* peak velocities. (Stimulated histogram not shown.) Solid and dashed blue vertical lines indicate mean and standard deviation of unstimulated distribution, respectively. Each red tick mark (bottom) gives the mean peak velocity for stimulated trials for a single stimulation site. Note that all red tick marks lie within one standard deviation of the mean of the unstimulated distribution.

Supplementary Figure 4



Supplementary Figure 4: Saccade latencies are unchanged by stimulation

First saccade latencies are shown in (**A**), second saccade latencies in (**B**). Conventions are the same as in **Supplementary Figure 3**. Latencies were also not significantly changed by stimulation. (Note: for the second saccade, latencies are measured from the offset of the fixation point. However, animals likely anticipated the time of their saccades - the 350ms earlier fixation requirement was constant – and likely caused relatively short latencies and lowered variance.

Chapter 3:

Delay activity in the frontal eye field encodes multiple remembered locations and their sequence

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Abstract

During the delay period of an oculomotor task, areas in both parietal and frontal cortex show similar persistent activity. We show that in the frontal eye fields (FEF), neurons are tuned for more than one cue simultaneously, in contrast to parietal cortex where persistent activity seems to encode only the upcoming movement. We demonstrate that it is possible to decode the locations of two cues from the delay activity of a population of FEF cells, and that there is sequence tuning during the delay. Prior work has given contradictory results about the role of the FEF in sequences of eye movements. We suggest that this is due to varying memory requirements of different tasks. It is likely that the FEF does not contribute to coding of motor sequence but carries information about sequences of visual cues and maintains sequential memories.

Introduction

The world that we live in changes both in space and in time. Our brains must thus take into account the temporal aspect of sensory input and motor output in order to control nearly all behavior. One central question about temporal processing is how the memory of temporally-ordered stimuli are used to guide sequential movement (Lashley, 1951).

Short-term, "working" memory for a single item has been well-studied. When monkeys are asked to remember a spatial or object cue over a short memory delay period and then use the memory to move to the remembered location or match a test stimulus, it is thought that persistent neural activity encodes the memory of the cue (Major and Tank, 2004). That is, a given cells fires selectively for a particular stimulus, and this firing rate is maintained throughout the delay, ceasing after the response has been made and the memory is no longer needed. Thus, information about the cue is carried by these neurons over the memory delay period. This sort of persistent activity has been found in many areas of the brain of the monkey (Fuster, 1995; Quintana and Fuster 1999), including in the parietal cortex (Gnadt and Andersen, 1998; Batista and Andersen, 2001), the temporal cortex (Miller and Desimone, 1994), and the frontal cortex (Fuster, 1973; Kojima and Goldman-Rakic, 1982).

To study how multiple memories could be encoded in persistent activity, we recorded the activity of neurons in the frontal eye field (FEF), while monkeys performed an oculomotor task requiring them to remember two spatial locations over a brief delay. We find that the persistent activity seen during the delay period in FEF encodes both

locations. This is in contrast to delay activity found in temporal and parietal areas, where persistent activity after a visual response seems to encode only a single item (Mazzoni et al, 1996; Miller and Desimone, 1994).

We recorded neurons from the frontal eye fields, an area found on the rostral bank of the arcuate sulcus in macaques (Robinson and Fuchs, 1969; reviewed by Tehovnik et al., 2000). Neurons here fire selectively for certain saccades (Bizzi, 1967), while microstimulation at low currents can evoke saccadic vectors, and long stimulation trains evoke saccade staircases (Robinson and Fuchs, 1969). The FEF is highly interconnected with the supplementary eye field, another frontal cortical area thought to be involved in oculomotor learning and motor sequencing, (Huerta et al. 1987; Huerta and Kaas, 1990) and various subcortical regions including the superior colliculus and the brainstem oculomotor nuclei (Huerta et al., 1986).

The role of oculomotor areas in the production of eye movement sequences has inspired much recent interest. It is thought that an area on the dorsomedial surface of the frontal lobe, the supplementary eye field (SEF) is important for oculomotor sequencing. Lu et al. (2000) demonstrated that the SEF contains neurons which fire selectively when sequences of saccades are being executed. This activity is often sequence-selective: that is, the activity of a neuron depends not only on the current saccade but also other saccades in the sequence, which can be several seconds in the past or future. More recently, Isoda and Tanji (2003) directly compared neurons in the FEF and SEF in a sequence task. They found that SEF neurons were much more sequence-selective, at the

time of a saccade, than neurons in the FEF. The SEF also lies near other dorsomedial motor areas important for sequencing of skeletal movements: the supplementary motor area, or SMA, and the pre-SMA (Tanji, 2001). Thus, it is thought that the SEF is important for sequencing eye movements, while recent reports show that the FEF seems to encode only the current movement being executed.

Our results expand upon these ideas. We show that persistent delay activity in the FEF actually carries sequence information: the order of cue presentation affects the responses of FEF neurons. At the time of a saccade, however, sequence selectivity in the FEF is reduced. Thus, the FEF may carry sequence information about visual cues and maintain this information over a delay, but it is likely that the SEF plays a stronger role in motor sequencing, showing more sequence information at the time of a saccade.

Results

We trained monkeys to perform a task which required them to remember two spatial locations over a memory delay period. The task is schematized in Figure 1. While animals fixated a central fixation point, two peripheral targets were illuminated in succession. After a one-second memory delay period, the animals were required to saccade to the remembered target locations in the order in which they appeared. On a given trial, the first cue could appear at any of six locations, spaced equally around the fixation point at a distance of 8 or 10 degrees of visual angle (for monkeys A and B, respectively). For a given first cue, the second cue could appear at either of the two adjacent locations; we restricted these locations so that each response saccade would be of the same amplitude. This gave twelve possible combinations of first and second cue.

Most cells in the FEF that gave visual responses were selective for both cues, and Figure 2 shows an example cell displaying this behavior. Here, all twelve combinations of first and second cue are shown in six panels. Each panel represents two trial types --- the two possible second cues, keeping the first cue fixed. In the upper-left panel (Figure 2A and 2B) the first cue always appears at 120°, up and to the left, while the second cue appears at 60°, up and to the right (green) or 180°, directly left (orange). For each panel, a raster and corresponding histogram is shown, and the trial time periods are identified in Fig. 2C. This cell shows a visual response for both the first cue (compare the upper-left panel with the lower-left panel), and the second cue (compare the orange and green trials in the middle-right panel). Furthermore, the firing rate over the delay period is a complex

function of the first and second cues. Compare the trials where the first cue appeared at 120° and the second cue at 180° (upper-left panel, orange) with the trials where these cues appeared in the reverse order: first cue at 180°, second cue at 120° (middle-left panel, green). In the former case, the cell's firing rate was ~ 35Hz throughout the delay period, while in the latter, the average rate over the delay period was ~ 15Hz. This cell shows visual responses and delay period activity that is selective for both cues, and furthermore, gives information about the sequence in which the two cues appeared.

Figure 3 summarizes this effect of dual tuning across the population. For each cell, we computed two selectivity indices, measuring the strength of selectivity to the first and second cues. These were constructed from statistical tests (see Methods). The two tuning indices measured the difference in median firing rates caused by the first or second cues. Larger values mean the cells carry more information about the cues. The top row of Figure 3 shows the tuning indices for all of the single units recorded from the FEF. The left column shows the tuning during the visual response periods and the right column the same index calculated during the delay period using a time interval of the same length as the visual responses. Each point is a cell, the first cue tuning index is plotted on the Y axis and the second cue tuning index is plotted on the X axis. We could compute the threshold at which the tuning was statistically significant at the $P<0.05$ level; that threshold is plotted for each axis as a dotted line, and regions where cells were statistically significant are shaded. The cell shown in Figure 2 is outlined in red in each figure. Figure 3A shows the tuning indices during cue presentation for single units. The cells' tuning indices are significantly correlated, as indicated by the regression line,

plotted in black ($P<10^{-14}$ using least-squares regression). In other words, cells tend to be tuned for both cues if they show visual responses. This trend continues through the delay period, as shown in figure 3B. Although tuning strength is slightly reduced, many cells carry information for both the first and second cues over the delay period. Finally, Figures 3C and 3D show that the effects seen for single units also exist for recorded multi-units, though the strongest tuning is seen in single units.

To assess whether it was possible to decode the identity of the cues from the neural activity, we first used a classifier, shown in Figure 4. We used a support vector machine to classify all twelve types of trials using the data from all single and multi-units recorded in the FEF. Each point shown gives the classifier performance, computed using leave-one-out cross-validation (see Methods), over a time window 200ms in length centered at that point. Using a classifier of this sort ensures that correlations between cells do not spuriously increase our ability to classify, as data recorded across cells and timepoints is not assumed to be independent (as e.g. a maximum-likelihood or Bayesian method would require). If the cells carry no information about the locations of the first or second cues, we would expect the classifier performance to be ~8.3% correct (1 in 12). This is what is seen over the baseline and fixation periods. Once the second cue appears, the classifier performance rises near 50% correct (1 in 2), which is expected as the identity of the first cue reduces the possible second cue choices to 2. Finally, during the second cue presentation, the classifier performs near 100%, indicating that nearly all single trials could be correctly classified based on the arrangement of both cues. A high performance rate is maintained over the delay period. The performance of the classifier shows that FEF neurons contain information for both cues throughout the delay period.

We also developed a way to explicitly decode the direction of the first and second cues based on the neural activity at a given timepoint in a trial. This technique, a sequential population vector decoding, is a slight modification of the population vector technique first used by Georgopoulos et al. (1986). It is able to recover two vector directions from a given set of neural activities. This technique is diagrammed in Figure 5. Fig. 5A shows a

standard population vector decoder. This type of decoder is applied twice in sequence, to the same data, to recover the first and second vector directions (Figure 5D). The first cue is decoded using a standard population vector decoder, and the cells' firing rates are predicted from the first vector direction. Then, the predicted rates are subtracted from the actual rates, and a second standard population vector decoder is used to recover the second cue direction from the differences. The results of this decoding are shown in Figure 6 for the first cue, and Figure 7 for the second cue. Figure 6 shows that the first cue direction can be decoded starting with the first cue presentation, and continuing throughout the delay. In Figure 7, the second cue direction begins to be reflected in the neurons beginning with the second cue presentation, and is then also maintained over the delay period. Thus, from every timepoint, it is possible to decode two quantities using this procedure: the first cue direction and the second cue direction.

Finally, in Figure 8, we address the issue of presaccadic responses in these cells. Isoda and Tanji (2003) found that neurons in the frontal eye field showed little sequence tuning during the 200ms preceding each saccade in a three-element sequence, when sequences were repeated in blocks. However, Tian et al. (2000) studied presaccadic responses of FEF neurons in a similar task to ours (trials randomly intermixed) and found that cells tended to be tuned for both of two stimuli when animals had to direct a saccade to one or the other. In addition, they found some sequence tuning (e.g. in their Figures 3 and 7). Our data shows that sequence tuning is still somewhat evident during this pre-saccadic period, but is weaker than during the visual and delay periods. An example cell showing this sequence tuning is plotted in Figure 8A-C. (This is the same cell as in Figure 2.)

The vertical solid black line in each histogram shows the time of the start of the saccade; trials are aligned on this time. The cell in Fig 8A-C is likely what Tian et al. call a "quasi-visual" cell, as it shows visual responses for both cues without a prominent presaccadic burst. It is evident that this cell shows some sequence tuning, that is, the response during the 200ms before the first saccade depends on the identity of the *second* cue. . This is seen in the fifth panel from the top; the green and orange trials share the same first cue but differ in the second cue location, and the cell's pre-saccadic response is different in the green and orange trials (i.e. when the second cue differs). Figure 8D and 8E display this effect in population scatter plots of first and second cue tuning indices using the same conventions as in Figure 3. It is also apparent that some cells develop pure motor tuning during this period: they show selectivity for a saccade in the period immediately preceding that saccade. This is seen here as cells which are selective for the first cue without selectivity for the second. These cells fall to the left of the vertical threshold line and lie in the pink region of Figures 8D and 8E.

Discussion

These results show that neurons in the frontal eye field display visual sequence selectivity in a working memory task. FEF cells respond selectively to both cues presented in a dual spatial memory task and maintain this selectivity over a delay period. We see strong sequence tuning over the visual and delay periods, and weaker sequence tuning immediately preceding the first saccade. We have also demonstrated that it is possible to decode the locations of the two cues given the memory activity of a population of recorded FEF cells. This implicates the FEF in the visual storage of multiple spatial locations and their sequence, when this memory is used to plan and guide a sequential movement.

The persistent activity that we observed encodes both spatial cues and their sequence, which makes frontal cortex unique. In the parietal lobe, the lateral intraparietal area (LIP) is heavily interconnected with the FEF and is known to be involved in oculomotor delayed response tasks (Gnadt and Andersen, 1988, Quintana and Fuster, 1999). In fact, the responses observed in a spatial delayed response task with a single cue are very similar LIP and FEF are directly compared in the same animal (Chafee and Goldman-Rakic, 1998). Mazzoni et al. (1996) used a similar task to this one, a spatial delayed response task with two cues, and recorded neurons in LIP. They concluded that delay activity in LIP encodes only the next movement the animal is going to make. In temporal cortex, persistent memory activity is also observed when images are remembered in a delayed match to sample task (Miller and Desimone, 1995), but this activity is abolished

by the presentation of a second object, even when it is not relevant to the task the animal is performing. Thus, a unique property of persistent activity in the frontal eye fields is the ability to encode multiple locations simultaneously.

The frontal eye fields are immediately rostral to what is regarded as prefrontal cortex (PFC), Brodmann's areas 9/46 and 12 in the macaque. Prefrontal cortex proper also seems to be able to encode multiple items in delay activity. Ninokura et al. (2003) have shown visual-sequence selectivity in a delayed-match-to-sample task, and Barone and Joseph (1989) have seen sequence selectivity during a delay in a reaching task. This supports the conclusion that delay activity in these two frontal areas can encode multiple memories, while other (parietal and temporal) brain areas seem to only encode a single item in their delay activity.

A recent modeling study (Mitchell and Zipser 2003) proposes that the FEF may encode multiple targets and makes predictions about the type of cell responses that should be observed. Our results confirm their hypothesis --- that the FEF does indeed store the memory of multiple spatial locations --- but argues against the details of their model. Notably, their memory output neurons are always narrowly tuned for a particular location in space and respond to only a single target. Multiple targets are encoded by having multiple sets of memory output neurons active. We rarely found cells that were selective for only the second cue without encoding the first cue. (Cells that encoded the first cue without encoding the second were found; they might also have been encoding the first saccade). The neurons we recorded encoded multiple items *simultaneously* in their firing

rate. That is, the activity of a single neuron multiplexed information about both cues. From an information point of view, these two types of coding are equivalent: we could perform decoding of both cues regardless of whether distinct populations encode each cue or whether single neurons carry information for both cues. Our results suggest that an explicit memory output for both cues is not found in the frontal eye fields; information for both cues is stored in activity across the population.

On their face, our results, which show sequence selectivity in FEF, appear to be at odds with the findings of Isoda and Tanji (2003), who found little sequence modulation there. However, it appears that the two experiments examine different aspects of sequential behavior. Our work has focused on the memory delay period, after the cue presentations, while the animal is fixating and before the first saccade has occurred. Isoda and Tanji analyzed the 200ms period before the saccades occurred. Thus, the FEF may carry sequence information during the memory, or motor planning, period, while it carries less sequence information during the saccade execution. However, there may be an additional difference between our two experiments. Isoda and Tanji used a task where sequences were presented in blocks, and they observed little delay activity. In fact, they report that on the first trial of a block, delay activity is observed, but as trials are repeated less and less persistent activity is seen during the delay. In this work, different sequences were randomly intermixed on every trial, and we see robust delay activity in many cells. This suggests an interesting possibility for the function of persistent activity. When the cues to be remembered change after every response, persistent activity is used to encode their locations, as we observed. However, when the same pattern must be remembered over

several trials, a temporal interval on the order of minutes, and is used for several responses, the memory may *not* be encoded in persistent activity, as seen by Isoda and Tanji. One possibility is that the connections between neurons in the network change over time to encode the sequential response, so persistent firing is no longer needed.

One role of the FEF may be to serve as a spatial memory buffer which is used to guide eye movements, storing multiple retinotopic locations and remapping these retinotopic fields after an eye movement. Our data shows that neurons can encode multiple remembered locations simultaneously. Vector saccades elicited by stimulation (Robinson and Fuchs, 1969) and physiology results (Bruce and Goldberg, 1985; Umeno and Goldberg 1997; Tian et al 2000), provide evidence that the FEF uses a retinotopic code. Recent work (Sommer and Wurtz, 2002; Sommer and Wurtz, 2004) suggests that these retinotopic fields are remapped by eye movements using a motor feedback signal coming from the colliculus. After lesion of the FEF (Schiller and Chou, 1998) or inactivation of the colliculus (Sommer and Wurtz, 2002), animals have difficulty executing motor sequences that require updating of eye position after the eyes are moved.

Here we use two different decoding methods to recover sequence information over the delay period: a classifier which measures discriminability at each timepoint, and a population vector decoder which, when trained with the second cue *visual* responses, recovers the two cue directions during the *delay* period. Essentially, the former method shows that there is sequence tuning during the delay period and the latter method shows that the delay period sequence tuning is similar to that during the second cue period.

Although these methods demonstrate one way that this information can be recovered from neural activity, they are not meant to imitate the way in which the brain might read out this information from neurons in the FEF. Various methods have been proposed for how population codes are read out and used for computation in the brain (Salinas and Abbott, 1994; Zemel et al., 1998), and there has been recent interest in how population codes deal with multiplicity or sequence (Sahani et al., 2003). These two lines of research --- experimental studies on sequence and theoretical work on sequence encoding --- have the potential to cooperatively explain not only how sequence is encoded, but why it is encoded in that way, and how the brain "reads the code".

Materials and Methods

Physiological and experimental methods

We trained two rhesus macaques (*Macaca mulatta*) to perform the task: monkey A (15kg, male) and monkey B (5kg, female). All animal procedures conformed to NIH guidelines and those of the MIT Institutional Animal Care and Use Committee. Animals were surgically implanted with a titanium head-holding device and a recording chamber placed stereotaxically over the medial frontal lobe, secured to the skull with cortical bone screws (Synthes, Inc.). A craniotomy was done inside the recording chamber, and tungsten epoxy-coated electrodes (FHC, Bowdoinham, ME) were inserted transdurally to record neural activity (electrode impedance post-stimulation >= 100kOhm at 1kHz). We recorded spike activity and LFP data (not examined in this report) using equipment from Plexon (Dallas, TX). Surgeries were conducted under isoflurane anesthesia and animals were given analgesics as part of post-operative care. Animals received all their liquid intake in the laboratory during training and experimental periods; they were rewarded with drops of apple juice or water. To reduce biases in our measurement of neural activity, we made no effort to select neurons based on their responses to the task. We lowered the electrodes, in parallel, into the cortex until a well-isolated cell was found and then began data collection.

We used microstimulation and anatomical MRI to identify the location of the FEF. In monkey A we recorded from left FEF, and in animal B from right FEF. On most electrode penetrations, at the conclusion of recording, we applied stimulation to each

recording site to elicit saccades. We used pulse trains of 150-250ms, consisting of biphasic square cathodal-first current pulses, each phase 200μS in duration, at 250Hz. Any site where vector saccades could be elicited at thresholds ≤50μA was considered a FEF site; when long pulse trains were used, saccade staircases could be elicited at FEF locations. The FEF was found on the anterior bank of the arcuate sulcus as verified with structural pre-surgical MRI. Data from electrode penetrations rostral to this were classified as part of prefrontal cortex and not included in this report.

Behavioral methods

Animals sat in a primate chair facing a computer monitor, 34cm away, on which stimuli were presented. An LCD monitor (NEC LCD1850E) was used to avoid flicker associated with CRTs. Pixel rise time to half-maximum intensity was 6.1±0.4ms, mean±standard deviation. All visual stimuli (fixation points and cue stimuli) were round white spots. The fixation spot (0.3° diameter) was presented at a central position and cue stimuli were displayed in the periphery; each was the same distance from the fixation point. Two cue stimuli, always adjacent to one another, were presented on any given trial. Trials analyzed here were randomly intermixed with other trials where the fixation point appeared in the periphery; data from these peripheral fixation trials are not reported here.

Stimuli were presented and animals' responses monitored with Cortex (NIH, Salk Institute: http://www.cortex.salk.edu). Eye position was monitored with the Eyelink II system (SR Research Inc., Mississauga, Ontario, Canada). The position of the left eye was sampled digitally by the eye tracker at 500Hz, recorded as an analog signal at 2kHz,

smoothed with a 2.5ms moving-average (uniform) filter, and downsampled to 500Hz for off-line analysis. The time of occurrence of visual stimuli was recorded with a photodiode and amplifier. All digital and analog signals were recorded using a Plexon system (Dallas, TX, USA).

Data analysis

Tuning indices for the first and second cue were constructed from non-parametric statistical tests; we used these because they capture tuning defined in a signal-to-noise sense (consistent with Shannon information measures), and their distributional properties have been well-studied. We first counted the number of spikes in the desired time window. For the first and second cue visual tuning indices, the window was the first cue and second cue presentation, respectively (500ms duration in each case). For the delay tuning indices, we used a window of the same 500ms duration in the middle of the delay: 250ms to 750ms from the start of the delay. For the motor indices, we used a 200ms window before the start of each saccade, following Isoda and Tanji (2003). The rank test with the greatest power for detecting cue 1 selectivity was the Kruskal-Wallis test. The Kruskal-Wallis test statistic, W, measures the difference in medians between the responses for each cue direction, normalized by a noise or spread (Zar, 1999). To measure second cue tuning while ingnoring the effect of the first cue, we used a set of 6 Wilcoxon rank-sum tests to measure the difference in rate when the first cue was the same but the second cue differed. There were 12 combinations of first and second cue; we divided them into six groups, each sharing the same first cue. We then computed the Wilcoxon test statistic (Z; see Zar, 1999) between the two groups of trials where only the

second cue was different. This resulted in 6 test statistics; we took the most significant, the maximum. For calculation of statistical significance, we applied the Bonferroni correction for multiple comparisons. Under the null hypothesis of no tuning, the Wilcoxon Z is distributed as a standard normal random variate, and the Kruskal-Wallis W as a chi-squared, or the square of a standard normal. Thus, to make the units of the indices more comparable, the first cue tuning index was defined as the square root of W, and the second cue tuning index was defined as the absolute value of the maximum W (over the 6 first cues). Note that the power of the first cue test was greater than that of the second cue test and so we were able to discern first cue tuning with greater sensitivity than second cue tuning.

We performed classification using a support vector machine, using the libSVM software (http://www.csie.ntu.edu.tw/~cjlin/libsvm/, Chang and Lin, 2001). We used the C-SVC SVM type, a radial basis function projection kernel, and leave-one-out cross-validation to produce probabilities of correct classification. We used all recorded single and multi-units, computed their spike counts over a 200ms time window centered at the desired time, and normalized the counts with an Anscombe transformation, which tends to reduce the change in variance with mean rate of a count random variable (Anscombe, 1948; Agresti, 2002). Each cell's activity was a variable, and there were 12 different groups: the different configurations of first and second cue. When there was a missing value (<0.05% of trials), due to too few correct trials on that configuration on the corresponding experimental day, we imputed the value by taking the mean rate over all trials that day.

To recover the locations of the first and second cues in a way that better generalizes to continuous cue locations, we used population vector decoding. As above, we used all recorded single and multiunits, counted spikes in a 200ms window, and normalized the counts using the Anscombe transformation. Below, we call the transformed counts the cells' "activities". For each trial, there was a direction value for the first cue and a direction value for the second cue, and we used each value sequentially, both for computing tuning properties over the second cue period and for decoding. To decode, we found the population vector for the first cue at each timepoint, by taking each cell's activity, subtracting the mean second-cue period rate, and multiplying the scalar result by by the cell's tuning vector. We then added all the cell tuning vectors to produce the first cue population vector. We found the nearest true first cue vector, and used that to predict the firing rate at the same timepoint. This was done by reading off the amplitude of the first-cue tuning curve at that first cue direction. For each cell, this value was subtracted from the true activity at the same time point. Then, the second cue was decoded in the same way, using second cue tuning parameters, also calculated over the second cue period. To balance contraversive tuning biases, we duplicated each neuron into a second neuron with the same tuning strength but direction mirrored over the vertical axis. In figures 6 and 7, we display the population decoding output by adding together the output of all six first cue directions after rotating them to point in the 0° direction. Also, we debiased each decoded output by using a bootstrapping procedure to compute bias at each timepoint. To compute tuning properties, we used a 500ms window, covering the entire 2nd cue presentation period, and fit cosine curves to the activities for the first cue (regardless of the direction of the second cue). Then, we subtracted the activities from

the fitted curve values at the first cue directions, and fit cosines to the differences. In the two fitting steps, we produced two sets of parameters for each cell: mean activities and cell tuning vectors for both the first and second cue.

Figures

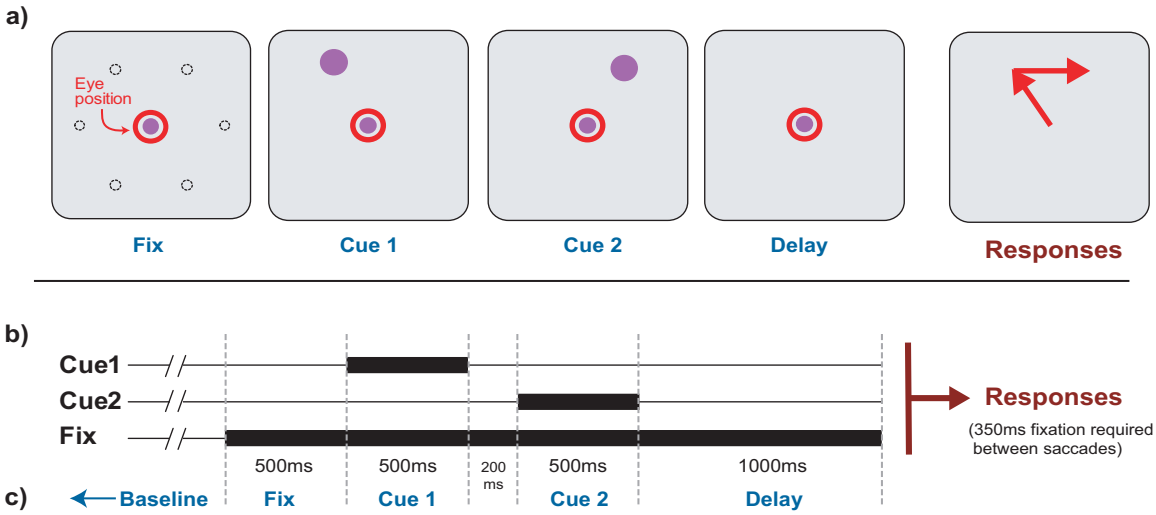

Figure 1: Task

The task performed by the animals is represented in (**A**). First, a central fixation point was illuminated and the monkey was required to move its eyes to this location and hold fixation there. The red circle signifies the position of the eyes, and small dotted circles represent possible cue locations. Second, a cue was illuminated briefly at one of the six possible locations (Cue 1 period), followed by a second cue (Cue 2 period). A one-second delay ensued, and its end was signified by the offset of the fixation point. Animals were then required to saccade to the remembered locations of the cues in the order in which they appeared. The timecourse of the task is shown in (**B**). A baseline period of 2-2.5sec preceded each trial, followed by the fixation period which lasted 500ms. Each cue was presented for 500ms, with a 200ms fixation period interposed between them. After the delay, animals saccaded to each remembered location; they were required to pause at the endpoint of the first saccade for 350ms.

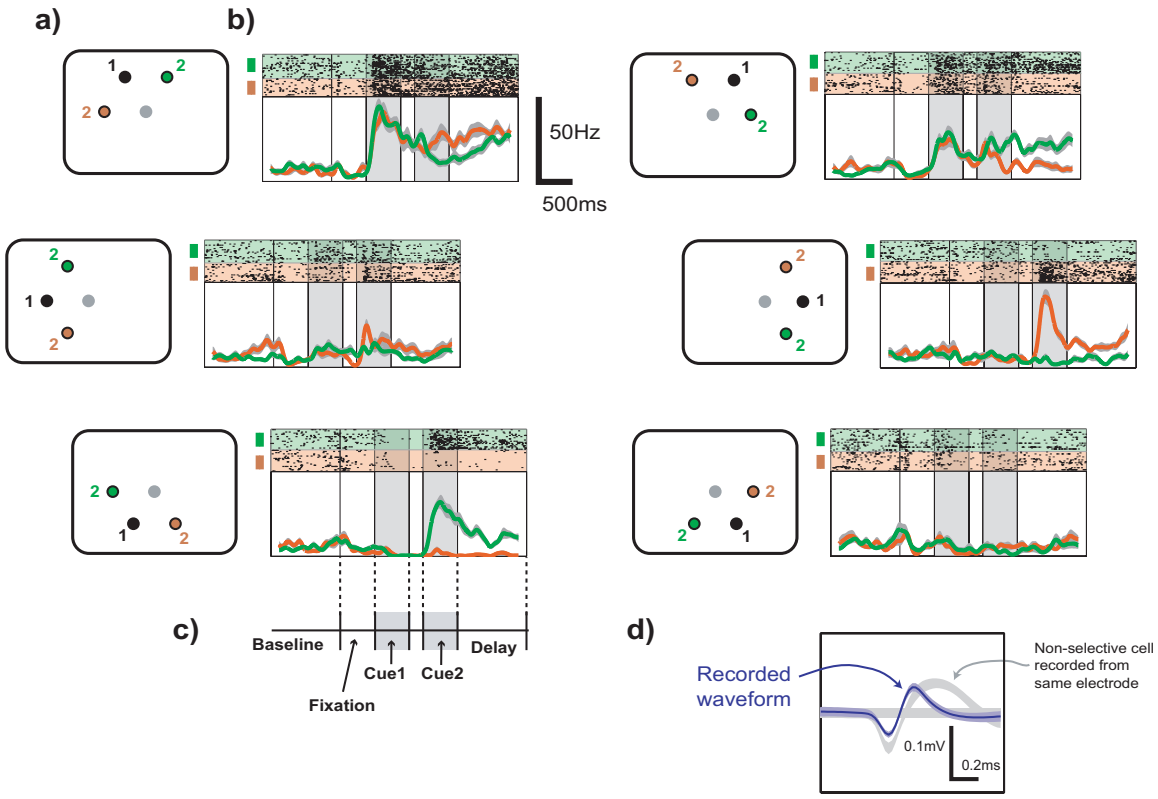

Figure 2: single cells are modulated by both cues

An example of a cell recorded from the FEF is shown here. Each of the six rows represents two different cue arrangements, each sharing the same first cue and differing in the second cue location. The orange and green colors label the two different second cue locations and their corresponding rasters and histograms. The cue arrangements are shown schematically in (**A**). In the top row, for example, the first cue always occurs at 0 degrees (3 o'clock), while the second cue appears at 60 degrees (orange) and -60 degrees (green). The cell's responses are shown in (**B**). For each row, the plots in (**B**) show rasters (upper) and their corresponding histograms (lower) for the two sets of cues indicated in (**A**). Time in trial is shown in **(C),** corresponding to the task intervals in Figure 1. (**D**) shows this cell's spike waveform. The purple region gives the mean waveform ± one standard deviation. The horizontal gray bar gives the mean noise level ±

1 s.d., and a cell with a higher-amplitude waveform recorded from the same electrode is plotted in gray (mean ± 1 s.d.). The cell plotted in gray was non-responsive in this task.

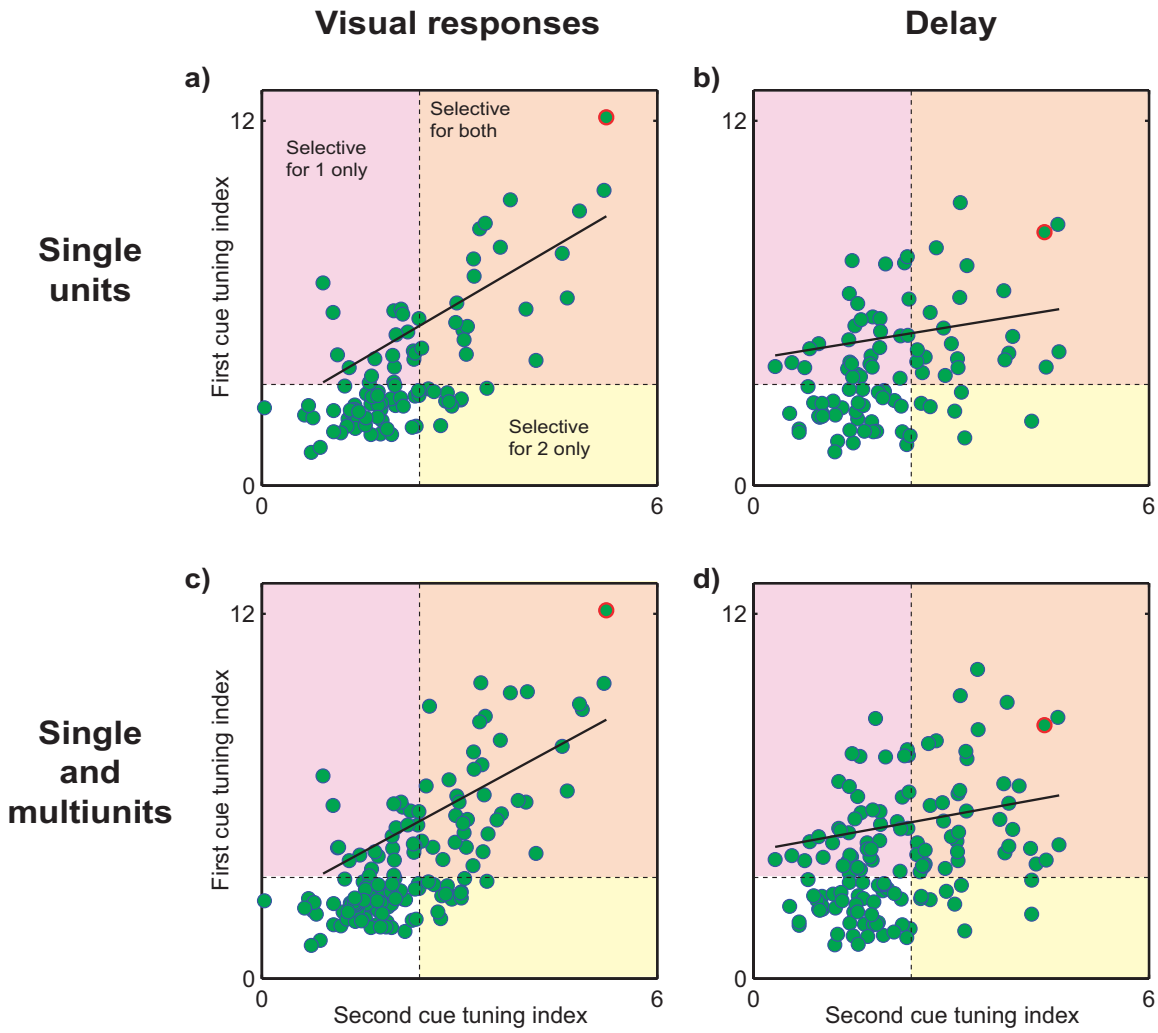

Figure 3: The population of recorded cells is tuned for both cues

This plot shows that single units that give tuned visual responses for the first cue are also usually tuned for the second. Each point is a cell, the first cue tuning index (computed during the Cue 1 period) is plotted on the Y axis and the second cue index (computed during the Cue 2 period) is plotted on the X axis. The solid black line shows a robust regression fit; a robust regression was used to decrease the influence of outliers (see Methods). Regression R^2: 0.61, significant at $p < 10^{-7}$.

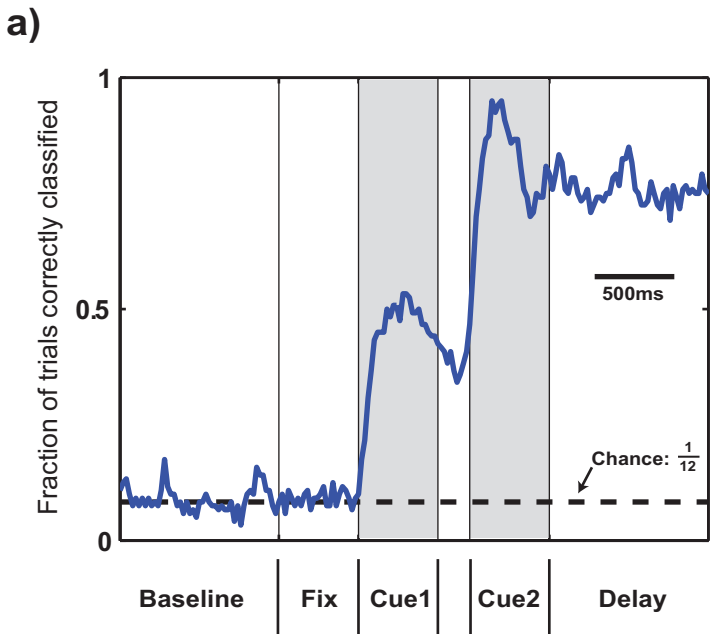

Figure 4: A classifier can be used to recover the first and second cue locations

This plot shows the performance of a classifier used to decode the type of trial given only neural responses in a given time window. There are 12 different types of trials comprising the different locations of the first and second cues. The X axis gives time in trial, with the trial epochs indicated below the plot. Gray shaded regions indicate the times that the first and second cue were displayed. The Y axis gives the probability of correct decoding of the trial number by a support vector machine classifier, computed using leave-one-out cross-validation (see Methods). The input data to the classifier was the activity of all recorded single and multiunits over a 200ms time window centered at the plotted X value. Black dashed line: chance performance of the classifier (what would be expected if the cells' responses gave no information about the trial type. Solid blue line: actual classifier performance. During the baseline and fixation period, the classifier performed at chance (i.e., there was no information in the neurons). During the first cue

presentation, the classifier performed at approximately 50% correct, the upper bound given that knowledge of the first cue restricted the possible second cue to one of two positions (60° clockwise or 60° counterclockwise of the first cue). During the second cue presentation, classifier performance peaked around 95% and stayed constant at around 75% correct throughout the delay period.

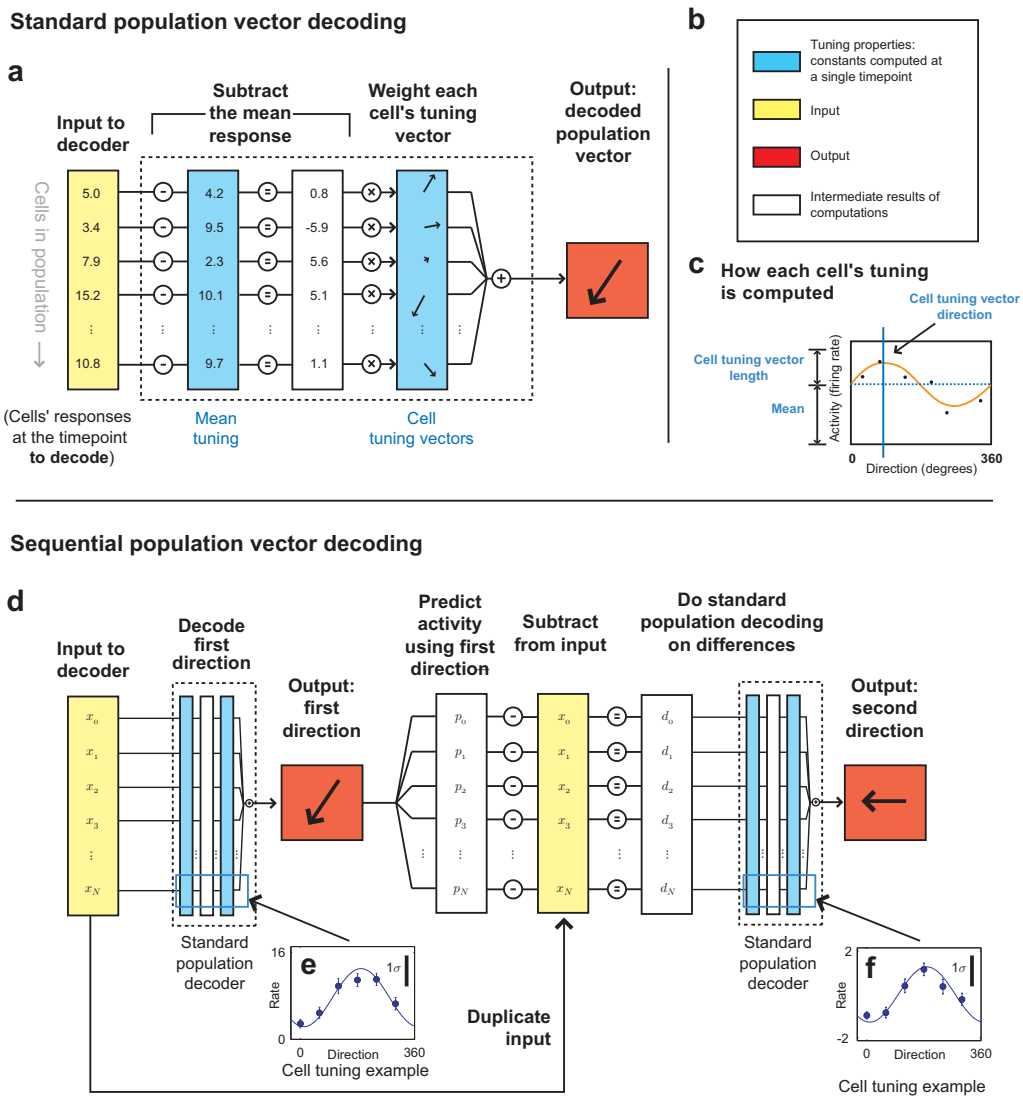

Figure 5: Sequential population vector decoding procedure

This figure shows a schematic of the sequential population vector decoding we used to recover both cues during the delay period. A standard population vector decoder is

shown in (**A**). Input data (yellow background) is used to weight each cell's tuning vector, and these cell vectors are summed to produce an output population vector (red). The cell tuning vectors are computed by fitting cosine curves to the cells' responses, shown schematically in (**C**). Instead of using this method, we extended it to allow decoding of two quantities (see Methods). This sequential population vector decoding is shown in part (**D**). Cells' responses at a given timepoint are used as input to a standard population vector decoder, which gives the first cue direction as its output. This direction is then used to predict the cells' responses to the first cue. The input data is used again; the predicted responses are subtracted from the actual responses to produce a set of differences. These differences are provided as input to another standard population vector decoded, which outputs the second cue direction. Cells' responses during the second cue presentation were used to construct both decoders shown in (**D**). An example of cell responses used to compute cell tuning vectors is shown in (**E**) and (**F**). These two cosine curves were computed from the responses of the cell in Figure 2.

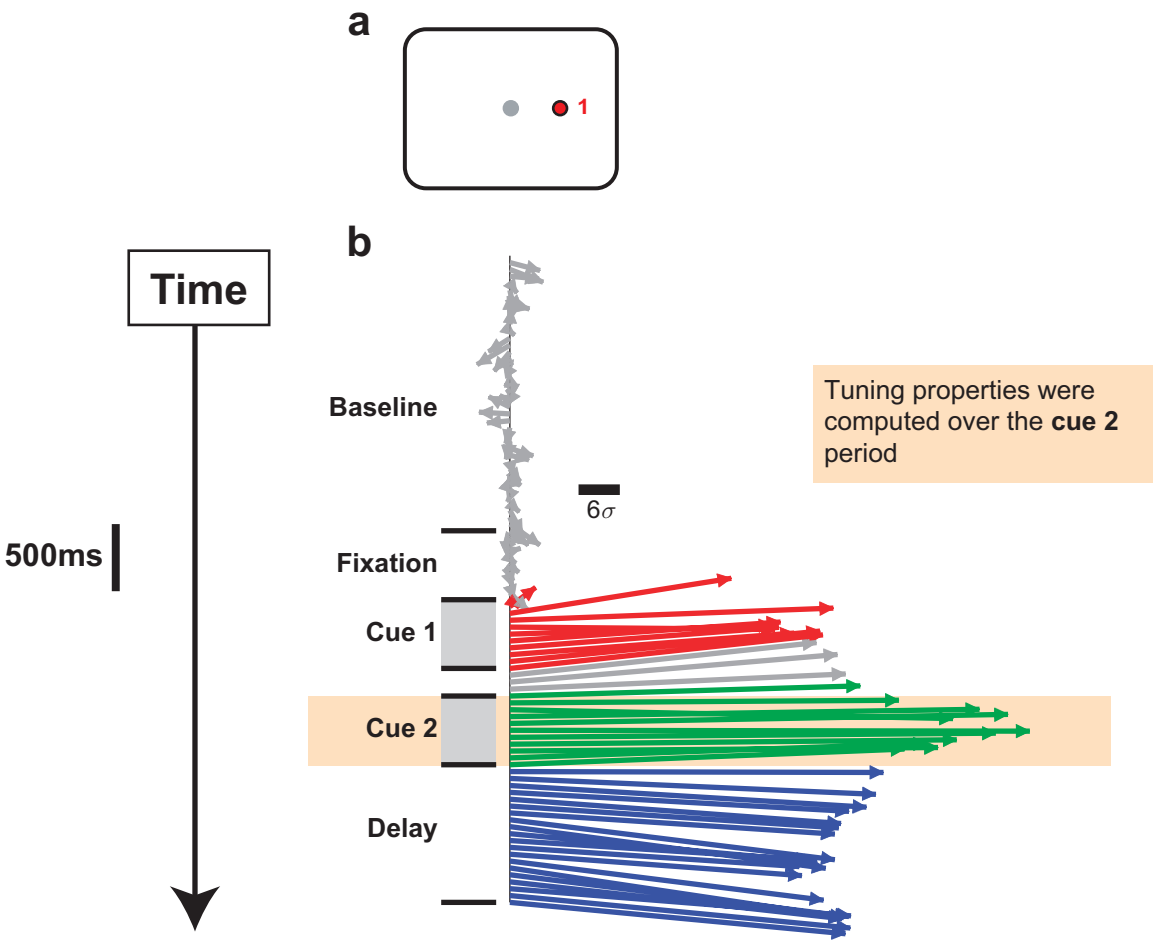

Figure 6: The first cue can be decoded during the delay

This figure shows the first cue output from the sequential decoder (diagrammed in Fig. 5D). The first cue actual location is shown in (**A**). See Methods for more detail. (**B**) shows the decoded output, drawn a set of vectors. Time in trial increases down the page. The base point (origin) of each vector is the center of a 150ms window in which the decoding was performed. Red arrows: first cue presentation. Green arrows: second cue presentation. Blue arrows: delay period. During the delay period, the output of the decoder accurately reflects the first cue direction.

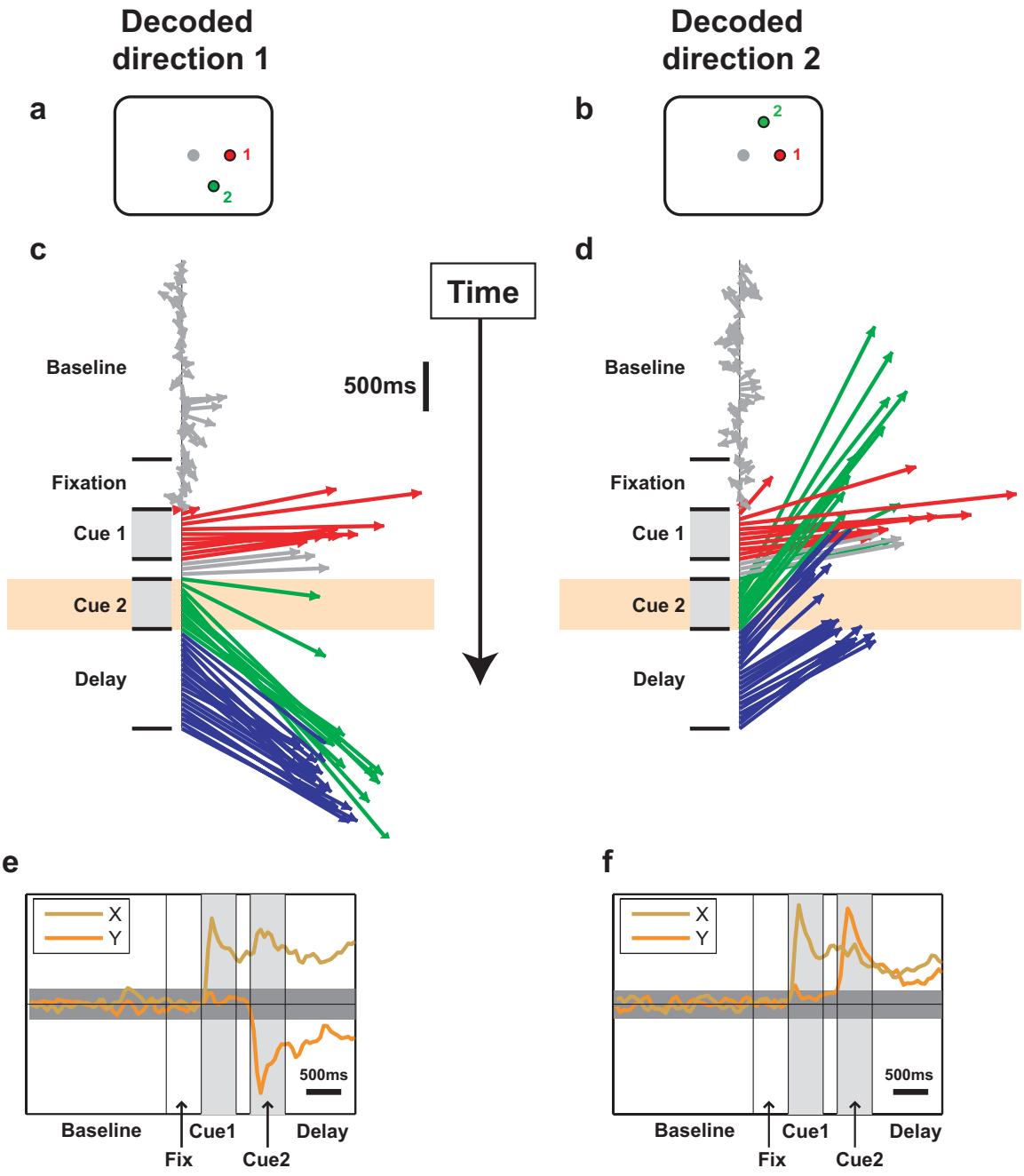

Figure 7: The second cue can be decoded during the delay

This shows the second cue output from the sequential decoder (diagrammed in Fig. 5D). The two columns each show a different second cue direction. The locations of the cues for each column are shown in (A) and (B). In both cases, the first cue is at 0° (3 o'clock).

In the left column, the second cue is at -60° (below the first cue), and in the right column, the second cue is at +60° (above the first cue). The output of the decoder in each of the two cases is shown in (C) and (D). Conventions are the same as in Figure 7. The X and Y components of the vectors plotted in (C) and (D) are shown in (E) and (F). Here, time is plotted on the X axis and the decoded vector components are plotted on the Y axis, in arbitrary units. The gray shaded region measures the noise; it is a ±3 std. dev. bound around the mean during the baseline period. This sequential vector decoding procedure, using tuning properties computed during a visual presentation, can recover the 2nd vector direction during the delay period.

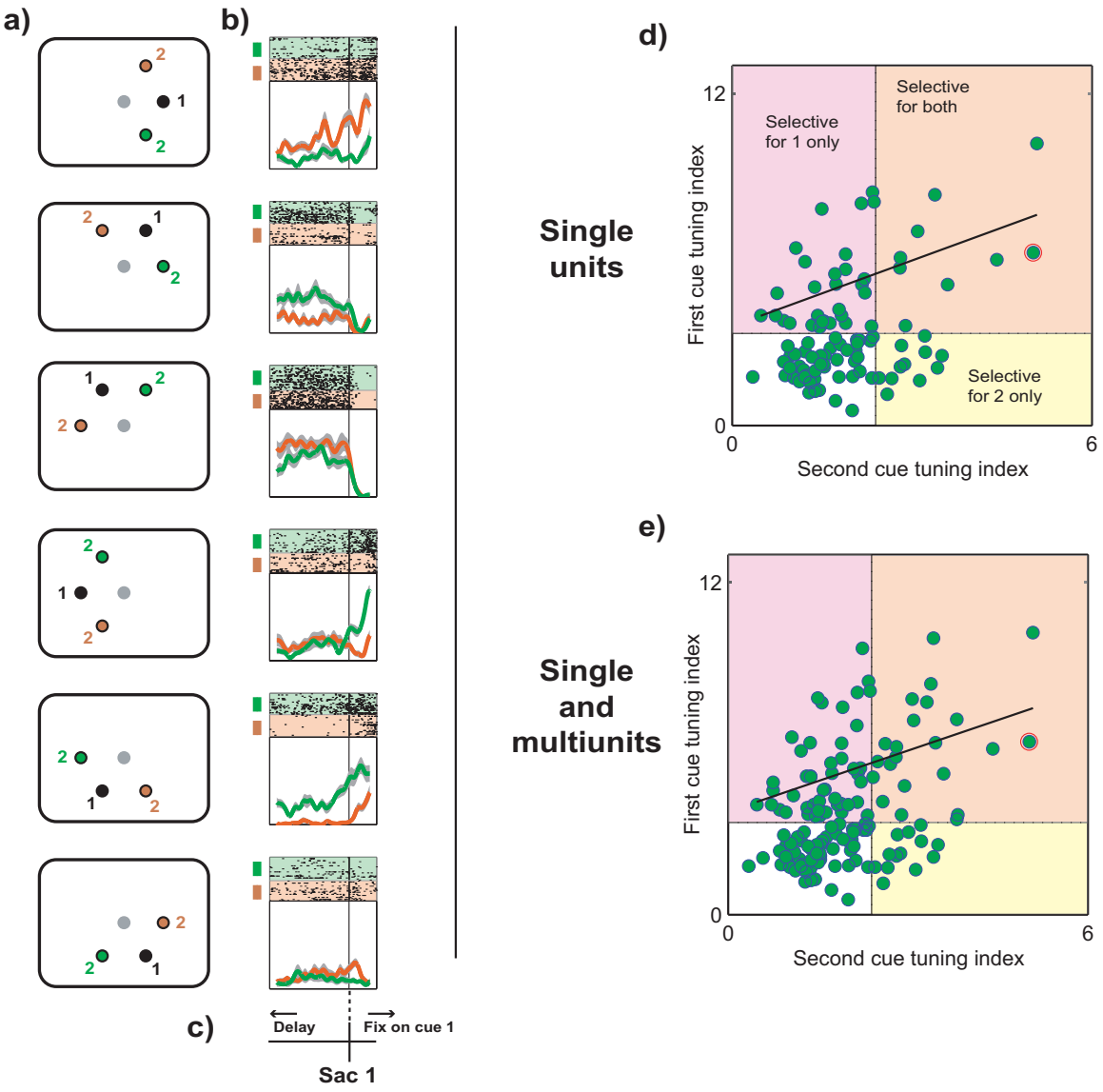

Figure 8: motor responses show weaker sequence tuning

During the interval just prior to the first saccade, cells show some tuning for the second saccade (sequence tuning), but it is weaker than during the visual period. Schematics of the cue configurations are shown in (A) and corresponding rasters and histograms are shown in (B). Conventions are the same as in Figure 2. The time periods are labeled in (C); the black vertical line shows the time of the start of the saccade to the first target. Before this the delay period and the offset of the fixation point occur, after the saccade

the animal is fixating the first cue location. There is tuning for both the first and second cues here. Comparing the third panel from the top (first cue up and left) to the 6th panel from the top (first cue down and right) shows first cue tuning. Comparing the green and orange trials (differing second cue) in panel 5 shows that there is also second cue tuning. This is summarized across the population by the scatterplots in panels (D) and (E). The tuning indices were computed in a 200ms period just prior to the start of the first saccade. Conventions are the same as in Figure 3. Sequence cells lie in the orange region in the upper-right quadrant of the plot, and comparing to Figure 3 shows that sequence tuning is slightly weaker here. Also, an increase in motor tuning is seen by the increase in the number of cells that are selective for only the first cue at the time of the first saccade (pink region).

Chapter 4:
Discussion

Summary of results: SEF stimulation affects order but not location, while FEF cells code the memory of multiple locations

In the experiments described in Chapters 2 and 3, we have examined the role of two frontal lobe oculomotor areas in sequential behavior when the sequences are planned from working memory.

First, we have demonstrated that subthreshold microstimulation of the SEF during a memory delay period can bias the order in which animals respond to two sequentially-cued spatial locations. While stimulation appears to change the remembered order of the locations, it has little effect on the locations themselves or the metrics or dynamics of

saccades to the targets. Furthermore, the SEF appears to organize sequences by specifying their endpoint.

Second, we found that neurons in the frontal eye fields of monkeys can encode several spatial locations and their order over a delay period. This distinguishes the persistent activity seen in the frontal eye fields from that seen in other areas of the brain, including parietal cortex (LIP), studied in an extremely similar task, and the temporal cortex.

Relation to prior work: how the FEF and SEF may interact in sequential planning

As discussed in Chapter 1, both the FEF and SEF have been implicated in sequence planning, using a variety of experimental approaches. Our results, taken together with these prior studies, suggest the following hypothesis about sequential planning from working memory:

> While FEF acts as a spatial memory buffer, storing retinotopic locations over brief delays and updating after eye movements, the SEF influences the sequential order of the responses, specifying entire sequences by their endpoint.

First, we review the evidence for FEF serving as a primary memory buffer for spatial locations. A form of this idea has been proposed before, in a computational model of the FEF (Mitchell and Zipser, 2003), but as discussed in Chapter 3, there are some differences between our experimental data and their proposals. It is first necessary to discuss the coding scheme used in the FEF for memory: FEF contains neurons which

appear to encode spatial locations retinotopically. If the animal is remembering a target at a given retinal location, and that location is moved into the receptive field of a cell by a saccade, the cell fires as if the receptive field location had been cued with no eye movement. In some sense, the receptive field goes to the remembered location of the target. This has been reported by Goldberg and Bruce (1990), Umeno and Goldberg (1997, 2001) and Tian et al. (2000). Tian et al. called these neurons "quasi-visual" cells after Mays and Sparks (1980). These results are strong evidence that the FEF uses a retinotopic code for memories, and work by Sommer and Wurtz (2002, 2004a, 2004b) confirms this, suggesting that the signal for updating comes from the superior colliculus via mediodorsal thalamus. Their work also suggests that visual signals in the FEF arrive with short latencies from visual areas, as visual information comes from the colliculus with latencies too long to explain FEF visual tuning (Sommer and Wurtz, 2004a). In summary, the FEF receives visual signals with short latencies, maintains them over delay periods, and updates them over eye movements.

Our results distinguish the memory properties of the FEF from those seen in the parietal and temporal cortices. Mazzoni et al. (1999), examined activity of neurons in LIP, a principal parietal eye movement area, using a delayed double-step task very similar to the one we used. They concluded that delay activity in this area represented only the upcoming single movement the animal was going to perform. That is, delay activity in LIP does not encode both locations or both movements simultaneously. Delay activity is also seen in inferotemporal (IT) cortex, an temporal lobe area that has shown to be important for high-level visual object recognition. Here, Miller and Desimone (1994)

found that when objects are presented in a sequence, IT cells show delay activity reflecting the most recently presented object. This activity is abolished by the presentation of any additional objects, even if they are not relevant to the task the animal is performing. The same group contrasted IT cortex with prefrontal cortex, where delay activity is not modified by the presentation of irrelevant objects (Miller et al, 1996). Our results show another way that delay activity in the frontal cortex, specifically the FEF, is different than that found in other brain regions: it can encode multiple memories simultaneously.

In the SEF, we observed that subthreshold stimulation could have an effect on sequential order and had little effect on location. Taking our FEF and SEF results together with prior work, we can speculate on the role of both areas in sequential movement planning. It is likely that the FEF is mainly responsible for the maintenance of spatial targets while the SEF can specify the order of a sequential movement. This is consistent with the prior lesion and inactivation work showing major sequence deficits in the FEF in memory-guided tasks only. Since in a double-step saccade task, the memory of one or both locations is required to successfully execute the sequence, sequential saccade performance will be strongly affected if spatial working memory ability is impaired. FEF lesions or inactivation may, in addition, affect the ability of the FEF to remap memory locations after saccades. Both possibilities are consistent with the proposed role of the FEF as a multiple spatial memory buffer.

Task issues: different mechanisms for memory and execution?

Our results also complement the prior physiological results on sequencing (Lu et al 2002, Isoda and Tanji, 2003) in the FEF and SEF. Both studies used a variant of skilled motor task where animals repeated sequences in blocks. In the Isoda et al. task, little delay activity was seen in either the FEF or SEF, even though animals performed the task from memory. In contrast, at the time of saccade execution, SEF neurons showed sequence selectivity while FEF neurons did not. This further argues that our FEF sequence-selective delay activity reflects a visual memory signal. Isoda and Tanji also report that on the first one or two trials of a block, some delay activity was observed, but as trials were repeated, the delay activity was attenuated. It could be the case that longer-term spatial memories are stored in the strength of connections between cells while short-term "working" memories are stored in persistent activity. It would be interesting to further study the connection between these different types of memory.

One other issue is why we produced strong sequence effects in the SEF with subthreshold stimulation while prior lesion and inactivation studies observed only weak sequence effects. We believe that this is due to two reasons: the frequency of errors in the task and the sensitivity of curve shifts to stimulation. First, the design of our task is such that behavioral correction of the effects of stimulation is difficult. Animals made sequence errors on 15-20% of trials without stimulation due to the varying difficulty of the task at different SOAs. Even the largest stimulation effects rarely increased this overall error percentage above 25-30%. Thus, since ordering errors were made frequently even in the absence of stimulation, a small increase in their rate due to stimulation was difficult for

the animal to discern. From trial to trial, it was nearly impossible for the animal to discern which errors were due to stimulation and which due to small SOAs, to allow for efficient error correction. Tehovnik el al. (1999) have shown that behavioral influences can have a large effect on the results of stimulation in SEF. Also, we observed anecdotally that when stimulation was applied during a version of the task using only large SOAs, only a few sequence errors resulted before stimulation ceased being effective, presumably due to the animal's ability to suppress the effects of stimulation. The second likely reason that we were able to induce large effects is that our task was sensitive to changes in behavior. By constructing psychophysical curves and looking for shifts, we could detect even small effects of stimulation. Since we performed the experiments at several locations over the course of a day, our discrimination power was not as high as e.g. that of Salzman et. al (1990), but we could reliably detect curve shifts of about 50ms (see Fig. 4C-D, Chapter 2).

Codes for sequence in the SEF and elsewhere

There are similarities between the role of SEF in the oculomotor system and premotor areas in the skeletal movement system. SEF occupies a position on the dorsolateral surface of the frontal lobe just rostral to skeletal premotor and supplementary motor areas. Skeletal premotor areas, defined as those that project to primary motor cortex (M1) (Dum and Strick, 2002), have been shown, through physiology, to be involved in sequential movements (Tanji, 2001), and also to code for the endpoint of certain movements. Similarly, the SEF has been shown to have a functional projection to the eye movement centers in the brain stem (Shook et al., 1990). Stimulation of premotor cortex

at relatively low currents can evoke arm movements that are convergent --- they share a single endpoint position or posture regardless of starting position. (Graziano et al. 2002). Our work provides evidence that SEF may specify a movement goal by reordering individual movement components. We suggest that one key function for SEF and premotor cortex is that they encode the goals of movements.

However, we are not suggesting that goal-directed movement coding is the sole role of the SEF. SEF neurons have been shown to be modulated in many different tasks, and various investigators have studied the role of the SEF in e.g. object-centered coding (Olson, 2003), selection of desired action (Amador and Fried, 2004), and reward prediction or error correction (Stuphorn et al. 2000). Why then, if SEF has other roles as well, might stimulation have such a profound effect on sequential ordering? The electrical stimulation we used passively spreads through the area at the tip of the electrode, and likely activates a large number of neurons; at 50μA high and low-threshold units are activated over an estimated radius of, respectively, 0.1-0.5mm (Stoney et al. 1968, reviewed by Tehovnik, 1996). In addition, it is probable that there is additional active propagation due to activation of local circuits through lateral interconnections (Tolias and Logothetis, submitted). Thus, our stimulation may selectively trigger a subset of local circuits (possibly those relying on lateral connections), which when activated specify the endpoint of a planned movement.

Our conclusion based on the pattern of biases observed as a result of SEF stimulation was that the stimulation was affecting the endpoint of the movement. There were two other

possibilities that our data did not support. First, *a priori,* it is possible that stimulation might inhibit the first movement rather than having a facilitatory affect on the endpoint. That is, instead of biasing the endpoint contraversively, stimulation inhibited the performance of the first saccade in the contraversive direction. If this were the case, we would not have seen the convergent pattern of endpoints that we observed. Also, previous investigators have reported that some neurons show inhibitory pre-saccadic responses, but they occur with low (<15%) frequency (Schlag and Schlag-Rey, 1987), in contrast to what we observed where the vast majority of biases were in the endpoint-contraversive direction. Furthermore, Tehovnik and Schiller (2001) have observed that stimulation of the SEF during a visual target selection task biases animals to make single saccades in the direction of the saccade elicited by suprathreshold stimulation, that is, contraversively. Thus, because sub-threshold SEF stimulation often produces contraversive single saccades, we think it is likely that the effect we observed is a facilitatory effect, making saccades with contraversive endpoints more likely. The second explanation we rejected as insufficient is that SEF stimulation influences the second saccade rather than the endpoint of the sequence. Due to the fact that we used only two cues, the endpoint and the vector of the second saccade are correlated. However, we think it unlikely that SEF stimulation would affect the second or last movement in a sequence rather than the endpoint. First, if SEF stimulation had an effect on only the second saccade, we would expect the direction of the first saccade to be unchanged by stimulation, leading to selection of incorrect targets by the second saccade. This was not observed; stimulation affected the order in which animals chose the targets but not which targets were chosen. Second, if SEF stimulation was indeed affecting one component

movement --- in this case, the second component --- it is likely that simulation would also sometimes affect the first movement, and we would expect a more evenly distributed direction of shift than we observed. In fact, neurons recorded during the performance of saccade sequences seem to be selective for all components of a sequence; they do not strongly favor the final movement. Finally, the fundamentally convergent or goal-directed nature of single saccades that can be elicited from many sites in the SEF (Schlag and Schlag-Rey, 1987) supports our notion that sequences of saccades are also coded in a goal-directed way.

We also note that our stimulation was typically applied in the upper layers of the cortex. In other areas of the brain, Schiller and Tehovnik (2001) have shown that stimulation in superficial vs. deep layers can have profoundly different effects. Many of the locations from which saccades can be evoked at low threshold from the SEF are in the deep layers, which probably explains why we could evoke saccades from only a minority of sites where SEF subthreshold stimulation caused ordering effects. Although we do not understand how the layered structure of cortex contributes to the contributions that it performs, (but see Shadlen and Newsome, 1998; Larkum et al, 1999 for proposals) perhaps some of the different functions reported in SEF may be preferentially represented in different layers of cortex.

The SEF likely uses a different sequence code than some other areas of the brain. In the hippocampus, it is thought that the precise timing of different neurons' spikes relative to a field potential oscillation plays a role in sequence encoding (O'Keefe and Recce, 1993;

Mehta and Wilson, 2002; Melamed et al, 2004). The train of stimulation pulses that we applied is likely to synchronize neurons near the tip of the electrode. Our results thus suggest that in the SEF, small timing differences between neurons' spikes are unlikely to code sequence information. Future experiments exploring the temporal relation of spiking activity in the SEF to underlying rhythms in the local field potential would be exciting.

Future work

A wide variety of interesting questions are raised by this work; we expand on three particular questions here. To test the hypothesis we proposed above about the roles of FEF and SEF in sequencing, one interesting manipulation would be to stimulate for part of a delay period in the SEF, verify that there was still an ordering effect, and examine the activity in FEF for the remainder of the delay. If our hypothesis is correct, we would expect to see the FEF activity on order error trials change so as to encode the reverse order that was cued. Evaluation of the effects of subthreshold stimulation in FEF would also be interesting. We predict that FEF stimulation would have an effect on the order of the response, but would not preferentially affect the sequence endpoint. This could be tested directly for each site by mapping the motor field, as saccades can be evoked from nearly every site in the FEF. Finally, we observed that delay activity in FEF encodes multiple spatial locations, which is a strong contrast to the otherwise-similar delay activity in LIP. One area whose delay activity has not been studied in a sequence task, to our knowledge, is the superior colliculus. Collicular neurons also show delay activity, and it would be interesting to test whether delay activity there encodes multiple spatial

locations or just the single upcoming saccade. Based on the work of Sommer and Wurtz (2004a), showing that visual activity in FEF does not arise from the colliculus and that the thalamic pathway between the two areas filters out delay activity, we would predict that collicular neurons would encode only a single saccade.

Conclusion

In this thesis, we report the results of an experiment showing that the SEF plays a role in serial organization, coding compound movements by their goals. As stimulation does not affect the individual movement components, it is likely that they are stored elsewhere. The FEF is a likely candidate to store these memories of spatial location. We propose that these two areas interact to control sequences of movements based on memory: the FEF stores memory for locations while the SEF codes the entire movement through its endpoint.

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