Muscimol-Induced Inactivation of Monkey Frontal Eye Field: Effects on Visually and Memory-Guided Saccades

ELISA C. DIAS AND MARK A. SEGRAVES
Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois, 60208

Dias, Elisa C. and Mark A. Segraves. Muscimol-induced inactivation of the monkey frontal eye field: effects on visually and memory-guided saccades. J. Neurophysiol. 81: 2191–2214, 1999. Although neurophysiological, anatomic, and imaging evidence suggest that the frontal eye field (FEF) participates in the generation of eye movements, chronic lesions of the FEF in both humans and monkeys appear to cause only minor deficits in visually guided saccade generation. Stronger effects are observed when subjects are tested in tasks with more cognitive requirements. We tested oculomotor function after acutely inactivating regions of the FEF to minimize the effects of plasticity and reallocation of function after the loss of the FEF and gain more insight into the FEF contribution to the guidance of eye movements in the intact brain. Inactivation was induced by microinjecting muscimol directly into physiologically defined sites in the FEF of three monkeys. FEF inactivation severely impaired the monkeys’ performance of both visually guided and memory-guided saccades. The monkeys initiated fewer saccades to the retinotopic representation of the inactivated FEF site than to any other location in the visual field. The saccades that were initiated had longer latencies, slower velocities, and larger targeting errors than controls. These effects were present both for visually guided and for memory-guided saccades, although the memory-guided saccades were more disrupted. Initially, the effects were restricted spatially, concentrating around the retinotopic representation of the center of the inactivated site, but, during the course of several hours, these effects spread to flanking representations. Predictability of target location and motivation of the monkey also affected saccadic performance. For memory-guided saccades, increases in the time during which the monkey had to remember the spatial location of a target resulted in further decreases in the accuracy of the saccades and in smaller peak velocities, suggesting a progressive loss of the capacity to maintain a representation of target location in relation to the fovea after FEF inactivation. In addition, the monkeys frequently made premature saccades to targets in the hemifield ipsilateral to the injection site when performing the memory task, indicating a deficit in the control of fixation that could be a consequence of an imbalance between ipsilateral and contralateral FEF activity after the injection. There was also a progressive loss of fixation accuracy, and the monkeys tended to restrict spontaneous visual scanning to the ipsilateral hemifield. These results emphasize the strong role of the FEF in the intact monkey in the generation of all voluntary saccadic eye movements, as well as in the control of fixation.

INTRODUCTION

The frontal eye field (FEF) of monkeys has been known to be associated with eye movements for more than a century (Ferrier 1875). The FEF contains neurons that fire before saccadic eye movements and electrical stimulation with minimal current evokes eye movements with short latency, suggesting the FEF has a causal role in their generation (Bruce and Goldberg 1985; Bruce et al. 1985; Goldberg and Bushnell 1981; Mohler et al. 1973). In addition, the FEF is extensively interconnected with other cortical and subcortical oculomotor areas (Andersen et al. 1985; Barbas and Mesulam 1981; Huerta et al. 1986, 1987; Schall et al. 1993, 1995; Stanton et al. 1988a,b, 1993) and has been shown, in imaging studies, to be activated by oculomotor tasks in humans (Darby et al. 1996; Luna et al. 1998; O’Driscoll et al. 1995; Paus 1996; Petit et al. 1996).

Surprisingly, however, both permanent removal or temporary inactivation of the FEF in monkeys has produced mainly subtle deficits in oculomotor behavior. The main deficits were an initial neglect of the contralateral hemifield, a subtle increase in latencies of saccades, and a deviation of fixation, all of which recovered within a few weeks (Keating and Gooley 1988; Latto and Cowey 1971; Lynch 1992; Schiller et al. 1980, 1987), though larger and more permanent effects have been reported recently (Schiller and Chou 1998). Additional deficits were uncovered when monkeys with FEF removed or inactivated were tested with more cognitive tasks. Monkeys were more impaired when required to make a saccade to a remembered location than when they made the same saccade to a visual target (Deng et al. 1985; Dias et al. 1995; Sommer and Tehovnik 1997). FEF removal also was found to impair the normal oculomotor scanning of the environment, that is, the ordering of successive saccades (Collin et al. 1982; Latto 1978; Schiller et al. 1980, 1998).

The oculomotor deficits seen after removal of the monkey FEF were much stronger if other areas involved in controlling eye movements also were removed surgically. For example, lesions of both the FEF and more caudal eye-movement related areas (including the caudal portion of the inferior parietal lobule and the adjacent dorsal prestripate cortex) caused much greater impairment than individual lesions of either the FEF or the posterior areas (Lynch 1992). Also, lesions of the FEF and the superior colliculus (SC) greatly impaired visually guided saccades, whereas lesions of either region alone caused only slight impairments in oculomotor behavior (Schiller et al. 1980, 1987). These observations suggest that the FEF is part of a network of cortical and subcortical areas that controls eye movements. Removal of one component of the network causes an initial deficit that soon is compensated for by the remainder of the network. When several nodes within the network are removed, however, the deficits become more permanent.

In an attempt to better understand the contributions of the
FEF to this network, we have acutely and reversibly inactivated regions within the FEF of monkeys with injections of muscimol. Muscimol is a high affinity GABA\(_A\) receptor agonist that has been shown to inhibit cortical neurons (Andrews and Johnston 1979). Analysis of the deficits in oculomotor performance after acute inactivation is likely to provide a better picture of the functions that require FEF involvement than is provided by permanent lesions. The oculomotor network has very little time to recover or adapt to the effects of inactivation in the short time between injection and behavioral testing. In addition, injections can be made in small subregions of the FEF, allowing for comparison of the characteristics of saccades represented by the injection site versus saccades controlled by the noninjected portions of the FEF in the same cortical hemisphere.

Preliminary data from this project have been published (Dias et al. 1995).

**Methods**

**Subjects**

Three adult macaque monkeys (Macaca mulatta) were used in this study. All were females (7–9 kg) and were in good health. All experimental procedures followed the guidelines of the Northwestern University Animal Care and Use Committee.

**Surgery**

Each monkey was implanted surgically with several devices to allow for chronic recording of cortical neurons and microstimulation while the monkey was awake and performing an oculomotor task. The surgery was performed under aseptic conditions. Anesthesia was induced with sodium thiamylal and maintained with halothane. A subconjunctival wire coil for the measurement of eye position with the magnetic search coil technique was implanted in one eye (Judge et al. 1980; Robinson 1963). Trephine holes were made through the skull over the intended recording sites. Stainless steel recording cylinders, a receptacle to fix the monkey’s head during recording sessions, and the connector for the eye coil were fixed in place and bonded to the skull with dental acrylic. Immediately after surgery and during the following week, the monkey was given prophylactic antibiotics (Panalog) to prevent infection and delay bone formation over the dura (Collins 1987). See Segraves (1992) for a more detailed description of surgical procedures.

**Behavioral training**

Before the surgery, the monkeys were trained to sit in a primate chair that was placed in the recording room. The primate chair was positioned in front of a tangent screen where light stimuli were back-projected. By gradual behavioral shaping, the monkey was taught a bar-press task where she began a trial by pressing a metal bar in front of her. The bar press caused a target light to be turned on. After a variable period of time (500–1,000 ms), the target light was dimmed, and the monkey was required to signal the detection of the dimming by releasing the metal bar to receive a liquid reward. This training facilitated postoperative training by exposing the monkey to the experimental setup and teaching the behavioral relevance of visual stimuli that were presented.

After the surgery each monkey received additional training in several visuomotor tasks. Because after surgery it was possible to accurately measure eye position with the search coil technique, the monkey was required to meet predetermined criteria for eye position relative to target position and for saccade amplitude and direction to receive the liquid reward. The room was usually dimly lit (0.033 cd/\(\text{m}^2\)), but sometimes the lights were turned off to test spontaneous eye movements in the dark (luminance <0.007 cd/\(\text{m}^2\)). The visual stimuli consisted of light stimuli originating from red laser diodes rear projected onto the screen in front of the monkey (luminance = 15.4 cd/\(\text{m}^2\)). A PDP 11–73 minicomputer controlled the experimental equipment and recorded behavioral and neuronal events.

To motivate the monkeys to perform the tasks, water was restricted in their home cages, and the monkeys obtained their daily water requirements during the training or experimental sessions. Whenever water access was controlled, the monkeys’ weight was monitored daily, and they were examined for signs of dehydration. They received additional liquids in their cages to supplement their water intake when necessary and were regularly given dried fruit and treats. A staff veterinarian did periodic surveys of the general health of the monkeys.

**Oculomotor tasks**

Two oculomotor tasks were used to obtain the results described in this paper.

In the **visually guided saccade task**, the monkey initiated the trial by fixating a spot of light in the center of the tangent screen. After a variable delay (0.9–1.5 s), the central spot disappeared and simultaneously a target spot appeared at another part of the screen. The monkey was rewarded for making a saccadic eye movement to the target. During recordings, this task was used to test for saccade-related activity of the neuron and during behavioral testing to verify the monkey’s ability to make visually guided saccades. The peripheral target could be positioned anywhere on the screen to test neurons for preferred saccade vectors or to verify the range of the oculomotor deficits.

The **memory-guided saccade task** also began with the monkey fixating a central spot. In this task, however, the target was flashed only briefly (0.3–0.5 s) in the periphery while the central spot was still on, and the monkey was required to maintain fixation on the central spot or the task was aborted. Only after the disappearance of the fixation spot, which occurred after a variable delay (0.1–0.7 s), was the monkey allowed to make a saccade toward the location where the peripheral target was flashed. This task was used to isolate the visual and motor components of neuron activity and to test the effects of inactivation on short-term memory. We could vary the memory delay in different blocks of trials to test memory effects. This task was more difficult than the visually guided saccade task, but the monkeys eventually learned to perform it well, successfully completing the trial in >90% of the cases.

The monkeys always were presented with trial blocks of the same task, and the saccadic target location could be fixed or, more commonly, vary from trial to trial in a pseudorandom fashion following a list of 2–48 target positions. Usually the locations of the targets were not more than 20° from the center of the target screen.

In this study, the location of visual stimuli and targets are expressed in polar coordinates, radius, and angle. The radius is equal to the distance in degrees of arc of the target or stimulus from the fixation point. An angle of 0° describes a rightward horizontal direction, and a 90° angle describes an upward vertical direction.

**Electrophysiology**

Neuronal activity in the cerebral cortex was recorded using tungsten microelectrodes (A-M Systems), which were introduced into the brain through the recording cylinder. The electrodes were lowered into the brain using a hydraulic microdrive (Narishige). The same electrodes were used for recording neuronal activity and for microstimulation. Multiple recordings on different days were reliably made at the
FRONTAL EYE FIELD INACTIVATION

same sites by use of a grid system (Crist et al. 1988). This grid was used to hold transdural guide-tubes through which we could introduce recording microelectrodes or the injection cannula and direct them to specific positions in the brain. This system allowed for the tip of the injection cannula to be positioned at a location that already had been investigated with a microelectrode. When the guide tube was not in use, a wire matched to its inside diameter was coated with antibiotic (3% tetracycline HCl ointment) and inserted into the guide tube. The grid system was left in place between experiments.

The electrodes were connected to a standard recording setup consisting of preamplifiers, amplifiers, and filters, as well as to a stimulator. Electrical stimulation using standard parameters (70 ms trains of 0.2-ms biphasic pulses at 333 Hz, current ≤100 μA) was used to test for low-threshold movements of the eyes, confirming that the electrode or cannula tip was located in the FEF (Bruce et al. 1985).

During an experiment, the FEF initially was mapped with a microelectrode. Neuron activity and thresholds for electrically evoking eye movements were recorded along the whole depth of the FEF. This information allowed us to later reconstruct the saccade vectors that might have been affected by the inactivation.

An example of a penetration along the anterior bank of the arcuate sulcus in monkey MK2, and the location of an injection site within that penetration is shown in Fig. 1. Notice how the direction of the evoked saccades varied in an orderly manner as the electrode was moved down the bank of the arcuate sulcus (Bruce et al. 1985).

**Injections**

Analysis of recording and stimulation data were used to select a site for injection. Our main criteria were that the region contain neurons that were active before eye movements and that stimulation with currents ≤50 μA evoke eye movements (Bruce et al. 1985). We usually chose sites that represented saccade vectors with amplitudes >5° because alterations of some of the parameters, such as amplitude and velocity, would be more evident (Table 1). We did, however, make injections in a few sites representing smaller saccade vectors (for example, the case in Fig. 8).

The injections were made with an injection system described in more detail elsewhere (Dias and Segraves 1997). Briefly, an injection cannula made with 30-gauge stainless steel hypodermic tubing was introduced into the brain through the transdural guide tube. Recordings of neural activity and stimulation were made through the cannula by means of an insulated 0.05-mm-diam stainless steel wire protruding from its end. This allowed us to directly characterize the position of the center of the injection site. Once the tip of the cannula was positioned at the desired site in the brain, an injection was made using pressure controlled by a pneumatic picopump (WPI). The amount of substance injected was measured by using an operating microscope with calibrated eye-piece reticule to quantify the movement of the meniscus of fluid in a glass capillary tube located between the injection cannula and the tubing from the picopump.

Usually a volume of 1.0 μl of a 5 μg/μl solution of muscimol was injected in 0.1-μl steps during a 10-min period. When analyzing the behavioral data after the injection, we considered the end of the injection as time = 0. In one case where we injected only 0.5 μl, an additional injection of 0.5 μl of muscimol was made 35 min later. The injections always were made on different days to ensure complete recovery at the injection site. In our experiments, the effects of muscimol injections disappeared within 24 h. Injections of 1.0 μl of sterile saline were made twice in MK1 and MK2 to control for the possibility that the effects we were observing were due to pressure on the cortical neurons or dilution of the extracellular matrix. Behavioral controls from the same monkey, with no injection, were collected on the days before or after a muscimol injection or both.

Although the range of amplitude and direction of eye movements affected by our injections gave us some indication of the spread of muscimol within the FEF, we did not directly test the spread of the drug in the cortex and have relied on published data for this measure. Martin (1991) has shown that in the rat cortex and brain stem, 1 μl of radioactive muscimol (1 mg/ml, [3 H]muscimol) spreads over a region with a radius of ~1.7 mm, and radioactivity can still be measured 2 h after injection. Martin also showed that the hypometabolism due to the inactivation of the neurons after the muscimol injection (recorded as glucose uptake) is strongest at a central core with a radius of ~1.0 mm. Our 1-μl injections were made with a higher concentration of muscimol (5 mg/ml), so we assume that the spread was somewhat larger.

As we injected the muscimol, we monitored the drug’s effect on the activity of connected neurons. After the injection was completed, we recorded the monkey’s eye movements during the oculomotor tasks, and the spontaneous eye movements produced both in the dark and in dim light, for as long as the monkey would work (2–5 h). The same tasks were repeated several times during the entire period to examine.

**FIG. 1.** Sample of an electrode penetration through the anterior bank of the arcuate sulcus, including a site of frontal eye field (FEF) injection. This figure illustrates the orderly variation of the vectors of saccades elicited by micro-stimulation as an electrode moved from surface to fundus along the anterior bank of the arcuate sulcus. Numbers to the left represent the depth from dural surface at which electrical stimulation was made. At each position, saccades were elicited with currents equal to or above the thresholds shown at the right of each stimulation site. Each sequence of dots represents a saccade’s trajectory, with each dot representing eye position recorded at 1 kHz. The saccade initiation point is marked by a circle for cases where saccades were elicited when the monkey was fixating the central fixation point, and by a square when saccades were elicited when the monkey was not required to fixate (positions 4.2 and 6.8 mm). In the latter cases, the starting locations for saccades began at different eccentricities are superimposed. Site chosen for injection mu7 is indicated between depths 5.3 and 5.6 mm.
the progression of the deficits resulting from the inactivation. The monkey’s general behavior was monitored throughout the experiment by means of a video camera.

All muscimol and saline injections were made in the FEF in the anterior bank of the arcuate sulcus. This was ascertained by electro-anatomical methods, not anatomic, but there is an abundance of published data supporting the good correlation between the two techniques for determining the location of the FEF in monkeys (Bruce et al. 1985; Stanton et al. 1988a, 1989).

We have histological confirmation of the injection locations in the brain of one of the monkeys; the other two monkeys still are being used for ongoing experiments in our laboratory.

**Data analysis**

The main laboratory computer, a PDP 11–73, which controlled the behavioral aspects of the experiments, also generated on-line displays of rasters and histograms of the neuronal activity being recorded. Both single-unit activity and behavioral data were stored on computer disks.

Saccades were analyzed off-line on a UNIX workstation. The start of a saccade was recognized as 20 ms before the eye velocity went above 100°/s and the end as 20 ms after eye velocity went below 10°/s. In a few cases, after inactivations, when saccades velocity was decreased, we lowered the criteria for recognizing the beginning of a saccade to 80 or even 70°/s. Latencies were always calculated as the time between the offset of the fixation point (movement cue) and the beginning of the eye movement. All saccade identifications were checked by the experimenter before the data were processed further.

For our data analysis, we only considered saccades that began <1,000 ms after the movement cue and were directed to the correct hemifield. That is, we tried to eliminate saccades that were not clearly target driven. In addition, we only looked at the first saccade the monkey made after the cue to begin the eye movement. Additional corrective saccades, occurring in the visually guided saccade task, were not analyzed. We did not observe corrective saccades occurring in the memory-guided task.

Additional analysis and graphic plotting of the data were accomplished with the commercially available software MATLAB (The Mathworks). Saccades were separated by target location, and plots of latencies, velocities, targeting error, amplitudes, and scatter of beginning and ending positions of the saccades were made. The experimental data were compared statistically with controls from the same monkey. In most cases, a comparison was made by performing single- or two-factor ANOVA. In some cases, this was followed by a protected two-tailed t-test. We compared the data for each target location after the injection with the data for the same target location without the injection, to test if there was a significant variation in specific directions. In a few cases, where noted, a regression analysis was made.

Because of the variability of the effects of muscimol injections over time, due probably to the difference in the injected sites (i.e., more superficial versus deeper, more medial versus more lateral), and the variability in amplitude and direction of saccade vectors represented at different injection sites, it was inappropriate to combine data from different experiments to do general statistical tests. Instead, we quantified the data for each individual injection.

We calculated systematic and variable targeting errors of the saccades and fixation using the same procedures described by White and collaborators (1994). In brief, the targeting errors, or saccade errors, were calculated for each target location by comparing the eye position at the beginning or end of each saccade with the position of the fixation spot or target (systematic error). We did not recalibrate the eye coil signal after the injection because this might conceal slow, drug-induced changes in eye position. As a result, we cannot exclude the possibility that some of the observed changes in eye position were due to slow drift in the eye movement recording system. This possibility underlines the importance of comparing changes for all directions of eye movements, including those represented by the injection site as well as those represented outside of the injection site in both ipsilateral and contralateral directions.

The saccadic/fixation scatter (variable error) was calculated for each target location by first calculating the mean of eye positions at the beginning or end of each saccade to that target for a particular block of trials and then calculating the absolute value of the distance between each eye position at the end/beginning of the saccade to this mean.

**RESULTS**

A total of 12 muscimol injections into the FEF were analyzed in detail for this report, 3 in monkey MK1 (mu1–mu3), 8 in monkey MK2 (mu4–mu11), and 1 in monkey MK3 (Table 1). In addition, MK1 and MK2 received two saline injections into the FEF each, as injection controls (sa1–sa4). We placed the injections in different subregions of the FEF, choosing sites representing different saccade vectors, ranging from small saccades (mu3: 3°, Fig. 8) to large saccades (mu11: 30°, Fig. 15).

Injection of muscimol immediately inactivated the FEF neurons surrounding the injection cannula tip. In a few cases, saccades still could be evoked by electrical stimulation of the injection site but only with higher thresholds than before. On only one occasion, we could record from a neuron after the injection even though the background neuronal activity disappeared. We can speculate that this neuron, which showed visuomotor presaccadic activity, received strong excitatory input from outside the region affected by the injection.

We tested the monkeys’ oculomotor performance after the injections and compared it with performance when no injections were made (1–4 days from the injection). Saline injections produced no observable changes in oculomotor performance.

**Effects of FEF inactivation with muscimol**

After FEF inactivation with muscimol, the monkeys had difficulty performing both visually and memory-guided saccades, mainly toward the visuotopic representation of the region injected. The range of amplitudes and directions of saccades first affected by the injection was restricted. The monkey still could make relatively normal saccades to sites outside of

<table>
<thead>
<tr>
<th>Injection</th>
<th>Monkey</th>
<th>FEF</th>
<th>Size*</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>mu1</td>
<td>MK1</td>
<td>R</td>
<td>Small</td>
<td>Down-left</td>
</tr>
<tr>
<td>mu2</td>
<td>MK1</td>
<td>R</td>
<td>Small</td>
<td>Down-left</td>
</tr>
<tr>
<td>mu3</td>
<td>MK1</td>
<td>R</td>
<td>Small</td>
<td>Down-left</td>
</tr>
<tr>
<td>mu4</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Down-right</td>
</tr>
<tr>
<td>mu5</td>
<td>MK2</td>
<td>L</td>
<td>Small</td>
<td>Up-right</td>
</tr>
<tr>
<td>mu6</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Horizontal-right</td>
</tr>
<tr>
<td>mu7</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
<tr>
<td>mu8</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
<tr>
<td>mu9</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
<tr>
<td>mu10</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
<tr>
<td>mu11</td>
<td>MK2</td>
<td>L</td>
<td>Large</td>
<td>Horizontal-right</td>
</tr>
<tr>
<td>mu12</td>
<td>MK3</td>
<td>R</td>
<td>Medium</td>
<td>Horizontal-left</td>
</tr>
<tr>
<td>sa1</td>
<td>MK1</td>
<td>R</td>
<td>Small</td>
<td>Up-left</td>
</tr>
<tr>
<td>sa2</td>
<td>MK1</td>
<td>R</td>
<td>Small</td>
<td>Down-left</td>
</tr>
<tr>
<td>sa3</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
<tr>
<td>sa4</td>
<td>MK2</td>
<td>L</td>
<td>Medium</td>
<td>Up-right</td>
</tr>
</tbody>
</table>

FEF, frontal eye field. *Size criteria: small, <10°; medium, >10°, <20°; large, >20°.
this representation. The cellular and behavioral effects of the injection lasted several hours, and even when the monkeys were tested 4 h after the injections these effects were present and generally enhanced. The oculomotor deficits were gone completely by the next day.

An example of monkey MK2’s oculomotor performance of visually and memory-guided saccades ~2 h after a muscimol injection in the FEF is shown in Fig. 2. Stimulation of the site before the injection consistently elicited a horizontal contraversive saccade ~10° to the right (Fig. 2A). The most obvious deficit in the monkey’s performance of the visually guided saccade task after the injection was the reduced number of saccades made to the right, particularly at polar angles of 0° and 315° (Fig. 2B). Although all targets were presented a similar number of times, the monkey would usually not initiate a saccade toward the retinotopic location represented at the injection site. In addition, the saccades toward regions flanking the affected region, for example down to the right, were slower than normal and smaller in size. This reduction in velocity is evident in the bottom panels of Fig. 2B. Saccades directed toward the ipsilateral hemifield were not as strongly affected by the inactivation, although they differed from controls in some respects. They reached peak velocities that were comparable with controls but were less accurate in reaching the target. The latencies of ipsilateral saccades were not as strongly affected by the injection as were the latencies of contralateral saccades. Plots of control data collected when no injection was made are included in Fig. 2B, right, and demonstrate that the monkey performed the visually guided task well under normal conditions. There was also a greater scatter of the end points of these saccades when compared with controls. In addition, eye positions during fixation before the start of saccades were deviated ipsilaterally and were scattered, whereas, in the controls, fixation positions overlapped closely and were centered on the fixation spot. These effects are presented in greater detail later in this paper.

While the monkey was showing difficulty in generating
visually guided saccades 2 h after the injection was made, she was completely unable to make memory-guided saccades into the hemifield contralateral to the injection site (Fig. 2C, left). She could, however, still make memory saccades into the ipsilateral hemifield, indicating that she was alert and motivated. Ipsilateral saccades had normal velocities, but many times were initiated before the cue to start the saccade, resulting in an error (see following text). Like the visually guided saccades, the memory-guided saccades also had scattered saccade starting positions, an indication of the monkey’s difficulty in accurately maintaining fixation of the central light. Under control conditions, the monkey made accurate, reproducible, memory-guided saccades (Fig. 2C, right).

Monkey MK1 also had difficulties performing saccades directed toward the retinotopic location represented at the injection site after an FEF inactivation (Fig. 3). After 37 min the saccades in the affected direction were clearly hypometric, and slow and fixation was deviated upward although not ipsilaterally (Fig. 3B). Two hours later fixation was deviated both upward and ipsilaterally (to the right) (Fig. 3C). The monkey missed many saccades that should have been directed down to the left. At this time, all contraversive saccades were slower than ipsiversive saccades. The accuracy and latencies were also deteriorating for these contralateral saccades. Ipsilateral saccades were much less affected by the injection. The changes that occurred over time after the injection are described further in the following text.

To look at these effects in greater detail, we quantified the latencies and peak velocities for both injection and control data. Saccadic latencies were increased systematically after the
FEF inactivation both in the visually guided and in the memory-guided tasks. This was a robust finding and was observed after every injection for all oculomotor tasks tested. Peak velocities were decreased, mainly for saccades directed to the retinotopic representation of the area injected. This also was observed for both visually and memory-guided saccades, although velocity decreases were greater for the memory-guided saccades.

**Latencies**

An example of these observations is shown in Fig. 4. For the visually guided saccade task, the effects of this muscimol injection became pronounced ≥1 h after the time of injection, and for this illustration, we analyzed data collected 2 h after injection. We found saccadic latencies increased, in comparison with controls, particularly for directions including and for this illustration, we analyzed data collected 2 h after injection. We found saccadic latencies increased, in comparison with controls, particularly for directions including and adjacent to that represented by the injection site. These differences were significant, and this was verified first by a two-factor ANOVA (factor 1: drug x control, $F_{1,48} = 97.6, P < 0.01$; factor 2: target directions, $F_{7,48} = 16.5, P < 0.01$; interaction: $F_{7,48} = 16.5, P < 0.01$), which indicated that there was a significant difference between the latencies in the drug case and the control case, there was also a significant difference between latencies for the different directions tested, and there was a significant change in the effects of the drugs depending on the direction tested (interaction). To verify which directions were significantly affected, we performed protected $t$-tests comparing the values for each direction with and without the drug (Fig. 4, *).

These same effects were observed closer to the time of the injection, when the monkeys performed the memory-guided saccade task. One to 2 h after the injection was made, the monkeys usually could not make memory-guided saccades in the affected directions. As a result of this degree of impairment, we quantified data from earlier trials when the saccades were impaired but still were being initiated by the monkey. For the data included in Fig. 4, we looked at memory-guided saccades recorded 40 min after the time of injection with the task having a memory-delay period of 300 ms. The latencies for contraversive memory-guided saccades were increased significantly compared with controls, and there was a significant interaction between drug condition and direction (factor 1: drug x control, $F_{1,48} = 6.51, P < 0.05$; factor 2: directions, $F_{7,48} = 5.174, P < 0.01$; interaction: $F_{7,48} = 4.075, P < 0.01$). Again, the directions most affected were adjacent to the direction represented at the injection site.

**Velocities**

The peak velocities of the saccades were decreased significantly both for visually guided saccades (two-factor ANOVA, factor 1: drug x control, $F_{1,48} = 11.392, P < 0.01$; factor 2: directions, $F_{7,48} = 4.4201, P < 0.01$; interaction: $F_{7,48} = 3.4529, P < 0.01$) and for memory-guided saccades (factor 1: drug x control, $F_{1,48} = 42.204, P < 0.01$; factor 2: directions, $F_{7,48} = 27.192, P < 0.01$; interaction: $F_{7,48} = 15.256, P < 0.01$; Fig. 4). This decrease in velocities occurred mainly in the directions adjacent to the direction represented at the site. Occasionally, a statistically significant increase was observed for visually guided saccades in the opposite direction.

The profiles of velocity over time for both visually and memory-guided saccades appeared abnormal after muscimol injection (Fig. 5). Postinjection saccades showed more variability in the velocity profiles than controls with velocities sometimes peaking later than for controls. In some cases, the monkey made a very slow saccade in a contralateral direction, which, after several intervening saccades to other targets, could be followed by a saccade in the same direction that had a relatively normal velocity profile. This was particularly true.
when the effects of the inactivation were just beginning to be noticeable. By the time the data illustrated in Fig. 5 were recorded, however, the memory-guided saccades had reduced peak velocities and abnormal velocity profiles for all contraversive saccades (Fig. 5B, inset).

The decrease in peak velocity and diminished velocity profiles did not appear to be due to a tendency to make smaller amplitude saccades after the injection. Plots of peak velocity versus amplitude for both memory-guided and visually guided saccades show that most contraversive saccades fell well below the main sequence following the injection (Fig. 6A). In this example, the monkey made 10° visually or memory-guided saccades the peak velocities of which varied from 250 to 700°/s, depending on their direction. Saccades with directions similar to the direction represented at the site were the slowest, saccades moving in the opposite direction were fastest, and saccades with intermediate directions (i.e., up and down) had intermediate velocities. For any given amplitude of saccade, contraversive saccades were always slower than ipsiversive.

Because there was very little variability in the amplitude of the saccades in the controls for these tasks, a comparison of the regression lines could be misleading. Thus we compared the peak velocities in muscimol cases to controls with a t-test. After the muscimol injection, saccades directed contralateral were significantly slower than saccades to the same targets in controls ($P < 0.001$). Saccades to ipsilateral targets were not significantly different from controls. This was true both for visually and memory-guided saccades.

This effect became even clearer when we tested the monkey with a block of trials where only two potential targets existed (Fig. 6B). There was a clear dissociation between the points corresponding to the peak velocity of saccades made in the ipsilateral direction (crosses), which were statistically indistinguishable from the controls, and those made in the contralateral direction (circles), which were significantly different from the controls ($P < 0.001$). Note that, unlike the controls, there was no overlap for the memory-guided saccades and very little overlap for the visually guided saccades.

This reduction in velocity both in the memory-guided saccades and in the visually guided saccades was observed for all muscimol injections in both monkeys.

**Saccade targeting error and scatter**

The accuracy of saccades decreased after FEF inactivation (Fig. 7). Both the targeting error for each saccade (distance between target location and the end point of the saccade), and the saccade scatter (amount of separation between the end points of saccades made to the same target) increased for both visually and memory-guided saccades after FEF inactivation, particularly for the direction corresponding to the movement vector represented at the injection site (Fig. 7, C and I). These increases in the magnitude of the targeting errors and the scatter of saccade end points were observed after all muscimol injections and tended to increase with time after the injection.

In the example shown in Fig. 7, the difference in targeting error between inactivated versus control conditions was assessed by a two-factor ANOVA (visually guided saccades: factor 1: drug $\times$ control, $F_{1,48} = 25.547$, $P < 0.01$; factor 2: directions, $F_{7,48} = 3.311$, $P < 0.01$; interaction: $F_{7,48} = 2.475$, $P < 0.05$; memory-guided saccades: factor 1: drug $\times$ control, $F_{1,112} = 41.084$, $P < 0.01$; factor 2: directions, $F_{7,112} = 7.771$, $P < 0.01$; interaction: $F_{7,112} = 9.357$, $P < 0.01$). Protected t-tests then were performed to verify which directions were showing significant changes. For visually guided saccades, the increases were significant in most directions but were higher in the directions similar to the one represented at the site. For the memory-guided saccades, only the directions close to the direction represented at the site were significant.

The scatter of the end points of saccades for each target
location also increased after inactivation and increased systematically with time. In the example, there was an increase in the scatter of the end points of saccades after FEF inactivation for both visually and memory-guided saccades (2-factor ANOVA—visually guided saccades: factor 1: drug control, $F_{1,48} = 52.708, P < 0.01$; factor 2: directions, $F_{7,48} = 9.026, P < 0.01$; interaction: $F_{7,48} = 4.238, P < 0.01$; memory-guided saccades: factor 1: drug control, $F_{1,112} = 12.355, P < 0.01$; factor 2: directions, $F_{7,112} = 4.238, P < 0.01$; Fig. 7, E and K). Protected t-tests showed that the directions that presented most scatter were similar to the direction represented at the injection site.

After the inactivations, there was a generalized shift in the end points of the saccades toward the ipsilateral side. For example, in Fig. 7A, most of the end points of the saccades were to the left of the target positions. This was not the case for the controls (Fig. 7B). As a result, the increases in targeting errors that were observed reflected, in part, the shift toward the ipsilateral hemifield, as if the whole frame of reference of the monkey was deviated ipsilaterally. This could explain why targeting errors for saccades directed toward the ipsilateral hemifield were so increased (Fig. 7C).

This ipsilateral shift also could be observed in the eye positions at the start of the saccades made after the injection (Fig. 7, F and L). The ipsilateral shift of eye position during the memory-guided task appeared to be of lesser magnitude than that observed for the visually guided task. This is a reflection...
of the fact that the memory-guided saccade data were collected earlier than the visually guided saccade data (40 min for memory-guided saccades vs. 2 h for the visually guided saccades).

For comparison, we analyzed the variation in amplitude of the saccades for all directions tested. In the example, there was a slight, but not statistically significant, decrease in saccade amplitude [2-factor ANOVA: visually guided saccades: factor 1: drug × control, $F_{1,48} = 0.041$, $P > 0.05$; factor 2: directions, $F_{7,48} = 0.431$, $P > 0.05$; interaction: $F_{7,48} = 1.322$, $P > 0.05$; memory-guided saccades: factor 1: drug × control, $F_{1,112} = 0.781$, $P > 0.05$; factor 2: directions, $F_{7,112} = 9.84$, $P < 0.01$] but not enough to account for the increase in targeting error that was observed. In other experiments, however, significant decreases in amplitudes could be observed, mainly in memory-guided saccades (for example, see Fig. 10), but always accompanied by even larger errors.

**Retinotopic extent of the deficits**

The deficits after the muscimol injection usually began by affecting saccades within a small retinotopic region, generally corresponding to the motor field of neurons at the injected site. This effect was seen most easily for saccade direction, which was tested most extensively in our experiments. For some of our injections, however, we also tested the effects on saccade amplitude. There was also an initial specificity of the deficit for this dimension as well (Fig. 8).

In the example in Fig. 8, all targets were presented close to the horizontal meridian in either left or right visual hemifield. The separation between adjacent target locations was 2°. The target that fell in the center of the location represented by the injection site was at 3° to the left of fixation point. The most
pronounced deficits were for saccades 1 and 3° to the left, although saccades to the targets located 5 and 7° to the left and 1° to the right also were impaired. The percentage of saccades initiated was smaller for these targets, and saccadic latencies were increased (Fig. 8C). Leftward saccades with larger amplitudes or saccades directed rightward toward the ipsilateral hemifield were much less impaired at the time these data were recorded, 50 min after the injection. Two hours after the injection all saccades directed contralaterally were impaired.

Temporal effects of the muscimol injection

The effects of the muscimol injection became stronger with time (Fig. 9). Initially, saccades toward the retinotopic representation at the injection site still could be made but with longer latencies, slower peak velocities and reduced accuracy. Eventually, usually after ≥1 h, the monkey was almost never able to make an saccade to the most affected direction, and the increase in latency expanded to flanking locations.

In most cases, it was difficult to detect a deficit in the visually guided saccade task a few minutes after the injection. Between 1 and 2 h after the injection, the deficits were much stronger. The monkeys rarely made a saccade toward the target in the retinotopic representation of the site. The saccades made were hypometric and slow, and their latencies were increased. After 2–3 h (depending on the injection site), the monkey rarely made any saccade into the contralateral hemifield. Even though the monkeys had this very pronounced deficit for making saccades toward the contralateral hemifield, they still could make saccades to ipsilateral targets. There was a scatter of the eye positions at the start and end of the saccades, but otherwise the saccade dynamics were well preserved. The preservation of the dynamics of the saccades directed ipsilaterally confirms that the monkey was still alert and motivated to perform the task. This can be seen both in Figs. 3 and 9.

The saccadic deficits were observed much sooner in the memory-guided task. Shortly after the injection, the monkeys were already making very slow, hypometric contralateral saccades and often did not initiate a saccade at all to a target in that hemifield. The monkeys also began to have difficulty maintaining fixation for the period required by the memory task; this resulted in many aborted trials. After ~1 h, these deficits increased and the monkeys did not make contralateral saccades. Eventually, usually after a couple of hours, the monkeys were unable to perform either contraversive or ipsiversive memory-guided saccades.

This progression of deficits was also evident in the increase of saccadic latencies with time (Fig. 9). In a control experiment, the monkey made saccades in eight directions to targets located 10° from the fixation point very consistently, with saccadic latencies of ~200 ms and initiating saccades with a target located at 3,185°. After ~1 h, these deficits increased and the monkeys did not make contralateral saccades. Eventually, usually after a couple of hours, the monkeys were unable to perform either contraversive or ipsiversive memory-guided saccades.

After the injection, there was a gradual increase in latency for saccades directed up and to the right, equivalent to the retinotopic representation of the injection site (Fig. 9). After 2 h some saccade latencies had doubled, and after 4 h the saccade latencies were sometimes quadrupled. Notice, however, that as time elapsed, the percentage of saccades initiated decreased, mainly for contralateral saccades but also for ipsilateral saccades. This is a reflection of the difficulty the monkey had in maintaining fixation, which was necessary to initiate the task. This resulted in many aborted trials in all directions. Nonetheless, the percentage of saccades initiated toward the contralateral side was always smaller than the percentage of saccades initiated toward the ipsilateral hemifield.
Because of the small number of saccades initiated in some directions on the later cases (for example, there was only 1 trial completed in the direction down to the right in the 240 min case), we used one-way ANOVAs to establish the difference between the experimental and control groups, with the significance levels adjusted for multiple comparisons (Bonferroni correction; for 5 comparisons, \( P < 0.01 \) to allow rejection of the hypothesis of no difference between the populations). All comparisons produced significant differences between controls and experiments (20 min: \( F_{129} = 11.67, P < 0.001 \); 100 min: \( F_{121} = 42.45, P < 0.001 \); 130 min: \( F_{135} = 35.58, P < 0.001 \); 180 min: \( F_{81} = 12.25, P < 0.001 \); 240 min: \( F_{73} = 15.3, P < 0.001 \)). Thus, we were able to proceed with protected \( t \)-tests, comparing each direction of each experimental set to the control for that direction, to verify which directions were most affected. The ones that were significant are marked (*). Noticeably, latencies of saccades toward the contralateral hemifield increased with time, whereas latencies of saccades toward the ipsilateral hemifield were less changed with time. In the case recorded at 20 min after the injection, the latency of saccades directed to the ipsilateral horizontal target actually decreased significantly.

**Effects of increasing delay time in memory-saccade performance**

Another factor influencing the performance of the monkey in the memory-guided saccade task after an FEF inactivation was the duration of the memory-delay period. This was the time imposed between the disappearance of the peripheral target and the cue to begin the eye movement (Fig. 10A). In the example shown, when the delay period was increased from 100 to 700
ms, the monkey’s accuracy in acquiring the remembered target decreased. Notice how some saccades to the targets horizontal-right and up-right are alternatively hypo- or hypermetric, suggesting a problem in remembering the target distance or the correct saccade to acquire the target. The monkey still was able to perform comparatively well in a visually guided saccade task at the time these data were collected, although contraversive visually guided saccades were somewhat hypometric and slower than controls. The saccades in the memory-guided saccade task with 700-ms delay were recorded before both the saccades in the memory-guided saccade task with 100-ms delay and the saccades in the visually guided saccade task, and thus the difference in performance could not be attributed to an increase in the inactivation effects over time.

The performance of the monkey on these tasks under control conditions shows that the monkey had no problem performing these tasks without the FEF inactivation (Fig. 10, controls). It is interesting to note in the control data that the memory-guided saccades to the lower hemifield were hypometric and the saccades to the upper hemifield were hypermetric, resulting in large targeting errors, which increased with the duration of the delay. This is in good agreement with studies that quantified memory-guided saccades in monkeys and showed that there is a general tendency for an upward shift in the end points of memory-guided saccades that increases with the delay (Gnadt et al. 1991; White et al. 1994). Because of these large systematic errors, both in controls and muscimol conditions, the difference in targeting error between the muscimol and control experiments was not statistically significant. In fact, for saccades directed to targets up and into the ipsilateral hemifield, the targeting errors were reduced after the muscimol injection, mainly because the saccades in the muscimol condition were reduced, and thus they did not overshoot the targets quite as much as they did in the control condition. There were significant differences of the targeting errors

![diagram](image_url)
between the three different tasks, but no interaction between drug condition and task (2-factor ANOVA: factor 1: drug × control, $F_{1,330} = 0.62, P > 0.5$; factor 2: task, $F_{2,330} = 74.13, P < 0.01$; interaction: $F_{2,330} = 1.08, P > 0.5$). Note, however, the increased standard deviation for the directions most affected. Their large values indicate that not only the monkey made large errors, but these saccades varied in accuracy from trial to trial.

**FIG. 11.** Temporal progression of the frequency of premature saccades in the memory-guided saccade task. A: premature saccades in the memory-guided saccade task (delay = 300 ms) 40 min after injection mu6. Left: memory-guided saccades made ≥170 ms after the cue to initiate the movement. Right: premature saccades. Bottom: saccadic velocity. B: scatterplot of the percentage of premature saccades in 7 injections in monkey MK2, with linear regression line superimposed. Symbol enclosed within a square represents data in A; symbols within the circles represent data shown in C. C and D: frequency of premature saccades increased over time. Memory-guided saccade task with memory-delay period of 300 ms. Injection mu11. C: saccade trajectories for memory-guided and premature saccades 1.25 and 4 h after the injection. D: saccade latencies. Time and associated task events are indicated on the $y$-axis. Graph space is divided into 3 temporally distinct parts. Saccades that began in the hatched area (0–500 ms, while the target is on) were considered visually guided prematures; saccades beginning in the gray area (500–970 ms, from target off to 170 ms after fixation point off) were considered anticipatory prematures; saccades that were initiated in the white part of the graph were considered nonpremature memory-guided saccades.
### Factor 1: Drug

A significant interaction of these two factors (2-factor ANOVA: factor 1: drug \* control, F_{1,330} = 12.90, P < 0.01; factor 2: task, F_{2,330} = 21.46, P < 0.01; interaction: F_{2,330} = 6.89, P < 0.01). Because of these statistically significant results, we were able to perform protected \(t\)-tests and obtained significance for the longer delays when the saccades were in directions similar to the direction represented at the injection site (Fig. 10C, *). Saccadic latencies were also significantly different in the muscimol and control experiments, but there was no significant interaction between drug and task (2-factor ANOVA: factor 1: drug \* control, F_{1,330} = 43.37, P < 0.01; factor 2: task, F_{2,330} = 47.11, P < 0.01; interaction: F_{2,330} = 1.16, P > 0.5; Fig. 10D). In fact, the latencies were highest for the memory-guided saccades when the task had the shortest memory delay. This appears to be a consequence of the 100-ms data being recorded later than the 700-ms data. As shown earlier, latencies increase with time (Fig. 9).

The peak velocities of saccades decreased with the longer memory delay, and there was a larger spread in the distribution of peak velocity plotted against saccade amplitude, particularly for the contralateral saccades in the 700-ms delay.

The performance on the memory-guided tasks with longer delays became progressively worse with time in all cases we studied. Over time, the monkey first would lose the ability to perform the longer delay tasks, followed by the shorter delay tasks, until finally it was incapable of performing any contraversive memory-guided saccade. This suggests that the mnemonic representation of saccadic target locations in relation to the fovea became progressively less stable after the FEF inactivation.

### Premature Saccades

A striking observation was that while performing the memory-guided saccade task the monkeys make many premature saccades to targets in the ipsilateral hemifield (Fig. 11). The memory-guided task required that the monkey maintain fixation when the target was flashed in the periphery, only making a saccade toward the location of the flash after the disappearance of the fixation spot. After the inactivation, the monkeys made many saccades that began before the fixation light was turned off, resulting in an aborted trial. Note that these premature saccades appeared to have normal velocity profiles (Fig. 11A).

**FIG. 12.** Effect of reward on the performance of a visually guided saccade task. Muscimol injection mu11. Each block of saccade trajectories includes the percent correct saccades (top numbers) and the latency ± SD (bottom numbers) for each direction. Time after the injection at which each block of data were recorded is included (left). The reward system for the monkey accidentally was turned off 120 min after the injection. 

**A:** saccades made to 2 target locations presented in random order (n = 19, in each direction). 

**B:** single saccade made to presentations of the rightward target alone (n = 9). 

**C:** saccades made to each of 10 presentations of the leftward target alone (n = 10). 

**D:** saccades made to targets presented both left (n = 16) and right (n = 17), in random order, after the reward system was turned back on (at 180 min after the injection). All targets were presented 10° from the central fixation point.

This was investigated further with an analysis of the saccade scatter. There was a statistically significant difference in saccade scatter, both for the drug condition and for the task, with a significant interaction of these two factors (2-factor ANOVA: factor 1: drug \* control, F_{1,330} = 19.46, P < 0.01; factor 2: task, F_{2,330} = 68.37, P < 0.01; interaction: F_{2,330} = 4.47, P < 0.01). Because of these statistically significant results, we were able to perform protected \(t\)-tests and obtained significance for the longer delays when the saccades were in directions similar to the direction represented at the injection site (Fig. 10C, *).

Saccadic latencies were also significantly different in the muscimol and control experiments, but there was no significant interaction between drug and task (2-factor ANOVA: factor 1: drug \* control, F_{1,330} = 43.37, P < 0.01; factor 2: task, F_{2,330} = 47.11, P < 0.01; interaction: F_{2,330} = 1.16, P > 0.5; Fig. 10D). In fact, the latencies were highest for the memory-guided saccades when the task had the shortest memory delay. This appears to be a consequence of the 100-ms data being recorded later than the 700-ms data. As shown earlier, latencies increase with time (Fig. 9).

The peak velocities of saccades decreased with the longer memory delay, and there was a larger spread in the distribution of peak velocity plotted against saccade amplitude, particularly for the contralateral saccades in the 700-ms delay.

The performance on the memory-guided tasks with longer delays became progressively worse with time in all cases we studied. Over time, the monkey first would lose the ability to perform the longer delay tasks, followed by the shorter delay tasks, until finally it was incapable of performing any contraversive memory-guided saccade. This suggests that the mnemonic representation of saccadic target locations in relation to the fovea became progressively less stable after the FEF inactivation.

**FIG. 13.** Effect of the number of targets on the performance of a visually guided saccade task. Injection mu11. Same conventions as for Fig. 12. 

**Top:** saccades generated with 2 possible target locations presented in random order (n = 14, in each direction). 

**Middle:** saccades generated with 8 possible target locations (n = 7, in each direction).

**Bottom:** same task as **top** (repeat experiment) (n = 7, in each direction).
is significant linear regression of the points (Fig. 11B, $r = 0.534$, $t$-test, $P < 0.05$). In the plot, each point represents a block of trials from a memory-guided saccade task. The exact time course of the increase in percentage of prematures depended on several factors, including the exact location of each injection and the practice the monkey had in the task at the time of the injection. Although the experiments were not initiated before the monkeys were trained to criterion, they continued practicing the same tasks every day for months, and it is possible that this somehow enabled them to increase their control of fixation in this task. For example, in the initial experiments (mu5 and mu6), the monkey made a large percentage of premature saccades very soon after the injection, whereas the same percentage was only reached later for the injections made at later dates (mu10 and mu11). We interpreted this result to be a consequence of the monkey’s increased training. In addition, because the monkeys rarely initiated memory-guided saccades after 2 h, there were fewer data available at those times.

Because of these confounding factors, we chose to analyze the variations observed for single injections. An example is shown in Fig. 11C. Although this experiment was unusual, because the monkey was capable of initiating memory-guided saccades for a very long period of time after the injection, it illustrates well the variation in percentage of prematures. After 1.25 h, the monkey made a number of premature saccades in the ipsilateral direction but also was able to make saccades with appropriate starting times for this direction (Fig. 11C). This was not the case several hours after the injection was made. After 4 h, the monkey only initiated nonpremature memory-guided saccades to the targets presented at angles of 45 and 315°, both in the contralateral hemifield. In spite of the target location, saccades to these targets were directed vertically up or down instead of obliquely toward the targets, resulting in large targeting errors. All saccades to targets presented ipsilaterally or on the vertical meridian were premature.

These premature saccades could be divided into two groups. Long-latency premature saccades began after the disappearance of the peripheral target and ≤170 ms after the disappearance of the fixation light (Fig. 11D, gray area). These saccades still may be considered as memory guided, but they also anticipated the movement cue and showed a great deal of variability in starting times. The timing of these saccades suggests that the monkey was still capable of suppressing, if temporarily, a visually guided saccade to the flashed target. Short-latency premature saccades began while the target was still present on the screen (Fig. 11D, hatched area). These saccades were visually guided and showed much less variability in starting time than was seen for the anticipatory premature saccades, indicating that the monkey was incapable of withholding the unwanted saccade.

With increasing time after the injection, there was a change in which group of premature saccades was more common. After 1.25 h, we observed more anticipatory premature saccades, whereas later, after 4 h, most of the premature saccades were visually guided. This effect was dependent on target location.

Effects of reward on the monkey’s performance

One serendipitous observation we made was that the incentives provided to the monkey for performing a task could influence the monkey’s behavior after the inactivation (Fig. 12). During one experiment, there was a period of time when the monkey’s liquid reward system accidentally was turned off. Surprisingly, the monkey continued to perform the visually guided saccade task, but her performance depended on where the target was presented. The monkey still made saccades to the ipsilateral target (Fig. 12, A and C) but did not make saccades to the contralateral target (Fig. 12, A and B). In the block of trials illustrated in Fig. 12A, only two target locations were presented in random order. Under these conditions, the monkey made contraversive saccades to only 3 of 19 presentations of the contralateral target. Even when the contralateral target was the only one presented, the monkey made only one saccade for nine presentations of the target (Fig. 12B). In spite of the absence of a reward, the monkey continued to make ipsilateral saccades with success rates of 95 and 100% (Fig. 12, A and C). After the reward system was turned back on, the monkey began to make saccades to both ipsilateral and contralateral target locations (Fig. 12D). Note that the contraversive saccades had longer latencies than ipsiversive saccades both with and without a reward present. In addition, ipsiversive saccades generated in the absence of a reward had longer latencies than when the same saccades were made with reward provided.

Effect of the number of target locations

We observed that performance improved for targets presented within the retinotopic representation of the injected site when the total number of potential targets in a block of trials was reduced (Fig. 13). For the example illustrated in Fig. 13, with only two potential targets, performance was at a 78% level for a target presented within the area represented by the injection site (Fig. 13, top). When the number of targets was increased to eight, performance for this same target location dropped to 28% (Fig. 13, middle). Note that saccadic latency did not appear to be affected by target number. This effect of the number of potential targets was observed in the two monkeys in which specific tests were performed.

Fixation

In all muscimol injections, there was a significant increase in the variability or scatter of the initial fixation positions in comparison with controls ($P < 0.01$). There was a tendency for this scatter to increase with time after the inactivation in seven of eight cases ($P < 0.05$; Fig. 14). In 60% of the cases, the slopes of the regression lines for scatter were significantly different from those of the saline control ($P < 0.05$). Examples of the scatter of initial fixation positions in a single block of trials have been shown earlier (Figs. 2, 3, and 7, F and L).

Spontaneous eye movements after the injection

We recorded the monkey’s spontaneous eye movements outside the context of a task both after inactivation of the FEF and during no-injection controls. An example is shown in Fig. 15.

The control data demonstrate the natural pattern of eye movements for this monkey (Fig. 15A). The monkey preferred to keep her eyes near the horizontal meridian but frequently scanned a large portion of the visual field. An injection was placed at a site representing large saccades directed up and to
the right (Fig. 15B). After this injection, the monkey tended to avoid looking at the contralateral hemifield and concentrated her gaze on the lower left quadrant of visual space (Fig. 15B).

In addition, while attempting to fixate, the monkey would occasionally make a spontaneous eye movement to the location diametrically opposite to that represented at the injection site. In those cases, she would maintain her eyes stationary at that position for a while, sometimes until she was distracted, for example, by the experimenter’s voice. In addition, as we previously have reported, the monkeys tended to avoid spontaneously making eye movements of the same dimensions as the one represented at the injection site (Dias et al. 1995). This was particularly clear when the monkey was returning its eye to the central fixation position, after a saccade to an ipsilateral target. Without the injection, the monkeys make a quick return saccade to refixate the central spot. After the injection, the monkey avoided making saccades represented by the injection site, and made two or three alternative saccades to reach the center of the tangent screen.

**DISCUSSION**

Acute inactivation of the FEF strongly impaired the monkeys at making both visually and memory-guided saccades to the retinotopic representation of the injected site and also in maintaining fixation. These results showed that the FEF has an important role in the initiation and control of voluntary saccadic eye movements to both remembered and visible targets. They are particularly relevant because most studies have shown that permanent lesions of the frontal eye fields cause only minor deficits in a monkey’s oculomotor performance and thus do not emphasize the importance of this area for the generation of eye movements. A portion of the current findings have been confirmed independently by two different studies of FEF inactivation in monkeys (Shi et al. 1998; Sommer and Tehovnik 1997). In combination, the results of chronic and acute inactivation of the FEF provide support for the hypothesis that a network of interconnected brain regions is responsible for the control of eye movements. This form of organization is similar to what has been proposed for other brain functions including attention, memory, and language (Lynch 1992; Mesulam 1981, 1998; Tian and Lynch 1996, 1997). On the basis of this pattern of organization, the sudden loss of one component or node within the network initially would destabilize the network. After some time, however, the remaining oculomotor areas would tend to compensate for the lost component.

Several parameters of the saccades were changed after the injections, and these are summarized schematically in Fig. 16 along with the effects on fixation and the occurrence of premature saccades in the memory-guided saccade task. For clarity, we divided the saccades into four categories: contraversive saccades in the direction represented at the injection site (A), contraversive saccades in other directions (B), ipsiversive saccades in the direction opposite to the direction represented at the injection site (C), and ipsiversive saccades in other directions (D). Most notably, for saccades toward the retinotopic representation of the injected site (Fig. 16A), latencies and targeting errors were increased, velocities were decreased, and fewer saccades were initiated. Independent of target location, fixation was deviated ipsilaterally, and for the direction opposite to the one represented at the site, there was a high percentage of inappropriate, premature saccades during performance of the memory-guided saccade task.

The monkey was motivated to perform the task throughout our data collection, and this was controlled permanently by having targets in the ipsilateral hemifield randomly intermixed with targets in the contralateral hemifield. This was an important control because, as we showed in the experiment where the monkey was not being rewarded (Fig. 12), the monkey’s interest in the task could significantly alter several saccadic parameters.
Drug spread and time course of the effects

We chose to use muscimol as an inactivating agent in this study for several reasons. Muscimol has the same effects on cortical neurons as GABA itself, that is, it hyperpolarizes the neuronal membrane, but the effects of muscimol last for several hours, whereas injections of GABA are only effective for a few minutes (Feldman et al. 1997). Lidocaine, a local anesthetic that also has been used frequently in inactivation studies, inactivates the cortex for ~30 min (Martin 1991; Tehovnik and Sommer 1997). Because we were interested in recording the effects of FEF inactivation on different tasks and on variations of those tasks, we chose the drug that had longer-lasting effects. A final reason for our choosing muscimol over other available drugs was that relatively small amounts of muscimol are sufficient to strongly inactivate the neurons surrounding an injection site. Small injection size minimizes the possibility of damage to the cortex. For example, we usually injected 1 μl of muscimol, whereas Sommer and Tehovnik found it necessary to inject 18 μl of lidocaine to obtain reliable inactivation of the FEF (Sommer and Tehovnik 1997; Tehovnik and Sommer 1997). The smaller injections also allowed us to inactivate smaller areas of cortex and observe effects that were restricted to a portion of the FEF topographic map.

Because we did not directly measure the spread of the drug, we have used an estimate for muscimol spread in the cortex based on autoradiographic experiments done in rats (Martin 1991). In that study, there was a mean spread of 1.7 mm radius after injection of 1 μl of muscimol. The concentration of muscimol used for the Martin study was 1 μg/μl compared with 5 μg/μl for our own experiments. We should assume a larger spread in our case, and thus we must have inactivated at least 20% of the FEF area with each injection, including all the cortical layers.

Martin’s study also showed that the maximal spread of muscimol in the cortex was achieved within the first 20 min after the injection and that the width of the inactivated region remained constant for ~120 min, suggesting that increases in effects beyond this time probably were mediated by neuronal

<table>
<thead>
<tr>
<th>Target location</th>
<th>Saccades correctly initiated</th>
<th>Latency</th>
<th>Velocity</th>
<th>Error</th>
<th>Premature saccades on memory task</th>
<th>Fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>- - -</td>
<td>+++</td>
<td>- -</td>
<td>+++</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>nsa+</td>
<td>nsa</td>
<td>nsa</td>
<td>+</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>nsa</td>
<td>nsa</td>
<td>nsa</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

(*) Not significantly altered

FIG. 15. Effects of muscimol injection on spontaneous eye movements. Eye position was recorded from monkey MK2 under dim lighting conditions. There was no task for the monkey to perform; she was free to look wherever she wished. Both plots of spontaneous eye movements include eye position recorded at 1 kHz (dots were connected, for clarity) during 10 intervals of 10-s duration. Monkey was rewarded at the end of each 10-s interval. A: eye position recorded under control conditions. B: eye position recorded 3 h after injection mus11. Left: saccades that were evoked by microstimulation at the injection site.

FIG. 16. Schematic summary of the effects of partial inactivation of a site in the left FEF on performance of saccades. Except where noted ("Premature saccades on memory tasks") the effects were present for both memory- and visually guided saccades. Top, saccade represented at the injection site was horizontal to the right.
interactions (Martin 1991). We consistently found that the magnitude of effects of FEF inactivation on every parameter we tested increased with time, suggesting that there might be a progressive influence of the injection on cortical interactions with both cortical and subcortical structures. Because it is highly unlikely that the drug spread to the opposite FEF, the deficits in ipsilateral saccades were probably a consequence of these interactions.

The range of directions of saccades that were disrupted increased with time in all cases. Initially, the saccades most impaired corresponded roughly in amplitude and direction with the saccades evoked at the site of the injection before inactivation (Fig. 16A). With time, the monkeys became progressively impaired also at initiating saccades with vectors of similar directions and amplitudes (Fig. 16B), and finally they would not initiate any contraversive saccade. As was shown in Methods (Fig. 1), saccade directions vary in a continuous manner as one moves down the bank of the arcuate sulcus. If there was a spread of the drug, it would be expected that the directions of the saccades most affected would be those flanking the direction represented at the site of the injection. Notice that in Fig. 1 we are showing only one dimension (depth) of a three-dimensional structure. Because the drug spread is three dimensional, the drug spread probably affects an even larger range of directions, and also of amplitudes, than we could predict by recordings and stimulations along the penetration.

Model of the pathways affected by FEF inactivation

A simplified schematic model including some of the pathways that probably are affected by the partial inactivation of the FEF is shown on Fig. 17. In this model, we have represented the FEF, other oculomotor-related areas (including the supplementary eye field and the posterior parietal cortex), the superior colliculus (SC), and the basal ganglia (caudate and substantia nigra pars reticulata) as having serial and parallel connections to the brain stem saccade generator. We have purposely not included several other areas (i.e., thalamus and cerebellum) and connections for simplicity.

The first observation from the model is that by inactivating the FEF we also deprive several other eye movement-related areas of their normal inputs. This is probably one of the reasons why the effects of inactivation of the FEF were strikingly similar to those observed after inactivation of the SC (Hikosaka and Wurtz 1985a).

We can use this model to examine what happens when the monkey is required to make a visually guided saccade of the dimensions represented at the injection site (see also Fig. 16A). Without the inactivation, FEF neurons produce a strong presaccadic burst (Bruce and Goldberg 1985; Wurtz and Mohler 1976). This activity is transmitted to the other cortical oculomotor areas, to the SC, both directly and indirectly through the basal ganglia (Hikosaka and Wurtz 1985b; Segraves and Goldberg 1987), and to the brain stem saccade generator, both directly and by way of the superior colliculus (Raybourn and Keller 1977; Segraves 1992). Presumably, this activity integrates with the activity of other saccade-related regions, and when a threshold is reached, a saccade of normal metrics is produced.

A muscimol injection in the left FEF, in a site representing horizontal rightward saccades, will inactivate the neurons surrounding the site (Fig. 17, black region) and probably affect to a lesser extent saccades in flanking directions (Fig. 17, dark gray region). When the monkey is required to make a horizontal saccade, the FEF neurons in that region do not become active, as we attested with our recording electrode at the site of the injection. Thus the oculomotor regions downstream from the FEF, i.e., the basal ganglia, the superior colliculus, and the brain stem saccade generator, will not receive FEF input, and their activities consequently will be less than they would be with the FEF input. Because these activities are diminished, it is conceivable that it will take longer for the saccade generator to reach the threshold to initiate a saccade and that there will be a decrease in the likelihood that this threshold is reached at all. This reasoning would explain our findings of increased latencies of the saccades in the direction represented at the injection site and decrease in the number of saccades initiated after the injections in the FEF.

In support of this model, recent experiments in our laboratory have shown that the presaccadic burst of activity of superior colliculus neurons have a reduced frequency soon after a FEF inactivation with muscimol (Helminski and Segraves 1997). In some of these neurons, the duration of the presaccadic burst increased after 1 h, suggesting that the reduced collicular activity had to be present for a longer time for the saccade generator to reach its threshold. This indicates that the SC neurons, with the help of input from other eye movement-related areas including posterior parietal cortex and sup-
plesserive direction, latencies and velocities were not as
always being performed in the dark, whereas the experiment
and it is possible that the slower velocities seen in the Sommer
dark are slower than saccades made in the light (Becker 1989),
than in the present study. It is known that saccades made in the
injections were smaller in the Sommer and Tehovnik study
both studies, the difference in the velocities before and after the
contraversive saccades after the inactivations were similar in
saccades generated after injections. Because the velocities of
velocities observed both in our controls as well as for ipsiversive
saccades before the injection were much slower than the ve-
delays. In their paradigm, however, the peak velocities of
velocities for saccades to briefly flashed or permanent targets
mild and inconsistent decreases in velocities of saccades after
lesions of the FEF had reported that saccadic ve-
neurons could lead to lower firing rates, which would explain
A decreased input to the brain stem saccade generator also
would result in slower velocities. The firing rate of burst
neurons in the brain stem is well correlated with saccade
velocity (Hepp et al. 1989). A decreased level of input to these
neurons could lead to lower firing rates, which would explain
the slower velocities we observed. This decrease in velocities
after FEF inactivation was unexpected because neuron activity
in the FEF does not seem to be strongly associated with
saccadic dynamics including velocity (Segraves and Park
1993). In agreement with the present experiments, studies of
permanent lesions of the FEF had reported that saccadic ve-
locities were lowered when the monkey was scanning a board
for pieces of apple (Schiller et al. 1980) or making saccades to
remembered target locations (Deng et al. 1985). Also, Schiller
and Chou (1998) recently have reported slower velocities for
saccades directed contralateral after a unilateral FEF lesion.
Remarkably, Sommer and Tehovnik (1997) reported only
mild and inconsistent decreases in velocities of saccades after
FEF inactivation in their paradigm. They reported diminished
velocities for saccades to briefly flashed or permanent targets
(see their Fig. 14) but not for memory saccades with longer
delays. In their paradigm, however, the peak velocities of
saccades before the injection were much slower than the ve-
locities observed both in our controls as well as for ipsiversive
saccades generated after injections. Because the velocities of
contraversive saccades after the inactivations were similar in
both studies, the difference in the velocities before and after the
injections were smaller in the Sommer and Tehovnik study
than in the present study. It is known that saccades made in the
dark are slower than saccades made in the light (Becker 1989),
and it is possible that the slower velocities seen in the Sommer
and Tehovnik study were a consequence of their experiments
always being performed in the dark, whereas the experiment
room was kept dimly lit during task performance in the present
study.

Ipsilateral saccades

When the monkey was required to make a saccade in the
ipsiversive direction, latencies and velocities were not as
strongly affected as when the saccade was in contraversive
directions (Fig. 16, C and D). It is conceivable they even could
have been improved because of a disinhibition of the FEF
coding saccades in the opposite direction (represented by the
lighter area in the opposite FEF). We did not, however, find a
consistent improvement of ipsiversive saccadic latencies and
velocities in our experiments. In only a few cases, saccades
made in the direction opposite to that represented at the injection
site had shorter latencies than controls (an example can be
seen on Fig. 9B, 20 min) and faster velocities (Fig. 4, visually
guided saccades). Interestingly, Schiller and Chou (1998) also
reported increased peak velocities for saccades to ipsilateral
targets after FEF lesions.

This lateralization of effects is in contrast with human studies
in which there is evidence that unilateral FEF lesions interfere with latencies bilaterally, possibly reflecting a cere-
bral dominance of one hemisphere (Pierrot-Deseilligny 1994;
Pierrot-Deseilligny et al. 1991; Rivaud et al. 1994).

Topographical organization of the FEF

A striking observation was that soon after the injection the
monkeys predominantly were impaired in the making of sac-
cades with vectors similar to those represented at the injection
site. This was not observed in surgical lesion studies in mon-
keys because in those studies the complete FEF region was
removed, and all contralateral saccades were impaired. Simi-
larly, Sommer and Tehovnik (1997) did not report deficits
restricted to a subset of contralateral saccades, probably be-
cause in most of their inactivation cases, they infused large
volumes of lidocaine that spread to include the entire FEF.
This is an important observation because of the complex
topographical organization of the FEF. Whereas in some brain
areas there are point-to point topographical maps, for example
in striate cortex (visual map) and in the superior colliculus
(visual and motor maps), in the FEF this may not be the case.
There is a topographic representation of saccade size with
small saccades represented laterally and large saccades repre-
sented more medially (Bruce et al. 1985). Polar direction,
however, is probably mapped in columns, with neighboring
columns coding saccades with similar directions (Bruce et
al. 1985). The direction varies continuously, covering the com-
plete range of contralateral saccades, but the same direction
can be repeated in different locations in the FEF (for example,
see on Fig. 1 how saccades directed down and to the right were
electrically elicited at the depths of 4.6 and again at 6.8). There
is, for now, no detailed description of the topographical orga-
nization of saccade direction in the whole FEF. It is possible
that saccade direction is organized in the FEF in a manner
similar to the distribution of orientation columns in striate
cortex, that is, there is a topographical organization of the
visual space, but columns with different orientation repeat
throughout the area.
So did we see one main direction affected? It is a
well-known fact that if a site in the FEF, representing a certain
direction, is stimulated electrically, a saccade with a similar
direction occurs (Bruce et al. 1985; Robinson and Fuchs 1969),
even though there are several other regions representing the
same direction that are not being stimulated. If, however, you
inactivate just one of the sites where this direction is repre-
sented, you should not disrupt all saccades in that direction
because there are several other regions representing that direction, and they should be able to code the saccade. But, as mentioned earlier, that is not what happens, and inactivation of one region of the FEF affects the triggering of all saccades in a certain direction.

In striate cortex Gilbert and Wiesel (1989) have shown that distant columns are interconnected by long horizontal connections that branch only at columns of the same orientation. Horizontal connections with occasional branching, anatomically very similar to those in striate cortex, have been shown to exist in the prefrontal cortex, in a region just anterior to the FEF (areas 45 and 9) (Kritzer and Goldman-Rakic 1995; Levitt et al. 1993; Pucak et al. 1996). Although they have not yet been shown in the FEF, it is very likely they are present in this area as well. It is possible that columns in the FEF representing the same saccadic directions are anatomically interconnected by these long horizontal connections, which branch specifically at those columns representing the same direction. Thus when a site coding a specific saccadic direction is inactivated, the effects we observe are not only from inactivating the neurons in that region but also from removing input to neurons in other columns representing the same direction. This way the total activation for that direction is weaker, and saccades in that direction are not made, or are slower, and occur later.

As mentioned in the preceding text, there are reports of a topographical organization in the FEF according to saccade size. In the injections we made into sites representing small saccades, we observed that after the inactivation the monkey was impaired in making small saccades with similar direction to the one represented at the site but could still make larger saccades in that same direction. This was temporary, and as the effects increased, the monkey would avoid initiating saccades, or make inaccurate and slow saccades, to all targets in the affected direction. We have not yet done the complementary experiment, which would be to inactivate a site representing large saccades and compare the monkey’s performance on an array of targets including smaller saccades, but we predict that, at least initially, the smaller saccades would be less impaired than the larger saccades.

**Mnemonic representations in the FEF**

After FEF inactivation there seems to be a stronger need for a visual target for accurate coding of saccadic metrics. Accuracy and trajectories of saccades were more disrupted in the memory-guided saccades, where no target was present when the saccade was initiated, than in the visually guided saccades, where the target was present when the saccade began. This indicates that the FEF, at least in the monkey, participates in coding saccades to remembered spatial locations, probably by maintaining a mnemonic representation of the target’s position relative to the fovea. This idea was supported by our observation that the longer the delay imposed on the task, the more inaccurate the saccades. It is possible that with the longer delays the already weak memory signal became degraded further, resulting in larger saccadic errors.

Deficits in the generation of memory-guided saccades were seen much sooner after the injection than were the visually guided saccade deficits. As was the case for visually guided saccades, effects of an injection on memory-guided saccades at first were limited to a relatively narrow range of directions and then spread to include the complete range of contralateral directions.

Permanent lesions of the FEF of monkeys also have been shown to interfere with the learning and performance of memory-guided saccades (Deng et al. 1985). It is important to keep in mind, however, that the FEF is connected to several different areas and thus inactivating part of the FEF also interferes with the activity of other areas (Fig. 17). Accordingly, there have been reports of manipulations in different brain areas that impaired the monkey’s ability to perform memory-guided saccades. Lesions of the prefrontal area adjacent to the FEF, area 46, also interfered with monkeys’ performance of memory-guided saccade tasks (Funahashi et al. 1993). The same was observed after inactivation of posterior parietal cortex (Li et al. 1995). Inactivation of the SC also disrupted performance of memory-guided saccades in monkeys (Hikosaka and Wurtz 1985a), and the authors of this last study suggested that this was probably a result of the disruption of the cortico-nigral-collicular pathway.

In the FEF there are neurons that fire during the delay in the memory-guided saccade task (Bruce and Goldberg 1985; Funahashi et al. 1989). Interestingly, each neuron is active only when the monkey is remembering a saccade to a specific target location, indicating that this short-term, or working, memory is organized topographically. It is possible that the inactivation of these neurons with memory-related activity by the muscimol injection causes the initially restricted, in terms of size and direction, memory deficits we observe. This last observation supports the hypothesis of topographically distributed memory-fields in the prefrontal cortex (Goldman-Rakic 1996).

It is not known how this memory-related neuron activity originates. It may arise from intrinsic networks, but more likely it is part of a larger network. A circuit involved in the generation and maintenance of the memory responses could involve the cholinergic system. We recently have shown that the cholinergic input from the nucleus basalis of Meynert to the FEF probably is involved in the maintenance of a memory of target location because FEF injections of scopolamine, a cholinergic antagonist, selectively impairs memory-guided saccades (Dias et al. 1996). There are also studies showing that dopamine innervation is also important. Dopamine antagonists injected in area 46 also selectively disrupted memory-guided saccades (Sawaguchi and Goldman-Rakic 1994).

Another reported effect of permanent FEF removal is a deficit in the ordering of saccades, such as that necessary for organized scanning of visual scenes. In the weeks after an unilateral FEF lesion, monkeys had difficulty performing a two-step sequence of saccades in the contralateral direction, whereas they were not impaired when saccades were in the ipsilateral direction (Schiller and Chou 1998). In another experiment, monkeys trained to recover small pieces of food in an array of covered holes had a tendency to return their fixations more times to holes that already had been seen after a FEF lesion than before the lesions (Collin et al. 1982), suggesting that the FEF could be involved in internally organized visual scanning. Also humans with frontal lesions have a less-organized scanning strategy when looking at a picture (Luria et al. 1966). Interestingly, the same patients also had difficulty scanning the same picture in the dark with a narrow beam from a flashlight, indicating that the patient might have not only a deficit in oculomotor scanning but a more general
inability to organize systematic scanning of the environment (Collin et al. 1982). Further evidence that the FEF is involved in organizing sequences of saccades is provided by a recent study by Burman and Segraves (1994), who recorded neuronal activity in the FEF of monkeys preceding saccades made during visual scanning.

This scanning deficit can be seen as a deficit in short-term memory of target locations. It is possible that the subjects were impaired in retaining the location of the previous saccadic target to “program” the next saccades in their visual search. If the subjects could not remember where they have looked before, they tended to return to the same place repeated times, showing disorganized scanning strategies.

Premature saccades and the role of the FEF in controlling fixation

Another important observation after the muscimol injection in the FEF was that the monkeys, when tested with the memory-guided saccade task, had difficulty suppressing premature saccades made toward targets in the ipsilateral hemifield (Fig. 11) (see also Dias et al. 1995; Sommer and Tehovnik 1997). This deficit began with the monkey making anticipatory saccades toward the location of the target before the cue to begin the saccade, and with time the monkeys became increasingly incapable of withholding saccades to the visual target when it was flashed on the screen, and most premature saccades were visually guided.

Human patients with unilateral frontal lesions also have difficulty suppressing unwanted saccades to suddenly appearing visual targets. Guitton and his colleagues (1985) tested patients with an antisaccade task in which the patient was instructed to make a saccade to the direction opposite to that of the visual target. They showed that the patients frequently made an initial reflexive saccade to the visual target when it came on, just the opposite of what they were instructed to do. These effects were mostly bilateral, unlike the unilateral ones we observed in the monkey, again probably reflecting the greater lateralization of brain function in humans.

Another study, however, reported that patients with unilateral ischemic lesions more restricted to the FEF region show an increase in the latency of saccades when tested with the antisaccade task, but they do not report reflexive saccades to the visual target (Rivaud et al. 1994). Rivaud and colleagues suggest that the discrepancy between their results and those of Guitton’s group could be due to the fact that the cortical damage in their patients was more restricted to the FEF than that of patients examined by Guitton and colleagues and that other areas in the frontal cortex, not damaged in their patients, might be responsible for suppressing the premature saccades. It is interesting to note, however, that with our very restricted injections on monkey FEF, we frequently observed premature saccades, and this was confirmed by the study of Sommer and Tehovnik (1997). It will be interesting to verify, with future experiments, which of these results are a consequence of interspecies variability and which are a result of the location and extent of cortical damage.

Overall, these studies in both monkeys and humans indicate that removal of the FEF interferes with the control of fixation, in some instances by diminishing the control over unwanted saccades and in others by interfering with the process of disengagement of fixation (Dias et al. 1995; Guitton et al. 1985; Rivaud et al. 1994; Sommer and Tehovnik 1997). The scatter in initial eye position observed in our study further emphasizes the monkey’s difficulty in maintaining fixation.

According to our model, when we inactivate a region of the FEF on one side of the brain, a corresponding region in the contralateral FEF coding saccades of similar sizes but opposite direction is “disinhibited” (Fig. 17, lighter area in the right FEF), and this disinhibition is transmitted to oculomotor areas downstream. In our experiments, we observed in a few cases a tendency for the monkey to move its eyes to a position corresponding to the exact opposite of the saccade represented at the injection site, suggesting an augmented “drive” for that saccade. Additional support for this idea is provided by a recent report that showed that if a saccade is elicited by stimulating one FEF, the neurons in the other FEF are inhibited if their preferred directions are different from that of the elicited saccade and excited if they are similar (Schlag et al. 1998).

If this disinhibition occurs, it is easy to imagine that it will raise the activity of the oculomotor areas downstream from the FEF such that when a visual target is flashed in the ipsilateral hemifield, there will be a strong drive to initiate a saccade toward it and a premature saccade will be initiated. The consistency with which we observed premature saccades into the ipsilateral hemifield after the FEF inactivations further supports this hypothesis.

An involvement of the FEF in fixation mechanisms had been previously suggested by Latto and Cowey (1971) when studying monkeys with FEF lesions. They showed that unilateral removal of FEF caused deficits in fixating contralateral targets and produced a consistent, transient, shift in fixation of ≤10° ipsilateral that recovered within a couple of weeks. Removal of the other FEF after recovery produced an even larger shift in the opposite direction. A difficulty in fixing contralateral targets after acute inactivation of the FEF also was shown by Sommer and Tehovnik (1997). We also observed that after the FEF inactivation the monkeys preferentially would maintain their gaze directed toward the ipsilateral hemifield (Fig. 15).

One possibility for this progressive deviation in fixation could be that each FEF contributes to a drive to look into the contralateral hemifield. Under normal conditions, the two FEF balance this drive, and the monkey fixates in the center. When one FEF is disabled by inactivation or lesion, there is an imbalance in this drive with the result that the intact FEF causes the eyes to be deviated ipsilaterally. A similar suggestion was made by Hikosaka and Wurtz (1985a) to explain why their monkeys were fixating ipsilaterally after SC inactivations. They proposed that inactivation of the SC reduced the probability of saccades of a particular direction and amplitude. If the average orbital position depended on the probability of saccades in all directions, then reducing the probability in one direction would shift the average orbital position ipsilaterally.

In support of a role of the FEF in the control of fixation, recent functional imaging experiments in humans show that the cortical region believed to be analogous to the monkey’s FEF is activated during fixation tasks (Anderson et al. 1994; Pet et al. 1995). There is also ample evidence that neurons in the FEF of monkeys have activity related to fixation (Bizzi 1968; Bizzi and Schiller 1970; Bruce and Goldberg 1985; Suzuki and Azuma 1977), and these neurons have projections both to the SC (Segraves and Goldberg 1987) and to the pons (Segraves
FRONTAL EYE FIELD INACTIVATION

2213

1992). The activity of many presaccadic neurons in the FEF also carry a signal representing the disengagement of fixation ( Dias and Bruce 1994). These neurons will be suppressed during FEF inactivation. Because the inactivation is restricted to a subregion of the FEF and the other hemisphere is not inactivated, the monkey is still able, to a certain extent, to maintain fixation at the center of the screen and perform oculomotor tasks after the inactivation, although the precision of fixation gets progressively worse.

In conclusion, we have demonstrated with these experiments that acute inactivation of the FEF produces profound changes in oculomotor behavior in monkeys. This very dramatic impairment, compared with previous lesions studies, is probably due to the acute nature of the injections, which limits reorganization and recovery of function within the oculomotor network. These results implicate that the FEF has a stronger role in coding and triggering saccades than the previous lesion studies suggested. Future experiments, with more specific drugs that only inactivate parts of the circuit, will allow us to dissect further the mechanisms of saccade generation in the FEF.

We are grateful to M. Kiesau for participating in the initial experiments of this project and to P. Ko, D. Compaan, and the staff of Northwestern University’s Center for Animal Resources for animal care. We thank M. Sommer for many interesting discussions of the results and C. J. Bruce and A. Ragin for help with the statistical analysis. We also thank our anonymous reviewers for their contributions to the paper.

This work was supported by National Eye Institute Grants EY-08212 and EY-06716.

Present address of E. C. Dias: Program in Cognitive Neuroscience and Schizophrenia, Nathan Kline Institute, 140 Old Orangeburg Rd., Orangeburg, NY 10962.

Address for reprint requests: M. A. Segraves, Dept. of Neurobiology and Physiology, 2153 N. Campus Dr., Northwestern University, Evanston, IL 60208-3520.

Received 8 December 1998; accepted in final form 4 February 1999.

REFERENCES


HUERTA, M. F., KRUBITZER, L. A., AND KAAS, J. H. Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and


Keating, E. G. and Gooley, S. G. Saccadic disorders caused by cooling the superior colliculus or the frontal eye field, or from combined lesions of both structures. Brain Res. 438: 247–255, 1988.


